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Tridimensional roughness and morphology of sound dentin surfaces after papain-gel treatment

Paula Maciel Pires, Paula Martins Bravo Miranda, Paula Helena de Accioly Costa, Amanda Souza Nunes Monteiro, Adílis Kalina Alexandria, Lucianne Cople Maia, Aline de Almeida Neves

Universidade Federal do Rio de Janeiro, Universidade Federal Fluminense, Universidade Esadual do Rio de Janeiro, Brazil

Abstract

PURPOSE: To investigate the effect of chemomechanical caries removing agents (CCRAs) based on papain (Papacárie Duo Gel® and Brix3000®) over dentin surfaces compared with 37% phosphoric acid and 11.5% polyacrylic acid.

MATERIALS AND METHODS: Sound human molars were sectioned at the crown level, resulting in 48 dentin blocks, which were randomly divided into 4 groups (n=12): 1) Papacárie Duo Gel®; 2) Brix3000®; 3) 11.5% polyacrylic acid solution and 4) 37% phosphoric acid gel. All products were applied for 30s. Ten blocks per group were analyzed by a non-contact 3D profilometer before and after treatments for linear (Ra) and volumetric roughness (Sa). The superficial morphology of the remaining blocks in each group (n=2) was evaluated by scanning electron microscopy (SEM). Normality was rejected for the data (Shapiro-Wilk test) and therefore, Kruskal-Wallis test, followed by Dunn's proof or Wilcoxon signed rank test with its respective effect size calculation were used to compare the results with α=5%.

RESULTS: Ra and Sa values for specimens submitted to Papacárie Duo Gel® and Brix3000® were statistically similar to baseline values. Application of phosphoric and polyacrylic acid resulted in a statistically increase in roughness compared to the CCRAs. SEM evaluation showed that Papacárie Duo Gel® resulted in surface debris. Polyacrylic acid and Brix3000® resulted in partial opening of the tubules but dentin exposed to polyacrylic was able to remove more smear layer than Brix3000®, while phosphoric acid resulted in total opening of the dentinal tubules.

CONCLUSION: Both Papacárie Duo Gel® and Brix3000® did not result in roughness changes when applied in sound dentin.

KEYWORDS: dental caries; chemomechanical caries removal; dental materials; dentin; papain; pediatric dentistry

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Email: aline.dealmeidaneves@gmail.com

Introduction

Non-treated caries lesions are still a burden to dental services around the world [1]. Chemomechanical caries removal agents (CCRAs) gained interest over the last twenty years due to many factors, including: 1) increased dental tissue preservation due to selective tissue removal; 2) ability to provide a more objective caries removal threshold; 3) reduced need for anesthesia during operative procedures and 4) possibility of undertaking operative procedures in non-dental settings [2-4].

Initially, hypochlorite-based CCRAs such as Carisolv® (RLS Global, Mölndal, Sweden) where developed, in which good effectiveness and a selective action in removal of carious dentin has been demonstrated [4]. In Brazil however, papain-based CCRAs became more commercially available and therefore, much commonly used

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(Papacárie Duo Gel®, Fórmula & Ação, São Paulo, SP, Brazil). This product is a gel containing 3% papain, 0.5% chloramine and toluidine blue and has been also found to be effective in removing infected carious dentin [5] with a user-friendly technique [6] and being more comfortable to the child dental patient than the drilling method [7]. More recently, another papain-based CCRA has been developed (Brix3000®, Brix Medical Science, Carcarañá, Santa Fe, Argentina) which allegedly contain an increased papain concentration (3.000 U/mg or 10%). According to the manufacturer, in this gel, the enzyme is protected by a buffer emulsion technology, which immobilizes and stabilizes it at an ideal pH, releasing papain only at the time of collagen proteolysis, resulting in an increase of the enzymatic activity by up to 60%. Differently from Papacárie Duo Gel®, Brix3000® does not contain chloramine in its composition [8].

From the currently available minimally invasive caries removal methods, CCRAs agents result in the best threshold for conservative caries removal [9,10]. Papain is an enzyme extracted from papaya fruit with broad proteolytic, bactericidal, bacteriostatic and anti-inflammatory activity [11]. Papain is able to breakdown infected or necrotic tissues because degraded collagen lacks α1-anti-trypsin, which normally inhibits protein digestion in healthy tissues [12,13], guaranteeing thus, the selectivity of the CCRA. The lack of anti-trypsin in infected/necrotic tissues will allow papain to further breakdown this substrate, enabling easy removal with hand instruments [14].

The concept of minimal invasive dentistry (MID) means removing only infected and irreversibly destroyed dentin and leaving, as residual substrate for further adhesive procedures, a slightly demineralized caries-affected dentin [15]. Like other active substances in CCRAs, papain is supposed to acts exclusively on the breakdown of the partially degraded collagen in carious dentin, without damaging intact collagen fibrils [16], leaving thus, sound dentin intact. Keeping with the MID approach, CCRAs are constantly used over sound dentin surfaces, especially if the enamel-dentin junction is prepared to “scratchy” dentin to ensure appropriate sealing of the restoration [17]. While hypochlorite-based CCRAs have been investigated on its topographic effects over sound dentin [18,19], the possible effects of papain-based CCRAs sound dentin surfaces have not yet been investigated.

To evaluate the effect of Papacárie Duo Gel® and Brix3000® on the superficial morphology and roughness of sound dentin compared to commonly used dentin conditioning agents (37% phosphoric acid and 11.5% polyacrylic acid), which purposely cause exposure of dentin collagen. Surface roughness and morphological features have been investigated by non-contact profilometry measurements and scanning electron microscope, respectively.

Material and Methods

Sound human third molars, extracted for clinical reasons were used after the patient has given written consent. This protocol has been approved by the institution’s ethical committee and it is registered at “Plataforma Brazil” (17389213.5.0000.5257). The teeth were selected according to the following inclusion criteria: absence of caries lesions or restorations/sealing material. After sectioning, the specimens were examined under a stereomicroscope for presence of any defects or areas of sclerotic dentin, and if present, they were excluded from the sample. Excluded teeth were discarded via standardized protocol for biological material.

Sample size was estimated for comparison of differences in more than two experimental groups considering a 0.05 alpha value and a 0.9 power of the test, based on results of a previous study on the effect of a hypochlorite-based chemo-mechanical caries removing method on the roughness of sound dentin [18]. The following information was input to the software (BioEstat v.5.3, Instituto Mamirauá, Manaus, AM, Brazil): minimum difference between mean of groups = 0.002; standard deviation

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of the experimental error = 0.001, which resulted in a minimum of 8 specimens per group.

The tooth crowns were cut, with the aid of a diamond disk mounted on a low speed cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) into dentin blocks (approximately 4 X 4 X 2 mm) in which the occlusal dentin was selected as the test surface.

Forty-eight dentin blocks were selected among those obtained and attached with sticky wax to polyethylene devices with the test surface exposed at the top. The specimens were further polished with 400, 800 and 1200 grit sandpaper, in this order, under water-cooling with the aid of a semi-automatic polishing machine (PLF, Fortel, São Paulo, SP, Brazil). After that, twelve specimens were randomly allocated to each four experimental groups: 1) application of Papacárie Duo Gel® (Fórmula & Ação, São Paulo, SP, Brazil); 2) application of Brix3000® (Brix Medical Science, Carcarañá, Santa Fe, Argentina); 3) application of a 11.5% polyacrylic acid solution (Vitro condicionador, DFL, Rio de Janeiro, RJ, Brazil) 4)

application of a 37% phosphoric acid gel (Condac, FGM, Joinville, Santa Catarina, Brazil). Application time for all products was standardized in 30s. After each application time, and for all groups, the specimens were washed in distilled water for 60s and stored under 100% humidity until further analysis.

Before and after the treatment regimens, 10 specimens in each group were analyzed in a 3D non-contact chromatic confocal optical profilometry (Nanovea PS50 Optical, Nanovea Inc., Irvine, California, United States). Linear roughness (Ra) was obtained by averaging three linear readings from each sample while volumetric roughness (Sa) was obtained from one volumetric reading over each specimen.

The remaining two blocks per group were used for surface topographic analysis. The blocks were fixed on stubs with double-faced carbon tape, gold-sputtered (30µm) and analyzed in a scanning electronic microscope (6460LV, JEOL, Tokyo, Japan) in secondary electrons mode after the

treatment regimens.

Shapiro-Wilk's test was used to evaluate normality of the data. As normality was rejected, Kruskall-Wallis test, followed by Dunn's proof was used to disclose statistical significance among the roughness values within the experimental time (baseline or treated) and an epsilon squared effect size was calculated [19]. Wilcoxon signed rank test was used to test differences in each experimental group between time (baseline or treated) and the effect size was calculated as the *r* estimate [19, 20]. The α-level chosen was 5% and all analysis were undertaken using BioEstat v.5.3 (Instituto Mamirauá, Manaus, AM, Brazil).

Results

Both surface roughness parameters evaluated (Ra and Sa) were similar among the groups at baseline. For the treated specimens, polyacrylic acid and phosphoric acid treated specimens showed statistically significant higher Ra and Sa values compared to both papain-treated groups, with large effect sizes (Tables 1 and 2).

Intra-group comparison of tridimensional surface roughness analysis disclosed that specimens submitted to the papain-based caries removing gels did not show significant changes in both roughness parameters tested (Ra and Sa) after the treatments (Tables 1 and 2). On the other hand, specimens treated with phosphoric acid or polyacrylic acid resulted in a statistically significant higher linear (Ra) and volumetric (Sa), with very strong or strong effect sizes.

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Table 1: Mean (±SD) of linear (Ra) roughness measurements (µm) before and after treatments in each experimental group.

Experimental Group	Ra		
	Baseline	Treated	Difference
Phosphoric acid	0.45 ± 0.11 $^{a, A}$	1.34 ± 0.48 $^{a, B}$	0.89 ± 0.53 ^{a}
Polyacrylic acid	0.48 ± 0.04 $^{a, A}$	1.84 ± 0.79 $^{a, B}$	1.37 ± 0.79 ^{a}
Papacárie Duo Gel®	0.41 ± 0.09 $^{a, A}$	0.47 ± 0.09 $^{b, A}$	0.07 ± 0.05 ^{b}
Brix3000®	0.40 ± 0.04 $^{a, A}$	0.39 ± 0.04 $^{b, A}$	0.03 ± 0.03 ^{b}

* Different lowercase superscript letters indicate statistical significance in the same column (Kruskal-Wallis followed by Dunn's test, $p<0.05$). Effect size was 0.76 (very strong) and 0.69 (very strong) for the column "treated" and "difference", respectively [19]. Different uppercase superscript letters indicate statistical significance between baseline and treated specimens in the same group (Wilcoxon signed rank test). Effect size was 0.83 (large) and 0.84 (large) for phosphoric acid and polyacrylic acid respectively [20].

Table 2: Mean (±SD) of volumetric (Sa) roughness measurements (µm) before and after treatments in each experimental group.

Experimental Group	Sa		
	Baseline	Treated	Difference
Phosphoric acid	0.61 ± 0.51 $^{a, A}$	2.12 ± 1.38 $^{a, B}$	1.51 ± 1.42 ^{a}
Polyacrylic acid	0.88 ± 0.25 $^{a, A}$	4.70 ± 3.75 $^{a, B}$	3.82 ± 3.65 ^{a}
Papacárie Duo Gel®	0.59 ± 1.79 $^{a, A}$	0.65 ± 0.16 $^{b, A}$	0.06 ± 0.17 ^{b}
Brix3000®	0.96 ± 0.62 $^{a, A}$	1.21 ± 1.69 $^{b, A}$	0.25 ± 1.30 ^{b}

* Different lowercase superscript letters indicate statistical significance in the same column (Kruskal-Wallis followed by Dunn's test, $p<0.05$). Effect size was 0.63 (strong) and 0.51 (strong) for the column "treated" and "difference", respectively [19]. Different uppercase superscript letters indicate statistical significance between baseline and treated specimens in the same group (Wilcoxon signed rank test). Effect size was 0.84 (large) and 0.81 (large) for phosphoric acid and polyacrylic acid respectively [20].

SEM analysis of the superficial morphology of the dentinal tubules revealed that phosphoric acid resulted in full opening of the dentin tubules (Figure 1A). Polyacrylic acid and Brix3000® resulted in partial opening of the dentin tubules (Figure 1B and 1D) while Papacárie Duo Gel® resulted in the presence of debris, probably resulting from the polishing procedures undertaken during specimen preparation which remained over the tubules (Figure 1C).

Discussion

The results of the present study revealed no statistically significant changes on linear (Ra) and volumetric (Sa) roughness in specimens submitted to the papain-based CCRAs before and after the treatments. Specimens treated with phosphoric acid or polyacrylic acid resulted in a statistically significant increase in both Ra and Sa. These results are in agreement with previous investigations of the effect of Carisolv® in sound dentin surfaces. Roughness parameters has also not

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been changed after Carisolv® application but increased substantially when phosphoric acid was applied in this substrate [18]. Carisolv® is a CCRA based on chloramine, while the CCRAs used in the present study are based on papain. Therefore, an investigation on the effect of these on the sound topography of dentin was warranted.

The morphology of the dentin surface as revealed by SEM showed that phosphoric acid resulted in full opening of the dentin tubules (Figure 1A). Polyacrylic acid and Brix3000® resulted in partial opening of the dentin tubules (Figures 1B and 1D) but dentin exposed to polyacrylic acid resulted in less smear layer than Brix3000®. Papacárie Duo Gel® resulted in a relatively smeared dentin surface the presence of debris (Figure 1C).

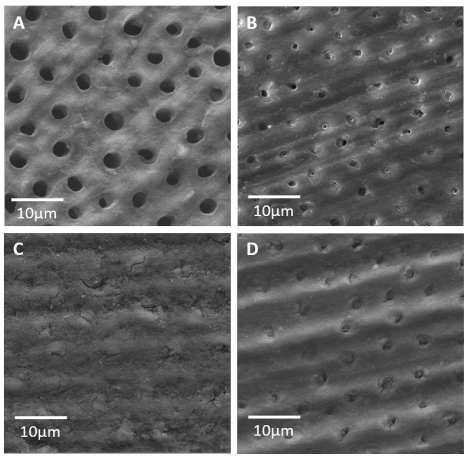

Figure 1. *SEM images of dentin surfaces after experimental treatments. A) 37% phosphoric acid treatment. B) 11.5% polyacrylic acid treatment. C) Papacárie Duo Gel® treatment. D) Brix3000® treatment.*

A previous SEM study comparing Carisolv® and other dentin conditioners over sound dentin also showed that this CCRA was unable to remove smear layer and completely open dentin tubules [21]. However, when Carisolv® has been used over carious dentin, the surface generally appeared clean, with open tubules [22]. The same is likely to be true for Papacárie, since many studies have also reported similar residual carious dentin surface morphology after it use, with open tubules and reduced smear layer [23-25]. Nonetheless, one study showed presence of smear layer and debris partially occluding the dentin tubules in residual carious primary teeth dentin after caries removal with Papacárie Duo Gel® [26].

It has been claimed that chloramine present in Papacárie Duo Gel® would be responsible for opening the dentinal tubules of the outer surface of the carious dentin, softening it chemically and facilitating its removal [27]. In the present study however, sound dentin surfaces treated with this product still showed a smear layer covered surface.

The main function of phosphoric acid 37% and polyacrylic acid 11.5%, used during conditioning of dentin surfaces prior to composite and glass ionomer restorations, is to guarantee removal of the smear layer, which is accompanied by the superficial mineral dissolution of the dentin, exposure of collagen fibers and opening of the superficial dentin tubules to allow composite tag formation [28]. The SEM observations of the present study corroborate the effect of both phosphoric and polyacrylic acid on sound dentin. Although complete removal of the smear layer is frequently seen with phosphoric acid [29], this is invariably followed by great peritubular dentin dissolution and exposure of collagen fibers [30]. Polyacrylic etched sound dentin surfaces normally retain part of the smear layer [31] and also result in calcium-enriched surfaces [29].

There have been claims that non-mineralized type I collagen fibrils would be partially degraded (without fiber rupture) by a papain-gel, as shown by atomic force evaluation of the surface of collagen fibers in sound dentin [32], what could be evidence of a detrimental effect of the gel in sound dentin. Others, however, using Fourier-transformed infrared spectroscopy claimed no collagen degradation to occur [33] and no removal of calcium of sound dentin after Papacárie application [34]. Regarding surface roughness and morphological characteristics, the results of the present study support the selective action of papain gels in carious dentin.

The restoration of cavities using a MID approach requires adhesive materials such as composite resins or glass ionomer cements, which directly bond to the dentin surfaces. Several studies have reported that papain-based gel treatment does not interfere with the bond strength of the adhesive restorative materials to

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sound and demineralized dentin [35-37]. However, these CCRAs do not promote enough surface roughness in sound dentin; thus, it's use probably does not replace the use of conditioning agents before restoration. More studies are indeed, necessary to investigate possible ultrastructural changes in sound and carious dentin after use of papain-based CCRAs in order to optimize its use in light of the MID approach.

Limitations of this study include the fact that the dentin quality among the experimental groups were not fully standardized. Factors such as age of the patient, anatomical location of the tooth or distance of the cut from the dentin-pulp junction, might result in morphological differences at the dentin level that could have influenced the results (i.e., the high variability of data obtained). However, as distribution of the dentin specimens was randomized among the groups in the present study, this effect was probably evenly distributed among the groups.

Papain-based caries removing gels did not induce significant roughness changes in sound dentin. Regarding the morphologic characteristics of the surfaces, application of Brix3000® resulted in partially opening of dentin tubules, similar to that caused by polyacrylic acid treatment but this removed more smear layer. Papacárie Duo Gel® did not remove smear layer and left dentin tubules obliterated. This study has shown that papain-based gels are harmless when applied in sound dentin surfaces.

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