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Effect of Two Matrix Metalloproteinase Inhibitors on the Color Stability of a Nanofilled Resin Composite

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Clinical Relevance

Accelerated aging induced perceptible color change in a nanofilled resin composite whether or not matrix metalloproteinase inhibitors were used.

SUMMARY

Objectives: This *in vitro* study evaluated the effect of two matrix metalloproteinase (MMP) inhibitors on the color stability of two shades of a nanofilled resin composite.

Methods and Materials: A total of 60 sound human molars were used in this study. Flat dentin surfaces were obtained by wet grinding the occlusal surfaces. Following acid etching, the molars were divided into three equal groups according to the MMP inhibitor used: Group 1: no inhibitor (control group), group 2: chlorhexidine digluconate based (CHX; Consepsis, Ultradent, South Jordan, UT, USA); group 3: doxycycline based (MTAD; Biopure, Dentsply TulsaDental, Johnson, TN, USA). Adper Single Bond 2 Adhesive (3M ESPE, St Paul, MN, USA) was applied to the treated dentin surfaces. Each group was then subdivided into two equal subgroups of 10 molars each, according to the shade of the resin composite (Filtek Z350 XT, 3M ESPE) used, either B1 or A3. The color was assessed for each subgroup at three times: baseline (after 24 hours); after aging using a total energy of 600 kJ/m^2 (Weather-Ometer Ci35A, Atlas Electronic Devices Company, Chicago, IL, USA); and then after a second period of aging, for a total energy of 1200 kJ/m^2. Color assessment was carried out using a spectrophotometer. Color change (ΔE) was calculated according to the Commission Internationale de l'Eclairage L*a* b* color scale, comparing each aging period with the baseline color measurement. Data were analyzed using repeated measures analysis of variance and Tukey *post hoc* test.

Results: All tested subgroups showed greater discoloration than the clinically acceptable

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level (3.3). MTAD induced the highest statistically significant color change, followed by CHX, whereas the control groups showed the lowest statistical ΔE values with both tested shades. Shade B1 subgroups showed higher ΔE values when compared with shade A3 subgroups.

Conclusion: Accelerated aging caused color change in a nanofilled resin composite regardless of MMP inhibitor used. Furthermore, lighter shades showed less color stability when compared with darker shades.

INTRODUCTION

The formation of a perfect resin-infiltrated hybrid layer, composed of collagen fibrils embedded by methacrylate-based resins, has been thought essential to provide durable and successful adhesion to human dentin. However, bonding to dentin is still a problem. The breakdown of resin-bonded interfaces has been directly related to the loss of stability of the hydrophilic resin components that comprise the hybrid layers and incomplete resin infiltration to the depth of the hybrid layer. This was associated with the breakdown of the naked collagen fibrils at the base of the hybrid layer. The degradation of these collagen fibrils affects the durability and longevity of the adhesive joint.[1,2] Several authors have attributed this phenomenon to the activation of the host-embedded enzymes in the dentin matrix, known as matrix metalloproteinases (MMPs).[1-4]

These host-derived proteases are thought to play an important role in numerous physiological and pathological processes occurring in dentin, including the degradation of collagen fibrils that are exposed by suboptimally infiltrated dental adhesive systems after acid etching.[2] Collagenolytic/gelatinolytic activity of these endogenous enzymes has recently been shown to be suppressed by using nonspecific protease inhibitors, such as chlorhexidine- and doxycycline-based products. Apart from their antibacterial property, these products also have an inhibitory effect on the MMP activity in dentin. This effect can be useful in preventing collagen degradation and disintegration of the bonding interface over time. Enhancement of the bond durability by using MMP inhibitors has been demonstrated by many authors.[2,5-6]

It has been assumed that both chlorhexidine- and doxycycline-based MMP inhibitors possess a degree of dentin substantivity.[7] However, the resin composite restorative material is identified as a semitransparent material. In this respect, the color of the underlying tissues can influence the esthetics of the restoration.[8]

This raised the question as to whether the use of MMP inhibitors can affect the color stability of resin composites, especially with lighter shades and when used under thin restorations, as in the case of laminate veneers.

Color stability is among the factors contributing to the success of esthetic restorations. Color alteration of resin composites is a multifactorial phenomenon and is associated with intrinsic discoloration and extrinsic staining that can occur during clinical use.[9]

Evaluation of color stability in tooth-colored restorations can be carried out by either visual assessment with shade guides or by the use of a digital spectrophotometer. Color can be considered a complex phenomenon, and several factors, such as lighting conditions, translucency, opacity, light scattering, and the human eye, may influence the overall perception of tooth color. Color assessment can be measured with a reflection spectrophotometer using the Commission Internationale de l'Eclairage (CIE) L*a*b* color measuring system, which is considered the most widely accepted color-notation system. According to the system, L* indicates the lightness of color (high L* value denotes increased lightness, whereas low value denotes increased darkness), a* indicates the red-green axis (a high a* value indicates increased redness, whereas a low value indicates increased greenness), and b* indicates the yellow-blue axis (a high b* value denotes increased yellowness, whereas a low value indicates increased blueness).[9-11] The color differences in this system can be expressed in units that can be related to visual perception and clinical significance; it is also well suited to determine small color differences.[10,11]

Thus, this current study was conducted to verify the effect of two MMP inhibitors on the color stability of a nanofilled resin composite restoration. The hypothesis tested that the use of MMP inhibitors does not affect the color stability of a nanofilled resin composite.

METHODS AND MATERIALS

Materials

Two types of MMP inhibitors, chlorhexidine (Consepsis, Ultradent, South Jordan, UT, USA) and doxycycline (Biopure MTAD, Dentsply TulsaDental, Johnson, TN,, USA) were evaluated. Two shades of visible light-activated nanofilled resin composite (shades B1 and A3, Filtek Z350, 3M ESPE, St Paul,

Table 1: *Materials' Specification, Composition, Manufacturers, and Lot Numbers*

Material	Specification	Composition	Manufacturer	Lot Number
Filtek Z350 XT Universal Restorative Material (shades B1 and A3)	Visible-light-activated nanofilled resin composite	Organic part: Bis-GMA, UDMA, TEGDMA, PEGDMA, and Bis-EMA resins. Inorganic part: 72.5% by wt (55.6% by volume) The fillers are a combination of a nonagglomerated/ nonaggregated 20 nm silica filler, a nonagglomerated/ nonaggregated 4-11 nm zirconia filler, and an aggregated zirconia/silica cluster filler (composed of 20 nm silica and 4-11 nm zirconia particles). Average cluster particle size of 0.6-20 μm.	3M ESPE, St Paul, MN, USA	Body B1: N194214 Body A3: N279581
Adper Single Bond 2 Adhesive	Adhesive system	Bis-GMA, HEMA, dimethacrylates, ethanol, water, a novel photoinitiator system, and a methacrylate functional copolymer of polyacrylic and polyitaconic acids. Incorporates 10% by weight of 5-nm-diameter spherical silica particles.		N271533
Fine Etch	Etchant	37% phosphoric acid semigel.	Spident Co Ltd, Incheon, Korea	FE11004
Consepsis	Chlorhexidine-based matrix metalloproteinase inhibitors	2.0% chlorhexidine gluconate, 98% water.	Ultradent Product Inc, South Jordan, UT, USA	U015
Biopure™ MTAD	Doxycycline-based matrix metalloproteinase inhibitors	mixture of a broad spectrum antibiotic (3% doxycycline hyclate which is a tetracycline isomer), 4.25% Citric Acid, detergent (0.5% Tween 80)	Dentsply Tulsa Dental specialities. Dentsply international Inc., Rolling Hills Drive, Johnson City	100255

Abbreviations: Bis-EMA, bisphenol A polyethylene glycol diether dimethacrylate; Bis-GMA, bisphenol A diglycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; PEGDMA, poly(ethylene glycol) dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

MN, USA) were used. The adhesive system used was Adper Single Bond 2 (3M ESPE). The material composition, manufacturer, and lot number are presented in Table 1.

Specimen Preparation

A total of 60 sound, noncarious lower first and second human molars were used. The teeth were washed, scrubbed, and scaled to remove any remnants of blood, mucous, shreds of periodontal ligaments, plaque, and calculus. The teeth were then stored in distilled water at room temperature for a maximum period of one month.[11]

The molars were embedded vertically in an autopolymerized acrylic resin (Acrostone Dental Factor, Cairo, Egypt) inside plastic tubes (with an internal diameter of 16 mm and a height of 15 mm) so that the occlusal surface faced upward to the level of the cervical line. Following setting of acrylic resin, the specimens were removed from the plastic tubes. Flat dentin surfaces were obtained by wet grinding the occlusal surfaces of the specimens using a 180-grit silicon carbide paper on a grinding machine (Emmevi SPA, Badia Polesine, Italy) with continuous water irrigation to produce a clinically relevant smear layer.[12]

The dentin surfaces were etched with 37% phosphoric acid gel (Fine Etch 37, Spident Co Ltd, Incheon, Korea) for 15 seconds, rinsed thoroughly, and then blot dried.

The samples were divided into three groups of 20 specimens each, according to the surface treatment used: group 1: no pretreatment (control group); group 2: application of a chlorhexidine digluconate based MMP inhibitor (Consepsis, Ultradent) for 60 seconds, and then the surfaces were blot dried; group 3: application of a doxycycline based MMP inhibitor

(Biopure MTAD, Dentsply TulsaDental) for 60 seconds, and then the surfaces were blot dried.

For each specimen, two consecutive coats of Adper Single Bond 2 adhesive (3M ESPE) were applied to the etched surface for 15 seconds with gentle agitation using a fully saturated brush applicator. The adhesive was gently air thinned for five seconds to evaporate the solvents. The adhesive coat was then light cured for 10 seconds, according to manufacturer's instructions, using an LED light with 1000 mW/cm^2 intensity and an effective wavelength of 420-480 nm (PenCure, J Morita Mfg Corp, Kyoto, Japan).

Each group was then subdivided into two equal subgroups of 10 teeth each according to the shade of resin composite used. In the first subgroup, shade B1 was used; in the second subgroup, shade A3 was used.

A specially fabricated metallic holder was used to hold the block and tooth during resin composite packing. The holder was constructed from a hollow stainless steel cylinder with a 30-mm length, 22-mm external diameter, and 16-mm internal diameter. The upper end of the holder was machined to create a 19-mm internal diameter for a split Teflon mold. A circular split Teflon mold was fabricated with a 2-mm thickness, 19-mm external diameter, and a central hole with a diameter of 5 mm. The lower end of the holder was designed to permit an adjustable stainless steel screw to move inward and outward to displace the acrylic block with the tooth until the flat tooth surface was flush with the split Teflon mold, which would allow adequate resin composite packing. After application of the adhesive resin, each block was placed inside the metallic holder and the height adjusted using the adjustable screw at the lower end of the ring. The Teflon mold was placed and the resin composite was then bulk packed using a Teflon condenser. A Mylar strip was placed on top of the mold, further covered by a glass slab, and pressed to extrude the excess material and obtain a uniformly smooth specimen surface. With the light-tip guide placed as close to the surface as possible, the specimens were cured for 20 seconds, according to manufacturer's instructions, using an LED light with a 1000 mW/cm^2 intensity and an effective wavelength of 420-480 nm (PenCure, J Morita Mfg Corp). A diagram for sample preparation is presented in Figure 1. Great attention was given to have samples as similar as possible to one another, so no polishing techniques were used because they would modify the surface.[13] The prepared specimens were removed from the stainless steel holder and stored in distilled water at room temperature for 24 hours[11] after labeling each specimen on the base of the acrylic mold to make sure that each specimen was a control for itself, using a repeated-measures approach.

Color Measurement and Artificial Aging of the Specimens

Specimens were removed from the distilled water and dried using filter paper. Baseline color assessment was carried out using a spectrophotometer (Shimadzu, UV-3101 PC Schimadza Corporation, Tokyo, Japan). The specimens were fixed to the hole of a specially fabricated holder that had a 5-mm wide aperture. Scanning of specimens was done in reflectance mode over a wavelength range of 380-780 nm (the visible spectrum range).The spectrophotometer consisted of a photometer unit and a computer. The light beam from a tungsten-halogen lamp was chopped by a chopper mirror into a sample beam and reference beam, and then it was passed through the sample compartment to the detector.

The light beam was detected by a photomultiplier sensitive to the visible/ultraviolet region of light. An integrating sphere 60 mm in diameter was attached and installed in the sample compartment of the spectrophotometer to measure diffuse reflectance of the sample. The integrating sphere opening was 18 mm in diameter on the reflection side. The sample beam was at an incident angle of 0° and the reference beam, 8°.

The spectrophotometer was calibrated for diffuse reflection 0/d geometry using a set of ceramic tiles calibrated at National Physical Laboratory, Teddington, England.

Colorimetric values of the specimens were determined using the CIE L* a* b* color scale.

After baseline measurements, specimens were aged for 50 hours with a total energy of 600 kJ/m^2 in an accelerated aging chamber (Weather-Ometer Ci35A, Atlas Electronic Devices Company, Chicago, IL, USA) using 1400-W xenon long-arc lights with a sophisticated controlled irradiance system, ensuring uniform specimen exposure.

Spectral power distribution was specified between 290 and 800 nm; relative humidity: 50%; chamber air temperature: 38°C; water spray: 18 minutes wet / 102 minutes dry; water temperature: 50°C; black-panel temperature: 70°C (light) and 38°C (dark); dry bulb temperature: 47°C (light) and 38°C (dark). Color

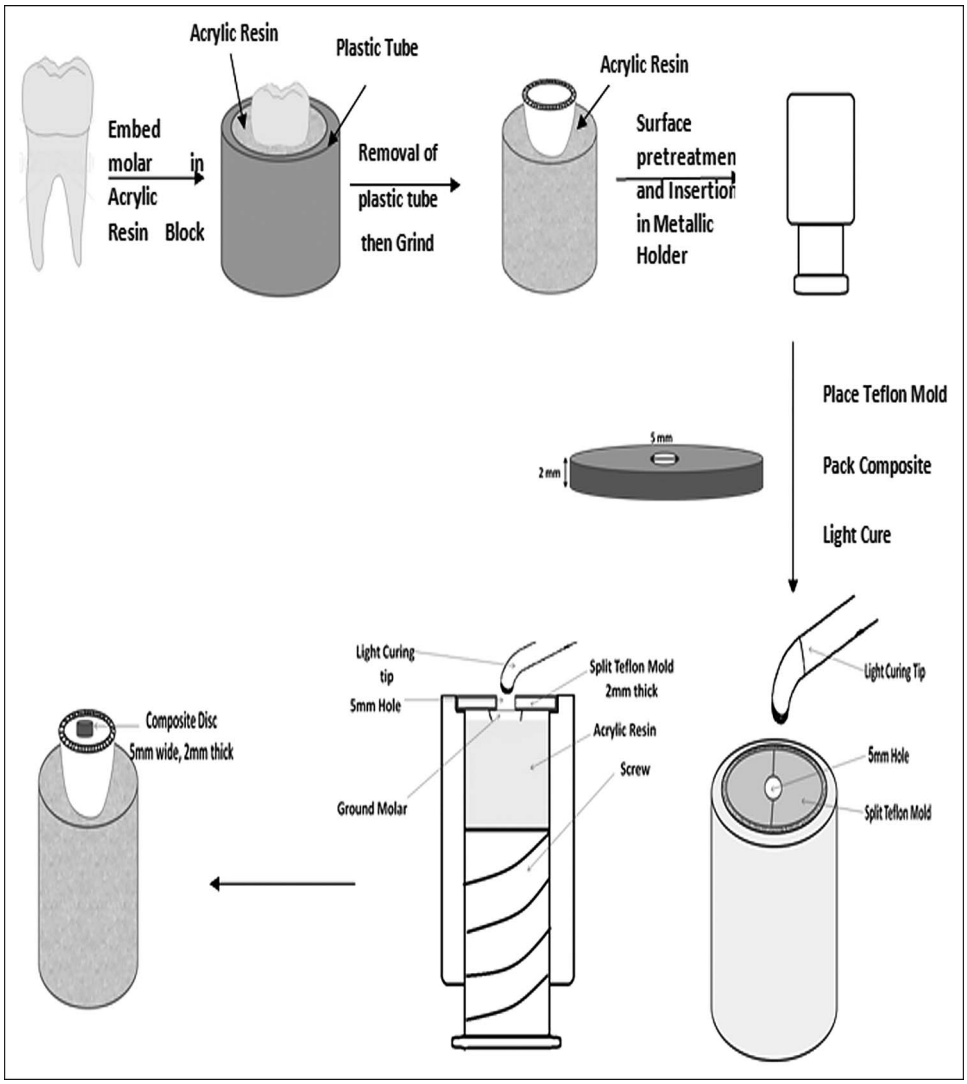

Figure 1. *Illustrative diagram for sample preparation.*

assessment was then performed. The specimens were then aged again using parameters similar to those described earlier. Therefore, the specimens received a total energy of 1200 kJ/m^2 for 100 hours.

The color change was calculated twice from the following equation:

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

where ΔE_1 represents the color change between the artificial aging for 50 hours (600 kJ/m^2) and the baseline color measurement, and ΔE_2 represents the color change between artificial aging for 100 hours (1200 kJ/m^2) and the baseline color measurement.

Statistical Analysis

Statistical analysis was performed with an IBM computer (IBM Corporation, Armonk, NY, USA) and SPSS (SPSS Inc, Armonk, NY, USA) version 20 for Windows.

The ΔE data were presented as mean and standard deviation (SD) values. A regression model using repeated-measures analysis of variance (ANOVA) was used in testing the significance for the effect of inhibitor, shade, aging, and their interactions on ΔE. Detailed comparisons between inhibitors were performed using a one-way ANOVA. The Tukey *post hoc* test was used for a pairwise comparison between the mean values when the ANOVA was found to be significant. Detailed comparisons between shades were performed using

Table 2: *Repeated Measures ANOVA Results for the Effect of Different Variables on ΔE*

Source of Variation	Sum of Squares	*df*	Mean Square	*p*-value*
Inhibitor	10	2	5	<0.001
Shade	70.9	1	70.9	<0.001
Aging	49.6	1	49.6	<0.001
Inhibitor × Shade × Aging	0.3	2	0.15	<0.001

Abbreviations: ANOVA, analysis of variance; df*, degrees of freedom (n-1);ΔE, color change.*
** Significant at* $p \leq 0.05$.

the Student *t*-test. The significance level was set at $p \leq 0.05$ for all analyses.

RESULTS

For the color analysis, a repeated-measures ANOVA was used to detect the effect of different variables (MMP inhibition, shade, and accelerated aging) and their interaction on mean ΔE values. The results showed that all variables and their interactions had a significant effect on the color change (Table 2).

The changes on the mean lightness and chromaticity coordinates and the total color change (ΔL*, Δa*, Δb*, and ΔE), along with the associated standard deviations, are presented in Table 3. It was evident that accelerated artificial aging of all tested subgroups induced perceptible color changes (ΔE≥3.3). The ΔE values ranged from 3.83 ± 0.27 to 7.19 ± 0.54 for the control group, from 4.14 ± 0.23 to 8.72 ± 0.49 for the CHX-treated group, and from 4.34 ± 0.48 to 9.61 ± 0.58 for the MTAD-treated group.

The effects of the MMP inhibitors on mean ΔE color changes in all tested subgroups was performed using a Tukey *post hoc* test (Table 4). It was evident that with both tested shades (B1 and A3), the doxycycline-based MMP inhibitor (MTAD) induced the highest statistically significant ΔE values, followed by the CHX-based inhibitor. The control groups showed the lowest statistically significant ΔE values.

A Student *t*-test revealed that, under all testing conditions, shade B1 showed a statistically higher perceptible color change when compared with shade A3 (Table 5).

DISCUSSION

A strong bond between dentin and resin composite materials is a main goal of adhesives in restorative dentistry. The degradation of collagen fibrils at the base of the hybrid layer is one of the causes of bond failure that has been attributed to MMP enzymes, which are naturally found in the dentin matrix.[1,4,14] Because resin composites are used for esthetic restorations, color stability in the environment is also a major concern. After years of research, MMP inhibitors were found to preserve the adhesive bond between resin composite and dentin and thus decrease the rate of failure at the restoration interface.[2,5-6] Thus, it seemed important to evaluate the effect of these inhibitors on the color stability of resin composites.

One type of resin composite material was used to avoid material dependent color changes. Two shades were tested: a dark shade (A3) and a lighter shade (B1). Color is a complex phenomenon, and several factors may influence the overall perception of tooth color; these include lighting conditions, translucency, opacity, light scattering, and the human eye. To eliminate potential subjective errors in color assessment, a spectrophotometer was used in the current study because it can numerically specify the per-

Table 3: *Mean (± SD) of ΔL, Δa, Δb, ΔE Values of All Tested Subgroups*

Subgroup	Aging for 50 Hours Mean (±SD)				Aging for 100 Hours Mean (±SD)			
	ΔL	Δa	Δb	ΔE_1	ΔL	Δa	Δb	ΔE_2
Control shade B1	−4.9 (±0.49)	1.18 (±0.15)	2.00 (±0.26)	5.43 (±0.43)	−6.51 (±0.58)	1.38(±0.16)	2.71 (±0.33)	7.19 (±0.54)
Control shade A3	−3.37 (±0.31)	1.01 (±0.07)	1.49 (±0.08)	3.83 (±0.27)	−4.08 (±0.4)	1.15(±0.07)	1.96 (±0.16)	4.67 (±0.39)
CHX shade B1	−5.56 (±0.52)	1.54 (±0.22)	2.63 (±0.45)	6.36 (±0.55)	−7.70 (±0.51)	1.94 (±0.2)	3.55 (±0.48)	8.71 (±0.49)
CHX shade A3	−3.69 (±0.29)	1.00 (±0.13)	1.57 (±0.4)	4.14 (±0.24)	−4.44 (±0.25)	1.11 (±0.14)	2.21 (±0.25)	5.09 (±0.23)
MTAD shade B1	−5.84 (±0.54)	1.89 (±0.21)	3.09 (±0.3)	6.89 (±0.43)	−8.35 (±0.65)	2.35 (±0.19)	4.11 (±0.25)	9.61 (±0.58)
MTAD shade A3	−3.88 (±0.52)	1.02 (±0.09)	1.63 (±0.08)	4.34 (±0.48)	−4.87 (±0.54)	1.18 (±0.07)	2.32 (±0.18)	5.52 (±0.52)

Abbreviations: CHX, chlorhexidine-based group; Δa, red-green axis; Δb, yellow-blue axis; ΔE, change in color; ΔL, lightness; MTAD, doxycycline-based group.

Table 4: *Mean and Standard Deviation of Mean ΔE Values Representing the Effect of Matrix Metalloproteinase Inhibitors on All Tested Groups*

Shade	Artificial Aging	Control (No Inhibitor)		CHX-based MMP Inhibitor		MTAD-based MMP Inhibitor		*p*-value*
		Mean	SD	Mean	SD	Mean	SD	
B1	50 hours of aging (ΔE_1)	5.43 C	0.44	6.35 B	0.55	6.89 A	0.43	<0.001
	100 hours of aging (ΔE_2)	7.19 C	0.54	8.72 B	0.49	9.61 A	0.58	<0.001
A3	50 hours of aging (ΔE_1)	3.83 C	0.27	4.14 B	0.23	4.34 A	0.48	<0.001
	100 hours of aging (ΔE_2)	4.67 C	0.39	5.09 B	0.23	5.52 A	0.52	<0.001

Abbreviation: CHX, chlorhexidine-based group; ΔE, color change; MMP, matrix metalloproteinase; MTAD, doxycycline-based group.
** Significant at $p \leq 0.05$. Different letters within the same row indicate significant statistical differences.*

ceived color of an object.[18] Thus, it was possible to compare the color change after aging using the ΔE parameter of the CIE L*a*b* system.[15-17]

The L*a*b* system scale, developed by CIE, is commonly used to describe color characteristics of an object based on three parameters: L*, a*, and b*. Color change is described quantitatively in ΔE* units, which combines changes in each of the individual parameters into a single value representing the distance between two colors. When ΔE is less than 3.3, the restoration might be considered clinically acceptable and does not necessitate replacement.[15-17]

After initial chromatic analysis, the samples were subjected to an artificial aging process that simulated extreme environmental conditions. The aging process consisted of UV light and water condensation. The temperature range of aging used in the present study included a black-panel temperature of 70°C (light) and 38°C (dark), as well as a dry bulb temperature of 47°C (light) and 38°C (dark). These two procedures acted together to result in the color change of the specimens. A total exposure of 150 kJ/m^2 corresponds to approximately three months of clinical service.[19-21] In this current study, the specimens were aged for 600 kJ/m^2, which is equivalent to 12 months, and then further aged for another 600 kJ/m^2, for a total of 24 months (two years).

In addition, artificial aging of both shades of resin composites induced a perceptible color change ($\Delta E > 3.3$). This observation is in agreement with several authors.[20-27]

In the present study, shade B1 showed a higher mean ΔE value (7.37 ± 0.47) than did shade A3 (4.60 ± 0.42). This indicates that lighter shades present more color change than darker shades after artificial aging. This was in agreement with Uchida and others[23] and Lee and Powers.[19] Those authors suggested that this may be due to environmental effects on the retention and/or stability of pigments and other additives in the polymer formulation (physical-chemical reaction within the material itself).

These current results contradict the findings of De Carvalho Pires-de-Souza and others,[27] Vichi and others,[28] Kim and Lee,[29] and Lee.[30] Those authors found no significant difference between darker and lighter shades with regard to color change. This contradiction may be due to differences in chemical composition of the examined resin composites and the use of different aging devices.

It was observed that the difference between the MTAD and control groups after 100 hours of

Table 5: *Mean and Standard Deviation of Mean ΔE Values Representing the Effect of Two Different Shades on All Tested Groups*

Group	Aging	B1		A3		*p*-value*
		Mean	SD	Mean	SD	
Control	50 hours of aging (ΔE_1)	5.43	0.44	3.83	0.27	<0.001
	100 hours of aging (ΔE_2)	7.19	0.54	4.67	0.39	<0.001
CHX-based MMP inhibitor	50 hours of aging (ΔE_1)	6.35	0.55	4.14	0.23	<0.001
	100 hours of aging (ΔE_2)	8.72	0.49	5.09	0.23	<0.001
MTAD-based MMP inhibitor	50 hours of aging (ΔE_1)	6.89	0.43	4.34	0.48	<0.001
	100 hours of aging (ΔE_2)	9.61	0.58	5.52	0.52	<0.001

Abbreviation: CHX, chlorhexidine-based group; ΔE, color change; MMP, matrix metalloproteinase; MTAD, doxycycline-based group.
** Significant at $p \leq 0.05$.*

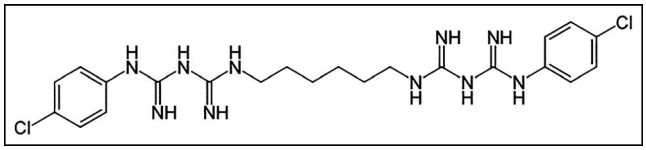

Figure 2. *Chemical structure of chlorhexidine digluconate.* [45]

accelerated aging was ≈2.4 for shade B1 and ≈0.85 for shade A3, and between the CHX and control groups it was ≈1.5 for shade B1 and ≈0.42 for shade A3. Although these differences might not be of clinical significance, as they all induced perceptible color change $\Delta E \geq 3.3$, they were of statistical significance. Thus, the null hypothesis is partially accepted.

It was found that MTAD induced the highest significant change, followed by CHX in all tested groups. Taking into account the elements that lead to color change in the control group, additional factors might be involved leading to greater color change in these groups. These factors could be due to the effect of the MMP inhibitors on both the dentin substrate and composite or interface.

The substantivity of both MMPs examined on the dentin substrate made them further susceptible to chemical changes due to the aging conditions. Chlorhexidine and doxycycline were found to bind with dentin. Chlorhexidine has the potential to bind to both organic (collagen) and inorganic (hydroxyapatite) components in dentin when applied after acid etching on the prepared tooth surface, whereas doxycycline is adsorbed to dentin. These chemical reactions with the dentinal substrate might have modified the color of the dentin, which might in turn influence the color of the final composite restoration, especially if lighter shades are used.[7,31-33] Sartori and colleagues[34] conducted a clinical study and found that CHX dentin pretreatment induced a statistically significantly higher degree of marginal discoloration after 36 months of clinical service compared with the baseline.

The color of a resin composite restoration is controlled by the optical properties of the material selected, the thickness of the material placed, and the color of the background. The background of the resin composite restoration is commonly composed of a hybrid layer of demineralized collagen and polymerized resin monomers induced by the adhesive agents applied. Therefore, the stability of the adhesive bond and the polymer network is a crucial contributing factor for the color stability of resin composite restorations. Also, because the composite

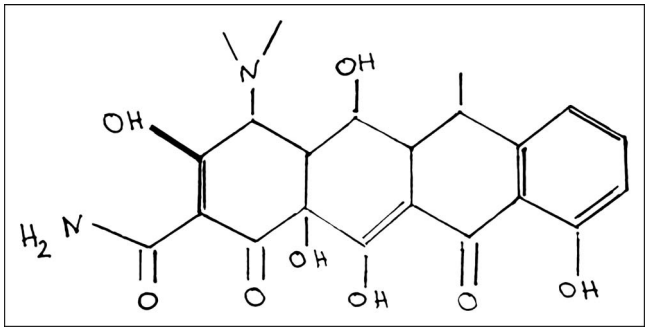

Figure 3. *Chemical structure of doxycycline hyclate.*

is a semitransparent (translucent) material, the color of the underlying tissue can influence the color of the restoration, especially if used in thin restorations.[8,19]

Given that the color of resin composite restorations is affected by the background color, color changes due to CHX or MTAD will affect the color of the final composite restoration. The oxidation of CHX and MTAD results in a color change. This might occur by the reaction of oxygen with the free amino and hydroxyl groups abundant in both structures, as presented in Figures 2 and 3. However, CHX has been found to be more stable than doxycycline.[35-40] This may explain why MTAD induced a higher significant change than CHX did.

According to Gaintantzopolou and others,[8] the quality of the resin/dentin interface is an important factor that affects the color stability of the composites. Poor adhesive infiltration into the demineralized dentin can create nanospaces in the hybrid layer, allowing for water infiltration and promoting degradation of the monomers. At the same time, Hiraishi and others[41] and Stanislawczuk and others[42] found that priming the dentin substrate with MMP inhibitors negates the bond quality. These data corroborate the results of the present study, given that greater alteration in the color was observed following treatment of the dentin substrate with MMP inhibitors. A clinical study performed by Dutra-Correa and others[43] found that the application of CHX prior to the dentin adhesives did not influence their clinical performance for up to 18 months of service.

Color change may also be attributed to the effect of any of the MMP inhibitors on the degree of polymerization. The presence of residual monomers negatively affects the color stability of resin composite restorations. Incorporating CHX into the resin could hinder the polymerization process and result in a higher level of residual monomers.[44]

The current study also showed that 100 hours of aging showed significantly higher ΔE values when compared with 50 hours of aging. This is in agreement with Lee and Powers,[19] who studied the color changes of resin composite in reflectance and transmittance modes after accelerated aging. This can be attributed to the cumulative effect of the artificial aging conditions.

CONCLUSIONS

Under the parameters of the present study, the following conclusions can be drawn:

1. Accelerated artificial aging caused color changes in a nanofilled resin composite, regardless of the MMP inhibitors.
2. Doxycycline-based MMP inhibitors produced a statistically significantly greater color change when compared with the chlorhexidine-based alternative.
3. Color stability of a nanofilled resin composite is shade dependent, because a lighter shade composite (B1) showed less color stability when compared with a darker alternative (A3).

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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REFERENCES

1. Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, Tjaderhan L, Visintini E, Cadenaro M, Tay FR, Dorigo E, & Pashley D (2010) Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer *Dental Materials* **26(6)** 571-578.
2. Breschi L, Mazzoni A, Nato F, Carrilho M, Visintini E, Tjäderhane L, Ruggeri A Jr, Tay FR, Dorigo E, & Pashley D (2010) Chlorhexidine stabilizes the adhesive interface: A 2-year *in vitro* study *Dental Materials* **26(4)** 320-325.
3. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, & Tezvergil-Mutluay A (2011) State of the art etch-and-rinse adhesives *Dental Materials* **27(1)** 1-16.
4. Carvalho RM, Manso AP, Geraldeli S, Tay FR, & Pashley D (2012) Durability of bonds and clinical success of adhesive restorations *Dental Materials* **28(1)** 72-86.
5. Cadenaro M, Pashley DH, Marchesi G, Antoniolli F, Mazzoni A, Tay FR, Di Lenarda R, & Breschi L (2009) Influence of chlorhexidine on the degree of conversion and E-modulus of experimental adhesive blends *Dental Materials* **25(10)** 1269-1274.
6. Shafiei F, & Memarpour M (2010) Effect of chlorhexidine application on long-term shear bond strength of resin cements to dentin *Journal of Prosthodontic Research* **54(4)** 153-158.
7. Rasimick BJ, Wan J, Musikant BL, & Deutsch AS (2010) Stability of doxycycline and chlorhexidine absorbed on root canal dentin *Journal of Endodontics* **36(3)** 489-492.
8. Gaintantzopoulou M, Kakaboura A, Loukidis M, & Vougiouklakis G (2009) A study on color stability of self-etching and etch-and-rinse adhesives *Journal of Dentistry* **37(5)** 390-396.
9. De Carvalho Pires-de-Souza F, Garcia L, Roselino L, & Naves L (2011) Color stability of silorane-based composites submitted to accelerated artificial ageing—An *in situ* study *Journal of Dentistry* **39(Supplement 1)** e18-e24.
10. Sarafianou A, Iosifidou S, Papadopoulos T, & Eliades G (2007) Color stability and degree of cure of direct composite restoratives after accelerated aging *Operative Dentistry* **32(4)** 406-411
11. Lee YK, Yu B, Lim HN, & Lim J (2011) Difference in the color stability of direct and indirect resin composites *Journal of Applied Oral Science* **19(2)** 154-160.
12. Yiu CKY, Hiraishi N, Chersoni S, Breschi L, Ferrari M, Prati C, King NNM, Pashley DH, & Tay FR (2006) Single-bottle adhesives behave as permeable membranes after polymerisation. II. Differential permeability reduction with an oxalate desensitizer *Journal of Dentistry* **34(2)** 106-116.
13. Vichi A, Ferrari M, & Davidson C (2004) Color and opacity variations in three different resin-based composite products after water aging *Dental Materials* **20(6)** 530-534.
14. Zhou J, Tan J, Chen L, Li D, & Tan Y (2009) The incorporation of chlorhexidine in a two-step self-etching adhesive preserves dentin bond *in vitro Journal of Dentistry* **37(10)** 807-812.
15. Fontes S, Fernandez M, Moura C, & Meireles S (2009) Color stability of nanofill composite: Effect of different immersion media *Journal of Applied Oral Science* **17(5)** 388-391.
16. Ruyter IE, Nilner K, & Moller B (1987) Color stability of dental composite resin materials for crown and bridge veneers *Dental Materials* **3(5)** 246-251.
17. Brook AH, Smith RN, & Lath DJ (2007) The clinical measurement of tooth color and stain *International Dental Journal* **57(3)** 324-330.
18. Gupta R, Parkash H, Shah N, & Jain V (2005) A spectrophotometric evaluation of color changes of various tooth colored veneering materials after exposure to commonly consumed beverages *Journal of Indian Prosthetics* **5(2)** 72-78.
19. Lee YK, & Powers J (2007) Color changes of resin composites in the reflectance and transmittance modes *Dental Materials* **23(3)** 259-264.
20. Paravina R, Ontiveros J, & Powers J (2004) Accelerated aging effects on color and translucency of bleaching-shade composites *Journal of Esthetic and Restorative Dentistry* **16(2)** 117-127.
21. Lee YK, Lu H, & Powers J (2006) Changes in opalescence and fluorescence properties of resin accelerated aging *Dental Materials* **22(7)** 653-660.

22. Powers JM, Fan P, & Raptis C (1980) Color stability of new composite restorative materials under accelerated aging *Journal of Dental Research* **59(12)** 2071-2074.

23. Uchida H, Vaidyanathan J, Viswandhan T, & Vaidyanathan T (1998) Color stability of dental composites as a function of shade *Journal of Prosthetic Dentistry* **79(4)** 372-377.

24. Schulze K, Marshall S, Gansky S, & Marshall G (2003) Color stability and hardness in dental composites after accelerated aging *Dental Materials* **19(7)** 612-619.

25. Furuse A, Gordon K, Rodrigues F, Silikas N, & Watts D (2008) Colour-stability and gloss retention of silorane and dimethacrylate composites with accelerated aging *Journal of Dentistry* **36(11)** 945-952.

26. Powers J, Barakat M, & Ogura H (1985) Color and optical properties of posterior composite under accelerated aging *Dental Materials Journal* **4(1)** 62-67.

27. De Carvalho Pires-de-Souza F, Garcia L, Hamida H, & Casemiro L (2007) Color stability of composites subjected to accelerated aging after using either a halogen or a light emitting diode source *Brazilian Dental Journal* **18(2)** 119-123.

28. Vichi A, Corciolani G, Davidson C, & Ferrari M (2007) Color and opacity variations in three different resin-based composite products after UV aging *International Dentistry South Africa* **9(1)** 58-66.

29. Kim I, & Lee Y (2007) Changes in color and color parameters of dental resin composites after polymerization *Journal of Biomedical Materials Research Part B: Applied Biomaterial* **80(2)** 541-546.

30. Lee YK (2005) Influence of color coordinates on the color changes after polymerization and thermocycling of dental resin composites *Journal of Materials Science* **40**(**11**) 2855-2860.

31. Rosenthal S, Spångberg L, & Safavi K (2004) Chlorhexidine substantivity in root canal dentin *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics* **98(4)** 488-492.

32. Carrilho MR, Carvalho RM, Sousa EN, Nicolau J, Breschi L, Mazzoni A, Tjäderhane L, Tay FR, Agee KA, & Pashley DH (2010) Substantivity of chlorhexidine to human dentin *Dental Materials* **26(8)** 779-785.

33. Kim J, Uchiyama T, Carrilho M, Agee KA, Mazzoni A, Breschi L, Carvalho RM, Tjaderhane L, Looney S, Wimmer C, Tezvergil-Mutluay A, Tay FR, & Pashley DH (2010) Chlorhexidine binding to mineralized versus demineralized dentin powder *Dental Materials* **26(8)** 771-778.

34. Sartori N, Stoff SC, Silva SB, Lopes GC, & Carrilho M (2013) Influence of chlorhexidine digluconate on the clinical performance of adhesive restorations: A 3-year follow-up *Journal of Dentistry* **41(12)** 1188-1195.

35. Singh H, Kapoor P, Meshram GK, & Warhadpande MM (2011) Evaluation of substantivity of chlorhexidine to human dentin and its application in adhesive dentistry *Indian Journal of Dentistry* **2(2)** 8-10.

36. Victor S, Sharma C, & Sreenivasan K (2011) Use of quartz crystal nanobalance to study the binding and stabilization of albumin and doxycycline on a thin layer of hydroxyapatite *Journal of Applied Surface Sciences* **258(5)** 1666-1669.

37. Tay F, Mazzouni A, Pashley D, Day T, Ngob E, & Breschi L (2006) Potential iatrogenic tetracycline staining of endodontically treated teeth via NaOCl/MTAD irrigation: A preliminary report *Journal of Endodontics* **32(4)** 354-358.

38. Wuelfing WP, Kosuda K, Templeton AC, Harman A, Mowery MD, & Reed RA (2006) Polysorbate 80 UV/vis spectral and chromatographic characteristics—Defining boundary conditions for use of the surfactant in dissolution analysis *Journal of Pharmaceutical and Biomedical Analysis* **41(3)** 774-782.

39. Yuan F, Hu C, Hu X, Wei D, Chen Y, & Qu J (2011) Photodegradation and toxicity changes of antibiotics in UV/H_2O_2 process *Journal of Hazardous Materials* **185(2-3)** 1256-1263.

40. Bjorvatn K, Skaug N, & Selvig KA (1985) Tetracycline-impregnated enamel and dentin: Duration of antimicrobial capacity *Scandinavian Journal of Dental Research* **93(3)** 192-197.

41. Hiraishi N, Yiu CK, King NM, & Tay FR (2009) Effect of 2% chlorhexidine on dentin microtensile bond strengths and nanoleakage of luting cements *Journal of Dentistry* **37(6)** 440-448.

42. Stanislawczuk R, Reis A, & Loguercio AD (2011) A 2-year *in vitro* evaluation of a chlorhexidine-containing acid on the durability of resin-dentin interfaces *Journal of Dentistry* **39(1)** 40-47.

43. Dutra-Correa M, Saraconi CH, Ciaramicoli MT, Kiyan VH, & Queiroz CS (2013) Effect of chlorhexidine on the 18-month clinical performances of two adhesives *Journal of Adhesive Dentistry* **15(3)** 287-292.

44. Anusavice KJ, Zhang NZ, & Shen C (2006) Controlled release of chlorhexidine from UDMA-TEGDMA resin *Journal of Dental Research* **85(10)** 950-954.

45. Yikrazuul (unknown year) "Chlorhexidin" - Own work. Licensed under Public domain via Wikimedia Commons – Accessed 30 July, 2014 from http://commons.wikimedia.org/wiki/File:Chlorhexidin.svg#mediaviewer/File:Chlorhexidin.svg