Comparative effect of the extended use of acids for surface treatment of osseointegrated implants. A laboratory study

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TO CITE THIS ARTICLE
Mendes GN, Takeshita WM, Brasileiro BF, Trento CL. Comparative effect of the extended use of acids for surface treatment of osseointegrated implants. A laboratory study.J Osseointegr 2024; 16(1): 65-71.

DOI 10.23805/JO.2024.621

KEYWORDS Dental Implants, Surface properties, Treatment Surface, Metallurgy Industry.

ABSTRACT

Aim The aim of this study is to comparatively analyze the effects of extended acid usage, up to the fifth time, on the treatment of dental implant surfaces on their apex, middle, and neck regions.
Materials and Methods Implant samples (n=10) were analyzed using a scanning electron microscope, and their captures by implant region (apex, body, and neck) were processed using ImageJ software. The generated Ra and Rq data of the samples were assessed using the Kruskal-Wallis test and the Dunn post-hoc test.
Results The treatment groups showed no statistical significance compared to the control group up to the fourth use. Therefore, extended acid usage is possible, but it is conditioned upon the number of acid reuses. However, in the 5th use, there was significant variation in the mean values of Ra and Rq in the Dunn post-hoc test.
Conclusion Based on the obtained results, it can be concluded that using the same acid for dental implant surface treatment up to four times did not alter the dental implant surface roughness property. Nonetheless, further studies are necessary, particularly because there is limited data regarding this unexplored research topic in the implantology literature.

INTRODUCTION

The success of implants for oral rehabilitation varies according to intrinsic and extrinsic factors related to the material. Some extrinsic factors include low bone density, occlusal overload, and patient hygiene, which contribute to peri-implant bone loss and the presence of bacteria in microgrooves. Intrinsic factors are inherent to the material's composition, biocompatibility, and surface treatment applied in the industry, which directly affects osseointegration (1).
In order to reduce failures related to dental implant osseointegration, their surfaces can be optimized through hydrophilic and rough surface treatments. Titanium implants can have their surfaces modified to improve biological performance, with alterations in roughness and/or the application of bioactive coatings to enhance wettability and surface tension, which can improve biochemical bonds capable of accelerating the initial phases of bone tissue formation (2).
According to Scarano et al. (2021), the wettability, hydrophilicity, and roughness of the implant contribute to initial bone formation and effective bone/implant contact. To ensure these beneficial characteristics for osseointegration, various surface treatment methods are applied for surface maintenance and characterization. Some manufacturers perform treatment with acid attack (single or double), alumina or TiO_2 blasting, calcium phosphate blasting, and anodization (1,3,4).
Scientific advancements in surface treatment aim to increase corrosion resistance and accelerate osseointegration. These modifications alter surface energy, wettability, and cellular adhesion, and all of these surface characteristics are related to roughness. Roughness is well-established in the literature, and treatment protocols must be strict to achieve a standard and obtain uniformity in implant surface (5,6).
Thus, modifications in surface treatment protocols, such as the reuse of acids, should be evaluated to avoid impairing surface characterization and ensure the safety and effectiveness of the acid expansion process. The present study aims to investigate the effect of expanding acid use in the apex, middle, and

neck regions of titanium alloy dental implant surface treatment compared to the same surface treatment method with single-use of acids.

METHODS

Sample Characterization

The dental implant samples (SINGULAR Implants ®, Paramirim, Brazil, 2023) consisted of 10 type IV titanium screws, received factory surface treatment and were divided into 5 groups, the sample size is in accordance with the proposed objectives of this preliminary study. The control group and the amplification groups are based on the number of reuses. Group 01 represents the first reuse, group 02 entailed implants with the second reuse, and successively for groups 3 and 4. The samples were characterized as follows: The implants were immersed in a 30% sulfuric acid solution in an Ultrasonic Washer (Ultratec UTC 25/7, São Paulo, Brazil, 2018) with preheated water at 60±7 °C for 120 minutes, after which they were rinsed with running water. After completing the first step, the implants were immersed in a 30% Nitric Acid solution in an Ultrasonic Washer at 60±7 °C for 30 minutes. Once the process was finished, the implants were taken to an oven at 60±7 °C for 90 minutes after washing with distilled water. All these steps were repeated for the following samples using the same acids. The protocols of implant surface treatment and acid reuse were developed by the dental implant manufacturer.

Implant surface analysis

The surface topography of the control samples and treatment samples 1, 2, 3, and 4 were analyzed to identify the physical properties of the implants using scanning electron microscopy (SEM) with a JEOL JSM-6510LV benchtop electron microscope (JEOL USA, Inc). The control group and treatment group samples were fixed on a metal base and photographed at magnifications of 500x, 1000x, 2000x, and 4000x.

FIG. 1 Representation of the three regions (neck, body and apex) of a dental implant studied.

Images were obtained of the apex, middle, and neck implant regions (Figure 1).

Roughness analysis

The ImageJ software (National Institutes of Health, Bethesda, MD, USA, 1997), an open-source scientific image processing program (v1.54d) was used, along with the SurfaceJ roughness calculation plugin, to analyze the images and calculate the average roughness (Ra) and root mean square roughness (Rq) of all SEM images in TIF format. The 3D image tool, Surface Plot, was also used for surface topography. Before analysis, image processing was performed, using a median filter to calculate the median of pixel values in a defined area to reduce noise. After image preparation, the pixel scale was adjusted to µm, as measured from the SEM images. For roughness analysis, a diagonal line was traced at both ends of the image, and the sampling length used for image processing was 90.0 µm for 500x, 40.0 µm for 1000x, 20.0 µm for 2000x, and 10.0 µm for 4000x. This ensured surface measurements were proportional to the image magnification for all samples (7,8).

Statistical analysis

After obtaining Ra and Rq values from the software, these data were exported and saved in spreadsheets, with subdivisions for capture magnification, and surface treatment acid usage groups. Regions of the implants were excluded from the analysis. For Ra and Rq measurements, with a significance level ($p>0.05$), the Kruskal-Wallis test followed by Dunn's post-hoc test was applied to compare different Ra and Rq values. All statistical procedures were computed with SPSS 25.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The 3D images obtained with ImageJ correspond to captures of implant regions in SEM (Figure 2), exported in TIFF format at magnification levels of 500x, 1000x, 2000x, and 4000x (Figures 3 to 5). Roughness analysis was primarily based on Ra and Rq values, representing average roughness and root mean square roughness, respectively. The values corresponding to each implant region are presented in Tables 1 to 6.

For the apex region of the dental implants, different Rq values were analyzed using the Kruskal-Wallis test, and the p-value of 0.007 was obtained. Therefore, the Dunn post-hoc test was performed (Table 1).

Ra values were also assessed for the apex region. The Kruskal-Wallis test was applied and the p-value was 0.007. Therefore, the Dunn post-test was performed (Table 2).

The body region of the dental implant was tested for different Rq values using the Kruskal-Wallis test, which obtained a p-value of 0.005. Thus, the Dunn post-hoc test was performed (Table 3).

To investigate the body region of the dental implants for

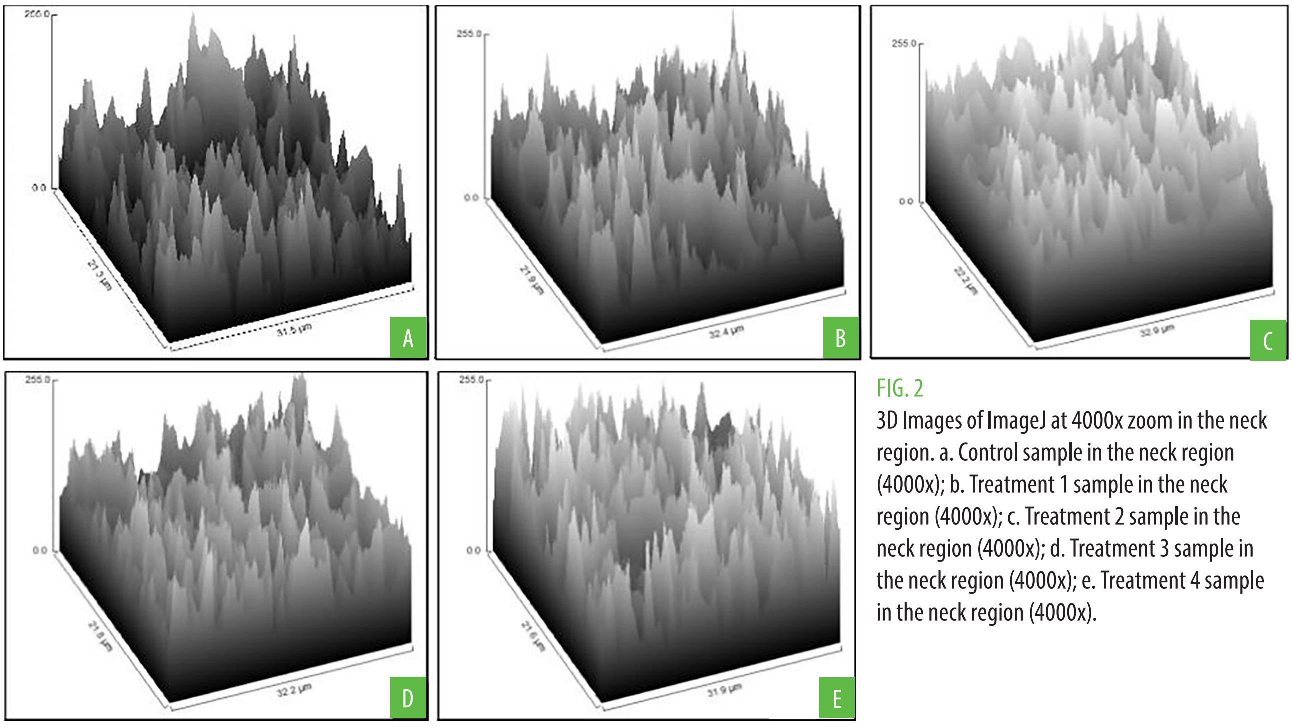

FIG. 2
3D Images of ImageJ at 4000x zoom in the neck region. a. Control sample in the neck region (4000x); b. Treatment 1 sample in the neck region (4000x); c. Treatment 2 sample in the neck region (4000x); d. Treatment 3 sample in the neck region (4000x); e. Treatment 4 sample in the neck region (4000x).

	Mean (Rq)	Standard Deviation	Median	1st percentile	3rd percentile	Dunn's post-test*
Control	42.23	11.62	37.04	35.60	54.03	A
Treatment 1	39.12	1.97	38.54	37.62	41.21	A
Treatment 2	44.04	1.40	43.93	42.80	45.39	AB
Treatment 3	48.86	2.39	47.75	47.52	51.32	AB
Treatment 4	62.44	3.32	61.33	60.05	65.94	B

*different letters indicate statistically significant difference between the groups (p<0.05). Ra values were also assessed for the apex region. The Kruskal-Wallis test was applied and the p-value was 0.007. Therefore, the Dunn post-test was performed (Table 2).

TABLE 1 Mean values, standard deviation, median, percentiles, and Dunn post-test for Rq measurements.

	Mean (Ra)	Standard Deviation	Median	1st percentile	3rd percentile	Dunn's post-test*
Control	33,56	10,56	29,73	26,65	44,29	A
Treatment 1	28,38	1,57	3,10	29,75	33,02	A
Treatment 2	35,70	1,57	35,76	34,16	37,18	AB
Treatment 3	40,29	1,94	39,35	39,26	42,24	AB
Treatment 4	52,22	3,05	51,17	50,03	55,47	B

*different letters indicate statistically significant difference between the groups (p<0.05). The body region of the dental implant was tested for different Rq values using the Kruskal-Wallis test, which obtained a p-value of 0.005. Thus, the Dunn post-hoc test was performed (Table 3).

TABLE 2 Mean values, standard deviation, median, percentiles, and Dunn post-hoc test.

different Ra values, the Kruskal-Wallis test was applied, and the p-value was 0.004. Therefore, the Dunn post-hoc test was performed (Table 4).

Similarly, the neck region of the dental implants was investigated for different Ra and Rq values. The Kruskal-Wallis test was applied, and the p-values obtained were

	Mean (Rq)	Standard Deviation	Median	1st percentile	3rd percentile	Dunn's post-test*
Control	41,71	1,70	41,36	40,31	43,45	AB
Treatment 1	44,83	8,36	48,26	36,19	50,04	AB
Treatment 2	30,02	5,47	32,20	24,24	33,62	A
Treatment 3	33,97	1,16	34,04	32,85	35,02	AB
Treatment 4	53,17	1,80	52,57	51,86	55,08	B

different letters indicate statistically significant difference between the groups (p<0.05). To investigate the body region of the dental implants for different Ra values, the Kruskal-Wallis test was applied, and the p-value was 0.004. Therefore, the Dunn post-hoc test was performed (Table 4).

TABLE 3 Mean values, standard deviation, median, percentiles, and Dunn post-test.

	Mean (Ra)	Standard Deviation	Median	1st percentile	3rd percentile	Dunn's post-test*
Control	33.971	0.9473	33.9027	33.6131	34.2605	AB
Treatment 1	36.415	7.2704	39.3316	35.4686	40.278	AB
Treatment 2	23.9072	4.3129	25.465	22.4563	26.9159	A
Treatment 3	27.2492	1.6083	27.6077	26.8581	27.9988	A
Treatment 4	44.0713	1.2162	43.7701	43.1968	44.6446	B

different letters indicate statistically significant difference between the groups (p<0.05).
Similarly, the neck region of the dental implants were investigate for different Ra and Rq values. The Kruskal-Wallis test was applied, and the p-values obtained were 0.008 and 0.003 respectively. The Dunn post-hoc test was performed for both variables (Table 5 and 6).

TABLE 4 Mean values, standard deviation, median, percentiles, and Dunn post-test.

	Mean (Ra)	Standard Deviation	Median	1st percentile	3rd percentile	Dunn's post-test*
Control	39.6099	1.813	40.1488	38.9001	40.8586	AB
Treatment 1	43.6571	0.2146	43.625	43.4824	43.7997	AB
Treatment 2	36.1296	5.8606	34.9188	32.5363	38.5121	AB
Treatment 3	31.1139	5.3435	33.3587	30.268	34.2046	A
Treatment 4	44.6651	0.8406	44.7839	44.1264	45.3225	B

different letters indicate statistically significant difference between the groups (p<0.05).

TABLE 5 Mean values, standard deviation, median, percentiles, and Dunn post-hoc test.

	Mean (Rq)	Standard Deviation	Median	1st percentile	3rd percentile	Dunn's post-test*
Control	31.885	1.2395	32.0093	31.4634	32.4309	AB
Treatment 1	35.124	0.4452	35.3183	35.0635	35.3789	AB
Treatment 2	28.5257	3.823	28.141	26.0762	30.5906	AB
Treatment 3	25.2017	4.6116	27.1816	24.4942	27.8891	A
Treatment 4	35.9037	1.0285	36.1028	35.2835	36.723	B

different letters indicate statistically significant difference between the groups (p<0.05) Description of comparative results among the different regions of the implants, regardless of the acid attack group (Table 7).

TABLE 6 Mean values, standard deviation, median, percentiles, and Dunn post-hoc test.

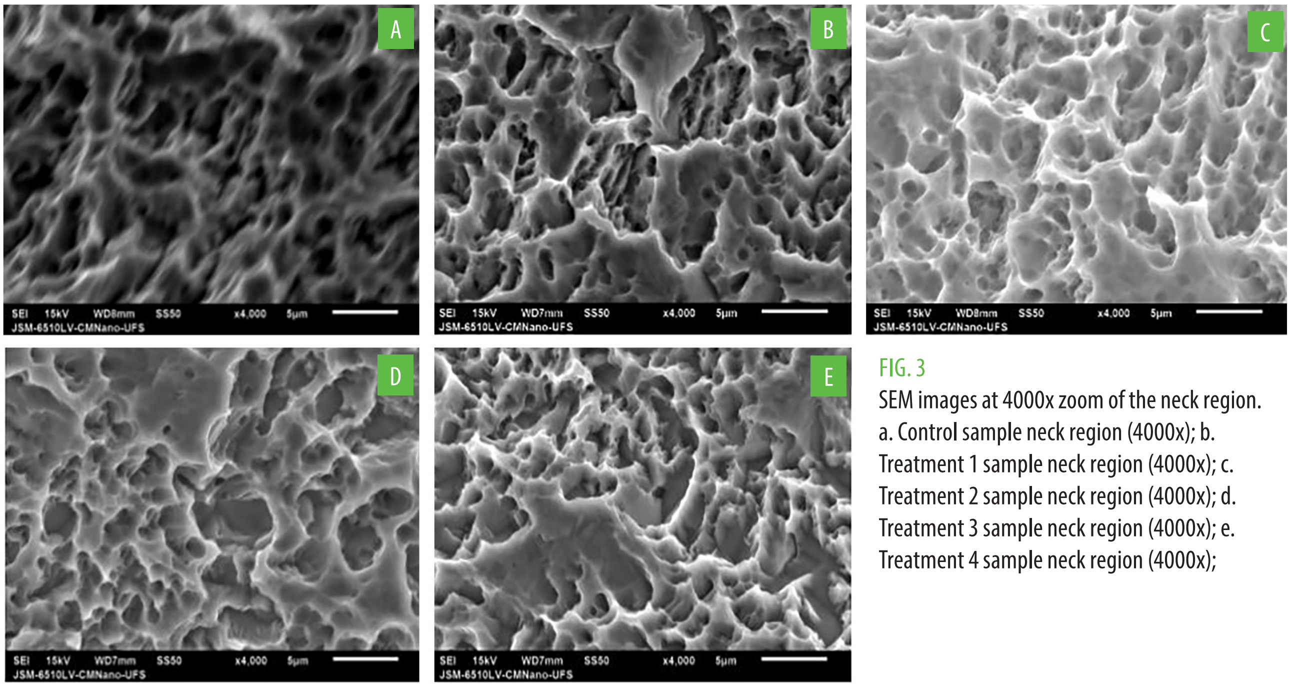

FIG. 3
SEM images at 4000x zoom of the neck region. a. Control sample neck region (4000x); b. Treatment 1 sample neck region (4000x); c. Treatment 2 sample neck region (4000x); d. Treatment 3 sample neck region (4000x); e. Treatment 4 sample neck region (4000x);

	Region	Mean	SD	SE	Tukey's post-test
Ra	Apex	47.3	9.77	2.18	A
	Body	40.7	9.33	2.09	AB
	Neck	39.0	6.07	1.36	B
Rq	Apex	38.6	8.88	1.99	A
	Body	33.1	8.05	1.80	AB
	Neck	31.3	4.81	1.08	B

**different letters indicate statistically significant difference between the groups ($p<0.05$).*

TABLE 7 Mean values, standard deviation and Tukey's post-test

0.008 and 0.003 respectively. The Dunn posthoc test was performed for both variables (Tables 5 and 6). Description of comparative results among the different regions of the implants, regardless of the acid attack group (Table 7).

DISCUSSION

The results between the control and treatment groups 1, 2, 3, and 4 showed that the values of Ra and Rq varied according to the dental implant region tested. Some authors have supported that the variation in roughness between implant regions is beneficial, as rougher surfaces have more bacterial adhesion. However, other authors argued that despite this adhesion, bacterial colonies proliferate on both smooth and rough surfaces (9,10). Therefore, preventing bacterial adhesion goes beyond the implant surface treatment, macrogeometry, and chemical composition. Contaminant elimination during surgery and patient hygiene are determinants for the development of peri-implantitis. The roughness average values (Ra and Rq) exhibited similarity, as the highest mean values for both Ra and Rq were found in the apex, followed by the body, and lastly the neck. This suggests that the resemblance between Ra and Rq values in the analyzed regions may indicate consistency and uniformity in implant surface roughness. Based on the statistical results, we can identify that the apex region has the highest mean values for Ra and Rq, but also the greatest variability in values. Furthermore, the apex and body regions are statistically different from each other, while the neck region is statistically distinct from the other two regions (Table 7).
The results of the treatment group, compared to the control group, did not show significant differences until the 3rd reuse. Therefore, expanding the use of acids is possible, but it is conditioned to the number

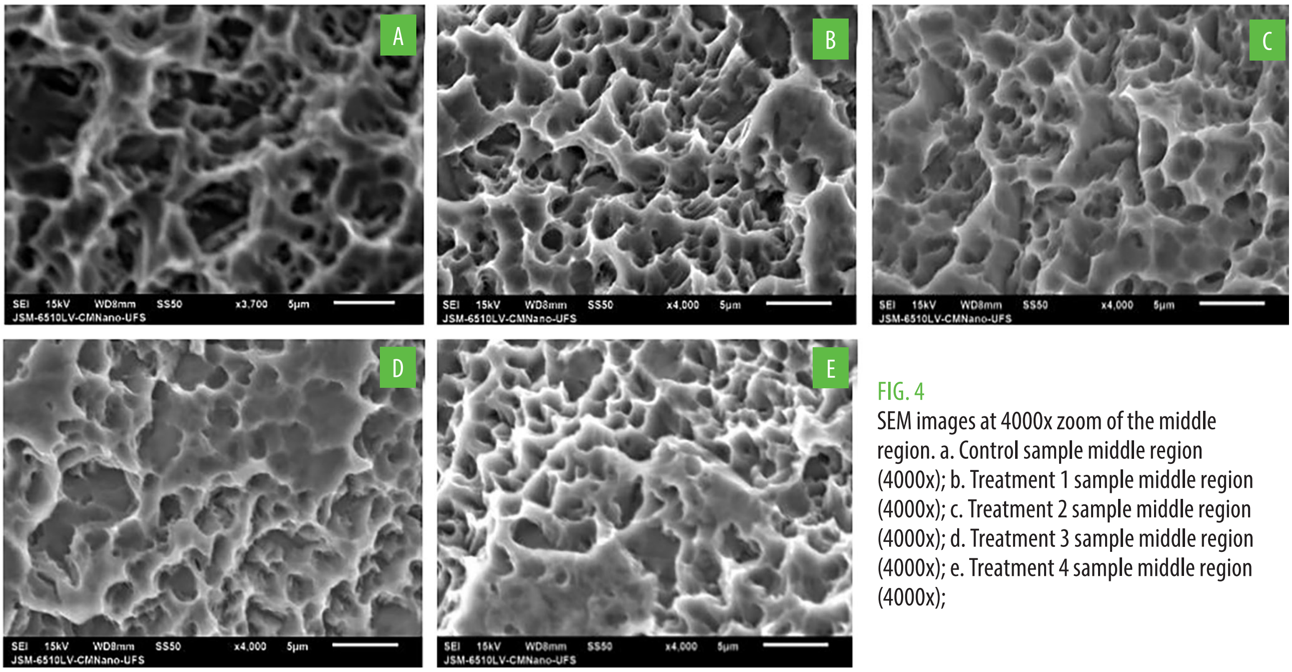

FIG. 4
SEM images at 4000x zoom of the middle region. a. Control sample middle region (4000x); b. Treatment 1 sample middle region (4000x); c. Treatment 2 sample middle region (4000x); d. Treatment 3 sample middle region (4000x); e. Treatment 4 sample middle region (4000x);

of reuses. Once the 4th reuse was reached, treatment group 4 showed statistically significant variation in the mean values of Ra and Rq in the Dunn post-hoc test (Table 1, 2, 3, and 4). It is important to emphasize that the reuse of acids depends on control of concentration, time, and temperature, and any change in these factors can alter the quality of surface treatment or interfere with the protocol to obtain the proposed results.
The reuse of acids occurs in the steel industry during the pickling process nowadays and can have important implications for dental implantology since both industries work with metal alloys (11-13). Acid reuse in the steel industry occurs after the purification process. When reusing acid without proper purification, unwanted substances and contaminants can remain in the solution, especially the release of Fe, which renders reuse impractical and inactivates the acid's action, affecting the integrity of the metal and compromising its strength and stability (14-16). However, titanium metal differs from iron in terms of resistance to pickling, making the reuse of acid possible in the dental implant industry. Because of these factors, it is essential to ensure proper purification of the acid before considering its reuse of dental implant surface

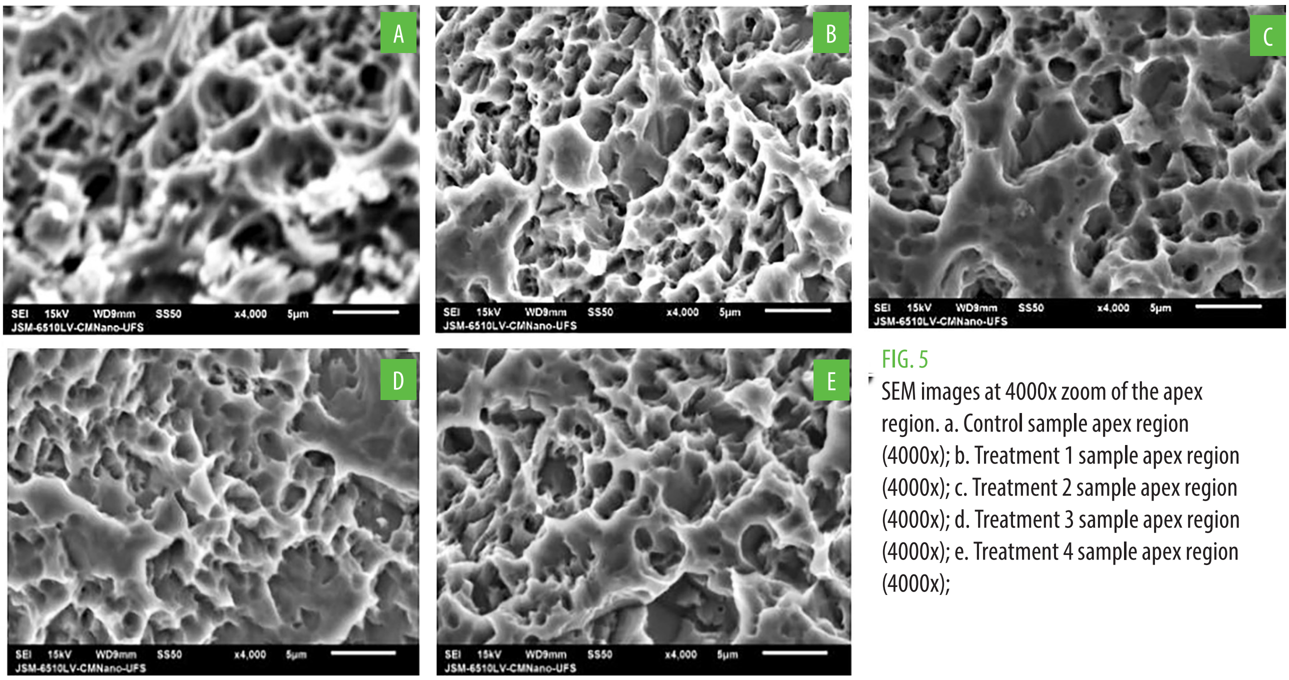

FIG. 5
SEM images at 4000x zoom of the apex region. a. Control sample apex region (4000x); b. Treatment 1 sample apex region (4000x); c. Treatment 2 sample apex region (4000x); d. Treatment 3 sample apex region (4000x); e. Treatment 4 sample apex region (4000x);

treatment after the fourth reuse. The samples tested maintained similar results, differing only in the 4th acid use of this experiment. It leads to the hypothesis of the limit of acid reuse expansion before purification. Factors that support this effect are that the metal alloy used in implants differs from that used in the steel industry, which indicates the need for multiple acid attacks per batch of implants before purification.
The results of this study suggested that cost reduction of the dental implant surface treatment could be achieved by the reuse of acids without compromising the quality of hardware preparation (17-20). The industry could benefit from acids being reused for up to four batches, maintaining the same uniform and homogeneous implant surface, and ensuring greater adhesion to the bone surface. Although further research is needed to determine the effectiveness of acid reuse, this possibility should be explored as part of a more sustainable approach to dental implant development.

CONCLUSION

Based on the results obtained from this research, it can be concluded that using the same acid up to four times did not affect the roughness properties of the implant surface, which may be of interest to implant manufacturers and implantologists to guarantee material quality for osseointegration and cost reduction. More studies on the topic of multiple acid reuse for dental implant surface treatment are necessary, especially due to a limited number of previous studies, and its potential financial and environmental relevance has not yet been addressed in the dental literature.

Acknowledgments
We would like to express our gratitude to the Multiuser Nanotechnology Center at UFS (CMNano-UFS) for providing the facilities for this research. The National Multiuser Center for Research is supported by the Financier of Studies and Projects (FINEP). We appreciate the technical support provided by the CMNano-UFS teams during the experiments outlined in proposal 085/2023. The generous provision of implant samples was made possible by SINGULAR Implantes®. This work was conducted with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001.

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