



Effect of Different Mouthwashes on Stain Susceptibility of Resin Composite in Contact with Beverages

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ABSTRACT

Objectives: This study aimed to investigate the effect of common mouthwashes on the color change of a nanohybrid composite and its further stain susceptibility to Coke and coffee.

Materials and Methods: One hundred and fifty composite discs were prepared and initial color values were measured using a spectrophotometer. The specimens were grouped based on the 24h exposure to common mouthwashes including Listerine, chlorhexidine, two types of fluoride mouthwashes (alcohol free and alcohol containing) as well as distilled water as control (N=30). The color change ($\Delta E1$) values were calculated to show the amount of color change caused by mouthwashes. Subsequently, the specimens in each group were subdivided and immersed in the secondary colorant solution (coffee, Coke and, distilled water) for seven days. The total color change (ΔE total) values were obtained to show the stain susceptibility. Statistical analysis was conducted using ANOVA and Tukey's post hoc test. A P-value less than 0.05 was considered statistically significant.

Results: The calculated $\Delta E1$ of resin composite was significantly higher for all mouthwash groups than that of the control group; however, all were in the clinically acceptable range. Exposure to Listerine and distilled water caused more staining effects compared to other mouthwashes after immersion in secondary colorant solutions. Regardless of primary mouthwash type, coffee and distilled water caused the highest and the least total discolorations, respectively ($P < 0.001$).

Conclusion: All mouthwashes caused a clinically acceptable color change in resin composite; however, further stain susceptibility depended on the mouthwashes but was not higher than distilled water.

Keywords: Composite Resins; Mouthwashes; Color

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INTRODUCTION

In recent years, composite restorations have been widely used by dentists for various reasons including the improvement of bonding procedures, growing patients' demand for aesthetic restorations, as well as the quality and strength of the composites, which has been improved significantly [1]. A vast variety of composite materials are currently available in the market to choose from. Color stability of a

composite over time is one of the most important factors drawing the attention of dentists and patients [2].

Different factors affecting the color stability of a composite restoration can be categorized into intrinsic and extrinsic factors. Intrinsic factors include resin matrix structure, type and percentage of the filler particles, as well as the way resin matrix and filler are connected. Extrinsic factors include colorant foods and drinks, such as

tea and coffee, some mouthwashes like chlorhexidine, some medications, and physical stimuli, such as ambient light [3-6].

Mouthwashes are used as an adjunct to mechanical methods of biofilm control to prevent and treat oral diseases. Mechanical biofilm removal methods may be difficult to perform or insufficient to prevent oral diseases in some people including those who use fixed orthodontic appliances, those who have recently had oral or gum surgery, people with disabilities, and people at high risk of dental caries. Considering these factors, mouthwashes are widely used as a chemical method [7,8]. However, the ingredients used in these materials could cause discoloration of the composite restorations and lead to restoration replacement [9-11].

Different studies have investigated the effect of mouthwashes on the color change of composite restorations; however, they have reported different results [12-14]. On the other hand, although mouthwashes affect the color of the composites, other factors, such as food and drinks, bacteria, as well as salivary pellicle may also have additional effects. Therefore, the color changes of composite restorations are not solely due to mouthwashes, themselves. Additionally, the use of different mouthwashes can probably change the hardness and roughness of the composites and affect their color stability [3-6]. Composite discoloration caused by mouthwashes in clinical situations may be influenced by other chromogenic materials, such as different food and drinks which may intensify the chromogenic potential of mouthwashes. However, there is limited study on this issue and most studies have been performed in the absence of additional chromogenic materials [15]. Therefore, the present study aimed to investigate the effect of exposure of a nano-hybrid composite to common mouthwashes on the color change of composites in two stages. The first stage investigates discoloration caused by mouthwashes and the second stage studies the stain susceptibility or the discoloration caused by subsequent immersion in colorant solutions, such as Coke and coffee. Choosing these mouthwashes was based on the commercial availability of them [10]. Moreover, as it said that alcohol content of mouthwashes

may be related to composite discoloration [10,15] and as two types of fluoride mouthwashes are widely available, both alcohol containing and alcohol-free fluoride mouthwashes included in this study. The null hypothesis was that there are no differences in the color change of the composite after exposure to different mouthwashes and that exposure to mouthwashes cannot affect the stain susceptibility of the composite to Coke and coffee.

MATERIALS AND METHODS

Specimen preparation:

This study was approved by the ethics committee of Ardabil University of Medical Sciences (code number: IR.ARUMS.REC.1398.620). In this experimental study, 150 discs of a nano-hybrid composite (Estelite Σ quick [Tokuyama, Japan]) were made in A2 shade using a cylindrical mold. For this purpose, the composite material was filled into a Teflon mold with the inner diameter and height of 7mm and 2mm, respectively. A polyester strip was placed on top and bottom of the mold, and the whole set was positioned and pressed between two glass slabs to eliminate excess composite and produce a flat surface. After that, the glass slabs were removed and the composite was cured for 20 seconds from both sides using a light-emitting diode (LED) light source [Radii Plus (SDI, Australia)] with an output intensity of ~ 1000 mW/cm².

Immediately after curing, both sides of the specimens were polished using 1000, 1200, and 2000 grit silicon carbide abrasive paper. The specimens were then rinsed in an ultrasonic bath for 5 minutes to detach any residues. The thickness of the specimens was measured with a digital caliper [Mitutoyo (Mitutoyo, Japan)], and those with a thickness deviation of 0.05mm were removed. The specimens were immersed in distilled water and incubated at 37 °C for 24 h to complete the polymerization process.

Immersion in mouthwashes and colorant solutions:

The samples were randomly divided into five groups according to the studied mouthwashes including an essential oil mouthwash (Listerine Cool Mint [Johnson & Johnson GmbH, Italy]), an alcohol-containing fluoride mouthwash (Vi-one General [Rojin Cosmetic, Iran]), an alcohol-free

fluoride mouthwash (Vi-one General Alcohol-free [Rojin Cosmetic, Iran]), chlorhexidine (Chlorhexidine 0.2% [Najo, Iran]), and distilled water as the control. The specimens were immersed in 200mL of the solutions in opaque containers at 37°C for 24 hours [14].

Samples in each group were further divided into three subgroups according to the secondary colorant solutions after exposure to mouthwashes in the previous stage. The specimens

were kept in 200mL Coke (Khoshgovar, Iran), coffee (Nescafe Classic [Nestle Iran P.J.S, Iran]), and distilled water at 37°C for 7 days [16] which were changed daily.

The coffee solution was prepared using 15 g of instant coffee and 200 ml of hot water according to the instructions provided by the company and cooled to room temperature. Table 1 tabulated the composition of the resin composite and mouthwashes used in this study.

Table 1. The summary of the composition of resin composite and mouthwashes used in the study

Material	Composition
ESTELITE Σ QUICK (Tokuyama, Japan)	Matrix: bis-GMA, TEGDMA Fillers: 82% wt, 71% v, 0.2 μ m mean size of silica-zirconia filler and Composite filler
Chlorhexidine 0.2% (Najo, Iran)	10% ethanol, 0.2 g chlorhexidine gluconate per each 100 mL
Vi-one General (Rojin Cosmetic, Iran)	Sodium Fluoride, Deionized Water, Sorbitol, Glycerin, Ethanol, Poloxamer 407, PEG 40 Hydrogenated Castor Oil, Flavor, Cetyl Pyridinium Chloride, Sodium Methylparaben, Sodium Propylparaben, Citric Acid, Sodium Saccharin, Menthol, C.I.42090
Vi-one General Alcohol-free (Rojin Cosmetic, Iran)	Sodium Fluoride, Deionized Water, Sorbitol, Glycerin, Poloxamer 407, PEG 40 Hydrogenated Castor Oil, Flavor, Sodium Methylparaben, Sodium Propylparaben, Cetyl Pyridinium Chloride, Sodium Benzoate, Citric Acid, Sodium Saccharin, Menthol, C.I.42090-C.I.47005
Listerine Cool Mint (Johnson & Johnson GmbH, Italy)	Aqua, Propylene Glycol, Sorbitol, Poloxamer 407, Sodium Lauryl Sulfate, Eucalyptol, Benzoic Acid, Sodium Benzoate, Methyl Salicylate, Thymol, Sodium Saccharin, Sodium Fluoride, Menthol, Sucralose, Aroma, Cl 42053. Contains Sodium Fluoride (220 ppm F)

Color measurement:

The color of specimens was measured according to CIE L^*a^*b system using a reflection spectrophotometer (YS3020 diffuse8 [3nh, China]) relative to CIE standard illuminant D65 over a black background ($l=1.72$, $a=0.05$, $b=0.08$). An average of three measurements was recorded for each specimen. Color difference values were calculated using ΔE formula (as follows) in which l represents the lightness of the color, and a and b indicate the chromaticity values of the color (a : red-green value and b : yellow-blue value; $-a$ =green, $+a$ =red, $-b$ =blue, $+b$: yellow) [17]:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

The baseline color of specimens was measured after 24h incubation period, the second

measurement was conducted after immersion in mouthwashes, and the third measurement was done after the second immersion in colorant solutions. Before the color measurement, the specimens were removed from containers and rinsed with distilled water, and dried with a drying paper. Two values of ΔE were obtained as follow:

$\Delta E1$: The differences between the baseline color and the color after exposure to the mouthwashes, ΔE total: The differences between the baseline color and the final color after final exposure to the secondary solutions.

It should be noted that ΔE values greater than 3.3 was considered visually perceptible and clinically unacceptable.

Statistical analysis:

Data were analyzed using SPSS software (Version 23; IBM, USA). Descriptive statistics, including mean and standard deviation (SD) of ΔE values,

were reported. The $\Delta E 1$ values were compared using one-way ANOVA. Two-way ANOVA was used to analyze the interaction effect of mouthwashes and secondary solution types on total color changes (ΔE total). Pairwise comparisons were done using Tukey's post hoc test and $P < 0.05$ was considered statistically significant.

RESULTS

Table 2 presents the mean values of $\Delta E 1$. The results of one-way ANOVA showed a significant difference among different mouthwash groups. Pairwise comparisons using post hoc Tukey test revealed that all mouthwashes caused significant color change compared to distilled water; however, there were no differences between different mouthwashes. Considering the clinically acceptable threshold, all mouthwashes caused the acceptable color change.

Figure 1 presents the ΔE total values of the composite based on colorant solution and the primary exposure to mouthwashes.

Two-way ANOVA was utilized to evaluate the effect of primary exposure to mouthwashes and the secondary colorant solutions. The result of analysis showed that the interaction effect of these two factors along with each individual factor were significant ($P < 0.001$). Post hoc Tukey test showed that the primary exposure to chlorhexidine and alcohol-containing fluoride mouthwashes caused less stain susceptibility

to secondary colorant solution compared to exposure to control group ($P = 0.001$, $P = 0.028$, respectively). However, Listerine and alcohol-free fluoride mouthwashes were not significantly different compared to control group in term of stain susceptibility ($P = 0.747$, $P = 0.374$, respectively).

Moreover, there were no significant differences in paired wise comparisons between total discoloration of groups that were exposed to chlorhexidine, alcohol containing fluoride, and alcohol-free fluoride mouthwashes ($P > 0.05$).

Post hoc test revealed that, coffee and distilled water respectively caused the highest and lowest total discolorations ($P < 0.001$) regardless of primary mouthwash type. Moreover, exposure to coffee resulted in clinically perceptible discolorations in all subgroups.

Table 2. Mean \pm standard deviation (SD) of primary color changes after the composite was exposed to mouthwashes and distilled water (N=30)

Mouthwash	Mean \pm SD of $\Delta E 1^*$
Chlorhexidine	1.16 \pm 0.45 ^A
Fluoride	1.12 \pm 0.39 ^A
Alcohol free fluoride	1.3 \pm 0.45 ^A
Listerine	1.11 \pm 0.49 ^A
Distilled water	0.42 \pm 0.28 ^B
P	<0.001

* Values shown by different superscript letters are statistically different

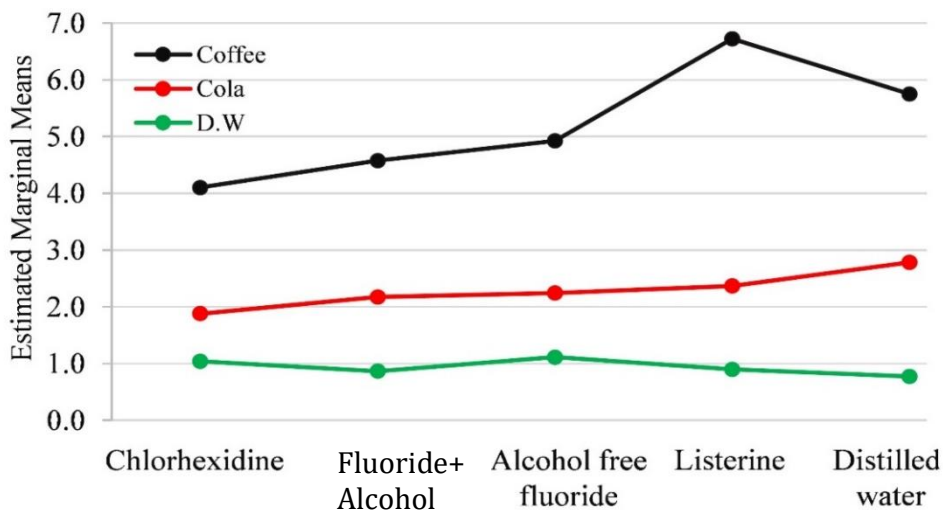


Fig. 1. Estimated marginal means of ΔE total values

DISCUSSION

The present study investigated the effect of some commercially available mouthwashes on the color change of a nanohybrid resin composite and its further stain susceptibility to the colorant solutions including Coke and coffee. The results rejected the null hypothesis of the study since all mouthwashes caused a statistically different amount of discoloration in the resin composite compared to distilled water and the exposure to some mouthwashes affected the subsequent stain susceptibility of the composite.

The exposure time is a critical factor affecting the results of *in vitro* experiments, and protocols that simulate at least one year or more daily use of mouthwashes are expected to produce significant differences. It should be noted that 24h immersion time in the mouthwashes selected in this study equaled two minutes of daily use for two years [14,18]. The results indicated that although the mouthwashes caused higher primary discoloration compared to distilled water, the color changes caused by different mouth-washes were not statistically different. Moreover, none of them could cause visually perceptible color changes and all the color changes were in the clinically acceptable range.

In a systematic review of 22 studies most of the included studies reported that mouthwashes did not cause clinically unacceptable color changes in resin composites which was in line with the results of the present study [15]. However, it was not possible to do the meta-analysis due to non-uniform methodologies of studies in the review. In addition, the review showed that in different studies, the comparison of the effect of different mouthwashes rendered controversial results [15]. For instance, Diab et al. [10], Lee et al. [9] and Gürdal et al. [11] reported that chlorhexidine mouthwash did not cause significant discoloration while fluoride mouthwash caused the greatest color change. It seems that several factors may have contributed to the differences observed in color stability values in the literature, including the type of resin composite, mouthwash formulation, and immersion time [15].

The common ingredients inside mouthwashes include chlorhexidine gluconate, ethanol, essential oils, detergents, anti-inflammatories, anesthetics, antibacterial agents, antifungals, antihistamines, remineralizing agents, moisturizers, flavorings, colorings, and stabilizers [19] and all of these agents can influence the final effect of mouthwash on the color change of restorative materials. Due to the staining effect of some ingredients, such as chlorhexidine, some manufacturers incorporate other ingredients to minimize this effect [18]. As an illustration, cetylpyridinium chloride is an old active material that has been added to mouthwashes and shows less severe staining compared to chlorhexidine [20].

On the other hand, because of its effect on the solubility of dimethacrylate-based resin composites the alcohol content of a product gained attention in terms of the staining effect. Some studies reported similar color changes in alcohol-containing and alcohol-free mouthwashes [13]. While others reported greater color change in resin composites exposed to alcohol-free mouthwashes compared to those containing alcohol [14, 21-24]. In the present study, the analysis resulted in similar values of $\Delta E1$ in all groups of mouthwashes regardless of the content of alcohol. It seems that other ingredients in different commercial products can have more impact on the staining effect of a mouthwash compared to the content of alcohol. Another study simulated six months of daily use of mouthwashes and showed that alcohol-containing mouthwashes did not induce morphological, ultrastructural, or biochemical change in resin composite [25]. This can be attributed to the higher impact of other contents, such as active ingredients, as well as pH of the mouthwash rather than alcohol content of mouthwashes per se which could affect the surface properties of resin composites [26].

It was reported that mouthwashes can cause surface degradation of the resin composite due to ingredients such as alcohol, detergent, emulsifier, and organic acids [27]. Although it was expected that exposure to mouthwashes makes the composite more susceptible to

staining by the secondary colorant solutions [15], in the present study, the chlorhexidine and alcohol-containing fluoride mouthwashes showed less total discoloration compared to ones recorded in distilled water and Listerine groups. It was demonstrated that the ultimate effect of chlorhexidine mouthwashes could change with the colorant food and beverages [20]. However, there is a lack of data to compare the results due to the difficulty of simulating the influencing factors in an in vitro environment [24]. Essential oils, such as eucalyptol, thymol, menthol, and methyl salicylate can facilitate the process of erosion/destruction of acrylic and thermo-plastic resins [28], wearing of filler surfaces, and debonding of fillers and increase the water sorption and solubility of resin composite [29]. The result of a study conducted by Yilmaz et al. showed that mouthwashes containing both essential oils and alcohol (21.6%) caused the most surface roughness in the nanohybrid composite [28]. They explained that the mouthwashes containing alcohol and essential oils had the lowest pH (pH=3.92) in addition to the highest alcohol level (21.6%); therefore, they can cause the most surface deterioration [28]. Despite this higher roughness showed in the studies [28], Listerine mouthwash in the current study expected to induced higher stain susceptibility but the stain susceptibility in Listerine group was equal to that of distilled water but, although was higher than other mouthwashes.

On the other hand, based on the results of the study performed by Urbano et al. [30] the alcohol concentration of mouthwash is not high enough to increase the surface roughness of a nanofilled composite. In the current study, alcohol-containing mouthwashes did not induce stain susceptibility in the composite. It can be concluded that the effect of mouthwashes on the surface properties of the resin composites is material dependent [28,31].

Based on the claims of some studies, the effect of mouthwashes on the color change of resin composite is related to the function of water itself [32, 33]; therefore, the water content of

mouthwashes can be a determining factor in deteriorating the surface of a resin composite and the subsequent staining susceptibility of the composite. When a composite is immersed in water, water sorption can cause a hygroscopic expansion in the resin network which can lead to such damages as color change, degradation of the filler/matrix combination, reduction of hardness, and wear resistance [34]. This can justify the observation that in this study distilled water (control group) induced more stain susceptibility compared to CHX and alcohol containing fluoride mouthwashes. However, it should be noted that in the current study, distilled water is used as a control and that the effect of saliva in the clinical situation must not be ignored. It has been shown that the distillation method and the utilized device also have an effect on the behavior and the staining effect of distilled water [17]. Moreover, based on the results of another study, the water sorption can be two times higher in distilled water than in artificial saliva [35]. Moreover, the resin composite used in this study contained BisGMA and TEGDMA that have higher water affinity, higher solubility, and a relatively higher degree of absorption and discoloration compared to other monomers [36].

Based on the results of the current study, the color change of composite caused by coffee was relatively higher (and in the clinically unacceptable range) compared to that caused by Coke and distilled water, regardless of the type of mouthwashes. It should be noted that although coffee has a higher pH compared to Coke (nearly 7 versus 2.7) [37], the yellowish color of the coffee penetrates the composite material and causes a higher color change. Therefore, the pH of the solutions plays a minor role in the composite discoloration. This result is in good agreement with the previous studies [38, 39]. Another interesting result in the present study was that the interaction effect of the mouthwashes and secondary solutions on total discoloration of composite was statistically significant. In other words, it can be said that the effect of mouthwashes on the color change of resin composite was influenced by the type of

different foods and beverages consumed. Therefore, a single in vitro study could not predict the exact clinical behavior of mouthwashes in terms of restorative material staining, and further studies are needed.

CONCLUSION

Based on the obtained results in the present in vitro study, all groups of the studied mouthwashes caused higher composite color change compared to that caused by distilled water; however, these color changes were in a clinically acceptable range. Furthermore, mouthwashes behaved differently in terms of inducing stain susceptibility in nanohybrid resin composites, with the higher amount belonging to the Listerine.

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CONFLICT OF INTEREST STATEMENT

None declared.

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