Efficacy of smear layer removal by cavity cleaning solutions: an atomic force microscopy study

Eficácia da remoção da lama dentinária por soluções de limpeza cavitária: estudo de microscopia de força atômica

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Abstract

Purpose: To evaluate the efficacy of smear layer removal by cavity cleaning agents by the use of atomic force microscopy (AFM).

Methods: Five intact human third molars were sectioned in the coronal portion to obtain dentin disks, which were ground with 600-grit abrasive paper for 10 s. Serial longitudinal sections were made perpendicular to each other to create four specimens, from each tooth. The specimens were divided into four treatment groups: GI, 2% chlorhexidine; GII, calcium hydroxide solution; GIII, 1.23% fluoride solution; and GIV, 37% phosphoric acid. The solutions were applied with a brush for 60 s, with the exception of the 37% phosphoric acid, which was applied for 15 s and rinsed with distilled water for 60 s. The specimens were examined by AFM.

Results: All of the specimens in GI and GII showed 100% of the dentin tubules obliterated by the smear layer. However, all of the specimens in GIII and GIV showed 0% of the dentin tubules obliterated by the smear layer.

Conclusion: The 1.23% fluoride solution was effective in removing the smear layer and can be used as a cavity cleanser.

Key words: Smear layer; chlorhexidine; sodium fluoride

Resumo

Objetivo: Avaliar a eficácia da remoção de lama dentinária por agentes de limpeza cavitária através da análise de microscopia de força atômica (MFA).

Metodologia: Cinco terceiros molares humanos hígidos foram seccionados transversalmente na porção coronária, obtendo discos de dentina de 2 mm, que foram desgastados com lixas de granulação 600, por 10 s.Foram feitos cortes seriados longitudinais e perpendiculares entre si, obtendo-se quarto espécimes de cada dente. Os espécimes foram divididos em 4 grupos: GI- clorexidina a 2%; GII- água de hidróxido de cálcio; GIII- solução fluoretada a 1,23%; GIV - ácido fosfórico 37%. As soluções foram aplicadas com pincel por 60 s, com exceção do ácido fosfórico que foi aplicado por 15 s e lavado com água destilada pelo mesmo tempo. Os espécimes foram analisados por MFA.

Resultados: Todos os espécimes de GI e GII apresentaram 100 % dos túbulos dentinários obliterados por lama dentinária. Os espécimes de GIII e GIV apresentaram 0 % dos túbulos obliterados por lama dentinária.

Conclusão: A solução de fluoretada a 1,23 % foi eficiente na remoção de lama dentinária, podendo ser empregada na limpeza de cavidades.

Palavras-chaves: Camada de esfregaço; clorexidina; fluoreto de sódio

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Introduction

The mechanical preparation of a cavity with rotating or manual instruments produces a semi-porous layer formed by small particles from the cutting debris; the thickness, composition and morphology of the smear layer depends on the instrumentation and dentin location from which it was created (1). However, this layer adheres firmly to the dentin surface and can interfere in resin-dentin bonding (2).

The development of self-etching primers raised the possibility of incorporating the original smear layers into hybrid layers (2). Acidic monomers of self-etching adhesives partially dissolve and infiltrate the smear layer and hydroxyapatite to generate a hybrid layer (3). However, there is some concern that self-etching primers may not be able to penetrate through thick smear layers (4). The acidity of the primer could also be buffered by the mineral components of the smear layer to the extent that the potential for primer penetration into the underlying sound dentin might be reduced, resulting in gaps in adhesive restorations (2). Because of these considerations, the treatment of the smear layer can be an important influence on the good performance of restorations (1).

Different kinds of chemical solutions have been employed to clean cavities and remove the smear layer for restorative procedures (5,6). When dentin is etched with acid solutions, such as phosphoric acid, the smear layer and smear plug are completely removed and the surface of the dentin is morphologically changed (7). Because non- or slightly-demineralizing agents, e.g., chlorhexidine digluconate, calcium hydroxide solution and some fluoride solutions, act by the simple mechanical action of washing and scrubbing, they also may result in partial removal of the smear layer (8).

To analyze the process of smear layer removal, it is necessary to monitor the dentin surface after application of the cleaning materials (9). The atomic force microscope (AFM) is a member of the scanning probe microscopy family of instruments, which also includes the scanning tunneling microscope (10). The AFM offers the opportunity to image the 3-dimensional surface topography of biological specimens with high spatial resolution under a wide variety of conditions. These conditions include exposure to air, water and other storage solutions at elevated or reduced temperatures (11). Due to its mechanism of image formation, there is no need for staining, dehydration, thin film covering or a vacuum environment. Hence, dental tissues, such as dentin, can be measured directly (12). However, there are only a few studies that employ AFM to investigate the removal of the dentin smear layer (9, 12).

The aim of this *in vitro* study was to evaluate the efficacy of smear layer removal by different cavity cleaning agents, 2% chlorhexidine digluconate, calcium hydroxide solution, 1.23% fluoride solution and 37% phosphoric acid (positive control), through analysis by AFM. The null hypothesis tested was that there would be no difference in the effectiveness of smear layer removal among the cleaning solutions.

Methods

Five unerupted, caries-free third molars were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the local Internal Review Board (116/2008). The selected teeth were stored in 0.01% thymol solution and used within one month after extraction. The occlusal enamel and deep dentin were removed by cutting two parallel sections at right angles to the long axis of the tooth using a slow-speed saw on a Labcut 1010 machine (Extec Corp, Enfield, CT, USA) under water cooling at 300 rpm to obtain 2-mm-thick disks of mid-coronal dentin. Additionally, all the disks were longitudinally sectioned in both the x and y directions across the center of the dentin surface resulting in four dentin specimens from each tooth. All the specimens were ground with 600-grit silicon carbide paper (SiC) for 60 seconds to create a uniform smear layer.

The specimens were randomly allocated into four groups (n=5) by Excel software (Excel 2003, Microsoft Corporation, One Microsoft Way, Redmond, WA, USA) according to the following treatment solutions: GI, 2% chlorhexidine digluconate; GII, calcium hydroxide solution; GIII, 1.23% fluoride solution; and GIV, 37% phosphoric acid solution as a positive control (Table 1). The cavity cleaning solutions were applied with a micropipette (50 µl) and agitated on the entire dentin surface with an applicator microbrush (KG Brush; KG Sorensen, Cotia, SP, Brazil). All the solutions were rubbed for 60 seconds except for phosphoric acid, which rubbed for 15 seconds and was rinsed for 60 seconds with distilled water. Excess of all solutions were removed with absorbent paper.

Table 1. List of materials used in the present study and their composition and manufacturer.

Material	Composition	Manufacturer (#Batch)
2% chlorhexidine digluconate	2% chlorhexidine digluconate, deionized water and volatile surfactant	FGM, Joinvile, SC, Brazil (#031109)
calcium hydroxide solution	Calcium hydroxide and distilled water	Pharmacy School – Federal University of Ceara, Brazil
1.23% fluoride solution	Sodium fluoride, phosphoric acid and distilled water	Pharmacy School – Federal University of Ceara, Brazil
37% phosphoric acid solution	phosphoric acid, colloidal silicon dioxide, methylene blue and deionized water.	DFL, Rio de Janeiro, RJ, Brazil (#0322)

After treatment, the specimens were fixed in a metallic device using double-sided tape (3M do Brasil Ltda, São Paulo, SP, Brazil). Following this, measurements were collected with an AFM (Nanoscopy IIIa AFM – Digital Instruments, Santa Barbara, CA, USA) using tapping mode. Tapping mode operates by scanning across the sample surface using pyramidal tips of Si_3N_4 attached to the end of a vibrating cantilever so that it is in intermittent contact with the surface. The cantilever amplitude is maintained constant by altering the vertical position of the piezoelectric scanner. Images were recorded at a slow scan rate (1 Hz). Each dentin disk was scanned across the central area. The scanning area was 50 x 50 μm^2.

Results

GI and GII micrographs showed dentin surfaces with dentin tubules completely covered by the smear layer, and ripples created by the action of SiC abrasives were visible (Fig. 1 and 2). However, in GIII micrographs, it is possible to clearly identify the openings of the dentin tubules, indicating that the smear layer was completely removed from the dentin surfaces. In GIV micrographs (Fig. 1 and 2), the presence of peaks and valleys formed from the expansion in diameter of the opening of the dentin tubules was also visible.

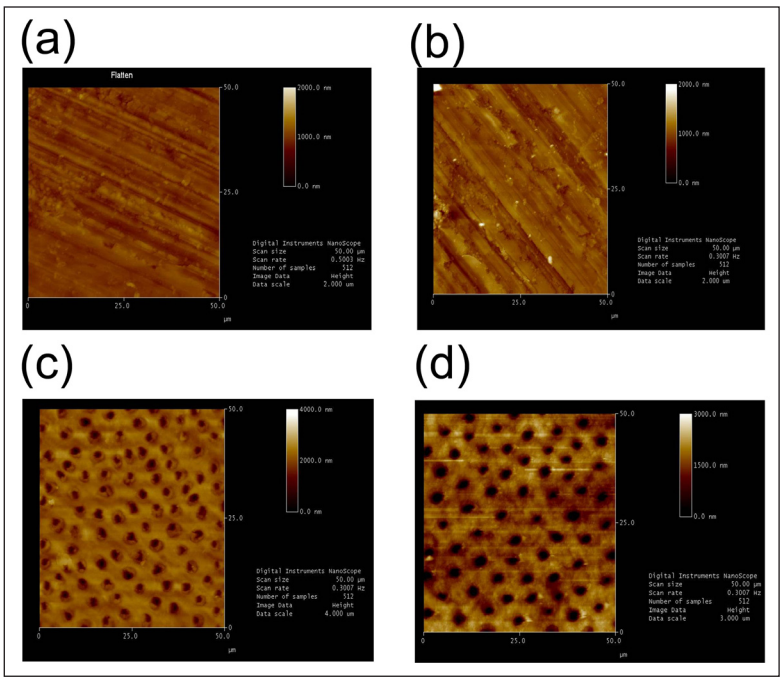

Fig. 1. AFM micrographs of the top of human dentin across a 50 μm x 50 μm scan area:
(a) 2% chlorhexidine digluconate;
(b) calcium hydroxide solution;
(c) 1.23% fluoride solution;
(d) 37% phosphoric acid solution.

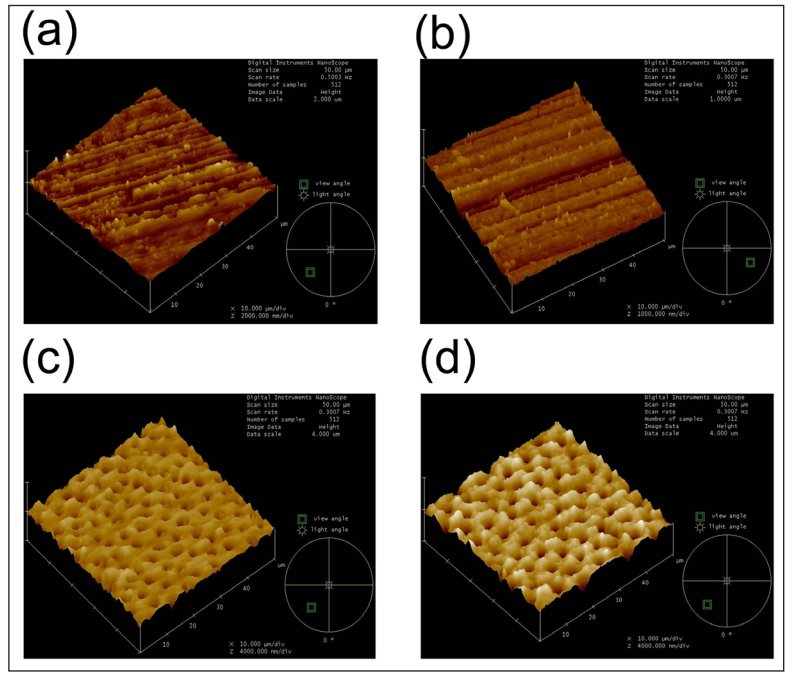

Fig. 2. Three-dimensional AFM micrographs of human dentin across a 50 μm x 50 μm scan area:
(a) 2% chlorhexidine digluconate;
(b) calcium hydroxide solution;
(c) 1.23% fluoride solution;
(d) 37% phosphoric acid solution.

Table 2. Score distribution with regard to smear layer removal in each experimental group (n = 5/group).

Groups	Score		
	A (100% of tubules obliterated)	B (50% of tubules obliterated)	C (0% of tubules obliterated)
GI	5	0	0
GII	5	0	0
GIII	0	0	5
GIV	0	0	5

The results of a visual assessment of the specimens, according to the images, are presented in Table 2. The chlorhexidine digluconate and the calcium hydroxide solution did not remove the smear layer from the dentin surface, while the 1.23% fluoride solution and the 37% phosphoric acid solution completely removed the smear layer from the specimens.

Discussion

Tapping mode AFM has been used in some dental research, dealing mainly with dentin alterations, such as in hybrid layer analysis (13) and dentin demineralization effects (14). Marshall et al. (15) were pioneers in the investigation of acid etching of dentin surfaces with AFM, and recently, other studies have adopted AFM to analyze dentin surfaces rather than using scanning electron microscopy (SEM), which has been traditionally used (9,16,17). The advantages of AFM are beyond the supply of high quality 3-dimensional images; this technique allows the analysis of nonconductive samples without the requirement for prior preparation, which is fundamental to analysis using SEM (12). In SEM, sample preparation is complicated and invasive, which may change the primary structure of samples. In contrast, AFM is noninvasive, and it can work in the air or in physiological conditions without causing irreversible damage to the samples (17). However, AFM also has some critical limitations. The main problems were related to sample surface height variations, and to image acquisition speed (10).

The use of non- or slightly-demineralizing agents on dentin surfaces promotes changes in the smear layer but does not expose the opening of dentin tubules (18). Luz et al. (8) observed changes in the structure of the smear layer and slight smear layer removal in specimens treated with biologic detergents when compared with air/water spray, but it was not completely removed. These results can be explained by the active methods in which the solutions were applied; it is possible that the mechanical actions of the procedures modified the results of the chemical treatments. The cleaning of the cavity with the calcium hydroxide solution or 2% chlorhexidine digluconate also did not possess the ability to remove the cutting debris responsible for the obliteration of dentin tubules, as observed in this study.

Chlorhexidine is used as a cavity cleaning agent because of its proven antibacterial properties and its substantivity (19). Previous studies have shown that the application of chlorhexidine as a cleaning agent after dentin acid etching does not promote immediate adverse effects on the adhesive bond strength between the composite and the dentin (20, 21). According to Zhou et al. (22), the use of chlorhexidine added to an adhesive primer for self-etching in two steps had no adverse effect on the bond strength to dentin tested immediately. However, Ercan et al. (23) found that the use of 2% chlorhexidine in disinfecting a cavity significantly reduces the shear strength of dentin when associated with self-etching adhesive systems. De Castro et al. (21) found that 2% chlorhexidine, applied before or after acid etching of dentin, does not affect the bond strength of composite-to-dentin treated with the adhesive systems Prime & Bond NT, Single Bond or Clearfil SE Bond. Carrilho et al. (24) found that the application of chlorhexidine inhibited the action of matrix metalloproteinases (MMPs) on collagen exposed by acid etching that was not covered by the adhesive monomers, so there was a preservation of the adhesive bond strength of composite to dentin when using adhesive systems associated with total conditioning.

The results observed from the application of the 1.23% fluoride solution (pH 3.6) were similar to the 37% phosphoric acid in removing the smear layer and exposing the dentin tubules. This result can likely be attributed to the low pH of the solutions and the active application of these cleaning agents. However, these results were not observed in other studies involving other types of fluoride, such as 1.27% acidulated phosphate and 2% sodium fluoride; the use of these cleaning agents did not expose the dentinal tubules, although they did disorganize the smear layer (18,25). The likely hypothesis for the differences among these data can be attributed to the use of fluoride in gel form, which reduces the penetration of these agents into the smear layer; the sodium fluoride used had a neutral pH, therefore it had no demineralizing action.

According to the results from our study, the null hypothesis was rejected because there was a difference observed in the effectiveness among the cavity cleaning agents. The 1.23% fluoride solution promoted the complete removal of the smear layer, differing from the calcium hydroxide solution and the 2% chlorhexidine digluconate. However, additional studies are necessary to clarify the effects of the 1.23% fluoride solution on the bond strength of resin to dentin and immediately and after periods of artificial aging.

Conclusion

AFM is a useful alternative method to visualize changes in the microstructure of dentin after the application of cleaning agents; thus, it indicated the efficacy of sodium fluoride in removing the smear layer.

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