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Effect of desensitizing dentifrices on eroded dentin wettability

Efeito de dentifrícios dessensibilizantes na molhabilidade da dentina erodida

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ABSTRACT

Objective: To analyze the wettability on the surface of eroded dentin in teeth submitted to abrasive wear with desensitizing dentifrices. **Material and Methods:** Bovine dentin specimens were polished and immersed in 10 mL of citric acid (pH=3.2) for 2 h. The eroded specimens were submitted to mechanic brushing according to the 4 dentifrices adopted: Colgate Total (control); Colgate Sensitive Pro-Relief; Sensodyne Repair & Protect; or Sensodyne Rapid Relief. Afterwards, it was conditioned in 37% aqueous phosphoric acid solution. Wettability of 80 specimens (n=10) brushed for 7 or 21 days was evaluated by measuring the contact angle between the dentin surface and a drop of the adhesive Single Bond Universal® (3M) with a goniometer. Changes in the surface morphology of 12 specimens (n = 3) brushed for 21 days were followed by confocal laser scanning microscopy (CLSM). Data were analyzed by two-way ANOVA and Tukey test ($p > 0.05$). **Results:** Groups treated with desensitizing dentifrices did not differ significantly ($p \leq 0.05$). Surface treatment and abrasive wear did not interact significantly ($p \leq 0.05$). Brushing along 7 days gave the smallest contact angle value ($p \geq 0.05$). CLSM images showed morphological changes for all the groups. **Conclusion:** The desensitizing dentifrices did not interfere in eroded dentin wettability after brushing along 7 or 21 days. Brushing with any of the dentifrices along 21 days promoted open dentinal tubules.

KEYWORDS

Dentifrices; Desensitizing; Toothbrushing; Dentin; Wettability.

RESUMO

Objetivo: Analisar a aplicação de um adesivo na superfície de dentina erodida em dentes submetidos ao desgaste abrasivo com agentes dessensibilizantes. **Material e Métodos:** Espécimes de dentina bovina foram polidos e imersos em 10 mL de ácido cítrico (pH=3,2) por 2 h. Os espécimes erodidos foram submetidos à escovação mecânica de acordo com os 4 dentifrícios adotados: Colgate Total (controle); Colgate Sensitive Pro-Alívio; Sensodyne Repair & Protect; ou Sensodyne Rápido Alívio. Em seguida, foram condicionados em ácido fosfórico a 37%. A molhabilidade de 80 espécimes (n=10) escovados por 7 ou 21 dias foi avaliada medindo-se o ângulo de contato entre a superfície dentinária com uma gota do adesivo Single Bond Universal® (3M) por um goniômetro. Alterações na morfologia da superfície de 12 espécimes (n = 3) escovados por 21 dias foram seguidos por Microscopia Confocal de Varredura a Laser (MCVL). Os dados foram analisados por ANOVA dois fatores e teste de Tukey ($p > 0,05$). **Resultados:** Os grupos tratados com dentifrícios dessensibilizantes não diferiram significativamente ($p \leq 0,05$). O tratamento de superfície e o desgaste abrasivo não interagiram significativamente ($p \leq 0,05$). A escovação ao longo de 7 dias apresentou o menor valor de ângulo de contato ($p \geq 0,05$). As imagens do MCVL mostraram alterações morfológicas para todos os grupos. **Conclusão:** Os dentifrícios dessensibilizantes não interferiram na molhabilidade da dentina erodida após escovação ao longo de 7 ou 21 dias. A escovação com qualquer um dos dentifrícios ao longo de 21 dias promoveu a abertura dos túbulos dentinários.

PALAVRAS-CHAVE

Dentífricos; Dessensibilizante; Escovação; Dentina; Molhabilidade.

INTRODUCTION

In order to treat dentin hypersensitivity, a very common condition, desensitizing toothpastes can be used [1-8]. However, as they act by depositing minerals, such as calcium and silicon, inside the dentinal tubules and on the dentin surface, they can change the morphology and composition of the dentin and interfere with the wettability of the substrate, altering the dispersion of dentin adhesives used in dental restorations [9-11].

Restorative treatments are effective when the adhesive material adheres satisfactorily to the dental substrate [12-15]. Close contact between the adhesive material and the dental substrate is necessary for adhesion to occur [13-17]. The surface contact area between a liquid (adhesive) and a solid (dental surface) increases when substrate wettability by the liquid adhesive is good [18-20].

In dental procedures, dentin wettability is important directly related to the adhesion of a restorative material to the tooth, mediated by physical and chemical interactions that hold the material and substrate together, mediated by the use of an adhesive [20-22]. The wettability is quantified by determining the angle between the adhesive and the dentin surface [20,21]. The smaller the contact angle, the closer the contact between the adhesive and the dental surface, which indicates increased wetting degree and greater adhesion efficiency [18,20,22,23]. The wettability of a solid is mainly related to its chemical composition and surface topography [20,24].

How the protective layer formed by desensitizing agents affects the adhesion between dental substrates and restorative materials must be considered [25-27]. Dentin wettability by resinous monomers of the adhesive system is fundamental to establish adhesion [7]. Unfortunately, studies about how desensitizing dentifrices interfere in adhesive spread are lacking. Therefore, this study analyzes the wettability and the morphology of eroded dentin treated with different desensitizing agents by brushing along 7 or 21 days.

The first null hypothesis is that treatment with different desensitizing dentifrices does not interfere in eroded dentin wettability. The second null hypothesis is that the brushing time does not affect eroded dentin wettability.

METHODOLOGY

Experimental design

The 92 specimens were randomly divided in individual containers without labels, being named after the cutting, and they were divided according to the experimental factors: desensitizing dentifrices at four levels (Colgate Total – control; Colgate Sensitive Pro-Relief; Sensodyne Repair&Protect and Sensodyne Rapid Relief) (Table I), and time of abrasive wear at two levels (brushing along 7 and 21 days). The response variable was the contact angle between dentin and the adhesive at 7 days (n = 10) and at 21 days (n=10), and additional analysis of surface morphology analysis by confocal laser scanning microscopy (CLSM) at 21 days (n=3).

Table I - Composition, batch, manufacturer, and manufacturing location of the desensitizing dentifrices

Material	Composition	Batch	Manufacturer
Colgate® Total 12	Active ingredients: 0.32% sodium fluoride (1450ppm F) and 0.3% triclosan	4129BR123D	Colgate-Palmolive Industrial LTDA (São Bernardo do Campo/ SP- Brazil)
Colgate® Sensitive Pro-Relief™	Active ingredients: arginine 8% and 1.1% sodium monofluorophosphate (1450 ppm F)	5290BR122C	Colgate-Palmolive Industrial LTDA (São Bernardo do Campo/ SP- Brazil)
Sensodyne® Repair & Protect	Active ingredients: sodium monofluorophosphate (1426ppm F), 5% sodium and sodium phosphosilicate.	295F	SmithKline Beecham ConsumerHealthcare (Maidenhead/Berkshire- UK)
Sensodyne® Rapid Relief	Active ingredients: sodium fluoride (1040 ppm F) and strontium acetate	UP0308V	GlaxoSmith-Kline Brasil LTDA (Rio de Janeiro/RJ- Brazil)

Selection of teeth and preparation of dentin specimens

Bovine incisors stored in 0.1% thymol solution at 9 °C were rinsed in natural mineral water for 24 h to eliminate thymol residues. Eighty incisors with no fracture lines or crown-deep cracks were selected. The teeth were transversely sectioned at the cement/enamel junction with a low-speed water-cooled diamond saw (Struers A/S, Ballerup, Denmark). Then, the dental crowns were sectioned, and one specimen measuring 7 mm width, 7 mm long and 2.5 mm depth was obtained from each crown. In which, after obtaining the cut, dentin was exposed.

Immediately after, the specimens were fixed in a Teflon matrix with melting wax (Kota Industria e Comercio Ltda, SP, Brazil). Then, the specimens were flattened and polished on a water-cooled polishing machine (Arotec S/A Ind. Com, SP, Brazil); 320- and 600-grit sandpapers were used to flatten the lateral walls. To flatten the dentin surface and to standardize smear layer formation, 1200- and 2000-grit (Hermes Abrasives Ltd., VA, USA) sandpapers were used for 10 s [27]. After polishing and changing the sandpaper, an ultrasonic bath was performed for 10 minutes with deionized water.

Erosion-like lesion formation

Erosion-like lesions were created according to a previously described method [28]. Each dentin specimen was immersed in a beaker containing 20 mL of 0.3 wt% citric acid (pH = 3.2) and placed in a shaker (CT155, Cientec, Piracicaba, SP, Brazil) under constant stirring at 50 rpm for 2 h. Then, the specimens were rinsed with distilled water and individually stored in vials containing artificial saliva at 37 ºC for 24 h. Artificial saliva was composed of methyl phydroxybenzoate (2.0 g), sodium carboxymethylcellulose (10.0 g), KCl (0.625 g), $MgCl_2.6H_2O$ (0.059 g), $CaCl_2.2H_2O$ (0.166 g), K_2HPO_4 (0.804 g), and KH_2PO_4 (0.326 g) in 1000 mL of distilled water [29,30].

Surface treatment and Time of abrasive wear

Abrasive wear was performed in an automatic toothbrushing machine (MAVTEC – Com. Peças, Acess. and Serv. Ltda. ME, Ribeirão Preto, São Paulo, Brazil). This device allows 12 samples to be brushed at 356 rpm simultaneously, simulating the horizontal brushing technique [31].

One soft nylon bristle toothbrushing head (Condor S.A., São Bento do Sul, Santa Catarina, Brazil) was used for every two specimens. The brush cables were cut so that they could be fitted and fastened to the screws located on the sides and top of the machine. A matrix made from auto-polymerized acrylic resin was employed to accommodate the specimens in a standard position. The specimens were fixed with melted wax and positioned alongside the bristles of the dental brush; a load of 200 g was focused on them.

Each dentifrice was diluted in enough amount of water (1:1 volume) to cover the specimens [31]. The specimens were submitted to 1025 (corresponding to brushing along one week, three times a day) or 3075 (corresponding to brushing along three weeks, three times a day) of brushing cycles [31]. Then, the specimens were washed in deionized water for 30 s, immersed in artificial saliva, and stored at 37 °C.

After 24 h, all specimens were conditioned with 37% phosphoric acid (Condac 37®, FGM, Dentscare LTDA, Joinville - Santa Catarina, Brazil) for 15 s, rinse in distilled water for 30 s, and dried with absorbent paper.

Wettability analysis

Eroded dentin wettability was determined by measuring the contact angle (θ) between the eroded dentin surface and a drop of the adhesive; a goniometer (OCA 20-DataPhysics Instruments GmbH, Filderstadt, Germany) was used, as depicted in Figure 1. Each specimen was placed on a mobile platform adjusted with screws. Then, 10 μL of adhesive for dental restorations (Single Bond Universal Adhesive, 3M ESPE, St. Paul, EUA) was dropped on the dentin surface with a micropipette. The micropipette tips were changed after each drop, to prevent the adhesive from being polymerized by ambient light. Through a lighting system with a tungsten lamp and a Charge-Coupled Device (CCD) camera, the image

Figure 1 - Representation of the contact angle (θ) between the eroded dentin surface and a drop of adhesive.

of the drop on the dentin surface was captured for 2 min, at 1-ms intervals [19]. The (θ) values were analyzed with software (SCA20-Software for OCA e PCA – Data Physics Instruments GmbH, Wurttemberg, Germany); the Young-Laplace fitting was applied. All the (θ) measurements were performed in a closed environment at controlled room temperature of 25 ± 0.5 °C and with standard ambient light.

Confocal Laser Scanning Microscope (CLSM)

Additional twelve eroded dentin specimens of (n = 3) submitted to brushing with desensitizing dentifrices along 21 days were analyzed by Confocal Laser Scanning Microscopy (OLS4000 LEXT by Olympus, Center Valley, PA, USA). The specimens were observed for obliteration of the dentinal tubules by the dentifrice before and after etching the dentin with 37% phosphoric acid, under a 100x magnification.

Data analysis

The (θ) values were submitted to the Shapiro–Wilk test and presented normal distribution. Thus, the data were analyzed by using two-way ANOVA with pos-hoc Tukey's test ($p \leq 0.05$). The data were analyzed with the Assistat software (beta version 7.7).

RESULTS

Eroded dentin wettability

ANOVA showed no significant difference for any of the groups ($p \geq 0.05$). Thus the desensitizing dentifrices and the brushing did not affect the θ° values (Table II). The average contact angle ranged from 24.69° ± 5.8° to 32.68° ± 7.1°.

Surface morphology analysis

We recorded CLSM images for eroded dentin before and after etching the dentin with 37% phosphoric acid. It was possible to observe that Colgate® Total 12 is deposited on the dentin in a subtle way, obliterating few dentinal tubules (Figure 2-A1) and none after etching with phosphoric acid (Figure 2-A2).

Table II - Mean and standard deviation of contact angle measurements (θ°) for the interaction between desensitizing dentifrice x abrasive wear

	7 days	21 days
Colgate Total	24.6 ± 5.8	34.2 ± 9.3
Colgate Sensitive Pro-Relief	26.7 ± 9.9	30.7 ± 8.3
Sensodyne Repair & Protect	28.2 ± 9.9	32.6 ± 7.1
Sensodyne Rapid Relief	27.2 ± 7.1	30.0 ± 11.6

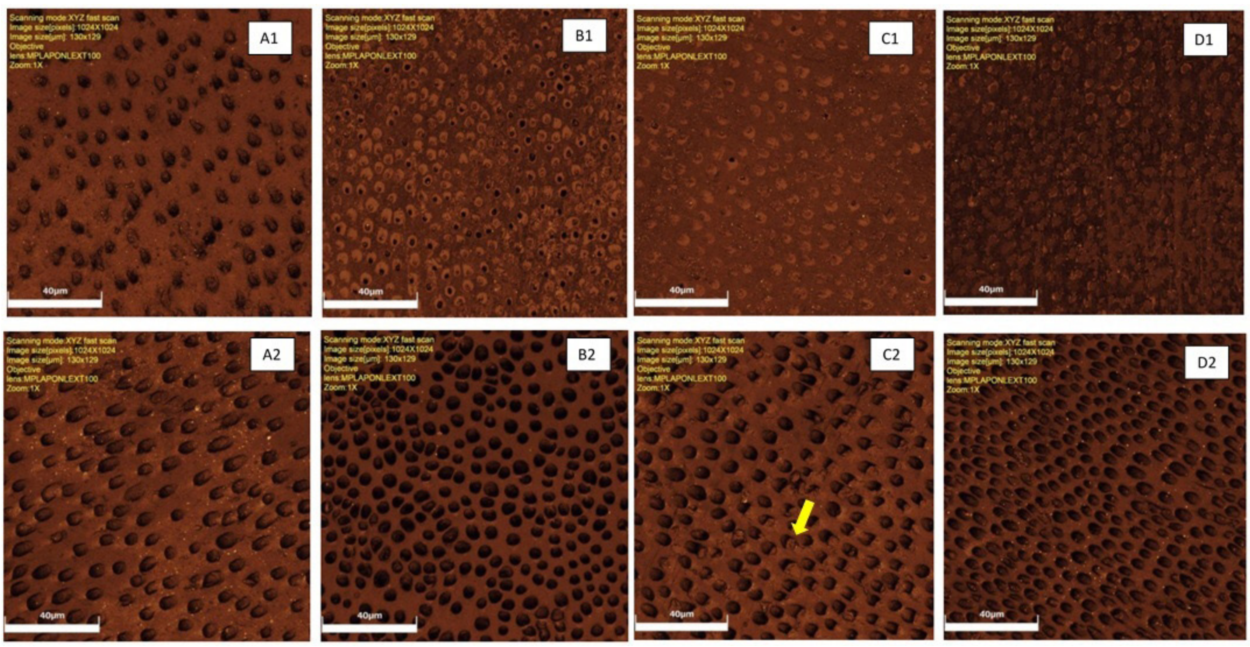

Figure 2 - Surface morphological analysis by CLSM after brushing along 21 days with: (A) Colgate® Total 12; (B) Colgate® Sensitive Pro-Relief™; (C) Sensodyne® Repair & Protect; and (D) Sensodyne® Rapid Relief. A1, B1, C1 and D1: before conditioning with 37% phosphoric acid. A2, B2, C2 and D2: same specimen after acid etching dentin for 15 seconds. X100 magnification.

The toothpaste whose desensitizing agent is 8% arginine associated with calcium carbonate (Pró-Argin® - Colgate® Sensitive Pro-Relief™) obliterated most of the dentinal tubules (Figure 2-B1) after a brushing time of 21 days. After phosphoric acid conditioning, it was possible to observe that most of the previously obliterated tubules were reopened (Figure 2-B2).

Toothpaste with calcium sodium phosphosilicate (Novamin® Sensodyne® Repair and Protect) and toothpaste with 8% strontium acetate (Sensodyne® Rapid Relief) visually block almost 100% of the dentinal tubules just after 21 days of brushing (Figures 2-C1 and 2-D1). After conditioning with 37% phosphoric acid, it was possible to observe that, despite the majority of the dentinal tubules being reopened, there are still some partially obliterated dentinal tubules (Figures 2-C2 and 2-D2), possibly characteristic of residues from the toothpastes themselves (indicated by the arrow).

These deposits obliterated the dentinal tubules only in the group treated with Sensodyne Repair & Protect (Figure 2-C2) as compared to the groups treated with Colgate Total (Figure 2-A2), Colgate Sensitive Pro-Relief (Figure 2-B2), or Sensodyne Rapid Relief (Figure 2-D2).

DISCUSSION

Desensitizing dentifrices act by depositing minerals on the dentin surface and inside the dentinal tubules, obliterating them [1,2,8,24]. However, how this affects the dental surface characteristics, such as dentin wettability by adhesives, has not been elucidated yet.

We confirmed the null hypothesis that treatment with different desensitizing dentifrices does not interfere in eroded dentin wettability. As well as the study by Ururahy et al. [27], in which there was no significant difference even in the control group (non-eroded dentin). This may have happened because all the specimens received acid etching on dentin before the adhesive was applied, which resulted in all the specimens having similar surface due to substrate demineralization. We conditioned dentin with 37% phosphoric acid for 15 s as part of the traditional adhesive protocol preceding adhesive application in restorative treatments [32]. According to a previous study [11], conditioning with acid increases wettability by up to 35% because it increases substrate roughness and surface energy.

The interaction between treatment with desensitizing dentifrices and abrasive wear did not affect eroded denting wettability probably because the dentin surfaces after brushing with the different dentifrices are morphologically similar.

Some studies have demonstrated that sodium, calcium, phosphate, and silica ion deposition by dentifrice containing calcium sodium phosphosilicate lead calcium phosphate to precipitate, to generate nucleation sites. Initially, these sites crystallize, forming individual hydroxycarbonapatite particles. Over time, these particles are degraded, obliterating the dentinal tubules and promoting a more homogeneous dentin surface [33-36].

Here, the CLSM images showed that brushing with Sensodyne Repair & Protect along 21 days promoted calcium sodium phosphosilicate deposition on acid-etched dentin. Indeed, calcium-strontium-apatite can penetrate the dentinal tubules and remineralize the dentin surface [1,37-39]. Arginine is attracted to the negatively charged dentin surface, while calcium carbonate is attracted and adhered to dentin collagen fibers. These compounds infiltrate the tubules, making the environment alkaline. This facilitates calcium and phosphate ion precipitation, blocking the dentinal tubule entrance [12,19,24,35,39,40]. Although the desensitizing dentifrices used herein have different mechanisms of action, all of them promote dentinal tubule obliteration and substrate remineralization [1,24].

The similarity between the results obtained from toothpastes with desensitizing agents (Colgate® Sensitive Pro-Relief™, Sensodyne® Repair & Protect and Sensodyne® Rapid Relief) and the control toothpaste (Colgate® Total 12) may be related to the presence of abrasives such as silica, which are able to obliterate the dentinal tubules [38]. The fluoride, present in the control toothpaste and toothpaste containing calcium and sodium phosphosilicate, as well as in saliva, acts as a remineralizing agent through the precipitation of calcium phosphate and the formation of fluorohydroxyapatite in dentin and enamel; however, it is less stable in acid solutions [35].

Here, CLSM revealed that the dentifrice deposits were unstable when dentin was acid-etched with phosphoric acid, which promoted open dentinal tubules. Studies evaluating the resistance of mineral deposits from desensitizing dentifrices inside dentinal tubules against 37% phosphoric acid are lacking. However, several studies have shown the resistance of these deposits against acid challenges from diet, such as grape and orange juices and soft drink, like Coca-Cola [40-43]. According to Pashley et al. [44], acid attack underlies dentinal demineralization to depths between 0.5 and 7.5 μm. Nevertheless, the depth that desensitizing agent mineral deposits can reach or the dentin mineral composition after treatment with desensitizing agents, followed by phosphoric acid application, are unknown.

Dentin wettability is an important factor to consider because it is directly related to adhesion between the tooth and restorative materials. This process is mediated by physical and chemical interactions that keep the material and substrate together, mediated by an adhesive [711,26]. The degree of adhesive spread on dentin depends on the degree to which the substrate is wetted. Wettability is quantified by measuring the contact angle between the adhesive and the surface, which in turn depends on surface roughness and substrate composition and surface energy [26,45]. This study has been able to highlight the effect of desensitizing dentifrices on eroded dentin wettability and morphological changes on the dentin surface.

CONCLUSION

It is concluded that desensitizing dentifrices do not interfere in eroded dentin wettability after brushing along 7 or 21 days. Our morphological findings revealed open dentinal tubules for all the desensitizing dentifrices after brushing along 21 days.

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Author's Contributions

RC: Author of project, responsible for writing the article, design and for all aspects guaranteeing the project precision and integrity. MMA: Contributing participant of the project, responsible for the selection of teeth and preparation of dentin specimens. FACZ: Contributing participant of the project, responsible for the wettability analysis and surface morphology analysis. SAMC: Contributing participant of the project, responsible for the Confocal Laser Scanning Microscope (LSCM). TCD: Contributing participant of the project and responsible for writing the article. LPAA: Contributing participant of the project and responsible for writing the article. APR: Contributing participant of the project, final reading of the article and co-orientation of the project. ABCEBC: Project tutor, responsible for the study design and guidance during its development.

Conflict of Interest

The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Regulatory Statement

Not applicable.

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