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Titanium Surface Analysis after Instrumentation with Different Burs Simulating the Implantoplasty Technique: A Pilot In Vitro Experimental Study

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Abstract: (1) Background: The present pilot in vitro study evaluated, physically and biologically, the effects produced by the wear of the titanium surface using different drill models. (2) Methods: Titanium disks were subjected to wear using four different burs and accordingly divided into the following test groups (n = 12 disks per group): Tungsten Burs (TB group), Tungsten Carbide Burs (TCB group), Coarse-Grained Diamond Burs (CGB group), and Fine-Grained Diamond Burs (FGB group). As a control group (CON group), titanium disks with a smooth surface (machined) were used. The samples were subjected to atomic force microscopy (AFM), profilometry analysis, scanning electron microscopy (SEM), and cell viability and adhesion assessments. (3) Results: The values of the measured roughness parameters showed statistical differences among the five groups (p = 0.0035 for Ra and p = 0.0010 for Rz). All test groups showed an important difference statistically (p = 0.0032) to the CON group for the cell viability and adhesion analysis. The data of cell absorbance at 570 nm were 0.4122 ± 0.05 for the CON group, 0.1354 ± 0.02 for the TB group, 0.123 ± 0.01 for the TCB group, 0.1414 ± 0.02 for the CGB group, and 0.1216 ± 0.03 for the FGB group. Additionally, the cell count showed the following adherence percentages: 57.6 ± 4.6% for the CON group, 22.9 ± 3.3% for the TB group, 23.4 ± 2.9% for the TCB group, 22.5 ± 3.1% for the CGB group, and 23.7 ± 3.3% for the FGB group. However, no statistical differences were found among the four test groups analyzed (p = 0.3916). (4) Conclusions: The results showed that the changes produced on the surface by the four different bur models altered the topography characteristics and affected the cell viability and adhesion in comparison with the control group.

Keywords: titanium surface; implantoplasty; cell viability and adhesion; peri-implantitis; titanium instrumentation; fibroblast cells

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1. Introduction

Peri-implantitis is the most serious disease in implantology and often has severe consequences for rehabilitation treatments with implants [1]. To date, there is no defined and/or predictable treatment for this condition [1–3]. Peri-implantitis is an inflammation of the tissues around an implant, characterized by progressive loss of the peri-implant tissue level [1,4–6]. The loss of bone support caused by peri-implantitis can vary among individuals; in addition, this loss may increase over time, usually progressively and non-linearly [1,4,7].

Regarding the incidence of peri-implant diseases, there is limited information in the literature about their real prevalence [8]. Peri-implantitis has a multifactorial origin and is

not well-determined [4]. According to Valente and Andreana [7], the overall frequency of peri-implant mucositis and peri-implantitis was 63.4% and 18.8%, respectively. There are also reports in the literature indicating that the incidence of peri-implantitis can vary from 12% to 40% [9].

Numerous methods to decontaminate implant surfaces have been used by clinicians. Comparisons of decontamination methods have not revealed any statistically significant difference in treatment results [10–12]. The establishment of a clean implant surface has been suggested as a prerequisite for successful regenerative treatment of peri-implantitis [13]. Contaminants such as bacteria and their secretions, calculus, and soft tissue cells should be removed without modifying the implant surface. However, it is still unknown to what extent these contaminants must be removed to achieve a successful treatment result [14].

An alternative used by some professionals in the treatment of contaminated implants that have peri-implantitis is the smoothing (wear) of the rough and irregular surfaces of the implant with rotating instruments [15]. Implantoplasty is performed to reduce new bacterial adhesion and to optimize cleaning, thus preventing the recurrence of peri-implantitis in the portion of the oral cavity which will be exposed later. When performing implantoplasty, different rotary instruments, such as carbide burs and diamond burs, can be used, and good polishing is important [15,16]. However, studies have shown that mechanical resistance is affected with this type of treatment [17,18]. The surface morphology of titanium, regardless of the roughness parameters, affects cell viability and adhesion. Any type of mechanical treatment performed on a dental implant will modify its physical–chemical structure [19].

The peri-implant tissue adhesion on the implant surface is a fundamental condition to restore the health of these areas after treatment. In this study, we used four different bur models (simulating implantoplasty techniques) to evaluate the effect of titanium instrumentation (wear) on the topography characteristic alterations as well as on cellular viability and adhesion in these conditions.

2. Materials and Methods

2.1. Titanium Disks and Group Formation

Sixty disks with a 5 mm diameter and a 2 mm thickness were produced from commercially pure titanium grade 4, according to ASTM-F67 (Figure 1). The chemical composition is: titanium (Ti) $\geq$ 98.6%, Iron (Fe) $\leq$ 0.50%, Oxygen (O) $\leq$ 0.40%, Carbon (C) $\leq$ 0.080%, Hydrogen (H) 0.015%, and other 0.3%.

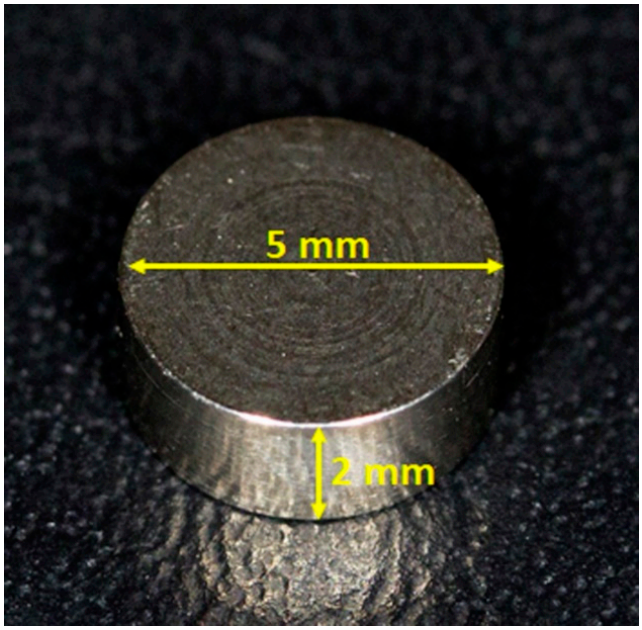

Figure 1. Representative image of the titanium disk used in the study.

All disks were manufactured by Implacil De Bortoli (São Paulo, Brazil) using titanium bars for dental implants. The disks were divided into four test groups according to the bur model used to wear the surface and a control group (n = 12 per group). The control group disks had a smooth surface, but had the same conditions (sterilization and physicochemical state) as commercialized implants. In one group, the disks were instrumented using a Tungsten Bur (TB group) tapered design at length of 4.4 mm and diameter of 1.2 mm of the active part, and granulometry of 91–126 µm; in another group, the disks were instrumented using a Tungsten Carbide Bur (TCB group) tapered design at length of 6.5 mm and diameter of 1.0 mm of the active part, and granulometry of 30 µm; in another group, the disks were instrumented using a Coarse-Grained Diamond Bur (CGB group) tapered design at length of 6.5 mm and diameter of 1.2 mm of the active part, and granulometry of 151 µm; and finally, in one group, the disks were instrumented using a Fine-Grained Diamond Bur (FGB group) tapered design at length of 6.5 mm and diameter of 1.0 mm of the active part, and granulometry of 46 µm. All tested burs were manufactured and commercialized by the same company (KG Sorensen, Cotia, Brazil) (Figure 2), and a new bur was used to wear each disk. Each disk was worn across the whole surface area (5 mm).

Figure 2. Representative images of burs used in each group to wear the disks.

The sample size was based on a power level of 85% to obtain a *p*-value of 0.05, calculated by using a software program (SigmaStat 4.0; Systat Software Inc., San Jose, CA, USA). To obtain a desired power level of 85%, with differences between the means and standard deviations of each group, the minimum sample size for each group under each condition was six samples.

The disks were clamped into a mechanical lathe machine Model BV-20 Ferrari (São Paulo, Brazil) with the purpose of obtaining a uniform wear of all disks (Figure 3a). All disks had worn a thickness of 0.5 mm. Figure 3b shows a disk after the instrumentation. The wearing was performed under intense water cooling at a speed of 30,000 rpm. The average time for each disk to wear out was 60 s.

After the instrumentation, all samples were washed in water, air-dried, and sterilized in an autoclave. Six disks for each group were used for the cell growth assay and six for atomic force microscopy (AFM) and scanning electron microscopy (SEM).

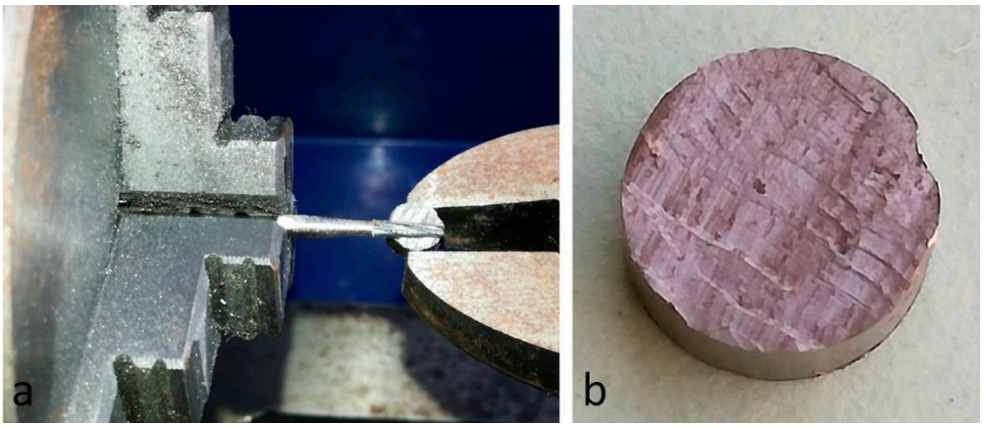

Figure 3. Representative images of a disk during (**a**) and after (**b**) the surface instrumentation.

2.2. Morphological Surface Analysis

For the measurement of the surface roughness and the description of the topographic characteristics of each group, six disks per group were submitted to scanning electronic microscopy (SEM, Philips XL30, Eindhoven, The Netherlands) at ×150, ×1000, and ×5000 to record a series of images based on secondary electrons (SEs). After this examination, all samples were analyzed by atomic force microscopy (AFM, Nanoscope IIIa, Veeco, Santa Barbara, CA, USA) to assess the 3D surface topography. Then, two roughness parameters, the absolute values of all profile points (Ra) and the arithmetic mean of the depths of the five deepest valleys (Rz), were measured using an optical laser profilometer (Mahr GmbH, Gottingen, Germany). All measurements were performed in three different areas of each disk (0.5, 2, and 4 mm from the disk edge), and an average was calculated for each sample.

2.3. Cellular Assay

The sample disks of each group were put into a well of a 48-well plate and 20,000 VERO cells (ATCC CCL-81) were seeded in each one. The cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% fetal calf serum, penicillin, and streptomycin and incubated in a 5% CO_2 atmosphere at 37 °C for 24 h. Cell proliferation was assessed by MTT (3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide) bioassay, which is based on the conversion of MTT to water-insoluble formazan crystals inside the cells by mitochondrial dehydrogenases. MTT was dissolved in phosphate-buffered saline (0.1 M PBS) at 5 mg/mL and added to the wells (0.02 mL). After 4 h of incubation at 37 °C, the medium with MTT was completely removed from the wells and DMSO (0.1 mL) was added to solubilize the mitochondrial formazan. The plates were gently shaken for 10 min. The optical density was read at 570 nm (Thermo Scientific Varioskan® Flash Multimode, www.thermo.com, (accessed on 5 April 2022)). Each experiment was repeated three times. The mean values ± standard deviation are reported for each group.

To evaluate differences in the morphology/adhesion pattern of cells to different tested surfaces, red LNCaP adherent epithelial cells, derived from a human prostate cancer cell line stably transfected with pEZX-MR02 plasmid (GeneCopoeia, Rockville, MD, USA), were used. These LNCaP cells with red phenotype were maintained in an RPMI 1640 medium supplemented with 10% FBS and penicillin/streptomycin in a 5% carbon dioxide atmosphere at 37 °C. Images were acquired using the ZOE Fluorescent Cell Imager (BioRad, Hercules, CA, USA). After 24 h, cells were washed with 1 mL 0.1 M PBS three times to remove unattached cells. The cells remaining after this procedure were counted manually using the ImageJ program (National Institute of Health, Bethesda, ML, USA).

2.4. Statistical Analysis

Statistical comparison was performed using a one-way analysis of variance (ANOVA) to determine the differences among all groups, followed by Bonferroni's multiple comparison test to determine individual differences between the groups. In all cases, $p < 0.05$ was

considered significant. The data were analyzed with the GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

For SEM analysis, images of the disks were obtained at ×150, ×1000, and ×5000 based on secondary electrons (SEs). The SEM images show different topography of the surfaces after wear for each group. At low magnification (×150), the wear produced a uniform and even surface in all groups. However, at higher magnifications (×1000 and ×5000), it was possible to observe large irregularities in the form of the non-uniform sulcus, and, in some places, poorly cut spikes of titanium, in comparison with the disks of the CON group (which showed a uniform distribution of the sulcus). In these images at greater magnification, the TCB and CGB groups showed greater irregularities compared to the TB and FGB groups. Figure 4 shows representative SEM images of the magnifications proposed for each group.

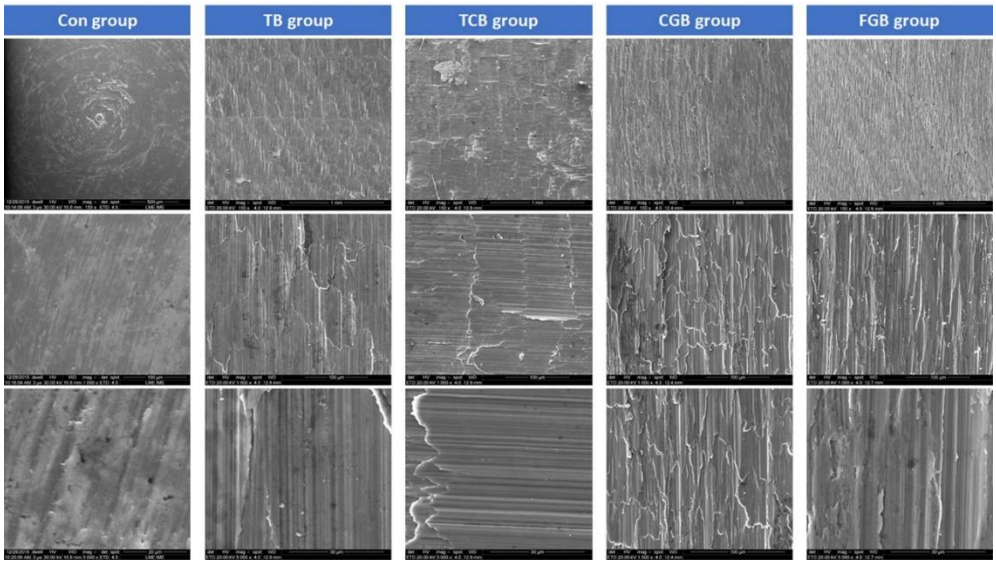

Figure 4. Representative SEM images of the magnifications proposed for each group: ×150, ×1000, and ×5000 magnifications.

With regard to the roughness analysis of the surfaces of the groups, the obtained values for the roughness parameters evaluated for Ra and Rz are presented in Table 1. In the comparison among all groups, the one-way ANOVA statistical test showed differences among them: $p = 0.0035$ for Ra and $p = 0.0010$ for Rz.

Table 1. The mean and standard deviation of the measured values (in μm) of surface roughness parameters analyzed (Ra and Rz) for each group.

Roughness Parameters	CON Group	TB Group	TCB Group	CGB Group	FGB Group
Ra	0.77 ± 0.14	0.45 ± 0.10	0.75 ± 0.16	0.83 ± 0.16	0.51 ± 0.14
Rz	4.32 ± 0.66	3.68 ± 0.64	5.61 ± 0.74	6.03 ± 0.71	3.98 ± 0.67

The AFM images of the surface morphology showed different irregularities of the surfaces after implantoplasty (instrumentation) procedures for the groups (Figure 5).

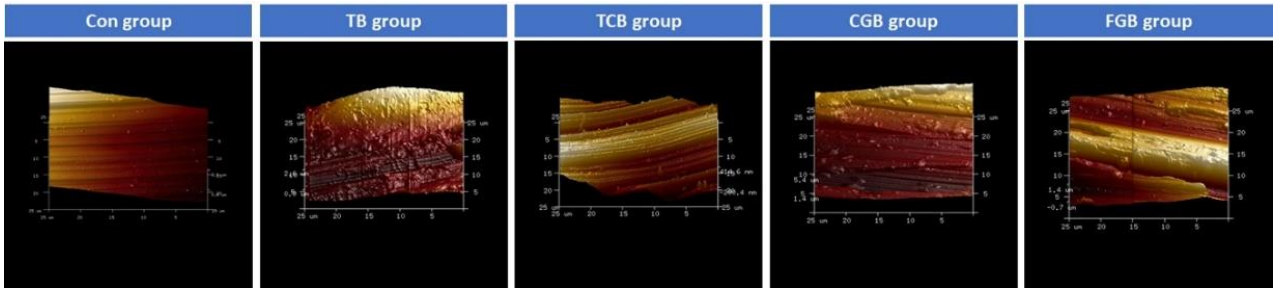

Figure 5. AFM 3D images of the surface morphology of each group.

To evaluate the influence of different surfaces on the growth of cells, a fibroblast-like cell line was used in an in vitro experiment where, after culturing for 24 h, fibroblast mitochondrial activity was evaluated through MTT assay. The groups showed an important statistical difference among them (p = 0.0032). However, we compared the four groups with wear on the disks, and no significant differences were found among these groups (p = 0.3916). The bar graph of Figure 6 shows the comparison among the groups.

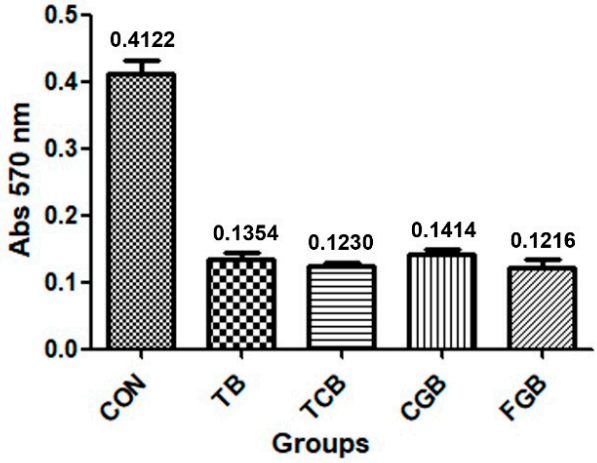

Figure 6. Viability assay of cells growing in the four groups of worn titanium disks assessed by MTT bioassay. Absorbance at 570 nm. Mean values, expressed as the mean ± standard deviation, are represented.

The Bonferroni's multiple comparison test between the groups is presented in Table 2.

Table 2. Bonferroni's multiple comparison test to compare the growth of cells between the groups.

Group Comparison	Mean of Diff.	*p*-Value	95% CI
CON vs. TB	0.2768	0.0022 *	0.2262 to 0.3274
CON vs. TCB	0.2892	0.0022 *	0.2385 to 0.3398
CON vs. CGB	0.2708	0.0022 *	0.2201 to 0.3214
CON vs. FGB	0.2906	0.0022 *	0.2400 to 0.3412
TB vs. TCB	0.01238	0.2403	−0.03825 to 0.06302
TB vs. CGB	−0.006019	0.6991	−0.05666 to 0.04462
TB vs. FGB	0.01382	0.4848	−0.03682 to 0.06446
TCB vs. CGB	−0.01840	0.1727	−0.06904 to 0.03223
TCB vs. FGB	0.001436	0.6884	−0.04920 to 0.05207
CGB vs. FGB	0.01984	0.3939	−0.03080 to 0.07048

Diff. = differences; * p-value summary for $p < 0.05$; CI = confidence interval.

Adherence of fibroblast cells with red phenotype to the five surface groups was investigated. The number of adherent cells remaining on the surface of the worn titanium disks was quantified and calculated as a percentage of the initial cell number deposited on

each surface. The cell count showed the following adherence percentages: 57.6 ± 4.6% for the CON group, 22.9 ± 3.3% for the TB group, 23.4 ± 2.9% for the TCB group, 22.5 ± 3.1% for the CGB group, and 23.7 ± 3.3% for the FGB group. Thus, the fibroblast cells showed equal ability to adhere in all groups, indicating a similar low adhesion capacity on worn surfaces. Figure 7 shows representative images of the adhered cells from a sample of each group.

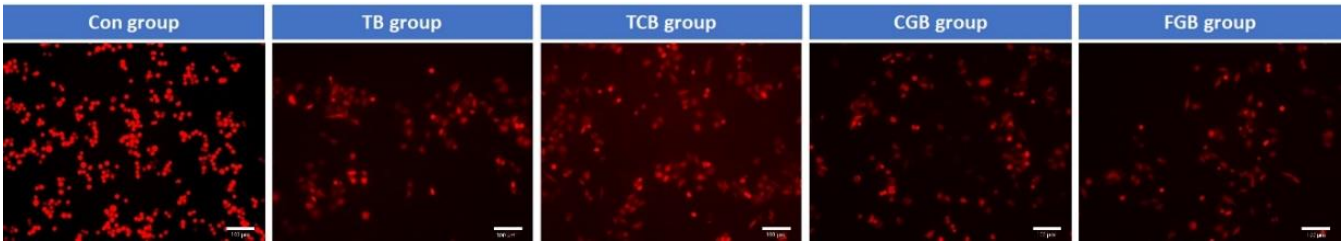

Figure 7. Representative images of the adhered cells from a sample of each group.

4. Discussion

Peri-implantitis is an inflammatory disease that affects the longevity of rehabilitation treatments involving dental implants. Different treatments have been proposed but without much predictability in their results [10–12,20,21]. However, there seems to be a consensus among researchers and clinicians that an adequate decontamination of the surface of the affected implants is of fundamental importance to obtain a satisfactory result [22]. Therefore, different approaches have been suggested and studied, such as cleaning by ultrasound, sandblasting, curettage, and, more recently, wear of the implants (implantoplasty) [20,22–24]. Our main objective was to study the effects of four different drills commonly used in dentistry on the wear of the titanium surface. We evaluated the topographic characteristics of the surface as well as the behavior of these "new surfaces" with respect to cell contact. The results, both in the evaluations of superficial morphology and in relation to cellular behavior, showed that regardless of the type of bur used, a favorable scenario was not obtained in any situation.

The health of peri-implant tissues depends on bacterial control, so the need for a microbiological seal is of fundamental importance to the long-term survival of implants [21]. A proper union between the peri-implant tissues and titanium is dependent on the physical and chemical conditions of its surface. When bone tissue has been lost around implants due to the occurrence of peri-implantitis, it is difficult to regenerate, even using guided bone-regeneration techniques. Therefore, after adequate treatment of peri-implantitis, the contact of the cervical implant portion will be with the mucosal tissue due to bone loss caused by this disease [25,26]. Thus, in our present study, fibroblast cells were used for the viability and adhesion tests. The results showed that, regardless of the type of drill used for disk wear, cell viability was low for all proposed test groups. When compared with the control group (with a smooth surface), but with their surfaces physically and chemically unaltered (similar to a ready-to-use implant), the results obtained for the test groups were much higher for the CON group. The disks worn by the burs had fibroblast cell viability and adhesion test values that were, on average, 65% lower than those of the machined disks (CON group).

Recent studies have demonstrated that smooth titanium surfaces can promote good cellular adhesion [27], including electrochemical polishing protocols, which have been proposed and incorporated into some processes for manufacturing implants and abutments [28]. Our surface analyses, after wear with the different types of burs, showed a very irregular surface topography. However, based on the classification of the degree of roughness proposed by Wennerberg and Albrektsson [29], and the values obtained for roughness parameters after disk wear, these surfaces can be considered as smooth. In accordance with a recent report by Gehrke et al. (2016) [18], we can suggest that the changes in the surface

characteristics and the surface topography regularity are responsible for the low cellular response found in our study.

As for the type of drill used, our results showed that the cutting power (coarse grain burs) is inversely proportional to the result obtained. Other studies have shown that, for implantoplasty, the use of conical carbide cutter burs had the best results [15]. Our results showed that there was no significant difference in results when using diamond burs compared to carbide burs. Moreover, the bur performance was significantly determined by the average cutting speed and its diameter [30]. Regarding the cost–benefit of using burs for implantoplasty, it is a very inexpensive method as this type of drill has a low price for its acquisition; however, in terms of benefit, the technique showed little addition to the cell activities tested (viability and adhesion).

Several problems associated with the surgical treatment of peri-implantitis have been investigated, including methods for decontamination of the implant surface, and various protocols have been suggested. Only the loss of bone support, in response to peri-implantitis, considerably decreased the resistance of the implants [31]. As demonstrated in this study, a loss of 3 mm of insertion decreased resistance by 37.2%, and a 5 mm loss of insertion decreased resistance by 53.8%. When an implant is worn (implantoplasty), the loss of resistance can reach 40% depending on the implant design [18]. Thus, implantoplasty can have consequences, both mechanically and biologically, that are not favorable to the longevity of treatment with implants. However, other authors have shown that changes in superficial morphology promoted by implantation associated with surgical treatment, such as the depth of the peri-implant pouch, purulent secretions, and tissue bleeding, can positively influence implant survival and clinical results [32]. Rough surfaces and threads of the implant facilitate the attachment of bacteria and biofilm and are difficult to clean [33–35], unlike smooth surfaces which are easier to clean [35]. However, the treatment of peri-implantitis is palliative, as cited by Schwarz et al. [36], "complete disease resolution was commonly not obtained". Its main objectives are to reduce and/or control the presence of microorganisms on the surface of the implants.

A few important limitations of the present study can be reported, such as the fact that the wears were performed on disks with a flat surface, which is different from implants (which are cylindrical). However, other recent studies have used titanium discs for different experiments; these include evaluation of the efficiency of protocols (mechanical and/or chemical) for bacterial decontamination simulating peri-implantitis [37–41] and simulating the implantoplasty [41,42]. Furthermore, performing cellular assays directly with implants is more complex due to its shape; as it is a round screw, it is difficult to properly stabilize the implant. The ease of access to perform the procedure outside the oral cavity and the possibility of direct viewing of the location of wear and tear can also be considered limitations. In addition, for the cellular assay, the disks were previously sterilized, which is not possible when this type of procedure is performed on patients in a contaminated environment.

5. Conclusions

The instrumentation (wear) of the disks with the four bur models tested produced roughness patterns (Ra and Rz) which were considered as a smooth surface. In the TCB and CGB groups, the roughness values presented were similar to those of the control group. However, all new surfaces obtained after wear showed changes in the surface that hindered the activity of fibroblastic cell viability and adhesion on the disks, in comparison with the control group. Despite the limitations reported—including the standardization of wear for the four models of drills tested, which would be different from the clinical setting where the implants are inserted into the tissues and not flat surfaces like the disks we used—we conjecture that the finish of the wear produced would be less regular. Our results showed that the cell viability and adhesion are not related to the roughness produced on the surface, but to the altering of other surface characteristics of titanium. Further studies are needed to understand the possible causes that hinder cellular activity on instrumented (worn) titanium surfaces.

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