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DOI: <https://doi.org/10.31979/etd.tbfe-k455>

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SURFACE AND BIOCOMPATIBILITY STUDY
OF ELECTROPOLISHED Co-Cr Alloy L605

A Thesis

Presented to

The Faculty of the Department of General Engineering
San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Hokuto Aihara

August 2009

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SURFACE AND BIOCOMPATIBILITY STUDY
OF ELECTROPOLISHED Co-Cr Alloy L605

By

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ABSTRACT

SURFACE AND BIOCOMPATIBILITY STUDY OF ELECTROPOLISHED Co-Cr Alloy L605

by Hokuto Aihara

Electropolishing has been widely used as a surface-modification technique to enhance tissue-to-material interaction by generating a smooth hydrophilic surface and forming a new protective oxide layer. The current study was undertaken to investigate the effect of temperature and time on the surface characteristics and biocompatibility of Co-Cr alloy electropolished in 15% (v/v) phosphoric acid. As the bath temperature was decreased from 35°C to 0°C, a lower and wider current plateau was generated on the characteristic I-V curve. The electropolishing rate decreased proportionally with a decrease in bath temperature. As the electropolishing time increased, the surface roughness decreased with an increase in the contact angle. Cr_2O_3 and $\text{Cr}(\text{OH})_3$, as detected by XPS analysis, and cell density (cells/ml) increased proportionally with an increase in electropolishing time. Controlled electropolishing was found at a lower bath temperature, and the formation of Cr_2O_3 increased with respect to electropolishing time, resulting in no cytotoxic effects.

ACKNOWLEDGEMENTS

The author wishes to acknowledge Dr. Guna Selvaduray for his patience, guidance, and continuous support in completing this research, and Dr. Raymond Yee for serving on the thesis committee. The author would like to thank Dr. John Turn for advising and giving guidance on electropolishing, in addition to serving on the thesis committee. The author also thanks Rolled Alloy, Inc. for donating the Co-Cr alloy L605 used in this research and Dr. Angela Y. Craig and John Moskito for graciously conducting the XPS and AES analyses, respectively. The author would also like to thank Mr. Cormia for his kind assistance with the AFM analysis. A special thanks to Utako Aihara, the author's mother, and Brian Hutchinson, for their support throughout the entire process.

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CHAPTER ONE

INTRODUCTION

Heart disease is the leading cause of death in the U.S. for both men and women. In 2002, 696,947 people died from heart disease worldwide, of whom 29% were from the U.S.[1].

In order to minimize heart attacks and arterial blockages, stents were introduced as a biomedical device to prevent coronary arteries in the heart from collapsing. In 2003, the coronary stent market was estimated at approximately \$3.0 billion and was dominated by four large companies: Johnson & Johnson Inc, Medtronic Inc, Boston Scientific, and Abbott Laboratories [2].

When a stent is deployed in the artery, the surface of the stent is exposed to the artery lumen walls. Surface modification processes are therefore used to improve tissue-to-material interaction. Vascular implants such as cardiovascular devices, vascular stents, and pacemakers are commonly electropolished as a surface-finishing process to improve their biocompatibility [3].

The main reason for electropolishing implants is to produce a smooth surface and form a passive oxide layer to reduce the corrosion rate; electropolishing also relieves residual stress. Specific formulations of electrolytes are necessary when electropolishing different alloys and metals.

Conventional coronary stents are fabricated from either medical-grade stainless steel 316L or NiTi. Co-Cr alloy L605 has been introduced in the coronary stent market as a bare stent. A comparison of the mechanical properties of the two metals helps in

understanding the material application for stents. The specific mass (g/cm^3), elastic modulus (GPa), and yield strength (MPa) for 316L stainless steel and L605 are 7.95 and 9.1 g/cm^3 , 193 and 243 GPa, and 340 and 500 MPa, respectively [4]. Co-Cr alloy L605 exhibits superior mechanical and material properties. Therefore, coronary stents can be fabricated with thinner struts without compromising the radial strength, while improving flexibility and deliverability [4].

The current investigation was undertaken to determine the surface characteristics and biocompatibility of electropolished Co-Cr alloy L605. In order to establish a process current for electropolishing, a current plateau was first experimentally generated for each temperature and electrolyte. With the process current defined, test specimens were electropolished under different conditions and the surfaces characterized.

Co-Cr alloy L605 and the mechanism of electropolishing are introduced in Chapter Two. The research objectives are explained in Chapter Three. Electropolishing methodology is covered in Chapter Four, followed by Results, while the current findings are summarized in Chapter Five. The experimental findings and biomedical applications are discussed in Chapter Six. The conclusion and recommendations for future research are presented in Chapters Seven and Eight, respectively.

CHAPTER TWO

ELECTROPOLISHING

The first observation of electropolishing was documented by Madsen in 1925 during the production of high quality electrodeposition [5]. Three years later, Burns and Warner applied for a patent for an electro-cleaning process using 70 -100% (v/v) phosphoric acid and a current density of 100 amp/ft². They recorded a brightened finish on a steel surface after the experiment. The development and enhancement of the electropolishing process was extensively studied in 1935 by Jacquet [6], who electropolished copper and other metals using a mixture of acetic and perchloric acid of varying concentrations to test the surface brightening effect, as an alternative to mechanical polishing.

2.1 Electropolishing Background

The electropolishing process has been shown to be effective in enhancing tissue-to-material interaction; a passive layer that protects the bulk material from further oxidation is formed [7]. Smooth and hydrophilic high surface-energy surfaces that prevent adhesion of platelets and endothelial cells are produced. The prevention of platelet and endothelial cell adhesion ultimately reduces the cases of thrombosis* [8]. Methods and challenges of obtaining smooth and hydrophilic surface on the test specimen by electropolishing is elucidated in the next section.

* Thrombosis is the formation of a clot (thrombus) inside a blood vessel, which reduces and ultimately prevents the flow of blood, causing conditions such as heart attack or stroke [8].

Although electropolishing has numerous benefits, there are also several disadvantages that can be categorized into two types: 1) difficulties in handling the electrolyte, and 2) difficulties in selecting an appropriate chemical solution for multi-phase alloys. Since most chemical solutions are hazardous and poisonous, care must be taken in handling the electrolyte. The material for the mounting unit should not react with the electrolyte used nor should it be coated to avoid unwanted side reactions. Because the rate of electropolishing is low, deep scratches or gouges from prior material processing may not be fully removed if the material is not electropolished for a sufficient length of time. The processing time is a critical factor in any electropolishing process.

Another disadvantage is the difficulty in selecting electrolytes for multi-phase alloys. The efficacy of electropolishing is greatly influenced by the process parameters. Electropolishing two different alloys with the same electrolyte does not guarantee the same quality of surface finish.

The electropolishing process is driven by the potential difference between the anode and the cathode. If the anode is a multiphase alloy, then the local potential differences can be dispersed during electropolishing. If the second phase of the work piece is anodic, the dissolution of the metal occurs in the second phase rather than in the first phase; if the second phase is cathodic, the material surrounding the second phase is polished, potentially detaching the cathode and leaving pits on the surface [8]. Despite its complexity, electropolishing has been used widely.

Industries that utilize electropolishing range from food and beverage to medical, pharmaceutical and electronic, in which clean and smooth surfaces are desirable [9]. The

formation of a passive film during electropolishing not only improves corrosion resistance but also reduces bacterial growth [10]. Therefore, vascular, dental, and orthopedic implants manufactured from Co-Cr alloys, 316L stainless steel, and NiTi are electropolished [3].

2.2 Electropolishing Mechanism

During electropolishing, the dissolution of metal ions occurs at the anode, and metal ions are released in the electrolyte and deposited on the cathode. The surface finish is characterized by smoothening and brightening. The smoothening occurs at a macroscopic level, highly dictated by the thickness of the anodic film formed during electropolishing; brightening occurs at a microscopic level, dictated by the formation of a thin oxide layer on the anode [11]. The work pieces are submerged in an aqueous acidic electrolyte. As shown in Figure 1, a DC current is delivered through the rectifier to the anode.

During this process, polarization occurs at the site of the anode, and metal ions dissolve from the anode [6]. Hydrogen gas is also formed at the cathode, and oxygen gas may evolve from the anode, depending on the voltage.

The material removal from electropolishing can be explained using Faraday's Law of Electrolysis, as shown in Equation 1 [12],

$$W_{loss} = \frac{ItM}{nF} \quad \text{Equation [1]}$$

where W_{loss} is the total material loss, n is the valence of the metal ion, F is Faraday's constant (96,500 Columnb), M is the molecular weight of the anode, I is the process current, and t is the polishing time. M , n , and F are constants. The two variables that affect the total material loss during electropolishing are process current (I) and electropolishing time (t).

Figure 1. Schematic diagram of the apparatus and material involved in electropolishing. J.B. Durkee, "Another Kind of Cleaning: Electropolishing I," *Metal Finishing*, 101(11), Nov. pp.59-63 (2003). Reprinted with permission of the author.

According to Kuhn [13], anodic dissolution is determined by the mass transport of the metal ions. The three equations that denote the chemical reaction at both the anode and the cathode during electropolishing of chromium are shown below:

Anode Dissolution

Equation [2]

Oxygen Formation

Oxide Layer Formation

Chromium oxidation, where metals on the surface are oxidized resulting in dissolution for mass transfer through the aqueous medium, is shown in Equation 2. The rate of electropolishing is greatly dependent on the tendency for the metal ions to dissolve from the anode into the medium. Therefore, the higher the current density, the more readily the metal ions dissolve from the anode. The oxygen gas evolution at the anode, which occurs during electropolishing, is shown in Equation 3 [14]. The formation of the oxidation layer, which occurs on the anode surface, is shown in Equation 4 [2]. The passivation of the anode plays a role in reducing the corrosion rate and protecting the bulk material from further oxidation.

2.3 Defining Process Current from an I-V Curve

A characteristic I-V curve needs to be generated by varying both temperature and electrolyte composition prior to electropolishing the work piece. An I-V curve is generated by altering the DC voltage and plotting it against the current. The purpose of the I-V curve is to determine whether the temperature and electrolyte combination exhibit a current plateau. As can be seen in Figure 2, the current plateau is where changes in the voltage no longer induce flux in the process current density, providing better control over the rate of electropolishing.

During electropolishing, hydrogen and oxygen gas are produced from the cathode and anode, respectively, as shown in Figure 3 [14]. The low rate of metal removal is the limiting factor. Because electropolishing occurs at approximately $1 \mu\text{m}/\text{min}$. under certain circumstances, damages caused during the material drawing process may not be fully removed if electropolishing is conducted for a short time. As indicated by Faraday's Law of Electrolysis, polishing time is one of the factors that determines the amount of metal removed from the surface.

Figure 2. An ideal characteristic I-V curve for the determination of the process current and associated voltage.

Furthermore, due to the low diffusive mechanism, a thick viscous film is formed on the surface of the anode. This retards the electrochemical reaction, forming a current plateau on the I-V curve [14]. The polishing of the anode surface occurs within the

viscous film. Electropolishing also occurs in the absence of the current plateau; however, due to the current flux, controlled electropolishing may be difficult to attain.

For electropolishing 316L stainless steel, the characteristic I-V curve was generated by Lee and Lai [14]. They determined the voltage range and the process current density from the I-V curves. By electropolishing with the current on the current plateau, they have shown that the anode surface becomes enriched with chromium with a reduction in the surface roughness to $0.03\text{ }\mu\text{m}$ while electropolishing with a distance greater than 5 mm. An experiment conducted by Lee [15] showed that polishing of the surface occurs even when a current density is selected outside the current plateau. Lee showed that a smooth surface was obtained when the current density was above 8 A/cm^2 . The surface roughness decreased proportionally with an increase in the current density. However, the variability in the measured surface roughness was not discussed.

Figure 3. Schematic diagram of electropolishing principle. E.-S. Lee, "Machining Characteristics of the Electropolishing of Stainless Steel (STS316L)," Int J Adv Technol, 16, 591-599 (2000). Reprinted with permission of the author.

An I-V curve was also generated by Murali et al. prior to electropolishing Al-7Si-0.3Mg [11]. The electrolyte used for the experiment was a combination of various concentrations of perchloric acid, methanol, nitric acid, and glycerol. They showed that the current-voltage relationship was heavily influenced by two parameters, acid concentration and bath temperature. At a bath temperature of 26°C, a high current density and a short current plateau were found. When the temperature of the bath was reduced from 26 to -33°C, there was a current density reduction of 79 mA/cm² from 94 mA/cm² to 15 mA/cm². The reduction in the current density indicated that the rate of electropolishing was reduced. As the temperature was reduced, the I-V curve showed a lower current density over a wide voltage range. A low current density of 15 mA/cm² indicated a low and controlled dissolution of the metallic ions in the electrolyte solution.

2.4 Electropolishing Parameters

2.4.1 Effect of Bath Temperature on Surface Characteristics

Bath temperature is one of the more important variables that affects the surface characteristics. Murali et al. [11] electropolished Al-7Si-0.3Mg in a 70% (v/v) methanol and 30% (v/v) perchloric acid mixture while varying the temperature of the acid bath at 26, 5, -22, -33, and -65°C. By using an external bath composed of liquid nitrogen, Murali et al. [11] reduced the bath temperature below ambient temperature. The current density decreased with a decrease in the bath temperature. The lowest surface roughness of 35 µm was obtained when the bath temperature was reduced to -33°C and a current density of 15 mA/cm². At -65°C, low surface roughness was not obtained due to the low

current density of less than 2 mA/cm^2 . With such a low current density, the effect of polishing and smoothing the surface was limited, which resulted in a relatively higher surface roughness than the test specimens that were electropolished at a higher current density.

Another experiment conducted by Lee [15] showed that the surface roughness increased as the bath temperature was decreased. Type 316L stainless steel was electropolished in a 50% (v/v) phosphoric acid, 20% (v/v) sulphuric acid, and 30% (v/v) distilled water mixture. The total electropolishing time was set at 400 s and the current density was set at 9 A/cm^2 . The electrode gap distance and the bath temperature were at 0.5 mm and 40°C , respectively. The average surface roughness decreased from approximately 2 to $0.5 \text{ }\mu\text{m}$, after 150 s of electropolishing. When the test specimen was electropolished for an additional 250 s, the average surface roughness decreased to $0.2 \text{ }\mu\text{m}$. At the completion of 400 s of electropolishing, the average surface roughness decreased from 2 to $0.2 \text{ }\mu\text{m}$. Therefore, the surface roughness decreased with an increase in the electropolishing time.

Although bath temperature alone affects the surface characteristics, Lin and Hu [16] showed that the interaction of temperature with electropolishing time also affected the surface roughness. Type 304 stainless steel was electropolished in a 2:1 phosphoric acid:sulfuric acid solution with the current density held constant at 0.5 A/cm^2 . When the bath temperature and electropolishing time were set at 80°C and 10 min., respectively, the highest surface roughness of $44.7 \text{ }\mu\text{m}$ was obtained. The lowest surface roughness of $8.0 \text{ }\mu\text{m}$ was obtained when the bath temperature and the electropolishing time were

decreased to 30°C and 5 min. When the bath temperature and the electropolishing time were decreased to 5°C and 3.75 min., the surface roughness increased to 13.5 μm . As both bath temperature and electropolishing time were decreased, the surface roughness decreased to a minimum. The surface roughness increased as the bath temperature and the electropolishing time were further decreased.

As the temperature decreases, the process current decreases. The decrease in the bath temperature, that correlates to a decrease in the process current, and the electropolishing time affect the total material removed from the surface. When less material is removed from the work piece, the surface roughness increases. The increase in the surface roughness resulted from the decrease in the two critical variables: process current and electropolishing time.

2.4.2 Effect of Electropolishing Time on the Surface Characteristics

Electropolishing time is one of the two variables in Faraday's Law of Electrolysis for calculating the total material removed from the work piece (anode). As electropolishing time is increased, more material is removed from the anode surface. The effect of electropolishing time on surface roughness was explained by Lee [15]. When he varied the electropolishing time from 0 to 400 s, the average surface roughness decreased with an increase in the polishing time [15]. As the polishing time increased from 120 to 200 s, protuberances and damaged layers were removed and the microstructures were revealed. When the polishing time was increased to 200 to 400 s, the characteristic microstructures of the 316L stainless steel were revealed. Another notable study that

characterized the relationship between surface roughness and electropolishing time was conducted by Lee and Lai [14]. 316L stainless steel was electropolished in a phosphoric acid, sulfuric acid, glycerin and DI water mixture at 60°C, 0.09 A/cm² with an anode and cathode distance of 7 mm. Electropolishing times of 3, 5, and 10 min. were used. The initial average surface roughness was set at 0.1 µm. The average surface roughness at 3 min. of polishing time was 0.110 µm. As the polishing time increased to 5 min., the average surface roughness decreased to 0.033 µm. At 7 min. of polishing time, an average surface roughness of 0.030 µm was obtained. The results of this study were in accordance with Lee [15]. The average surface roughness decreased when the polishing time was increased.

2.4.3 Effect of Acid Concentration on Surface Characteristics

Electropolishing is conducted in a bath composed of one or several acid mixtures with the addition of moderators and other additives [11]. Murali et al. [11] electropolished Al-7Si-0.3Mg in 5, 10, 20, 30, and 40% (v/v) of perchloric acid. As the concentration was increased to 30% (v/v), a wider process voltage range was obtained, from 4.0 to 20.5 V. As the concentration increased to 40% (v/v), while maintaining the bath temperature constant at -33°C, the region of the current plateau decreased. The current density also decreased from 30 mA/cm² to 15 mA/cm². The widest current plateau on the I-V curve was found at 30% (v/v) perchloric acid. This research indicated the significance of the acid concentration for the generation of an electropolishing region on

the I-V curve. More than one type of acid mixture can be used for electropolishing, that highlights the versatility of the process.

2.5 Surface Characterization

Surface analysis techniques are used to reveal nano-scale surface characterization differences on the electropolished specimens. The surface roughness and contact angle measurements affect cellular adhesion. Surface chemistry is important to assess corrosion resistance by determining the surface oxide layer produced during electropolishing.

Monitoring and quantifying surface characteristics are the critical areas of interest for medical implants due to tissue-material interaction. In the case of coronary stents, material surface characteristics play a critical role in preventing the development of thrombosis, restenosis[†] or neointimal hyperplasia[‡] [7]. Combinations of these surface characterizations can be used to predict the tissue-to-material interaction between implants and the surrounding cells.

2.5.1 Effect of Surface Roughness Post Electropolishing

According to Mani et al. [7], susceptibility to thrombogenicity was greater on rougher surfaces than smoother surfaces. Therefore, a smooth surface for stent applications is desired to reduce the risk of inducing thrombosis. Kang and Lee [17]

[†] Restenosis is the re- formation of a clot (thrombus) inside a blood vessel, which reduces and ultimately prevents the flow of blood, causing conditions such as heart attack or stroke [7].

[‡] Neointimal hyperplasia is a body response to an injury or complication of arterial reconstruction, e.g., in-stent restenosis [7].

electropolished AISI type 316L stainless steel with phosphoric acid, glycerol and water (34: 47:19 wt%) for 3 min. at 1 A/cm²; the average surface roughness was 1.4 nm ± 0.4 nm compared to the average surface roughness of 116.4 nm ± 26.3 nm of the unpolished specimen. The surface roughness profile before and after electropolishing is shown in Figures 4 (a) and (b). Kang and Lee [17] showed that a smooth and hydrophilic surface reduced the protein absorption, compared to a rougher hydrophobic surface.

2.5.2 Contact Angle Measurements

Thrombogenicity increases with an increase in the surface energy [7]. For implants such as stents, a low energy surface is desired in order to prevent the adhesion of platelets and endothelial cells. The hydrostatic interaction of the surface-modified specimen can be determined by using the sessile drop method and measuring the contact angle of a single droplet of DI water on the specimen surface [18].

The contact angle is the angle between the tangent line and the baseline on the solid surface. As can be seen in Figure 5, γ_{LV} is the tangent line and the baseline is represented by γ_{SV} and γ_{SL} [19]. The contact angle is measured to determine the hydrostatic property of the material surface. The force equilibrium for determining the contact angle is shown in equation [5].

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta \quad \text{Equation [5]}$$

γ_{LV} is the interfacial energy between solid and vapor, γ_{SL} is the interfacial energy between solid and liquid, γ_{LV} is the interfacial energy between liquid and vapor, and θ is the

contact angle between the liquid and the solid. The wettability of the surface increases with a decrease in the contact angle, that also correlates to a decrease in the surface energy [19].

Figure 4. (a) Surface roughness profile of bare stainless steel. (b) Surface roughness profile of electropolished stainless steel. C.-K. Kang and Y.-S. Lee, "The Surface Modification of Stainless Steel and The Correlation Between The Surface Properties and Protein Adsorption," J Mater Sci Mater Med, 18, 1389-1398 (2007). Reprinted with permission of the author.

Kang and Lee [17] electropolished AISI type 316L stainless steel in phosphoric acid, glycerol, and water (34:47:19 wt%) for 3 min. and with a current density of 1 A/cm². The contact angles of the electropolished specimens were compared to that of bare stainless steel using the sessile drop method. The average contact angle roughness of the bare stainless steel was $78.8^{\circ} \pm 1.9^{\circ}$. Electropolished specimens exhibited an average contact angle less than 10° and a surface roughness of $1.4 \text{ nm} \pm 0.4 \text{ nm}$. The contact angle measured after electropolishing indicated high hydrophilicity. The electropolished 316L stainless steel specimens that exhibited greater wettability and smoother surfaces had a decrease in protein absorption, which correlates to a reduction in thrombogenicity.

Figure 5. Schematic diagram of contact angle created with a liquid droplet and the material surface and the equilibrium of forces of vapor, liquid, and solid. K. Neuking, A. Abu-Zarifa and G. Eggeler, "Surface Engineering of Shape Memory Alloy/Polymer-Composites: Improvement of the Adhesion Between Polymers and Pseudoelastic Shape Memory Alloys," Mater Sci Eng A, 481-482, 606-611 (2008). Reprinted with permission of the author.

Chu et al. [20] electropolished 10 mm x 10 mm x 1 mm NiTi samples in a 21% (v/v) perchloric acid and 79% (v/v) acetic acid electrolyte at 10 V for 6 min. The water contact angle with water was determined to be $43.5 \pm 4.8^\circ$. The low contact angle was attributed to an increase in the formation of the Ti-OH group after electropolishing. The electropolished samples were tested for hemocompatibility. The results showed that both wettability and hemocompatibility increased after electropolishing.

2.5.3 Theories behind Oxide Formation

Through electropolishing, the old oxide layer is replaced by a new uniform oxide film while the metal surface is polished. The newly formed oxide layer and the stability of the oxide layer play a role in improving corrosion resistance. The formation of an oxide layer is dependent on numerous variables, such as electropolishing time, process current, bath temperature, acid type, and concentration. When electropolishing alloys,

more than one type of surface oxide can be formed. In order to determine the primary surface oxide, the Standard Gibbs Free Energy of Formation [21] is used to determine the oxides that will preferentially form on the work piece (anode). The Pilling Bedworth ratio is used to determine the adherence and protective properties of the surface oxides. In this section, theories behind the formation and stability of the surface oxide layer are considered.

2.5.3.1 The Standard Gibbs Free Energy of Formation

The stability of the oxides formed can be theoretically determined using the Standard Gibbs Free Energy of Formation [21], according to Equation [6]

$$\Delta G = \Delta G_f^\circ + RT \ln Q \quad \text{Equation [6]}$$

where Q is the reaction quotient and R is the universal gas constant. (8.31451 J/K mol). T represents temperature in Kelvin. ΔG is the free energy difference of reactants and products. ΔG_f° is the standard free energy of formation. At equilibrium, ΔG equals zero because there is no tendency for the reaction to proceed in either direction, resulting in Equation [7],

$$\Delta G_f^\circ = -RT \ln K \quad \text{Equation [7]}$$

where K is the equilibrium constant for the reaction. When $\Delta G_f^\circ < 0$, the reaction is spontaneous and favors the product. When $\Delta G_f^\circ > 0$, the reaction is not spontaneous and favors the reactants [21].

2.5.3.2 Pilling Bedworth Ratio

Electropolishing produces a protective oxide layer. The Pilling-Bedworth ratio (P-B ratio) can be used to determine the adherence of the passive oxide layer, and can be calculated according to Equation 8 [22].

$$PB_{ratio} = \frac{(M_{C_2O_3})(\rho_{Cr})}{n(M_{Cr})(\rho_{C_2O_3})} \quad \text{Equation [8]}$$

where M represents the atomic or molar mass, n is the number of metal atoms in the oxide, and p is the density. When the P-B ratio is between 1 and 2, the oxide film is nonporous and adherent, thus providing protection to the underlying bulk material. A ratio above 2 indicates an oxide film that is non-adherent and non-protective and would not protect the underlying bulk metal. A ratio below 1 exhibits an oxide film that is porous and would not protect the underlying bulk material.

2.5.4 Effect of Electropolishing on Biocompatibility and Corrosion

The surface property changes induced by electropolishing have been reported to affect corrosion resistance and biocompatibility. Trépanier et al. [23] compared the corrosion characteristics of electropolished (EP), passivated (PA), air aged (AA), and

heat treated (HT) NiTi (50.8 at% Ni) 4 mm in diameter and 14 mm in length. A potentiodynamic anodic polarization test was used to investigate the corrosion resistance of the different surface modifications. Auger Electron Spectroscopy (AES) was used to determine the oxide layer thickness and X-ray Photoelectron Spectroscopy (XPS) was used to determine the Ni/Ti ratio before and after the corrosion test. The corrosion experiment was conducted in a 37°C solution. AES analysis showed that the oxide thickness increased in the order of PA ($300 \pm 10 \mu\text{m}$) < EP ($340 \pm 140 \mu\text{m}$) < AA ($2400 \pm 780 \mu\text{m}$) < HT ($9110 \pm 270 \mu\text{m}$).

As shown in Figure 6, the Ni/Ti ratio decreased after the corrosion test among specimens that were electropolished (EP), air aged (AA), and passivated (PA), but not those that were heat treated (HA). Trépanier et al. [23] proposed that the TiO_2 on the HA specimen was non-stoichiometric and promoted the diffusion of Ni to the surface. Specimens that were treated with EP, AA, and PA showed that the Ni diffusion to the surface was minimized even after the corrosion test. As can be seen in Figure 7, the breakdown potential of the EP specimen was greater than that of bare NiTi. According to the corrosion test, surface modified specimens showed an increase in corrosion resistance.

Cisse et al. [24] compared the NiTi corrosion resistance of mechanical polishing (MP), electropolishing (EP), chemical passivation (CP), straw-colored oxide finishes (SCO), and blue-colored oxide finishes (BO) in Hanks solution. The Ni release decreased as a function of time, that was in accordance with the finding by Trépanier et al. [25]. Ni release from the specimens was monitored after 9 and 25 days. The amount

of Ni ions released increased in the order of EP < MP < CP < BO < SCO. 30 ppm was required to trigger a cytotoxic response *in vitro*.

Figure 6. Ni/Ti ratio of NiTi specimens before and after surface treatments: electropolished (EP), air aged (AA), heat treated (HT) and passivated (PA). C. Trépanier, M. Tabrizian, L.H. Yahia, L. Bilodeau and D.L. Piron, "Effect of Modification of Oxide layer on NiTi Stent Corrosion Resistance," J Biomed Mater Res, 43(4), 433-440 (1998). Reprinted with permission of the author.

The release of Ni for SCO was 1.2 ppm after 25 days, that was below the threshold [24]. The thickness of the oxide layer was measured by AES. EP did not produce the thickest oxide layer. The oxide thickness increased in the order of CP (650 μm) < EP (1300 μm) < MP (2000 μm) < BO (7300 μm) < SCO (10600 μm). A cyclic polarization test was also conducted. For the EP sample, there was no hysteresis loop formed during cyclic polarization. Therefore, the authors concluded that there was no chance for pitting corrosion to occur for electropolished NiTi.

Figure 7. Breakdown potential of surface modified NiTi specimens: passivation (PA), electropolishing (EP), heat treatment (HT), air aged (AA), and no treatment (NT). C. Trépanier, M. Tabrizian, L.H. Yahia, L. Bilodeau and D.L. Piron, "Effect of Modification of Oxide layer on NiTi Stent Corrosion Resistance," J Biomed Mater Res, 43(4), 433-440 (1998). Reprinted with permission of the author.

Tepe et al.[26] conducted an *in vitro* experiment to measure the thrombogenicity of NiTi stents subjected to various surface treatments, namely mechanical polishing, passivation, and electropolishing. They measured the thrombocyte activation and tissue inflammatory response, as well as the platelet count and the level of β -TG, TAT, and PMN-elastase after 120 min. of blood exposure. Reduced thrombogenicity was found on the electropolished NiTi specimen, in a short-term *in vitro* test with 120 min. of blood exposure. However, the susceptibility to thrombosis for longer term experiments is still under investigation.

An *ex vivo* experiment on thrombogenicity involving New Zealand white rabbits was conducted by Shih et al. [27]. The experiment was conducted to determine the stability of passivated 316L stainless steel stents *in vivo*. The material surface was

electropolished in 80% (v/v) phosphoric acid and 20% (v/v) glycerin electrolyte mixture at 65°C for 30 s. Thrombosis was determined by measuring the percentage increase in thrombus weight. The *ex vivo* surface-treated 316L stainless steel stents were implanted for 4 weeks. Corrosion pits and copious amounts of white thrombus were found in the electropolished stents. The white thrombus was composed mainly of platelets and fibrin, that were found primarily in the strut intersections.

The *ex vivo* experiment on the thrombogenicity of electropolishing 316L stainless steel stents contradicted the result of Tepe et al. [30]. NiTi and 316L stainless steel resulted in contradictory results, indicating the variability caused by material selection. Due to various factors that influence the onset of thrombosis, a cytotoxicity experiment alone may not provide concrete evidence of how surface modified specimens would react with the anatomy *in vivo*, but this method could be utilized as a primary process in understanding the tissue-to-material interaction.

Alloys containing Ni or Cr may have an adverse biological effect due to the leaching of Ni or Cr ions *in vivo*. Leaching of particular metals can potentially cause inflammatory response, impede cell proliferation, and may also cause allergic reactions that provoke carcinogenic reactions. The accumulation of Ni has been known to affect the metabolism of DNA synthesis [25]. Therefore, a problem may occur in establishing biocompatibility for alloys containing Ni. A cytotoxicity study with the objective of evaluating the biological response to surface-treated NiTi in *in vivo* animal implantation assays and in *in vitro* cell proliferation tests was conducted by Trépanier et al. [25]. The study compared the cytotoxicity of surface-treated NiTi when electropolished (EP), air

aged at 450°C (AA), heat treated in salt at 500°C (HT), and after passivation in nitric acid solution at STP (PA). After 6 days of incubation, a decrease in the cell proliferation was observed in the well containing electropolished NiTi test specimen.. This result was verified by a decrease in the doubling time (time required for fibroblast cells to double in number) of the fibroblast cells. The cell impedance could be explained by the possible leaching of Ni in the cell culture. The Ni leaching was minimized by the formation of a TiO₂ oxide layer for NiTi. The thickness of the layers were $340 \pm 140 \mu\text{m}$, $300 \pm 10 \mu\text{m}$, $2400 \pm 780 \mu\text{m}$, and $9110 \pm 2700 \mu\text{m}$ for EP, PA, AA, and HT, respectively. PA, AA, and HT did not show any significant differences between the proliferation rates during incubation. It was, however, difficult to determine whether the impedance of cell proliferation was dependent on the thickness or the uniformity of the oxide layer.

During the *in vivo* experiment, the surface-treated NiTi samples were implanted in the dorsal of New Zealand white rabbits. The location of the implants exhibited inflammation and fibrous capsule thickening under optical microscopy. The inflammation and the fibrous capsules decreased in thickness as a function of implantation time. After 2 weeks, the inflammation around the implantations had subsided. The impedance of cell attachment on the electropolished specimens supported the possible biocompatibility. The NiTi surface treated by EP, PA, AA, and HT exhibited cytocompatibility.

One of the contributions toward biocompatibility is the development of an oxide layer. The uniformity of the oxide layer and the corrosion resistance contribute to the implant biocompatibility. The purpose of the oxide film for biocompatibility is the

hindrance of metal ions leaching from the bulk material. Oxide films contribute to biocompatibility by increasing the stability of the surface layer by protecting against further oxidation of the bulk material, and creating a chemical barrier against Ni depletion [24].

2.6 Co-Cr Alloy L605 Background

Co-based alloys are mainly derived from either Co-Cr-W or Co-Cr-Mo triphasic compositions, that are grouped into alloys with three distinctively different attributes [28]: wear-resistance, corrosion-resistance, and heat-resistance. Co-based alloys were first investigated by Elwood Haynes in the early 1900s. During World War I, cobalt-based tools were commonly used and in 1922 wear-resistant Co-based alloys were used for plowshares, oil well drilling bits, dredging cutters, hot trimming dies, and in internal combustion engines [29]. In the early 1940s, corrosion-resistant and high-temperature Co-based alloys were introduced. High-temperature Co-based alloys were utilized for both air and land gas turbines for their strength at elevated temperatures and resistance in thermal fatigue, oxidation, and sulfidation [29]. They were also incorporated in the gas turbine industry, in fabrication of assemblies and ductwork, and in furnace applications. Co-Cr alloy L605, also known as Haynes 25, is considered a high temperature-resistant alloy. Thus, many of the applications for L605 have been in turbines. Other Co-based alloys have a long history of orthopedic, cardiac, and dental applications. However, L605 has rarely been used in vascular implants. In 2004, Guidant Corporation introduced a vascular stent manufactured from L605. This opened a variety of opportunities for the

application of L605 in vascular implants. Not only do implants need to have excellent mechanical properties, they also have to be biocompatible and exhibit corrosion-resistance.

L605 is a Co-based alloy with Cr, W, and Ni as the main alloying elements. Cr improves corrosion and sulfidation resistance. W is integrated as a solid-solution strengthener and as a carbide former [30]. The addition of Ni in the alloy stabilizes the FCC structure by reducing the allotropic phase transformation temperature [31]. Due to the difficulty in fabrication, the implementation of Co-Cr alloys as medical implants has been limited [32]. In recent years, Co-based alloys have been utilized as orthopedic, cardiac, and dental implants [33]. MP35N is used for cardiovascular pacing leads, catheters and orthopedic cables [18], and L605 for heart valves and coronary stents [34]. As can be seen from Table 1, L605 surpasses MP35N in all mechanical properties.

Table 1. Mechanical and material property comparison of MP35N and L605.

Alloy Description	Specific Mass	Elastic Modulus	UTS	Yield Strength
MP35N	8.43 g/cm ³	233 GPa	930 MPa	414 MPa
L605	9.1 g/cm ³	243 GPa	1000 MPa	500 MPa

L605 has a higher elastic modulus, ultimate tensile strength (UTS), and yield strength. Because of the high elastic modulus and high specific mass, L605 has excellent radial strength and radiopacity. Also, coronary stents fabricated from L605 are MRI compatible [4]. Co-based alloys are now widely accepted in the biomedical industry as a high-strength alloy that can be used in implants.

2.7 Mechanical Properties of Co-Cr Alloy L605

The majority of coronary stents in the market are manufactured from either NiTi or AISI 316L stainless steel. Those made from AISI 316L stainless steel are categorized as balloon expandable stents. Coronary stents manufactured from Co-Cr alloy L605 fit in the same category.

A comparison of the mechanical properties of the two alloys, as shown in Table 2, helps in understanding their material application as stents. L605 surpasses AISI 316L stainless steel in all aspects of mechanical properties, including specific mass, elastic modulus, ultimate tensile strength (UTS), yield strength, and tensile elongation [4]. Due to a high relative specific mass density compared to AISI 316L stainless steel, L605 exhibits high radiopacity and high visibility under fluoroscopy during stent deployment. The superior mechanical properties of L605 enable the fabrication of stents with thinner struts that have many advantages, including lower profile, smaller stent volume, and improved flexibility and deliverability [4]. L605 coronary stents with thinner struts can be fabricated without compromising the radial strength.

Poncin et al. [4] indicated that stents with low yield strength are desired to accommodate the pressure from balloon catheter expansion. A high tensile strength material usually exhibits higher yield strength, that is needed for maintaining a greater radial force. However, the higher tensile strength can potentially exhibit an undesired balloon coil during deflation. Therefore, a balance between tensile and yield strength is needed for stents that do not exhibit recoiling of the balloon catheter but at the same time do not compromise radial strength.

Table 2. Mechanical properties of L605 test specimen compared with stainless steel 316L [4].

Alloy Description	Specific Mass	Ultimate Tensile Strength	Yield Strength	Maximum Elongation
L605 Test Specimen	9.1 g/cm ³	979 GPa	458 KPa	52%
316L	7.95 g/cm ³	670 GPa	340 KPa	48%

2.8 L605 as a Coronary Stent

A clinical study conducted on restenosis by Kastrati et al. [35] investigated the effect of strut width of coronary stents in blood vessels of diameters greater than 2.8 mm. The result yielded a favorable reduction in restenosis in a long-term assessment of intracoronary stenting. This finding was also supported by Saskia et al. [36] in a clinical study on the impact of strut thickness for the long-term assessment of late luminal loss. The clinical trial was also conducted in a reference vessel with a diameter of approximately 2.8 mm. The study conducted by Kastrati et al. [35] demonstrated a reduced restenosis rate of 14.7% for the thin strut and 25.3% for the thick strut. The experiment conducted by Saskia et al. [36] reported a restenosis reduction of 11% for the thin strut and 17% of the late luminal area loss for the thick struts. Although these two clinical trials demonstrated a significant reduction in the occurrence of restenosis, the limitation of the study was the applicability of the reduced late luminal loss for a vessel diameter of 2.8 mm or greater.

Another clinical trial conducted by Briuori et al. [37] demonstrated the applicability of thinner struts with vessel diameters smaller than 2.8 mm. Although stents

with thinner strut thickness showed a substantial reduction in the restenosis rate, strut thickness failed to reduce the restenosis rate for vessels smaller than 2.8 mm.

Nevertheless, the application of L605 for the reduction of strut thickness showed a benefit in reducing the restenosis rate and late luminal loss. In order to assess the biocompatibility of L605 in the human body, Kereiakes et al. [38] conducted a clinical trial on the biocompatibility of Co-Cr alloy stents. The experiment showed no evidence of adverse effect induced by the Co-Cr alloy stent in a 6-month clinical trial. The past literature showed the applicability of Co-Cr alloy L605 in vascular implants, reducing restenosis and late luminal loss, and demonstrating an improvement in biocompatibility.

2.9 Summary

Electropolishing produces a smooth surface that also increases the wettability of the surface, as explained by Kang and Lee [17]. Surface roughness decreased as the electropolishing time was increased [15]. Electropolishing also forms an oxide layer that protects the bulk of the material from corrosion, as described by Trépanier et al. [23]. The formation of the protective oxide layer by electropolishing reduces the leaching of Ni from NiTi specimens. Oxide films contribute by protecting the surface against the further oxidation of the bulk material, and by creating a chemical barrier against Ni depletion [24]. Coronary stents from L605 have the advantages of increased radiopacity and flexibility, and can be made with thinner struts without compromising the radial pressure.

Much of the previous surface engineering research was geared toward the surface modification of NiTi and 316L stainless steel for enhanced biocompatibility. Co-Cr alloy

L605 has been gaining attention as a biomaterial for coronary stents. Surface characterization and biocompatibility of electropolished L605 have not been extensively studied.

CHAPTER THREE

RESEARCH OBJECTIVE

Electropolishing is known to produce smooth and passivated surfaces. NiTi stents that were electropolished have been shown to result in a reduction in thrombogenicity in a short-term *in vitro* experiment. However, the effect of electropolishing time and bath temperature on the biocompatibility of electropolished Co-Cr alloy L605 has not been extensively studied.

The purpose of this study was to investigate the effects of electropolishing on the surface characteristics and biocompatibility of Co-Cr alloy L605. The surface characteristics of electropolished specimens are dependent on process parameters such as process current, electropolishing time, and bath temperature. The specific goals of this research were:

1. Determine the electropolishing process conditions for that a current plateau can be obtained.
2. Determine the effect of process current, electropolishing time, and bath temperature on surface roughness and contact angle.
3. Determine the effect of electropolishing time and bath temperature on surface chemistry and surface oxide thickness.
4. Determine the effect of electropolishing time and bath temperature on the cytotoxicity of Co-Cr alloy L605, as a preliminary method of assessing biocompatibility.

CHAPTER FOUR

EXPERIMENTAL METHODOLOGY

4.1 Overview

The experimental work was divided into six categories. The process flow diagram undertaken in the present investigation is shown in Figure 8.

1. Surface preparation of Co-Cr alloy specimen for electropolishing.
2. I-V curve generation by varying time, temperature, and acid concentration.
3. Electropolishing, with temperature, time, and current as variables.
4. Surface analysis of each test specimen and calculation of rate of electropolishing.

Contact angle, surface roughness and thickness and chemical composition of the surface oxide layer were determined.

5. Cytotoxicity tests of electropolished specimen and control.
6. Comparison of qualitative and quantitative toxicity of electropolished test specimens.

4.2 Experimental Materials

0.0159 m x 0.0159 m (5/8" x 5/8") test specimens were sheared from a 0.305 m x 0.305 m (12" x 12") Co-Cr alloy L605 sheet donated by Rolled Alloy, Inc. The heat analysis for the specimens is shown in Table 3.

Three different concentrations of phosphoric acid were used in the experiment: 85, 50, and 15% (v/v). Each concentration was obtained by adding a set amount of acid

to DI water to obtain the desired acid concentration. DI water was used to prevent introducing Cl and other mineral contaminants in the solution.

Figure 8. Flow diagram of process undertaken to characterize the surface properties and determine the biocompatibility of electropolished Co-Cr alloy L605.

4.3 Experimental Apparatus

Electropolishing was conducted in a Betmann Cleaning-Electropolishing System, (Model E782EP, ESMA, Inc., South Holland, IL) with a 304 stainless steel bath.

Titanium (Ti) was used as the cathode, that was incorporated in the Betmann Cleaning-Electropolishing System. The apparatus and the equipment used for electropolishing are shown in Figure 9.

Table 3. Chemical analysis of Co-Cr alloy L605 from Rolled Alloy, Inc. [30].

C	Co	Cr	Fe	Mn	Ni	P	S	Si	W
0.11%	BAL	20%	2.3%	1.5%	10%	0.01%	0.004%	0.25%	14.7%

Figure 9. Electropolishing apparatus and its respective equipment utilized for the experiment.

An electrolyte volume of 1L was used for electropolishing. The E782EP system contained a 25 amp. electropolishing unit, a dip tank, an ultrasonic cleaning tank, and an ultrasonic rinse tank. A dip and flow chiller (Beckman Co.) was to cool the acid solution when electropolishing below ambient temperature. The chiller cooled the external bath that housed the internal bath filled with 1 L of acid mixture. Two separate digital thermometers were used to monitor the temperature of both the external and internal baths.

Two RS232 compatible Digital Multi Meters (DMM) were used to acquire current and voltage simultaneously. A simplified circuit diagram is shown in Figure 10. The voltage meter was positioned parallel to the circuit, while the current meter was positioned in series with the circuit. The letter R signifies the electropolishing process.

Figure 10. A simplified circuit diagram of electropolishing equipment and the DMM positions.

4.4 Specimen Preparation

Test specimens were cleaned in accordance with ASTM B322-99. The specimen edges were deburred by mechanically polishing for 5 s at a constant force with 600 grit

SiC paper. The test specimens were then mechanically polished for 1 min. at constant force with 600 grit SiC paper, with DI water as the lubricant and coolant, in order to establish a standard start surface roughness. The specimens were then cleaned by sonication in a 70% IPA bath for 5 min., followed by sonication in DI water for an additional 5 min. before air drying.

Post-cleaning was conducted by following the procedures specified in ASTM B912-02. After electropolishing, test specimens were dipped in 100 ml DI water to remove the acid and the test specimens were sonicated in 100 ml DI water for 3 min. to remove any residual acid and surface contaminants. The specimens were then air dried.

4.5 Generation of I-V Curve

An I-V curve was generated prior to conducting the electropolishing experiment. acid concentration, bath temperature and electropolishing time were varied to generate the I-V curve. As shown in Table 4, the phosphoric acid concentrations used were 15, 50 and 85% (v/v). The bath temperatures were 0, 25, 35, and 45°C. Time was set at 1, 2, 3, and 10 min.

From the I-V curve, a voltage range and a process current were established. After the I-V curves were generated, parameters that did not generate a current plateau were eliminated from the test matrix.

- 1) The temperature of the electropolisher was set at the specified temperature.
- 2) 1L of phosphoric acid having the appropriate concentration was added to the electropolishing bath.

- 3) The bath was then permitted to reach equilibrium at the specified temperature $\pm 3\text{ }^{\circ}\text{C}$.
- 4) Once the electropolishing temperature was reached, the test specimen was inserted in the acid. The specimen was positioned with the largest area facing the cathode.
- 5) A digital hand-held timer was used to measure the electropolishing time.
- 6) Steps 1-5 were repeated for parameters shown in Table 4. Five replications were conducted for each parameter.

I-V curves were generated for each set of condition by plotting the current and corresponding voltage.

Table 4. Electropolishing parameters used to generate the I-V curve.

I-V Curve Parameters		
Temperature	Acid Concentration	EP time
0 $^{\circ}\text{C}$	15% (v/v)	1 min.
25 $^{\circ}\text{C}$	50% (v/v)	2 min.
35 $^{\circ}\text{C}$	85% (v/v)	3 min.
45 $^{\circ}\text{C}$	N/A	10 min.

4.6 Process Parameters for Electropolishing

4.6.1 Process Current Effect on the Surface Characteristics

Electropolishing was conducted while varying the process current and EP time. Both control and fixed parameters are shown in Table 5. Three currents were used in the experiment. The first current was chosen to be the minimum current, the second current was the mid-point of the current plateau, and the third current was located above the

current plateau. The three process currents were determined from the characteristic I-V curve. Electropolishing times of 1, 2, and 3 min. were used. The electrode distance, cathode, anode, and bath temperature were fixed.

Table 5. Control and fixed parameters utilized to characterize the surface characteristics.

Control Parameters	
Current (A)	Ia
	Ib
	Ic
EP Time (min.)	1 min.
	2 min.
	3 min.
Fixed Parameters	
Temperature (°C)	25°C
Cathode	Ti
Anode	L605
Electrode Distance (cm)	2.54 cm
Surface Preparation	600 grit

4.6.2 Time and Temperature Effect on the Surface Characteristics

Further investigation was conducted to ascertain the effect of electrolyte temperature and electropolishing time (EP time) on the surface characteristics. As can be seen in Table 6, EP time and the bath temperature were the control parameters investigated. The bath temperatures were set at 35, 25, and 0°C. The electrode distance, cathode, anode, and process current were fixed. Further investigation was conducted to determine the surface characteristics of specimens electropolished for durations of 30 and 60 min. at 0°C, while keeping all other parameters constant.

4.7 Surface Analysis

4.7.1 Surface Roughness AFM Analysis

A Nano-R AFM (Pacific Nano Technology, Santa Clara, CA) was used to quantify surface roughness. All AFM surveys were characterized and processed with the same parameters, as shown in Table 7. The images were processed and analyzed for Root Mean Square surface roughness by NanoRule+ (version 2.5.03, Pacific Nano Technology), an analytical software for the Nano-R AFM. The captured images were leveled by using a 2nd order polynomial. The average surface roughness of 5 test specimens was determined. 5 separate surface roughness was determined for each specimen. The surface roughness acronyms are shown in Appendix A.

Table 6. Control parameters, temperatures and EP times used for the electropolishing.

Control Parameters	
Temperature (°C)	35 °C
	25 °C
	0 °C
EP Time (min.)	3 min.
	10 min.
Fixed Parameters	
Current (A)	I (current plateau)
Cathode	Ti
Anode	L605
Electrode Distance (cm)	2.54 cm
Surface Preparation	600 grit

4.7.2 Surface Morphology by SEM

The SEM used in the experiment was a FEI QUANTA 200 environmental scanning electron microscope equipped with an energy dispersive x-ray spectroscope

(EDS). The Quanta 200 was used to characterize the surface morphology of the test specimens.

Table 7. AFM scanning parameters.

Scan Parameters	
Scan Rate	1 Hz
Size	25 μm x 25 μm
Z-offset	150 μm
Leveling	2 nd order Polynomial
Pixel Density	256
Scanning Mode	Contact Mode

4.7.3 Surface Chemical Analysis by XPS and AES

X-ray Photoelectron Spectroscopy (XPS) was performed, using a Physical Electronic Instrument (PHI) Quantum 2000, to measure the surface chemistry of the specimen. The parameters used to analyze the test specimens are shown in Table 8.

Table 8. Parameters for the XPS analysis.

XPS Analysis Parameters	
Instrument	PHI Quantum 2000
X-ray Source	Monochromated Al α 1486.6 eV
Acceptance Angle	$\pm 23^\circ$
Take-Off Angle	45°
Analysis Area	1400 x 300 μm
Charge Correction	C1s 284.8 eV

Auger Electron Spectroscopy (AES) was performed, using a PHI SMART-200 Scanning Auger Nanoprobe, to characterize the depth profile of the surface oxide layer. The thickness of the oxide layer and the surface contaminants were determined by using this analytical technique. The parameters used to analyze the electropolished specimens are shown in Table 9.

Table 9. Parameters for the AES analysis.

AES Analysis Parameters	
Instrument	PHI SMART-200 Scanning Auger Nanoprobe
Analyzer Type	CMA
Electron Beam Energy	10keV, 20 μ A
Tilt	30° to sample nominal
Ion Gun Etch Rate	75 & 14 Å/min.

4.7.4 Electropolishing Rate Calculation

Electropolishing rates were calculated for each set of parameters. The weight of the specimen was measured before and after electropolishing. A scientific precision scale, Mettler Toledo series AL with an resolution of 0.0001 g, was used to measure the weights. The electropolishing rate was calculated according to Equation 9.

$$\left[\frac{(Weight_{beforeEP} - Weight_{afterEP})}{Area} \right] / Time = EP_{rate} \quad \text{Equation [9]}$$

where $Weight_{beforeEP}$ is the weight of the test specimen before electropolishing and after surface preparation and $Weight_{afterEP}$ is the weight after electropolishing and post-

cleaning. Area is the total area of the test specimen (1.58 cm x 1.58 cm) and Time is the electropolishing time in s (seconds). The units of the EP rate were $\text{gram}/\text{cm}^2 \text{ min}$. The EP rate was calculated using Equation 9.

4.8 Contact Angle Measurement by Sessile Drop Method

The sessile drop method was used to measure the contact angle. A micro-pipette was used to administer a single droplet of 50 μl DI water on the specimen surface. The droplet image was captured using a CCD camera within the first 5 s of the dispensation to avoid the gradual loss of contact angle due to water spread. Adobe Photoshop ver. 5.5, a graphic editorial software, was used to measure the contact angle at a magnification of 150X. The contact angle was measured one time for each of the 5 specimens and the average was calculated.

4.9 Cytotoxicity Determination

The test closely followed the direct-contact cytotoxicity test described in ISO 10993-5. The test specimens and the control were directly in contact with cells, grown in near confluence in flasks. A minimum of three replicates were done. The test specimens were incubated in the well for an additional 24 hours. The negative control (biomaterial that exhibits non-toxicity) was glass and the positive control (biomaterial that exhibits toxicity) was Ni Foil. The toxicity of the biomaterial was characterized both quantitatively and qualitatively, as described in Appendix B.

The cytotoxicity test procedure consisted of 5 separate steps: culture media preparation, initial culturing step, subculture process, growing cells in wells to be tested, and cell evaluation. Each process is covered in detail in Appendix B.

4.9.1 Cell Evaluation

4.9.1.1 Cytotoxicity Qualitative Assessment

1. The qualitative assessment was conducted for specimens while viewing the cell culture under the inverted microscope.
2. The assessment was conducted by categorizing the confluence of the cells using Table 10. Cell culture that exhibited confluence was considered to have a cytotoxicity of 0. A cytotoxicity of 3 was assigned to cell cultures that did not exhibit viable cells, and a cytotoxicity of 2 was assigned when the cell culture exhibited major gaps in the cell confluence, an indication of cell lysis. A cytotoxicity of 1 was assigned when the cell culture exhibited minor gaps in the cell confluence.

Table 10. Table used to translate the degree of cytotoxicity to a numeric cytotoxicity scale.

Cytotoxicity Scale	Interpretation
0	Noncytotoxic
1	Mildly Cytotoxic
2	Moderately Cytotoxic
3	Severely Cytotoxic

4.9.1.2 Cytotoxicity Quantitative Assessment

1. 20 μ l of the cell culture was dispensed and mixed with another 20 μ l of typhon blue. The two solutions were mixed thoroughly by pipetting approximately 10 times.
2. 20 μ l of the mixed solution was dispensed on the bright-line™ hemacytometer from Warner-Lambert Technologies Inc. Cells that fell in squares in the 4 corners were counted.
3. The total was then multiplied by 5×10^4 to determine the cell density (cells/ml).

CHAPTER FIVE

RESULTS

Overall, electropolishing modified the surface characteristics of L605. Both surface roughness and contact angle with water decreased with an increase in the electropolishing time (EP time). The decrease in the contact angle indicated that the surface became more hydrophilic with processing time. The electropolishing rate (EP rate) increased with an increase in the bath temperature. Both a decrease in the bath temperature and an increase in the electropolishing time showed an improvement in the biocompatibility. The formation of Cr in the oxidized form obtained after electropolishing and the Cr rich surfaces are thought to have contributed to the improved biocompatibility.

5.1 Effect of Electropolishing Parameters on the I-V Curve

I-V curves were generated to establish a process current for electropolishing. Current plateaus were obtained for bath temperatures of 0, 25, and 35°C, with an acid concentration of 15% (v/v). As the bath temperature decreased a lower current plateau over a wide voltage range was obtained. A constant current on the I-V curve indicated a controlled electropolishing with minimal current flux. Parameter combinations that exhibited the current plateau are shown in Table 11. No further experimentation was conducted on the parameter combinations that failed to generate the current plateau, as shown in Table 12.

A one-way ANOVA was conducted to compare the currents obtained from the I-V curves for EP times of 1, 2, and 3 min. for acid concentrations of 85, 50, and 15% (v/v). As can be seen in Table 13, the p-value of 0.995 indicated that no statistically significant difference was found among the currents on the I-V curve for EP times of 1, 2, and 3 min., at the 95% confidence interval, for the acid concentration of 85% (v/v) and the bath temperature of 45 °C. The one-way ANOVA tables for acid concentrations of 85, 50, and 15% (v/v) and bath temperatures of 0, 25, 35, and 45 °C are shown in Appendix C.

Table 11. Acid concentration, bath temperature, and EP time combinations that exhibited a current plateau on the characteristic I-V curve.

Acid Concentration	Bath Temperature	EP Time
15% (v/v)	0°C	3 min.
	25°C	10 min.
	35°C	30 min.
	-	60 min

Table 12. Acid concentration, bath temperature, and EP time combinations that did not exhibit a current plateau.

Acid Concentration	Bath Temperature	EP Time
85% (v/v)	25°C	1 – 3 min.
	35°C	
	45°C	
50% (v/v)	25°C	
	35°C	
	45°C	
15% (v/v)	45°C	

The I-V curve for 85% (v/v) phosphoric acid, 35°C, and EP time of 1, 2, and 3 min. is shown in Figure 11. These parameters resulted in a linear line with varying slopes without a current plateau. Since the process current did not stabilize, electropolishing rate fluctuations may occur, resulting in an undesired surface finish with a high surface roughness due to the formation of pits.

Table 13. One-way ANOVA of currents at 1, 2, and 3 min. on the I-V curve with 85% (v/v) phosphoric acid at 45 °C.

Source	DF	SS	MS	F	P
Time	2	0.002	0.001	0	0.995
Error	18	4.445	0.247		
Total	20	4.447			
S = 0.4969 R-Sq = 0.05% R-Sq(adj) = 0.00%

Figure 11. I-V curve of 85% (v/v) phosphoric acid, 35°C, and EP time of 1, 2, and 3 min. with 1 min. intervals.

The voltage and the process currents on the I-V curves at various electropolishing times were compared. There was no statistically significant difference at the 95% confidence interval among the voltages or the currents due to the change in EP time. The same trend was obtained when electropolishing the specimens in 50 and 15% (v/v) phosphoric acid at all temperatures for the respective EP times.

As can be seen in Figure 12, the I-V curve generated for 50% (v/v) phosphoric acid at 35°C did not show a current plateau. The current plateau was not observed for EP times of 1, 2, and 3 min. The changes in the EP time had no statistically significant effect on the I-V curve at the 95% confidence interval. This finding was in accordance with the I-V curve found with 85% (v/v) phosphoric acid. The current plateau was not generated for 50% (v/v) phosphoric acid at all temperatures ranging from 25 to 45°C with a 10°C interval. Therefore, no further experiments were conducted on the parameter combinations for 50 and 85% (v/v) phosphoric acid. I-V curves for 50 and 85% (v/v) phosphoric acid for various temperature and EP time are shown in Appendix D.

A current plateau was found for 15% (v/v) phosphoric acid at bath temperatures 0, 25, and 35°C, electropolished for 3 min., as shown in Figure 12. There was no plateau at 45°C. The current plateau was also obtained for EP times of 1, 2, 3, and 10 min. The changes in the EP time had no statistically significant effect on the slope of the I-V curve at the 95% confidence interval because a stable current reading was obtained within the first minute.

As can be seen in Figure 13, a wider and more distinct current plateau was obtained at 25°C and 0°C than at 35°C. The widest current plateau was obtained at the

bath temperature of 0°C. Depending on the bath temperature, different current plateau values were found.

Figure 12. I-V curve of 50% (v/v) phosphoric acid, 35°C, and EP time of 1 to 3 min. with 1 min. intervals.

As the bath temperature decreased, the current plateau became wider (wider voltage range) and the value of the current plateau decreased. The lowest current of 0.3 A was obtained at 0°C, while the highest current of 0.62 A was obtained at 35°C. The decrease in the process current with a decrease in the bath temperature was in accordance with Murali [11].

Murali [11] electropolished Al-7Si-0.3Mg cast alloy in a 30% (v/v) perchloric and 70% (v/v) methanol mixture. As the bath temperature decreased from 5 to -65°C, there was a decrease in process density from 15 mA/cm² to 2 mA/cm². The conspicuous current plateau on the 15% (v/v) phosphoric acid at 0°C demonstrated that a lower temperature provided a more robust condition for electropolishing.

Figure 13. I-V curve of 15% (v/v) phosphoric acid, 3 min. EP time, and process temperatures of 0, 25, 35, and 45°C.

5.2 Effect of Process Parameters on Electropolishing Rate

5.2.1 Effect of Process Current on Electropolishing Rate

The process currents and voltages were 0.12 A, 0.39 A, 0.68 A, 2.24 V, 3.42 V, and 5.02 V, respectively, as shown in Figure 14. The average EP rate increased with an increase in the process current. There was no statistically significant effect on the average EP rate among EP time of 1, 2, and 3 min.

The electropolishing rate was calculated on test specimens electropolished at 25°C in 15% (v/v) phosphoric acid. The EP rate of the test specimen electropolished for 3 min. at 0.12 A resulted in an average EP rate of 0.00015 g/cm² min. The EP rate of 0.01165 g/cm² min. was calculated when the current was increased to 0.39 A, that was located on the current plateau. An EP rate of 0.02259 g/cm² min. was calculated at the

process current of 0.68 A at the temperature of 35°C. A two-way ANOVA analysis was conducted to determine the relation between the process current and EP time. As shown in Table 14, the EP rate was influenced by an increase in the process current, that increased linearly with an increase in the process current. The process current had a statistically significant effect on the EP rate, at the 95 % confidence interval.

Figure 14. Average (n=3) EP rate (g/cm² min.) of test specimen, electropolished in 15% (v/v) phosphoric acid, 25°C and 1 to 3 min. EP times with 1 min. intervals.

Table 14. Two-way ANOVA Analysis: Average (n=4) EP rate (g/cm² min.) versus current (A), time (min.).

Source	DF	SS	MS	F	P
Current (A)	2	0.000692	0.000346	1522.89	0
Time (min.)	2	0.0000017	0.0000008	3.65	0.125
Error	4	0.0000009	0.0000002		
Total	8	0.0006946			
S = 23.99 R-Sq = 91.32% R-Sq(adj) = 89.29%					

5.2.2 Effect of EP Time and Bath Temperature on Electropolishing Rate

Electropolishing rate was proportional to the bath temperature. EP rate decreased when the bath temperature decreased, as shown in Figure 15. The EP rate of $0.0300 \pm 0.0017 \text{ g/cm}^2 \text{ min.}$ was calculated for the test specimen electropolished at 35°C for 10 min. at a process current of 0.62 A. As the bath temperature decreased to 25°C , the process current decreased to 0.39 A and the EP rate decreased to $0.0113 \pm 0.0004 \text{ g/cm}^2 \text{ min.}$ At 0°C and 0.3 A, the lowest EP rate was calculated to be $0.0041 \pm 0.0002 \text{ g/cm}^2 \text{ min.}$ The EP rate data from the experiment for 15% (v/v) phosphoric acid at 35, 25, and 0°C is shown in Appendix E.

Figure 15. Average ($n=3$) EP rate ($\text{g/cm}^2 \text{ min.}$) of test specimens electropolished at 15% (v/v) phosphoric acid at the current plateau for bath temperatures 35, 25, and 0°C for various EP times.

Not only did the average EP rate decrease with a decrease in the bath temperature, the standard deviation of the EP rate decreased from 0.0017 to 0.0002 g/cm² min. This reduction in the EP rate variability among test specimens could indicate a more robust electropolishing process at lower temperatures.

A multi-variant design analysis was done to characterize the effect of time and bath temperature on the EP rate. As can be seen in Table 15, temperature had a statistically significant effect on the EP rate, at the 95% confidence interval. There was no statistically significant effect on the EP rate when the electropolishing time was varied, at the 95% confidence interval.

Table 15. Multilevel factorial design general linear model: Average (n=3) EP rate (g/cm² min.) versus temperature (°C) and EP time (min.).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temperature	2	0.0022377	0.0022377	0.0011188	3686.79	0
Time	1	0.0000003	0.0000003	0.0000003	0.98	0.342
Temperature*Time	2	0.0000017	0.0000017	0.0000009	2.85	0.097
Error	12	0.0000036	0.0000036	0.0000003		
Total	17	0.0022433				
S = 0.000550883 R-Sq = 99.84% R-Sq(adj) = 99.77%						

Electropolishing was also done for 30 and 60 min. at 0°C to determine the EP rate at longer EP times. At 30 and 60 min., the calculated EP rates were 0.0046 ± 0.0002 and 0.0044 ± 0.0002 g/cm² min. One-way ANOVA analysis was done to determine the effect of EP time on the EP rate at 0°C. As shown in Table 16, the four EP times did not show

a statistically significant effect on the EP rate, at the 95% confidence interval. Therefore, it was concluded that the EP rate was influenced primarily by the bath temperature.

The EP rate decreased with a decrease in the bath temperature. According to the I-V curve analysis, the bath temperature and the process current were proportional to each other. A decrease in the bath temperature resulted in a decrease in the process current.

Table 16. One-way ANOVA: Average (n=4) EP rate (g/cm² min.) versus EP time (min.).

Source	DF	SS	MS	F	P
EP time (min.)	3	0.0000006	0.0000002	3.57	0.067
Error	8	0.0000004	0.0000001		
Total	11	0.000001			
S = 0.0002300 R-Sq = 57.24% R-Sq(adj) = 41.20%					

Therefore, as the current increased, a greater EP rate was obtained. The higher current was obtained when the specimen was electropolished at 35°C in 15% (v/v) phosphoric acid. The value on the current plateau was mainly affected by the temperature of the bath. The bath temperatures that were tested were 35, 25, and 0°C. The EP rate decreased with a decrease in bath temperature.

5.3 Surface Roughness

5.3.1 Effect of Process Current on Average Surface Roughness

The average surface roughness decreased when the process current increased. As shown in Figure 16, the highest average surface roughness of 175 nm was obtained when

the test specimen was electropolished at a process current of 0.12 A for 3 min. with a bath temperature of 25°C. The average surface roughness of the test specimen electropolished with a process current of 0.12 A increased when the EP time was increased. An average surface roughness of 112.8, 142.9, and 159.4 nm was obtained for EP times of 1, 2, and 3 min., respectively. Similarly, the average surface roughness of the test specimens electropolished at a process current of 0.39 A increased between EP time of 1 to 2 min. at 25°C. However, the surface roughness did not increase between 2 and 3 min. An average surface roughness of 46.9, 56.5, and 55.8 nm was obtained for EP times of 1, 2, and 3 min., respectively.

Figure 16. Average (n=5) root mean square (nm) of test specimen electropolished at 15% (v/v) phosphoric acid, 25°C, and EP times of 1, 2, and 3 min.

The average surface roughness of the test specimen electropolished at a process current of 0.68 A increased from 32.3 to 88.5 nm with an EP time of 1 and 2 min. At 3

min., the average surface roughness decreased to 27.0 nm. Although the average surface roughness increased with an increase in EP time, no statistically significant difference was found using a two-way ANOVA analysis, at the 95% confidence interval, as shown in Table 17.

The average surface roughness decreased for specimens electropolished for 1 and 3 min., when the process current was increased at 25°C. The same trend was not obtained for the test specimen electropolished for 2 min.

Table 17. Two-way ANOVA: Average (n=5) RMS surface roughness Sq (nm) versus current (A), time electropolished with 15% (v/v) phosphoric acid at 25°C.

Source	DF	SS	MS	F	P
Current (A)	4	64927.7	16231.9	28.19	0
Time	2	878.8	439.4	0.76	0.497
Error	8	4605.7	575.7		
Total	14	70412.2			
S = 23.99 R-Sq = 93.46% R-Sq(adj) = 88.55%					

5.3.2 Effect of EP Time and Bath Temperature on Surface Roughness

The surface roughness (RMS) of the test specimens decreased with an increase in the EP time and increased with a decrease in bath temperature. As can be seen in Figure 17, the surface roughness decreased when the bath temperature was decreased. The surface roughness on the test specimen electropolished for 3 min. increased from 55.2 ± 12.1 nm, 59.5 ± 11.3 nm, to 61.3 ± 5.62 nm, when the bath temperature was decreased from 35 to 25°C, and 0°C.

Test specimens electropolished for 10 min. exhibited an increase in the surface roughness of 45.2 ± 3.50 nm, 47.6 ± 8.3 nm, to 47.9 ± 6.29 nm at bath temperatures of 35, 25, and 0°C , respectively. The surface roughness data of the test specimens electropolished at various process currents, bath temperatures, and EP times are shown in Appendix F. According to the multilevel factorial design analysis shown in Table 17, the bath temperature had a statistically significant effect on the surface roughness at the 95% confidence interval.

Figure 17. Surface roughness root mean square RMS (nm) and the standard deviation of test specimens electropolished in 15% (v/v) phosphoric acid at the current plateau at various temperatures ($^{\circ}\text{C}$) and EP times (min.).

The surface roughness decreased when the EP time was increased. When the test specimen was electropolished for 3 and 10 min. at 35°C at 0.62 A, the surface roughness decreased from 55.2 ± 12.1 nm to 45.2 ± 3.50 nm. When the test specimen was electropolished at 25°C at 0.39 A for 3 and 10 min., the surface roughness decreased

from 59.1 ± 11.4 nm to 47.6 ± 8.3 nm. When the test specimen was electropolished at 0°C at 0.30 A for 3 and 10 min., the surface roughness decreased from 61.3 ± 5.62 nm to 47.9 ± 6.30 nm. According to the multilevel factorial design analysis shown in Table 18, EP time did not affect the average surface roughness, at the 95% confidence interval. In order to further understand the effect of EP time on the surface roughness, test specimens were electropolished for an additional 30 and 60 min. The result from the one-way ANOVA is shown in Table 19. According to the analysis, EP time had a statistically significant effect on the average surface roughness.

Table 18. Multilevel factorial design general linear model: Surface roughness root mean square (n=5) versus temperature ($^{\circ}\text{C}$) and EP time (min.).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temperature	2	4735.5	4735.5	2367.8	12.46	0
EP Time	1	303.7	303.7	303.7	1.6	0.222
Temperature*Time	2	880.2	880.2	440.1	2.32	0.127
Error	18	3421.6	3421.6	190.1		
Total	23	9341.1				
S = 13.7874 R-Sq = 63.37% R-Sq(adj) = 53.19%						

Table 19. One-way ANOVA: RMS Sq (nm) versus EP time (min.).

Source	DF	SS	MS	F	P
Time (min.)	3	1302	434	5.91	0.01
Error	12	881	73.4		
Total	15	2183			
S = 8.568 R-Sq = 59.64% R-Sq(adj) = 49.56%					

The average surface roughness of test specimens electropolished at 0°C with 0.30 A for 3, 10, 30, and 60 min. were 61.3 ± 5.62 nm, 47.9 ± 6.30 nm, 23.1 ± 10.3 nm, and 48.5 ± 11.3 nm. The surface roughness decreased with respect to an increase in the EP time. The lowest average surface roughness of 23.1 ± 10.3 nm was obtained for a test specimen electropolished at 0°C for 30 min. under a constant current of 0.30 A. At a prolonged EP time of 60 min., there was an increase in the surface roughness. A surface topography was generated for the test specimen electropolished for 60 min. at 0.30 A, as shown in Figure 18. Pits with an approximate size of $2.49 \mu\text{m}$ were obtained on the specimen surfaces, that may have increased the surface roughness at EP time of 60 min.

Figure 18. AFM surface survey of Co-Cr test specimen electropolished at 0°C for 60 min. under a constant current.

5.4 Contact Angle

5.4.1 Effect of Process Current on Contact Angle

As can be seen in Figure 19, the average contact angle decreased with a decrease in EP time for the test specimens electropolished with the process current of 0.12 A, 0.39 A, and 0.68 A. The contact angle data of the test specimens electropolished at various

process currents, bath temperatures, EP times are shown in Appendix G. As the electropolishing time was increased from 1 to 3 min., the contact angle decreased from 60.7 to 52.5° for the test specimen electropolished at the process current of 0.12 A, resulting in the specimen surface becoming more hydrophilic.

Figure 19. Contact angle of test specimens (n=5) electropolished in 15% (v/v) phosphoric acid at 25°C in various currents (A) with EP times of 1, 2, and 3 min.

As shown in Table 20, both current and EP time had a statistically significant effect on the contact angle, at the 95% confidence interval. A two-way ANOVA analysis was conducted to characterize the effect of the current and EP time. The average contact angle increased with respect to an increase in the process current. While the process current increased from 0.12 to 0.39 A and to 0.62 A, the contact angle increased from

63.7, 73.1, and 79.3° for the test specimen electropolished for 2 min. at 25°C. As the current increased, the specimen surface became more hydrophobic.

5.4.2 Effect of EP Time and Bath Temperature on Contact Angle

The average contact angle increased with a decrease in the bath temperature. As can be seen in Figure 20, test specimens electropolished in 15% (v/v) phosphoric acid for 3 min. at the bath temperatures of 35, 25, and 0°C showed an increase in the average contact angle from $62.8 \pm 1.72^\circ$, $68.9 \pm 2.73^\circ$, to $73.0 \pm 3.02^\circ$, respectively.

Table 20. Two-way ANOVA: Contact angle (n=5) of test specimens electropolished with various currents (A) and EP times (min.).

Source	DF	SS	MS	F	P
Current (A)	2	1047.3	523.65	26.87	0
EP Time (min.)	2	376.39	188.196	9.66	0.001
Interaction	4	59.14	14.785	0.76	0.565
Error	18	350.76	19.487		
Total	26	1833.59			
S = 4.414 R-Sq = 80.87% R-Sq(adj) = 72.37%					

When the test specimen was electropolished for 10 min. at 35, 35, and 0°C, the average contact angle increased from $59.7 \pm 1.91^\circ$ to $67.5 \pm 2.21^\circ$. The contact angle data for test specimen electropolished various bath temperature and EP time are shown in Appendix G.

The increased contact angle can be correlated to the total material removed. According to Faraday's Law of Electrolysis, the total removed from the anode is

dependent on the process current and the EP time. While the EP time was kept constant at 10 min., the only factor that affected the total material removed was the process current.

Figure 20. Contact angle ($n=5$) and the standard deviation of test specimens electropolished in 15% (v/v) phosphoric acid at temperatures 35, 25, and 0°C for various EP times (min.).

The experiment showed that as the bath temperature decreased from 35 to 25°C, and to 0°C, the process current decreased from 0.62, 0.39, to 0.30 A. Therefore, the total material removed from the test specimen decreased as the bath temperature decreased, that increased the contact angle. Less material was removed from the surface at 0°C than at 35°C.

A greater contact angle was found at an EP time of 10 min. at the bath temperature of 0°C than 35°C. Therefore, the surface became more hydrophobic as the bath temperature decreased at a constant EP time. A multilevel factorial design analysis

was conducted to determine the effect of EP time and bath temperature on the average contact angle. As can be seen in Table 21, the increase in the average contact angle with respect to a decrease in time resulted in a statistically significant effect at the 95% confidence interval.

Table 21. Multilevel factorial design general linear model: Contact angle (n=5) versus temperature (°C) and EP times (min.).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
EP Time	1	1.37	1.37	1.37	0.26	0.613
Temperature	2	951.29	951.29	475.64	90.63	0
EP Time*Temperature	2	178.95	178.95	89.48	17.05	0
Error	30	157.44	157.44	5.25		
Total	35	1289.05				
S = 2.29087 R-Sq = 87.79% R-Sq(adj) = 85.75%						

As EP time increased, the contact angle decreased for test specimens electropolished at 35 and 0°C for 3 and 10 min., respectively. The decrease in the contact angle was due to the increase in the total material removed from the specimen surface. The decrease in the contact angle with an increase in the EP time did not result in a statistically significant difference, at the 95% confidence interval.

In order to further understand the effect of EP time on the average contact angle, specimens were electropolished for an additional 30 and 60 min. at 0°C. The average contact angle for the test specimens electropolished at 0°C for 3, 10, 30, and 60 min. were $73.0^\circ \pm 3.02^\circ$, $66.2^\circ \pm 1.96^\circ$, $63.7^\circ \pm 3.33^\circ$, and $57.5^\circ \pm 2.84^\circ$, respectively.

The contact angle decreased with a decrease in EP time, that indicated that the specimen surface became more hydrophilic. A one-way ANOVA analysis was done to

determine the effect of EP time on the contact angle. As can be seen in Table 22, the decrease in the average contact angle with an increase in the EP time was statistically significant, at the 95% confidence interval. The decrease in the contact angle was positively correlated to the total material removed from the anode. As EP time increased, more material was removed from the surface, at a constant process current. While more material was removed from the surface, the test specimen surface became smoother, resulting in a decrease in the contact angle. This decrease in the contact angle indicated that the test specimen surface became more hydrophilic with an increase in the EP time.

Table 22. One-way ANOVA Analysis (n=5): Contact angle versus EP times (min.).

Source	DF	SS	MS	F	P
Group	3	745.18	248.39	31.08	0
Error	20	159.83	7.99		
Total	23	905.01			
S = 2.827 R-Sq = 82.34% R-Sq(adj) = 79.69%					

5.5 Surface Morphology

Surface morphology was examined and characterized using both a stereo microscope and scanning electron microscopy (SEM). Electropolished test specimens revealed a smooth surface with the presence of grain structures under the stereo microscope and SEM. As shown in Figure 21, the surface morphology of the test specimen electropolished with 0.30 A for 10 min. at 0°C revealed a smooth surface with detection of pits at 300X under a stereo microscope. The size of one pit was confirmed as 34.575 μm in diameter. The existence of the pits was also confirmed by SEM survey.

As can be seen in Figure 22, pits were also detected at 400X magnification. In contrast, the pit found at 400X magnification on the SEM image had a diameter of approximately 5 μm .

Surface morphology was also examined on a specimen electropolished at 0°C for 3 min. with a process current of 0.30 A. A smooth surface without major defects was found. As shown in Figure 23, the stereo microscope survey at 200X magnification revealed a smooth surface and grain structures. A closer image was taken at 500X magnification using the SEM. The smooth surface and the grain structures were confirmed by the SEM survey, as shown in Figure 24.

Figure 21. Stereo microscope survey of Co-Cr test specimen electropolished at 0.30 A for 10 min. at 0°C under 300X magnification.

Figure 22. SEM survey of Co-Cr test specimen electropolished at 0.30 A for 10 min. at 0°C under 400X magnification.

Figure 23. Stereo microscope survey of Co-Cr test specimen at 200X electropolished at 0°C for 3 min. under a constant current.

Figure 24. SEM survey of Co-Cr test specimen at 500X electropolished at 0°C for 3 min. under a constant current.

Figure 25. SEM survey of Co-Cr test specimen at 2000X electropolished at 0°C for 3 min. under a constant current.

Even though a smooth surface was obtained when the test specimen was electropolished at 0°C for 3 min. with a process current of 0.30 A, etch pits were still found. As shown in Figure 25, under 2000X magnification, an etch pit with an approximate diameter of 3 μm was confirmed with SEM analysis.

Also, a wavy morphology was confirmed using the AFM surface survey, as shown in Figure 26. This specific test specimen was electropolished for 3 min. at 0°C with a process current of 0.30 A. From the peaks and valleys revealed in the surface topography, it was thought that the surface did not reach a homogenous smoothness with an EP time of 3 min. at 0°C. As illustrated in Figure 27, a relatively smooth and homogeneous surface was obtained when the specimen was electropolished at 0°C for 30 min. with a process current of 0.30 A. When the test specimens were electropolished for 60 min. with the same parameters, a greater number of pits were revealed by the AFM analysis, as shown in Figure 28.

Figure 26. AFM surface survey of Co-Cr test specimen electropolished at 0°C for 3 min. under a constant current.

Figure 27. AFM surface survey of Co-Cr test specimen electropolished at 0°C for 30 min. under a constant current.

Figure 28. AFM surface survey of Co-Cr test specimen electropolished at 0°C for 60 min. under a constant current.

5.6 Surface Chemistry

5.6.1 Effect of Process Current on the Surface Chemistry

X-ray Photoelectron Spectroscopy (XPS) was used to examine the surface chemistry of the test specimens electropolished in 15% (v/v) phosphoric acid for 3 min. at 25°C with process currents of 0.68, 0.39, and 0.12 A. A control test specimen

underwent the XPS analysis in order to compare the surface chemistry with that of electropolished specimens. The purpose of the XPS analysis was to characterize the chemical state of the elements found on the specimen surfaces at different process currents. The test specimens that underwent XPS analysis are shown in Table 23.

Table 23. Test specimens electropolished in 15% (v/v) phosphoric acid for 3 min. at 25°C with process currents of 0.68, 0.39, and 0.12 A.

Description	Temperature	Current	Voltage	EP time
Specimen 1	25°C	0.68 A	5.02 V	3 min.
Specimen 2		0.39 A	3.42 V	
Specimen 3		0.12 A	2.24 V	
Control	-	-	-	-

As shown in Figure 29, all test specimen surfaces contained primarily chromium (Cr) with a maximum concentration above 5.3 at%. Detection of Cr decreased with an increase in the process current. The electropolished test specimens resulted in a greater Cr content on the surface, as compared to the control. As the process current increased from 0.30, 0.39, and to 0.62 A, Cr detected on the surface decreased from 5.4, to 3.8, and 3.1 at%, respectively. As the process current increased, the atomic concentration of W decreased from 1.3, 1.0 to 0.8 at%. Ni was present at a low atomic concentration of 0.1 and 0.2 at% for process currents of 0.30 and 0.39 A, respectively. At the process current of 0.62 A, Ni was not detected.

The atomic concentration found on the control and electropolished test specimens is shown in Table 24. The majority of the detection was from carbon and oxygen. The electropolished specimen surfaces also contained higher concentration of Co, Cr, and W

than the control. Low concentration of nickel, nitrogen, sodium, magnesium aluminum silicon, zinc, calcium, potassium and phosphorous were also detected on the surface.

Figure 29. Atomic concentration of Co-Cr alloy L605 electropolished at 25°C for 3 min. with various process currents. The graph shows the atomic concentration of the chemical state for Cr, Co, Ni, and W.

Table 24. Atomic concentration of Co-Cr alloy L605 electropolished at 25°C for 3 min. with currents 0.12, 0.39, and 0.68 A.

Parameters	C	N	O	Na	Mg	Al	Si	P	
Control	43.7	1.7	42.0	0.4	-	0.2	0.6	10.9	
3 min. 2.24 V 0.12 A	42.4	1.4	41.1	0.3	0.4	0.6	1.4	2.0	
3 min. 3.42V 0.39 A	32.7	1.1	44.5	0.5	0.6	-	2.4	6.0	
3 min. 5.02V 0.68 A	28.2	1.1	45.1	-	0.7	-	3.3	6.8	
	Cl	K	Ca	Cr	Co	Ni	Cu	Zn	W
Control	-	0.2	0.2	-	0.3	-	-	-	-
3 min. 2.24 V 0.12 A	0.3	-	1.8	5.4	0.5	0.1	0.4	0.6	1.3
3 min. 3.42 V 0.39 A	0.4	-	2.5	3.8	1.7	0.2	0.8	2.0	1.0
3 min. 5.02 V 0.68 A	0.9	-	4.5	3.1	1.2	-	1.3	3.2	0.8

A high resolution scan was done to characterize the chemical states of Co, Cr, and W at the three process currents. For each element, the oxide and metallic states were characterized. Cr(OH)_3 and Cr_2O_3 were detected from the high resolution scan. As can be seen in Table 25, the detection of Cr in the oxidized form decreased from 99.0 to 95.3% between process currents of 0.12 and 0.68 A. The percentage of the Cr in the oxidized form did not significantly change when the specimen was electropolished with the process current of 0.39 and 0.68 A. There was a 0.2 % difference between the Cr in the oxidized state. The chemical states of both W and Co were also determined. The metallic state was detected for both W and WO_3 and WO_2 were found as the oxides. Both metallic and oxidized formed of Co were determined from the high resolution scan, however, the specific chemical state of Co could not be determined. The W oxide percentage changed from 100, 87.1 to 90% with an increase in process current and the Co in the oxidized form changed from 100, 79.8 to 93.4%. When the specimen was electropolished at 0.12A for 3 min., there was a more Cr, W, and Co in the oxidized formed was found, as compared to the control.

The following contaminants were detected on the surfaces: Phosphorous (P), Calcium (Ca), Sodium (Na), Aluminum (Al), Silicon (Si) and Carbon (C). According to the chemical analysis by Haynes International, low levels of C, P, and Si are readily found on the Co-Cr alloy L605.

Other elements, such as Na, Al, and Ca, were considered as contaminants, and were excluded from the analysis. The electropolishing solution and the DI water used to clean the test specimens were considered as the possible source of contaminants. A low

resolution scan was conducted on both the DI water and the electropolishing solution in order to characterize the solutions. As shown in Figures 30 and 31, no trace of Ca or Al was detected in the electropolishing solution. No trace of Al and only 0.3 at% of Ca were detected in the DI water. Therefore, the possibility of contaminants being caused by the two solutions was considered minimal.

Table 25. Chromium, Tungsten, and Cobalt chemical state % contribution found on the test specimen surface with varying process current and control.

Temperature	Current Voltage	Chromium		Tungsten		Cobalt	
		Cr Metal	Cr Oxide	W Metal	W Oxide	Co Metal	Co Oxide
Control	0 A 0 V	18.8%	81.2%	42.1%	57.9%	55.3%	44.7%
25°C	0.12 A 2.24 V	1.03%	99.0%	0%	100%	0%	100%
	0.39 A 3.42 V	4.50%	95.5%	12.9%	87.1%	20.2%	79.8%
	0.68A 5.02 V	4.74%	95.3%	10%	90%	6.43%	93.6%

5.6.2 Effect of EP Time and Bath Temperature on the Surface Chemistry

As shown in Figure 32, Cr, W, and Co detected on the test specimen electropolished at 0.30 A in a 0°C acid bath increased with an increase in the EP time. The detection of Cr, W, and Co increased from 1.1 to 3.4 at%, 0.9 to 2 at%, 0.2 to 1.0 at%, respectively. There was no increase in the Ni detection. When the specimen was electropolished at 25°C with a process current of 0.39 A, the detection of Cr, W, and Ni increased; however, the detection of Co decreased with an increase in EP time from 3 to 10 min. The atomic concentration of Cr, W, and Ni increased from 3.8 to 5.2 at%, 1 to

2.3 at%, and 0.2 to 0.3 at%, respectively. The high resolution XPS scan of Cr and W are shown in Appendix H.

Figure 30. Low-resolution XPS scan of electropolishing solution composed of 15% (v/v) phosphoric acid.

Figure 31. Low-resolution XPS scan of DI water used to clean the test specimens.

Figure 32. Atomic concentration (at %) of Chromium, Tungsten, Cobalt, and Nickel with respect to each parameter.

The Co detection decreased from 1.7 to 1.5 at%. The atomic concentration of Cr exhibited the greatest difference of 2.3 and 2.7 at% with respect to an increase in the EP time and bath temperature. As shown in Table 26, a two-way ANOVA analysis was conducted to characterize the effect of EP time and temperature on the Cr at% found on the specimen surface. Although at% of Cr was influenced by both the bath temperature and the EP time, no statistically significant difference was obtained at the 95% confidence interval.

The Cr concentration was further investigated to determine the effect of EP time and bath temperature on the surface chemistry. A 2-level 2-factorial Taguchi DOE (Design of Experiment) was conducted to characterize the Cr at%. As can be seen in Figure 33, the concentration of Cr increased with EP time from 3 to 10 min., however, less Cr was detected when the bath temperature was decreased from 25°C to 0°C.

Table 26. Two-way ANOVA: Cr at% versus EP temperature (°C) and EP time (min.).

Source	DF	SS	MS	F	P
Temperature	1	5.76	5.76	64	0.079
EP Time	1	4	4	44.44	0.095
Error	1	0.09	0.09		
Total	3	9.85			
S = 0.3 R-Sq = 99.09% R-Sq(adj) = 97.26%					

The chemical states of Cr, W, and Co were further characterized using the high resolution XPS analysis. As seen in Table 27, metallic Cr, W, and Co on the surface of the control specimens was 18.8, 42.1, and 55.3%, respectively.

Figure 33. Main effects graph of Cr at% using 2-level 2-factorial Taguchi DOE.

Cr, W, and Co in the oxidized state for the control was 81.2, 57.9, and 44.7%, respectively. When the test specimens were electropolished, Cr, W, and Co were found primarily in their oxidized form. 95.5% of the Cr was found in the oxidized state when the test specimen was electropolished at 25°C for 3 min. When the test specimen was

electropolished for 3 min. at 25°C, the Cr, W, and Co found in its oxidized form increased relative to that of the control to 95.5, 87.1, and 79.8%, respectively. When the bath temperature was reduced to 0°C, the oxidized state of Cr decreased to 85.2%, W increased to 96.8%, and due to a weak signal, the Co in the oxidized state form was not determined.

Table 27. Proportion of Chromium, Tungsten, and Cobalt in metallic and oxide state with respect to electropolishing parameters.

Temperature	Current Voltage	EP Time	Chromium		Tungsten		Cobalt	
			Cr Metal	Cr Oxide	W Metal	W Oxide	Co Metal	Co Oxide
25 °C	0.39 A 3.42 V	3 min.	4.5%	95.5%	12.9%	87.1%	20.2%	79.8 %
		10 min.	6.8%	93.2%	6.6%	93.4%	28.9%	71.1%
0 °C	0.30 A 3.10 V	3 min.	14.8%	85.2%	3.2%	96.8%	Weak Signal	
		10 min.	14.4%	85.6%	2.6%	97.4%	2.7%	97.3%
		30 min.	9.3%	90.7%	8.2%	91.8%	37.7%	62.3%
		60 min.	9.1%	90.9%	5.0%	94.9%	27.0%	73.0%
Control	-	-	18.8%	81.2%	42.1%	57.9%	55.3%	44.7%

A 2-level 2-factorial Taguchi DOE was conducted to characterize the time and temperature effect on the Cr in the oxidized form on the specimen surface. As shown in Figure 34, time and temperature are inversely proportional to each other. An increase in the temperature resulted in an increased detection of Cr in the oxidized form.

The detection of Cr oxide decreased with respect to EP time. There was a decrease in the Cr oxide percentage for the test specimen electropolished at 0.39 A in a 25°C acid bath for 3 and 10 min. The Cr oxide detection decreased from 95.5 to 93.2% with an increase in the EP time. In contrast, when the test specimen was electropolished

at 0.30 A in a 0°C acid bath for 3 and 10 min., the Cr oxide detection increased by 0.4% from 85.2 to 85.6%. Cr oxide detection decreased with respect to a decrease in the bath temperature. When the test specimen was electropolished for 3 min. at 25 and 0°C, the Cr oxide detected on the surface decreased from 95.5 to 85.2%. Similarly, when the test specimen was electropolished for 10 min. at 25 and 0°C, the Cr oxide decreased from 93.2 to 85.6%. The decrease in the Cr oxide with a decrease in bath temperature was mainly due to the difference in the process current.

Figure 34. Main effects graph of Cr found in the oxidized form using 2-level 2-factorial Taguchi DOE.

As more material was removed from the test specimen surface, new Cr oxide was formed. According to Faraday's Law of Electrolysis, the total material removed from the surface is proportional to EP time and process current. While keeping EP time constant, the amount of process current contributes to the total material removed from the surface. The process current decreased with respect to a decrease in the bath temperature. For 25

and 0°C, the process currents were 0.39 and 0.30 A, respectively. Therefore, more material was removed from the test specimen electropolished at 25°C, ultimately forming more Cr oxides.

A 2-level 2-factorial Taguchi DOE was conducted on the W oxide %. As shown in Figure 35, W oxide increased with respect to an increase in EP time and a decrease in the bath temperature. When the test specimen was electropolished at 0.39 A in a 25°C bath for 3 and 10 min., the W oxide increased from 87.1 to 93.4%. Similarly, when the test specimen was electropolished at 0.30 A in a 0°C bath for 3 and 10 min., the W oxide increased from 96.8 to 97.4%. W found in the oxide state also increased with a decrease in the bath temperature. The W detected in an oxide state increased from 87.1 to 96.8% when the test specimen was electropolished for 3 min. in a 25 and 0°C bath. Similarly, a 4% increase from 93.4 to 97.4% was detected on the test specimen electropolished for 10 min. with a decrease in the bath temperature from 25 to 0°C. While keeping the process current constant, the only factor that affected the total material removed from the surface was the EP time. Therefore, more material was removed by electropolishing for 10 min. than 3 min., which resulted in the W formation as an oxide on the specimen surface.

Further investigation was conducted to characterize the Cr oxide formation with respect to EP time. A high resolution scan was conducted to analyze the chemical composition of the oxide form of Cr found on the specimen surface. XPS analysis determined that the oxide form of Cr was $\text{Cr}(\text{OH})_3$ and Cr_2O_3 .

The test specimens were further electropolished for 30 and 60 min. in a 0°C bath to determine the effect of EP time on the percentage of C on the surface. The percentage

of Cr found in the oxide state on the surface increased with respect to an increase in EP time. At EP times of 3, 10, 30, and 60 min., Cr found in the oxide form increased from 85.2, 85.6, 90.7, to 90.8%. The formation of Cr oxides and electropolishing time is proportionally related. There was an increase in the formation of Cr oxides as the electropolishing time increased. As the electropolishing time increased, more material was removed from the specimen surface, which linearly correlated with the formation of new Cr oxides.

Figure 35. Main effects graph of W % oxide using 2-level 2-factorial Taguchi DOE.

5.7 AES Analysis

Electropolished L605 test specimens exhibited Cr, W, and Co on the surface with a reduced detection of Ni, as shown in Figure 36. The high amount of O on the surface indicated that the three elements found on the surface were in the oxide state. Traces of Ca, P, and C were found on the surface, which are thought to be contaminants. The amount of trace elements found on the surface was negligible and did not hinder the outcome of the analysis. As EP was increased, more Cr was found on the surface, that

indicated that the surface became Cr rich, as shown in Figure 37. The AES analysis depth profile for EP time of 10 and 60 min. at 0°C and 3 and 10 min. at 25°C are shown in Appendix I.

5.7.1 Effect of EP Time and Temperature on the Oxide Thickness

AES analysis was conducted to determine the oxide thickness. Specimens that underwent AES are shown in Table 28. A 2-level 2-factorial Taguchi DOE was conducted to determine the effect of temperature and EP time on the thickness of the Cr oxide layer. The test specimens were electropolished for 3, 10, 30, and 60 min. at 0°C. Another set of test specimens was electropolished for 3 and 10 min. at 25°C. As can be seen in Figure 38, the maximum oxide thickness of 1100 nm was obtained on the test specimen electropolished in a 0°C bath for 3 min.

Table 28. Test specimen parameters that underwent AES analysis.

Voltage	Current	Temperature	EP Time
3.42 V	0.39 A	25°C	3 and 10 min.
3.10 V	0.30 A	0°C	3, 10, 30, and 60 min.

The thickness of the oxide layer decreased with respect to EP time. The Cr oxide thicknesses on the test specimens electropolished in a 0°C bath for 3, 10, 30, and 60 min. were 1100, 950, 300, and 330 nm, respectively. The oxide thickness decreased with respect to an increase in EP time.

Figure 36. AES analysis of test specimen electropolished in a 0°C 15% (v/v) phosphoric acid for 3 min.

Figure 37. AES analysis of test specimen electropolished in a 0°C 15% (v/v) phosphoric acid for 30 min.

Figure 38. Oxide thickness (nm) of test specimens electropolished at various temperatures (°C) and EP times (min.).

A 2-level 2-factor Taguchi DOE was conducted to understand the effect of temperature and time on the thickness of the oxide layer. The oxide thickness increased with respect to a decrease in the bath temperature. When the test specimen was electropolished for 3 min. in a 25°C and 0°C bath, the Cr oxide thickness increased from 520 to 1100 nm. Similarly, when the test specimen was electropolished for 10 min. in a 25°C and 0°C bath, the Cr oxide thickness increased from 400 to 950 nm.

As can be seen in Figure 39, time had a limited influence on the oxide thickness at a constant temperature. However, the oxide thickness had a strong dependency on the temperature of the electropolishing bath. As temperature decreased, there was an increase in the thickness of the oxide layer. Conversely, as temperature increased, there was a decrease in the oxide thickness.

Figure 39. Main effects graph of the oxide thickness on the test specimen surface using 2-level 2-factorial Taguchi DOE.

5.8 Cytotoxicity

5.8.1 Qualitative Assessment

Visual qualitative assessment was conducted on the test specimens. Glass was the negative control and was assigned a toxicity of 0. Ni Foil, the positive control, was assigned a cytotoxicity of 3. A specimen that was not electropolished was the control with a cytotoxicity of 1.5. The test specimens, that were electropolished in 15% (v/v) phosphoric acid at 0, 25, and 35°C bath for 3 and 10 min. with 4 replications, were compared with the negative, positive and the specimen controls. Test specimens electropolished in 25 and 35°C bath for 3 and 10 min. were assigned a relative toxicity of 1.5. The cells were confluent around some of the edges; thus, no cell lysis was found. However, no cell growth or limited cell growth that indicated a lack of confluence around the edges were also found, as shown in Figure 40.

A toxicity of 0.5 was assigned to test specimens electropolished for 3, 10, 30, and 60 min. in a 0°C bath. Confluent cell growth around the specimen electropolished in a 0°C bath for 3 min. is shown in Figure 41.

Figure 40. Inverted microscope survey of HEP 2 cells. Test specimen electropolished at 25°C for 3 min. at 0.39 A.

Figure 41. Inverted microscope survey of HEP 2 cells. Test specimen electropolished at 0°C for 3 min. at 0.30 A.

5.8.2 Quantitative Assessment

The cells were quantified using a hemocytometer. The average from 4 replications was calculated. The cell density was calculated using the following equation [10],

$$\left[\frac{(N_1 + N_2 + N_3 + N_4) * (5 * 10^4)}{4} \right] = \rho \quad \text{Equation [10]}$$

where N is the cell count on the four corners of the hemocytometer and ρ is the cell density (cells/ml). The cell densities on the electropolished test specimens were significantly greater than the positive control and lower than the negative control. Therefore, it was concluded that electropolished specimens exhibited minor toxicity if any, rather than severe toxicity. The cell density increased with respect to an increase in EP time.

As seen in Figure 42, the cell densities of the test specimens electropolished for 3 min. at 35, 25, and 0°C were $42,750 \pm 8,340$ cells/ml, $46,660 \pm 3540$ cells/ml, and $58,130 \pm 11,170$ cells/ml, respectively. There was an increase in the cell density with respect to a decrease in the bath temperature.

The cell density of the test specimens electropolished for 10 min. at 35, 25, and 0°C were $40,380 \pm 9,660$ cells/ml, $44,750 \pm 12,480$ cells/ml, and $60,750 \pm 10,620$ cells/ml, respectively. Similarly, the cell density increased with respect to a decrease in the bath temperature when the test specimens were electropolished for 10 min.

A multilevel factorial design analysis was conducted to determine the effect of the bath temperature and the EP time on the cell density. As shown in Table 29, a statistically significant difference was found in the cell density due to a different bath temperature, at the 95% confidence interval. As the bath temperature decreased, cell

density increased. Although cell density increased with an increase in EP time, there was no statistically significant increase in the cell density at the 95% confidence interval.

Figure 42. Cytotoxicity quantitative analysis (cells/ml) of HEP2 Cells with test specimens electropolished at various bath temperatures (°C) and EP times (min.).

Table 29. Multilevel factorial design general linear model: Average (n=4) cell density (cells/ml) versus temperature (°C) and EP time (min.).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temperature	2	1400303333	1400303333	700151667	7.44	0.004
EP Time	1	1870417	1870417	1870417	0.02	0.889
Temperature*Time	2	30603333	30603333	15301667	0.16	0.851
Error	18	1694292500	1694292500	94127361		
Total	23	3127069583				
S = 9701.93 R-Sq = 45.82% R-Sq(adj) = 30.77%						

A two-sample T-test was conducted to compare the cell density with the control.

The cell density of the test specimens electropolished at 0°C for 3 and 10 min. did not

show a statistically significant difference. As can be seen in Table 30, the cell density on specimens electropolished at 0°C for 30 and 60 min. exhibited a statistically significant difference at the 95% confidence interval. Therefore, electropolishing for 30 to 60 min. enhanced the biocompatibility of Co-Cr alloy L605.

Table 30. Two-sample T-test of the average (n=4) cell density (cells/ml) compared to the control. The test specimens were electropolished at 0°C for the respective EP times (min.).

EP Time	3 min.	10 min.	30 min.	60 min.
Test specimen cell density	58.1 x10 ³ cells /ml	60.8 x10 ³ cells /ml	62.1 x10 ³ cells /ml	64.0 x10 ³ cells /ml
Control cell density	46 x10 ³ cells /ml			
P-Value	0.166	0.100	0.039	0.047

5.9 Summary of Results

A current plateau on the I-V curve was determined using 15% (v/v) phosphoric acid at the bath temperatures of 0, 25, and 35°C. A decrease in the current and a wider current plateau occurred with a decrease in the bath temperature. The current plateau was not obtained when 50 and 80% (v/v) phosphoric acid was used to generate the I-V curves; hence, no further electropolishing experiment was conducted.

The average EP rate decreased with a decrease in the bath temperature, which was also linearly correlated to a decrease in the process current. Therefore, the decrease in the process current had an influence in decreasing the EP rate. The surface roughness decreased with an increase in the EP time until 30 min. At a longer duration of electropolishing at EP time of 60 min., the surface roughness increased and pits formed.

The surface roughness increased with a decrease in the bath temperature. The contact angle decreased with a decrease in the EP time until 30 min. and then increased at an EP time of 60 min. The contact angle increased with a decrease in the bath temperature.

According to the surface chemistry analysis, Cr in the oxidized form increased with an increase in the EP time and an increase in the bath temperature. The thickness of the Cr oxides increased with a decrease in the bath temperature. An overall decrease in the oxide thickness was obtained with an increase in the EP time. L605 test specimens exhibited an increase in biocompatibility with an increase in the EP time and a decrease in the bath temperature. As EP time increased, there was a statistically significant increase in cell density as compared to the control, which confirms that electropolishing improves the biocompatibility of Co-Cr alloy L605.

CHAPTER SIX

DISCUSSION

6.1 Effect of Process Current on the Surface Characteristics

The electropolishing rate (EP rate) increased with an increase in the process current. The EP rate at process currents of 0.12, 0.39, and 0.68 A were 0.00015, 0.01165, and 0.02259 g/cm² min., respectively. The test specimens were electropolished at a 25°C bath temperature for 3 min. As was described in Chapter 2, the increase in the electropolishing rate can be deduced from Faraday's Law of Electrolysis, shown in Equation 1.

$$W_{loss} = \frac{ItM}{nF} \quad \text{Equation [1]}$$

where W_{loss} is the total material loss, n is the valence of the metal ion, F is Faraday's constant (96,500 Columnb), M is the molecular weight of the anode, I is the process current, and t is the EP time. M , n , and F are constants. The two variables that affect the total material loss during electropolishing are process current (I) and electropolishing time (t), as shown in Equation 1. The electropolishing rate was measured by the total material removed over EP time, and is shown in Equation 11.

$$\frac{W_{loss}}{t} = \frac{IM}{nF} \quad \text{Equation [11]}$$

The only variable that affected the electropolishing rate was the current, as shown in Equation 11. Therefore, the electropolishing rate increased with an increase in the process current.

The surface roughness increased with an increase in electropolishing time, for a process current set at 0.12 A. An average surface roughness of 112.8, 142.9, and 159.4 nm was obtained for EP times of 1, 2, and 3 min. The surface roughness increased from 112.9 to 159.4 nm with an increase in EP time from 1 to 3 min. This finding was contradictory to that of Lee [15]. According to Lee [15], average surface roughness decreases with respect to an increase in EP time. Surface roughness decreased from approximately 9 to 0.2 μm when type 316L stainless steel was electropolished for 400 s. The decrease in the surface roughness can be explained by the electropolishing effect. When the current is applied to the anode and anode potential becomes greater at the current plateau, high specific gravity, highly viscous and insulating anodic film covers surface. The anodic film covers the lower peaks and valleys preventing dissolution; while higher peaks with high charge concentration are protruded above the anodic films dissolve more readily [15]. In conjunction, more material from the surface is removed with an increase in electropolishing time, as deduced from Faraday's Law of Electrolysis.

The increase in the surface roughness on test specimens electropolished at 0.12A may have been due to the low process current that was located at the base of the I-V curve with low anode potential. Due to the low anode potentials, the protective anodic film failed to form on the specimen surface that would have prevented the dissolution of

material from low peaks and valleys [15]. The surface was etched when the anode potential was low, that may have contributed to the increase in the surface roughness when the test specimens were electropolished at the low process current.

6.2 Effect of EP Time and Bath Temperature on the Surface Characteristics

Electropolished test specimens became hydrophilic with respect to an increase in the electropolishing time (EP time) while keeping the bath temperature constant. There was a parallel finding between the surface roughness and the contact angle as the EP time increased. The surface roughness and the contact angle decreased with an increase in EP time, which was in accordance with that of Lee [15], who electropolished a type 316L stainless steel in a 40°C sulfuric acid, phosphoric acid, and water mixture at the process current of 9.0 A/cm². The surface roughness decreased from approximately 2.0 to 0.2 μm after an electropolishing time of 400 s. Similarly, in the current investigation, the surface roughness of the Co-Cr test specimen decreased from 92.6 to 23.0 nm after 30 min. of electropolishing in 0°C 15% (v/v) phosphoric acid at the process current of 0.30 A. The limitation of the experiment by Lee was that the electropolishing time was limited to 400 s. Therefore, the effect of an EP time greater than 400 s was not captured in the experiment. The current research showed that the surface roughness increased at an EP time of 3600 s or 60 min. due to the formation of pits, which was found from the three-dimensional profile of the test specimens. The test specimen surface became smoother as more material was removed from the surface. As was explained in Chapter 5, the process current decreased with a decrease in the bath temperature. Therefore, as EP time

increased, more material was removed from the specimen surface, and this resulted in a lower surface roughness and a decrease in the contact angle, making the specimen surface more hydrophilic.

In comparison, the surface roughness decreased and the contact angle increased as the bath temperature decreased while maintaining a constant EP time. As was described in Chapter 5, the bath temperature and the process current are proportional to each other. Therefore, the decrease in the bath temperature was an indication that the process current decreased proportionally. According to Faraday's Law of Electrolysis, the total material removed decreased with respect to a decrease in process current. Since less material was removed from the test specimen, the surface roughness was greater when the test specimen was electropolished at a higher temperature, with a fixed EP time. This finding was in accordance with that of Lin and Hu [39]. Surface roughness was determined on a 4 cm x 12 cm 304 stainless steel mesh electropolished in a phosphoric acid sulfuric acid mixture. A 2^{5-1} fractional factorial design was analyzed to determine the effect of acid ratio, amount of glycerol, temperature, current density and electropolishing time on the specimen surface roughness. Lin and Hu [39] showed that the average surface roughness increased with respect to a decrease in the process current from 1.0 to 0.5 A/cm². However, there was an increase in the surface roughness with an increase in the bath temperature from 30 to 70°C. The current study showed that as the process current decreased, the surface roughness increased, while maintaining a constant EP time. As EP time increased, the surface roughness decreased..

The surface energy determines the polar and dispersive forces at the surface of a material and it also characterizes the behavior of fluids on the surface. [40]. According to Palmaz [40], hydrophilic and hydrophobic surfaces are high and low energy, respectively. When a droplet of DI water is administered on a high-energy surface, the droplet spreads; however, on a low-energy surface, the droplet of water forms a beaded shape. The interaction between the hydrophobic surface and the blood plasma protein heavily denatures the protein molecules and results in a relatively thrombogenic surface[40]. In contrast, a hydrophilic surface increases the affinity for water molecules and decreases the affinity for plasma proteins and cells, reducing the susceptibility of the material surface to become thrombogenic. Therefore, a hydrophilic surface is highly favored on implants.

The test specimen surface became more hydrophilic with an increase in EP time. The cell density (cells/ml) increased with an increase in the EP time. The result was compared with the cell density (cells/ml) of the control by using a two-sample t-test. A statistically significant difference was not determined at EP times of 3 and 10 min. As EP time was increased to 30 and 60 min., a statistically significant difference was obtained at the 95% confidence interval. Therefore, the research showed that a smooth hydrophilic surface resulted in an increase in the cell density (cells/ml).

6.3 Effect of Faraday's Law of Electrolysis on the Formation of Cr Oxides

As was explained by Farady's Law of Electrolysis, the total material removed from the test specimen during electropolishing is controlled by both time and current.

When the specimen was electropolished at 0°C for 3, 10, 30, and 60 min., the total material removed increased with respect to time; however, the average material removal rate from the anode did not change. Therefore, the EP rate, i.e., the average material removed per EP time, had no significant difference in the average rate of material removal.

The current value on the current plateau was influenced by the bath temperature. The highest current can be obtained at the highest temperature tested that exhibited the greatest average EP rate. The increased EP rate at the highest temperature can be deduced from Faraday's Law of Electrolysis. Since the current is one of the parameters for calculating the total material removed, the higher the current, the greater the total material removed. Therefore, the test specimen electropolished at the highest temperature with the highest current exhibited the greatest EP rate. The bath temperature and current were proportional to each other; the lowest current was obtained at 0°C. Since the current at 0°C was the lowest, relatively less material was removed at 0°C compared to 35°C, while keeping the time constant. The specimen electropolished at 0°C exhibited the lowest EP rate.

The long-term biocompatibility of implants depends on the ability of metals and alloys to form a passive oxide layer that protects the bulk from further oxidation [41]. According to the X-ray Photoelectron Spectroscopy (XPS) analysis, Cr in the oxidized state was determined as the primary oxide state on the electropolished Co-Cr test specimen. The formation of the Cr oxides was influenced by bath temperature and EP time. As the test specimen was electropolished for 3 min. in a 25 and 0°C bath, the

amount of Cr detected in the oxidized state decreased from 95.5 to 85.2%. Similarly, when the test specimen was electropolished for 10 min. in the same bath temperatures, the detection of Cr oxides decreased from 93.2 to 85.6%. The formation of the new oxides depends on the total material removed from the test specimen surface that is explained by Faraday's Law of Electrolysis. The total material removed from the surface was correlated with the process current and the EP time. While maintaining a constant EP time, the only factor that influenced the total material removed was the process current. The bath temperature and the process current were also correlated. The process currents at 25°C and 0°C were 0.39 A and 0.3 A, respectively. Therefore, at the bath temperature of 25°C, more material was removed from the surface, that removed the old oxide layer, providing more surface area for new oxides to form.

The detection of Cr in the oxidized state for the test specimens electropolished at 0°C for 3 and 10 min. were 85.3 and 85.6%, respectively. When the EP time was increased to 30 and 60 min., the amounts of Cr in the oxide state were 90.7 and 90.9%, respectively. Cr detected in the oxidized form increased with respect to an increase in the EP time. The cytotoxicity of the electropolished test specimen was influenced by the increase in the Cr in the oxidized state.

The cell density (cells/ml) determined from the cytotoxicity experiment increased with an increase in the Cr in the oxidized state. The result was compared with the cell density (cells/ml) of the control by using a two-sample t-test. A statistically significant difference in the cell density (cells/ml) was not determined for EP times of 3 and 10 min. at the 95% confidence interval. The cell density (cells/ml) of the control was compared

to that of test specimens electropolished for 30 and 60 min. at 0°C. At the EP time of 30 and 60 min., there was a statistically significant difference in the cell density (cells/ml) with the control specimen, at the 95% confidence interval. Therefore, the biocompatibility of the electropolished Co-Cr specimens was enhanced with respect to an increase in the formation of the Cr in the oxidized state.

CHAPTER SEVEN

CONCLUSION

The wide current plateau on the I-V curve indicated a constant current was obtained at lower temperatures. Since polishing rate and current are correlated, by keeping the current constant it can allow for greater control over the electropolishing process. The presence of a current plateau was found to be dependent on the temperature of the acid bath.

The EP rate showed a strong dependence on the temperature. At a high temperature, there was an increase in the current, resulting in more material being removed from the specimen.

Surface roughness showed a strong dependence on temperature. The bath temperature and the surface roughness were proportionally to each other. EP time had a limited influence on the surface roughness. Contact angle and bath temperature were inversely proportional to each other. Therefore, the surface roughness and the contact angle were also inversely correlated. Greater hydrophobicity was obtained with a decrease in the bath temperature. However, as the EP time increased, the surface roughness decreased and the specimen surface became more hydrophilic.

According to the AFM analysis, electropolishing resulted in a wavy surface topography. Etch pits were found on the specimen surface under SEM analysis, that correlated closely with an increase in EP time. An increase in EP time resulting in greater etch pits was confirmed by both AFM and SEM analysis. The etch pits found at

longer EP rates exhibited a greater surface roughness, that ultimately showed a decrease in contact angle, making the surface more hydrophilic.

The formation of metal oxides was proportional to the process current and electropolishing time. Higher concentrations of Cr_2O_3 on the surface were obtained at a longer electropolishing time and a higher process current. Under these conditions, more material was removed from the specimen and there was a greater tendency for the metals on the specimen surface to oxidize.

From the qualitative analysis, electropolished specimens were given the toxicity of 1. Cell density increased with respect to a decrease in the bath temperature and an increase in the EP time. According to the quantitative analysis, the cell density increased with respect to an increase in EP time. The specimens electropolished for 30 and 60 min. showed a statistically significant increase in cell density, as compared to the control. The cell density was lower than the negative control and higher than the positive control. Therefore, electropolishing only had a limited influence on the biocompatibility of the alloy.

Electropolishing L605 in 15% (v/v) phosphoric acid alters the surface characteristics. The nature of the surface characteristics is dependent on the EP time, bath temperature, and process current. Smooth and hydrophilic surfaces were obtained with an increase in EP time, which is beneficial in improving the tissue-to-material interaction. In addition to surface characteristics, surface chemistry also changes as a result of electropolishing. The available Ni on the specimen surface decreases, thus minimizes the potential toxic effect of L605 contains traces of Ni.

CHAPTER EIGHT

FUTURE WORK

The research covered the surface characterization of electropolished L605 resulting from changes in temperature, current, and electropolishing time. Future work should be conducted to investigate the effect of other critical process parameters (e.g. electrolyte ingredient) to determine the surface characteristics and biocompatibility of L605. The current research showed that as the bath temperature increased, the current plateau on the 15% (v/v) phosphoric acid disappeared. As the bath temperature decreased, a distinct and wider current plateau was generated. It would be of interest to characterize the current plateau for a higher concentration of phosphoric acid.

Other future work should entail leach testing of the electropolished L605, rather than the cytotoxicity test per ISO10993-05. The current biocompatibility experiment only assessed the viability of the HEP 2 cells quantitatively and qualitatively. It would be of interest to characterize the metallic ions that leach from the electropolished test specimens.

Parallel to the leaching test, it might prove beneficial to conduct cell adhesion experiments to assess the effect of cell adhesion on the hydrophilic electropolished specimens.

By using XPS and AES data obtained from the current experiment, a corrosion test should be conducted to characterize the oxide thickness and the corrosion properties.

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APPENDIX A

SURFACE ROUGHNESS DEFINITIONS

Table 31. Definitions of surface roughness properties.

Roughness Parameters	Definition
Total Area	Entire surface area including peaks and valleys
Sa	An arithmetic roughness average relative to the centerline that divides the valleys from the hills
Sq	Root mean square of the surface roughness relative to the centerline
Sz	Ten point height: The average of five highest local peaks plus the average of the five lowest local peaks
Sr	Range: Highest roughness on a highest range

APPENDIX B

CYTOTOXICITY METHODS

Culture Media Preparation

1. Making complete media

- a. A sterile premixed/stock DMEM solution with sodium bicarbonate and without glutamine was obtained.
- b. The following material and volume was added to the 500 ml media.
 - i. 5 ml glutamine
 - ii. 1 ml Pen-Strep
 - iii. 44 ml NCS

Initial Culturing Step

1. 1 ml HEP-2 cells were obtained from the liquid nitrogen container.
2. The cells were thawed in a 37°C water bath for approximately 10 min.
3. A 75 cm² flask was obtained.
4. 10 – 15 ml media was dispensed in the flask
5. The cells were pipetted in the flask
6. The flask was gently swirled to equally distribute the cells.
7. The flask was incubated in a 37°C incubator for 3-4 days.

Subculture Process

1. Cell culture media and trypsin was pre-warmed in a 37°C water bath.
2. Flask from the incubator was obtained and the cells were inspected under the inverted microscope to make sure they were healthy.

3. Media was removed from the flask and rinsed with 2 ml Hanks Solution (without Calcium and Manganese). Step 3 was repeated twice to ensure that the cells were rinsed thoroughly.
4. After moving the Hanks solution, 2 ml of trypsin was added to the 75 cm² flask, and incubated in the 37°C incubator for 10 min.
5. While waiting for the cells to detach from the flask surface, a new flask (25 cm²) was prepared with the appropriate label (cell type, number of cells, date, and operator's initials). 5 ml of complete media was added to a 25 cm² flask.
6. After 10 min., cells were inspected to make sure they were coming off the flask surface.
7. 2 ml of complete media was added to the flask to inhibit the action of trypsin.
8. The cell culture was aggregated repeatedly by pipetting 10 to 20 times against the side of the flask.
9. Cells were counted using the hemocytometer.
 - a. 20 µl of the cell culture was dispensed and mixed with another 20 µl of typhon blue. The two solutions were mixed thoroughly by pipetting approximately 10 times.
 - b. 20 µl of the mixed solution was pipetted on the hemocytometer and the 4 large squares on the corners were counted. The total was taken and multiplied by 5×10^4 to determine the number of cells per ml.
10. From the cell concentration (cells/ml) that was determined in step 9, a dilution was made to acquire 10^5 cells/ml and was dispensed in the 25 cm² flask. The

determination of the appropriate volume of cell culture to dispense is shown by

Equation 13. Cell count is the qty. of cells recorded by the hemocytometer.

$$\frac{(5 * 10^5)}{(CellCount * (5 * 10^4))} = V_{cellMedia} \quad \text{Equation [13]}$$

Cell Count: Quantity of cells from hemocytometer

$V_{cellMedia}$: Volume of cell culture needed to make 10^5 cell/ml

11. The flask was incubated in a 37°C incubator for 3 – 4 days.

Grow Cells in Wells

1. Two 24-well titer plates were obtained. Each column was labeled in order to distinguish the difference test specimens.
2. 1 ml of complete media was dispensed in each well.
3. 0.5ml cell culture was obtained.
4. The titer plates were incubated for 1 week in order for the cells to grow confluent.
5. Culture medium was removed and discarded and new fresh culture medium was added (1 ml).
6. Each test specimen was autoclaved and was placed in an appropriate well.
7. The flask was then incubated for an additional 1 week.
8. After 1 week, the toxic effect was determined.

Cell Evaluation

Cytotoxicity Qualitative Assessment

1. The qualitative assessment was conducted for specimens while viewing the cell culture under the inverted microscope.
2. The assessment was conducted by categorizing the confluence of the cells using Table 32.

Table 32. Table used to translate the degree of cytotoxicity to a numeric cytotoxicity scale.

Cytotoxicity Scale	Interpretation
0	Noncytotoxic
1	Mildly Cytotoxic
2	Moderately Cytotoxic
3	Severely Cytotoxic

Cytotoxicity Quantitative Assessment

1. 20 μ l of the cell culture was dispensed and mixed with another 20 μ l of typhon blue. The two solutions were mixed thoroughly by pipetting approximately 10 times.
2. 20 μ l of the mixed solution was dispensed on the hemocytometer. Cells that fell in squares in the 4 corners were counted. Each sample was physically counted using the hemocytometer.
3. The total was then multiplied by 5×10^4 to determine the number of cells per ml.

APPENDIX C

ONE-WAY ANOVA ANALYSIS

Table 33. One-way ANOVA of currents at 1, 2, and 3 min. on the I-V curve in 85% (v/v) phosphoric acid at 35°C.

Source	DF	SS	MS	F	P
Time (min.)	2	0.018	0.009	0.34	0.713
Error	24	0.6279	0.0262		
Total	26	0.6459			
S = 0.1617 R-Sq = 2.78% R-Sq(adj) = 0.00%					

Table 34. One-way ANOVA of currents at 1, 2, and 3 min. on the I-V curve in 85% (v/v) phosphoric acid at 25°C.

Source	DF	SS	MS	F	P
Time (min.)	2	0.0225	0.0112	0.45	0.641
Error	24	0.5941	0.0248		
Total	26	0.6165			
S = 0.1573 R-Sq = 3.64% R-Sq(adj) = 0.00%					

Table 35. One-way ANOVA of currents at 1, 2, and 3 min. on the I-V curve in 50% (v/v) phosphoric acid at 45°C.

Source	DF	SS	MS	F	P
Time (min.)	2	0.001	0	0	0.997
Error	24	3.546	0.148		
Total	26	3.547			
S = 0.3844 R-Sq = 0.02% R-Sq(adj) = 0.00%					

Table 36. One-way ANOVA of currents at 1, 2, and 3 min. on the I-V curve in 50% (v/v) phosphoric acid at 35°C.

Source	DF	SS	MS	F	P
Time (min.)	2	0.037	0.019	0.07	0.935
Error	21	5.791	0.276		
Total	23	5.828			
S = 0.5251 R-Sq = 0.64% R-Sq(adj) = 0.00%					

Table 37. One-way ANOVA of currents at 1, 2, and 3 min. on the I-V curve in 50% (v/v) phosphoric acid at 25°C.

Source	DF	SS	MS	F	P
Time (min.)	2	0.065	0.033	0.08	0.922
Error	24	9.579	0.399		
Total	26	9.645			
S = 0.6318 R-Sq = 0.68% R-Sq(adj) = 0.00%					

Table 38. One-way ANOVA of currents at 1, 2, and 3 min. on the I-V curve in 15% (v/v) phosphoric acid at 45°C.

Source	DF	SS	MS	F	P
Time (min.)	2	0.003	0.002	0.01	0.993
Error	30	6.739	0.225		
Total	32	6.742			
S = 0.4739 R-Sq = 0.05% R-Sq(adj) = 0.00%					

Table 39. One-way ANOVA of currents at 1, 2, and 3 min. on the I-V curve in 15% (v/v) phosphoric acid at 35°C.

Source	DF	SS	MS	F	P
Time (min.)	2	0.001	0	0	0.998
Error	45	7.785	0.173		
Total	47	7.786			
S = 0.4159 R-Sq = 0.01% R-Sq(adj) = 0.00%					

Table 40. One-way ANOVA of currents at 1, 2, and 3 min. on the I-V curve in 15% (v/v) phosphoric acid at 25°C.

Source	DF	SS	MS	F	P
Time (min.)	2.00	0.00	0.00	0.00	1.00
Error	39	1.8064	0.0463		
Total	41	1.8064			
S = 0.2152 R-Sq = 0.00% R-Sq(adj) = 0.00%					

Table 41. One-way ANOVA of currents at 1, 2, and 3 min. on the I-V curve in 15% (v/v) phosphoric acid at 0°C.

Source	DF	SS	MS	F	P
Time (min.)	2	0.001	0.0005	0.01	0.993
Error	39	2.6558	0.0681		
Total	41	2.6568			
S = 0.2610 R-Sq = 0.04% R-Sq(adj) = 0.00%					

APPENDIX D

I-V CURVES

85% (v/v) Phosphoric Acid

Figure 43. I-V curve of 85% (v/v) phosphoric acid at 25°C at EP times 1, 2, and 3 min.

Figure 44. I-V curve of 85% (v/v) phosphoric acid at 35°C at EP times 1, 2, and 3 min.

Figure 45. I-V curve of 85% (v/v) phosphoric acid at 45°C at EP times 1, 2, and 3 min.

50% (v/v) Phosphoric Acid

Figure 46. I-V curve of 50% (v/v) phosphoric acid at 25°C at EP times 1, 2, and 3 min.

Figure 47. I-V curve of 50% (v/v) phosphoric acid at 35°C at EP times 1, 2, and 3 min.

Figure 48. I-V curve of 50% (v/v) phosphoric acid at 45°C at EP times 1, 2, and 3 min.

15% (v/v) Phosphoric Acid

Figure 49. I-V curve of 15% (v/v) phosphoric acid at 0, 25, 35, and 45°C for 1 min.

Figure 50. I-V curve of 15% (v/v) phosphoric acid at 0, 25, 35, and 45°C for 2 min.

Figure 51. I-V curve of 15% (v/v) phosphoric acid at 0, 25, 35, and 45°C for 3 min.

APPENDIX E

TABLE OF ELECTROPOLISHING RATE

Table 42. Average EP Rate ($\text{g}/\text{cm}^2 \text{ min.}$) and standard deviation of test specimens electropolished in 15% (v/v) phosphoric acid at process currents of 0.12, 0.39, and 0.68 A for the respective EP times of 1, 2, and 3 min.

Temperature	Current and Voltage	EP Time	Average EP Rate ($\text{g}/\text{cm}^2 \text{ min.}$)	Standard Deviation
25°C	0.12 A 2.24 V	1 min.	0.00016 $\text{g}/\text{cm}^2 \text{ min.}$	0.00014
		2 min.	0.00012 $\text{g}/\text{cm}^2 \text{ min.}$	0.00011
		3 min.	0.00015 $\text{g}/\text{cm}^2 \text{ min.}$	0.00019
	0.39 A 3.42 V	1 min.	0.01034 $\text{g}/\text{cm}^2 \text{ min.}$	0.00096
		2 min.	0.01078 $\text{g}/\text{cm}^2 \text{ min.}$	0.00024
		3 min.	0.01165 $\text{g}/\text{cm}^2 \text{ min.}$	0.00092
	0.68 A 5.02 V	1 min.	0.02077 $\text{g}/\text{cm}^2 \text{ min.}$	0.00064
		2 min.	0.02151 $\text{g}/\text{cm}^2 \text{ min.}$	0.00020
		3 min.	0.02259 $\text{g}/\text{cm}^2 \text{ min.}$	0.00082

Table 43. Average EP Rate ($\text{g/cm}^2 \text{ min.}$) and standard deviation of test specimens electropolished in 15% (v/v) phosphoric acid at bath temperature of 35, 25, and 0°C for the respective EP times.

Temperature	Current and Voltage	EP Time	Average EP Rate	Standard Deviation
35°C	0.62 A 5.20 V	3 min.	0.029 $\text{g/cm}^2 \text{ min.}$	0.0011
		10 min.	0.030 $\text{g/cm}^2 \text{ min.}$	0.0017
25°C	0.39 A 3.42 V	3 min.	0.012 $\text{g/cm}^2 \text{ min.}$	0.0009
		10 min.	0.011 $\text{g/cm}^2 \text{ min.}$	0.0004
0°C	0.30 A 3.10 V	3 min.	0.005 $\text{g/cm}^2 \text{ min.}$	0.0004
		10 min.	0.004 $\text{g/cm}^2 \text{ min.}$	0.0002
		30 min.	0.005 $\text{g/cm}^2 \text{ min.}$	0.0003
		60 min.	0.004 $\text{g/cm}^2 \text{ min.}$	0.0002

APPENDIX F

SURFACE ROUGHNESS ANALYSIS

Table 44. Average surface roughness (nm) and standard deviation of test specimens electropolished in 15% (v/v) phosphoric acid at process currents of 0.12, 0.39, and 0.68 A for the respective EP times of 1, 2, and 3 min.

Temperature	Current and Voltage	EP time	Average Surface Roughness	Standard Deviation
Control		N/A	85.20 nm	43.595
25°C	0.12 A 2.24 V	1 min.	112.8 nm	72.96
		2 min.	142.9 nm	39.47
		3 min.	159.4 nm	58.84
	0.39 A 3.42 V	1 min.	46.9 nm	11.36
		2 min.	56.5 nm	8.72
		3 min.	55.8 nm	9.39
	0.68 A 5.02 V	1 min.	32.3 nm	17.23
		2 min.	88.5 nm	24.34
		3 min.	27.0 nm	16.99

Table 45. Surface Roughness (nm) and standard deviation of test specimens electropolished in 15% (v/v) phosphoric acid at bath temperatures of 35, 25, and 0°C for the respective EP times.

Temperature	Current and Voltage	EP Time	Average Surface Roughness	Standard Deviation
Control		N/A	92.6 nm	20.8
35°C	0.62 A 5.20 V	3 min.	55.2 nm	12.1
		10 min.	45.2 nm	3.5
25°C	0.39 A 3.42 V	3 min.	53.7 nm	11.4
		10 min.	68.8 nm	8.3
0°C	0.30 A 3.10 V	3 min.	37.8 nm	5.6
		10 min.	35.4 nm	6.3
		30 min.	23.1 nm	10.3
		60 min.	48.5 nm	11.3

APPENDIX G

CONTACT ANGLE MEASUREMENTS

Table 46. Average contact angle and standard deviation of test specimens electropolished in 15% (v/v) phosphoric acid at process currents of 0.12, 0.39, and 0.68 A for the respective EP times of 1, 2, and 3 min.

Temperature	Current and Voltage	EP Time	Average Contact Angle	Standard Deviation
Control		N/A	44.2°	4.5
25°C	0.12 A 2.24 V	1 min.	60.7°	6.2
		2 min.	57.8°	6
		3 min.	52.5°	2.9
	0.39 A 3.42 V	1 min.	71.0°	2.1
		2 min.	72.0°	1.2
		3 min.	66.1°	3.7
	0.68 A 5.02 V	1 min.	73.7°	5.6
		2 min.	75.3°	3.9
		3 min.	63.0°	5.1

Table 47. Average contact angle and standard deviation of test specimens electropolished in 15% (v/v) phosphoric acid at bath temperatures of 35, 25, and 0°C for the respective EP times.

Temperature	Current and Voltage	EP Time	Average Contact Angle	Standard Deviation
Control		N/A	44.2°	4.5
35°C	0.62 A 5.20 V	3 min.	62.9°	1.7
		10 min.	59.7°	1.9
25°C	0.39 A 3.42 V	3 min.	68.9°	2.7
		10 min.	67.4°	2.2
0°C	0.30 A 3.10 V	3 min.	73.0°	3
		10 min.	67.5°	2
		30 min.	63.7°	3.3
		60 min.	57.5°	2.8

APPENDIX H

XPS ANALYSIS

Figure 52. A high-resolution XPS scan of control L605 test specimen at the Cr peaks.

Figure 53. A high-resolution XPS scan of L605 test specimen at the Cr peaks. The test specimen was electropolished in a 0°C bath at a process current of 0.3 A for 3 min.

Figure 54. A high-resolution XPS scan of L605 test specimen at the Cr peaks. The test specimen was electropolished in a 0°C bath at a process current of 0.3 A for 10 min.

Figure 55. A high-resolution XPS scan of L605 test specimen at the Cr peaks. The test specimen was electropolished in a 0°C bath at a process current of 0.3 A for 30 min.

Figure 56. A high-resolution XPS scan of L605 test specimen at the Cr peaks. The test specimen was electropolished in a 0°C bath at a process current of 0.3 A for 60 min.

Figure 57. A high-resolution XPS scan of L605 test specimen at the W peaks.

Figure 58. A high-resolution XPS scan of L605 test specimen at the W peaks. The test specimen was electropolished in a 0°C bath at a process current of 0.3 A for 3 min.

Figure 59. A high-resolution XPS scan of L605 test specimen at the W peaks. The test specimen was electropolished in a 0°C bath at a process current of 0.3 A for 10 min.

Figure 60. A high-resolution XPS scan of L605 test specimen at the W peaks. The test specimen was electropolished in a 0°C bath at a process current of 0.3 A for 30 min.

Figure 61. A high-resolution XPS scan of L605 test specimen at the W peaks. The test specimen was electropolished in a 0°C bath at a process current of 0.3 A for 60 min.

Figure 62. A high-resolution XPS scan of L605 test specimen at the Co peaks. The test specimen was electropolished in a 0°C bath at a process current of 0.3 A for 30 min.

Figure 63. A high-resolution XPS scan of L605 test specimen at the Co peaks. The test specimen was electropolished in a 0°C bath at a process current of 0.3 A for 60 min.

APPENDIX I

AES ANALYSIS

Figure 64. AES depth profile of L605 test specimen Control.

Figure 65. AES depth profile of L605 test specimen electropolished in 25°C 15% (v/v) phosphoric acid at a process current of 0.12 A for 1 min.

Figure 66. AES depth profile of L605 test specimen electropolished in 25°C 15% (v/v) phosphoric acid at a process current of 0.68 A for 3 min.

Figure 67. AES depth profile of L605 test specimen electropolished in 0°C 15% (v/v) phosphoric acid at a process current of 0.30 A for 3 min.

Figure 68. AES depth profile of L605 test specimen electropolished in 0°C 15% (v/v) phosphoric acid at a process current of 0.30 A for 10 min.

Figure 69. AES depth profile of L605 test specimen electropolished in 0°C 15% (v/v) phosphoric acid at a process current of 0.30 A for 30 min.

Figure 70. AES depth profile of L605 test specimen electropolished in 0°C 15% (v/v) phosphoric acid at a process current of 0.30 A for 60 min.

Figure 71. AES depth profile of L605 test specimen electropolished in 25°C 15% (v/v) phosphoric acid at a process current of 0.39 A for 3 min.

Figure 72. AES depth profile of L605 test specimen electropolished in 25°C 15% (v/v) phosphoric acid at a process current of 0.39 A for 10 min.