

***In vitro* effects of alcohol-containing mouthwashes on human enamel and restorative materials**

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Abstract: The objective of this study was to evaluate the *in vitro* effects, including surface morphological characteristics and chemical elemental properties, of different mouthwash formulations on enamel and dental restorative materials, simulating up to 6 months of daily use. Human enamel samples, hydroxyapatite, composite resin, and ceramic surfaces were exposed to 3 different mouthwashes according to label directions – Listerine® Cool Mint®, Listerine® Total Care, and Listerine® Whitening – versus control (hydroalcohol solution) to simulate daily use for up to 6 months. The samples were analyzed using scanning electron microscopy (SEM), infrared spectrophotometry (μ -Fourier transform infrared microscopy), energy-dispersive X-ray (EDX) spectroscopy, and color analysis before and after exposure. No relevant changes were observed in the morphological characteristics of the surfaces using SEM techniques. The physical and chemical aspects of the enamel surfaces were evaluated using mid-infrared spectroscopy, and EDX fluorescence was used to evaluate the elemental aspects of each surface. There was no variation in the relative concentrations of calcium and phosphorus in enamel, silicon and barium in composite resin, and silicon and aluminum in the ceramic material before and after treatment. No relevant changes were detected in the biochemical and color properties of any specimen, except with Listerine® Whitening mouthwash, which demonstrated a whitening effect on enamel surfaces. Long-term exposure to low pH, alcohol-containing, and peroxide-containing mouthwash formulations caused no ultra-structural or chemical elemental changes in human enamel or dental restorative materials *in vitro*.

Keywords: Mouthwashes; Composite Resins; Hydroxyapatites; Ceramics; Microscopy, Electron, Scanning.

Introduction

Biofilm control is paramount in preventive dentistry and directly reflects the oral health of individuals. Various methods, such as mechanical procedures, chemical agents, or a combination of both, can be used to achieve adequate biofilm control.^{1,2} Pathogenic microorganisms present in the biofilm are a contributing factor to dental caries and periodontal disease, suggesting that removal of biofilm can be essential to the prevention of such conditions. Mechanical control of the biofilm continues to be important

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in preventing periodontal diseases and maintaining oral health. In addition, the use of mouthwashes has shown substantial ability for biofilm control.

The prevention of oral diseases and the promotion of oral health must be comprehensively addressed to control the initiation and development of dental caries and periodontal diseases, which have been shown to compromise both oral and general health. The Council of Scientific Affairs (American Dental Association [ADA]) has adopted a program to evaluate the efficacy of various agents used to control gingivitis via biofilm control. According to the criteria set forth, such agents must be evaluated for over 6 months, by placebo-controlled clinical trials and demonstrate a significant improvement in gingival health when compared to a placebo group. To date, 2 mouthwash agents have been approved for use by the ADA to control dental plaque and gingivitis: chlorhexidine and a fixed combination of essential oils (eucalyptol [0.092%], menthol [0.042%], methyl salicylate [0.060%], and thymol [0.064%]).

The prevalence of gingivitis and periodontal diseases is high, even in young people.^{3,4} For a considerable proportion of individuals, mechanical methods alone are not sufficient to maintain gingival health and prevent the occurrence, recurrence or progression of periodontal diseases. The addition of chemotherapeutic agents for biofilm control can augment the effect of mechanical methods and help prevent the development of periodontal diseases.^{1,5,6} Though essential oil-containing mouthwashes have been proven safe and efficacious in daily use for over 100 years,⁷ there are still safety concerns regarding tooth enamel and dental restorative materials because of their low pH (< 5.5),^{8,9} the presence of peroxide (for tooth whitening), and alcohol¹⁰ (used as a solubilizer and preservative).¹⁰ Therefore, this *in vitro* study aimed to compare the effect of 3 different mouthwash formulations on human enamel, commonly used dental restorative materials, and hydroxyapatite, over a simulated 3- to 6-month period of daily use.

Methodology

Human teeth, comprising premolars and third molars that were extracted due to orthodontic or surgical indications, were obtained from the School of Dentistry

at the University of Taubaté. Teeth with abnormal features such as cracks, cavities, and fillings close to the testing area were excluded. In total, 20 teeth were used in this study, and the study protocol was approved by the ethics committee of the School of Dentistry at the University of Taubaté (Protocol# 388/10).

Teeth and specimen preparation

The crown of each tooth was separated from the root, and 4 samples from each crown surface (buccal, lingual, mesial, and distal), measuring 4.0 ± 0.2 mm \times 4.0 ± 0.2 mm with a thickness of 6.0 ± 0.2 mm, were obtained. The hydroxyapatite disks measured 5.0 ± 0.2 mm (diameter) \times 2.0 ± 0.2 mm (thickness) and were white in color with no cracks (Lot # 080109-5 mm; Hitemco Medical, Old Bethpage, USA). Both the composite resin samples (Tetric N-Ceran, Ivoclar Vivadent; Shade A3.5, K42776, Exp. 2011-14), light cured using LEC Prime (WL SN/528; MM Optics Code: 10.02307), and the feldspathic ceramic samples (shade A2) measured 6.6 ± 0.2 mm (diameter) \times 1.40 ± 0.2 mm (thickness). Irregular or stained samples were discarded. The selected samples were rinsed using distilled water and stored in artificial saliva for ≤ 7 days (40 mL of complete saliva, Lot # 32699, Northeast Laboratory, USA; Lab-Lemco powder, proteose peptone, yeast extract, type II hog gastric mucin, sodium chloride, calcium chloride, purified water, and 10% urea solution) at room temperature until used for treatment.

Surfaces evaluation by scanning electron microscopy

High-resolution $100\times$ and $3,000\times$ images were acquired using a scanning electron microscope (SEM) and formatted virtually by transcription of the electron beam path between the sample and the condenser lenses of the microscope, forming a 3-dimensional image that was used to evaluate the surface morphology. The samples were attached to the SEM stubs and sputtered with a mix of gold and palladium. The morphology of the samples was determined using the EVO LS SEM (Carl Zeiss SMT GmbH, Germany), operated at 20 kV under high vacuum at a working distance of 14–15 mm.

Mineral quantification by energy-dispersive x-ray fluorescence spectrometry

Energy-dispersive X-ray (EDX) spectroscopy is an analytical technique used for elemental analysis, such as observation of demineralization in tooth surfaces. Semi-quantitative elemental analyses of the samples were performed using an energy-dispersive micro-X-ray fluorescence spectrometer (μ -EDX 1300, Shimadzu, Kyoto, Japan), equipped with a rhodium X-ray tube and a liquid nitrogen-cooled Si (Li) detector. The equipment was coupled to a computer system for data acquisition and processing (Shimadzu μ -EDX MP ver. 1.03). The voltage was set at 15 kV with an incident beam diameter of 50 μ m. Data were obtained by scanning 1.0 \times 1.0 mm sections of each specimen. Semi-quantitative elemental analyses of calcium (Ca) and phosphorus (P) concentrations in enamel and hydroxyapatite, silica (Si), and barium (Ba) concentrations in composite resins. In ceramic samples, Si and aluminum (Al) concentrations were performed using the μ -EDX 1300, operating at 50 kV with an incident beam diameter of 50 μ m. Line mapping was performed using 40 \times 1 points with a 10- μ m step along each sample.

Infrared microscopy

The Fourier transform-Infrared Microscopy (FT-IRM) technique enables detailed observation of the surface through the reflection of the produced radiance, which is directly related to the observed chemical structure of the surfaces. Spectral FT-IR data were obtained using the Spectrum Spotlight 400 device (Perkin-Elmer, Wellesley, USA), equipped with a microscope fitted with both white light and infrared optics and a liquid nitrogen-cooled

mercury-cadmium telluride (MCT) detector. Spectra were collected at a 2 cm^{-1} resolution, in the range of 4000–750 cm^{-1} from 64 scans by using a reflectance technique with a point mode. The aperture size was 50 \times 50 μ m, and the spectra were baseline corrected, normalized, and transformed to the absorbance spectra.

Colorimetric analysis

The color measurements were determined by using a Minolta colorimeter (Konica Minolta model CR-400) and analyzing CIE $L^*a^*b^*$ parameters. A white-colored plate was used as a standard for calibration and as a background for color measurements. CIE $L^*a^*b^*$ values were evaluated based on reflectance measurements, where L^* indicates luminosity, a^* indicates redness, and b^* indicates yellowness. Three analyses were recorded before and after sample immersion.

Treatment

Table 1 presents the 4 treatment regimens that were randomly assigned to samples. Four different samples were immersed in 3 different mouthwashes as follows: Essential oils mouthwash (EO; LISTERINE® Cool Mint®), essential oils + fluoride (100 ppm) + zinc chloride mouthwash (EOFZC; LISTERINE® Total Care), 2.0% hydrogen peroxide mouthwash (HP; LISTERINE® Whitening Original Pre-brush Rinse), and 21.6% hydroalcohol control solution (H). Table 2 describes the mouthwashes used in this study. All samples were stored in artificial saliva between treatments and after each period of analysis: baseline, 1, and 3 months for HP, and baseline, 3, and 6 months for EO and EOFZC. Samples were immersed in the EO and EOFZC mouthwashes for

Table 1. Specimen distribution.

Groups	Enamel (E) (n = 5)	Hydroxyapatite (Ha) (n = 5)	Composite Resin (CR) (n = 5)	Ceramic (C) (n = 5)
Group 1 - Essential Oils (EO) Mouthwash (n = 20)	EO E1	EO Ha1	EO CR1	EO C1
Group 2 - Essential Oils + Fluoride + Zinc Chloride (EOFZC) Mouthwash (n = 20)	EOFZC E2	EOFZC Ha2	EOFZC CR2	EOFZC C2
Group 3 - 2.0% Hydrogen Peroxide (HP) Mouthwash (n = 20)	HP E3	HP Ha3	HP CR3	HP C3
Group 4 - Hydroalcohol Control Solution (H) (n = 20)	H E4	H Ha4	H CR4	H C4

3 cycles of 30 minutes each with three 30-minute intervals to simulate 3 months of daily use, and in 3 cycles of 60 minutes each with three 60-minute intervals to simulate 6 months of daily use. The total immersion time for simulating 3 months of daily use was 90 minutes. Similarly, the total immersion time for 6 months of daily use was 180 minutes (Table 3). The samples were immersed in the HP mouthwash in 3 cycles of 10 minutes each with three 10-minute intervals to simulate 1 month of daily use, and in 3 cycles of 30 minutes each with three 30-minute intervals to simulate 3 months of daily use. The total immersion time for simulating 1 month of daily use was 30 minutes. The total immersion time for 3 months of daily use was 90 minutes. The immersion time was based on a pilot study that aimed to simulate clinical use (Table 4).

Data analysis and statistical methods

Analyses were based on data from all samples, comprising human enamel, hydroxyapatite, composite resin, and ceramic samples treated with the study mouthwashes or control (H) solution without any major protocol violations. A 95% confidence interval of the least square (LS) means and Student's *t* test were used to compare the means obtained from the EDX and CIE L*a*b* color analyses.

For the primary analysis, use of EO, EOFZC, and HP mouthwashes and control (H) solution was assessed according to the following aspects:

- a. Possible overall morphological changes after an equivalent period of baseline and 3 and 6 months of treatment with EO and EOFZC compared with the initial morphological values of the tested samples.

Table 2. Ingredients and active components of the tested mouthwashes.

Active components	Brand name	Manufacturer	Ingredients
Essential Oils	LISTERINE® Cool Mint	Johnson & Johnson, São José dos Campos, SP, Brazil	Water, ethanol, menthol, eucalyptol, thymol, methyl salicylate, benzoic acid, poloxamer 407, and flavor
Essential Oils + Fluoride + Zinc Chloride	LISTERINE® Total Care	Johnson & Johnson, São José dos Campos, SP, Brazil	Water, ethanol, menthol, eucalyptol, thymol, methyl salicylate, benzoic acid, poloxamer 407, fluoride, zinc chloride, and flavor
2.0% Hydrogen Peroxide	LISTERINE® Whitening Original Pre-brush Rinse	Johnson & Johnson, São José dos Campos, SP, Brazil	Water, hydrogen peroxide, ethanol, menthol, eucalyptol, benzoic acid, poloxamer 407, and flavor
NA	Hydroalcohol Solution (21.6% alcohol)	Johnson & Johnson, São José dos Campos, SP, Brazil	Water and ethanol

Table 3. Immersion time per group for the essential oils (EO) and essential oils + fluoride + zinc chloride (EOFZC) mouthwashes.

G1/G2, minutes	A1	3 months	A2	6 months	A3
IP		30		60	
INT		30		60	
IP		30		60	
INT	Baseline / Analysis 1	30	Analysis 2	60	Analysis 3
IP		30		60	
INT		30		60	
TIP		90		180	

G1 = Group 1, G2 = Group 2, IP = immersion period, INT = interval, TIP = total immersion period, A1 = baseline, A2 = analysis 2, A3 = analysis 3.

Table 4. Immersion time per group for the 2.0% hydrogen peroxide (HP) mouthwash and control solution (H).

G3/G4, minutes	A1	1 month	A2	3 months	A3
IP		10		30	
INT		10		30	
IP		10		30	
INT	Baseline / Analysis 1	10	Analysis 2	30	Analysis 3
IP		10		30	
INT		10		30	
TIP		30		90	

G3 = Group 3, G4 = Group 4, IP = immersion period, INT = interval, TIP = total immersion period, A1 = baseline, A2 = analysis 2, A3 = analysis 3.

- b. Possible overall chemical changes after an equivalent period of baseline and 3 and 6 months of treatment with EO and EOFZC compared with the initial chemical values of the tested samples.
- c. Possible overall $L^*a^*b^*$ changes after an equivalent period of baseline and 3 and 6 months of treatment with EO and EOFZC compared with the initial $L^*a^*b^*$ values of the tested samples.
- d. Possible overall morphological changes after an equivalent period of baseline and 1 and 3 months of treatment with HP compared with the initial morphological values of the tested samples.
- e. Possible overall chemical changes after an equivalent period of baseline and 1 and 3 months of treatment with HP compared with the initial chemical values of the tested samples.
- f. Possible overall $L^*a^*b^*$ changes after an equivalent period of baseline and 1 and 3 months of treatment with HP compared with the initial $L^*a^*b^*$ values of the tested samples.

The regression models were adjusted for emphasis in effect screening and the analyses were performed using the JMP statistical software version 12 with the significance level set at $\alpha = 0.05$. Differences with p-values of < 0.05 were considered significant. Concerning colorimetric analysis, ΔE values < 3 were considered not clinically relevant since they are weakly perceptible to the eye.

Results

Scanning electron microscopy

No changes were observed in dental enamel, hydroxyapatite, and composite resin surfaces after immersion in the EO and EOFZC mouthwashes as well as the control solution. A superficial change was observed on the enamel surface at 1 month after using the HP mouthwash, which was reversed after 3 months of daily use. A trend toward change in erosion without demineralization was observed after exposure to the HP mouthwash and control solution. Most of the changes were regular circular erosions, measuring 2 to 3 μm , observed to a considerable extent on the ceramic samples with respect to the HP mouthwash, and regular

circular erosions, measuring 1 to 2 μm , at 1 month and $>10 \mu\text{m}$ at 3 months with the control solution. There was no change on the ceramic samples after exposure to the EO mouthwash. The ceramic samples exposed to the EOFZC mouthwash showed mild changes on the surface after 3 months, but showed normal appearance after 6 months of exposure (Figures 1–5).

Energy-dispersive X-ray

No demineralization was observed on the enamel and hydroxyapatite surfaces tested during any examination period. The EDX analysis showed that Ca and P concentrations remained the same before and after exposure to mouthwashes. Similar results were observed for composite resin and ceramic surfaces. Moreover, Si and Ba concentrations remained the same for composite resin surfaces and Si and Al concentrations remained the same for ceramic surfaces before and after exposure to mouthwashes (Table 5).

FT-IRM

A detailed observation of the surfaces was recorded using infrared microscopy, through the reflection of radiance to evaluate not only the visible image but also the image formed by infrared spectrum (chemical imaging). The spectra obtained from the studied surfaces corresponded to the pattern spectra of the enamel, ceramic, and composite resin; no change was observed even after simulating 6 months of daily use.

Colorimetric analysis

The color measurements (Konica Minolta model CR-400) were based on the CIE $L^*a^*b^*$ measurements. Luminosity (L^*)/coordinate a^* (variation between red and green)/coordinate b^* (variation between yellow and blue) were determined. No change was detected in the color properties of any specimen, except for the HP rinse, which had a whitening effect on the enamel surface (Table 6).

Discussion

The results showed that simulated long-term exposure (up to 6 months) to alcohol-containing mouthwashes (EO, EOFZC, and HP) caused no morphological, ultra-structural, or biochemical change

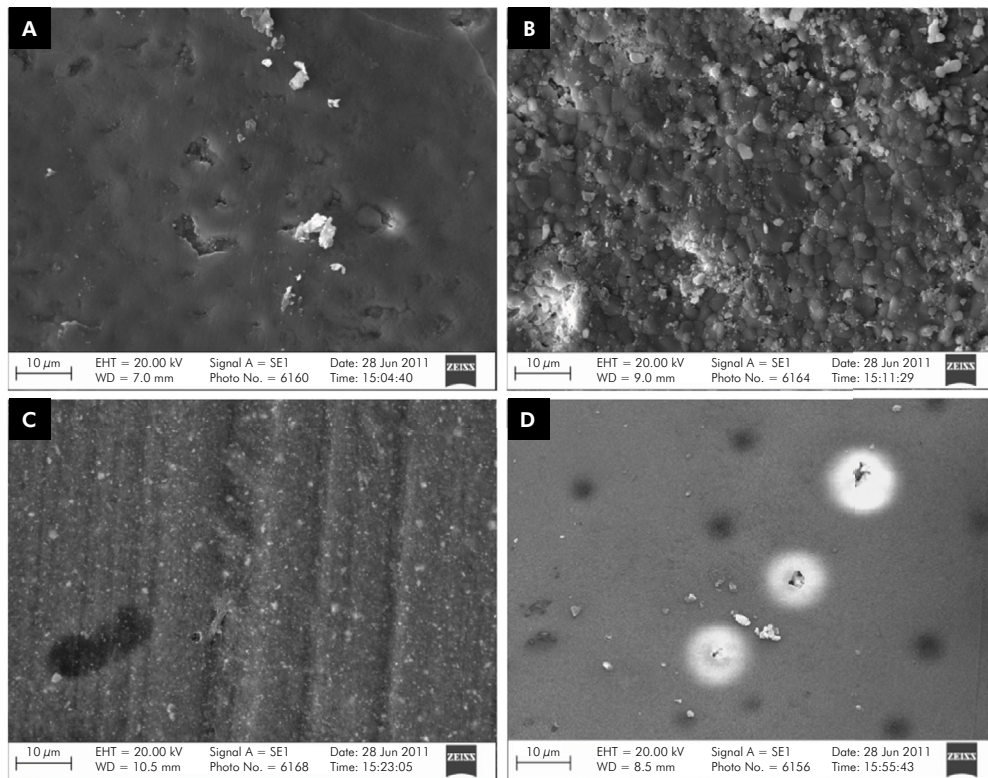


Figure 1. Baseline specimens without treatment: (A) enamel, (B) hydroxyapatite, (C) composite resin, and (D) ceramic (3,000×)

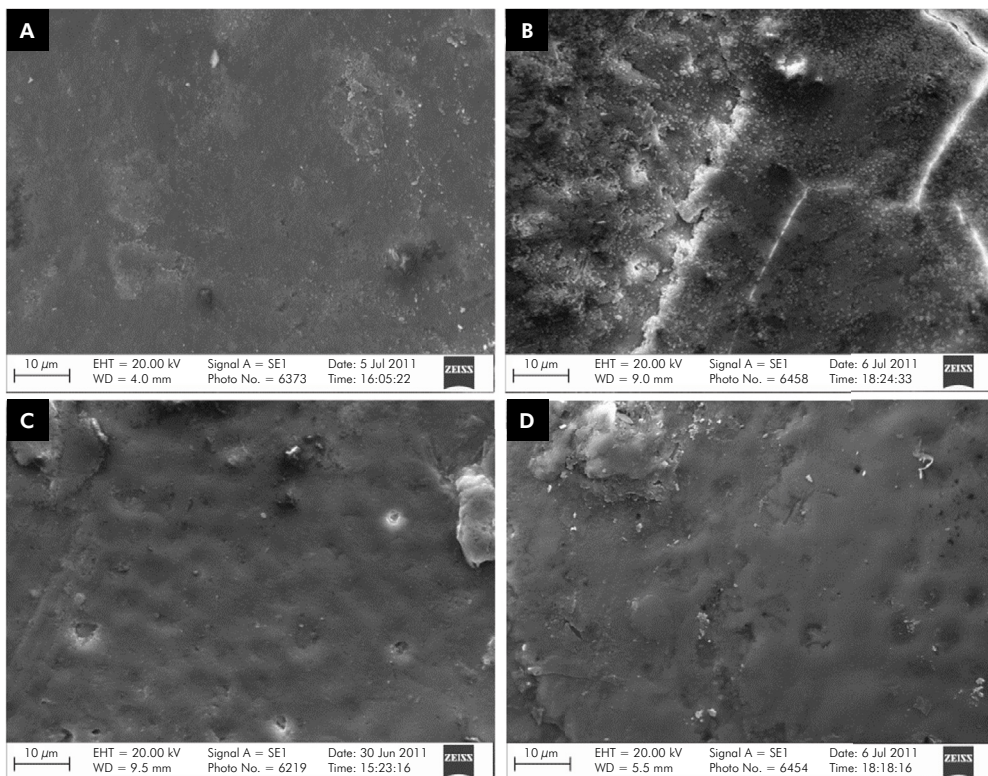


Figure 2. SEM images of enamel surfaces after each treatment period: (A) EO after 6 months, (B) EOFZC after 6 months, (C) HP after 3 months, and (D) H after 6 months (3,000×). EO, essential oils mouthwash; EOFZC, essential oils + fluoride + zinc chloride mouthwash; H, hydroalcohol control solution; HP, hydrogen peroxide mouthwash; SEM, scanning electron microscope

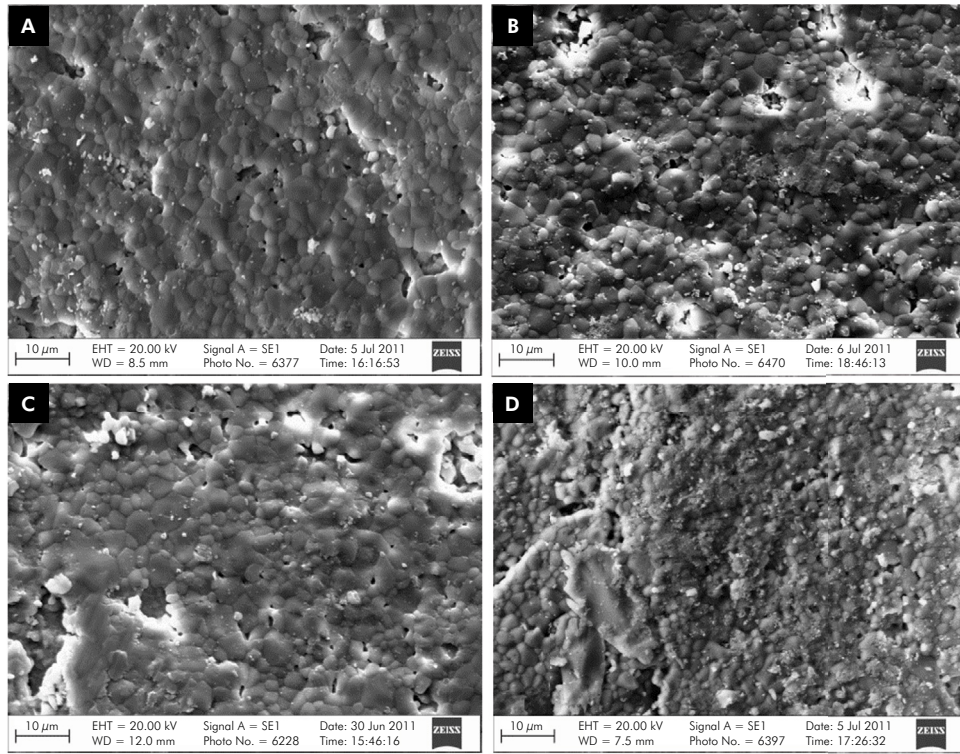


Figure 3. SEM images of hydroxyapatite surfaces after each treatment period: (A) EO after 6 months, (B) EOFZC after 6 months, (C) HP after 3 months, and (D) H after 6 months (3,000×). EO, essential oils mouthwash; EOFZC, essential oils + fluoride + zinc chloride mouthwash; H, hydroalcohol control solution; HP, hydrogen peroxide mouthwash; SEM, scanning electron microscope

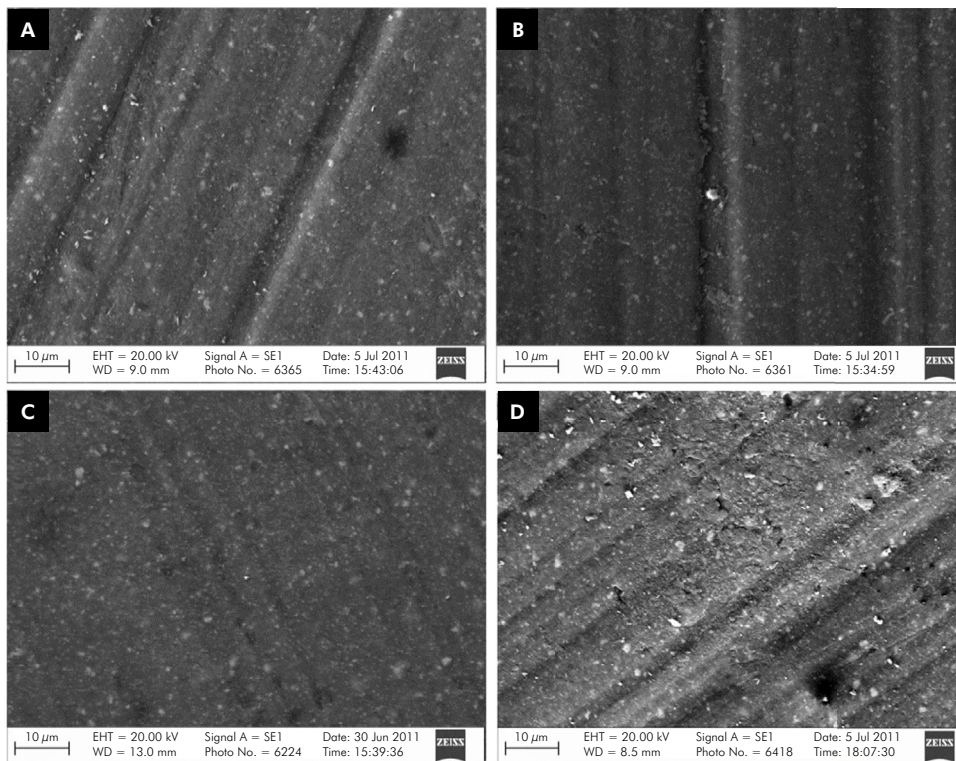


Figure 4. SEM images of composite resin surfaces after each treatment period: (A) EO after 6 months, (B) EOFZC after 6 months, (C) HP after 3 months, and (D) H after 6 months (3,000×). EO, essential oils mouthwash; EOFZC, essential oils + fluoride + zinc chloride mouthwash; H, hydroalcohol control solution; HP, hydrogen peroxide mouthwash; SEM, scanning electron microscope

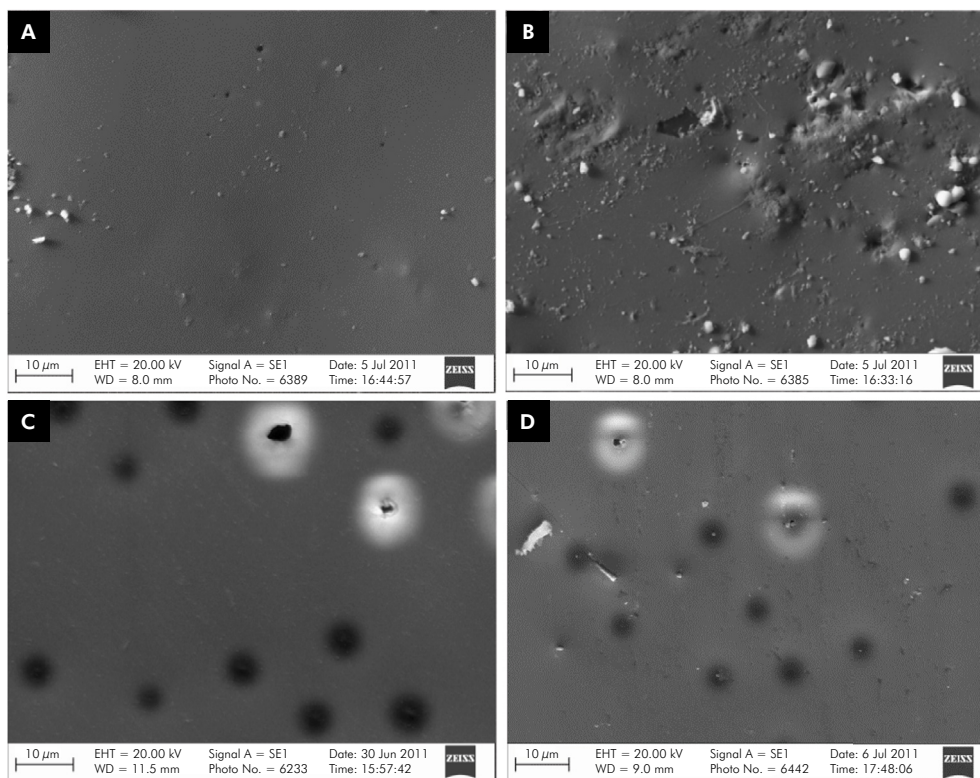


Figure 5. SEM images of ceramic surfaces after each treatment period: (A) EO after 6 months, (B) EOFZC after 6 months, (C) HP after 3 months, and (D) H after 6 months (3,000×). EO, essential oils mouthwash; EOFZC, essential oils + fluoride + zinc chloride mouthwash; H, hydroalcohol control solution; HP, hydrogen peroxide; SEM, scanning electron microscope

Table 5. Mean values for mineral elements in different surfaces and treatments obtained by EDX semi-quantitative mapping.

Time point	Treatment	Enamel	Hydroxyapatite	Resin	Ceramic
		Ratio (Ca)/(P)	Ratio (Ca)/(P)	Ratio (Si)/(Ba)	Ratio (Si)/(Al)
Baseline	NA	1.82 ± 0.13	1.86 ± 0.10	2.05 ± 0.28	1.06 ± 0.10
1 month	HP	1.78 ± 0.45	1.87 ± 0.11	2.01 ± 0.19	1.05 ± 0.11
3 months	HP	1.82 ± 0.01	1.82 ± 0.05	1.96 ± 0.03	1.09 ± 0.07
3 months	EO	1.75 ± 0.03	1.83 ± 0.07	2.09 ± 0.00	1.08 ± 0.13
3 months	EOFZC	1.79 ± 0.17	1.81 ± 0.05	2.07 ± 0.16	1.11 ± 0.12
3 months	H	1.76 ± 0.55	1.84 ± 0.14	2.01 ± 0.09	1.10 ± 0.10
6 months	EO	1.76 ± 0.20	1.87 ± 0.18	2.03 ± 0.19	1.00 ± 0.08
6 months	EOFZC	1.75 ± 0.27	1.82 ± 0.08	2.07 ± 0.12	0.99 ± 0.09
6 months	H	1.75 ± 0.06	1.82 ± 0.09	2.00 ± 0.15	0.99 ± 0.07

Note: Average obtained by surface mapping using 40 × 1 points with a step of 10 μm along each sample until 1.0 × 1.0 mm. The average was compared by LS means and Student’s t-tests and showed no significant variation.

Al = aluminum; Ba = barium; Ca = EDX = energy-dispersive X-ray spectroscopy, EO = essential oils mouthwash; EOFZC = essential oils + fluoride + zinc chloride mouthwash; H = hydroalcohol solution; HP = 2.0% hydrogen peroxide mouthwash; LS = least squares.

in human enamel, hydroxyapatite, composite resin, and feldspathic ceramic surfaces under the experimental conditions simulating label directions. Furthermore, the samples tested did not undergo remineralization phases that are typically experienced *in vivo*.¹¹ No

relevant change was seen as a result, although an overestimation of exposure time seemed likely.

Certain results from the present study are not consistent with findings from the available literature regarding the morphological and chemical stability as well

Table 6. ΔE^*ab , ΔL , and L^*a^*b mean and standard deviation values (n = 5).

Time point	Treatment	Material	Luminosity (L*)	Red/Green (a*)	Yellow/Blue (b*)	ΔL	ΔE^*ab
Baseline	No treatment	Ceramic	70.54 (\pm 0.51)	1.43 (\pm 0.33)	18.06 (\pm 0.63)		
		Enamel	69.34 (\pm 0.27)	0.46 (\pm 0.20)	7.84 (\pm 0.68)		
		Hydroxyapatite	89.25 (\pm 0.64)	0.35 (\pm 0.15)	1.67 (\pm 0.12)		
		Resin	80.06 (\pm 0.66)	1.30 (\pm 0.15)	17.29 (\pm 1.01)		
1 month	HP	Ceramic	70.37 (\pm 0.94)	1.36 (\pm 0.23)	18.36 (\pm 0.41)	-0.28 (\pm 0.37)	0.72 (\pm 0.24)
		Enamel	74.71 (\pm 1.27)	0.20 (\pm 0.03)	7.63 (\pm 0.09)	5.37 (\pm 1.27)	5.38 (\pm 1.27)
		Hydroxyapatite	89.40 (\pm 0.12)	0.39 (\pm 0.20)	1.63 (\pm 0.12)	0.07 (\pm 0.12)	0.23 (\pm 0.15)
		Resin	80.08 (\pm 0.08)	1.33 (\pm 0.03)	17.25 (\pm 0.06)	0.02 (\pm 0.08)	0.09 (\pm 0.07)
3 months	HP	Ceramic	70.63 (\pm 0.44)	1.45 (\pm 0.07)	18.25 (\pm 0.19)	0.09 (\pm 0.44)	0.46 (\pm 0.15)
		Enamel	76.80 (\pm 0.70)	0.17 (\pm 0.05)	6.96 (\pm 0.47)	7.53 (\pm 0.75)	7.46 (\pm 0.70)
		Hydroxyapatite	89.14 (\pm 0.29)	0.54 (\pm 0.16)	2.12 (\pm 0.33)	-0.18 (\pm 0.29)	0.63 (\pm 0.20)
		Resin	79.69 (\pm 0.05)	1.28 (\pm 0.04)	17.21 (\pm 0.49)	-0.37 (\pm 0.05)	0.56 (\pm 0.16)
3 months	EO	Ceramic	70.93 (\pm 1.70)	1.20 (\pm 0.11)	18.36 (\pm 0.32)	0.40 (\pm 1.70)	1.42 (\pm 0.93)
		Enamel	70.76 (\pm 0.64)	0.28 (\pm 0.07)	8.29 (\pm 0.05)	1.42 (\pm 0.64)	1.51 (\pm 0.61)
		Hydroxyapatite	89.08 (\pm 0.23)	0.42 (\pm 0.01)	2.44 (\pm 0.18)	-0.17 (\pm 0.23)	0.81 (\pm 0.23)
		Resin	78.58 (\pm 1.12)	1.25 (\pm 0.14)	16.56 (\pm 0.61)	-1.48 (\pm 1.12)	1.67 (\pm 1.67)
3 months	EOFZC	Ceramic	70.65 (\pm 1.00)	1.17 (\pm 0.29)	18.17 (\pm 1.33)	-0.22 (\pm 0.78)	1.19 (\pm 0.14)
		Enamel	72.82 (\pm 0.79)	0.28 (\pm 0.09)	9.33 (\pm 0.09)	3.20 (\pm 0.56)	3.56 (\pm 0.47)
		Hydroxyapatite	88.76 (\pm 0.15)	0.42 (\pm 0.02)	2.42 (\pm 0.17)	-0.49 (\pm 0.15)	0.90 (\pm 0.21)
		Resin	79.70 (\pm 0.57)	0.63 (\pm 0.10)	15.96 (\pm 0.42)	-0.36 (\pm 0.57)	1.61 (\pm 0.43)
3 months	H	Ceramic	70.23 (\pm 1.13)	1.32 (\pm 0.36)	18.40 (\pm 1.56)	-0.30 (\pm 1.13)	1.55 (\pm 1.06)
		Enamel	70.48 (\pm 0.78)	0.11 (\pm 0.06)	9.83 (\pm 0.08)	1.14 (\pm 0.78)	2.42 (\pm 0.31)
		Hydroxyapatite	88.14 (\pm 0.62)	0.43 (\pm 0.02)	2.27 (\pm 0.06)	-1.12 (\pm 0.62)	1.31 (\pm 0.52)
		Resin	77.70 (\pm 0.60)	0.89 (\pm 0.02)	16.08 (\pm 0.28)	-2.36 (\pm 0.60)	2.69 (\pm 0.66)
6 months	EO	Ceramic	70.23 (\pm 0.51)	1.31 (\pm 0.14)	18.23 (\pm 0.62)	-0.31 (\pm 0.51)	0.78 (\pm 0.27)
		Enamel	72.13 (\pm 0.72)	0.32 (\pm 0.03)	8.31 (\pm 0.06)	2.79 (\pm 0.72)	2.84 (\pm 0.72)
		Hydroxyapatite	89.03 (\pm 0.18)	0.42 (\pm 0.02)	2.40 (\pm 0.17)	-0.22 (\pm 0.18)	0.78 (\pm 0.21)
		Resin	79.30 (\pm 0.40)	1.71 (\pm 0.06)	16.16 (\pm 0.08)	-0.76 (\pm 0.40)	1.45 (\pm 0.27)
6 months	EOFZC	Ceramic	70.61 (\pm 0.54)	1.43 (\pm 0.14)	18.96 (\pm 0.67)	0.07 (\pm 0.54)	1.04 (\pm 0.67)
		Enamel	73.11 (\pm 0.92)	0.28 (\pm 0.07)	9.31 (\pm 0.07)	3.44 (\pm 0.61)	3.76 (\pm 0.53)
		Hydroxyapatite	88.80 (\pm 0.04)	0.42 (\pm 0.02)	2.31 (\pm 0.02)	-0.45 (\pm 0.04)	0.79 (\pm 0.04)
		Resin	79.73 (\pm 0.56)	0.71 (\pm 0.11)	16.16 (\pm 0.18)	-0.33 (\pm 0.56)	1.42 (\pm 0.09)
6 months	H	Ceramic	70.00 (\pm 0.78)	1.20 (\pm 0.23)	17.89 (\pm 1.01)	-0.54 (\pm 0.78)	1.20 (\pm 0.59)
		Enamel	71.51 (\pm 0.91)	0.29 (\pm 0.36)	9.75 (\pm 0.16)	2.17 (\pm 0.91)	2.98 (\pm 0.64)
		Hydroxyapatite	88.09 (\pm 0.41)	0.44 (\pm 0.04)	2.26 (\pm 0.04)	-1.16 (\pm 0.41)	1.33 (\pm 0.33)
		Resin	77.60 (\pm 0.55)	0.89 (\pm 0.05)	16.08 (\pm 0.27)	-2.47 (\pm 0.55)	2.78 (\pm 0.62)

EO = essential oils mouthwash; EOFZC = essential oils + fluoride + zinc chloride mouthwash; H = hydroalcohol solution; HP = 2.0% hydrogen peroxide mouthwash.

as durability of surfaces after using alcohol-containing mouthwashes. The literature on the detrimental effect or lack of effect of EO alcohol-containing mouthwashes on microhardness (SMH),^{12,13} surface roughness,^{13,14} surface strength,¹⁵ fluid absorption,^{16,17} color stability,^{13,17,18,19,20} and wear¹² of direct restorative materials have demonstrated that these findings were dependent on the type of material used, study design, and analytical methodology. It is well established that saliva provides protection against caries. Inclusion of artificial saliva in this study therefore increases the clinical relevance of the results.^{21,22}

Several published *in vitro* studies have detailed the detrimental effects or lack of effect of peroxide-containing tooth whitening products on enamel microhardness, enamel resistance to abrasion, dentin microhardness, dentin roughening, and restoration microhardness.^{23,24,25} The results are also based on the methodology used and the tested materials or products. Numerous recent studies have indicated that hydrogen peroxide- and carbamide peroxide-containing products confer no remarkable deleterious effects on SMH of human enamel and dentin.^{13,18}

According to studies, small chemical and elemental alterations from EO alcohol-containing mouthwashes in human enamel, ceramic, and hydroxyapatite can occur; therefore, it is important to consider the clinical relevance of this study on the daily use of mouthwashes and its implications on safety, durability, and esthetic aspects. The anti-calculus effect of an EO-containing zinc chloride formulation was previously evaluated using X-ray diffraction, infrared spectroscopy, and SEM.²⁶ The present study evaluated only the inorganic components of human dental calculus and the ability to inhibit calculus formation.

Degradation of restorative materials can occur because of chemical and mechanical factors from the oral environment, which may lead to changes on surfaces^{27,28} as well as on composite resin characteristics.¹⁶ This degradation not only affects the esthetics but can also facilitate an increase of plaque buildup, inflammation, and recurrent caries.²⁹ In the present study, no relevant morphologic, elemental, or chemical change was observed on composite resin surfaces during the examination periods. The ceramic surfaces showed minor changes, under 3,000× magnification, wherein certain erosions were observed. New analyses revealed that the erosions were present before the treatment and were thus mostly caused by factors such as the quality and preparation of the ceramic material. A superficial change was observed on the human enamel surface at 1 month after the use of whitening mouthwash, which was reversed after 3 months of use. This may have been due to the remineralizing effect of the artificial saliva used as storage solution.

CIE L*a*b* analysis was used to evaluate changes in color stability (ΔE). No alteration was detected in the color properties of the composite resins, with similar results to certain studies.^{15,17-20} The effects of peroxide bleaching on the surface texture and chemistry of the restoration are strongly dependent on the type of restoration.³⁰ Overall, a composite restoration seems to be more reactive to the effects of bleaching.^{23,31}

Regarding surface staining, studies have reported^{31,32,33,34,35} that ΔE values >3.3 are clinically

unacceptable. The present study showed ΔE mean values of 1.72 and 2.03 for LISTERINE® Cool Mint® and LISTERINE® Total Care, respectively, 0.70 for composite resin and 1.40 for ceramic restorations by simulating 6 months of daily use. LISTERINE® Whitening showed ΔE mean values of 1.28 for composite resin and 0.78 for ceramic at 3 months, suggesting its safety and color stability for restorative materials under experimental conditions. The only relevant colorimetric change was a whitening effect on the enamel surface with LISTERINE® Whitening due to its hydrogen peroxide content after simulating 12 weeks of daily use, which is in agreement with findings from the available literature.^{24,25}

Conclusion

Results of this *in vitro* study show that the ultra-structure and morphology of enamel and dental restorative materials did not present any meaningful change with any of the mouthwash treatments after up to 6 months of simulated daily use. No variation in either relative Ca or P concentrations of enamel, Si and Ba concentrations of composite resin, or Si and Al concentrations of ceramic were observed before and after all treatments. No change was detected in the biochemical and color properties of any of the specimens except the whitening rinse, which had a whitening effect on enamel surfaces. Long-term exposure of these alcohol-containing, low pH mouthwashes (<5.5) caused no ultra-structural and biochemical change on human enamel and restorative materials.

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