Submitted: 20 October, 2025 Accepted: 04 January, 2026 Published: 03 May, 2026 DOI:10.22514/jocpd.2026.081

Journal of Clinical Pediatric Dentistry

ORIGINAL RESEARCH

Effect of different mouthwashes on the color stability and surface roughness of a compomer and a resin-based fissure sealant: an *in vitro* study

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Abstract

Background: The aim of this study was to determine the impact of mouthwashes with varying chemical compositions on the color stability and surface texture of compomers and resin-based fissure sealants, which are frequently used in pediatric dentistry. **Methods**: A total of 80 disc-shaped samples were fabricated for each resin-based material—Nova Compomer Classic and I-Seal Light Curing (LC) Fissure Sealant. Each group was subdivided into four sets (n = 10) according to the immersion medium: an alcohol-free mouthwash (Listerine Total Care Zero, Johnson & Johnson, USA), a chlorhexidine-based rinse (Kloroben, Drogsan, Ankara, Turkey), ozonated water, and distilled water serving as the control. Samples were immersed twice daily for 2 minutes over a 28-day period. Color alterations and surface roughness were assessed at baseline and after 1, 7, 14, 21, and 28 days using a spectrophotometer and a profilometer, respectively, and the changes in color (ΔE00) and roughness (ΔRa) were calculated. Statistical analyses were performed using IBM SPSS version 25. The data were compared using non-parametric tests (Kruskal-Wallis and Friedman) with Bonferroni-adjusted *post hoc* comparisons. **Results**: Significant differences were detected between the compomer and fissure sealant groups in terms of both color and surface roughness changes ($p < 0.05$). The resin-based fissure sealant exhibited significantly greater color changes than the compomer, with the most pronounced discoloration following immersion in ozonated water and Listerine ($p < 0.05$). In contrast, the compomer demonstrated significantly greater surface roughness changes than the resin-based fissure sealant, particularly after immersion in Listerine and distilled water ($p < 0.05$). The effects of the immersion media on ΔE00 and ΔRa values varied over time ($p < 0.05$). **Conclusions**: While the resin-based fissure sealant showed greater susceptibility to color change, the compomer exhibited more pronounced surface roughness alterations.

Keywords

Mouthwashes; Color; Surface properties; Pediatric dentistry; Dental materials

1. Introduction

The increasing aesthetic demands in dental practice have encouraged the development of a wide variety of resin-based materials, each designed with distinct physical and chemical properties for clinical application [1]. As both children and their parents have become more attentive to dental appearance, maintaining the long-term color stability of resin-based materials has gained particular importance [2]. Pit and fissure sealants have been used for approximately 50 years to prevent and control caries lesions in primary and permanent teeth. Resin-based sealants are frequently the preferred materials [3, 4]. However, the clinical longevity of these materials relies not only on their mechanical strength, but also on their ability to resist discoloration and surface wear in the oral cavity. An increase in surface irregularities promotes bacterial adhesion and plaque retention, which consequently elevates the risk of secondary caries, gingival inflammation, and abrasion of opposing teeth [5]. The degradation of resin-based materials generally involves the loss of inorganic fillers from the resin matrix, resulting in the formation of surface voids that increase roughness and make the surface more prone to extrinsic discoloration [6]. Compomers contain a combination of resin monomers and polyacid-modified components, making them susceptible to water sorption and matrix softening, whereas fissure sealants primarily consist of Bisphenol A-Glycidyl Methacrylate (Bis-GMA)-based resin matrices, which may undergo pigment uptake and surface degradation when exposed to chemical agents [7].

Mouth rinses are commonly used as adjuncts to tooth brushing and flossing to reduce plaque accumulation, provide antimicrobial effects, and control halitosis [8]. Chlorhexidine gluconate (CHX) is regarded as the gold standard due to its well-documented and broad-spectrum antimicrobial activity. In general, mouthwashes are composed of water, antibacterial agents, salts, fluoride, surfactants, and organic acids. Regular or prolonged use may influence the oral microbial flora, cause transient taste disturbances, irritate soft tissues, and modify the surface properties of resin restorations [9]. Previous *in vitro* studies have reported that mouth rinses can induce perceptible color alterations and changes in surface roughness in resin-based materials [10, 11]. Although these effects have been evaluated in composite resins and, to a limited extent, in compomers, studies specifically examining resin-based sealants remain scarce. Furthermore, direct comparisons between compomers and resin-based sealants under identical conditions are lacking [5, 12]. In contrast, ozonated water has been extensively investigated for its antimicrobial activity, capacity to reduce plaques, and potential benefits in managing gingival inflammation [13–15]. However, despite its increasing clinical use, the effects of ozonated water on the optical or surface properties of resin-based pediatric materials have not been adequately studied. Most ozone-related literature focuses on soft tissue or microbial outcomes, and there is very limited *in vitro* evidence on the impact of aqueous ozone on the resin matrix or filler particles of restorative or preventive resin materials [16]. Listerine Cool Mint, an alcohol-containing rinse, has been the focus of numerous studies, whereas Listerine Total Care Zero—an alcohol-free formulation with added fluoride—is considered more suitable for pediatric patients [17]. Previous studies frequently evaluated only a single material or one type of mouthwash, used short immersion periods, or relied on single end-point measurements, which limit the clinical relevance of their findings [10, 18].

Therefore, the aim of the present *in vitro* study was to examine the influence of alcohol-free mouthwash, CHX mouthwash, and ozonated water on the color stability and surface roughness of a compomer and a resin-based fissure sealant frequently applied in pediatric dentistry. The null hypothesis proposed that there would be no significant differences in either color variation (ΔE00) or surface roughness change (ΔRa) among the materials and immersion solutions tested.

2. Materials and methods

2.1 Study design

This study was conducted at the Department of Pedodontics, Faculty of Dentistry, Bolu Abant İzzet Baysal University. An experimental *in vitro* repeated-measures study involving 2 materials (a compomer and a resin-based fissure sealant), 4 immersion solutions, and 6 time points (baseline, and days 1, 7, 14, 21, and 28) was designed. The dental materials used in the study were a compomer (Nova Compomer Classic; Imicryl, Konya, Turkey) and a resin-based fissure sealant (I-Seal LC; i-dental, Šiauliai, Lithuania). Forty disc-shaped specimens (6 mm diameter × 2 mm thickness) of each material were prepared and divided into four subgroups according to the mouthwash solution (n = 10 per subgroup). The working groups are shown in the flow chart in Fig. 1.

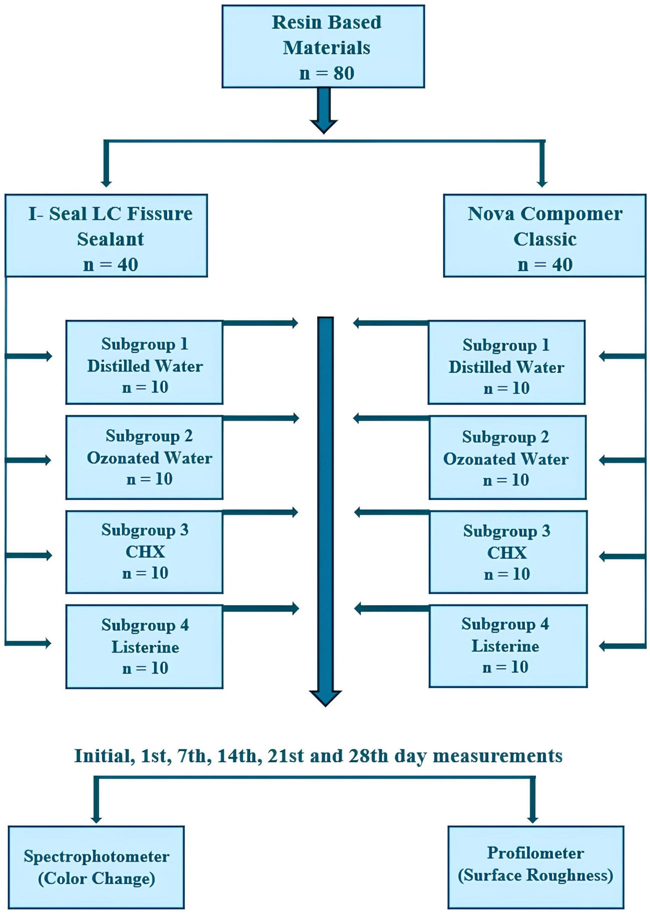

FIGURE 1. Flow chart illustrating grouping of specimens tested in the study. CHX: Chlorhexidine gluconate; LC: Light Curing.

2.2 Sample size determination

The number of specimens required for the study was estimated using the G*Power 3.1.9.2 software (Heinrich Heine University Düsseldorf, Düsseldorf, NRW, Germany) with a confidence level of 95%. Based on data from a previous study reporting a standardized effect size of 0.9158 [12], the minimum sample size per group was calculated as six, considering $\alpha = 0.05$ and a theoretical power of 0.95. To strengthen the statistical reliability, ten samples were included in each subgroup.

2.3 Specimen preparation

The disc-shaped specimens of the resin-based dental materials (Table 1) were fabricated using a stainless-steel mold placed between two sterile glass plates. Excess resin was eliminated by applying uniform pressure with glass plates lined with Mylar strips. Polymerization was achieved by exposing the material to a light-emitting diode (LED) curing unit (LED F, Woodpecker, Guilin, China) emitting 430–480 nm light at an intensity of 1200 mW/cm^2 for 20 seconds. The intensity output was verified at intervals using a radiometer (Demetron LED Radiometer, Kerr, Orange, CA, USA).

TABLE 1. Composition of composite resin materials.

Material	Brand	Content	Weight (wt%), Volume (vol%)	Particle Size	Manufacturer	Lot Number
Compomer	Nova Compomer Classic	Monomer matrix: Bis-GMA, UDMA, carboxylate-modified dimethacrylate, dimethacrylates, TEGDMA, and trimethacrylate. Fillers: ytterbium trifluoride, Sr-alumina-sodium fluoro-phosphor-silicate, strontium fluoride, catalyst, BHT, and pigments.	82 wt%, 62 vol%	Undeclared	Imicryl, Konya, Turkey	C211
Fissure Sealant	I-Seal LC Fissure Sealant	Ground glass 30–50%, methacrylate mixture 20–50%, silicon dioxide 1–5%, co-initiator <1%, photo-initiator 1%, inhibitor 1%, stabilizer 1%, opacifier 1%.	50.5 wt%, 31.9 vol%	Undeclared	i-dental, Šiauliai, Lithuania	170420

Bis-GMA: Bisphenol A-Glycidyl Methacrylate; UDMA: Urethane Dimethacrylate; TEGDMA: Triethylene Glycol Dimethacrylate; BHT: Butylated hydroxytoluene; LC: Light Curing.

Only one surface of each specimen was polished using a polishing disc system (Super Snap Rainbow disc system, Shofu Inc., San Marcos, CA, USA) with progressively finer abrasives applied in a single direction for 15 seconds at 10,000–20,000 rpm using a contra-angle handpiece. The discs were stored in distilled water at 37 °C for 24 h to complete polymerization. Subsequently, the specimens were air-dried, and the baseline color and surface roughness were evaluated. No sample loss occurred during the study, and all prepared specimens were included in the final analysis. The first investigator (DSÖÇ) randomized and coded the specimens, while the second investigator (GK) performed the immersion procedures and calculations in a blinded fashion to minimize bias. Randomization was performed using a simple random sampling method. Each specimen was assigned a unique identification number, and group allocation was determined by randomly drawing these numbers. This approach ensured that every specimen had an equal probability of being assigned to any of the groups. Fig. 2 shows the preparation of the disks and their separation into groups.

2.4 Mouthwash exposure protocol and measurements

The specimens of each material were immersed in the following solutions (n = 10):

(1) alcohol-free mouthwash (Listerine Total Care Zero, Johnson & Johnson Inc., USA);

(2) chlorhexidine gluconate mouthwash (Kloroben, Drogsan, Ankara, Turkey);

(3) ozonated water prepared freshly each day; and

(4) distilled water (control) (Table 2).

Ozonated water was produced using an ozone generator (Ozonette Dent; Sedecal, Madrid, Spain) operating at a flow rate of 30 L/h and a concentration of 50 μg/mL for 10 minutes [14, 16, 19]. To mimic oral rinsing conditions, specimens were manually agitated in their respective solutions for two minutes, twice daily, over a 28-day period.

2.5 Color measurement

Color changes were assessed on a standardized white matte background with a spectrophotometer (Easyshade Advance Vita; Vita Zahnfabrik, Bad Säckingen, BW, Germany). Three measurements were taken from each sample and the average of the resulting L*, a* and b* values were recorded. Measurements were obtained at baseline and on days 1, 7, 14, 21, and 28. Color difference (ΔE00) was calculated following the CIEDE2000 formula (Eqn. 1) [20]:

$$\Delta E00=\sqrt{\left(\frac{\Delta L'}{K_L S_L}\right)^2+\left(\frac{\Delta C'}{K_C S_C}\right)^2+\left(\frac{\Delta H'}{K_H S_H}\right)^2+R_T\left(\frac{\Delta C'}{K_C S_C}\right)\left(\frac{\Delta H'}{K_H S_H}\right)} \quad (1)$$

K_L, K_C and K_H values were accepted as 1. In the current study, the acceptable color change value was determined as 2.7 based on the study of Paravina *et al.* [21].

2.6 Surface roughness measurement

Average surface roughness (Ra, μm) was recorded using a mobile profilometer (Marsurf M300; Mahr GmbH, Göttingen, NI, Germany). Prior to each session, the device was calibrated with a standard reference sample. Measurements were taken

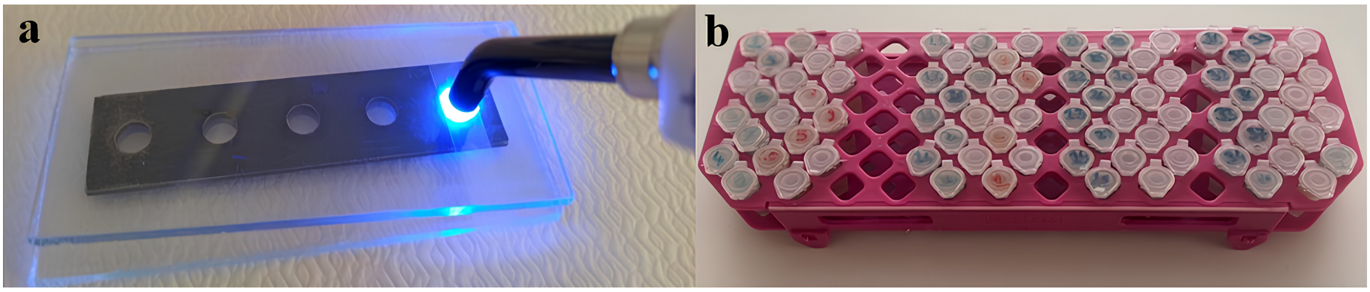

FIGURE 2. Preparation, grouping, and storage of disk specimens used in the study. (a) Preparation of the disks used in the study, (b) separation and storage of the disks according to the mouthwash groups used.

TABLE 2. The mouthwashes used in the study.

Mouthwash	Manufacturer	Composition	pH	Lot Number
Distilled Water	–	–	5.76	–
Ozonated Water	–	Ozonated distilled water	5.30	–
Kloroben	Drogsan, Ankara, Turkey	0.15% Benzydamine HCl, 0.12% Chlorhexidine Gluconate, sorbitol, propylene glycol, polysorbate 20, sucralose, peppermint oil, citric acid monohydrate, sodium citrate dihydrate, patent blue V, quinoline yellow, Ecocool MP (aroma), purified water	4.91	23M198211
Listerine Total Care Zero	Johnson & Johnson Inc., USA	Water, sorbitol, propylene glycol, poloxamer 407, sodium lauryl sulfate, flavor, eucalyptol, zinc chloride, benzoic acid, sodium benzoate, methyl salicylate, thymol, sodium saccharin, menthol, sucralose, Color Index 16035, Color Index 42090, sodium fluoride (220 ppm)	3.57	793530

HCl: Hydrochloride.

across a 4 mm tracing length with a cutoff of 0.8 mm. Each specimen was scanned four times, and the mean value was calculated. Surface roughness variations (ΔRa) were obtained at the same time points as color measurements. Measurements were obtained at baseline and on days 1, 7, 14, 21, and 28.

2.7 Statistical analysis

Data were summarized in the form of mean, standard deviation, and median. Due to the non-normal distribution in several comparisons, descriptive statistics were also presented as interquartile ranges (IQR) to appropriately reflect the distribution of non-parametric data. The Shapiro-Wilk and Levene tests were used to verify normality and homogeneity of variances respectively, while Mauchly's W test was used to evaluate sphericity. Depending on the distribution, parametric (independent and paired *t*-tests, one-way analysis of variance (ANOVA), repeated measures ANOVA) or non-parametric tests (Mann-Whitney U, Wilcoxon signed-rank, Kruskal-Wallis, Friedman) were employed. *Post hoc* comparisons were conducted using Bonferroni and adjusted Bonferroni corrections. All analyses were performed with IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Color measurements (△E00)

In the comparison of ΔE00 values between materials immersed in the different solutions for varying durations, significant differences were identified on day 1 for CHX, on day 7 for distilled water and ozonated water, on day 14 for ozonated water and Listerine, on day 21 for ozonated water and CHX, and on day 28 for ozonated water, CHX, and Listerine ($p < 0.05$). Furthermore, the ΔE00 values of the fissure sealant were higher than that of the compomer (Table 3).

Significant differences were observed between the ΔE00 of compomer specimens exposed to the different solutions for 1, 7, and 14 days ($p < 0.05$). In the fissure sealant group, significant differences were detected on days 7, 14, 21, and 28 ($p < 0.05$). The ΔE00 of compomer after one day of immersion in Listerine was higher than that observed with ozonated water; on day 7, the ΔE00 value was higher in the Listerine subgroup compared with the distilled water and CHX subgroups, and on day 14, distilled water resulted in higher ΔE00 compared with CHX and Listerine, while ozonated water achieved greater ΔE00 compared with Listerine (Table 3). In the fissure sealant group, the ΔE00 values of the Listerine and ozonated water subgroups were higher than that of the CHX subgroup on day 7. On day 14, the ΔE00 value of ozonated water was higher than that of distilled water, Listerine and CHX. On day 21, the ΔE00 values for distilled water and Listerine were higher than

TABLE 3. Distribution and comparison of △E00 measurements according to materials, solutions, and measurement times.

	Day 1	Day 7	Day 14	Day 21	Day 28	According to time	
	Mean ± SD (M; IQR)	Mean ± SD (M; IQR)	Mean ± SD (M; IQR)	Mean ± SD (M; IQR)	Mean ± SD (M; IQR)	Test St.	*p*
Compomer							
Distilled Water	$3.14 \pm 0.92^{a,b,c,A,B}$ (3.17; 1.04)	$2.67 \pm 0.46^{c,B}$ (2.57; 0.68)	$4.84 \pm 1.36^{a,A}$ (5.35; 2.11)	$2.97 \pm 0.81^{b,c}$ (2.81; 0.87)	$3.62 \pm 0.64^{a,b}$ (3.52; 0.78)	24.72¥	<0.001*
Ozonated Water	$2.26 \pm 0.55^{b,B}$ (2.33; 0.93)	$3.23 \pm 1.11^{a,b,A,B}$ (2.89; 0.70)	$4.48 \pm 1.50^{a,A,B}$ (4.41; 2.08)	2.60 ± 0.95^{b} (2.50; 0.98)	$3.07 \pm 0.95^{a,b}$ (2.99; 1.35)	25.04¥	<0.001*
CHX	$2.59 \pm 1.13^{A,B}$ (2.40; 1.67)	2.32 ± 0.87^{B} (2.02; 1.10)	2.36 ± 0.86^{C} (2.23; 1.54)	2.29 ± 1.16 (1.95; 0.84)	3.78 ± 2.68 (3.03; 1.57)	8.96¥	0.062
Listerine	$3.63 \pm 0.59^{a,b,A}$ (3.60; 1.06)	$4.66 \pm 1.13^{a,A}$ (4.76; 1.59)	$3.18 \pm 0.63^{b,B}$ (3.06; 0.93)	2.89 ± 0.55^{b} (2.81; 1.01)	3.21 ± 0.54^{b} (3.26; 0.94)	15.57	<0.001*
Test Statistic	5.23	20.93‡	10.13	7.31‡	4.51‡		
p	0.004*	<0.001*	<0.001*	0.063	0.211		
Fissure Sealant							
Distilled Water	2.76 ± 2.12^{c} (2.03; 1.96)	$4.91 \pm 2.06^{b,c,A,B}$ (4.34; 2.30)	$6.45 \pm 1.85^{a,b,B}$ (5.78; 1.99)	$7.27 \pm 1.83^{a,A}$ (7.01; 2.09)	$8.26 \pm 1.70^{a,A}$ (7.74; 1.68)	35.92¥	<0.001*
Ozonated Water	3.40 ± 1.77^{b} (2.94; 1.89)	$6.09 \pm 2.67^{b,A}$ (5.02; 4.76)	$9.82 \pm 3.00^{a,A}$ (9.62; 4.91)	$7.35 \pm 3.45^{a,b,A,B}$ (6.04; 5.28)	$8.57 \pm 3.43^{a,A,B}$ (7.19; 5.06)	30.96¥	<0.001*
CHX	$4.79 \pm 2.85^{a,b}$ (4.26; 4.65)	$2.66 \pm 1.70^{b,B}$ (2.07; 1.83)	$3.88 \pm 2.70^{b,B}$ (2.90; 3.49)	$4.31 \pm 2.06^{a,b,B}$ (4.01; 1.92)	$6.01 \pm 2.04^{a,B}$ (5.45; 1.83)	19.92¥	0.001*
Listerine	3.70 ± 1.09^{c} (3.36; 2.01)	$5.12 \pm 1.14^{b,c,A}$ (4.81; 1.59)	$5.82 \pm 1.25^{b,c,B}$ (5.47; 1.76)	$7.16 \pm 1.15^{a,b,A}$ (6.95; 0.74)	$8.28 \pm 1.16^{a,A}$ (7.99; 1.04)	36.96¥	<0.001*
Test Statistic	6.52‡	15.55‡	11.48	12.70‡	11.76‡		
p	0.089	<0.001*	<0.001*	0.005*	0.008*		
	Test Statistic/*p*	Test Statistic/*p*	Test Statistic/*p*	Test Statistic/*p*	Test Statistic/*p*		
Inter-Material Comparison							
Distilled Water	27.00†/0.089	4.00†/<0.001*	28.00†/0.105	0.00†/1.000	0.00†/1.000		
Ozonated Water	27.00†/0.052	7.00†/<0.001*	−5.04/<0.001*	−4.19/0.002*	−4.88/0.001*		
CHX	−2.26/0.043*	47.00†/0.853	31.00†/0.165	16.00†/0.009*	15.00†/0.007*		
Listerine	−0.19/0.846	−0.91/0.375	−5.96/<0.001*	0.00†/1.000	−12.53/<0.001*		

*Test St.: Test statistic value; *$p < 0.05$, Student t-test; †: Mann Whitney U test, One-Way ANOVA (Post-Hoc: Bonferroni adj. Student t test or Tamhane test); ‡: Kruskal Wallis test (Post-Hoc: Bonferroni adj. Dunn test), Repeated Measures ANOVA (Post-Hoc: Bonferroni adj. Paired Samples t test); ¥: Friedman test (Post-Hoc: Bonferroni adj. Dunn test).*
^{A,B,C}: indicate statistically significant differences between groups at the same measurement time, whereas ^{a,b,c}: indicate statistically significant differences within the same group over different measurement times. For both comparisons, different letters denote a statistically significant difference ($p < 0.05$), while the same letters indicate no statistically significant difference ($p > 0.05$).
M: Median; IQR: Interquartile Range; SD: Standard Deviation; CHX: Chlorhexidine gluconate.

that observed for CHX, and on day 28, the ΔE00 values for distilled water and Listerine were higher than that for CHX (Table 3).

When the time-dependent changes in color were measured according to materials and solutions, significant differences were detected between day 7 and days 14 and 28, and between days 14 and 21 for compomer specimens immersed in distilled water ($p < 0.05$). For ozonated water, significant differences were observed between day 14 and days 1 and 21 ($p < 0.05$). For Listerine, significant differences were detected between day 7 and days 14, 21, and 28 ($p < 0.05$) (Table 3).

In the fissure sealant group, significant differences were observed between day 1 and days 14, 21, and 28, and between days 7 and 28 for specimens exposed to distilled water ($p < 0.05$). For ozonated water, significant differences were observed between day 1 and days 14 and 28, and between day 7 and days 14 and 28 ($p < 0.05$). The ΔE00 values on days 14 and 28 were higher than that on days 1 and 7. For CHX, significant differences were detected between day 28 and days 14 and 7 ($p < 0.05$). For Listerine, the ΔE00 values showed significant differences between day 1 and days 21 and 28, and between day 28 and days 7 and 14 ($p < 0.05$) (Table 3). Fig. 3 shows the line graph of the distribution of ΔE00 measurements according to materials, mouthwashes, and measurement times.

3.2 Surface roughness (ΔRa)

When comparing ΔRa measurements according to materials, solutions, and measurement times, significant differences were observed between the materials on day 1 for Listerine, and on days 7, 14, 21, and 28 for distilled water and Listerine subgroups ($p < 0.05$). The ΔRa measurements of the compomer were higher than that of the fissure sealant (Table 4). However, no significant differences were detected between the ΔRa values of either material in the presence of the different solutions for all time points ($p > 0.05$).

The time-dependent ΔRa values were also compared for the different materials and solutions. In the compomer group, significant differences were observed for distilled water between day 28 and days 1, 7, and 14, and between day 1 and day 21 ($p < 0.05$). For ozonated water and CHX, the ΔRa values between day 28 and days 1, 7, and 14, between day 1 and days 14 and 21, and between days 7 and 21 showed significant differences ($p < 0.05$). For Listerine, significant differences were observed between days 21 and 28 and days 1, 7, and 14, between day 14 and days 1 and 7, and between days 7 and 1 ($p < 0.05$) (Table 4).

In the fissure sealant group, significant differences were observed for distilled water, ozonated water, and Listerine between day 28 and days 1, 7, and 14, between days 1 and 14 and 21, and between days 7 and 21 ($p < 0.05$). For CHX, significant differences were detected between day 28 and days 1, 7, and 14, and between day 1 and day 21 ($p < 0.05$) (Table 4). Fig. 4 shows the line graph of the distribution of ΔRa measurements according to materials, mouthwashes, and measurement times.

4. Discussion

The color stability and surface integrity of resin-based materials play a vital role in the aesthetics and the prevention of recurrent caries in pediatric dentistry. In the present study, different mouthwashes produced measurable changes in the color and surface roughness of the compomer and fissure sealant materials. Although the patterns of change varied between materials and solutions, the results suggest that the interactions between resin-based materials and daily-use rinses are influenced by both the chemical composition of the solutions and the intrinsic properties of the materials.

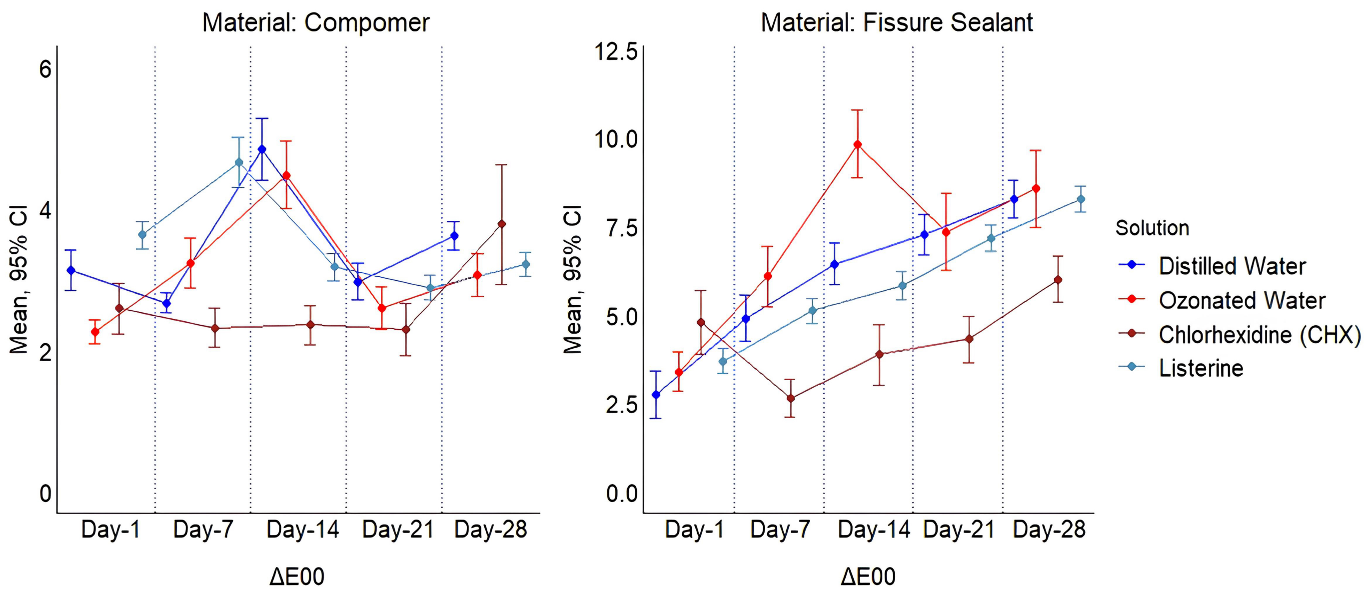

FIGURE 3. Line graph of the distribution of ΔE00 measurements according to materials, mouthwashes, and measurement times. CHX: Chlorhexidine gluconate; CI: Confidence Interval.

TABLE 4. Distributions and comparisons of ΔRA (μm) measurements according to materials, solutions, and measurement times.

	Day 1	Day 7	Day 14	Day 21	Day 28	According to time	
	Mean ± SD (M; IQR)	Mean ± SD (M; IQR)	Mean ± SD (M; IQR)	Mean ± SD (M; IQR)	Mean ± SD (M; IQR)	Test Statistic	*p*
Compomer							
Distilled Water	0.04 ± 0.04^{c} (0.03; 0.07)	0.14 ± 0.10^{b,c} (0.11; 0.11)	0.19 ± 0.13^{b,c} (0.17; 0.18)	0.26 ± 0.13^{a,b} (0.27; 0.18)	0.32 ± 0.16^{a} (0.31; 0.26)	39.28$^{¥}$	<0.001*
Ozonated Water	0.03 ± 0.03^{d} (0.01; 0.04)	0.16 ± 0.12^{c,d} (0.13; 0.19)	0.24 ± 0.18^{b,c} (0.17; 0.28)	0.34 ± 0.22^{a,b} (0.28; 0.46)	0.41 ± 0.21^{a} (0.35; 0.39)	40.00$^{¥}$	<0.001*
CHX	0.04 ± 0.05^{d} (0.02; 0.07)	0.15 ± 0.12^{c,d} (0.10; 0.14)	0.25 ± 0.15^{b,c} (0.19; 0.22)	0.33 ± 0.17^{a,b} (0.27; 0.28)	0.40 ± 0.16^{a} (0.37; 0.19)	40.00$^{¥}$	<0.001*
Listerine	0.06 ± 0.04^{d} (0.06; 0.07)	0.23 ± 0.12^{c} (0.20; 0.15)	0.30 ± 0.13^{b} (0.24; 0.17)	0.40 ± 0.15^{a} (0.37; 0.19)	0.53 ± 0.23^{a} (0.49; 0.30)	38.19	<0.001*
Test Statistic	2.42	4.58‡	1.88	4.78‡	4.61‡		
p	0.081	0.205	0.150	0.188	0.202		
Fissure Sealant							
Distilled Water	0.01 ± 0.00^{c} (0.01; 0.01)	0.05 ± 0.03^{c} (0.04; 0.04)	0.08 ± 0.05^{b} (0.08; 0.09)	0.11 ± 0.06^{a,b} (0.10; 0.12)	0.16 ± 0.07^{a} (0.15; 0.13)	40.00$^{¥}$	<0.001*
Ozonated Water	0.03 ± 0.03^{d} (0.01; 0.03)	0.10 ± 0.07^{c,d} (0.10; 0.09)	0.16 ± 0.14^{b,c} (0.14; 0.16)	0.22 ± 0.21^{a,b} (0.18; 0.23)	0.29 ± 0.24^{a} (0.23; 0.33)	40.00$^{¥}$	<0.001*
CHX	0.02 ± 0.02^{c} (0.01; 0.04)	0.09 ± 0.06^{b,c} (0.08; 0.08)	0.16 ± 0.11^{b,c} (0.14; 0.12)	0.27 ± 0.19^{a,b} (0.20; 0.26)	0.34 ± 0.23^{a} (0.27; 0.42)	39.28$^{¥}$	<0.001*
Listerine	0.01 ± 0.01^{d} (0.01; 0.01)	0.06 ± 0.03^{c,d} (0.07; 0.06)	0.10 ± 0.05^{b,c} (0.09; 0.08)	0.14 ± 0.06^{a,b} (0.12; 0.10)	0.21 ± 0.07^{a} (0.19; 0.07)	40.00$^{¥}$	<0.001*
Test Statistic	6.96‡	5.03‡	4.46‡	7.18‡	4.22‡		
p	0.073	0.169	0.215	0.066	0.238		
	Test Statistic/*p*	Test Statistic/*p*	Test Statistic/*p*	Test Statistic/*p*	Test Statistic/*p*		
Inter-Material Comparison							
Distilled Water	26.50†/0.075	14.00†/0.005*	20.00†/0.023*	14.00†/0.005*	18.00†/0.015*		
Ozonated Water	36.50†/0.315	30.50†/0.143	1.20/0.243	1.21/0.242	1.18/0.251		
CHX	1.36/0.198	35.00†/0.280	26.50†/0.075	35.50†/0.280	35.00†/0.280		
Listerine	3.91/0.003*	4.16/0.002*	4.70/0.001*	2.00†/<0.001*	3.00†/<0.001*		

**$p < 0.05$, Student t-test; †: Mann-Whitney U test, One-Way ANOVA; ‡: Kruskal-Wallis test, Repeated Measures ANOVA (Post-Hoc: Bonferroni adj. Paired Samples t test); $^{¥}$: Friedman test (Post-Hoc: Bonferroni adj. Dunn test). ^{a,b,c,d}: indicate statistically significant differences within the same group over different measurement times. Different letters denote a statistically significant difference ($p < 0.05$), while same letters indicate no statistically significant difference ($p > 0.05$).*

M: Median; IQR: Interquartile Range; SD: Standard Deviation; CHX: Chlorhexidine gluconate.

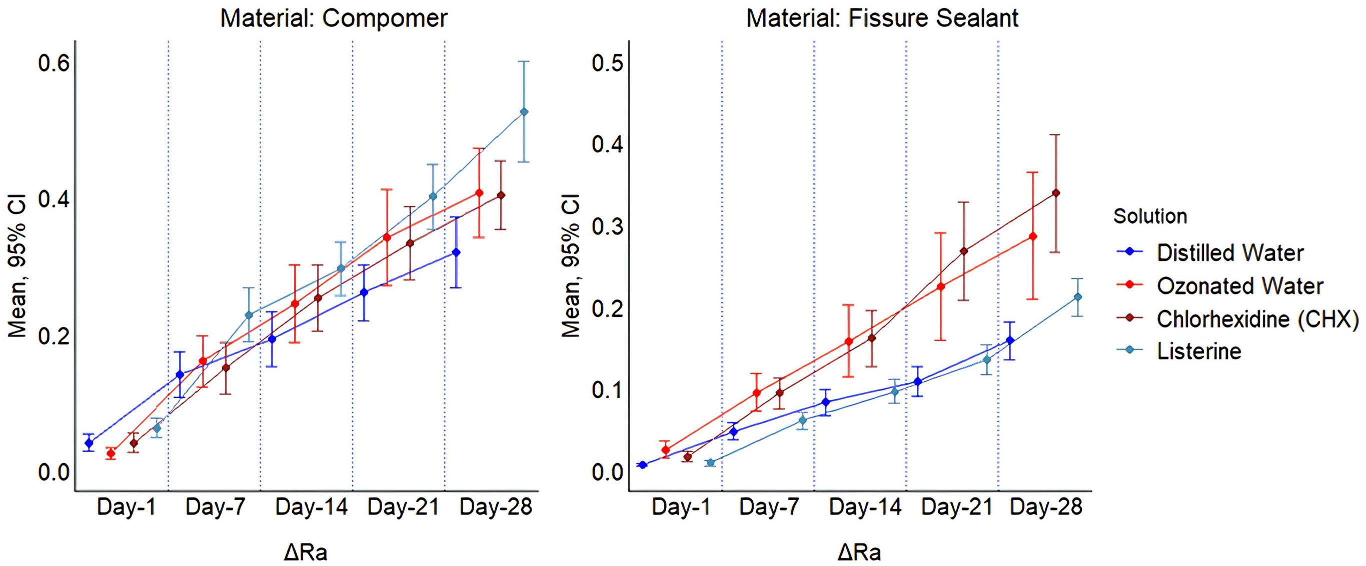

FIGURE 4. Line graph of the distribution of ΔRa measurements according to materials, mouthwashes and measurement times. CHX: Chlorhexidine gluconate; CI: Confidence Interval.

Although ozone dosage ranges in medical applications have been standardized, similar parameters in dentistry remain insufficiently defined. The Madrid Declaration provided the first guidelines for dental ozone therapy, emphasizing the challenges in establishing a precise therapeutic window and suggesting that recommendations should be updated as new evidence emerges [22]. Nicolini *et al*. [14] assessed the effects of ozonated water rinsing (70 μg/mL, 10 minutes daily) on early plaque accumulation and gingival inflammation *in vivo*. Similarly, Cosola *et al*. [19] compared the clinical efficacy of CHX and ozonated water for maintaining oral hygiene in orthodontic patients using ozonated water generated at a rate of 50 mg/h (20 °C) and 0.264 L/min, applied twice daily. Mon *et al*. [13] produced ozonated water at a concentration of 2.4 mg/L (>2 ppm) using an ozone generator and investigated its antimicrobial efficacy alongside herbal and CHX mouthwashes. Both gaseous and aqueous ozone have strong oxidizing potential and well-documented microbicidal activity [23]. In line with prior reports, ozonated water in the current study was prepared at 50 μg/mL for 10 minutes with a flow rate of 30 L/h. The ozone concentration and manual agitation protocol employed here were consistent with previous clinically relevant models [13, 14, 19]. Manual shaking was performed to simulate the rinsing motion typical of everyday mouthwash use. Microfluidic and viscosity-based simulation studies have demonstrated that such dynamic agitation closely approximates real oral rinsing conditions [24]. Therefore, the experimental design in this study can be considered a valid and clinically meaningful representation of actual mouthwash application.

Compomers are widely used for restoring primary teeth due to their favorable mechanical and aesthetic properties, including fluoride release, wear resistance, and low polymerization shrinkage [25]. Compomers are commonly formulated with dimethacrylate resin systems containing bisphenol A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), and triethylene glycol dimethacrylate (TEGDMA) [26], whereas resin-based fissure sealants are generally formulated with Bis-GMA-based dimethacrylate resin matrices, as reported in the literature [27]. Although pit-and-fissure sealants are protective rather than restorative materials, they share similar resin matrices with compomers. Due to their fluoride-releasing capabilities and adequate flow and bonding properties, compomers can even be used as fissure sealants in some clinical situations [3, 28]. Both materials are exposed to the same oral environment and commonly come into contact with the same mouth rinses in pediatric patients. Therefore, evaluating compomers and resin-based sealants under identical conditions provides meaningful insights into their optical and surface stability. Discoloration of sealants is also clinically important, as color changes may impair visual assessment of sealant retention, mask early caries activity, and affect parental esthetic expectations [29–31]. Greater color change was observed with the fissure sealant compared with the compomer for all three mouthwash types, which may be explained by differences in their resin matrix composition and filler content. Fissure sealants typically contain unfilled or minimally filled Bis-GMA-based resin matrices, which exhibit higher water sorption, solubility, and pigment uptake compared with the more highly filled resin-based materials. Materials with a higher organic matrix fraction tend to absorb more fluids and colorants, resulting in increased ΔE00 values following immersion in aqueous or staining solutions [32, 33]. In contrast, compomers contain higher filler loads and polyacid-modified resin systems, which enhance structural stability and reduce susceptibility to hydrolytic and discoloration processes. Overall, both materials exhibited unacceptable discoloration when immersed in distilled water (control), and the difference between them was statistically insignificant, which aligns with the results of several previous studies [12, 34, 35]. Although distilled water contains no chromogenic agents, previous studies have demonstrated that resin-based materials may still exhibit measurable discoloration when stored in distilled water. This effect has been attributed to hydrolytic degradation of the polymer matrix, water sorption-induced plasticization, and increased light scattering caused by microstructural changes within the resin network. Materials with a higher organic matrix content or lower filler load are particularly susceptible to these mechanisms, which may explain the color changes observed in the distilled water group in the present study [33, 36, 37]. After 28 days, the color change in fissure sealant samples exposed to CHX mouthwash was lower than that observed with ozonated water or Listerine Zero, although it remained above the clinical acceptability threshold (ΔE00 = 6.01). This result corresponds with earlier findings reporting the staining potential of CHX-containing rinses [10, 38]. The discoloration associated with CHX can be attributed to the release of parachloraniline, which interacts with metal sulfides and results in yellow-brown surface pigmentation [39]. In the case of Listerine Zero, the pronounced color change in fissure sealants may be linked to the product's low pH (3.57) and the hydrolysis of ester groups in dimethacrylate monomers (Bis-GMA, Bis-EMA, UDMA, or TEGDMA), which may compromise the resin matrix [40]. Ozonated water caused the most pronounced color alteration in fissure sealants. Magni *et al.* [41] proposed that the oxidative action of ozone on composite surfaces does not produce sufficient oxygen byproducts to alter the micromechanical structure; since ozone application did not influence composite microhardness after thermocycling, it was unlikely to induce significant surface chemical modification. In the present study, the use of liquid-phase ozone and variations in exposure parameters may have contributed to the observed differences. Moreover, ozone decomposition generates peroxide and hydroxyl radicals with strong oxidizing capacities. These radicals react with chromophore double bonds within organic pigments or the inorganic salt molecules in the enamel matrix, leading to altered light absorption and the formation of simpler, less light-reflective compounds. Consequently, visible surface color changes may occur [42]. This mechanism supports the likelihood that ozone exposure was responsible for the pronounced discoloration observed in both compomer and fissure sealant materials.

The compomer material exhibited a greater increase in surface roughness compared with the fissure sealant, particularly when exposed to Listerine. Sadaghiani *et al.* [43], reported similar findings, noting that Listerine Zero increased the surface roughness of compomers and resin-modified glass ionomers over time, likely due to its low pH. Castro *et al.* [44] also demonstrated that the acidity of fluoride products correlated with increased surface roughness in composite materials. Since Listerine Zero contains fluoride, its acidic nature likely contributed to the enhanced roughness observed in this study. No statistically significant differences in surface roughness were detected among the mouthwash groups for either material. Similar results have been documented by Aragão *et al.* [45], who compared the roughness of Filtek Z350 composite specimens immersed in various mouthwashes, and by Urbano *et al.* [46], who reported no significant roughness changes after 30 days of exposure to different rinses. These findings collectively suggest that while mouthwashes can influence surface properties, their effects vary by material composition and exposure conditions.

The *in vitro* nature of this study presents a major limitation, as it cannot fully replicate the intraoral environment that is influenced by salivary enzymes, pellicle formation, microbial activity, thermal changes, and dietary habits. Additionally, the 28-day immersion protocol may not entirely reflect the long-term and intermittent exposure patterns seen with real mouthwash use. Another constraint is the limited number of studies investigating ozonated water in pediatric dentistry, and the absence of standardized ozone concentration and application guidelines, which restrict direct comparison with existing literature. Finally, although efforts were made to minimize bias, complete blinding was not feasible due to the experimental design.

Nevertheless, it can be concluded from the findings that different mouth rinses exert material-dependent effects on the color stability and surface roughness of resin-based materials used in pediatric dentistry. Both the composition and acidity of mouthwashes can significantly affect the optical and mechanical performance of these materials. Therefore, the null hypothesis of this study—predicting no differences in color variation (ΔE00) or surface roughness (ΔRa) between materials and immersion media—was rejected, as statistically significant differences were identified.

5. Conclusions

Compomers and fissure sealants commonly used in pediatric dentistry are susceptible to color and surface alterations when exposed to mouthwashes. Compomers exhibited greater surface roughness changes, while fissure sealants demonstrated higher levels of discoloration. These findings indicate that the optical and mechanical behavior of resin-based materials is influenced by the composition of the material, as well as the characteristics of the mouthwash. Future studies should investigate these effects under *in vivo* conditions, where saliva, pellicle formation, microbial diversity, and dietary factors also interact with resin-based materials. Additional studies evaluating different material formulations and standardized ozone protocols are also needed to clarify the underlying mechanisms.

AVAILABILITY OF DATA AND MATERIALS

The corresponding author can provide the datasets generated and analyzed during this study upon reasonable request.

AUTHOR CONTRIBUTIONS

DSÖÇ—contributed to the study's conceptualization, methodological design, supervision, data analysis, and drafting of the original manuscript, as well as critically revising the final version. GK—participated in the conceptualization, methodological framework, data collection and interpretation, and statistical analysis, and also contributed to the preparation of the original draft. Both authors reviewed and approved the final manuscript prior to submission.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable, as this research was conducted entirely *in vitro* and did not involve human participants or animal subjects.

ACKNOWLEDGMENT

The authors gratefully acknowledge Imicryl (Konya, Turkey) for providing the compomer materials and Ozonette Dent (Sedecal, Spain) for supplying the ozone generator used in this investigation.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare that this study received non-financial support. The ozone generator used to produce ozonated water was provided by Ozonette Dent (Sedecal, Spain), and the compomer materials were donated by Imicryl (Konya, Turkey). No fees or financial payments were received from these companies, and they had no role in the study design, data collection, analysis, interpretation of the results, or manuscript preparation. The authors declare no other conflicts of interest.

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How to cite this article: Deniz Sıla Özdemir Çelik, Gamze Kaplan. Effect of different mouthwashes on the color stability and surface roughness of a compomer and a resin-based fissure sealant: an *in vitro* study. Journal of Clinical Pediatric Dentistry. 2026; 50(3): 263-273. doi: 10.22514/jocpd.2026.081.