Modification of titanium surfaces by cathodic polarization: a promising technique for enhancing osseointegration

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Abstract

Titanium implants represent successful alternatives for bone and dental replacement in healthy patients. Modifications on the surface can increase their bioactivity and bone regeneration, with a consequent impact on the implant survival rate, and the modification technique used can impact the cellular response. The aim of this study was to characterized titanium surfaces modified by the cathodic polarization method with different current densities and exposure times. Seventy-two blocks of pretreated pure titanium were used and randomly allocated in four experimental groups, being 18 samples for group. The cathodic polarization process was conducted in a solution of acetic acid and sodium acetate (pH 3), being the titanium specimens used as cathode and a rectangular platinum as anode at a controlled temperature of 22°C. The current densities were 1.6 mA/cm2 and 4.5 mA/cm2 and the exposure times were 2h and 4h. Samples were dried and maintained in a Nitrogen vacuum chamber. The modified titanium samples were topographically characterized using Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) and Energy Dispersion X-Ray Spectroscopy (EDX). Water contact angles formed in the surface of the titanium were measured with a tensiometer (ThetaLite TL101; Biolin Scientific Inc., Finland) using the sessile drop method, in four different times in minutes: t=0, t=15, t=30 and t=45. The contact angle formed between the water droplet and the surface was monitored in real time for 60 seconds. AFM and SEM analysis showed rougher surfaces in the modified groups. EDX data revealed the presence of sodium, carbon, and titanium mainly. The Wettability test indicated that all groups showed contact angles with less than 90°. The Control Group and Group 3 showed higher contact angles, around 76° to 71° and Groups 1 and 2 lower contact angles, around 66° to 63° respectively. The topography analysis showed higher roughness in the groups with higher current density and exposure time. The electrochemical cathodic polarization technique modified the titanium surfaces, as current density and exposure time influenced the roughness and wettability of the treated titanium surface, properties that could enhance osseointegration.

Keywords: Titanium, Osseointegration, Biocompatible materials, Electrochemical Technique.

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Introduction

Oral and craniofacial defects resulting from trauma, congenital anomalies or cancer problems represent a major challenge to be solved by the biomedical field, requiring complex intervention treatments that could alter functionality, aesthetics, self-assessment and social interaction status, important dimensions in the perception of patients quality of life[1-3].

Studies have shown that facial and maxillary injuries have increased worldwide due to various factors such as urban violence, traffic accidents, or falls[4,5]. Also, oral diseases such as caries, periodontal disease[6] and cancer[7], increase the proportion of edentulous and craniofacial defects in the population. For instance, edentulism is estimated to be 16.0% of oral disorders worldwide[8] demanded the need for dental repair or replacement in 85.0% of world population[9]. In Brazil, results from the last oral health survey reported high prevalence of caries, periodontal disease and edentulism, being increased the need for prosthetic treatment from 29.7% in 15-year-olds to over 50.0% in people over 60[10].

Biomedical implant devices represent successful alternatives for bone or dental replacement in healthy situations[11]. Titanium has been widely used in prostheses and implants worldwide for decades, presenting good corrosion resistance, biocompatibility and adequate mechanical properties[12-17]. Clinical results showed high success rates[15], which is related to the patient´s medical status (intrinsic response or diseases such as osteoporosis), bone status, material properties and treatment technical aspects[12-14]. Treatment complications such as infections, micromotions or stress shielding that could influence the healing process and osseointegration leading to bone resorption or destruction[16]. In order to solve some of this problems, surface modification of titanium have been performed to developed more reactive implants with bioactive or biomimetic coatings to induce specific cells and tissue responses or control molecules released directly onto the tissue-implant interface[17].

Implants surface modifications could promote the inclusion of organic or inorganic materials and controlled its exposure, concentration, retention or release from the implant and consequently improve its bioactivity, increase bone regeneration and reduce failure rates associated with systemic diseases, bone loss, chronic metabolic diseases, and damage due to degenerative trauma[19-21].

Modification techniques including plasma spraying, ion implantation, sol gel or electrochemical methods were employed to introduce in surface elements, biomolecules, or drugs or to modified its roughness characteristics, important factors to enhance cellular response and osseointegration[16]. Some studies showed the ability of the cathodic polarization method to modify the titanium surface, and apparently differences in the current density and exposure time used during the procedure could influence the mechanical and physical surface characteristics and also the biological response[22-25]. The aim of this study was to characterized titanium surfaces modified by the cathodic polarization method with different current densities and exposure times.

Materials and methods

The cathodic polarization process was conducted in an acidic solution of acetic acid and sodium acetate (pH 3), being the titanium samples used as cathode and a rectangular platinum as anode at a controlled temperature of 22ºC.

Samples and polishing pretreatment

This study used a Titanium (Ti) F67- UNS R50400 (Realum®) square-shape samples with diameter 1x1 cm and thickness 2.25 mm, commercially pure grade II Titanium. Surfaces were polished under metallographic sander machine (polishing) (model Aropol-E, brand ARATEC), with the rotation set up at 200 rpm with silicon carbide papers for metallographic grinding in the sequence of P400, P600, P1200, and P2000, two minutes each and finished under colloidal granular deagglomerated alumina suspension (1µm) for five minutes. After polishing, the Ti samples were washed by a ultrasonic bath for five minutes, then immersed for fifteen minutes each in Acetone, alcohol at 93,5%, and distilled water. Samples were afterward dried and stored under nitrogen cover gas chamber (Plas-Lab Quimis Q216-21) for four hours.

Acid pretreatment

After drying, the samples were extracted from the vacuum chamber and the sanded surface immediately placed in the petri dish in 2 mL of the mixture of 1 mL hydrochloric acid (18% HCl) and 1 mL H2SO4 sulfuric acid (48%) and placed in the muffle (EDG Equipment 3000) at a temperature of 125-130°C for six minutes. After heat treatment, the petri dishes with the samples were extracted from the muffle and quickly immersed in distilled water, placed in a Becker, washed once again in distilled water, and then brought to the vacuum chamber under nitrogen gas. Inside the chamber, the samples were removed from distilled water after ten minutes, immersed in 0.9% NaCl sodium chloride for six hours and finally exposed to drying for four hours.

Experimental Groups

Seventy-two blocks of pretreated pure titanium were used and randomly allocated in four experimental groups. Samples were randomly divided according to the experimental groups, as shown in Table 1, being 18 samples for group. For the cathodic polarization, all groups were treated in a CH3COOH-C2H3NaO2 buffer solution, Group 1 with a current density of 4,5 mA/cm2 for 4h, Group 2 with 1,6 mA/cm2 for 4h and Group 3 with 1,6 mA/cm2 for 2h. The Control Group was not treated with cathodic polarization.

Table 1. Experimental groups and coating deposition conditions by the cathodic polarization process on Ti surfaces.

Group	Surface pretreatment		Buffer synthesis	Equipment configuration parameters		n
	Mechanical polishing	Acid etching		Temperature	Equipment configuration	
GC	Yes	Yes	No	22°C	No	18
G1	Yes	Yes	Buffer CH_3COOH - $C_2H_3NaO_2$	22°C	3 pH / 4 hours / 4.5 mA/cm^2	18
G2	Yes	Yes	Buffer CH_3COOH - $C_2H_3NaO_2$	22°C	3 pH / 4 hours / 1.6 mA/cm^2	18
G3	Yes	Yes	Buffer CH_3COOH - $C_2H_3NaO_2$	22°C	3 pH / 2 hours / 1.6 mA/cm^2	18

Electrochemical Test by Cathodic Polarization

The equipment used to perform the electrochemical tests was a potentiostat (AUTOLAB Metrohm, AUT85833). The NOVA Version 1.1 software controlled parameters such as laboratory test time and current. In the electrochemical assay a double-walled three-electrode glass cell containing 200 mL of 2M acetic acid and sodium acetate buffer solution at pH 3 and pH 5 respectively was used. The cathode used was the titanium alloy samples placed in a copper sample holder and the anode was a rectangular platinum electrode. In groups 1 to 3, samples were assembled and inserted into the sample holder with the pretreated surface placed in front of the rectangular platinum covering the entire surface of the samples at a distance of 1.5 cm under a magnetic stirrer at 5 rpm and a controlled temperature of 22°C. The pH 3 was monitored every 30 minutes with MColorpHast™ pH (0-14) pH indicator paper. The samples were processed for 4 hours and for 2 hours depending on the group. After the process, the samples were dried in a vacuum chamber under nitrogen gas (Plas-Lab Quimis Q216-21) for 2 hours, and then stored in Eppendorf tubes for later characterization.

Samples surface characterization

The modified titanium samples were topographically characterized using Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) and Energy Dispersion X-Ray Spectroscopy (EDX - Ray Ny - EDX 720, Shimadzu), at [quali-quant] easy-metal mode, at two bands (Ti-U and Na-Sc) with 1mmX-ray beam. Water contact angles formed in the surface of the titanium were measured with a tensiometer (ThetaLite TL101; Biolin Scientific Inc., Finland) using the sessile drop method, in four different times in minutes: t=0 t=15, t=30 t=45. The contact angle formed between the water droplet and the surface was monitored in real time for sixty seconds.

Results

Titanium samples showed darker surfaces after treatments and Groups 1 to 3 showed more rough surfaces evident through simple observation, with the surface being covered by jagged lines with the appearance of cracks along its entire length, as the Control Group showed a more smooth surface. Figure 1 shows samples before any treatment (Figure 1a) and after polishing pretreatment (Figure 1b) and the acid pretreatment and cathodic polarization treatment (Figure 1c).

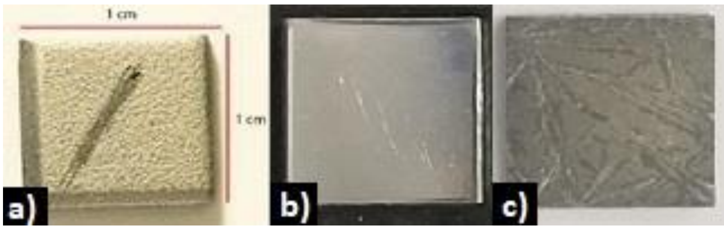

Figure 1. Representation of samples before and after treatment: (a) sample before any treatment; (b) sample after polishing pretreatment; (c) sample after acid pretreatment and cathodic polarization treatment.

Atomic Force Microscopy (AFM) analysis

AFM analysis of surfaces is showed in Figure 2. All groups presented rougher surfaces. Group 1 surface presented small rounded particles, high peaks and deep valleys with a uniform distribution on the surface (Figure 2b). Group 2 also showed high peaks and deep valleys with a uniform distribution on the surface, middle-sized rounded particles and some big hexagonal shape particles (Figure 2c). In Group 3, the titanium-modified surfaces presented mostly small rounded and some rectangular-shaped middle-sized particles distributed uniformly (Figure 2d). Control Group, showed lower roughness with small flat peaks and valleys and a few rounded pentagonal small-sized particles (Figure 2a). AFM analysis of the titanium surfaces showed higher roughness in the groups with higher current density and exposure time.

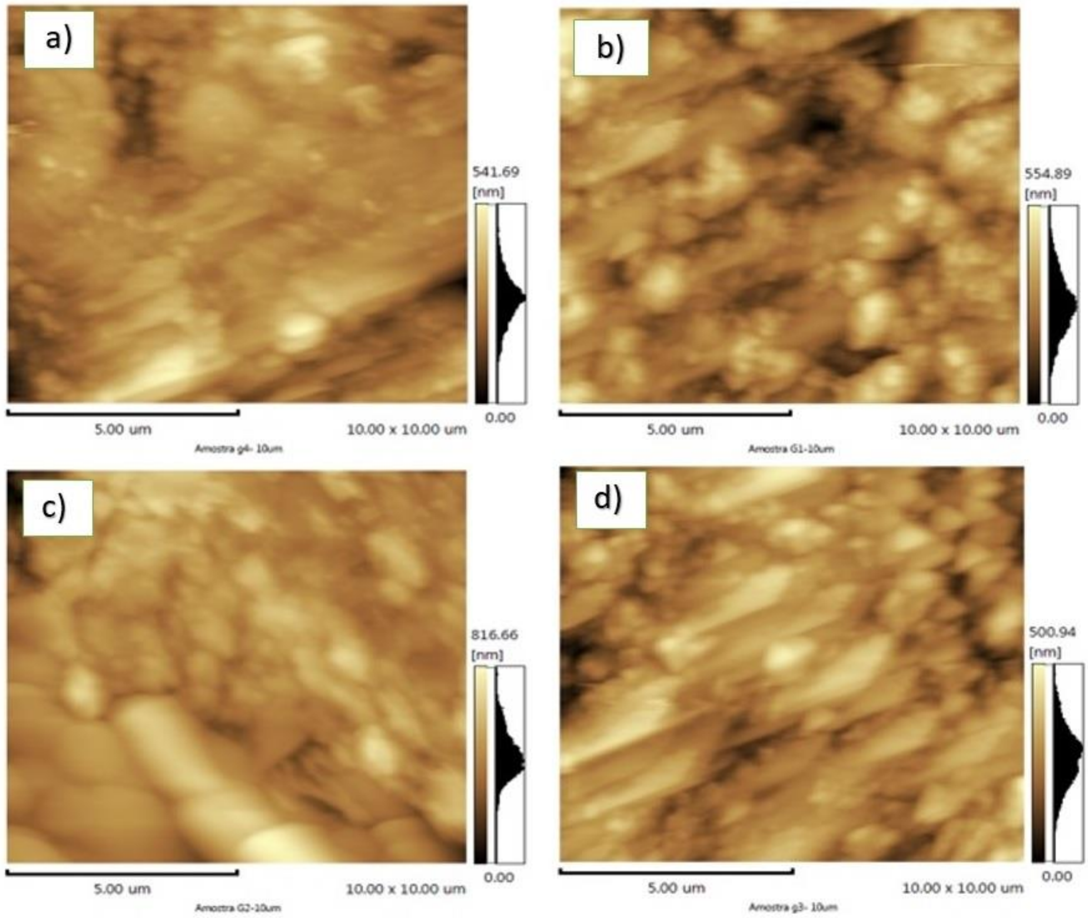

Figure 2. AFM analysis of modified titanium surfaces in groups: GC (a): AFM 10µm; G1 (b): AFM 10µm buffer solution (CH_3COOH-$C_2H_3NaO_2$) / 4.5 mA/cm^2, 4h; G2 (c): AFM 10µm buffer solution (CH_3COOH-$C_2H_3NaO_2$) / 1.6 mA/cm^2, 4h; G3 (d): AFM 10µm buffer solution (CH_3COOH-$C_2H_3NaO_2$) / 1.6 mA/cm^2, 2h.

Scanning Electron Microscopy (SEM)

SEM analysis (Figure 3) showed rougher surfaces in the modified groups, linear irregularities and rounded small prominences all heterogeneously distributed. Group 1 also presented some surfaces depressions. Group 2 surfaces showed depressions as well as some fewer spherical particles than Group 1. In Group 3 surface presented more rounded hexagonal shape prominences compared to the other groups. The Control Group presented a surface with only a few rounded small prominences without any significant irregularities.

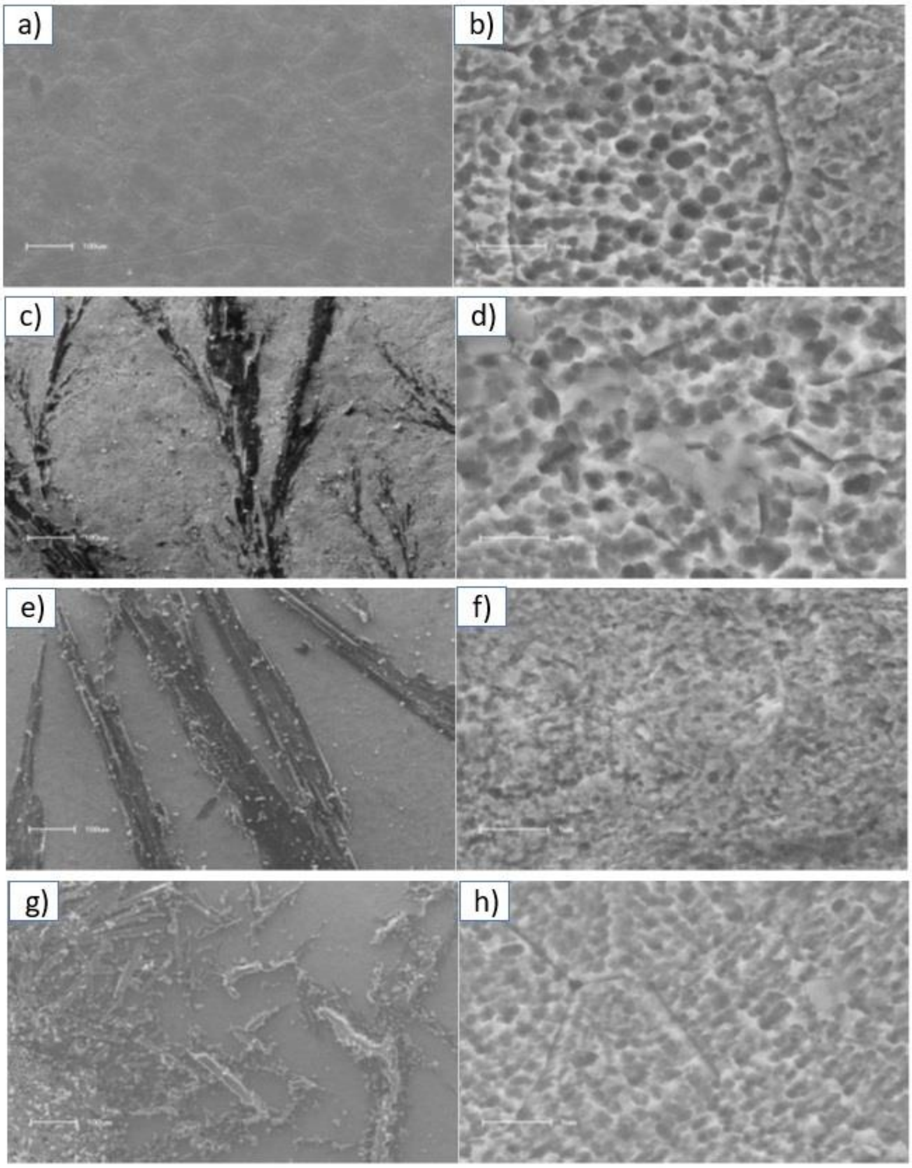

Figure 3. SEM micrograph after the cathodic polarization treatment of GC sample (a – 100x; b – 3000x); G1 sample (c – 100x; d – 3000x); G2 sample (e – 100x; f – 3000x) and G3 sample (g – 100x; h – 3000x).

Energy Dispersive X-Ray Analysis (EDX)

EDX analysis results are presented in Table 2. EDX data revealed the presence of carbon and titanium mainly. All groups presented sodium and aluminum. Surfaces in Group 2 also had Cu. Surfaces in the Control Group had Cl and it was the only group without the presence of carbon. Group 2 presented the highest intensity of Ti (93.069) on the surface and Group 3 presented the lowest intensity of Ti (41.315) compared to the other groups.

Table 2. Quantitative EDX analysis of titanium surface elements.

Group	Ti	Al	C	Cu	Na	Cl
GC	81.851	0.492	-	-	0.556	0.742
G1	92.501	0.591	0.809	-	0.625	-
G2	93.069	0.544	0.805	0.419	0.471	-
G3	41.315	0.457	0.741	-	0.991	-

Wettability

Results for the water contact angle formed with the modified titanium indicate similar patterns of wettability (Figure 4). All groups showed contact angles with less than 90°, indicating hydrophilic characteristics. Control Group and Group 3 showed higher contact angles, around 76° to 71° and Groups 1 and 2 lower contact angles, around 66° to 63° respectively. Contact angles were reduced over the time. G1 showed the lowest reduction between the contact angles after 30 minutes and 45 minutes with 36° and 21.5° (Figure 5).

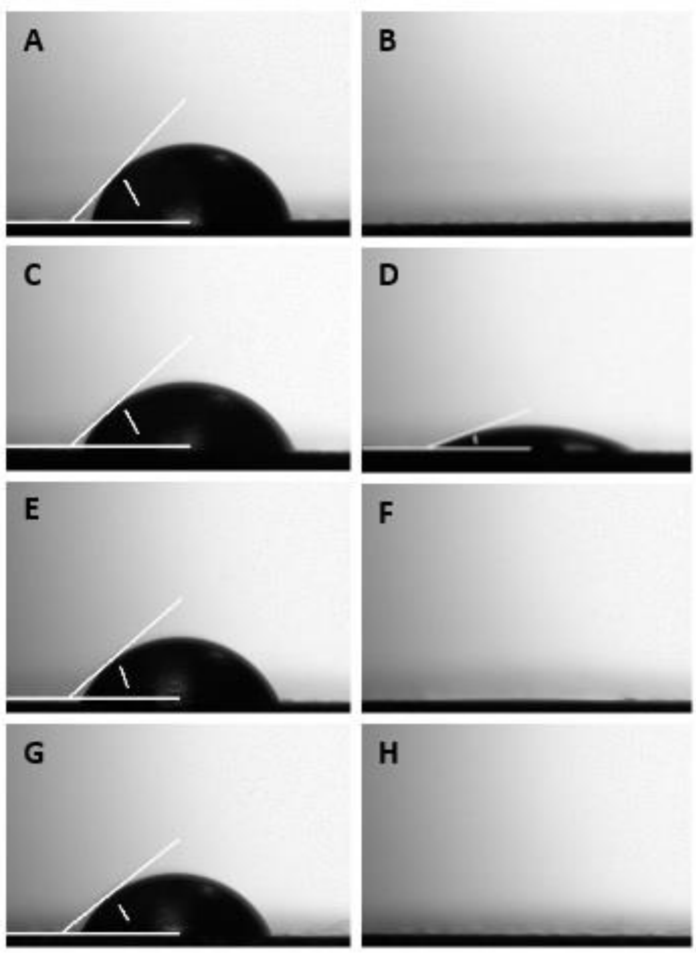

Figure 4. Wettabilitty test results in groups: GC: CA mean 76°, t=0 (A) and t=45 (B); G1: CA mean 66° and 22°, t=0 (C) and t=45 (D); G2: CA mean 63°, t=0 (E) and t=45 (F) and G3: CA mean 71°, t=0 (G) and t=45 (H).

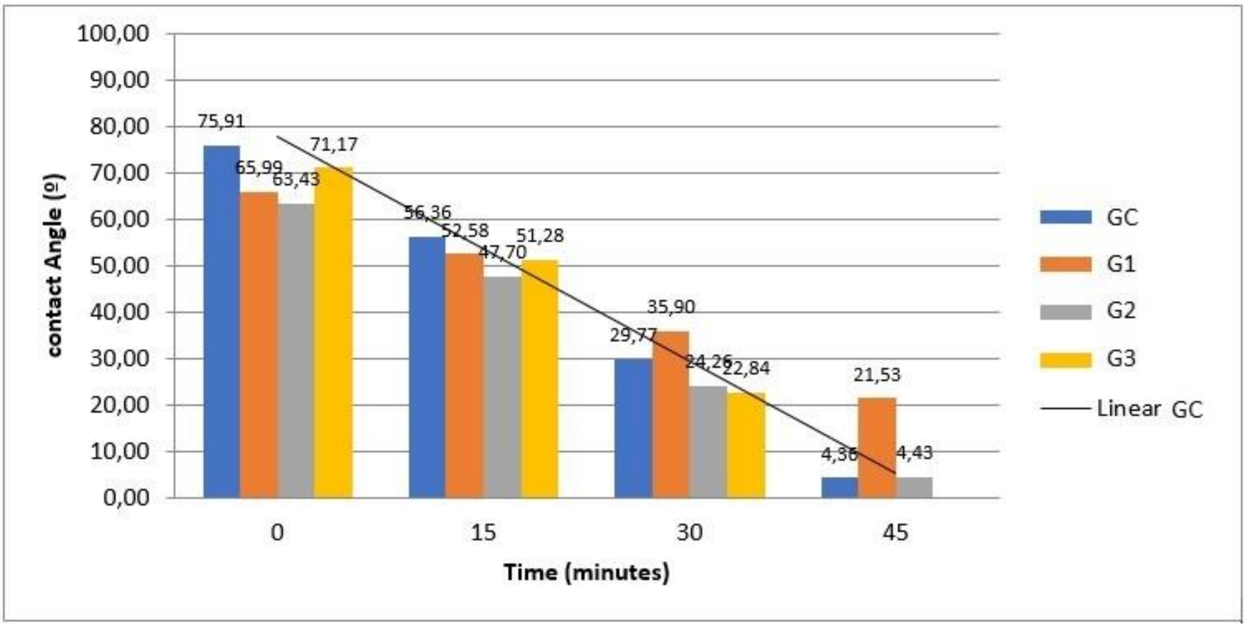

Figure 5. Results for the water contact angle formed with the modified titanium.

Discussion

This study showed that density and time of exposure during electrochemical cathodic polarization method influenced roughness and wettability of medical titanium surfaces. Group 2, with a current density of 1.6 mA/cm2 and 4 hours of exposure time, presented higher roughness (816, 66 nm) compared to the other groups and lower contact angle (63º). Titanium mechanical and physical properties can be exploited by modifying its surface, generating a satisfactory interaction between implant and tissue, therefore, increasing titanium biological activity, that could be demonstrated when tested in vivo[18, 24-27]. Studies showed that cathodic polarization modification process could influence positively cellular response and the osseointegration process by increasing biocompatibility, which depends on the physical and chemical properties of the surface, such as roughness, chemical composition and wettability[22, 26, 27].

This method uses acetic acid and sodium acetate buffer solution with controlled pH and temperature to modified the surface creating a hydride layer on titanium where charged biomolecules can easily anchor, as increase bone attachment and human gingival fibroblast proliferation, for example[23-25]. In our study, surfaces of medical titanium submitted to the cathodic polarization technique were successfully modified. Different patterns of roughness, wettability and the presence of ions on the surface were observed according to modifications in current density and time of exposure during the electrochemical method. Our results showed that roughness increased considerably in the groups treated by cathodic polarization, especially in those groups with higher current density and exposure time.

Rough titanium surfaces improve the biomechanical properties of bone-anchored implants by increasing their surface interlocking capacity[18]. When the micromechanical anchorage occurs, the bone repair process changes from a distant osteogenesis process to an osteogenesis by contact and a slow bone corticalization to a fast trabeculization around the implant surface, as this process ends up generating stronger implant fixation[22-25, 28].

Studies have shown that modifications during the electrochemical process, such as variation in exposure time and current density, could altered the surfaces in relation to chemical, physical and biological characteristics[22-25]. These modifications can determinate the format of the roughness alterations and the adhesion of substances, being these parameters important because the increase in the surface contact area directly influences the orientation of cells in the osseointegration process [28, 29].

Roughness is an important biological parameter that influence the cellular response and biocompatibility[30]. In vivo studies demonstrated a positive correlation between high titanium roughness, direct bone implant contact, higher strength and stability bone implant interface[31]. Surface roughness influence cell adhesion force to the surface, being the force related to the presence of different cells and the fibronectin existence and also in the connection between cells that influence the chemical communication important for cell differentiation. In this context the shape of the cells regulate their growth, genetic expression, protein secretion, differentiation and cell death. In homogenous and rough surfaces cells are round and grow in all directions[32]. Studies showed that Ra between 0.54 to 1.97um would be good roughness parameter for dental implants[31].

The current density can influence the formation of external layers and the mechanical, physical and biological characteristics on the titanium surface[23-25, 33]. Being able to determine the type and format of deposition and alteration on the surface, increasing or decreasing this density, in addition to influencing the particles size, which the smaller the better for cellular adhesion[34]. The hydride layer thickness, important for anchoring biomolecules and increasing bone attachment[23-25], was shown to improve with increasing the current density, as the surface roughness can also increase and generate a formation of more titanium, which was observed after the treatment with high current density[23]. The polarization time exposure can also stimulate the hydride layer formation and the complexity of the surface topography of the treated titanium, which may be positively related to greater proliferation of fibroblasts and connective tissue around the implant[24, 25].

Surface energy is also an important property that express chemical composition, residual stress spatial arrangement of atoms and implant bone contact that can be quantified by wettability[32]. Contact angles with less than 90° were observed among the experimental groups in this study, indicating hydrophilic characteristics, which is considered an important factor on osseointegration and biological response[36-38]. A hydrophilic surface can lead to higher bone-to-implant and better interactions between the surface of treated titanium and cells, biological fluids, tissues and the hydration shells around proteins and other biomolecules[35, 39, 40]. Osseointegration initiated when implant contact the blood[32]. Thus, the degree of wettability influences the adsorption of proteins present in the blood[41] and can also promote fibrin adhesion, as those guide osteoblasts in migration across the surface of the material, mediating cell adhesion, differentiation and proliferation[35, 42, 43]. Also, bacteria are hydrophobic which means that they prefer to adhere in such surfaces and some of those bacteria such as *Staphylococcus aureus* are related to orthopedic infection[16]. Even though studies found relationship between high roughness and reduced contact angles, other did not found such results attributed hydrophilicity to the surface chemistry[24].

The chemical composition of the treated titanium surface is important as it can improve cellular performance by stimulating osteoblastic differentiation, facilitating mineralization during the bone matrix formation process[33, 39]. In addition, chemical interactions on the surface can generate adsorption of organic and inorganic molecules that contribute to increased biocompatibility[44, 45]. EDX data in our study revealed a greater presence of titanium and carbon, but also aluminum, copper, sodium and chlorine. Titanium and carbon are the main elements present in the structural composition of the material, as the one used for this study (Titanium (Ti) F67-UNS R50400), increasing its composition due to the modifications caused by the surface treatment[33, 46]. The presence of titanium on the surface of the material can indicate greater bioactivity and ability to increase bone regeneration and osseointegration, besides all the beneficial properties already mentioned[18, 25]. For the carbon present, it is indicative of an unavoidable type of contamination unrelated to surface topography that can occur on modified titanium surface. Thus, the amount of this element can influence the activity of osteoblasts, suggesting a decomposition prior to implant placement, in order to increase its biocompatibility[47, 48]. However, in both cases, the cellular response should be investigated by biological tests. The alumina used in the polishing step is insoluble in acid and hard to remove from the surface, as it can explain the presence of Al. Although alumina is suspected to interfere on osseointegration, cellular response and corrosion resistance on titanium surfaces, this behavior is not expected and biological studies to support these affirmations are necessary[49, 50]. The presence of Cu on the surface of Group 2 can be explained by the use of the copper sample holder as the cathode and the dispersion of this element during the Electrochemical Cathodic Polarization process[18]. As after the acid pretreatment the samples were immersed in 0.9% sodium chloride, the presence of these elements (Na and Cl) can be detected as well[51].

Our study showed preliminary results that indicates that different exposure times and current density in the cathodic polarization method influenced roughness, contact angle and surface chemistry important properties influencing osseointegration process. However, this statement can only be validated by carrying out more mechanical, physical and biological tests and this recommendation remains here.

Conclusion

The cathodic polarization technique modified the titanium surfaces. Current density and electrochemical exposure time used during the modification process influenced roughness and wettability of the surface of the treated titanium, in addition to promoting adsorption of substances. Further research is necessary to study the biological response on the treated titanium surface.

Resumo

Os implantes de titânio representam alternativas bem-sucedidas para substituição óssea e dental em pacientes saudáveis. Modificações na superfície podem aumentar sua bioatividade e a regeneração óssea, com um consequente impacto na taxa de sobrevida do implante, sendo que a técnica de modificação utilizada pode influenciar a resposta celular. O objetivo deste estudo foi caracterizar superfícies de titânio modificadas pelo método de polarização catódica com diferentes densidades de corrente e tempos de exposição. Foram utilizados 72 blocos de titânio puro pré-tratado, alocados aleatoriamente em quatro grupos experimentais, com 18 amostras por grupo. O processo de polarização catódica foi conduzido em uma solução de ácido acético e acetato de sódio (pH 3), utilizando os espécimes de titânio como cátodo e uma platina retangular como ânodo, a uma temperatura controlada de 22°C. As densidades de corrente foram 1,6 mA/cm² e 4,5 mA/cm², e os tempos de exposição foram de 2h e 4h. As amostras foram secas e mantidas em uma câmara de vácuo com nitrogênio. As superfícies de titânio modificadas foram caracterizadas topograficamente usando Microscopia de Força Atômica (AFM), Microscopia Eletrônica de Varredura (SEM) e Espectroscopia de Raios X por Dispersão de Energia (EDX). Os ângulos de contato da água formados na superfície do titânio foram medidos com um tensiômetro (ThetaLite TL101; Biolin Scientific Inc., Finlândia) usando o método de gota séssil em quatro tempos diferentes, em minutos: t=0, t=15, t=30 e t=45. O ângulo de contato formado entre a gota de água e a superfície foi monitorado em tempo real por 60 segundos. As análises de AFM e SEM mostraram superfícies mais rugosas nos grupos modificados. Os dados de EDX revelaram a presença de sódio, carbono e titânio, principalmente. O teste de molhabilidade indicou que todos os grupos apresentaram ângulos de contato menores que 90°. O Grupo Controle e o Grupo 3 apresentaram ângulos de contato maiores, em torno de 76° a 71°, e os Grupos 1 e 2, ângulos de contato menores, em torno de 66° a 63°, respectivamente. A análise da topografia mostrou maior rugosidade nos grupos com maior densidade de corrente e tempo de exposição. A técnica de polarização catódica eletroquímica modificou as superfícies de titânio, sendo que a densidade de corrente e o tempo de exposição influenciaram a rugosidade e a molhabilidade da superfície de titânio tratada, propriedades que podem melhorar a osseointegração.

Palavras-chave: Titânio, Osseointegração, Materiais Biocompatíveis, Técnica Eletroquímica.

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