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Physical Changes Caused by Citric Acid Applied by Two Different Methods on Roughened and Unroughened Titanium Surfaces

İki Farklı Yöntemle Uygulanan Sitrik Asidin Pürüzlendirilmiş ve Pürüzlendirilmemiş Titanyum Yüzeylerinde Neden Olduğu Fiziksel Değişimler

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ABSTRACT

Objective: Treatment of peri-implant diseases is carried out by controlling the biofilm accumulated on implant surfaces. Decontamination of the surface with chemical agents contributes to the removal of biofilm from the surface more effectively. The aim of this study was to observe and compare the effects of citric acid decontamination protocol applied using two different methods for measuring of surface roughness and surface energy on titanium discs having two different surface characteristics.

Methods: 10% CA applied for 4 minutes by immersion or rubbing on 16 machined surface (MS) and 16 sand blasted/acid etched (SA) titanium discs. When the decontamination process and the surface characteristics of the discs were combined, 4 subgroups were created. Surface roughness (Ra, Rz and Rmax) and surface wettability measurements (theta) were made at baseline and post-application. Statistical analyses were conducted to test the significance of the difference between two measurements and the significance of the difference in the results obtained from the 4 groups (P<.05).

Results: Ra increased and the contact angle diminished in all discs in 4 groups (P=.000). Analyses revealed that there was no difference between the MS groups for Ra (P=1.000) and Rz (P=.970) and the change in the Rmax was statistically significant difference between the SA groups (P=.048).

Conclusion: Application of CA on the titanium surface causes different physical modifications. While rubbing on SA surfaces caused a decrease in surface roughness parameters, the contact angle decreased on both SA and MS surfaces, regardless of rubbing or immersion.

Keywords: Dental implant, surface roughness, contact angle, chemical detoxification, citric acid

ÖZ

Amaç: İmplant çevresi hastalıkların tedavisi, implant yüzeylerinde biriken biyofilmin kontrol altına alınmasıyla gerçekleştirilir. Yüzeyin kimyasal maddelerle dekontaminasyonu, biyofilmin yüzeyden daha etkili bir şekilde uzaklaştırılmasına katkıda bulunur. Bu çalışmanın amacı iki farklı yüzey karakteristiğine sahip titanyum diskler üzerinde iki farklı yöntemle uygulanan sitrik asit dekontaminasyon protokolünün yüzey pürüzlülüğü ve yüzey enerjisi ölçümüne etkilerini gözlemlemek ve karşılaştırmaktır.

Yöntemler: 16 adet işlenmiş yüzey (MS) ve 16 adet kumlanmış/asit ile aşındırılmış (SA) titanyum disk üzerine 4 dakika süreyle %10 CA uygulandı. Dekontaminasyon işlemi ile disklerin yüzey özellikleri birleştirildiğinde diskler 4 alt gruba ayrıldı. Başlangıçta ve uygulama sonrasında yüzey pürüzlülüğü (Ra, Rz ve Rmax) ve yüzey ıslanabilirlik ölçümleri (theta) yapıldı. İki ölçüm arasındaki farkın anlamlılığını ve 4 gruptan elde edilen sonuçlardaki farkın anlamlılığını test etmek için istatistiksel analizler yapıldı (P<,05).

Bulgular: Dört grupta tüm disklerde Ra arttı ve temas açısı azaldı (P<,001). Analizler, MS grupları arasında Ra (P=1.000) ve Rz (P=,970) açısından fark olmadığını ve Rmax'taki değişimin SA grupları arasında istatistiksel olarak anlamlı fark olduğunu ortaya çıkardı (P=,048).

Sonuç: CA'nın titanyum yüzeyine uygulanması farklı fiziksel değişikliklere neden olur. SA yüzeylerinde sürtünme, yüzey pürüzlülük parametrelerinde azalmaya neden olurken, sürtünme veya daldırmadan bağımsız olarak hem SA hem de MS yüzeylerinde temas açısının azaldığı gözlemlendi (P=,048).

Anahtar Kelimeler: Dental implant, yüzey pürüzlülüğü, temas açısı, kimyasal detoksifikasyon, sitrik asit

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INTRODUCTION

Oral implantology has become an integral part of today's dentistry. Dental implants are widely used to replace missing teeth, providing satisfactory treatment results in terms of compensating for aesthetic and functional loss, while also playing a helpful role in preserving bone structure.[1]

Titanium stands out among the materials used for dental implant production thanks to its mechanical strength. In addition, titanium is considered as a unique material in terms of its suitability for the expected integration between the oxide layer formed on the implant surface and the bone tissue.[2] This oxide layer forms naturally when the titanium surface encounters air or liquid media. While oxide layers make the material more resistant to corrosion, they also contribute positively to surface roughness.[3] Surface roughness is one of the most important elements for the stability of the implant in the surrounding bone tissue. A properly osseointegrated implant minimizes the possibility of subsequent complications related to occlusal forces.

Treatments involving dental implants have a high long-term success rate; however, but similar to periodontal tissues, peri-implant tissues are also susceptible to bacterial infections. If peri-implant mucositis, which begins as soft tissue inflammation around the implant, is not treated, it can evolve into peri-implantitis, which is a more severe condition.[4] Peri-implantitis is a process characterized by soft tissue inflammation and loss of supporting marginal bone. In this process, the local inflammatory host response is active and is triggered by biofilm accumulation around the implant. The methods used for years to successfully treat implants that develop peri-implantitis are based on evidence obtained for the treatment of periodontitis.[5] However, the relationship of implants with the surrounding tissues is quite different from that of the tooth root. Treatment of peri-implantitis and regeneration of marginal bone lost due to infection are among the interesting topics of dentistry. The ultimate goal of these treatments is to ensure re-osseointegration to the implant surfaces exposed due to marginal tissue loss after infection. For this purpose, treatment involves non-surgical and surgical procedures to control the biofilm accumulated on the surfaces to restore the health of the tissues around the implant.[6] Considering the macro and micro rough structure, it is almost impossible to completely remove the biofilm from the implant surface with mechanical cleaning methods alone.[7] In addition to mechanical debridement carried out with curettes, ultrasonic devices, titanium brushes or air-flow devices, decontamination of the surface using appropriate chemical agents contributes to the removal of biofilm from the area more effectively.[8,9]

The use of different chemical agents such as citric acid (CA), hydrogen peroxide (HP), chlorhexidine, tetracycline (CHX) and sodium chloride solutions (NaCl) has been suggested to decontaminate exposed implant surfaces.[10] Although there is no consensus on the most effective chemical agent as a surface decontaminant in the treatment of peri-implantitis, the most prominent one is CA. The antiseptic effect of CA has been attributed to its low pH, but low pH is also a factor that can negatively affect the physical and chemical properties of the Ti surface.[11] Additionally, since the acidic environment created by high concentration of CA may have toxic effects in direct contact with host cells, the use of CA solutions with concentrations below 40% has been recommended for application.[11,12]

The decontamination effect of citric acid is not only affected by application time or pH but also other factors. It has been shown in many studies that applying CA to the surface by immersing or rubbing can create different effects on the titanium surface.[13] Studies comparing the effects of decontamination protocols applied in the treatment of peri-implant diseases on surface roughness, surface wettability and corrosion of exposed surfaces, which are important factors for the osseointegration of the implant, have shown that the reduction in surface roughness achieved by rubbing can increase the long-term survival rate of the implant.[14,15]

The aim of this study was to observe and compare the effects of citric acid decontamination protocol applied with two different methods on the measurement of surface roughness and surface energy on of sandblasted-acid etched titanium discs and on machined surface titanium discs.

METHODS

Titanium Disc Preparation

For this study, 32 Grade 5 (Ti6Al4V) titanium discs with a cylindrical form of 7 mm in diameter and 1 mm in height were prepared. 16 of these discs were prepared as machined surfaces (MS) to simulate smoother implant surfaces. While preparing the machined surface discs, a circular sanding machine (Gripo 2V, Metkon, Bursa, Türkiye) and silica papers of different grain sizes (thinning down to 1200 grade) were used. The remaining 16 of the discs were subjected to sandblasting and acid etching (SA) to obtain a roughness equivalent to commercially available dental implants. For this roughening process, aluminum particles of 25-250 Nm in size were sprayed onto the disc surface to provide macrostructure. Then, the disc was immersed in hydrofluoric acid to obtain a micro-rough surface. After acid etching, the discs were washed with pure water. After the discs were prepared, they were stored in airtight containers.

Surface Measurements

In order to implement two different surface decontamination protocols, initially the discs were divided into two, each further subdivided into 2 subgroups consisting of 8 discs each. These group of discs were named as follows: machined surface immersing (MS-i), machined surface rubbing (MS-r), sand blasted-acid etched immersing (SA-i), and sand blasted-acid etched rubbing (SA-r). Initially, the discs were kept in an ultrasonic alcohol/ethanol mixture solution for 5 minutes to remove all dirt that might be on their surfaces, and were allowed to dry completely at room temperature for 30 minutes. Surface roughness values of all samples were measured using a profilometer (Perthometer M2, Mahr Gmbh, Göttingen, Germany). The measurements were completed as recommended by performing three repetitions from the outer edge of the disc to the center, and the Ra1, Rz1 and Rmax1 values of the disc were obtained by taking the arithmetic average of the three measurements. From these data, Ra1 was recorded as average roughness, Rz1 as average maximum surface height, and Rmax1 as maximum of all per-sampling length peak-to-valley heights. Surface energy measurement of the discs was conducted by the sessile drop method with distilled deionized water at room temperature. An optical goniometer (DSA100S; KRÜSS GmbH, Hamburg, Germany) was used for this purpose. During the sessile drop experiment, a 2µL drop of distilled deionized water (0.78 mm diameter) was placed on the disk. As suggested by Li et al. [16], 2 seconds after the drop touched the surface, the image was frozen and a photo of the drop on the titanium disk surface was taken. A digital photograph was processed with the Drop Shape Analysis DSA4 software (version 2.0-01; KRÜSS GmbH, Hamburg, Germany), the contact angle (theta value) between the water drop and the titanium surface was recorded. The measurement was taken in 3 repetitions and the arithmetic average of the 3 measurements was obtained as Theta1. The discs were left at air flow regulated cabin to dry

completely between repetitions of surface energy measurements. After the measurements, the discs continued to be stored in their own airtight containers.

Surface Modifications

In applying CA to titanium discs, the method suggested by Cordeiro et al. [17] was followed. The discs in the MS-i and SA-i groups constituted the immersed groups. All of these discs were immersed in 10% CA solution as soon as they were taken out of the box, kept in the solution and exposed to CA for 4 minutes. The discs in the MS-r and SA-r groups were rubbed 4 minutes by applying light pressure with a cotton pellet dipped in 10% CA solution. All discs in both the immersion and rubbing groups were washed with sterile saline and dried between applications and allowed to be exposed to air for 5 minutes. In immersion and rubbing processes, the CA solution was shaken for 30 seconds before each application to homogenize the solution.

After the surface modification processes were completed, the surface roughness and surface contact angle of the discs were measured by following the same steps as in the previous stage, and the Ra2, Rz2, Rmax2 and Theta2 values of the discs were obtained. Due to the serious differences in the surface properties of the MS and SA group discs, instead of analyzing the Ra and Theta change between measurements directly using their numerical values, the percentage equivalent of the change rate was calculated and recorded as Ra% and Theta%.

Statistical Analysis

The data obtained from all measurements were evaluated using statistical analysis software (SPSS, IBM, Bethesda USA). After the normal distribution of the data for the change in Ra, Rz, Rmax and Theta values between two measurements was evaluated with the Kolmogorov-Smirnov test, the paired samples t-test was conducted to analyze the significance of the difference. The Wilcoxon Signed-Rank test was used to evaluate the significance of the change in the Rmax value. The one-way ANOVA test was used to evaluate the significance of the difference of Ra, Rz and Theta values between the groups and the Bonferroni correction was used for post-hoc evaluation. Pairwise comparisons for the Rmax parameter were conducted with the Kruskal-Wallis test.

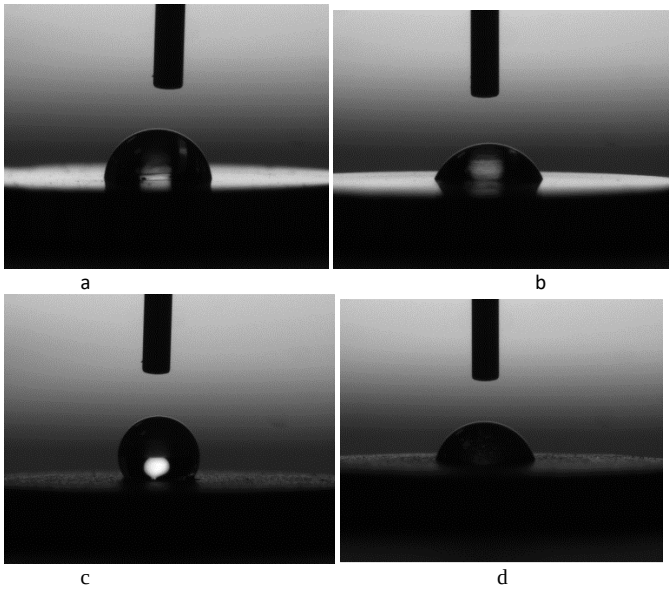


Figure 1. Contact angle measurement on machined surface at baseline (a), machined surface-rubbed (b), sand blasted and acid etched surface at baseline (c), sand blasted and acid etched surface-rubbed (d)

RESULTS

The results of measurements made on titanium discs with the profilometer and goniometer are shown in Table 1. While the initial Ra values of the discs in the MS group were measured between 0.138 and 0.152 (mean: 0.146±0.0039), the initial Ra values of the discs in the SA group were measured between 1.213 and 1.281 (mean: 1.256±0.021). After surface decontamination, Ra increased slightly in all discs across the 4 groups. Considering the Ra change percentages, a similar increase in roughness was observed in MS discs in both the immersion and rubbing groups, while the lowest proportional change occurred at the SA-i group. While the initially measured contact angle values of MS discs were between 73.20° and 78.00° (mean: 76.06°±1.273), whereas those of SA discs ranged between 111.50° and 116.10° (mean: 114.08°±1.327) (Figure 1). The measurements made after surface decontamination showed that the contact angle decreased in all discs, but the largest percentage decrease was observed in the SA-i group.

When the significance of the difference between the baseline and after decontamination measurements was evaluated, it was seen that the change in Ra and Theta parameters was significant (P<.001), but the change in Rz value was not significant (P=.494) (Table 2). When the change in Rmax value between the two measurements was analyzed with the Wilcoxon test, it was concluded that the difference was significant (P<.001).

Table 1. Baseline and post-decontamination values measured from all titanium discs

Sample No	Group	Ra1	Rz1	Rmax1	Ra2	Rz2	Rmax2	Ra-d%	Theta1	Theta2	Theta-d%
1	MS-i	0.142	0.84	1.04	0.183	1.09	1.36	28.64	74.80	48.70	34.89
2	MS-i	0.145	0.88	1.01	0.184	0.88	1.59	26.61	73.20	49.10	32.92
3	MS-i	0.145	0.89	1.00	0.186	0.94	1.22	28.21	75.00	47.20	37.06
4	MS-i	0.138	0.81	0.95	0.167	0.90	1.02	21.26	75.70	48.00	36.59
5	MS-i	0.150	0.83	0.99	0.190	1.02	1.17	26.61	76.30	48.10	36.96
6	MS-i	0.148	0.96	1.10	0.181	0.95	1.26	22.30	76.20	50.80	33.33
7	MS-i	0.145	0.84	1.16	0.184	0.95	1.25	26.61	77.10	47.80	38.00
8	MS-i	0.152	0.87	1.12	0.188	0.93	1.03	23.69	77.70	49.20	36.68
9	MS-r	0.152	0.89	1.14	0.193	1.07	1.20	26.98	76.10	41.30	45.73
10	MS-r	0.148	0.92	1.18	0.184	1.18	1.48	24.10	76.60	42.10	45.04
11	MS-r	0.147	0.86	1.19	0.178	1.05	1.15	21.32	77.30	40.30	47.87
12	MS-r	0.145	0.86	0.99	0.179	1.03	1.06	23.17	75.20	39.90	46.94
13	MS-r	0.147	0.87	1.06	0.186	1.02	1.38	26.53	77.30	40.90	47.09
14	MS-r	0.147	0.84	1.13	0.183	1.08	1.49	24.21	78.00	41.10	47.31
15	MS-r	0.141	0.82	1.03	0.174	0.97	1.08	23.40	75.60	40.70	46.16
16	MS-r	0.142	0.89	1.10	0.178	0.98	1.03	25.35	74.90	40.60	45.79
17	SA-i	1.271	7.89	9.53	1.394	8.45	9.76	9.68	115.00	91.50	20.43
18	SA-i	1.213	7.46	8.98	1.308	8.38	9.29	7.80	115.40	91.00	21.14
19	SA-i	1.269	7.09	9.08	1.384	8.20	9.24	9.03	115.10	91.80	20.24
20	SA-i	1.269	6.66	8.93	1.369	8.11	9.46	7.88	111.50	87.80	21.26
21	SA-i	1.244	7.59	9.38	1.365	7.99	9.43	9.75	114.80	89.00	22.48
22	SA-i	1.271	7.47	9.37	1.363	8.07	9.44	7.24	112.50	88.20	21.60
23	SA-i	1.255	8.19	9.54	1.368	8.87	9.55	9.00	113.70	88.80	21.90
24	SA-i	1.239	6.92	8.96	1.362	7.97	9.68	9.93	115.00	90.10	21.65
25	SA-r	1.224	8.36	9.36	1.476	8.01	9.28	20.56	116.10	76.70	33.94
26	SA-r	1.258	8.67	9.87	1.522	8.12	9.90	20.99	113.30	73.90	34.77
27	SA-r	1.260	9.22	9.68	1.524	8.52	9.58	20.95	113.90	74.60	34.50
28	SA-r	1.279	7.93	9.36	1.509	7.03	9.76	17.98	114.60	74.70	34.82
29	SA-r	1.271	8.39	8.96	1.496	7.21	8.97	17.70	112.30	73.60	34.46
30	SA-r	1.229	9.43	9.16	1.456	7.65	8.62	18.44	112.70	73.30	34.96
31	SA-r	1.281	9.30	9.69	1.503	8.81	9.63	17.33	115.50	75.30	34.81
32	SA-r	1.249	8.09	9.87	1.486	7.68	9.62	18.98	113.90	74.30	34.77

(Ra: average surface roughness, Rz: the arithmetic mean value of the single roughness depths of consecutive sampling lengths, Rmax: the maximum distance between the highest point and the lowest point on the profile, Theta: contact angle, parameters with 1: baseline measurement, parameters with 2: measurement after decontamination, Ra-d%: percentage of difference of Ra to baseline measurement, Theta-d% percentage of difference of Theta to baseline measurement, MS-i: machined surface immersion, MS-r: machined surface-rubbing, SA-i: sand blasted-acid etched-immersion, SA-r: sand blasted-acid etched-rubbing)

Table 2. Results of paired samples t-test used to analyze the difference between values obtained at two measurements of Ra, Rz and Theta

	Mean	Std deviation	t	*Sig.*
Ra	-0.105844	0.085160	-7.031	0.000
Rz	-0.08063	0.11651	-0.692	0.494
Theta	31.62188	6.31530	28.325	0.000

(*Ra: average surface roughness, Rz: the arithmetic mean value of the single roughness depths of consecutive sampling lengths, Theta: contact angle) (P<.05)*

One-way ANOVA was used to evaluate whether the change in Ra, Rz and Theta values measured from the discs in 4 different groups created a significant difference between the groups (Table 3). It was determined that there was no significant difference between the MS-i and MS-r groups in Ra and Rz values (*P*=1.000, *P*=.970, respectively), and the difference between the other groups was statistically significant. In the Kruskal-Wallis test it was determined that the change in the Rmax parameter created a statistically significant difference between the SA-i and SA-r groups (*P*=.048).

Table 3. Results of pairwise comparison analyses of differences in Ra, Rz, Rmax and Theta parameters between groups

	Sig. Ra*	*Sig.* Rz*	*Sig.* Rmax**	*Sig.* Theta*
MS-i / MS-r	1.000	1.000	1.000	0.000
MS-i / SA-i	0.000	0.000	1.000	0.000
MS-i / SA-r	0.000	0.000	0.102	0.000
MS-r / SA-i	0.000	0.001	1.000	0.000
MS-r / SA-r	0.000	0.000	0.395	0.000
SA-i / SA-r	0.000	0.000	0.017	0.000

(*MS-i: machined surface immersion, MS-r: machined surface-rubbing, SA-i: sand blasted-acid etched-immersion, SA-r: sand blasted-acid etched-rubbing, *: one-way ANOVA, **: Kruskal-Wallis test) (P<.05)*

DISCUSSION

Peri-implant mucositis and peri-implantitis, which cause inflammation and tissue damage in the tissues around the implant, are conditions triggered by biofilm accumulation and their treatment is to remove this accumulation from the implant surfaces.[18] Chemical, mechanical, laser treatments, or combinations of these can be applied to effectively remove biofilm from implant surfaces. While curettes and air-polishing systems are used for mechanical removal, detoxification of the surface in addition to debridement contributes positively to the treatment outcome.[19] Methods used to detoxify titanium implants include the use of chemical agents that contain high concentrations of fluoride or are very acidic, or laser application. Although there are different chemical agents used for detoxification, evidence supporting the use of adjuvant chemical agents for clinical improvement and/or tissue size regeneration is still lacking.[20] Although the primary criterion evaluated in many studies investigating surface detoxification is the removal of bacterial biofilm, less emphasis has been placed on the changes that may occur in the surface properties of implants when subjected to these treatment protocols.[21]

Chemical agents frequently used for detoxification of contaminated surfaces include saline, CA, CHX, HP, tetracycline, and doxycycline. These chemicals can be prepared in different concentrations and applied to the implant surface by various techniques. Although it cannot remove all of the biofilm accumulated on the surface of titanium, CA has shown to still be one of the most effective chemicals for detoxification.[22] CA, a weak acid, has the ability to penetrate the cells of microorganisms without dissociation, dramatically lowering the intracellular pH and disrupting cell structure. CA has been proven to be a chemical agent to be used to decontaminate implant surfaces after treatment minimally disrupting to biocompatibility.[23] It has been shown that solutions with low pH value (<3) or high fluoride concentrations deteriorate the titanium oxide layer after application to the titanium surface, and as a result, metal ions and residues separated from the surface may cause tattoo-like discoloration in the surrounding tissues and it also prevents the passivation of the oxide layer.[24,25] This is a serious problem that can lead to corrosion and local decomposition of titanium (pitting attack). In addition to providing a more suitable surface for integration of bone cells, it has been shown that an increase in surface roughness can facilitate the adhesion and colonization of bacteria, and make complete elimination of biofilm more difficult.[26] In a study evaluating the effectiveness of CA at concentrations of 1, 10, 20 and 40%, it was shown that CA at 10% concentration did not show direct cytotoxicity and showed a desired antimicrobial effect with 8-minute rubbing application, and that application at this concentration would not cause corrosion damage.[17] The biofilm removal capacities of CA solutions at different concentrations applied to titanium discs by two different methods, immersing and rubbing, were evaluated and the application of CA at 10% concentration by rubbing method for 4 minutes showed proper in vitro antibacterial action on both machined and SLA surfaces and prevented potential corrosion.

While there are studies demonstrating that rubbing CA on titanium surfaces effectively eliminates biofilm, there are also studies indicating that one method has no obvious superiority over the other.[26] In a study where they evaluated the changes caused by surface detoxification methods on the properties of titanium discs, Wheelis et al. reported that acidic rubbing was much more aggressive than the immersion method, and this was especially observed in high concentration solutions. It has been shown that decreasing the pH of the solution and increasing the application pressure also increases the dissolution of the oxide layer, which is vital for implant-tissue bonding and corrosion resistance. This situation can cause very serious complications in implants that remain in use despite exposed surfaces due to peri-implantitis, because the detoxified oxide layer encounters the mechanical forces that constantly occur in the mouth, making it impossible to passivate this layer again.[27]

When a liquid contacts a solid surface, it creates a certain angle. This is called the contact angle and varies depending on the material with which the solid or liquid is in contact. While the contact angle expresses the degree of wettability, it is also related to of surface energy. The size of this angle depends on the magnitude of the cohesion and adhesion forces. There are many methods to determine surface and interfacial tension. The sessile drop method is standardized (ASTM D7334-08[2022]) and has become frequently used in research. Although distilled deionized water is most commonly used as drop material, liquids such as glycol and diiodomethane as well as solvents can also be used when necessary, considering the characteristics of the surface to be tested.[28] In addition, although there are many different methods for measuring contact angle, in our study we preferred the circle-fitting method, which has high consistency in repeated measurements and is commonly used for contact angle measurement on the titanium surface.

Although goniometer is accepted as a standard for contact angle measurement and is frequently used in research, it should be supported by different methods to determine the surface characteristics that cause contact angle differences. It is possible to obtain detailed chemical analysis of the surface by examining it with devices such as an infrared microscope, atomic force microscope (AFM) or X-ray or photoelectron spectroscopy (XPS).[29,30] Additionally, the extent of damage caused by surface corrosion can be determined by measuring potentiodynamic

polarization using a potentiometer.[31] This measurement shows how much the passive and biocompatible oxide layer on the titanium surface dissolves during a corrosion attack.

The use of a profilometer device for surface roughness measurement is often preferred in research. This system is advantageous due to its simplicity, rapid measurements and low cost. However, correctly detecting the disks in the system requires some experience, and the working principle, which does not allow for measurements covering the entire area by measuring only on a thin line on the surface, has been a pioneering issue for the use of more advanced monitoring systems in research. It is possible to analyze the surface topography in detail with scanning electron microscopy.[30] In addition, the existence of derivatives that simultaneously allow for chemical surface analysis is an element that increases the depth of the data received. Moreover, as mentioned in a mini review article published in 2021, respectively 50% and 27% of the studies focusing on quantifying the microbial biofilm after decontamination employed scanning electron microscope (SEM) or confocal laser scanning microscopy (CLSM). MicroCT analysis, which has been used in many areas of dentistry research for the last 10 years, is frequently employed in research where the roughness of titanium surfaces is evaluated, especially, since it allows volume measurement on very small surfaces.[31] For example, Cui et al., in their study evaluating the osseointegration success of dental implants with different surface properties placed in the canine mandible, examined tantalum-coated titanium implants with different control groups using microCT and energy dispersive spectroscopy, and showed that tantalum-coated titanium implants can be used as new types of dental implants.[32] However, these two methods can be challenging due to their financial costs and long time requirements for analysis.

In addition to Ra, which shows the average roughness level of the evaluated surface, the profilometer device also measures Rz and Rmax values, which provide significant data about the surface characteristics. While the Ra value provides the absolute arithmetic average of surface fluctuation, the Rz value gives the average height difference between recesses and protrusions, and Rmax, the widest height difference detected. Although the studies have focused mostly on the Ra value, interpreting the change in Rz and Rmax values to understand the change in the surface profile. According to the findings of our study, there is a significant decrease in Rz on SA surfaces decontaminated with the rubbing method, and there is a significantly larger Rmax change in SA discs to which rubbing is applied, compared to the immersion method. The reason is the rubbing process performed on the roughened surface (SA) causes not only a chemical but also a physical profile change on the titanium surface, which is already affected by low pH. Although the rubbing process is a method that increases Ti separation from the surface, it also creates a polishing effect and causes the most protruding areas on the surface to become smooth. In addition, by facilitating the removal of free titanium particles/ions that separate from the surface during rubbing, this process can prevent issues such as stimulation of pro-inflammatory cytokines, infiltration of inflammatory cells, activation of osteoclasts and microbial dysbiosis that may arise from the presence of these particles in the peri-implant tissue.[33]

The results of studies evaluating the reduction of bacterial load after detoxification applications performed on titanium surfaces provide valuable data on controlling peri-implant diseases. Ntrouka et al.[10] evaluated the removal potency of each agent after performing surface cleaning process using EDTA, CA, cetylpyridinium chloride, H2O2 + CA, Ardox-X, CHX and water on Streptococcus biofilm and polymicrobial biofilm coated titanium disks. Although Ardox and H2O2 gave satisfactory results, the most successful results were obtained with CA in both killing and removing, in both Streptococcus and polymicrobial groups. In another study, the cleaning efficacy of sodium hypochlorite, HP, CHX, CA and two commercial mouthwashes on titanium implant samples was evaluated.[34] It was concluded that none of the antiseptics and agents other than sodium hypochlorite were sufficiently effective against Candida albicans, Streptococcus sanguis and Staphylococcus epidermis species in the 60-second detoxification process. The authors reported that adequate detoxification could not be achieved with a 40% concentration CA solution, which is known to have very high cytotoxicity. Although there is no consensus decision regarding the chemical to be used and the duration of the contact with the implant surface during decontamination, many studies have reported that the application time of CA is a minimum of 4 minutes for optimal results. This situation can be considered to be the reason of the lack of evidence regarding the biofilm removal efficacy of CA in the study mentioned. Kotsakis compared the effects of surface detoxification with CHX, CA, and saline on both the titanium surface and the cytotoxicity of the evaluated chemicals on the biofilm and the host.[22] CHX was not recommended for detoxification of implants due to the deterioration of the biocompatibility of the titanium surface. It has also been shown that residues of chemical agents that may remain on the surface may negatively affect the osteoblastic response, which is counterproductive to their intended use.

The aim of this study was to evaluate the changes that a 10% concentration of CA applied to titanium samples with two different surface properties by two different methods could have on the titanium surface in terms of surface roughness and surface wettability. The results of detoxification procedures with CA applied in both titanium disc groups are compatible with the existing literature. Using SEM or microCT scans, which enable measurement on the entire surface of the titanium disc, can make the data more reliable compared to scanning a limited area with a profilometer device for surface roughness measurement. Additionally, further studies should be designed to monitor physicochemical changes such as corrosion, resulting from the decontamination of exposed titanium surfaces with different methods. Additionally, more studies are needed to evaluate the effects of different decontamination protocols on biofilm accumulation.

Application of citric acid (CA) at 10% concentration to the titanium surface for decontamination purposes causes different physical modifications. A factor affecting these changes is the macro and micro indented surface caused by surface roughening processes applied to maximize tissue contact on implants. The way decontamination is applied can also lead to physical and chemical changes on the titanium surface. While decontamination by rubbing on sandblasted and acidified surfaces caused a decrease in surface roughness parameters, the contact angle decreased on both SA and MS surfaces, regardless of rubbing or immersion.

Ethics Committee Approval: Ethics committee approval was not obtained because no human subject. There is no procedure performed on humans in the study; the study was carried out entirely on titanium discs produced for this purpose.
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