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ORIGINAL ARTICLE

Influence of Er,Cr:YSGG laser, associated or not to desensitizing agents, in the prevention of acid erosion in bovine root dentin

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Abstract

This in vitro study evaluated the influence of the Er,Cr:YSGG laser, associated or not to desensitizing agents, in the prevention of acid erosion in bovine root dentin. Eighty dentin specimens were selected and divided into eight groups ($n = 10$): G1: negative control; G2: positive control (5% fluoride varnish-FV); G3: Er,Cr:YSGG laser; G4: FV + laser; G5: 3% potassium oxalate; G6: 3% potassium oxalate + laser; G7: biphasic calcium silicate/phosphate gel (gel); G8: gel + laser. Laser parameters: 0.5 W, 6.25 J/cm^2 at 1-mm distance. The erosive drink used was a cola soft-drink (pH = 2.42 at 4 °C), lasting 5 min, twice a day, with 6-h intervals between the challenges, during 14 days. Kolmogorov-Smirnov and Levene's tests were satisfied. The surface roughness data were submitted to ANOVA and Tukey post hoc tests. For the wear profile, Kruskal-Wallis and Dunn post hoc tests were used. Afterwards, the Spearman correlation test was performed. All statistical tests assumed a significance level of 5% ($\alpha = 0.05$). G1 presented the highest surface roughness value after the erosive challenge (3.586 μm^2 ± 0.205 μm^2) and the G7 presented the lowest surface roughness value after the erosive challenge (1.071 μm^2 ± 0.180 μm^2). For the lost volume, G4 presented the lowest percentage (9.7% ± 0.9%), while G1 had the highest percentage (41.8% ± 2.5%), both with $p < 0.05$. There was a weak correlation between the response variables ($\rho = 0.33$). All groups presented lower values of surface roughness and loss of volume when compared to the negative control group. For the surface roughness, the biphasic calcium silicate/phosphate gel presented the best result. For volume loss, the 5% fluoride varnish + Er,Cr:YSGG laser showed the best results compared to the other groups.

Keywords Er,Cr:YSGG laser · Dental erosion · Prevention · Dentinal hypersensitivity

Introduction

Dentin hypersensitivity (DH) or hyperalgesia is understood to be an acute pain, of short duration, manifesting in an uncomfortable way for the patient. This hyperalgesia occurs due to the presence of open dentinal tubules on an exposed dentin surface [1–3].

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Acid erosion has been pointed out as one of the main triggers of DH and can act alone or in association with some clinical situations [4–6].

There are several methods available for the treatment of DH [7–9], all with the same intention: to seal the dentinal tubules. Among these methods, we can mention use of fluoride varnishes, potassium oxalate, self-etching adhesive system, fluoride gel, laser, and special dentifrices such as biphasic gel with calcium silicate and sodium phosphate salts and slurries with sodium monofluorophosphate.

GAFFAR (1999) in his research with the fluoride varnish Duraphat observed the formation of calcium fluoride crystals, which prevented the opening of the dentinal tubules, and in conjunction promoting the remineralization of a lasting relief of the dentin hypersensitivity [10]. Potassium oxalate is a desensitizing agent that acts on tubule obliteration and depolarization of nerve endings. It is presented both in the form of dentifrices and in topical applications [11]. STEAD et al.

(1996) noted a decrease in dentin permeability due to obliteration of dentinal tubules, but this result was temporary because the crystals were partially dissolved in the saliva.

The calcium silicate gel and sodium phosphate salts combine and integrate to the tooth, regenerating the enamel, thus recovering its mineral composition. However, it acts in the early stages of erosion. The use of the Potentializer increases the effectiveness of advanced toothpaste by 43%, maximizing the regeneration power of the enamel, even in hard-to-reach places such as between the teeth [12]. In this way, it provides a targeted protection for the teeth against the effects of enamel erosion and acid attacks [13]. However, no studies of these agents have been found in dentin.

Recent research has shown promising results regarding the treatment of DH with the use of laser in the area of clinical dentistry, in which large varieties of procedures are performed with this device, such as cavity preparation, caries removal, restoration removal, surface treatment, dentin sensitivity treatment, caries prevention, and bleaching [14].

The application of the Er,Cr:YSGG laser on the dental surface causes an increase in temperature and changes its chemical structure, leaving the surface less soluble [15]. In dentin, this laser presents the advantage to obliterate dentinal tubules after the fusion and resolidification mechanisms, differently from other light sources [16]. However, there are no studies with this wavelength in the analysis of the wear profile using confocal laser scanning microscopy.

Therefore, it is important to study the adequate parameters for the use of Er,Cr:YSGG laser in order to increase the acid resistance of dentin, since it is a promising technique that can promote effective and long-lasting results, generating greater comfort to the patient, besides being a fast method and able to attend a great part of the population.

In vitro methods were proposed [17] for evaluation of dental erosion, including wear profile and surface roughness using the LEXT OLS4000 laser confocal microscope (Olympus Japan).

Surface roughness is one of the parameters most used to quantify dental surface changes for in vitro studies. This property was analyzed because the presence of superficial irregularities in the tooth entails the retention of bacterial biofilm, increasing the risk of caries and periodontal inflammation [18].

The volume loss is calculated as the volume ratio in μm^3 divided by the analyzed area, which allows to quantitatively seeing the lost volume at the surface of the tooth [19].

Therefore, the present study analyzed surface roughness (μm^2) and volume loss (μm^3), considering these two methods mentioned above to measure the percentage of lost volume of the treated area followed by erosion in relation to the untreated area and consequently the wear superficial.

The null hypothesis is that there would be no statistically significant difference in surface roughness and loss of volume in the different groups, independently of the employed treatment.

Objective

The aim of the present study was to evaluate the influence of Er,Cr:YSGG laser, associated or not to desensitizing agents, in the prevention of acid erosion in bovine root dentin, using the following methods of analysis: surface roughness (parameter Ra in μm^2) and evaluation of the wear profile (percentage of lost volume), performed by laser scanning confocal microscope.

Materials and methods

Experimental design

The factor under study was treatment of the specimens in eight levels: G1: no treatment (negative control); G2: duraphat varnish (positive control); G3: Er,Cr:YSGG laser; G4: duraphat varnish + laser Er,Cr:YSGG; G5: oxagel; G6: oxagel + laser Er,Cr:YSGG; G7: regenerate boost serum; G8: regenarate boost serum + laser Er,Cr:YSGG. All the irradiated groups used the following parameters: 0.5 W, 5 Hz, without water-cooling, and 55% of air. The sample of the experiment was 80 bovine root dentin specimens divided into these eight groups ($n = 10$). The quantitative response variables were surface roughness (parameter Ra in μm^2) and evaluation of the wear profile (percentage of lost volume).

Teeth selection

Forty bovine incisors were selected, without the presence of cracks and wear. The teeth were cleaned using a periodontal curette and then immersed in a 10% formalin solution (pH = 7) for 7 days for sterilization. These teeth were then washed and stored in distilled and deionized water at a temperature of 4 °C, exchanged daily for a period of 14 days.

Specimen preparation

The incisors were sectioned by separating the coronary portion from the root with the use of a diamond disk under refrigeration in the ISOMET® 1000 cutting machine (Precision Saw Buehler, Illinois, USA). The first cut was performed 1 mm below the enamel-cement junction. The second cut was performed in the mesio-distal direction, obtaining two halves (buccal and lingual). Each half was again sectioned to obtain specimens in the initial dimensions of 4.25 mm × 4.25 mm. The specimens had their sides polished on the Arotec APL-4 polishing machine (Series 41042, Arotec S.A. industry and trade), using sandpaper # 600 with water cooling to standardization at 4 mm × 4 mm, resulting in a surface area of 16 mm^2. The polishiment was not performed on the evaluated surface of the specimen. Changes in dimensions were allowed in 10%, for more or less.

Half of the surface of each specimen was covered with adhesive tape. Two layers of red nail cosmetic enamel and sculpt wax were applied, and their impermeabilization was performed. After this procedure, the insulation tape was removed and each specimen was left with half the free surface of the protection made with cosmetic enamel and wax. The specimens were stored in distilled and deionized water at a temperature of 4 °C until the proposed treatment was performed, where they were randomly divided into eight groups ($n = 10$) and each group received its treatment as described in Table 1.

Treatment of specimens

The fluoride varnish (5% sodium fluoride) used was Duraphat® (Colgate-Palmolive Ind. And Co., São Paulo, SP, Brazil) with a disposable applicator (microbrush), and after 4 min, the excess was removed with a sterile gauze. The laser device was the Er,Cr:YSGG (Waterlase Millennium, Biolase Technologies Inc., San Clemente, USA), with fiber containing 600 μm in diameter (tip model: ZipTip MZ6 3 mm), irradiated for 10 s in scan mode, at 1 mm irradiance distance (Table 2). The use of the laser without water cooling was based on previous study, where it was verified that the water could ablate the tissue, thus showing less effectiveness in the preventive treatment [15]. For the potassium oxalate, potassium oxalate monohydrate 3% was used, with disposable applicator (microbrush); it was maintained with slight excess in contact for 2 min. The dual regenerate gel composed of two parts: part A-calcium silicate, phosphate salts, and sodium monofluorophosphate and part B-sodium fluoride (activator gel) was applied with microbrush for 3 min.

Erosive challenge

After the treatments, the specimens were submitted to the erosive challenge: each group was placed separately in a becker and was immersed in Coca-Cola® (Cia. De Bebidas Ipiranga, Ribeirão Preto, SP, Brazil) with a pH of 2.42 at 4 °C for 5 min on a magnetic stirrer (ABC-LAB, model 221-1).

After this time, the erosive solution was discarded and the specimens were washed with distilled and deionized water for 10 s and stored again in that water. This procedure was performed twice a day, with 6-h intervals between the challenges, for a total period of 14 days. The specimens were stored at 4 °C immersed in distilled and deionized water until analysis. The enamel and wax (control area) of each specimen were removed using the lecron instrumentation. There was no contact of the instrument with the central surface of the specimen, only on the sides.

Analysis of surface roughness and wear profile

For these analyses, the specimens were placed parallel to the table of the confocal laser-scanning microscope LEXT (Olympus, Japan) with the aid of the parallelogram.

After selecting the central region of the 1 mm × 1 mm specimen, image acquisition was performed with a 20× magnification lens. After obtaining the images, these were analyzed for wear profile and surface roughness (parameter Ra). For the analysis of surface roughness, the central region was measured, encompassing the control region (reference) and the eroded area. The data, in μm^2, were acquired by means of a specific software (OLS4000®) (Fig. 1).

The wear profile was determined by the difference between the volume of the reference area and the eroded area between the midline of the graph. Data were obtained in μm^3, and for the statistical calculations, a comparison was made between the control area and the eroded area, transforming in percentage of lost volume.

Statistical analysis

The surface roughness data were submitted to the statistical analysis. Firstly, it was observed that the data presented normal distribution (Kolmogorov-Smirnov) and homogeneous (Levene). After this, we applied the analysis of variance (ANOVA) for comparison of the means and Tukey's post hoc for multiple comparisons. The wear profile data were submitted to Kruskal-Wallis test and the Dunn post hoc. Afterwards, the Spearman correlation test was performed. All statistical tests assumed a significance level of 5% ($\alpha = 0.05$).

Results

Surface roughness

The surface roughness values are described in Table 3.

For the reference area, all groups presented surface roughness values with statistical similarity ($p > 0.05$).

In the area submitted to the erosive challenge (treated + eroded), the surface roughness values of the negative control group were higher and with a statistically significant difference in the other groups ($p < 0.05$). There was no statistically significant difference between the groups: G2, G3, G4, G5, G6, G8 ($p > 0.05$).

The G7 group presented a statistically significant difference in relation to the other groups that had treatments ($p < 0.05$), evidencing a lower surface roughness of the analyzed area.

Table 1 Treatment of specimens/experimental groups ($n = 10$)

Groups	Treatments
G1	No treatment (negative control)
G2	Application of 5% fluoride varnish (positive control)
G3	Application of Er,Cr:YSGG (0.5 W; 5 Hz, 55% air)
G4	Fluoride varnish + laser Er,Cr:YSGG application (0.5 W; 5 Hz; air 55%)
G5	Application of 3% potassium oxalate
G6	Application of 3% potassium oxalate + laser Er,Cr:YSGG (0.5 W; 5 Hz; air 55%)
G7	Applying the calcium silicate/phosphate toothpaste
G8	Application the calcium silicate/phosphate toothpaste + laser Er,Cr:YSGG (0.5 W; 5 Hz; 55% air)

Wear profile

Regarding the percentages of volume lost, it was observed that G4 (duraphat varnish + Er,Cr:YSGG laser) had the lowest volume loss ($p < 0.05$), only 9.7%, when compared to the other groups. On the other hand, the greatest loss of volume was observed in G1 (negative control), which presented 41.8% (Fig. 2) and a statistically significant difference in relation to the other groups ($p < 0.05$) (Table 4).

Spearman correlation test

There was a weak correlation between the response variables (surface roughness × wear profile ($\rho = 0.33$)).

Discussion

This study was conducted to evaluate the possible increase of acid resistance in bovine root dentin after erosive challenge with Coca-Cola®. The results showed that the proposed treatments had a statistically significant difference ($p < 0.05$) for the two analyzed properties. Thus, the null hypothesis that different treatments would have no effect on the dentin acid resistance after erosive challenge was rejected.

The initial standardization of the specimens was confirmed by the surface roughness results of the reference area, as there was no significant difference between the groups ($p > 0.05$). In addition, this similar result indicates that the waterproofing of the specimens was effective, since the surface roughness values of the reference area were smaller than the treated area followed by erosion.

The choice of bovine teeth was made due to the higher standardization of these teeth and was based on previous studies in which tests were made that proved that the human and bovine dentine substrates had similar morphology [20, 21]. Furthermore, bovine teeth were used in several in vitro tests, such as erosion/abrasion test, shear bond strength test, among others [22].

The immersion of the specimens in Coca-Cola® was performed because of its erosive potential already studied and discussed in several scientific works [23, 24]. In addition to being a widely consumed beverage in the world, its pH of 2.42 (at temperature of consumption) is far below the critical pH of the dentin, unbalancing the process of demineralization-remineralization [25].

Table 2 Er,Cr:YSGG laser application parameters

Power	0.5 W
Irradiation distance	1 mm
Application time	10 s
Mode of application	Surface scanning
Wavelength	2.78 μm
Fiber diameter	600 μm
Energy density	6.25 J/cm^2
Water	Without water-cooling
Air	55%

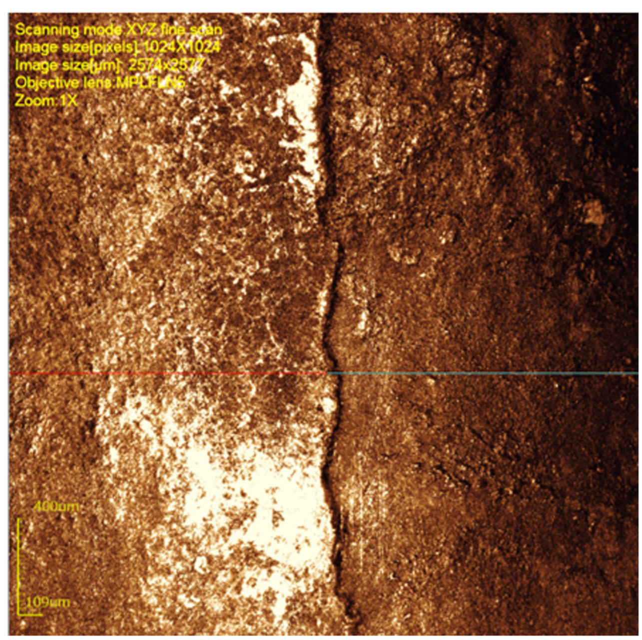

Fig. 1 Morphological differences between the control region (left side) and the treated followed by erosion region (right side)

Table 3 Mean values and standard deviation of the surface roughness of the groups, considering the control region and the treated + eroded area

Groups	Reference area	Treated area followed by erosion
G1: no treatment (negative control)	0.844 (0.081)[a]	3.586 (0.205)[c]
G2: application of 5% fluoride varnish (positive control)	0.821 (0.087)[a]	2.205 (0.084)[b]
G3: application of Er,Cr:YSGG (0.1 W; 5 Hz, 55% air)	0.875 (0.069)[a]	2.155 (0.090)[b]
G4: fluoride varnish + laser Er,Cr:YSGG application (0.1 W; 5 Hz; air 55%)	0.893 (0.071)[a]	1.961 (0.104)[b]
G5: application of 3% potassium oxalate	0.882 (0.091)[a]	2.104 (0.067)[b]
G6: application of 3% potassium oxalate + laser Er,Cr:YSGG (0.1 W; 5 Hz; air 55%)	0.849 (0.080)[a]	2.118 (0.099)[b]
G7: applying the calcium silicate/phosphate toothpaste	0.852 (0.057)[a]	1.071 (0.180)[a]
G8: application of the calcium silicate/phosphate toothpaste + laser Er,Cr:YSGG (0.1 W; 5 Hz; 55% air).	0.889 (0.092)[a]	1.956 (0.055)[b]

Equal letters represent statistical similarity between groups ($p > 0.05$)

In a study of dental erosion induced by different beverages, Coca Cola® was considered the most erosive agent among hot and cold drinks studied (37%). After 10 min of exposure to Coca Cola®, the exposed dental surface showed effects such as clearly visible enamel prisms, fissures, rough surfaces, showing signs of demineralization. In the SEM (scanning electron microscopy), it showed visible debris and exposed prisms and the hardness tends to decrease [26]. This corroborates with the results verified in the present study, in which dentin surface demineralization was observed after erosive challenge with Coca Cola®.

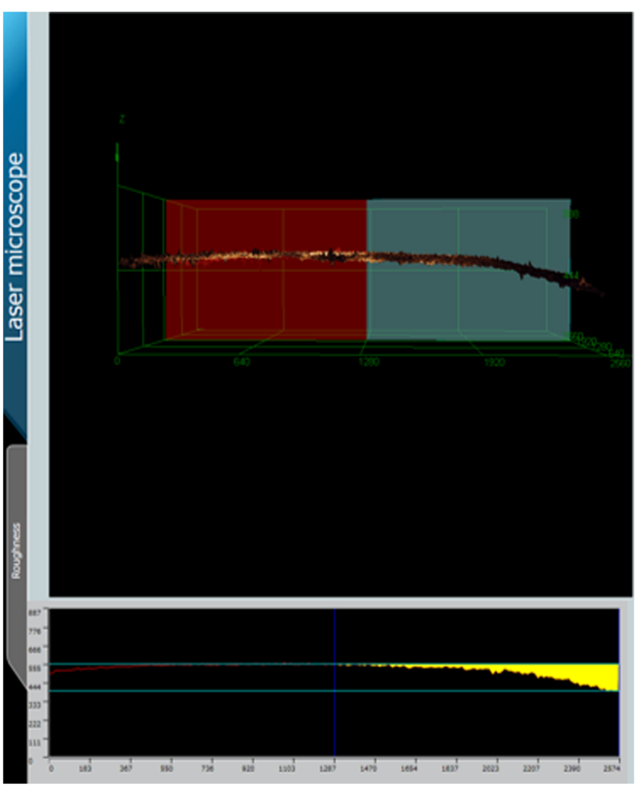

Fig. 2 3D profilometry, showing the control region (left side) and the treated followed by erosion region (right side). The volume lost is represented by the yellow color

G1 showed the highest value of surface roughness and volume loss in the treated area followed by erosion, demonstrating that the absence of any kind of preventive treatment enhances the action of Coca Cola®, that is, demineralization on the surface of the root dentin. In fact, analysis of atomic force microscopy and scanning electron microscopy have also reported changes in the dentin surface provoked by erosion in this soft-drink [27].

The obtained data for surface roughness test showed that, in the control region, there was no statistically significant difference between the groups ($p > 0.05$). The control region always had a lower roughness when compared to the treated and eroded area, for all groups.

In relation to the surface roughness, the groups G2, G3, G4, G5, G6, and G8 showed that there was no statistically significant difference after the erosive challenge with Coca Cola® among them, but it was more effective than G1 (negative control) that presented the highest surface roughness value (3586 μm). This explains the use of preventive treatments to avoid increasing surface roughness.

The G7 (application of the calcium silicate/phosphate toothpaste) showed a lower surface roughness (1071 μm) after the erosive challenge compared to the other groups. This can be explained because this treatment based on calcium silicate and sodium phosphate can provide protection to the enamel by several mechanisms: releasing calcium ions to the surrounding oral fluids under acidic conditions, thus increasing the local concentration of calcium, the degree saturation with respect to enamel hydroxyapatite, and inhibiting dissolution [28]. Considering that enamel and dentin are constituted mostly by hydroxyapatite, it is expected that this remineralizing agent also protects the root dentin with similar mechanisms described above for the enamel.

As regards to wear profile, the Er,Cr:YSGG laser showed to be effective, since it presented low values of lost volume when applied alone (G3) or in association with duraphat (G4), oxagel (G6), regenerate (G8), especially in the association of G4, which presented the lowest value of lost volume among

Table 4 Mean values and standard deviations of volume loss (%) in the groups studied

Groups	Lost volume (%)	Standard deviation
G1: no treatment (negative control)	4.8[d]	2.5
G2: application of 5% fluoride varnish (positive control)	27.8[c]	1.9
G3: application of Er,Cr:YSGG (0.1 W; 5 Hz, 55% air)	20.5[b]	1.4
G4: fluoride varnish + laser Er,Cr:YSGG application (0.1 W; 5 Hz; air 55%)	9.7[a]	0.9
G5: application of 3% potassium oxalate	29.2[c]	1.1
G6: application of 3% potassium oxalate + laser Er,Cr:YSGG (0.1 W; 5 Hz; air 55%)	19.4[b]	1
G7: applying the calcium silicate/phosphate toothpaste	29.1[c]	1.2
G8: application the calcium silicate/phosphate toothpaste + laser Er,Cr:YSGG (0.1 W; 5 Hz; 55% air)	19.7[b]	1.4

Equal letters represent statistical similarity between groups ($p > 0.05$)

all the studied groups (9.7%). These findings can be justified because the association of laser and fluoride increases the incorporation of fluoride ions in the dentin structure, increasing the acid resistance and consequently decreasing the percentage of lost volume [29]. G2, G5, and G7 presented lower lost volume only when compared to G1 that had the highest percentage among all groups (41.8%).

The Er,Cr:YSGG laser can be used in dentistry as a high and low power laser. For preventive purposes, it should be used with sub-ablative parameters to only modify chemically and morphologically the structures [30, 31]. Some manuscripts have been published using this enamel laser and have obtained positive responses for caries prevention [32, 33]. For this reason and based on previous studies that showed that the use of the laser emission of 2.78-μm wavelength is also effective in dentin, we opted for the choice of this laser in the present study. This wavelength coincides with the water and hydroxyapatite absorption peaks [33–35].

Consistent studies such as that of Zach and Cohen (1965) have already shown that healthy pulp tissue is not thermally affected if the temperature rise was less than 5.5 °C [36]. Thus, the use of laser to increase the acid resistance of dentin is safe and a new option for preventive treatment [6, 31].

Studies have proven that laser applications combined with fluoride may potentiate its effect. Gao et al. (2006) carried out a study to analyze the association of laser and fluoride application and observed that, when the laser is applied, there is a better absorption of fluoride in the root dentin, resulting in a better inhibition of demineralization [37].

Studies combining acidulated phosphate fluoride and Er,Cr:YSGG laser irradiation have shown that this association reduced enamel demineralization more than any fluoride treatment or laser treatment isolated [15, 38]. The results of this study showed that the association of laser irradiation with fluoride varnish duraphat was more effective, because the ion incorporation on the specimen surface was potentiated with treatment association, leaving the surface more resistant to acids, as demonstrated in G4.

In the literature, many techniques have been used to investigate the effects of erosive challenges on hard dental tissues. Micro-indentation, surface profilometry, microradiography, chemical analysis, and SEM were considered the most advanced in this evaluation [17]. The use of confocal laser scanning microscopy allows the qualitative understanding of dentin surface demineralization processes by observing the specific and structural morphology characterizing dentin [39], analyzing the loss of substance and surface characteristics of dentin. The use of the Olympus LEXT OLS4000 laser confocal microscope leads to higher resolutions of these captured images and allows the observation of superficial changes such as enamel prisms, dentinal tubules, and erosion areas, being compatible with demineralization, once it does not cause damage to the specimen's surface because there are no contact forces between them [19].

It would be interesting to submit these specimens to longitudinal microhardness analysis in future studies.

The present study demonstrated that these treatments might increase the dentin acid resistance after erosive challenge. However, further studies are important to ensure the use of these treatments over time, as well as periodic monitoring of the patient's oral health. In addition, dentists must recommend and instruct his patients to reduce the frequency of Coca Cola® ingestion.

Conclusion

Considering the obtained results and the limitations of an in vitro study, it was concluded that all groups had lower values of surface roughness and loss of volume when compared to the negative control group. For the surface roughness, the biphasic Regenerate gel presented the best result. For wear profile, the 5% fluoride varnish + Er,Cr:YSGG laser showed the best results compared to the other groups, suggesting a promising preventive treatment of dentin hypersensitivity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This project followed all the ethical principles for medical research, according to Declaration of Helsinki.

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