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Investigation of the Effects of Mouthwashes on the Microhardness and Color Stability of Bulk-fill Composite Resins

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ABSTRACT

Objective: The current study aimed to investigate the effects of mouthwashes on the microhardness and discoloration of bulk-fill resin composites by comparison with a conventional resin composite.

Methods: In the study, 22 disc-shaped samples (8*2 mm) were prepared from four different composite resins, Clearfil Majesty Esthetic (CME), Estelite Bulk-fill Flow (EBF), Filtek One Bulk-fill Restorative (FBF) and SonicFill-2 (SF2). Half of the samples for each material were stored in Listerine Cool Mint and the other half in Colgate Optic White mouthwashes for 24 hours (n=11). The color and microhardness of the samples were measured baseline and after 24 h incubation in mouthwashes. After calculating color change (ΔE00) and microhardness changes (ΔVHN), the obtained data were analyzed in SPSS software at α=.05 by Two-Way ANOVA, One-Way ANOVA, Post-hoc Tukey, Independent and Paired-Samples t test.

Results: The effect of composite material type (p=.016) and mouthwash type (p=.008) on color change was statistically significant. All materials were more colored in Listerine Cool Mint than in Colgate Optic White but statistically significant only for FBF (p=.017). Discoloration of the materials in both mouthwashes didn't exceed the 50:50% acceptability threshold (ΔEAT=1.8). For all materials, microhardness decreased significantly after storage in mouthwashes compared to baseline state (p<.05). There was no significant difference in ΔVHN between the mouthwashes (p>.05).

Conclusion: Although the discolorations of materials were at an acceptable threshold for the tested mouthwashes, a significant decrease in microhardness is very important for long-term clinical success of the materials. The uncontrolled use of mouthwash should be avoided.

Keywords: Bulk-fill, Color stability, Microhardness, Mouthwashes, Spectrophotometer

1. INTRODUCTION

Resin composites are frequently preferred in direct restoration of teeth (anterior/posterior) due to their aesthetic and mechanical properties (1,2). In recent years, bulk-fill composite resins have become popular, offering bulk application and curing of up to 4-5 mm thickness in a single step (3). Bulk-fill composites have the advantage of less polymerization shrinkage, preventing the formation of interlayer gaps and contamination risks in conventional methods (4). It also provides advantages for both the physician and the patient, as it reduces the time it takes to complete the restoration (5). Additionally, for the bulk-fill technique, concerns have been reported regarding aesthetics, mechanical properties, degree of conversion, and polymerization stress, as well as disadvantages such as less satisfactory handling compared to traditional resin composites (6).

There are different type of bulk-fill materials available on the market with low and high viscosity that may or may not require coating with a conventional resin composite (7). However, some companies have also produced materials with high viscosity that offer the advantage of use by reducing viscosity with sonic activation (8). In addition to the advantages they provide, materials are affected by oral environmental conditions at different levels. Controlled clinical studies examining bulk-fill composites over the long term are limited literatures (9,10), but existing studies have reported that they exhibit similar clinical performance to conventional composite resins (11-13). In general, fracture toughness, marginal adaptation, adequate wear resistance, microhardness, and color stability are important factors affecting the long-term success of composite resin restorations (14). Microhardness is influenced by the composition and degree of polymerization of the resin composite. A low degree of polymerization of the composite can cause secondary caries and discoloration due to reduced microhardness and increased plaque accumulation (15).

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Increased microhardness affects the clinical success by the increasing resistance to wear and surface scratching (16). Another prerequisite for clinical success is color stability (17). After restorations are made properly in accordance with the shade selection, it is expected that the restoration will maintain its color and optical properties for a long time (18). However, composite resins can easily deteriorate when exposed to various conditions due to their polymer base (19).

In oral conditions, oral hygiene practices such as tooth brushing and use of various mouthwashes can have an impact on the mechanical and optical properties of composite resins (20,21). The preventive use of mouthwashes, which is extremely important in maintaining public oral health, has increased in recent years, especially due to the restrictions on face-to-face treatment of patients after the Covid-19 pandemic (22). Mouthwashes can be used for various purposes, including the treatment of periodontal disease, as a caries preventive in cases of high caries risk such as orthodontic treatment, in special cases such as bad breath, and for the expectation of whitening (23). These products are easy to obtain, and their use is often outside professional supervision (24). A previous study suggested that more than half of adults aged 25 to 34, and nearly a quarter of all age groups, use mouthwash daily (25).

Mouthwash solutions contain various components such as organic acids, emulsifiers, detergents, dyes and alcohol (26). While some mouthwashes used for whitening purposes contain hydrogen peroxide as the active ingredient (27), some contain alternative substances such as tetrasodium pyrophosphate, sodium hexametaphosphate, phthalimido-peroxy-caproic acid, and sodium citrate for this purpose (28). Variations in mouthwash composition, including ingredient concentration and pH, can influence their effects on restorative materials (29). Previous studies have reported that mouthwashes with lower pH and higher alcohol content can reduce the microhardness of composite resins, affect the bond between the matrix and inorganic fillers, leading to reduced erosion resistance and increased staining of the material (26,29-31). In addition, the literature provides varying results regarding the effects of hydrogen peroxide (HP)-containing and HP-free mouthwashes on restorations (15,23). The differences in results make the evaluation of the effects of mouthwashes on resin-based materials clinically important and inevitable.

The effects of mouthwashes on resin-based materials have often been evaluated on conventional resin composites (15,21,23). Especially, studies on the effect of mouthwashes on bulk-fill composite resins are limited (32,33). In this context, the aim of this study was to evaluate the effects of two different mouthwashes on the microhardness and color stability of three bulk-fill composite resins compared to conventional composite resin.

The null hypotheses of the study were, 1. Mouthwash type and material type does not affect the color stability of the tested materials, 2. Mouthwashes does not affect the microhardness of the materials, and 3. There is no difference between the two mouthwashes in terms of microhardness change of the tested materials.

2. METHODS

In this in vitro study, the following restoration materials were used: nanohybrid universal composite (Control: Clearfil Majesty Esthetic (CME), Kuraray, Japan), supranano-filled flowable bulk-fill (Estelite Bulk-fill Flow (EBF), Tokuyama, Japan), nano filled high viscosity bulk-fill (Filtek One Bulk-fill Restorative (FBF), 3M ESPE, USA), and sonic-activated bulk-fill composite (SonicFill-2 (SF2), Kerr, USA). For mouthwashes, Listerine Cool Mint (Johnson & Johnson, Neuss, DE, Germany) and Colgate Optic White (Colgate–Palmolive Swidnica, Poland) were used. The chemical compositions of the composite resins and mouthwashes used in the study are presented in Table 1 and Table 2, respectively. The study design is summarized in Figure 1. Minimum sample size was calculated for 2 sub-experimental groups of each material with 0.85 power and 0.40 effect size in G Power 3.1.9.2 software. A total of 88 samples, 22 from each material and 11 from each sub-group, were found sufficient (n=11). In the current study, a total of 176 samples were prepared, 88 samples for color stability and 88 samples for microhardness.

Table 1. *Restorative materials used in the study*

Material	Type	Contents	Filler Load	Manufacturer
Clearfil Majesty Esthetic	Nanohybrid universal composite	Bis-GMA, TEGDMA, hydrophobic aromaticdimethacrylate Silanated barium glass filler, prepolymerized organic filler, Filler size: 0,7 µm Photoinitiator:CQ	78 wt% 66 vol%	Kuraray, Okayama, Japan LOT: 7E0228
Estelite Bulk-Fill Flow	Suprananofil flowable bulk – fill composite	Bis-GMA, Bis-MPEPP, TEGDMA, SiO2, and ZrO2 fillers Filler size:200nm Photoinitiator: RAP technology™	70 wt% 56 vol%	Tokuyama, Tokyo, Japan LOT: 011E97
Filtek One Bulk-Fill Restorative	Nanofill high viscosity bulk-fill composite	AUDMA, AFM, DDDMA, UDMA Silica (20 nm), zirconia (4–11 nm), ytterbiumtrifluoride (100 nm), zirconia/silica Photoinitiator: CQ	76,5wt% 58,4 vol%	3M, St.Paul, USA LOT: NA29615
SonicFill-2	Sonic activated hybrid bulk-fill composite	Bis-GMA, TEGDMA, Bis-EMA Zirconium oxide, glass Filler size: 25-400nm Photoinitiator: not disclosed	81,3 wt% 66 vol%	Kerr, Orange, USA LOT: 6689419

Bis-GMA: Bisphenol A-glycidyl methacrylate, Bis-MPEPP: Bisphenol A polyethoxymethacrylate, TEGDMA: Triethylene glycol dimethacrylate AUDMA: Aromatic urethane dimethacrylate monomer, AFM: Additional fragmentation monomer, DDDMA: 1,10-decanediol dimethacrylate, UDMA: Urethane dimethacrylate, Bis-EMA: Ethoxylated Bisphenol A dimethacrylate, RAP: Rapid amplified photopolymerization

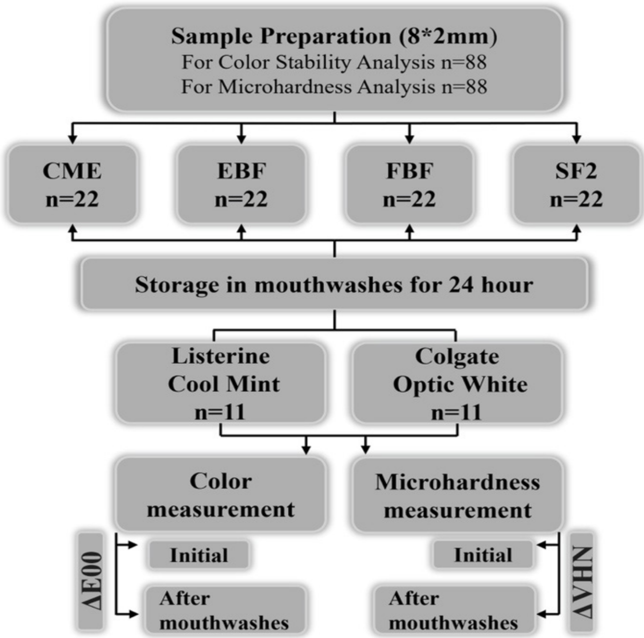

Figure 1. *Summary of the study design*

Table 2. *Characteristics of the mouthwashes used in the study*

Material	Type	Contents	pH	Manufacturer
Listerine Cool Mint	Whitening –	Aqua, Alcohol, Sorbitol, Poloxamer 407, Benzoic Acid, Sodium Saccharin, Eucalyptol, Methyl Salicylate, Thymol, Sodium Benzoate, Menthol, Aroma	3.89	Johnson & Johnson, Neuss, DE, Germany
Colgate Optic White	Whitening+	Aqua, Glycerin, Propylene Glycol, Sorbitol, Tetrapotassium Pyrophosphate, Polysorbate 20, Tetrasodium Pyrophosphate, Zinc Citrate, PVM/MA Copolymer, Aroma, Benzyl Alcohol, Sodium Fluoride, Sodium Saccharin	4.92	Colgate – Palmolive Swidnica, Poland

The samples were prepared in Teflon molds of 2 mm depth and 8 mm diameter. The resin composite was placed in the Teflon mold on a glass slide, gently pressed over the transparent strip and the excess resin was removed with a spatula. Then, a glass slide was placed on top, and the sample was polymerized. Polymerization was performed with a third-generation LED curing unit (Valo Grand, Ultradent Products, South Jordan, USA) in standard mode (1000 mW/cm^2 for 20 s). The tip of the curing device touched the glass to standardize the exposure distance during polymerization of all samples. For surface standardization, samples were polished by the same researcher using aluminum oxide discs (Sof-Lex Discs, 3M, St.Paul, MN, USA) of coarse, medium, fine and super fine sizes, respectively, for 5 seconds each at 10.000 rpm. The prepared samples were kept in lightproof bottles in distilled water at 37°C for 24 hours and the post-polymerization period was completed.

Prepared composite resin samples were divided into 2 groups according to mouthwashes. For each type of resin composite, half of the samples were stored in Listerine Cool Mint and the other half in Colgate Optic White (n=11). The samples were kept in lightproof bottles containing 20 ml of mouthwash solution for 24 hours. The bottles were numbered in order of sample. The pH of the mouthwashes was checked with a pH meter (Mettler-Toledo GmbH, Schwerzebbach, Switzerland) during the experiment (Listerine pH=3.89 and Colgate pH=4.92).

Color coordinates of the samples in the CIEL*a*b* system were measured from the upper surfaces with a spectrophotometer (VITA Easyshade V, VITA Zahnfabrik, Bad Säckingen, Germany) on a standard white background at baseline and after 24 h storage in the mouthwashes. L* indicates lightness and ranges from 0 for black to 100 for white, a* indicates green (a*<0) or red (a*>0) color components and b* indicates blue (b*<0) or yellow (b*>0) color components (21). To minimize the effect of environmental conditions, the measurements were performed by the same clinician under D65 standard light, with the tip of the device placed perpendicular (90°) to the sample surface, and the device was calibrated at each measurement. The color of the samples was measured 3 times and the average L, a, b values were recorded. Second color measurements followed the same protocol as the beginning. The following CIEDE2000 formula was used to calculate the color change (21):

$$\Delta E_{00}=\sqrt{\left(\frac{\Delta L}{K_L S_L}\right)^2+\left(\frac{\Delta C}{K_C S_C}\right)^2+\left(\frac{\Delta H}{K_H S_H}\right)^2+R_T\left(\frac{\Delta C}{K_C S_C}\right)\left(\frac{\Delta H}{K_H S_H}\right)}$$

In this formula, the letters "Δ_L", "Δ_C" and "Δ_H" indicate the differences in brightness, chroma and hue between the initial and subsequent measurements, respectively. S_L, S_C and S_H are functions used to calibrate the lack of visual inhomogeneity for the aspects of lightness (L), chroma (C) and hue (H). The environmental correction parameters used, K_L, K_C and K_H, were set to 1. RT is a rotation function included to consider the interaction between chroma and hue differences in the blue region.

Microhardness measurements of the samples were carried out baseline and after storage in mouthwashes using a Vickers device (Duramin, Struers, USA) under a load of 2.94N for 15 seconds at ×40 magnification. Three measurements were obtained for each sample, from the right, left and center of the upper surface and their averages were recorded. The change in microhardness was calculated with the formula $\Delta VHN=VHN_{after} - VHN_{before}$. Negative values in the results indicate a decrease in microhardness.

Data were analyzed in SPSS (v22.0, IBM-SPSS Inc. Chicago, Illinois, USA) software. In the evaluated parameters, the conformity of the data belonging to each group to normal distribution was determined with Shapiro Wilk Test, and the homogeneity was determined with Levene Test. The effects of composite material type, mouthwash type and composite*mouthwash interaction on color stability and microhardness were examined using Two-Way ANOVA. Differences of materials for each mouthwash in terms of ΔE00 and ΔVHN were examined with One-Way ANOVA. Pairwise comparisons were evaluated by Post-hoc Tukey and Post-hoc Tamhane's T2 test. Differences in color change between two mouthwashes were determined with Independent-Samples t test. The microhardness of each material at the baseline and after storage in the mouthwashes was examined with different Paired-Samples t test. (α=.05, statistically significant).

3. RESULTS

The color change (ΔE00) findings of the tested materials in the mouthwashes are listed in Table 3. The effects of material type (p=.016) and mouthwash type (p=.008) on color change were statistically significant, while the material*mouthwash interaction (p=.251) was not significant (Table 4).

Table 3. *Color changes (ΔE00) and microhardness change (ΔVHN) of tested materials in mouthwashes*

Parameter	Materials	Mouthwashes		
		Listerine Cool Mint	Colgate Optic White	p value*
ΔE00	CME	0.99±0.38 [a]	0.89±0.29 [ab]	.533
	EBF	1.62±0.75 [a]	1.39±0.79 [a]	.483
	FBF	1.48±0.90 [a]	0.71±0.37 [b]	.017
	SF2	1.34±0.45 [a]	1.08 ±0.35 [ab]	.148
	p value**	.151	.02	
ΔVHN	CME	-5.10±2.37 [a]	-3.60±1.16 [a]	.075
	EBF	-4.77±1.05 [a]	-4.10±3.23 [a]	.530
	FBF	-4.10±2.03 [a]	-4.05±1.60 [a]	.945
	SF2	-5.73±2.45 [a]	-4.64±2.03 [a]	.270
	p value**	.324	.730	

Different lowercase letters in the columns represent statistical significance, while at least one of the same letters indicates no significant difference (Separately for the ΔE00 and ΔVHN parameters). Lettering applies to columns only. Independent t test, **One-Way ANOVA. Statistical significance p<.05*

Table 4. *Two-Way ANOVA test results for color change and microhardness*

ΔE00	Sum of Squares	Mean Square	F value	p value
Materials type	3.725	1.242	3.637	.016*
Mouthwashes type	2.537	2.537	7.432	.008*
Materials*Mouthwashes	1.427	0.476	1.394	.251
VHN	**Sum of Squares**	**Mean Square**	**F value**	**p value**
Materials type	9730.496	3243.499	171.958	.000*
Mouthwashes type	3.641	3.641	0.193	.662
Materials* Mouthwashes	225.439	75.146	3.984	.011*

**Statistically significant. p<.05*

When the color change of the materials in the mouthwashes was examined, the difference between of the materials in Listerine Cool Mint mouthwash was not significant (p=.151), but the difference between of the materials in Colgate Optic White mouthwash was significant (p=.02). The least discoloration was obtained in FBF (0.71±0.37) kept in Colgate Optic White, which was not significantly different when compared to CME (0.89±0.29) and SF2 (1.08±0.35) (p>.05). This value was significantly lower compared to EBF (1.39±0.79) (p<.05) (Table 3).

Among the tested materials, discoloration of FBF was significantly lower in Colgate Optic White mouthwash (0.71±0.37) compared to Listerine Cool Mint (1.48±0.90) (p=.017). For the other materials, discoloration levels were slightly lower in Colgate Optic White than in Listerine Cool Mint, however, the differences were not statistically significant (p>.05) (Table 3)

The effects of material type (p<.001) and material*mouthwash interaction (p=.011) on microhardness were statistically significant, while the mouthwash type (p=.662) wasn't significant (Table 4). The microhardness of the tested materials at the baseline and after storage in mouthwashes are shown in Table 5. The microhardness of the materials decreased significantly after 24 hours of storage in both mouthwashes compared to the baseline state (p<.05). For both mouthwashes, the difference in microhardness between the materials was found significant (p<.05). The highest microhardness value was obtained in the Listerine Cool Mint group of SF2 (baseline: 74.34±3.34, after: 68.60±3.69, respectively), the lowest microhardness value was obtained in the Colgate Optic White group of CME (baseline: 45.14±2.22, after: 41.54±1.92). The microhardness order between the materials in Colgate Optic White mouthwash was SF2>FBF>EBF>CME. The order in Listerine Cool Mint was SF2>FBF>CME>EBF, however, the difference between the microhardness of CME and EBF in Listerine Cool Mint was not statistically significant (p>.05).

Table 5. *Microhardness values of the tested materials baseline and after mouthwashes*

Materials	Listerine Cool Mint		p value*	Colgate Optic White		p value*
	Baseline	After		Baseline	After	
CME	51.09±4.79 [a]	45.99±5.03 [a]	.000	45.14±2.22 [w]	41.54±1.92 [w]	.001
EBF	48.50±4.70 [a]	43.74±4.17 [a]	.000	51.88±3.10 [x]	47.77±3.18 [x]	.006
FBF	64.98±6.05 [b]	60.87±7.05 [b]	.000	67.27±3.31 [y]	63.21±4.00 [y]	.018
SF2	74.34±3.34 [c]	68.60±3.69 [c]	.000	72.94±4.91 [z]	68.30±3.85 [z]	.023

Different lowercase letters (a, b, c, d) in the same column indicate significant differences between the materials in Listerine Cool Mint mouthwash. Different lowercase letters (w, x, y, z) in the same column indicate significant differences between the materials in Colgate Optic White mouthwash. Lettering applies to columns only. Paired-samples t test. Statistical significance p<.05*

The changes in microhardness (ΔVHN) of the tested materials in the mouthwashes are listed in Table 3. The difference between the materials in terms of ΔVHN was not significant (p>.05). Besides, while the differences were not significantly different, the decrease in microhardness was greater in Listerine Cool Mint when compared to Colgate Optic White but it wasn't statistically significant (p>.05).

4. DISCUSSION

Mouthwashes are recommended to patients under the supervision of a dentist for the control of periodontal diseases and oral health (34); however, considering that these products are sold without a prescription in the dental market worldwide, they can often be used uncontrolled according to individuals' own preferences and application methods (24). Therefore, examining the effects of mouthwashes on resin composites, which are widely used restorative materials in the mouth, is an important issue in terms of the reliability of these products. According to the results of the current study, the first null hypothesis was rejected because the color stability of the tested materials was statistically significantly affected by the type of materials and the mouthwashes (Table 4).

All the tested composites tested in this study exhibited numerically greater discoloration in Listerine Cool Mint compared to Colgate Optic White; however, the increase in staining in Listerine Cool Mint mouthwash was statistically significant only in FBF (Table 3). Resin composites can be intrinsically or extrinsically colored due to aging from oral conditions or exposure to external agents such as mouthwashes (24). Previous studies have reported that acidic pH and alcohol content in mouthwashes can cause deterioration of the surface smoothness of composite resins and increased stainability, depending on the concentration (35-37). Staining after exposure to mouthwashes may occur internally due to rapid degradation of the polymer matrix resulting from the formation of alcohol and carboxylic acids resulting from hydrolysis of ester bonds in the materials due to the low pH (30), or externally due to adsorption of colored pigments from mouthwashes onto the surface of the composites (24,38). Both tested mouthwashes in this study were blue in color, however the pH of Listerine Cool Mint (3.89) was lower than that of Colgate Optic White (4.92). Additionally, it has been reported that tetra potassium pyrophosphate and tetrasodium pyrophosphate, which are also present in the composition of Colgate Optic White, are included in HP-free whitening mouthwashes to prevent stain adhesion (28). The greater staining observed in the materials tested in this study with Listerine Cool Mint mouthwash may be due to the lower pH and higher alcohol concentration (21.6%) of this mouthwash, as well as and the pyrophosphate content in Colgate Optic White, which prevents stain adhesion.

Regarding the findings on a per-material basis for color stability, no difference was observed between the materials in Listerine Cool Mint, while a significant difference was found between the materials in Colgate Optic White. The least discolored material was FBF, and its level of discoloring was significantly lower than EBF. The color change in the samples can be attributed to different resin formulations (26,39). It has been reported that materials using urethane dimethacrylate in the resin matrix exhibited more color stability than others due to their lower water absorption properties (40). Additionally, for the performance of resin composites in aqueous environments such as mouthwash solutions, uniform particle distribution throughout the polymer network in essential (41,42), as voids or unbonded areas at the filler/matrix interface can increase the water absorption of the composites (26). The smaller the filler particle, the less water absorbed by the polymer network, resulting in less disruption of the interface matrix/particle and hence lower color change (43). Among the tested materials, FBF, which showed the lowest coloration, was the only material containing UDMA without Bis-GMA in the resin matrix. Furthermore, the filler particle size (4-11nm) was lower than the other materials and the filler volume percentage (58.4%) was lower . This indicates that the particles are better compacted and can perform more stably in liquid environments. All of these may have contributed to the less staining of FBF than other materials in the current study.

The clinical perceptibility and acceptability of the coloration findings presented by the materials in the mouthwashes are important in terms of the contribution of the results to oral conditions. In this study, the 50:50% perceptibility threshold (ΔE_{PT}=0.8) and 50:50% acceptability threshold (ΔE_{AT}=1.8) reported by Paravina et al. were used to evaluate color changes (44). Morais Sampaio GA et al. reported in their systematic review that most studies under different in vitro conditions concluded that mouthwashes do not cause clinically unacceptable color changes in composite resins (42). Consistent with the results of previous studies, none of the materials in the current study exhibited unacceptable discoloration. In their study, where Toz Akalin et al. tested a Listerine-derived mouthwash with similar content to our study with a 12-hour immersion period, Sonicfill showed a color change above the clinically acceptable threshold, unlike the current study (45). The differences in results may be attributed to variations in the polishing procedures of the samples. Surface roughness can influence outcomes by facilitating the precipitation of coloring agents from the mouthwash onto the sample surface. The results of studies compare the discoloration levels of bulk-fill composites with conventional composites vary from lower (46) to higher (24). In this study, there was no significant difference in the discoloration levels of bulk-fill composites compared to the control group (CME), which was the conventional composite. Differences in results may be due to the composition of the tested materials and the behavior of the material in various mouthwashes. In addition, the fact that EBF with low viscosity is significantly more discolored than FBF with high viscosity bulk-fill is consistent with the results of previous studies in the literature (46). This may be related to the TEGDMA composition, which provides lower viscosity and at the same time causes more water absorption and solubility (43,46).

In the second step of the experiment, the effect of mouthwashes on the microhardness of the tested materials was investigated. The findings were that both mouthwashes significantly reduced the microhardness of the materials compared to the baseline (for all groups p<.05, Table 5), so the second null hypothesis of the study was rejected. It has been previously reported that mouthwashes contain water, essential oils, emulsifiers, alcohol and sometimes hydrogen peroxide (in the case of whitening mouthwashes), and this can result in hydrolysis of ester bonds in the polymer matrix of composite resins, softening of the polymer matrix and a decrease in microhardness (15,26,27,36). In this context, the decrease in microhardness of the tested materials after 24 hours of storage on both mouthwashes was an expected result. Considering the experimental procedure and material variety, it is difficult to compare the findings with previous studies; however, in line with our study, Alessa NA reported a significant decrease in microhardness in low pH mouthwashes post-immersion compared to pre-immersion (47). Similarly, Jyothi K. et al. (48) and Miranda Dde et al. (36) reported a significant decrease in microhardness, especially for alcohol-containing mouthwashes. Hamdy et al. (15) reported that Colgate Optic White mouthwash significantly reduced microhardness compared to artificial saliva, while Lepri et al. (39) reported that mouthwashes did not significantly differ from artificial saliva in terms of microhardness in their studies using Listerine. It has been reported that 24-hour storage under in vitro conditions simulates two years of clinical, assuming 2 minutes of daily mouthwash use is considered (49). When comparing the results with previous studies, it is important to note that the immersion protocol and duration are important factors for microhardness due to the aging of composite resins. In this study, the highest microhardness was obtained in SF2 both baseline and after storage in mouthwashes. The obtained result can be attributed to the increased filler content of SF2. The microhardness of the conventional composite CME was significantly lower than that of the other bulk-fill composites in the baseline and post-treatment measurements in two mouthwashes. This result may be related to the fact that bulk-fill composites contain translucent fillers to increase the depth of polymerization, which likely results in higher monomer conversion (7).

When examining the microhardness changes (ΔVHN) in the mouthwashes of the materials, the decrease in microhardness of all materials was slightly higher in Listerine Cool Mint compared to Colgate Optic White, but the difference between the two mouthwashes was not significant. Since the result for Listerine Cool Mint covers all materials, we can say that the third null hypothesis of the study was partially rejected. The lower pH and higher alcohol content of Listerine Cool Mint may have been effective in the obtained result. In addition, the HP-free form of Colgate Optic White was used in the study. Hamdy et al. (15) tested the HP-containing form of the same mouthwash and found a greater decrease in microhardness. Different forms of the same product on the market may cause different results due to chemical composition.

When the changes in microhardness (ΔVHN) were evaluated for each material, SF2 exhibited the greatest reduction in microhardness in both mouthwashes, although the differences were not statistically significant. It was an interesting finding that SF2 exhibited the highest hardness values both at baseline and post-immersion measurements and also demonstrated the greatest decrease in microhardness. Despite the increased filler content of Sonicfill2, it has been reported that sonic activation during placement significantly increased the internal void volume of the material (50). This may affect the matrix stability in liquid environments, leading to increased monomer release and a further decrease in microhardness, and may explain the results obtained in the present study.

Mouthwash exposure is an important factor to consider in the long-term performance of restorative materials. Although the mouthwashes tested in this study did not cause unacceptable changes in the color stability of the materials, they significantly reduced microhardness. Clinically, a decrease in microhardness may compromise the material's wear resistance, leading to increased surface roughness (42). This, in turn, can promote plaque accumulation, staining of the restoration, and ultimately reduce its longevity. Patients should be advised to avoid tooth brushing immediately after using mouthwashes, particularly those with low pH, to minimize potential adverse effects on restorative materials (15). When evaluating the clinical significance of the results obtained in this in vitro study, it is necessary to consider the limitations of the study such as the use of artificial saliva, the absence of brushing simulation, thermal cycles, and the lack of representation of patient' nutritional habits. Various intraoral factors and patient behaviors may either increase or mitigate the effects of mouthwashes on restorative materials.

5. CONCLUSION

Within the limitations of the study, the conclusions can be summarized as follows;

1. Although the color stability of the tested materials varied depending on the examined mouthwashes, the color changes were at an acceptable level for both mouthwashes. In this context, it can be suggested that the examined mouthwashes are safe in terms of color stability of the materials; however, the results should be validated by studies representing longer periods of clinical use.
2. The decrease in microhardness is a significant finding in terms of the effect of mouthwashes on the long-term clinical success of restorative materials. Therefore, the uncontrolled and prolonged use of mouthwashes should be avoided.
3. The discoloration and microhardness of the same material differed depending on the mouthwashes. Therefore, the restorative material selection should be based on the specific clinical situation, including the location of the restoration (anterior/posterior) and the expected performance properties.

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