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ORIGINAL RESEARCH

Influence of Erosion/Abrasion and the Dentifrice Abrasiveness Concomitant with Bleaching Procedures

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Purpose: The aim of this study was to evaluate the effect of erosive/abrasive cycles and two different levels of abrasiveness of dentifrices over enamel and dentin subjected to bleaching.
Methods: Enamel and dentin bovine specimens were prepared and submitted to an at-home bleaching treatment using 9.5% hydrogen peroxide gel, which was applied daily (30 min/14 days). Concomitant with bleaching, an erosive cycle was performed using citric acid (0.3%, pH 3.8, 5 mins, 3×/day), followed by immersions in artificial saliva for remineralization (30 mins). Abrasion was done with two (high and low abrasiveness) dentifrices (2×/day, 120 seconds) after the first and third erosive immersion each day. Enamel and dentin softening were assessed by microhardness and erosive tooth wear by optical profilometry. Data were submitted to repeated measures ANOVA, followed by the Tukey's test with a significance level of 5%.
Results: For the enamel and considering the erosive-abrasive cycle, significant differences were found between the groups tested, the bleaching, and the abrasiveness of the dentifrice tested; however, the final microhardness values were significantly lower than the initial ones. For dentin, differences were found between the eroded/abrasion and the non-eroded/abrasion groups, with the former presenting lower microhardness values compared with the latter. In addition, bleaching decreased the microhardness values only for the highly abrasive dentifrice, and the final values were lower than for the initial ones for all tested groups.
Conclusion: The use of high and low abrasiveness dentifrices during bleaching and concomitant with erosion/abrasion cycles is more harmful to dentin than to enamel.
Clinical Relevance: Although bleaching is considered a conservative treatment, it can cause deleterious effects to dental hard tissue. The association of an at-home bleaching technique with erosion and high- or low- abrasive dentifrices harms dentin more than enamel.

Keywords: bleaching, enamel, dentin, erosion, wear

Introduction

Bleaching is an effective noninvasive procedure for patients wishing to whiten their teeth[1,2] With the at-home technique, the patient applies a gel for a few days, but during the treatment the bleached enamel may be submitted to erosive cycles. Erosion is a multifactorial process derived from the close contact between tooth structures and acids from diet (extrinsic) or from gastroesophageal disorders (intrinsic) in the absence of biofilm.[3] Erosion first leads to a reduction in enamel and dentin microhardness, softening the surface, followed by tooth wear with continued contact with the acid.[2] In recent decades, changes in nutritional

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parameters associated with behavioral and environmental habits have increased the prevalence of erosive lesions in enamel and dentin.[3]

Bleaching gels can be slightly erosive causing chemical and morphological alterations such as increased surface roughness and reduced microhardness,[4–8] although reports have been controversial.[9–11] The association of hydrogen peroxide with calcium has been reported to reduce enamel mineral alterations when bleaching and erosion are combined.[12–16]

During the bleaching treatment, the patients should maintain their oral hygiene, in which dentifrices play an important role. Their use, together with a toothbrush, may increase or decrease enamel and dentin wear.[6] Enamel and dentin have been reported to be more susceptible to wear with the use of highly abrasive dentifrices,[17] but clinical evidence is still scarce. Recent studies have shown that whitening dentifrices might induce greater enamel wear,[18–20] but for dentin little is known. The association of dentifrice abrasiveness with erosive wear and bleaching has been a controversial topic.[5,21]

Therefore, the aim of this study was to evaluate the effect of erosive/abrasive cycles and two different levels of dentifrice abrasiveness over enamel and dentin subjected to bleaching. The null hypotheses tested were as follows: a) erosive/abrasive cycle models does not change microhardness and surface loss of bleached enamel and dentin; b) different abrasiveness of dentifrices does not change microhardness and surface loss of bleached enamel and dentin.

Materials and Methods

Experimental Design

This experimental in vitro study was conducted with extracted human molars after the approval of the local ethics committee (UTHSCSA - protocol number HSC20080233N) and performed in accordance with the principles of the Declaration of Helsinki, and all the patients signed a consent form for enrollment in the research and data publishing. The extracted teeth were cleaned with pumice and water and stored in 0.1% thymol solution at 5 ºC until use. Teeth with caries, hypomineralization, or incomplete root formation were not included in the study.

The three experimental factors in the study design were the presence and absence of bleaching gel; the presence and absence of erosive challenge; and the use of high- and low-abrasive dentifrice for abrasion. The experiments were conducted separately on enamel and dentin.

Specimen Preparation

Enamel blocks with dimensions of 3 mm × 3 mm × 2 mm (height × width × depth) were obtained from the buccal and lingual surfaces using a water-cooled diamond precision saw (Isomet, Buehler). Dentin specimens with the same dimensions were obtained from the root. The blocks were separately embedded in acrylic resin (SR Triplex Cold Acrylic Resin, Ivoclar Vivadent AG) using a silicone mold with dimensions of 5 mm × 5 mm × 5 mm, leaving the enamel or the dentin surface exposed.

The enamel and dentin specimens were polished flat using plain back diamond lapping film (grits 30 μm, 15 μm and 1 μm) in a MultiPrep™ Precision Polishing machine (Allied High Tech, CA, USA). At the end of the polishing procedures, the specimens were sonicated with distilled water for 3 min. Following this, two coats of an acid-resistant nail varnish were applied on the polished enamel and dentin surfaces of each block, leaving a central area of 1 × 3 mm exposed.

Initial Microhardness

Initial microhardness (SMHi) was measured on each test block surface by using a Vickers diamond indenter (HMV-2T Micro-Hardness tester, Shimadzu Scientific Instruments, Columbia, MD, USA), with a load of 0.25 N applied for 15 s. Three indentations were made at each surface at a distance of 100 μm from each other, and the latter was averaged to define the SMHi value for each test block. Additionally, these specimens were analyzed with an optical profilometer (Scantron Proscan 2000 V2, Taunton, England) to identify those with surface curvature below 0.3 μm, which were included in the study.

Group Division

After initial SMH measurements, the enamel and dentin specimens were divided into 8 groups (n=12) for each substrate according to the bleaching protocol (with and without), the erosive protocol (with and without), and the abrasive protocol (low and high abrasiveness). Sample size calculation was done based on data from a pilot study.

Bleaching Treatment and Erosive Protocol

Bleaching was performed on enamel and dentin according to the groups receiving this treatment. Each enamel and dentin specimen received a thin layer (20 μL) of a 9.5% hydrogen peroxide bleaching gel with neutral pH (Pola Day, SDI, Southern Dental Industries, Bayswater, Australia) for 14 days. The gel was placed for 30 min each day over the

specimens, according to the manufacturer instructions, and then washed with deionized water and dried with absorbent paper. Between each gel application, the specimens were kept in artificial saliva for remineralization. The composition of the artificial saliva used was: $MgCL_2.6H_2O$ (0.148 mmol/L), K_2HPO_4 (4.59 mmol/L), KH_2PO_4 (2.38 mmol/L), KCL (8.39 mmol/L), calcium lactate (1.76 mmol/L), fluoride (0.05 ppm), sodium carboxymethylcellulose (2.25 mmol/L), and methyl-4-hydroxybenzoate (13.14 mmol/L), with the pH adjusted to 7.2 with KOH.[19]

The model adopted for the specimens from the groups submitted to erosion-abrasion consisted of three daily immersions in citric acid (0.3%, pH 3.8) for 5 mins without agitation, followed by washing in deionized water and immersions in artificial saliva for 60 min for remineralization. Specimens were placed individually in a standard volume (10 mL) of acid and saliva. For groups without erosion, distilled water was used.

The abrasion was performed using either a low abrasive dentifrice (RDA < 70, Colgate Cavity Protection Regular™, Colgate-Palmolive Colgate, New Jersey, USA), or a high-abrasive dentifrice (RDA between 120–130, Colgate Advanced Whitening; Colgate-Palmolive Colgate, New Jersey, USA) from which a slurry was prepared (dentifrice: saliva - 1:3 weight ratio). The same standard toothbrush (Oral B indicator soft, Procter & Gamble, USA) was used for all the groups, and abrasion was performed 2×/day (15 s of brushing – 2 N load, followed by 105 s without brushing)[22] using a toothbrushing machine (Sabri Dental Enterprises, Downers Grove, IL, USA). The erosive/abrasive cycle was performed for 14 days. It consisted of 3 immersions in the acid and 2 abrasions, with immersions in artificial saliva for remineralization between them. Overnight the specimens were also kept immersed in the artificial saliva at room temperature (21ºC). Figure 1 shows the erosive/abrasive cycle used.

Final Microhardness

After the 14 days of bleaching treatment and erosive-abrasive cycles, the final microhardness (SMHf) was measured following the same protocol previously described. For dentin specimens, the measurements were made with the specimen moist, immediately after removing it from the artificial saliva and using absorbent paper to remove excess moisture.

Optical Profilometry Analysis

The enamel and dentin surface loss after the erosive/abrasive model was measured by optical profilometry

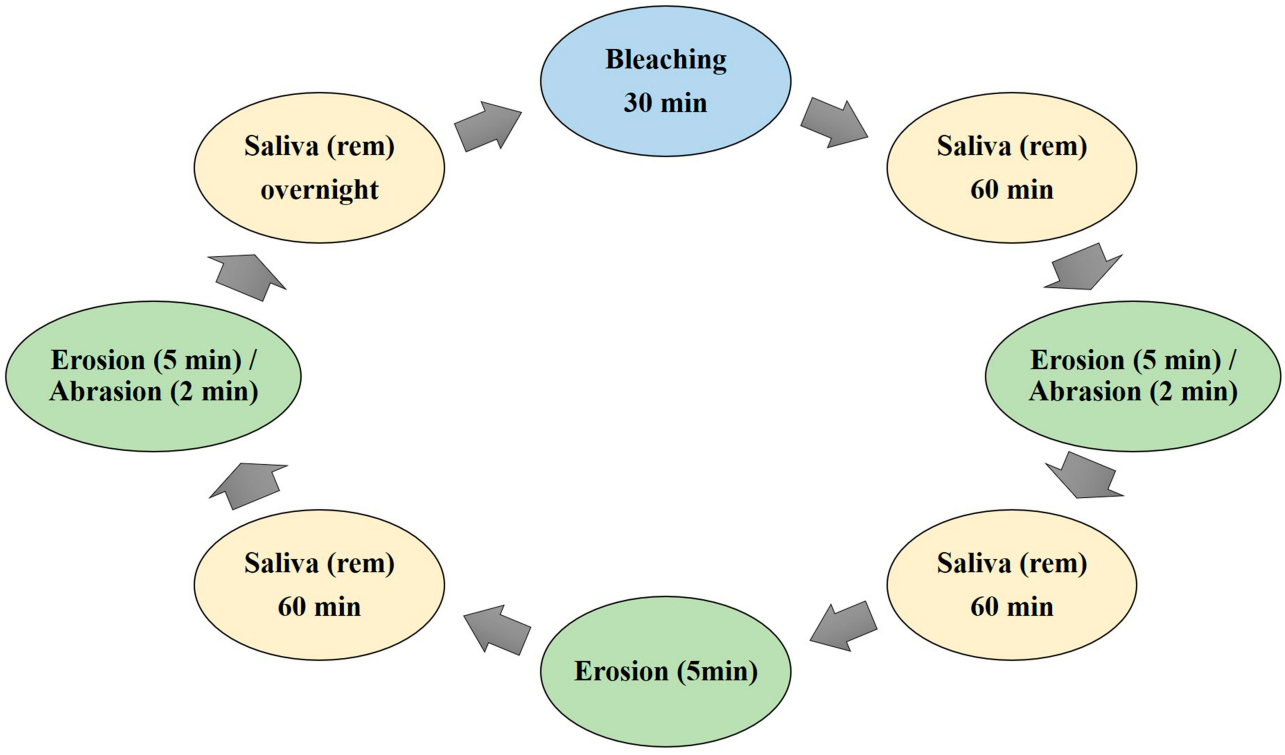

Figure 1 Schematic of the erosive/abrasive protocol used.

(Scantron Proscan 2000 V2, Taunton, England). For that, the varnish was removed with acetone, and an area of 2 mm in length and 1 mm in width was scanned, including the treated surface and the untreated one. The resolutions used for the readings were 0.01 µm at the x-axis and 0.05 µm at the y-axis. The images were analyzed by using dedicated software (Scantron Proscan 2000 V2.1.1.15D+, Taunton, England), and the surface loss calculated by subtracting the mean height from the reference area and the treated one; the final value was expressed in µm.

Statistical Analysis

The enamel and dentin substrates were separately analyzed. Data from surface loss and microhardness were nonparametric, so they were transformed to $\log_{10}$ to satisfy the assumptions of the ANOVA. Microhardness data were then submitted to repeated measures ANOVA followed by the Tukey's test, and the surface loss was submitted to 2-way ANOVA followed by the Tukey's test (α=0.05).

Results

Microhardness

For the enamel, no significant differences were found among the groups tested, considering the erosive-abrasive cycle, the bleaching, and the abrasiveness of the dentifrice tested. However, the final microhardness values were significantly lower than the initial ones (Table 1).

For dentin, differences were found between the eroded and the non-eroded groups, with the eroded dentin having lower microhardness values. In addition, bleaching was associated with a decrease in microhardness values only for the highly abrasive dentifrice, and the final values were lower than the initial ones for all the groups tested (Table 2).

Surface Loss

Significant differences were found in enamel surface loss among the factors studied ($p<0.05$). The highly abrasive dentifrice resulted in higher mineral loss, regardless of the group tested, and the groups submitted to the erosive/abrasive cycle model also had higher mineral loss. However, bleaching alone did not increase surface loss. For dentin, all three factors promoted significant differences, with higher abrasive dentifrices, the presence of the erosive/abrasive cycle, and bleaching being associated with higher surface loss (Table 3).

SEM images were obtained from one specimen of each group for qualitative analysis. Figure 2 shows examples of enamel samples submitted to bleaching with and without erosion, showing that bleaching and the use of high- or low-abrasive dentifrice were not harmful. The same was observed for dentin (Figure 3), except that the use of a highly abrasive dentifrice was associated with erosion with an increase in tubule exposure (Figure 3C) when compared with a low-abrasive dentifrice (Figure 3D).

Discussion

The null hypotheses tested were rejected, as the association between erosion and abrasion significantly reduced the microhardness and surface loss of bleached enamel and dentin; and higher abrasive dentifrices also led to a larger decrease in microhardness and more surface loss for dentin.

Table 1 Mean and SD for Enamel Vickers Microhardness Values

Abrasiveness	Bleaching	Erosion	Time	
			Initial	Final
Low	W/o	With W/o	346.58 (18.63) A 338.21 (17.70) A	266.04 (9.06) B 277.79 (7.09) B
	With	With W/o	342.12 (19.80) A 344.34 (22.37) A	257.79 (3.42) B 272.62 (7.47) B
High	W/o	With W/o	343.48 (20.76) A 348.60 (17.14) A	273.58 (17.22) B 275.60 (6.69) B
	With	With W/o	339.58 (16.96) A 339.66 (15.52) A	258.94 (3.95) B 275.04 (6.77) B

Notes: *Erosion/Abrasion* refers to the groups subjected to the erosive/abrasive cycle model, and *W/o* refers to those without erosion/abrasion. Uppercase letters show differences within lines between the initial and final measurements. There were no significant differences between bleaching and erosive/abrasive conditions ($p>0.05$) and also no significant differences between the abrasiveness tested ($p>0.05$).

Table 2 Mean and SD for Dentin Vickers Microhardness Values

Abrasiveness	Bleaching	Erosion	Time	
			Initial	Final
Low	W/o	With W/o	62.05 (4.60) Aa 63.14 (4.86) Aa	42.36 (2.30) Ba 61.23 (4.34) Ab
	With	With W/o	61.92 (3.81) Aa 62.29 (4.50) Aa	42.11 (2.57) Ba* 60.83 (2.12) Bb
High	W/o	With W/o	62.58 (3.30) Aa 62.47 (4.40) Aa	42.11 (1.67) Ba 60.64 (2.96) Ab
	With	With W/o	60.36 (4.29) Aa 62.29 (2.90) Aa	37.17 (2.63) Bc* 59.99 (2.57) Bb

Notes: *Erosion/Abrasion* refers to the groups subjected to the erosive/abrasive cycle model, and *W/o* refers to those without erosion/abrasion. Uppercase letters show differences within lines, between the initial and final measurements. Lowercase letters shows differences within columns, for the erosive/abrasive and bleaching conditions. The asterisk (*) shows differences between the abrasiveness (low × high) for the same condition (bleaching and erosion/abrasion) tested.

Table 3 Mean and Standard Deviation for Surface Loss (μm)

Substance	Bleaching	Erosion	Abrasiveness	
			Low	High
Enamel	W/o	With W/o	0.89 (0.47) Aa 0.06 (0.03) Ab	1.23 (0.56) Ba 0.08 (0.03) Bb
	With	With W/o	0.80 (0.15) Aa 0.06 (0.02) Ab	0.96 (0.24) Ba 0.08 (0.03) Bb
Dentin	W/o	With W/o	3.17 (0.55) Aa * 0.31 (0.10) Ab #	3.66 (0.63) Ba * 0.98 (0.16) Bb #
	With	With W/o	2.21 (0.42) Aa ** 0.22 (0.06) Ab ##	3.56 (0.77) Ba ** 0.31 (0.07) Bb ##

Notes: Uppercase letters show differences within lines between the low and high abrasiveness. Lowercase letters show differences within columns for enamel and dentin separately considering the erosive/abrasive cycle. For the bleaching factor, only dentin showed differences: asterisks show differences between the absence (*/#) and presence (**/##) of bleaching considering the same condition tested.

Hydrogen peroxide is the active ingredient in bleaching gels, whitening by releasing free radicals with a high capacity to oxidize the chromophore molecules responsible for tooth staining.[23] Although this redox generally occurs only at the organic stains without dissolving the enamel matrix, long exposure periods could result in the dissolution of this matrix.[12,24] This would have deleterious effects on the tooth surface, such as alterations in the enamel morphology, chemical composition, and microhardness values,[25] with a few studies also describing mild erosive effects promoted by acidic bleaching agents.[9,11,26,27]

The hydrogen peroxide gel used in the present study contained 9.5% hydrogen peroxide at a neutral pH and simulated the at-home technique with daily applications of 30 min, for 14 days. The use of hydrogen peroxide instead of carbamide peroxide reduces the daily use period. The outcomes from this study indicated that, for enamel, the bleaching treatment did not predispose it to greater erosive wear. The possible microstructural changes induced by the bleaching agent may have been repaired by the adsorption and precipitation of salivary calcium and phosphate derived from the artificial saliva applied before the erosive challenge.[10,21,28]

The use of high- or low-abrasive dentifrices did not cause greater harm to the enamel (Tables 1 and 3, and Figure 2), indicating that erosion per se is the main cause of enamel wear. Previous studies have reported that the abrasion resistance of enamel is reduced when the surface is eroded,[4] which is consistent with the findings of the present study; however, an increase in enamel loss with the increasing abrasiveness of the toothpaste[19] was not

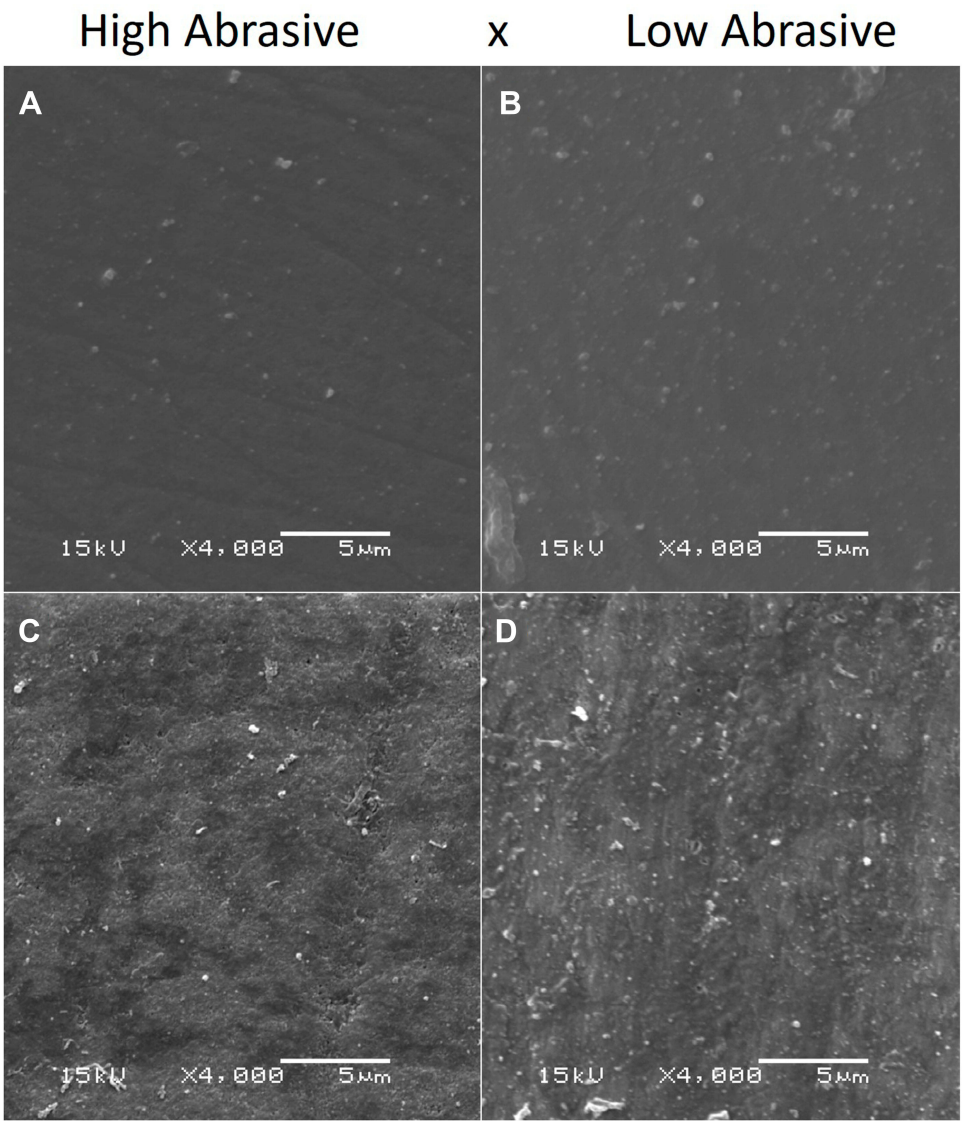

Figure 2 Enamel: (**A**) and (**B**) with bleaching and without erosion; (**C**) and (**D**) with bleaching and with erosion.

observed (Figure 2). As this finding is consistent with that of a previous study using carbamide peroxide,[5] both bleaching protocols (hydrogen peroxide and carbamide peroxide) might be considered safe for clinical use. Regarding the microhardness reduction found for enamel in the groups without erosion and bleaching (Table 1), the polishing procedure might have induced the formation of a slightly smooth surface, which was removed by the abrasion during the 14-day protocol used, and previously discussed.[28]

Although bleaching is not recommended directly on dentin, it was included to evaluate the potential harmful effect of bleaching gels in patients with dentin exposed by gingival recession. Safety regulations require bleaching gels to be tested in both enamel and dentin, and therefore, we chose to investigate the influence of bleaching with erosion and abrasion on both substrates.

The results indicated that dentin was more sensitive to microhardness and surface loss after erosion/abrasion cycles and after bleaching. For dentin, the abrasiveness of the dentifrice was relevant only for surface loss, with the highly abrasive dentifrice being associated with greater loss, consistent with previous findings.[19,29] The lower microhardness and higher surface loss found for the dentin groups with bleaching might indicate the oxidation of the organic matrix by the hydrogen peroxide, increasing surface loss. Consistent with the findings of the present study (Figure 3C and D), the use of a low or moderate RDA toothpaste,[30,31] without toothbrushing directly after acidic challenge,[32] has been reported to decrease dentin loss,

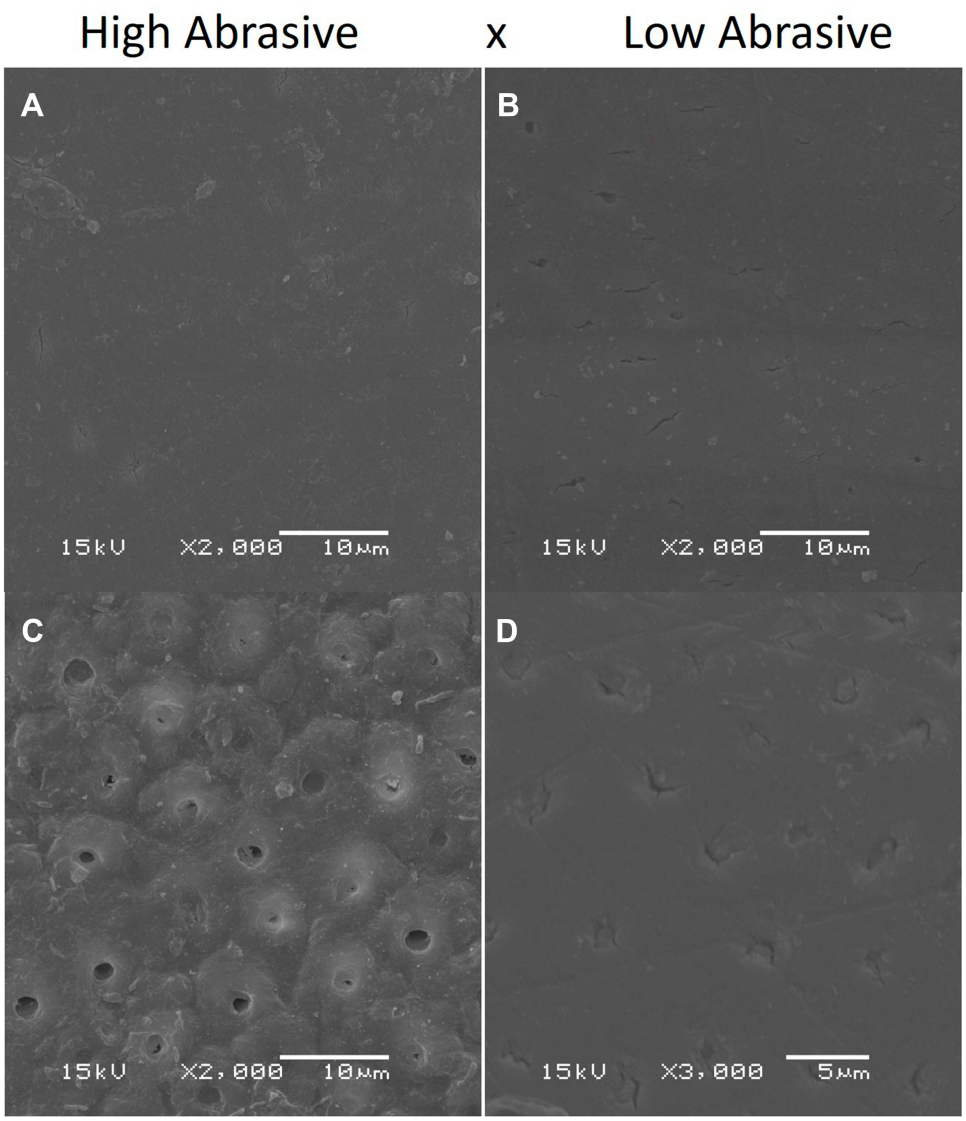

Figure 3 Dentin: (**A**) and (**B**) with bleaching and without erosion; and (**C**) and (**D**) with bleaching and with erosion.

indicating that the more abrasive dentifrice was more harmful to dentin. The presence of open tubules increases the risk for dentin hypersensivity,[33] and our results indicated that the use of highly abrasive dentifrices associated with erosion promoted more tubule exposure. Recommending a low abrasive dentifrice should be considered for patients with dentin sensitivity and a high risk of erosion, although bleaching is not contraindicated for these patients. Limitations of this in vitro study included that enamel rehardening in vitro is slow and rehardening of dentin may not occur.[29] Also, the use of artificial saliva might not reflect the same remineralization induced by human saliva,[34] and the absence of an acquired pellicle might have affected the findings.[35] Therefore, clinical studies are indicated to confirm the results.

Conclusions

The use of dentifrices with high and low abrasiveness during bleaching and concomitant with erosion/abrasion cycles does not interfere in enamel microhardness but increased its loss when highly abrasive dentifrice was used. For dentin, the association of erosion and abrasion increased loss and decreased microhardness, independently of the presence or not of bleaching. Dentifrices with low abrasiveness should be used when bleaching is performed.

Ethical Approval

The study was carried out in accordance with the principles of the Declaration of Helsinki, and all patients signed a letter of a written consent for enrollment in the research

and data publishing. The protocol was reviewed by the Institutional Review Board of The University of Texas Health Science Centre at San Antonio (UTHSCSA - protocol number HSC20080233N).

Disclosure

The authors report no conflicts of interest in this work.

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