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ORIGINAL ARTICLE

Effect of Toothbrushing with Different Dentifrices on the Surface of an Infiltrant Resin Used to Inactivate White Spot Lesions

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ABSTRACT
Objective: To verify whether an infiltrant resin used to inactivate white spot lesions (WSL) on enamel can resist the abrasive challenges promoted by tooth brushing with different dentifrices. **Material and Methods:** WSL was induced on bovine enamel samples (pH cycling) and then treated with an infiltrant resin (ICON). Afterward, half of the sample was protected. The other half underwent an abrasive challenge using an electric toothbrush with soft bristles., using dentifrices with different abrasiveness (n=10): Group 1: Colgate Maximum Cavity Protection; Group 2: Sensodyne Repair & Protect; Group 3: Colgate Sensitive Pro-Relief; Group 4: Colgate 2 in 1 Watermelon Flavor; e Group 5: Colgate Luminous White Advanced. The wear profile after brushing was evaluated by confocal laser scanning microscopy (µm), and the enamel/resin interface was observed in scanning electron microscopy (SEM). Profilometry data were analyzed using one-way ANOVA followed by Tukey's test (α=5%). **Results:** Brushing produced more significant wear on group 5 (3.96±0.26) when compared to groups 1 (3.45±0.21), 2(3.22±0,4) and 4(3.11±0.34). Data from Group 3 (3.82±0.6) was similar to G5 but higher than G1, G2, and G4. SEM analysis showed that the adhesive interface remained intact in all groups after the abrasive challenge. **Conclusion:** Dentifrices with a higher relative dentin abrasiveness produced more significant wear of the infiltrant, but the composite/enamel interface was not altered after the abrasive challenges.

Keywords: Dental Caries; Composite Resins; Dentifrices; Tooth Abrasion.

Introduction

Dental caries is a significant health problem that affects a large part of the population, promoting localized destruction of dental hard tissues [1]. The initial caries lesion is known as a white spot lesion (WSL), a white opacities seen on teeth after the subsurface layer of enamel becomes demineralized. They can be active when stains present a rough and white-opaque appearance with biofilm retention or inactive when they present a shiny appearance and smooth surface [2]. Early diagnosis of WSL allows the indication of a non-invasive treatment to inhibit the caries progression [3]. Although the most used technique to remineralize WSL is the topical application of fluoride gel or varnish, this method alone cannot paralyze the lesions. In an attempt to discontinue the progression of the WSL, some studies were carried out using an infiltrant resin, which would penetrate the demineralized enamel and interrupt the progression of caries [4,5].

This infiltrant is a low-viscosity composite that penetrates the enamel, creating a diffusion barrier inside the WSL, allowing replacement of the mineral lost by the composite [5]. This minimally invasive technique allows initial carious lesions to be treated in a single session without anesthesia, cavity preparation, or pain complaints. Furthermore, the infiltrant resin can mask white spot lesions, significantly improving the teeth' aesthetics [4]. To achieve clinical longevity, the infiltrant must resist chemical and abrasive challenges in the oral environment, such as abrasion resulting from toothbrushing, wear, corrosion, and erosion due to diet or intrinsic acids [6].

Several toothpastes on the market are indicated for different clinical situations, such as pediatric dentistry, tooth whitening, and the relief of dentin hypersensitivity. The effect of these dentifrices often depends on the abrasion of dental tissue, either to remove extrinsic staining from the enamel surface or to create a smear layer on the dentin surface to obliterate dentinal tubules [7,8]. However, these dentifrices may change the enamel/dentin surface or a dental restoration.

An increase in the surface roughness due to wear of the infiltrant may increase the risk of caries on that tooth, as it would lead to greater biofilm accumulation. Furthermore, the wear of the infiltrant can affect the enamel/resin interface, which would allow acids from cariogenic bacteria to penetrate the enamel. A proportional relationship exists between roughness on the surface of restorations and bacterial adherence, mainly *Streptococcus mutans* [9]. Furthermore, studies have shown that bacterial adherence is greater on resin surfaces than enamel. Thus, an irregular resin surface favors the formation of bacterial colonies, resulting in composite degradation [10]. This fact can reduce the hardness of the infiltrant and facilitate wear during an abrasive challenge. If there is volume loss of the infiltrant during dental brushing, WSL would be susceptible to the action of acids produced by cariogenic bacteria or intrinsic (gastroesophageal reflux disease) and extrinsic (diet) acids.

Therefore, this study aimed to analyze the wear profile and the adhesive interface of the infiltrant resin after toothbrushing with different dentifrices. The null hypothesis was that toothbrushing does not increase the wear of the infiltrant surface.

Material and Methods

Experimental Design

This research evaluated the wear profile of an infiltrant resin subjected to abrasive challenges using conventional, whitening, pediatric, and dentifrices indicated for dentin hypersensitivity. The experimental units consisted of 50 enamel specimens obtained from the buccal surfaces of bovine incisors (n=10). The response variable was surface wear, which was evaluated using a 3D confocal laser scanning microscope (OLS 4000 LEXT; Olympus, Japan). Furthermore, samples were observed using scanning electron microscopy to assess the interface between enamel and infiltrant.

Sample Preparation

Fifty non-carious bovine anterior teeth were obtained and visually inspected to exclude those with cracks or defects. After cleansing, samples were stored in buffered saline at 4°C until use. The crowns were separated from the roots at the cementoenamel junction. After, crowns were sectioned with a diamond disc mounted on a cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water cooling to generate 50 samples (5x5x3 mm). Samples were polished with 600, 1200, and 2400 grit sandpaper to standardize the enamel surface for 1 minute. In each sample, an area of 16.0 mm^2 was delimited with red nail varnish, where the treatments were performed.

White Spot Lesion Induction

Each sample was fixed with wax on the bottom of a plastic tube, leaving the exposed enamel surface at the top. Enamel demineralization was performed using a dynamic model of demineralization and remineralization, simulating the in vivo high-risk conditions for caries [11]. The demineralization solution (pH 4.3) consisted of 2.0 mmol/l Ca and 2.0 mmol/l phosphate in a buffered acetate solution of 0.075 mol/l. The remineralization solution (pH 7.0) consisted of 1.5 mmol/l Ca, 0.9 mmol/l phosphate, and 150 mmol/l potassium chloride. Each sample was cycled in 5.0 ml of each solution for eight hours in the demineralizing solution and 16 hours in the remineralizing solution. This procedure was carried out for 14 days at 37°C. At the end of each five consecutive days of cycling, the samples were immersed in remineralizing solution for two days.

Infiltrant Resin Treatment

After cleaning the samples with a Robinson brush at low speed and prophylactic paste, the infiltrating resin (ICON, DMG, Hamburg, Germany) was applied according to the manufacturer's instruction, as follows: the lesion was etched with 15% hydrochloric acid(Icon etch, DMG), rinsed after 2 min and dried with air for the 30s; on the second step, ethanol (Icon Dry, DMG) was applied for 30s and dried for 10s; finally the infiltrant resin (Icon Infiltrant, DMG) was applied and, after 3 minutes, the excess resin was removed with air-jet and light-cured for 40s. The resin infiltration step was repeated with a penetration time of 60 seconds to allow the resin to infiltrate the remaining porosities and be light-cured for 40 seconds. Finally, the polishing was done with polishing cups.

Abrasive Challenge

Half of the sample was protected with electrical tape, and the other half received the abrasive challenge. The wear was performed with an electric toothbrush (Oral-B Pro-Health Power, Procter and Gamble, São Paulo-SP, Brazil) attached to a standardized fixed support. The brush head has three sets of bristles of different shapes and positioned at various angles and heights. During brushing, the bristles contacted the sample's surface for 30 minutes, with a force of 1.96N, at room temperature. Brushing was performed on a digital precision scale to standardize the pressure on the sample. Each sample was fixed on the scale tray, and then this scale was set to zero (weight). Then, the bristles of the brush were positioned perpendicular to the sample, and the brush pressed down the sample until the weight of 196 g was reached on the scale display. Considering that an individual brushes each tooth three times a day, for 5 seconds on each face, this protocol simulated 120 days of toothbrushing [12]. A solution (slurry) was obtained by mixing each dentifrice (Table 1) and distilled water in a 1:2 weight ratio (200ml of distilled water and 100g of dentifrice - Specification ISO #14569-1). This solution was prepared every day 20 minutes before use. During the abrasive challenge, 1.0 ml of slurry was injected laterally into the sample, between the infiltrant and the bristles, every 30 seconds. Lastly, the excess dentifrice was removed from each sample under running water and cleaned in an ultrasound bath filled with distilled water for 3 minutes.

Table 1. Dentifrices used in each experimental group.

Group	Dentifrice	Composition	Manufacturer	RDA
1	Colgate – Maximum Anticaries Protection® (L3290CO1014)	1450 ppm of fluoride, calcium carbonate, sodium lauryl sulfate, sodium saccharin, tetrasodium pyrophosphate, sodium silicate, sorbitol, carboxymethylcellulose, methylparaben, propylparaben, flavor, and water. Contains: sodium monofluorophosphate - MFP.	Colgate-Palmolive Indústria e Comércio Ltda, São Bernardo do Campo, SP, Brazil.	70
2	Sensodyne Repair & Protect (164EO316)	Sodium potassium phosphosilicate, glycerin, silica, sodium lauryl sulfate, sodium monofluorophosphate, titanium dioxide, flavor, potassium acesulfame, carbomer	GlaxoSmithKline, Rio de Janeiro, RJ, Brazil.	104
3	Colgate Sensitive Pro-Relief (4164BR123C)	Arginine, Sodium Monofluophosphate, Sorbitol, Sodium Lauryl Sulfate, Cellulose Gum, Sodium Silicate, Titanium Dioxide, Xanthan Gum, Sucralose	Colgate-Palmolive Indústria e Comércio Ltda, São Bernardo do Campo, SP, Brazil.	125
4	COLGATE® 2 in 1 Watermelon Flavor - WATERMELON TOOTHPASTE	500 ppm fluoride, calcium carbonate, sodium fluoride 0.24%, sorbitol, water, hydrated silica, PEG-12, cellulose gum, sodium laurel sulfate, tetrasodium pyrophosphate, Cocamidopropyl betaine, sodium saccharin, xanthan gum, flavoring	Colgate-Palmolive Indústria e Comércio Ltda, São Bernardo do Campo, SP, Brazil.	50
5	Colgate Luminous White Advanced	Sodium fluoride (0.243%), sorbitol, glycerin, pentasodium triphosphate, PEG-12, tetra potassium pyrophosphate, sodium lauryl sulfate, sodium saccharin, and sodium hydroxide	Colgate-Palmolive Indústria e Comércio Ltda, São Bernardo do Campo, SP, Brazil.	175

RDA: Relative Dentin Abrasiveness, according to each manufacturer.

Wear Profile Analysis

The samples were positioned parallel to the reading table of the confocal laser scanning microscope (CSLM, LEXT; Olympus, Japan). Images were captured using an x5 objective lens, obtaining an x107 total optical zoom. For the wear profile analysis (Rv-μm), the control and experimental areas were calculated using the length of the wear line (in μm) between them.

Scanning Electron Microscopy (SEM) Observation

Three samples per group were randomly selected. Each sample was divided in half, exposing the interface between the enamel and the infiltrant resin. Afterward, each sample was immersed in water and placed in an ultrasonic machine for two 5-minute cycles. Dehydration was performed using a series of ethanol solutions of 25%, 50%, and 75% potency for 20 min each, 95% potency for 30 min, and 100% potency for 60 min. The samples were fixed on stubs using gold-plated metalized adhesive tape (SDC 050) with the interface turned upward for analysis. The SEM was operated at 20 kV (Phillips, XL30 FEGm Eindhoven, Holland). The samples were observed at 500x and 1000x magnification, and the interface was scanned to observe changes in that area. The most representative areas of each group were photographed at different magnifications.

Statistical Analysis

The data were analyzed for normality and homogeneity, and the data distribution was normal. Profilometry data were obtained in μm, and they were analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's test. The significance level adopted was 5%. Data were analyzed by BIOESTAT 5.3 (Instituto de Desenvolvimento Sustentável Mamirauá, Belém, PA, Brazil).

Results

Table 2 presents the wear profile found for all experimental groups after each abrasive challenge, showing statistical differences between the tested dentifrices. The abrasive challenge induced more significant wear on the samples of group 5 (3.96±0.26) when compared to groups 1 (3.45±0.21; p=0.007), 2 (3.22±0.12; p=0.001) and 4 (3.11±0.34; p=0.000005). Group 3 (3.82±0.6) showed similar wear to G5 (p=0.5858) but higher than that observed in G1 (p=0.029), G2 (p=0.0002), and G4 (p=0.0001). There were no statistical differences among groups 1,2 and 4.

Table 2. Wear profile (µm) found in different groups after brushing with abrasive dentifrices.

Group	Wear Profile
1	3.45±0.21[b]
2	3.22±0.12[b]
3	3.82±0.41[a]
4	3.11±0.34[b]
5	3.96±0.26[a]

The same letters indicate no statistical differences (α= 5%, F=10.55; p<0.01).

Figure 1A shows an image obtained on the confocal laser scanning microscope used for the wear profile analysis.

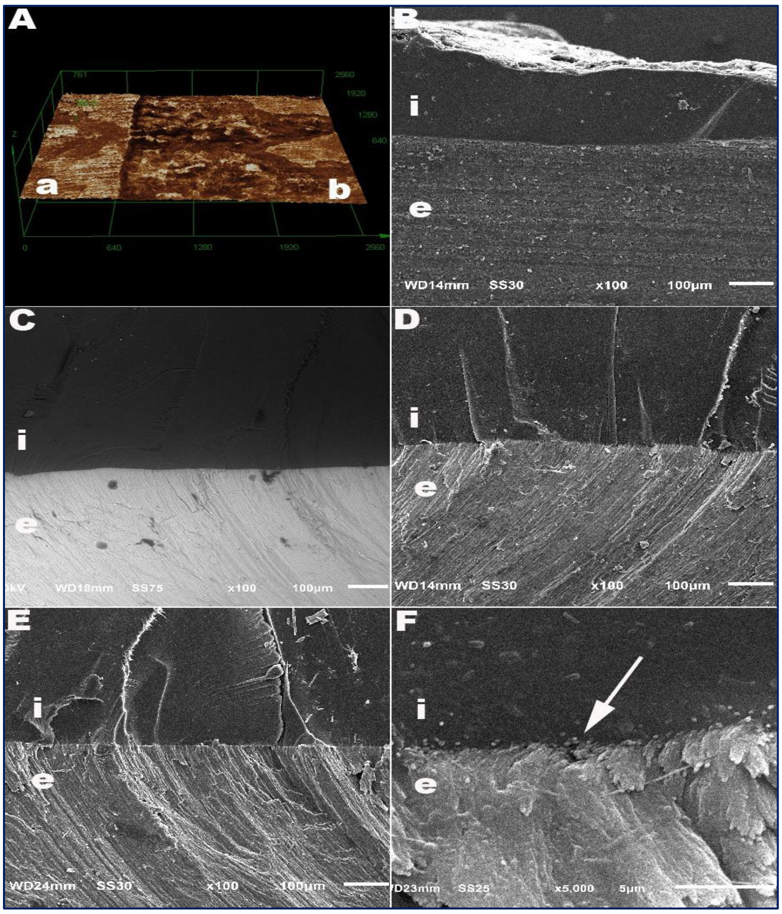

Figure 1. A- A representative image was used to analyze the wear profile obtained on the CSLM, suggesting the loss of volume in a brushed sample (a-control; b-experimental area). SEM images are represented in B (G1), C (G2), D (G3), E (G4), and F (G5) and illustrate the adhesive interface between Icon infiltrate resin (i) and dental enamel (e). Note that the adhesive interface appears intact in images B, C, D, and E. In D, a 5000x magnification was used to show a possible failure in the adhesive interface (arrow).

In this image, the wear of the infiltrant (b) is observed when compared to the control area (a). All groups presented a similar image, indicating that, in all cases, there was volume loss after abrasive challenges. Representative scanning electron microscopy images are illustrated in Figures 1B (Group 1), 1C (group 2), 1D (Group 3), 1D (group 4), and 1F (group 5). Those images show the interface between enamel and infiltrant resin, indicating that the abrasive challenges tested here did not cause significant damage to the enamel/infiltrant interface. In 1B, a change in the infiltrant surface is observed. Only in group 5 (Figure 1F) was a failure at the adhesive interface observed.

Discussion

This study aimed to evaluate whether toothbrushing promotes wear of the infiltrant resin and if the abrasive challenges alter the enamel/composite interface. The results showed that, despite the wear of the infiltrant after brushing, the adhesive interface remained preserved. The null hypothesis that toothbrushing does not increase the wear of the infiltrant surface was rejected.

Electric toothbrushing is suitable for simulating, *in vitro*, daily oral hygiene practices. For this, some procedures had to be standardized, such as brushing duration, frequency, amount of dentifrice used during brushing, and the amount of force applied on the samples during abrasive challenges [13]. The present study used an electric toothbrush with a soft bristle and a standardized brushing force of 1.96N. The brush head has three sets of bristles of different shapes and positioned at various angles and heights. Each sample was brushed for 30 minutes, and every 30 seconds, a new slurry solution was applied between it and the bristles of the brush. Considering that an individual brushes each face of the tooth with an electric toothbrush for around five seconds (15 seconds/day), the total simulated time in this study would be 120 days [12,14]. Although some dentifrices used for dentin hypersensitivity can be applied with fingers, the dentifrices used here are applied to the teeth using a toothbrush. The effect of the dentifrices used here on the teeth depends on their use with a toothbrush. Usually, individuals brush their teeth with specific toothpaste for each occasion, as dentists recommend (i.e., caries prevention, tooth bleaching, or treatment of dentin hypersensitivity). Therefore, we believe it would not make sense to check the effects of toothbrushing without dentifrices on the infiltrant surface.

Whitening or desensitizing dentifrices, in most cases, have greater abrasive power than conventional dentifrices or those used in pediatric patients. This happens because those toothpastes aim to remove extrinsic pigmentation from the teeth (whitening) or to obliterate the dentinal tubules (dentin hypersensitivity). Different dentifrices were used here to cover a greater number of possibilities, as the infiltrant resin can be used in patients of all ages who have teeth discolored not only by caries but also by fluorosis and enamel hypoplasia, among others.

The abrasiveness of dentifrices is measured by relative dentin abrasiveness levels (RDA) ranging from 0 to 250. Low-abrasive dentifrices have an RDA between 0-70, medium-abrasive dentifrices have an RDA of 71-100, high-abrasive dentifrices have an RDA of 101-150, and those considered potentially harmful to dentin have an RDA of 151-250. This information is provided by each manufacturer or by specific studies [15-17]. According to this classification, the dentifrices used in this study were of low and high abrasion. The gel-based dentifrices present the lowest RDA, containing silica as an abrasive agent. However, its abrasivity increases when associated with other abrasives, such as calcium carbonate, sodium pyrophosphate, titanium oxide, or sodium phosphate [15]. Each toothpaste's RDA number helped us differentiate the products tested here. Literature shows that RDA numbers may present slight divergences between manufacturers and studies published in the literature, as these analyses are conducted in different laboratories [16]. The RDA number was not calculated in the present research. However, as stated above, the range stipulated for defining the abrasive potential of each dentifrice (RDA) is broad, and, therefore, these slight divergences between laboratories would not change the abrasiveness classification of the dentifrices used here. So, these slight divergences were not significant in the present research. Based on the analysis of our results, kinds of toothpaste with a higher RDA promoted more substantial wear of the infiltrant resin, which agrees with previous studies [16,17].

Some dentifrices used here are indicated for the treatment of dentin hypersensitivity. Sensodyne® Repair & Protect has calcium and sodium phosphosilicate (NovaMin® or CSP), a bioactive ceramic glass composed of minerals in our body that react when they come in contact with saliva [18]. NovaMin® is used for the treatment of dentin hypersensitivity and dental erosion due to its mechanism of action through the precipitation of calcium and phosphate ions on enamel or dentin surface, assisting saliva in neutralizing acids, occluding dentinal tubules and assisting in the process of remineralization of enamel subjected to erosive challenge [18]. A previous study showed that this dentifrice promotes loss of tooth structure due to the action of the brush bristles and not due to the abrasiveness of the dentifrice, which agrees with the results obtained here since group 2 was similar to the control group (G1) [19].

Colgate Sensitive Pro-Relief contains the Pro-Argin™ formula, which represents the association of the amino acid arginine with calcium carbonate. This bioactive agent was developed in the form of a polishing paste and dentifrice for the treatment of dentin hypersensitivity through the formation of plugs within the dentinal tubules, which are stable and resistant to erosive challenges, in addition to enabling the deposition of high levels of calcium, phosphorus, oxygen, and carbonate on the dentin surface [20]. Combining that agent with other abrasives, such as titanium oxide and sodium phosphate, is considered a high abrasive dentifrice [21]. According to the literature, this dentifrice causes more wear on the tooth structure than Sensodyne® Repair & Protect [19].

Generally, composites show a particular wear pattern because their composition directly influences their wear resistance. Optimally, the loading force is ultimately transferred from the matrix to the filler particles. The wear characteristics of a dental composite are affected by the fillers' size, shape, and hardness, the bonding between fillers and polymer matrix, and the polymerization dynamics. The physical and mechanical properties of the composite, such as flexural strength, fracture toughness, hardness, modulus of elasticity, and cure depth, may be influenced by its composition [22]. According to the manufacturer, Icon infiltrant is a methacrylate-based resin matrix containing 70 to 95% TEGDMA (triethylene glycol dimethacrylate), a low molecular weight monomer. The use of that diluent monomer allows the infiltrant to be fluid enough to penetrate the porosities of the enamel white spot lesion [5]. As the volume of monomer is high, there is a low amount of inorganic filler (in volume) in the infiltrant, which makes it susceptible to abrasive wear [23].

Colgate Maximum Cavity Protection (RDA=70) and Colgate 2 in 1 (RDA=50) are considered to be low abrasive, the latter being indicated for dental hygiene in children. Therefore, it is likely that the results obtained in groups 1 and 4 were also affected by the action of the toothbrush bristles on the infiltrant resin. The same effect had already been observed in enamel and dentin [24].

The present research used a dentifrice indicated for tooth whitening (Colgate Luminous White Advanced). Since one of the main characteristics of whitening dentifrices is the presence of optimized abrasive particles, hydrated silica, associated with other types of abrasives, may have been responsible for the more significant wear observed in group 5. Although the abrasive is most often silica or its derivative, each dentifrice has a different amount of abrasive. Particulate hydrated silica, present in dentifrices at various concentrations, is the most often used abrasive today. Silica for dentifrices is a tasteless amorphous powder that is insoluble in water or acid, chemically inert and has good solubility with fluoride and other toothpaste unprocessed materials. Production conditions can influence the structure and particle size of silica. Three products are manufactured based on the distinct sorts of dentifrices: friction type, mixed type, and thickening type. Hydrated silica, with a molecular weight of 60.08, an average particle size of 4-8 m, and a molecular weight of 10-15% crystal water, is chemically inert and is compatible with fluoride and other components in dentifrices [25]. Additionally, combining pyrophosphate and silica (Colgate Luminous White Advanced) enhances the abrasive effect of whitening toothpaste [26]. Therefore, as the force used during brushing was standardized here, the wear was more significant, probably because Colgate Luminous White Advanced has greater abrasiveness than the dentifrices used in G1, G2, and G4.

As observed in SEM (Figure 1), although all dentifrices removed part of the infiltrant from the tooth surface, the abrasive challenges did not affect the integrity of the adhesive interface. If the adhesive interface is not affected, the infiltrant will likely remain inside the white spot lesion, which makes it difficult for bacterial acid to penetrate enamel. This suggests that the treatment's objective was achieved or partial protection of the enamel was achieved. However, new studies must be carried out to evaluate the joint action between acid and abrasive challenges and verify whether the infiltrant can resist both conditions. Literature reports that there may be variation in the penetration capacity of the infiltrating resin, as this directly depends on the size and diameter of the pores in the WSL because they could not be the same in the different areas of the lesion, which would lead to partial resin penetration in some areas [27,28]. In general, earlier studies have demonstrated that the hybrid layer generated between demineralized enamel and the infiltrant demonstrates stability when subjected to chemical or physical challenges, consistent with the results found here [27,29,30].

Therefore, the results achieved here suggest that the surface of the infiltrant did not resist abrasive challenges. However, the adhesive interface between the infiltrant and enamel has not been significantly affected. Then, the infiltrant resin can effectively protect demineralized dental enamel, mainly in preventing the progression of the carious lesion into the dental hard tissue. However, further studies are needed to verify whether there is a significant increase in surface roughness due to wear caused by toothbrushing. This increase could promote biofilm accumulation in the region treated with the infiltrating resin, leading to a greater risk of caries.

Conclusion

All abrasive challenges removed part of the infiltrant from the enamel surface. Despite the wear of the infiltrant resin, the integrity of the composite/enamel interface was not damaged.

Authors' Contributions

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All authors declare that they contributed to a critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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