



Tooth and Composite Discoloration After Photodynamic Therapy with Different Photosensitizers and Cleansers Under Accelerated Aging Conditions

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Abstract

Introduction: This study aimed to evaluate tooth and composite discoloration after photodynamic therapy using different concentrations of photosensitizers, combined with various cleaning agents (water, 2.5% sodium hypochlorite and 17% EDTA), immediately and after accelerated aging.

Methods: In this *in vitro* study, 104 bovine maxillary central incisors were selected. Enamel-dentin discs (7×7×2 mm) were prepared from the middle of the crown and subjected to an artificial demineralization and remineralization cycle for 14 days. The samples were divided into 13 groups: Photodynamic therapy using methylene blue or toluidine blue at two concentrations of 50 µg/ml and 100 µg/ml, each with three detergents (water, 2.5% sodium hypochlorite, or 17% EDTA), and a control group: disinfection with chlorhexidine 2%. The surface of the dentin was bonded, and the composite resin was applied. All specimens underwent 100 hours of xenon lamp exposure. Tooth and composite colors were measured at baseline, after photodynamic therapy, and after accelerated aging. Statistical analysis was performed using one-way ANOVA and Tukey's HSD.

Results: Color change caused by methylene blue (50 µg/ml) and rinsing with EDTA or sodium hypochlorite was below the perceptibility threshold ($\Delta E < 3.3$). According to the Tukey HSD test, no significant difference was observed between this group and the control group ($P > 0.05$). All groups demonstrated clinically perceptible composite discoloration.

Conclusion: Tooth discoloration after photodynamic therapy with 50 µg/ml methylene blue and rinsing with EDTA or sodium hypochlorite is not visually detectable. Photodynamic therapy with 100 µg/ml methylene blue or toluidine blue leads to the discoloration of composite restorations.

Keywords: Photodynamic therapy, Affected dentin, Composite resin



Introduction

Dental caries, which is caused by certain pathogenic bacteria (primarily *Streptococcus mutans*), is one of the most common chronic diseases worldwide. The complete removal of deep carious lesions may lead to pulp damage or pulp exposure, which requires procedures such as pulpectomy and RCT. These treatments are expensive and lead to the loss of vital pulp function.¹ The new conservative approach is to selectively remove caries, which includes complete removal of demineralized dentin in the peripheral area and superficial necrotic dentin on the pulp side of the cavity to reduce sensitivity after treatment, prevent pulp exposure, and remineralize the inner layer of dentinal caries.^{2,3}

The number of bacteria in the inner layer of carious dentin or affected dentin is less than the surface area.

However, these bacteria can demineralize dentin and proliferate, resulting in a concern about recurrent caries and sensitivity after treatment.⁴

Various methods have been used to reduce the number of bacteria in the cavity after the selective removal of caries. The oldest method uses chemical antimicrobial agents such as 2% chlorhexidine gluconate.⁵ Newer methods have been introduced to increase the amount and depth of the antimicrobial effect.⁶

Antimicrobial photodynamic therapy (aPDT) is a broad-spectrum antimicrobial treatment, which is effective against multidrug-resistant microorganisms. It can also act very specifically on the cell wall of bacteria, and unlike old methods, it does not lead to bacterial resistance.⁷ aPDT involves the use of a low-intensity light source, such as a laser, and a light-sensitive material containing

a non-toxic dye. When light with a specific wavelength (630–800 nm) is irradiated, light-sensitive agents directly react with oxygen molecules or biomolecules to produce cytotoxic substances such as free radicals (type 1 reaction) or singlet oxygen (type 2 reaction). This reaction leads to bacterial cell wall and cell membrane damage, reducing metabolism or causing cell death.⁸

aPDT is a promising option for treating dental caries.⁹ RCT studies have shown reduced *Streptococcus mutans* and *Lactobacillus* counts in deeply decayed dentin of the restorative cavity, reduced plaque formation on the tooth surface, and reduced bacterial counts in the dental biofilm after PDT.^{10,11} A clinical study has shown the effectiveness of PDT in reducing salivary *Streptococcus mutans* counts in children with severe early caries.² In addition, an animal study has shown the prevention of enamel demineralization in the presence of a cariogenic diet.¹² A meta-analysis showed that PDT has a potential indication in treating deep caries of primary teeth. However, in the case of permanent teeth, more studies with long follow-ups are necessary.¹³

Bisphenothiazine-based dyes such as toluidine blue and methylene blue are photosensitizers often used in aPDT with favorable results.⁹ However, these are colored substances that can change the tooth color and endanger the patient's beauty and mental well-being.¹³ Few studies have investigated the effect of these light-sensitive materials on the discoloration of teeth and restorations, and little information is available on how these light-sensitive materials are removed from the tooth after photodynamic therapy.¹⁴ Therefore, further investigations with other light-sensitive agents and irrigation solutions with different concentrations are necessary to determine a potential protocol for minimizing tooth discoloration and restoration after photodynamic therapy. Therefore, this study aimed to determine tooth and composite resin restoration color changes after photodynamic therapy with different concentrations of light-sensitive substances and irrigation with water, 2.5% hypochlorite, and 17% EDTA immediately and after accelerated aging.

Materials and Methods

Sample Size Calculation

The Fixed Effects ANOVA Power Analysis of PASS 11 software was used based on the results reported by Ayun. Considering $\alpha=0.05$ for two variables of light-sensitive substance with six levels with an effect size = 0.442 and a detergent with four levels and an effect size = 0.411, the sample size of each subgroup of the study was calculated at $n=8$ samples, with a statistical power of 0.999 to discover a significant difference for both independent variables ($\beta=0.001$).¹⁵

Preparation of the Samples

A total of 104 bovine maxillary central incisors were selected. The teeth with caries, cracks, hypomineralization,

and hypoplasia were excluded. Scaling and removal of debris were performed with a scaler, followed by pumice powder and a rubber cap. The samples were disinfected in a 1% chloramine T solution and kept at room temperature for up to one month in normal saline before the research process. A cubic block of enamel–dentin measuring $7 \times 7 \times 2$ mm was prepared from the middle region of the crown of each tooth using a diamond disk. The samples were washed with distilled water and dried. The enamel surface was covered with two layers of colorless nail polish and allowed to dry for an hour.

Demineralization and Remineralization Cycle

The samples were placed individually in 10 mL of a demineralizing solution (2.2-mM CaCl_2 , 50-mM NaH_2PO_4 , and 2.2-mM acetic acid) with pH = 4.8 for 8 hours, followed by immersion in a remineralizing solution (1.5-mM CaCl_2 , 0.9-mM NaH_2PO_4 , 0.15-mM KCl) with pH = 7 for 16 hours. After immersion, the samples were washed with distilled water, which continued for 14 days at room temperature without agitation. Then, the varnish was removed with acetone, and the samples were washed with distilled water and dried.

Groups

The samples were divided into 13 groups:

1. Methylene blue (Blue + M, Novateb Pars, Iran) with a concentration of 50 $\mu\text{g/mL}$ and rinsing with distilled water (M50W)
2. Methylene blue with a concentration of 50 $\mu\text{g/mL}$ and rinsing with 2.5% sodium hypochlorite (M50H)
3. Methylene blue with a concentration of 50 $\mu\text{g/mL}$ and rinsing with 17% EDTA (M50E)
4. Methylene blue with a concentration of 100 $\mu\text{g/mL}$ and rinsing with distilled water (M100W)
5. Methylene blue with a concentration of 100 $\mu\text{g/mL}$ and rinsing with 2.5% sodium hypochlorite (M100H)
6. Methylene blue (Blue + T, Novateb Pars, Iran) with a concentration of 100 $\mu\text{g/mL}$ and rinsing with 17% EDTA (M100E)
7. Toluidine blue with a concentration of 50 $\mu\text{g/mL}$ and rinsing with distilled water (TBO50W)
8. Toluidine blue with a concentration of 50 $\mu\text{g/mL}$ and rinsing with 2.5% sodium hypochlorite (TBO50H)
9. Toluidine blue with a concentration of 50 $\mu\text{g/mL}$ and rinsing with 17% EDTA 50 (TBO50E)
10. Toluidine blue with a concentration of 100 $\mu\text{g/mL}$ and rinsing with distilled water (TBO100W)
11. Toluidine blue with a concentration of 100 $\mu\text{g/mL}$ and rinsing with 2.5% sodium hypochlorite (TBO100H)
12. Toluidine blue with a concentration of 100 $\mu\text{g/mL}$ and rinsing with 17% EDTA (TBO100E)
13. Control group: application of 2% chlorhexidine to the surface for 30 seconds using an applicator, followed by drying with cotton pellets

Photodynamic Therapy

The samples were immersed in the coloring solution for 5 minutes and then underwent diode laser irradiation. For methylene blue, irradiation with a wavelength of 660 (Konftec, Taiwan) and a power of 150 mW was performed for 1 minute, and for toluidine blue, irradiation was carried out with a wavelength of 635 nm (Konftec, Taiwan) and a power of 220 mW for 1 minute. Then, the samples were immersed in the rinsing solution for 5 minutes, and agitation was performed every one minute.

Composite Resin Restoration

Universal bonding (G-Premio Bond, GC) was applied on the dentin surface according to the manufacturer's instructions. Then, composite resin (G-aenial Anterior GC) shade A1 with a thickness of 2 mm was placed on the surface using an index. The celluloid matrix tape was placed on the composite resin surface; then, a glass slab was placed on it and gently pressed to create a smooth surface. Then, the composite resin was irradiated at a light intensity of 1000–1100 mW/cm² for 40 seconds with a Lux (DTW) light-curing unit (Woodpecker, China).

Each sample was placed inside a tube containing normal saline and incubated at 37°C for 24 hours.

Accelerated Aging

The samples were placed in a metal case with a xenon lamp (Xeno Test Beta LM, Germany) to undergo xenon irradiation with a wavelength of 320 nm at a temperature of 35°C, humidity of 22%, and exposure of 95% in the on state and 5% in the off state for 100 hours.¹⁶

Spectrophotometry

Color measurements were performed on the external surface (enamel) of the tooth at baseline (T0), after photodynamic therapy (T1), and after accelerated aging (T2), and on the composite resin surface immediately after restoration (T1) and after accelerated aging (T2).

Color analysis was performed using a spectrophotometer (Vita Easy Shade V, VITA Zahnfabrik), which calculates CIEL*a*b* values for 2° observation and D65 illumination curve and is not significantly affected by different lighting conditions or sources. This device uses a probe with a diameter of 5 mm. The single-tooth measurement mode was selected. Calibration was performed by placing the probe tip on the calibration port before measuring each sample. The color measurements were standardized by using the same white background and one operator. A silicone index was used to vertically position the spectrophotometer at the center of each sample. The color coordinate data were calculated by the software of the device. In these calculations, the L* parameter represents the brightness axis, a* represents the red-green axis, and b* represents the yellow-blue axis. Overall color changes

between different time points were determined using the following formulae:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

$$\Delta L^* = L2 - L1$$

$$\Delta a^* = a2 - a1$$

$$\Delta b^* = b2 - b1$$

L*: lightness (-L* = black; +L* = white), a*: green-red (-a* = green; +a* = red), and b*: blue-yellow (-b* = blue; +b* = yellow)

The color changes of each sample were measured three times, and the mean value was reported as the final result.

Statistical Analysis of Data

Data were analyzed using SPSS 25 software. For this purpose, central dispersion indices were calculated and reported, including mean, standard deviation, minimum and maximum values of parameter b, and overall color changes (ΔE). Due to the normal distribution of data, statistical comparisons were performed with one-way ANOVA. Also, pairwise comparisons of the groups were performed with HSD Tukey tests. An acceptable type 1 error in this study was considered at 0.05.

Ethics Code

This study was approved with the code of ethics IR.TUMS.DENTISTRY.REC.1401.062 in the Ethics Committee of Tehran University of Medical Sciences.

Results

Tooth Discoloration

Tables 1 and 2 present the results of tooth discoloration. According to the results, the color change in the methylene blue group with a concentration of 50 µg/mL, followed by rinsing with EDTA and sodium hypochlorite, could not be detected by naked eyes ($\Delta E < 3.3$), while in other groups, the color change was clinically detectable.

According to HSD Tukey tests, the color change in the groups of methylene blue with a concentration of 50 µg/mL, followed by rinsing with EDTA and sodium hypochlorite, was not significantly different from that of the control group ($P > 0.05$), while the other groups had significantly more color changes than the control group ($P < 0.05$). The maximum color change was observed in the toluidine blue group with a concentration of 100 µg/mL, followed by rinsing with water and EDTA (Figures 1 and 2). The measurement of the amount of change in parameter b* (Table 3) showed that the color changes were toward decreased yellowness, but the tooth color remained in the yellow (positive) range in all groups.

Table 1. Means±SD of overall color change of the tooth after stain removal compared with baseline (ΔE_1)

Cleansing agent	Photosensitizing agent			
	M 50*	M 100**	TBO 50***	TBO 100****
Distilled water	4.80±3.46 ^{Ab}	4.36±2.55 ^{Aa}	6.66±1.70 ^{Ab}	8.04±3.06 ^{Ab}
EDTA	2.29±1.48 ^{Aa}	5.36±2.53 ^{Abc}	6.95±2.20 ^{Ab}	7.86±1.79 ^{Ac}
Sodium Hypochlorite	3.14±1.23 ^{Aa}	4.38±1.97 ^{Aa}	3.93±1.52 ^{Aa}	4.99±2.26 ^{Aa}
Chlorohexidine	0.26±0.13			

*=Methylene blue 50 µg/ml, **=Methylene blue 100 µg/ml, ***=Toluidine blue 50 µg/ml, ****=Toluidine blue 100 µg/ml.
Means labeled with the same uppercase letters are not significantly different, as compared in columns. Means labeled with the same lowercase letters are not significantly different, as compared in rows.

Table 2. Means±SD of overall color change of the tooth after accelerated aging compared with baseline (ΔE_2)

Cleansing agent	Photosensitizing agent			
	M 50*	M 100**	TBO 50***	TBO 100****
Distilled water	17.23±3.39 ^{ABa}	17.56±1.44 ^{Aa}	18.19±2.64 ^{Aa}	18.74±4.97 ^{Aa}
EDTA	18.73±2.33 ^{Aa}	15.54±3.18 ^{Aa}	9.19±3.16 ^{Bb}	18.90±1.54 ^{Aa}
Sodium Hypochlorite	13.55±0.97 ^{Ba}	14.35±2.05 ^{Ab}	12.60±3.98 ^{Ba}	18.60±1.49 ^{Ab}
Chlorohexidine	15.71±2.54			

*=Methylene blue 50 µg/ml, **=Methylene blue 100 µg/ml, ***=Toluidine blue 50 µg/ml, ****=Toluidine blue 100 µg/ml.
Means labeled with the same uppercase letters are not significantly different, as compared in columns. Means labeled with the same lowercase letters are not significantly different, as compared in rows.

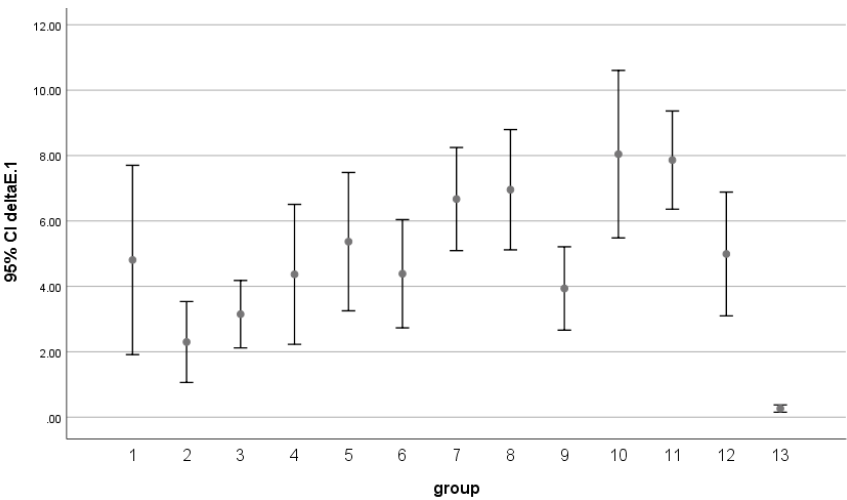


Figure 1. Means±SD of overall color change of the tooth after stain removal compared with baseline (ΔE_1)

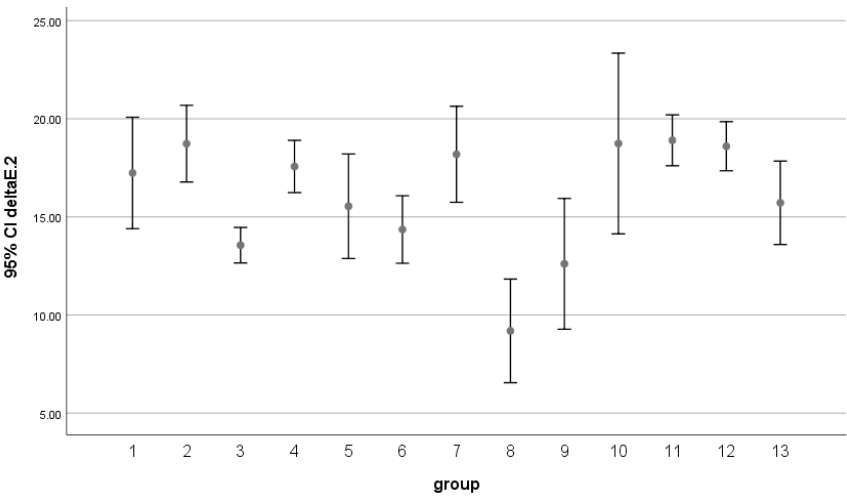


Figure 2. Means±SD of overall color change of the tooth after accelerated aging compared with baseline (ΔE_2)

Table 3. Means $\pm$ SD of color change in the yellow-blue axis of the tooth after stain removal (Δb_1) and after accelerated aging (Δb_2) compared with baseline and composite resin after accelerated aging (Δb_c)

Group	Δb_1	Δb_2	Δb_c
MB50 W	-2.35 $\pm$ 1.62	-5.66 $\pm$ 2.50	0.46 $\pm$ 1.10
MB50 E	-1.88 $\pm$ 1.64	-6.63 $\pm$ 1.14	0.56 $\pm$ 0.96
MB50 H	-2.40 $\pm$ 1.64	-6.35 $\pm$ 1.56	1.88 $\pm$ 1.31
MB100 W	-4.15 $\pm$ 2.65	-5.00 $\pm$ 1.68	-1.30 $\pm$ 2.01
MB100 E	-4.40 $\pm$ 2.32	-6.07 $\pm$ 0.47	-2.82 $\pm$ 1.47
MB100 H	-2.32 $\pm$ 1.65	-5.31 $\pm$ 1.80	-3.65 $\pm$ 1.26
TBO50 W	-5.44 $\pm$ 1.73	-6.15 $\pm$ 1.89	-4.74 $\pm$ 1.28
TBO50 E	-5.02 $\pm$ 1.54	-3.26 $\pm$ 0.71	-3.41 $\pm$ 1.47
TBO50 H	-3.11 $\pm$ 1.22	-4.63 $\pm$ 1.55	-6.08 $\pm$ 1.67
TBO100 W	-6.12 $\pm$ 2.93	-7.47 $\pm$ 1.01	-2.94 $\pm$ 2.05
TBO100 E	-6.05 $\pm$ 1.54	-6.77 $\pm$ 1.20	-0.31 $\pm$ 1.37
TBO100 H	-4.30 $\pm$ 2.59	-4.70 $\pm$ 2.35	-2.45 $\pm$ 2.04
Chlorohexidine	-0.02 $\pm$ 0.23	-3.61 $\pm$ 1.58	0.70 $\pm$ 1.32

M50W=Methylene blue 50 μ g/ml-water, M50E=Methylene blue 50 μ g/ml-EDTA, M50H=Methylene blue 50 μ g/ml- sodium hypochlorite, M100W=Methylene blue 100 μ g/ml- water, M100E=Methylene blue 100 μ g/ml- EDTA, M100H=Methylene blue 100 μ g/ml- sodium hypochlorite, TBO50W=Toluidine blue 50 μ g/ml- water, TBO50E=Toluidine blue 50 μ g/ml- EDTA, TBO50H=Toluidine blue 50 μ g/ml- sodium hypochlorite, TBO100W=Toluidine blue 100 μ g/ml- water, TBO100E=Toluidine blue 100 μ g/ml- EDTA, TBO100H=Toluidine blue 100 μ g/ml- sodium hypochlorite.

Color Change of Composite Resin

Table 4 shows color changes in composite resin. In all the groups, clinically detectable discoloration of the composite resin was observed (Figure 3). Examining the changes in parameter b* (Table 3) showed that the changes in the control group and in the methylene blue group with a concentration of 50 μ g/mL with all three rinsing solutions tended to increase yellowness, and they tended to decrease yellowness in the other groups.

Discussion

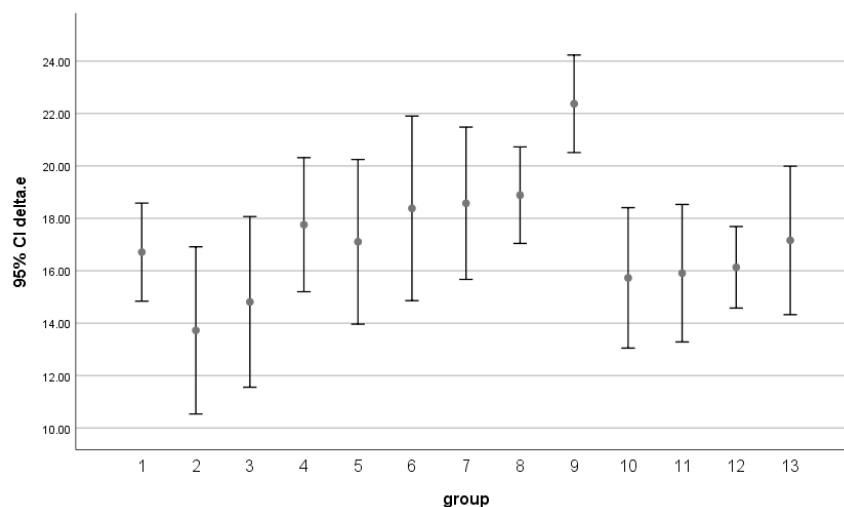
In this study, the effect of photodynamic therapy using methylene blue and toluidine blue at concentrations of 50 μ g/mL and 100 μ g/mL, followed by rinsing with water, sodium hypochlorite or EDTA, on tooth and composite resin discoloration, was investigated. The results showed that the discoloration of the teeth happened as a decrease in yellowness, although methylene blue at 50 μ g/mL, followed by rinsing with sodium hypochlorite or EDTA, resulted in no visible color change. The results of composite resin color changes after accelerated aging showed clinically unacceptable discoloration in all the groups. The composite resin color change in the control group and in the methylene blue group at a concentration of 50 μ g/mL was associated with an increase in yellowness, whereas in other groups a decrease in yellowness was noted.

Table 4. Means $\pm$ SD of overall color change of composite resin after accelerated aging (ΔE_c)

Cleansing agent	Photosensitizing agent			
	M 50*	M 100**	TBO 50***	TBO 100****
Distilled water	16.71 $\pm$ 2.23 ^{Aa}	17.75 $\pm$ 2.76 ^{Aa}	18.57 $\pm$ 3.14 ^{Aa}	15.72 $\pm$ 2.89 ^{Aa}
EDTA	13.72 $\pm$ 3.81 ^{Aa}	17.10 $\pm$ 3.75 ^{Aa}	18.88 $\pm$ 2.20 ^{Aa}	15.90 $\pm$ 3.13 ^{Aa}
Sodium Hypochlorite	14.81 $\pm$ 3.52 ^{Aa}	18.38 $\pm$ 4.21 ^{Aab}	22.37 $\pm$ 2.22 ^{Ab}	16.13 $\pm$ 1.86 ^{Aa}
Chlorohexidine	17.15 $\pm$ 3.38			

*=Methylene blue 50 μ g/ml, **=Methylene blue 100 μ g/ml, ***=Toluidine blue 50 μ g/ml, ****=Toluidine blue 100 μ g/ml.

Means labeled with the same uppercase letters are not significantly different, as compared in columns. Means labeled with the same lowercase letters are not significantly different, as compared in rows.

**Figure 3.** Means $\pm$ SD of overall color change of composite resin after accelerated aging (ΔE_c)

Light-sensitive materials are essential in photodynamic therapy. However, the saturation of the dentin structure with these materials has adverse effects, such as tooth discoloration. According to previous studies, this color change can be detected using digital methods and visual observation.¹⁷

The digital spectrophotometer uses the CIEL*a*b* system, which provides a sensitive and repeatable method. This system shows the color differences numerically and is in agreement with visual perception. For this reason, this method was used in the present study to investigate the color changes of teeth and composite resin. According to previous studies, $\Delta E = 3.3$ was considered the clinically acceptable threshold.¹⁸

Despite the effect of chlorhexidine mouthwash on the tooth and composite resin color, studies have shown that 2% chlorhexidine as a cavity disinfectant does not cause tooth and composite resin discoloration^{19–21}; therefore, 2% chlorhexidine was chosen as the control group in this study. It has been observed that toluidine blue achieves its maximum antibacterial effect at lower concentrations due to its higher solubility in the bacterial membrane compared to methylene blue. However, this effect may vary in different bacterial species, depending on their cell structure.²² A clinical study showed no significant difference in the antibacterial effect of PDT with methylene blue and toluidine blue at a concentration of 100 µg/mL and chlorhexidine on carious dentin of the pulpal floor of the cavity.²³ In the present study, the highest color change occurred with toluidine blue (100 µg/mL), followed by rinsing with water, with a significant difference compared to methylene blue. One study measured the diffusion rate of methylene blue into root dentin as 6.74×10^{-6} cm/s.²⁴ The molecular weight of toluidine blue is one-third of that of methylene blue, which can increase its penetration into the dentin.^{25,26} Therefore, due to its lower water solubility and greater penetration, it is expected that toluidine blue will induce greater color change, and its removal will be more difficult,²⁷ which was confirmed in our study. In the present study, tooth discoloration caused by methylene blue and toluidine blue was not significantly different after rinsing with 2.5% sodium hypochlorite. However, in the case of rinsing with water, the difference between methylene blue (100 µg/mL) and toluidine blue (100 µg/mL) was significant. Figueiredo et al. compared tooth discoloration induced by methylene blue (100 µg/mL) and toluidine blue (100 µg/mL) immediately after photodynamic therapy and concluded that after rinsing with 2.5% sodium hypochlorite, there was no significant difference between the two agents¹⁷; these findings are consistent with our study.

In our study, after accelerated aging, there was no significant difference in tooth discoloration between methylene blue (100 µg/mL) and toluidine blue (100 µg/mL). Costa et al. compared tooth color changes following

photodynamic therapy with methylene blue (100 µg/mL) and toluidine blue (100 µg/mL) after 60 days of water immersion and concluded that there was no significant difference between the two agents after rinsing with 1% sodium hypochlorite,²⁷ which is consistent with our study.

In our study, the change in tooth color after rinsing with water remained clinically unacceptable in all groups. Similarly, Souza et al,²⁸ Ramalho et al,²⁹ and Carvalho et al³⁰ showed that water rinsing alone could not effectively remove the discoloration caused by methylene blue (100 µg/mL), which is in agreement with our results.

Sodium hypochlorite, by producing free chlorine, can oxidize methylene blue and toluidine blue residues in the dentin structure and has been used as a rinsing agent after photodynamic therapy.^{31,32} In the present study, there was no significant difference between distilled water and 2.5% sodium hypochlorite in any of the groups immediately after the treatment. However, unlike water, sodium hypochlorite could reduce discoloration to clinically acceptable levels in the 50 µg/mL methylene blue group. The previous studies by Ramalho et al²⁹ and Carvalho et al³⁰ reported a significant difference between rinsing with 2.5% sodium hypochlorite and saline in removing methylene blue (100 µg/mL) from dentin immediately after treatment. These results are somewhat contradictory to our study. This discrepancy can be related to the rinsing time. In previous studies, samples were rinsed for 2–3 minutes; however, since tooth discoloration was not reduced to clinically acceptable levels in most of these studies,³³ a rinsing time of 5 minutes was selected in the present study. The chlorine released from sodium hypochlorite has a bleaching effect on the tooth structure.^{34–36} Due to the prolonged exposure to sodium hypochlorite in this study, a part of the color change can be attributed to the oxidation of dentin chromophores rather than residual photosensitizers.

In the present study, eight samples were excluded due to debonding after accelerated aging. All of these samples belonged to the groups rinsed with sodium hypochlorite. The effect of sodium hypochlorite on the bond strength of composite resin materials has been investigated in various studies, and it has been determined that its effect depends on the chemistry of the adhesive system used, the form (gel or solution), and the concentration of sodium hypochlorite and its application time.³⁷ The adverse effect of sodium hypochlorite on the bond strength, as seen in various studies, is related to its proteolytic properties and the interference of the residual free chlorines with free radical polymerization.³⁸ Several studies concluded that sodium hypochlorite adversely affects the bond strength of self-etch systems, with no effect on etch-and-rinse systems.^{39,40}

In a previous study, using 2.5% sodium hypochlorite for 1 minute significantly reduced the pull-out bond strength of self-adhesive cement to dentin.⁴¹ In the present study,

using 2.5% sodium hypochlorite for 5 minutes and a universal self-adhesive bonding system resulted in debonding in some samples. However, a few studies have suggested that when sodium hypochlorite is used at an optimal concentration and for an appropriate duration with etch-and-rinse systems, after etching and before bonding, it may enhance the bond strength to dentin. There is currently insufficient evidence to support this claim.³⁷

Ethylene diamine tetraacetate (EDTA) is an anionic and water-soluble material that dissolves inorganic sediments, including metal oxides and carbonates, and is used as a chelating agent in root canal therapy.⁴² Its effects on the dentin structure include the removal of the smear layer and smear plug and superficial, mild demineralization of peritubular and intertubular dentin.⁴³ It has been reported that EDTA has a whitening effect on dentin due to its ability to dissolve dentinal phosphoproteins.⁴⁴ In addition, it can act as a reducing agent to neutralize residual free chlorine.⁴⁵

Ramalho et al²⁹ reported that rinsing with 17% EDTA for 2 minutes was insufficient to remove discoloration caused by 100 µg/mL methylene blue. They suggested increasing the rinsing time, considering that the maximum effect of EDTA occurs after approximately 5 minutes. In the present study, rinsing with 17% EDTA was performed for 5 minutes. The results showed that in the 50 µg/mL methylene blue group, discoloration was reduced to a clinically acceptable level following EDTA application, while in the other groups, EDTA could not completely remove the discoloration.

In the present study, after accelerated aging, there was no significant difference in the degree of color change induced by methylene blue between the groups rinsed with water and those rinsed with 17% EDTA. Souza et al.²⁸ investigated tooth discoloration after photodynamic therapy with 100 µg/mL methylene blue and rinsing with distilled water or 17% EDTA for 3 minutes. The results showed no significant difference in color change between the two groups after 60 days of water immersion, which is consistent with our study.

In the present study, all water-rinsed groups showed significantly more color change compared to the control group. Karakas et al⁴⁶ observed that photodynamic therapy with 100 µg/mL methylene blue and rinsing with water caused significantly higher tooth discoloration compared to 2% chlorhexidine, which is consistent with our results.

The high concentration and prolonged application time of EDTA might be destructive for the dentin structure. Gandolfi et al. showed that 17% EDTA led to complete dentin demineralization and destruction of collagen bonds. They also reported that EDTA residues persisted in dentin and continued to destroy the tooth structure even after 24 hours. Therefore, they stated that the current guidelines regarding the use of EDTA,

including the concentration and time, should be revised.⁴⁷ Most studies have stated that 17% EDTA can be safely applied to the dentin surface for 1–2 minutes. However, longer application times are likely to result in structural damage.^{48,49} Therefore, although the 5-minute application was effective in eliminating discoloration caused by 50 µg/mL methylene blue, it raises concerns about the destruction of the tooth structure.

Ozkocak et al¹⁸ reported that the application of methylene blue and toluidine blue (100 µg/mL) on the surface of composite resin restorations resulted in significant discoloration, which could not be removed by water rinsing. In the present study, the photosensitizers were applied to the tooth surface and rinsed prior to composite resin restoration. In this study, the overall color changes of the composite resin were affected by multiple factors, including the presence of residual photosensitizers and detergents, as well as changes during accelerated aging. Each of these factors affects L*, a*, and b* parameters differently. To assess the amount of methylene blue and toluidine blue penetration, changes in the b* parameter were evaluated. It was observed that in the control group and in the 50 µg/mL methylene blue groups, the yellowness of composite resin was increased after accelerated aging, whereas in other groups, a decrease in yellowness was noted. This may suggest the penetration of blue pigment residues into the bulk of the composite resin. Further studies are warranted to clarify this phenomenon.

Limitations of the study include the use of bovine teeth and disc-like specimens. In a clinical situation, irregularities in the cavity wall may make it more difficult to remove pigments. Also, the 5-minutes rinsing time may not be feasible in a clinical situation. Furthermore, intraoral conditions, including temperature and pH changes, are not fully simulated by artificial aging.

Using a combination of cleaning agents may improve the efficacy of pigment removal while enabling a reduction in overall washing time. For future studies, it is recommended to implement a combined washing protocol consisting of a reduced exposure time to sodium hypochlorite, followed by the application of EDTA, in order to eliminate any residual pigments and neutralize the free chlorine released by sodium hypochlorite.

Conclusion

1. Tooth discoloration after photodynamic therapy with methylene blue at a concentration of 50 µg/mL and subsequent rinsing with EDTA or sodium hypochlorite cannot be detected by human eyes.
2. When sodium hypochlorite was used for rinsing, there was no significant difference between methylene blue and toluidine blue in terms of tooth color change.
3. The highest degree of tooth discoloration occurred after photodynamic therapy with toluidine blue at a

concentration of 100 µg/mL and subsequent rinsing with water or EDTA.

4. Photodynamic therapy in the restorative cavity using methylene blue at a concentration of 100 µg/mL or toluidine blue led to the color change of the composite resin restoration toward yellow.

Authors' Contribution

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Competing Interests

I certify that I have no commercial associations (consultancies, patent-licensing arrangements, equity interests) that might represent a conflict of interest in connection with the submitted manuscript.

Ethical Approval

This study was approved with the code of ethics IR.TUMS.DENTISTRY.REC.1401.062 in the Ethics Committee of Tehran University of Medical Sciences.

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