VOL.40 Nº4 2023
https://doi.org/10.22491/rmct.v40i4.12301.en

Analysis and characterization of titanium surfaces in different stages of the silanization process

André Aguiar Marques[1], andre.marques@ime.eb.br, ORCID: 0000-0003-1511-4038
Marcelo Henrique Prado da Silva[1], marceloprado@ime.eb.br, ORCID: 0000-0002-1182-5345
Álvaro José Boareto Mendes[1], boareto@ime.eb.br, ORCID: 0000-0003-1432-9045
Carlos Nelson Elias[1], elias@ime.eb.br, ORCID: 0000-0002-7560-6926
[1]Instituto Militar de Engenharia, Rio de Janeiro, RJ, Brasil

ABSTRACT: The main methods of physical, chemical and topographical modification of the surfaces of metallic implants have already been widely studied and documented. With advances in tissue engineering, new approaches to replacing or repairing organs and tissues are made possible by a new era of bioactive materials with the combination of biomaterials with biomolecules. The association of biomolecules with titanium surfaces can be carried out through the silanization method, which enables a covalent bond between the biomolecule and the silane anchored on the previously functionalized titanium surface. This study proposes the analysis, through scanning electron microscopy and characterization in laser interferometry, of titanium surfaces in three stages that make up the silanization process and precede the association of the biomolecule. For this, Grade 4 titanium discs with 6 mm in diameter and 2 mm in thickness were used and studied after the acid etching and alkaline etching stages and after the incorporation of silane. The titanium surfaces of the discs from the three mentioned stages of the preparation process were analyzed and characterized, comparing the uniformity standard and roughness and wettability parameters found. The results showed that all stages of the treatment maintained a micro-rough and uniform surface with adequate roughness parameters compatible with the parameters considered ideal. However, there was an important variation regarding wettability in the groups studied.

KEYWORDS: *Titanium, biomolecule, silanization, roughness, wettability.*

RESUMO: As principais modalidades de modificação física, química e topográfica das superfícies dos implantes metálicos já são amplamente estudadas e documentadas. Com os avanços da engenharia tecidual, novas abordagens de substituição ou reparo de órgãos e tecidos são possibilitadas por uma nova era de materiais bioativos com a combinação de biomateriais com biomoléculas. A associação de biomoléculas às superfícies de titânio pode ser realizada por meio do método de silanização, que viabiliza uma ligação covalente entre a biomolécula e o silano ancorado na superfície de titânio previamente funcionalizada. Este estudo propõe a análise, através de microscopia eletrônica de varredura e caracterização em interferometria a laser, de superfícies de titânio em três etapas que compõem o processo de silanização e precedem a associação da biomolécula. Para isso, foram utilizados discos de titânio Grau 4 com 6 mm de diâmetro e 2 mm de espessura e estudadas após as etapas de condicionamento ácido, de condicionamento alcalino e após a incorporação do silano. As superfícies de titânio dos discos das três etapas citadas do processo de preparação foram analisadas e caracterizadas, comparando o padrão de uniformidade e parâmetros de rugosidade e molhabilidade encontrados. Os resultados mostraram que todas as etapas do tratamento mantiveram uma superfície microrrugosa e uniforme, com parâmetros de rugosidade adequados e compatíveis com os parâmetros considerados ideais. No entanto, houve uma variação importante quanto à molhabilidade nos grupos estudados.

PALAVRAS-CHAVE: Titânio, biomolécula, silanização, rugosidade, molhabilidade.

1. Introduction

Although the success rate of titanium implants is in the order of 95-98%, new surface modification techniques are continuously developed to improve roughness, wettability, adhesion, cell fixation, cell proliferation, and osseointegration [1]. The treatments aim to obtain the appropriate roughness of the implants to increase bone anchoring and improve the biocompatibility of the implants [2]. At the same time, changes in the oxide layer of metallic surfaces, such as titanium, and surface functionalization techniques with bioactive materials have been investigated and developed [3]. The functionalization of metal surfaces is very relevant in the area of biomaterials, since it is able to control the wettability of the material [4], surface energy [5], protein adsorption [6], drug release [7], and the interaction of cells with the implant [8].

Surface functionalization by silanes is one of the most commonly used methods to prepare monolayers. The main advantage of using silanes on metal surfaces is the rapid formation of a covalent bond between the substrate and the anchoring group. This covalent bond stabilizes the monolayer and allows subsequent chemical modifications, such as the association of biomolecules, without compromising the integrity of the monolayer [9].

Faced with the possibility of conferring antimicrobial activity to the titanium surface, or even some level of bioactivity in a metal initially considered inert, this study performed field emission gun scanning electron microscope analysis (FEG-SEM) and laser interferometry characterization of titanium surfaces in three different stages of the silanization process, to analyze and characterize the uniformity pattern and roughness parameters found.

2. Literature review

several surface modifications of metal implants have been developed using subtractive and additive methods. Among these methods, the most commonly used are particle blasting, acid etching, and anodizing. These techniques increase roughness and improve clinical success, with faster healing rates and potentially shorter time intervals for loading. However, each procedure generates a rough surface with slightly different topographic characteristics, even when they have equal values in their arithmetic means of roughness (Ra). Moreover, as a result of these modifications, the wettability and chemical characteristics of the implant surface are also often altered and can dramatically change the initial cellular response to an implanted material [10].

Most of the studies available in the literature that analyze the influence of roughness on osseointegration conventionally use only Ra values. Some studies correlate the Ra value with other properties of surfaces, such as wettability, *in vitro* cell adhesion, and protein adsorption. In addition to the fact that the choice of Ra as a parameter for roughness analysis is not justified in these studies, the isolated evaluation of an implant roughness parameter is not the ideal analysis for its characterization, since surfaces with similar values of Ra may present different morphologies. Therefore, there is a need to correlate the various parameters that characterize roughness, as well as analyze interference with other surface properties of implants [11].

The parameters for roughness analysis and characterization determine the implant surface. Nevertheless, the specific role of each roughness parameter in osseointegration is not yet conclusive [11]. A better evaluation of roughness and cell adhesion can be obtained by associating the values of at least one height parameter, one spatial parameter, and one hybrid parameter [12]. Surface roughness can be divided into three levels depending on the scale of the features: macro, micro, and nano-sized topologies [13]. The macro level is defined for topographic features as being in the range of millimeters to tens of microns. This scale is directly related to the implant geometry, with threaded screws and macroporous surface treatments resulting in surface roughness greater than 10 μm. The high roughness results in mechanical interlocking between the implant surface and the adjacent bone. However, a big risk with high surface roughness may be an increase in peri-implantitis [14].

The micrometer roughness of dental implants is in the range of 1-10 μm. This roughness range maximizes the interlocking between the mineralized bone and the implant surface. On these surfaces, the irregularities allow osteogenic cells to join and deposit bone, producing the bone-implant interface; therefore, it is considered that microrugosities act at the cellular level of osseointegration [15].

Surface profiles in the nanometer range play an important role in protein adsorption, osteoblast cell adhesion, and the rate of osseointegration. However, reproducible surface roughness in the nanometer range is difficult to produce with chemical treatments. Furthermore, optimal surface nano-topography for selective protein adsorption leading to osteoblast cell adhesion and rapid bone apposition is still unknown [14].

Several methods have been developed to create a rough surface and improve the osseointegration of titanium dental implants. These methods use plasma spraying of titanium, sandblasting with ceramic particles, acid etching, and anodizing [10, 14].

Surface modification techniques can be used individually or combined, and can be classified into three categories: physical, chemical, and biological [2].

Nano-rough surfaces with characteristics similar to those of cell membrane receptors and proteins play a crucial role in improving implant performance and osseointegration. In a nanoscale condition, cell membrane receptors, integrins, and proteins are involved and improve the overall quality of osseointegration and other biological reactions between bone and implant [16].

Although metallic biomaterials may differ in diverse physical and chemical properties, many of them share the potential for surface functionalization by the reactivity of surface bonded -OH groups as anchor points for the formation of densely packed monolayers. Activation can be achieved by wet etching, dry etching, and plasma activation. The surface terminated by hydroxyl groups makes it possible to bond with other molecules by condensation reactions [9].

Several functional groups react with the terminal hydroxyl, leading to the formation of layers known as self-assembled monolayers (SAMs) or self-organizing monolayers. By specific chemical interactions, organic molecules can spontaneously organize themselves on various types of surface [17].

The preparation of silane monolayers from solution is currently standardized to more reproducibly and rapidly obtain silane SAMs by immersing a metal in a solution of the precursor APTES (3-aminopropyl) triethoxysilane at different concentrations and temperatures. APTES is commonly used to obtain amine-terminated surfaces that are applied to promote protein adhesion and cell growth in biological implants [9].

The reaction has the potential to form a modified surface or work as an intermediate in binding organic ligands to titanium surfaces [4].

Materials and methods

the samples were prepared jointly in the Biomaterials Laboratory – SE/8 and Biotechnological Processes Laboratory – SE/5 of the Military Institute of Engineering, RJ.

A total of 15 samples were divided into three groups with five grade 4 titanium discs, measuring 6X2 mm and initially with polished surface. The groups formed were: titanium after the acid etching step (Ti-Etc), titanium after the alkaline etching step (Ti-Alk), and titanium after the silanization step (Ti--Sil).

All discs were subjected to the same acid etching with a solution composed of H_2SO_4 / HF / HCl to form a uniform microrough surface similar to the microtexture of osseointegrable implants available on the market, this being, therefore, the initial working surface for this study. After this treatment, the discs were washed with distilled water, dried in N2.

The samples of the Ti-Alk and Ti-Sil groups were activated with alkaline treatment by immersion in 20 ml of solution with 5M NaOH for 8h at a temperature of 60°C for the formation of the hydroxyl functional groups (OH^-) required for covalent bonding between titanium and silane.

After this step, they were washed with distilled water for 30 min and dried individually in N2. Although this step makes it possible to bond the silane on the titanium surface, it has as a possible consequence the formation of an amorphous sodium titanate layer on the metal surface, which, if immersed in fluid with ideal ionic conditions, may undergo crystallization in apatite, thus conferring some level of bioactivity to the material.

For the silanization procedure, a silane, a base, and a solvent are required. The silane chosen was CPTES of 3(chloropropyl)-triethoxysilane, due to the presence of the terminal group chloro in its organofunctional portion, which facilitates the interaction of silane with the peptide at a pH of 11. Anhydrous pentane was the chosen solvent and N,N-diisopropylethylamine (DIPEA) was the chosen base. All reagents were purchased from Sigma-Aldrich Brasil.

For creating the Ti-Sil group, five previously activated samples were immersed in a solution containing 7 ml of anhydrous pentane, 1.2 ml of 3(chloropropyl)--triethoxysilane, and 0.6 ml of the base N,N-diisopropylethylamine (DIPEA) under a saturated N2 atmosphere for 1h. At the end of this period, the samples were washed three times with distilled water and dried with nitrogen.

Samples from the three groups were analyzed by scanning electron microscopy (SEM) and characterized in Zygo laser interferometry at the Biomaterials Laboratory of the Military Institute of Engineering (IME).

Wettability was quantified by measuring the contact angle with distilled water. In this study, the FTA100 goniometer (First Ten Angstroms, Portsmouth, VA, USA) was used. The smaller the contact angle, the higher the wettability. Reducing the contact angle indicates that the surface is more hydrophilic.

3. Results and discussion

after acid etching with the solution composed of H_2SO_4 / HF / HCl, all discs formed a microrough surface with Ra parameter approximately between 1 and 2 μm, similar to the microtexture of osseointegrable implants available on the market, as verified in **Figures 1 and 2**, which confirmed the uniformity of the treatment carried out throughout their entire length.

The surface of the samples activated by etching with NaOH (**Figure 3**) and samples subjected to silane incorporation (**Figure 4**) were also analyzed by FEG-SEM and characterized by Zygo laser interferometer, confirming the uniformity of surface microtexture and roughness parameters achieved after acid etching.

The FEG-SEM analysis of the Ti-Alk and Ti-Sil subgroups in greater magnification, especially in 40000X (**Figures 5 and 6**), showed surfaces with nanometric characteristics throughout the surface, which may mean better protein adsorption and an improvement in implant osseointegration.

Fig. 1 - Micrograph (FEG-SEM) of the surface of the Ti-Etc group titanium sample after acid etching. 500X, 1000X, 2500X, 5000X magnification.

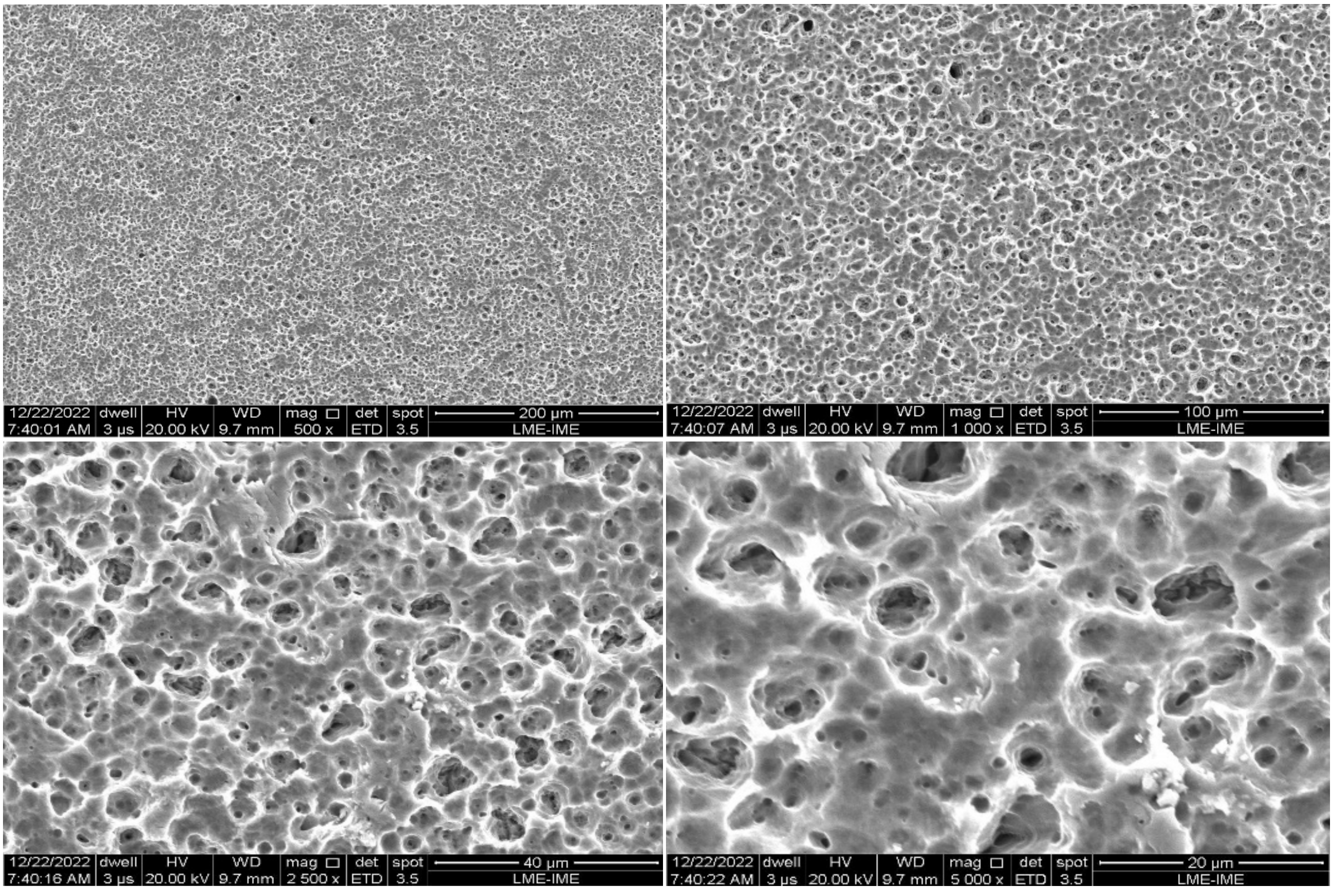


Fig. 2 - Micrograph (FEG-SEM) of the surface of the Ti-Etc subgroup titanium sample after acid etching. 10000X, 40000X magnification.

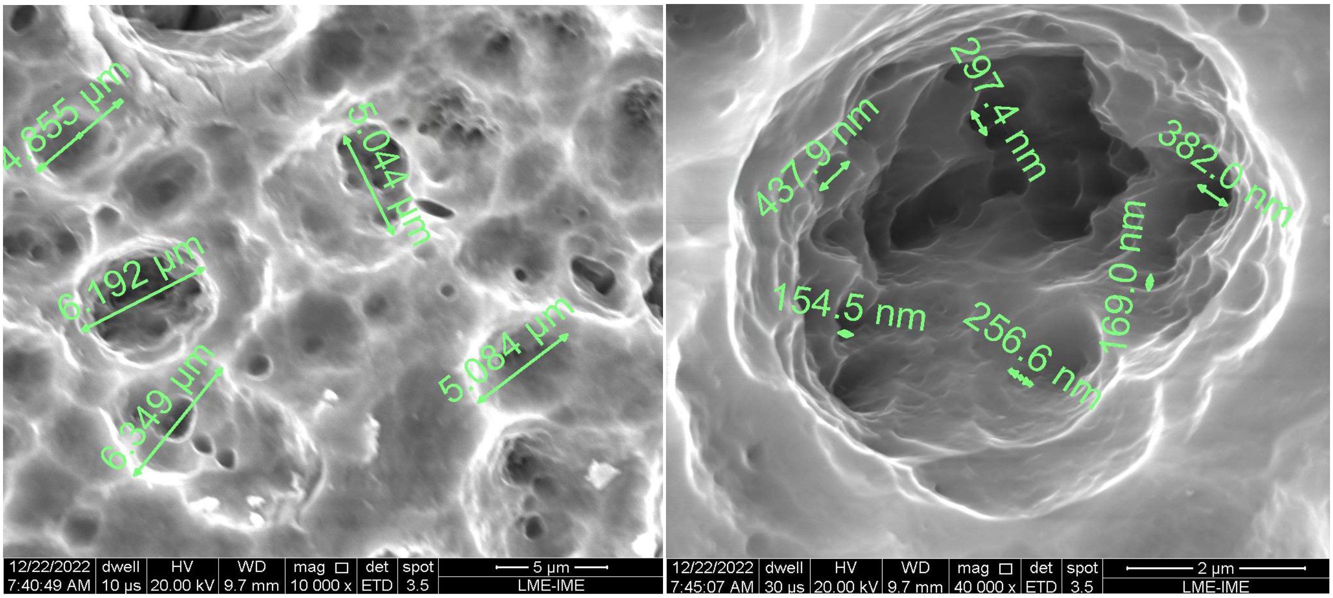


Fig. 3 - Micrograph (FEG-SEM) of the surface of the Ti-Alk group titanium sample after acid etching. 500X, 1000X, 2500X, 5000X magnification.

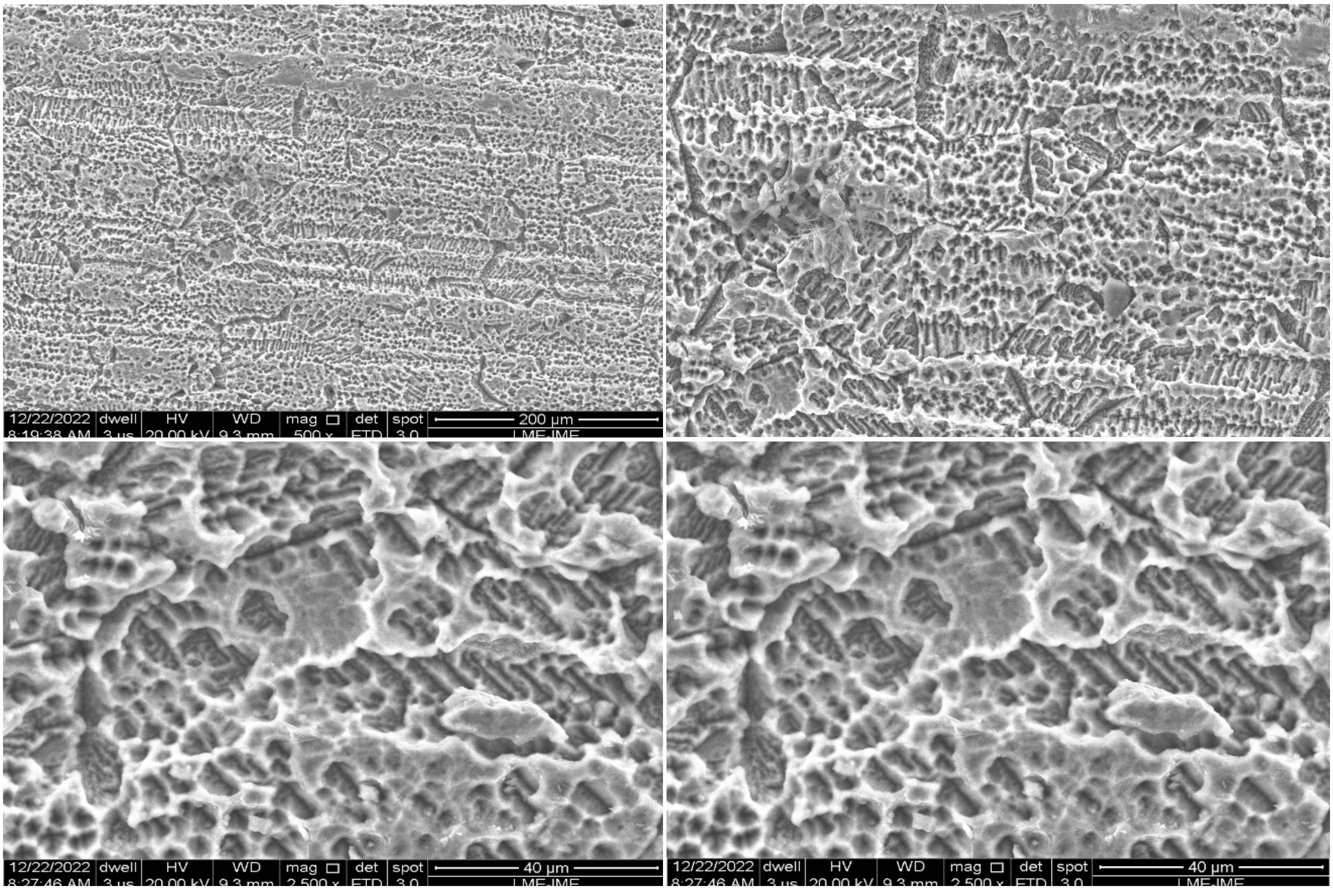


Fig. 4 - Micrograph (FEG-SEM) of the surface of the Ti-Sil group titanium sample after silanization. FEG--SEM. 500X, 1000X, 2500X, 5000X magnification.

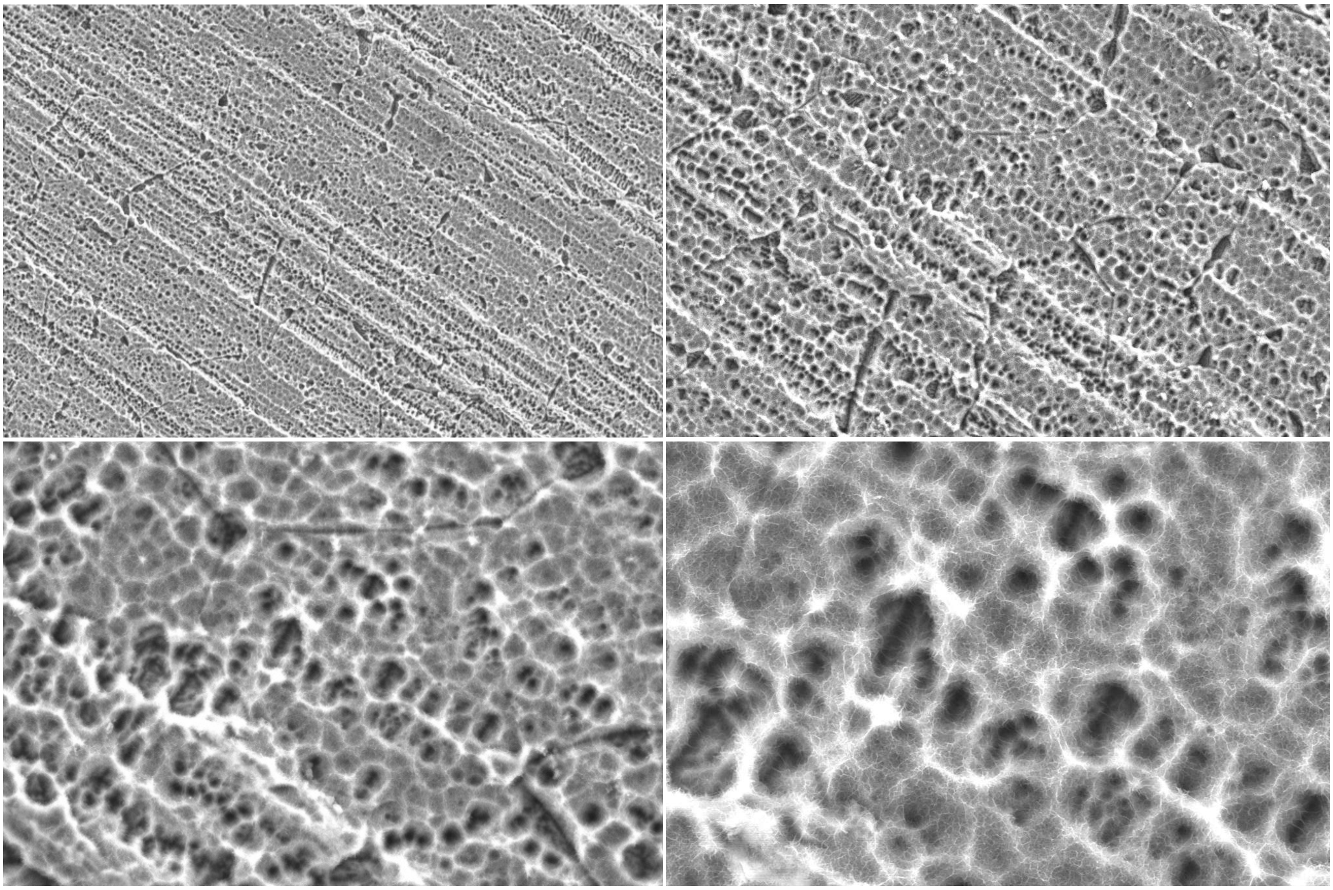


Fig. 5 - Micrograph (FEG-SEM) of the surface of the Ti-Sil group titanium sample after alkaline etching. FEG-SEM. 10000X, 40000X magnification.

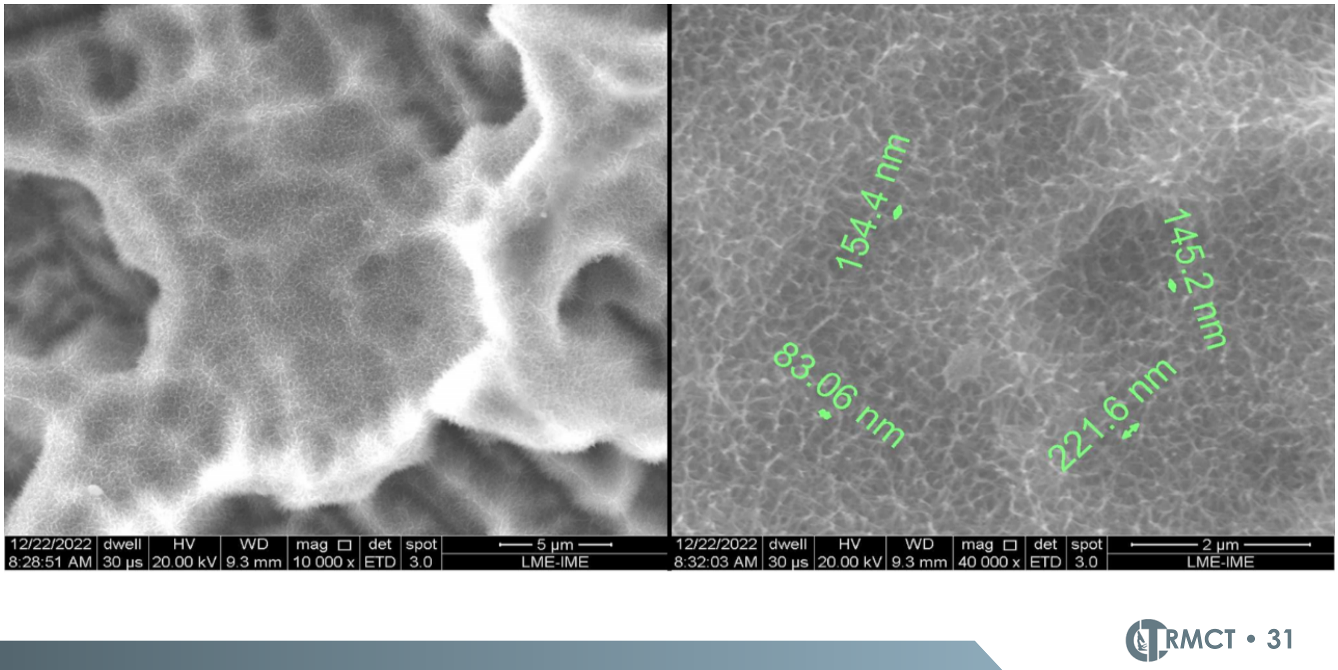
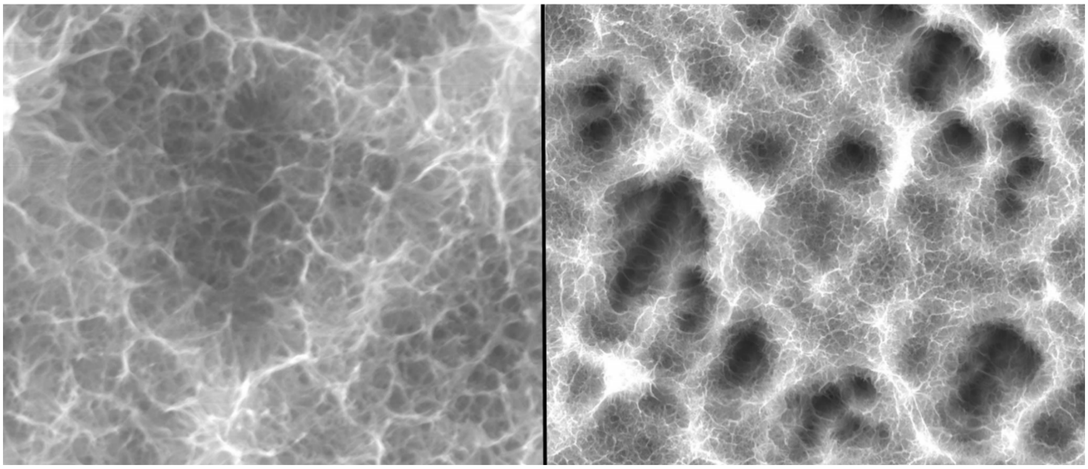


Fig. 6 - Micrograph (FEG-SEM) of the surface of the Ti-Sil subgroup titanium sample after silanization. FEG-SEM. 10000X, 40000X magnification.



The surface characterization in Zygo laser interferometer of the samples of the three groups studied (**Figures 7, 8, 9; Tables 1, 2, 3**) showed a surface with roughness parameters compatible with commercial osseointegrable implants, including an average Ra parameter between 1 and 2 μm.

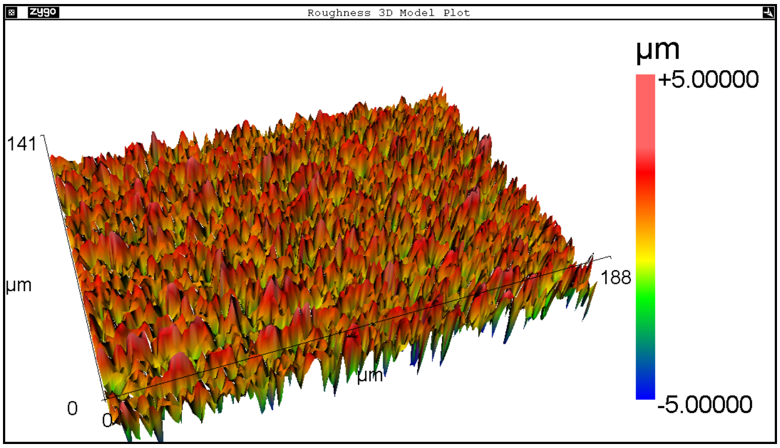
No significant difference was found between the means of the roughness parameters in the groups studied. However, statistical difference was observed between the other groups.

A comparative study in titanium samples with anodized surface, with acid etching and fluoride on the Ra value and contact angle, concluded that the contact angles were influenced by Ra in the sample groups. Despite the small difference between the Ra of the groups, the wettability had a tendency to reduce with the increase of Ra [18].

Different treatments applied to increase the surface roughness and wettability of titanium samples, and analyzed with profilometry and contact angle measurements, showed that wettability is not the critical parameter for cell adhesion and proliferation, and that surface topography plays the main role [19].

In this study, the highest mean Ra values were found in the Ti-Alk group, that is, after the alkaline treatment step, compared to the other two subgroups. Nevertheless, different from the results reported by Coutinho and Elias [18], the group that presented higher mean values of Ra also had lower values of contact angle and higher wettability.

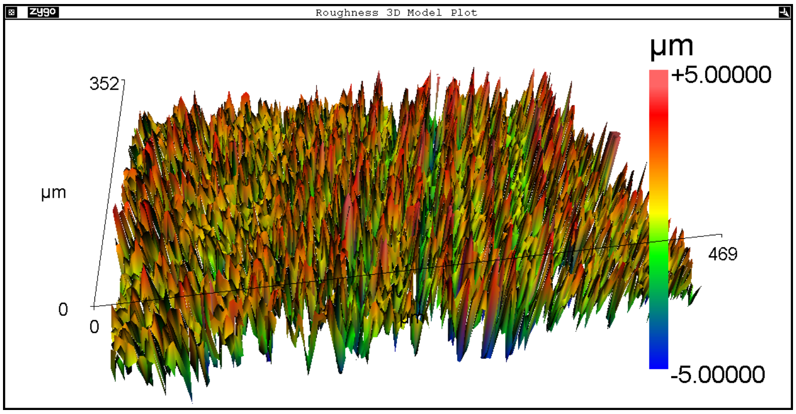
Fig. 7 - 3D image of sample surface microtopography after acid etching – Zygo Interferometer



Tab. 1 - Roughness parameters in five different regions on the sample surface after acid etching – Zygo Interferometer

	Ra	Rq	Peaks	Valleys	Peak density	Valley density	Peak distance
	µm	µm			1/mm²	1/mm²	µm
1	1.013	1.380	84	480	3,185.323	18,201.848	17.718
2	0.990	1.303	85	457	3,223.391	17,330.467	17.613
3	1.002	1.313	87	464	3,299.397	17,596.782	17.409
4	1.105	1.453	86	403	3,261.101	15,281.670	17.511
5	1.083	1.424	92	418	3,491.314	15,862.711	16.924
Mean	1.038	1.374	87	444	3,292.105	16,854.696	17.435
Deviation	0.052	0.066	3	32	119.188	1,229.869	0.308

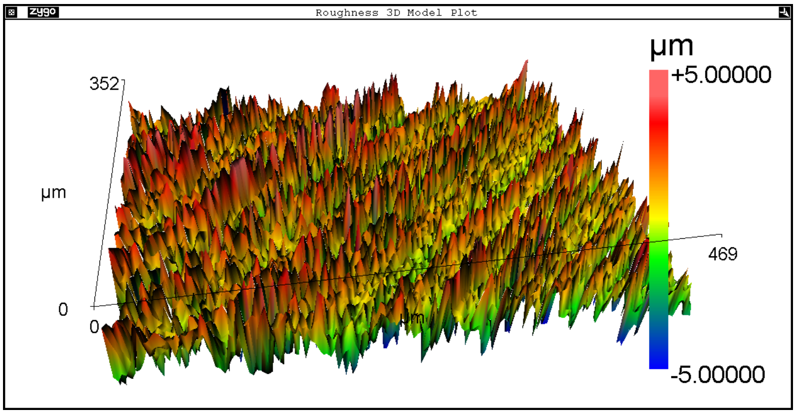
Fig. 8 - 3D image of sample surface microtopography after alkaline etching – Zygo Interferometer



Tab. 2 - Roughness parameters in five different regions on the sample surface after alkaline etching – Zygo Interferometer

	Ra	Rq	Peaks	Valleys	Peak density	Valley density	Peak distance
	µm	µm			1/mm²	1/mm²	µm
1	1.497	1.881	335	653	2,074.292	4,043.321	21.957
2	1.463	1.848	484	922	3,133.691	5,969.552	17.864
3	1.457	1.842	713	1374	4,980.133	9,597.060	14.170
4	1.522	1.895	396	569	2,429.805	3,491.310	20.287
5	1.550	1.927	295	626	1,797.977	3,815.368	23.583
Mean	1.498	1.879	445	829	2,883.180	5,383.322	19.572
Deviation	0.039	0.035	166	334	1274.508	2,546.225	3.687

Fig. 9 - 3D image of sample surface microtopography after silanization – Zygo Interferometer

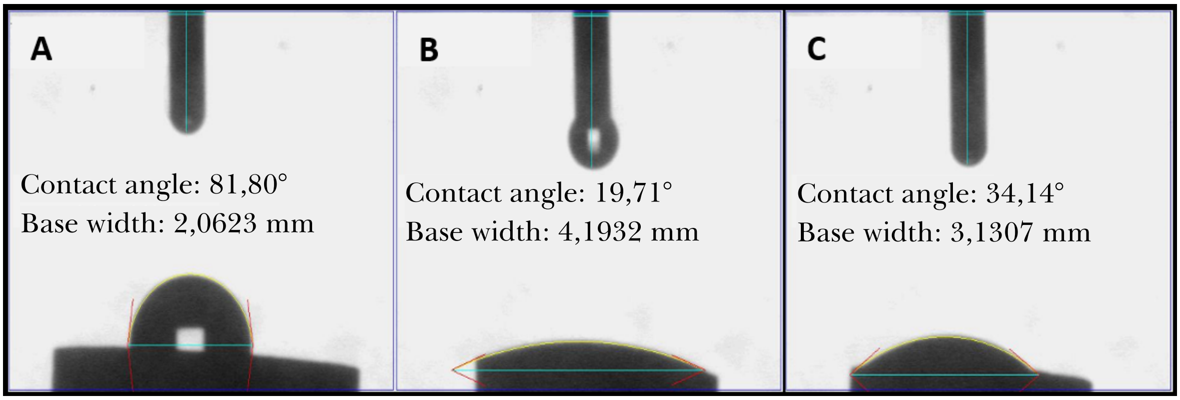


Tab. 3 - Roughness parameters in five different regions on the sample surface after silanization – Zygo Interferometer

	Ra	Rq	Peaks	Valleys	Peak density	Valley density	Peak distance
	µm	µm			1/mm²	1/mm²	µm
1	1.231	1.619	621	620	3,769.170	3,763.101	16.288
2	1.157	1.554	676	634	4,101.644	3,846.808	15.614
3	1.365	1.828	667	498	4,053.691	3,026.594	15.706
4	1.255	1.664	765	551	4,641.971	3,343.433	14.677
5	1.213	1.622	617	651	3,743.953	3,950.265	16.343
Mean	1.244	1.657	669	591	4,062.086	3,586.040	15.726
Deviation	0.076	0.103	60	64	362.253	388.538	0.673

The wettability quantified by measuring the contact angle with distilled water showed lower values on the surfaces of the Ti-Alk and Ti-Sil groups, causing the studied surfaces of these two steps to present more hydrophilic characteristics compared to the surfaces of the subgroup only with the acid treatment (**Figure 10**).

Fig. 10 - Wettability of the samples of the Ti-Etc (A), Ti-Alk (B), and Ti-Sil (C) groups.



5. Conclusion

According to the results found, it can be concluded that:

1. No significant difference was found between the means of roughness parameters in the surface samples of the three groups studied;
2. The samples of all groups studied presented mean roughness values, compatible with the parameters recommended in previous studies;
3. The samples of the Ti-Alk group showed significantly higher wettability than samples with only acid etching;
4. In the studied samples, the tendency of linear reduction of wettability with increasing roughness was not observed;
5. The characteristics found in the titanium surfaces after alkaline etching and silanization steps maintain adequate roughness parameters and also suggest an improvement in wettability, conferring greater hydrophilicity to these surfaces, compared to the initial group used as reference (Ti-Etc).

References

[1] JEMAT, A.; GHAZALI, M. J.; RAZALI, M.; OTSUKA, Y. Surface modifications and their effects on titanium dental implants. **BioMed Research International**, [*s. l.*], 2015. https://doi.org/10.1155/2015/791725

[2] LIU, Y.; RATH, B.; TINGART, M.; ESCHWEILER, J. Role of implants surface modification in osseointegration: A systematic review. **Journal of Biomedical Materials Research Part A**, [*s. l.*], v. 108, n. 3, p. 470-484. https://doi.org/10.1002/jbm.a.36829

[3] ZAVAGLIA, C. A. C.; PRADO-SILVA M. H. **Biomateriais**. São Paulo: Elsevier Inc., 2016.

[4] PESEK, J. J.; MATYSKA, M. T. Methods for the modification and characterization of oxide surfaces. **Interface Science**, [*s. l.*], v. 5, p. 103-117, 1997. https://doi.org/10.1023/A:1008648923962

[5] RYU, D. Y.; SHIN, K.; DROCKENMULLER, E.; HAWKER, C. J.; RUSSELL, T. P. A generalized approach to the modification of solid surfaces. **Science**, [*s. l.*], v. 308, n. 5719, p. 236-239, 2005. https://doi.org/10.1126/science.1106604

[6] LIU, X.; CHU, P. K.; DING, C. Surface nano-functionalization of biomaterials. **Materials Science and Engineering: R: Reports**, [*s. l.*], v. 70, n. 3-6, p. 275-302, 2010. https://doi.org/10.1016/j.mser.2010.06.013

[7] ZAFAR, M. S.; FAREED, M. A.; RIAZ, S.; LATIF, M.; HABIB, S. R.; KHURSHID, Z. Customized therapeutic surface coatings for dental implants. **Coatings**, [*s. l.*], v. 10, n. 6, p. 568, 2020. https://doi.org/10.3390/coatings10060568

[8] SAFONOV, V.; ZYKOVA, A.; SMOLIK, J.; ROGOWSKA, R.; LUKYANCHENKO, V.; KOLESNIKOV, D. Modification of implant material surface properties by means of oxide nano-structured coatings deposition. **Applied Surface Science**, [*s. l.*], v. 310, [*s. l.*], p. 174-179, 2014. https://doi.org/10.1016/j.apsusc.2014.04.110

[9] PUJARI, S. P.; SCHERES, L.; MARCELIS, A. T.; ZUILHOF, H. Covalent surface modification of oxide surfaces. **Angewandte Chemie International Edition**, [*s. l.*], v. 53, n. 25, p. 6322-6356, 2014. https://doi.org/10.1002/anie.201306709

[10] HOTCHKISS, K. M.; SOWERS, K. T.; OLIVARES-NAVARRETE, R. Novel in vitro comparative model of osteogenic and inflammatory cell response to dental implants. **Dental Materials**, [*s. l.*], v. 35, n. 1, p. 176-184, 2019. https://doi.org/10.1016/j.dental.2018.11.011

[11] LEITE, G. B.; FONSECA, Y. R.; GOMES, A. V.; ELIAS, C. N. Relação entre os parâmetros de rugosidade 3D e a molhabilidade do titânio com grãos micrométricos e sub-micrométricos. **Revista Matéria**, Rio de Janeiro, v. 25, n. 2, 2020. https://doi.org/10.1590/S1517-707620200002.1055

[12] WENNERBERG, A.; ALBREKTSSON, T. Effects of titanium surface topography on bone integration: a systematic review. **Clinical oral implants research**, [*s. l.*], v. 20, p. 172-184, 2009. https://doi.org/10.1111/j.1600-0501.2009.01775.x

[13] MATOS, G. R. M. Surface roughness of dental implant and osseointegration. **Journal of Maxillofacial and Oral Surgery**, [*s. l.*], v. 20, p. 1-4, 2021. https://doi.org/10.1007/s12663-020-01437-5

[14] LE GUÉHENNEC, L.; SOUEIDAN, A.; LAYROLLE, P.; AMOURIQ, Y. Surface treatments of titanium dental implants for rapid osseointegration. **Dental Materials**, [*s. l.*], v. 23, n. 7, p. 844-854, 2007. https://doi.org/10.1016/j.dental.2006.06.025

[15] WENNERBERG, A.; ALBREKTSSON, T.; ANDERSSON, B.; KROL, J. J. A histomorghometric study of screw-shaped and removal torque titanium implants with three different surface topographies. **Clinical Oral Implants Research**, [*s. l.*], v. 6, n. 1, p. 24-30, 1995. https://doi.org/10.1034/j.1600-0501.1995.060103.x

[16] LI, J.; ZHOU, P.; ATTARILAR, S.; SHI, H. Innovative surface modification procedures to achieve micro/nano-graded Ti-based biomedical alloys and implants. **Coatings**, [*s. l.*], v. 11, n. 6, p. 647, 2021. https://doi.org/10.3390/coatings11060647

[17] FREIRE, R. S.; PESSOA, C. A.; KUBOTA, L. T. Emprego de monocamadas auto-organizadas no desenvolvimento de sensores eletroquímicos. **Química Nova**, [*s. l.*], v. 26, n. 3, p. 381-389, 2003. https://doi.org/10.1590/S0100-40422003000300016

[18] COUTINHO, M. P.; ELIAS, C. N. Rugosidade e molhabilidade do titânio usado em implantes dentários com superfícies tratadas. **Revista Brasileira de Odontologia**, Rio de Janeiro, v. 66, n. 2, p. 234-238, 2010. http://dx.doi.org/10.18363/rbo.v66n2.p.234

[19] PORRELLI, D.; MARDIROSSIAN, M.; CRAPISI, N.; URBAN, M.; ULIAN, N. A.; BEVILACQUA, L.; MAGLIONE, M. Polyetheretherketone and titanium surface treatments to modify roughness and wettability–Improvement of bioactivity and antibacterial properties. **Journal of Materials Science & Technology**, [*s. l.*], v. 95, p. 213-224, 2021. https://doi.org/10.1016/j.jmst.2021.04.023