



ORIGINAL ARTICLE

Color stability, Roughness, and Microhardness of Enamel and Composites Submitted to Staining/ Bleaching Cycles



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Abstract Objective: To compare the effect of two bleaching systems (bleaching gel and whitening strips) on the color change, roughness, and microhardness of enamel and two resin composites.

Material and methods: Two cavities were prepared on bovine enamel specimens ($n = 16$) and restored with two composites: a nano-hybrid [Herculite Ultra (HU)] and a micro-hybrid composite [TPH Spectra (TS)]. Baseline color (CIE $L^*a^*b^*$), roughness (μm), and microhardness (kgf/mm^2) were measured using a spectrophotometer, optical profilometer, and Vickers microhardness (VHN) tester, respectively. The specimens were stained with coffee for 14 days, and randomized into two bleaching groups: gel and strips ($n = 8$), then submitted to a 10-day bleaching/staining test. Color, roughness, and microhardness were re-measured. The outcomes were analyzed using two-way ANOVA and Fisher's-PLSD test ($\alpha = 0.05$).

Results: Gel significantly improved the color (ΔE 4.9–8.3) and increased the roughness (Ra 0.04–0.08 μm) of all substrates ($p < 0.0001$) compared to strips. Enamel color was significantly improved (ΔE 5.4–8.3) compared to that of HU (ΔE 2.6–4.9) and TS (ΔE 2.0–4.9) with either gels or strips. TS roughness (0.03–0.08 μm) was significantly higher than that of enamel (0.01–0.05 μm) and HU

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(0.02–0.04 μm). Enamel had significantly reduced microhardness compared to HU ($p = 0.0144$).

Conclusion: Gels produced the greatest color improvement and roughness compared to strips. Enamel had significant color improvement but had the greatest decrease in microhardness.

Clinical significance: There was unacceptable color change between enamel and the composites after the combined cyclic effects of staining and bleaching.

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1. Introduction

Esthetic dental care has been one of the most common treatments in modern clinical practice in the last decade. Its increased demand can be due to the population's high esthetic perceptions of dental care, which results in optimum health, function, and improved esthetics (Ahmad, 2010). In restorative dentistry, resin composites are the most commonly used direct restorative materials due to their high aesthetic properties, preservation of tooth structure, durability, and micromechanical bond to tooth structures (Ferracane, 2011). However, different resin composite materials are susceptible to discoloration (Turkun and Turkun, 2004; Villalta et al., 2006; Alharbi et al., 2017).

Bleaching is considered the most common minimally invasive esthetic treatment for discolored natural teeth because it is safe, effective, predictable and economical (Carey, 2014; Kose et al., 2016). However, the effect of bleaching systems on resin composite restorations cannot be avoided during the bleaching treatment. Bleaching may adversely affect the physical properties of restorations and surface characteristics, altering their longevity and esthetic appearance such as color stability, surface roughness, and gloss (Jain et al., 2013; Mourouzis et al., 2013). Peroxides in dental bleaching increase the composite's color lightness and surface roughness (Silva Costa et al., 2009; Mourouzis et al., 2013). However, other studies have shown no effect on the surface roughness of composites (Gurbuz et al., 2013; Mourouzis et al., 2013). Similarly, the effects of peroxides on the microhardness of composites and color change after staining remain controversial (Campos et al., 2003; Mohammadi et al., 2012; Mourouzis et al., 2013).

During bleaching, optimum results can be achieved if patients refrain from consuming excessive colored beverages such as coffee, tea, and some dietary intake rich in artificial colors (Attia et al., 2009; Cortes et al., 2013). However, many people consume dark-colored foods and drinks in their daily diet, which makes it difficult for them to follow these recommendations. Most previous studies have either investigated cyclic staining or cyclic bleaching of either enamel or composites (Alharbi et al., 2017, 2018). This does not represent the actual clinical situation, as patients with esthetic direct restorations tend to consume colored beverages more than once during the day during the bleaching treatment. Attia and others (Attia et al., 2009) investigated the effect of coffee solution on enamel color during home bleaching applications. Although they reported a non-significant effect of coffee exposure during bleaching, the whitening effect was less stable (Attia et al., 2009). However, they did not investigate the effect of exposure to coffee during bleaching on different resin composites.

To the best of our knowledge, there has been no study on the combined cyclic effects of staining and bleaching on the color change, surface roughness, and microhardness of both substrates, i.e., enamel and composites. Therefore, the aim of this *in vitro* study was to compare the color stability, roughness, and microhardness of enamel, and a nano-hybrid and a micro-hybrid composite, treated with two different bleaching systems on a staining/bleaching cyclic model.

2. Materials and methods

2.1. Experimental design

This *in vitro* study model was designed for clinical reproduction of the physiological condition associated with home bleaching of different substrates during coffee consumption. Two experimental factors were investigated in the study: substrate at three levels (bovine enamel, nano-hybrid resin composite [Herculite Ultra (HU), Kerr Corporation, Orange, CA, USA], micro-hybrid resin composite [TPH Spectra (TS), Dentsply, York, PA, USA]) and bleaching systems at two levels (bleaching gel and whitening strips). The study outcomes were color change (ΔE), surface roughness (μm), and microhardness (Vickers microhardness [VHN]) measured at two time points: after baseline staining and after bleaching/staining cyclic treatment of all substrates.

2.2. Specimen preparation

Sixteen bovine enamel samples ($8 \times 8 \times 2 \text{ mm}^3$) were prepared using a low-speed diamond saw (IsoMet, Buehler, Lake Bluff, IL, USA) and finished using 1200-grit paper (MD-Fuga, Struers Inc., Cleveland, OH, USA) after local institutional review board approval (Indiana University IRB #NSO 911–07). The specimens were embedded in acrylic resin blocks ($10 \times 10 \times 8 \text{ mm}^3$, VariDur; high-performance mounting kit; Buehler), then two box-shaped cavities ($3 \times 2 \text{ mm}$) were manually prepared in each specimen using a diamond fissure bur (No. 835KR.31.008, Brasseler, Savannah, GA, USA) in a high-speed handpiece with air/water coolant.

One cavity preparation was located on the upper right part of the sample; the other was on the lower left part of the sample. The area opposite one cavity was used as the enamel sample. Each prepared cavity was etched with 35% phosphoric acid for 20 s (Ultra-Etch, Ultradent Products Inc., South Jordan, UT, USA), followed by application of a bonding agent (Opti-Bond Universal, Kerr Corporation) and light-cured for 15 s, based on the manufacturer's instructions, using a Demi Plus LED light-curing unit (Kerr Corporation) with a light output

of 625 mW/cm². Two esthetic composite restorative materials (shade A2), i.e., HU and TS, were placed in two increments (each, 1-mm deep) in each cavity so that each specimen had one cavity filled with HU and the other with TS. Each composite type was identified via an indentation on the side of the acrylic resin block. A Mylar strip was placed over the final increment, pressed with a glass slide, and a static load (0.53 kg) was applied using a heavy glass slab to allow excess material to extrude over the top of the cavity margins and to ensure that the material was flush with the surface of enamel, then polymerized according to the manufacturer's instructions for 40 s.

After 24-h storage at 37 °C in artificial saliva (2.20 g/l gastric mucin, 1.45 mM CaCl₂·2H₂O, 5.40 mM KH₂PO₄, 28.4 mM NaCl, 14.9 mM KCl, pH 7.0), the specimen surfaces were wet-ground and polished using a sequence of 500-, 1200-, 2400-, and 4000-grit silicon carbide paper.

2.3. Color assessment

Commission Internationale de l'Eclairage (CIE) L*a*b* values were obtained for the enamel and composite restorations of each specimen using a spectrophotometer (CR-241 Chroma Meter, Minolta Camera Co., Osaka, Japan) with a light beam focus of 0.3 mm. Color measurements were taken at baseline staining and after the bleaching/staining cyclic treatment. The baseline stain was used for randomization only.

All measurements were performed by one examiner and were repeated three times. The color difference (ΔE) was calculated using the following equation:

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

representing color changes after cycling

($\Delta E_{\text{Cycling}}$: bleaching/cyclic treatment – baseline staining).

Another calculation was made utilizing the combined L*a*b* coordinates of both the enamel and composites (staining and after bleaching/cyclic treatment) to measure the clinical perceptible color difference between both substrates:

$$(\Delta E_{\text{Bleaching_difference}} = \{(\Delta L_{\text{Enamel-composite}})^2 + (\Delta a_{\text{Enamel-composite}})^2 + (\Delta b_{\text{Enamel-composite}})^2\}^{1/2}$$

where ΔE is the color difference and ΔL^* , Δa^* , and Δb^* represent changes in lightness, red-green coordinate, and yellow-blue coordinate, respectively.

2.4. Surface roughness testing

The specimens were scanned by a 3D optical profilometer (Proscan 2000, Scantron, Taunton, England) using the S5/03 chromatic sensor. Roughness was measured at baseline and after bleaching/staining cyclic treatment in a 0.5 × 0.5 mm² area at the center of each restoration and the center of the enamel area on each side of the restorations (step size, 0.01 × 0.1; number of steps, 100) using 100 Hz frequency with full sensor speed (100%).

2.5. Vickers microhardness (VHN) testing

Microhardness was measured using an LM247 VHN tester (LECO, St. Joseph, MI, USA). Three indentations, spaced

200 μm apart, were performed on each specimen using a 25-g load and 20-s dwell time for the composites, and 50-g load and 15-s dwell time for the enamel. The indentations were measured using an optical microscope with a digital camera. Microhardness was measured at baseline and after bleaching/cyclic treatment, where the microhardness value for each specimen was obtained as the mean result of the three indentations.

2.6. Staining and bleaching cycling

All specimens were stored in a staining solution (dark roast coffee, Starbucks, Seattle, WA, USA) prepared based on the manufacturer's instructions. The staining period was 2 weeks and the coffee solution was replaced daily. After creating the baseline stain, the specimens were rinsed under running distilled water and gently dried. The L values from the baseline stain were used for stratified randomization of the specimens into the bleaching gel and whitening strips groups (n = 8 per group).

The staining/bleaching cycling was performed for 10 consecutive days (Mondelli et al., 2012). The specimens were stained with coffee for 3 h daily (Cortes et al., 2013), followed by treatment with the assigned whitening agents.

The first group was treated with an at-home bleaching protocol, using a 15% carbamide peroxide (CP) home bleaching agent (pH 6.5; Opalescence PF, Ultradent Products, Inc., South Jordan, UT, USA). The bleaching gel was applied on the top surface of each specimen (0.5–1.0 mm thick) and kept for 4 h each day in an incubator at 37 °C according to the manufacturer's instructions. The second group was treated with 10% hydrogen peroxide (HP) whitening strips (Crest 3D White WhiteStrips Vivid, power 2, Procter & Gamble, Cincinnati, OH, USA) positioned on the top of each specimen for 30 min each day according to manufacturer's instructions. After bleaching, specimens from both groups were rinsed under running distilled water for 1 min to remove the bleaching agents and were blotted dry. The specimens were kept in artificial saliva overnight at 37 °C after each cycle.

2.7. Statistical methods

Statistical analyses were performed using two-way analysis of variance (ANOVA), followed by Fisher's protected least significant difference test for multiple comparisons. All statistical analyses were carried out at a 5% significance level. Analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Color change

Between the two bleaching treatments, bleaching gel significantly changed the color ($\Delta E_{\text{Cycling}}$, $p < 0.0001$) compared to whitening strips for the three substrates tested (enamel, HU, TS). The change in color was perceptible when the three substrates were treated with the bleaching gel, and only when the enamel treated with the whitening strips ($\Delta E > 3.3$).

Comparison of the color change of the three substrates showed a statistically significant color change for enamel compared to the two composites (HU and TS) ($p < 0.0001$). No significant differences in color change were observed between the two composites (Table 1).

Table 1 Color change ($\Delta E_{\text{Cycling}}$)* mean values (standard-error) for enamel and composite resins.

Substrate	Gel	Sig.	Strips	Sig.
E	8.37 (0.51)	a/A	5.47 (0.75)	a/B
TS	4.99 (0.11)	b/A	2.09 (0.29)	b/B
HU	4.92 (0.10)	b/A	2.61 (0.16)	b/B

Different uppercase letters indicate significance among treatments (row, $p < 0.05$). Different lower case letters indicate significance among substrate (column, $p < 0.05$).

E: Enamel, TS: Micro-hybrid composite (TPH-Spectra). HU: Nano-hybrid composite (Herculite-Ultra).

* $\Delta E_{\text{Cycling}}$: Bleaching/cyclic treatment-staining.

3.2. Surface roughness

Between the two bleaching treatments, bleaching gel significantly increased the surface roughness for the three substrates tested compared to the whitening strips ($p = 0.0001$). Comparison of the surface roughness showed that TS was significantly rougher than enamel ($p = 0.023$) and HU ($p = 0.042$) following treatment with either the bleaching gel or whitening strips. No significant differences in surface roughness were found between enamel and HU after treatment with either the bleaching gel or whitening strips (Table 2).

3.3. Surface microhardness

Between the two bleaching treatments, no significant differences in microhardness was observed for all three tested substrates with either the bleaching gel or whitening strips. Comparison of the substrates showed that enamel had a significantly higher change in microhardness value than HU when treated with the bleaching gel and whitening strips ($p = 0.014$). No significant differences were found between enamel and TS and between TS and HU (Table 3).

4. Discussion

Dental composite materials have become an essential part of today's dental restorative treatment. With the presence of a huge number of materials used in the clinic, each patient's mouth may contain different types of resin composite restorations. Therefore, it is important to study the effect of bleaching materials on different substrates (enamel, and different composites), especially when patients do not refrain from ingesting certain beverages and artificial food colorants, which might affect the bleaching outcome of enamel and resin composites. In the present study, we used two resin composites (nano-composite and micro-hybrid composite) that represent the most commonly used composites for anterior esthetic restorations. Each specimen contained all of the three substrates, i.e., enamel, nano-composite, and micro-hybrid composite, and was subjected to staining, bleaching, and saliva storage intervals in a cyclic model to mimic the clinical situation. Different substrates may have differing responses to cyclic bleaching/staining episodes, which would be critical for their long-term esthetic outcomes. We aimed to obtain better understanding of the actual clinical situation to provide better recommendations regarding composite replacement after bleaching/staining cycles.

Coffee was selected as the staining medium because of its high popularity among patients and due to its intense staining effect on natural teeth and composites (Jain et al., 2013; da Silva et al., 2017). Coffee consists of anionic polyphenols (yellow colorants), which interact with the cationic salivary pellicles on the tooth surface and adsorb on composite into the organic phase due to the compatibility of the polymer phase with the yellow colorants of the coffee (Proctor et al., 2005). The baseline stain in the present experiment was performed for 2 weeks to maximize the stain absorption, as resin compos-

Table 2 Roughness (Ra) mean values (standard-error) for enamel and composite resins.

Substrate	Gel roughness (μm)				Strips roughness (μm)			
	Pre	Post	Change (SE)	Sig.	Pre	Post	Change (SE)	Sig.
E	0.046	0.097	0.051 (0.008)	b/A	0.057	0.067	0.010 (0.013)	b/B
TS	0.062	0.143	0.081 (0.007)	a/A	0.067	0.097	0.030 (0.012)	a/B
HU	0.052	0.097	0.046 (0.010)	b/A	0.057	0.078	0.021 (0.012)	b/B

Different uppercase letters indicate significance among treatments (row, $p < 0.05$). Different lower case letters indicate significance among substrate (column, $p < 0.05$).

E: Enamel, TS: Micro-hybrid composite (TPH-Spectra). HU: Nano-hybrid composite (Herculite-Ultra).

Table 3 Vickers microhardness change (VHN) and standard error for enamel and composites resin.

Substrate	Gel microhardness (kgf/mm^2)				Strip microhardness (kgf/mm^2)			
	Pre	Post	Change (SE)	Sig.	Pre	Post	Change (SE)	Sig.
E*	316.6 (4.2)	300.7 (4.1)	-15.9 (3.7)	b/A	312.6 (7.1)	307.1 (1.5)	-5.5 (6.7)	b/A
TS	77.0 (1.4)	70.2 (1.5)	-6.8 (2.1)	ab/A	73.9 (2.5)	72.5 (1.5)	-1.4 (1.9)	ab/A
HU	60.7 (1.3)	58.6 (1.1)	-2.2 (1.6)	a/A	59.8 (1.4)	58.2 (0.8)	-1.6 (1.7)	a/A

Hardness change values with different uppercase letters indicate significance among treatments (row, $p < 0.05$). Different lower case letters indicate significance among substrates (column, $p < 0.05$).

E: Enamel, TS: Micro-hybrid composite (TPH-Spectra). HU: Nano-hybrid composite (Herculite-Ultra).

ites rapidly uptake fluids within the first 2 weeks (Diaz-Arnold et al., 1992). Additionally, 2 weeks of coffee staining was used to establish the baseline stain, which represented 14-month coffee consumption based on the average daily intake of coffee (3.2 cups/person) assuming that each cup would take 15 min to be consumed, according to coffee manufacturers (Guler et al., 2005). Color was evaluated using the CIE Lab color system, which characterizes colors based on human perception, and to eliminate subjective errors (Hafez et al., 2010). In this system, a color difference value (ΔE) is considered clinically perceptible when it equals or exceeds 3.3 (Hafez et al., 2010). The baseline stain was clinically perceptible ($\Delta E > 3.3$) and was used to randomize the specimens before initiating the bleaching/staining cycles. Furthermore, a single composite shade (A2) was selected for all specimens to minimize the influence of color differences.

Although bleaching gels contain lower concentrations of peroxide compared to strips (15% CP versus 10% HP), the bleaching gel yielded a significant color improvement within all substrates ($\Delta E > 3.3$) compared to the whitening strips. The prompt color improvement by the bleaching gel could be explained by the longer exposure time (4 h versus 30 min), which was performed according to the manufacturer's instructions. This agrees with previous studies that suggested that longer enamel bleaching time yielded better color improvement regardless of the concentration of the bleaching agents (Suliman et al., 2006; Cortes et al., 2013; Al-Angari et al., 2019; Farawati et al., 2019). Furthermore, previous studies that used different CP and HP concentrations concluded that bleaching treatments significantly improve composite color when used for extended durations regardless of the concentration (Monaghan et al., 1992; Kurtulmus-Yilmaz et al., 2013; Al-Angari et al., 2019).

Throughout the testing period, the bleaching systems were significantly more effective in enamel (ΔE 5.4–8.3) compared to that in HU and TS (ΔE 2–4.9). This can be explained by the difference in the morphological structure and characteristics of both the enamel and the composites. Enamel histological structure is less resistant to acid challenges, i.e., peroxide bleaching agents, than the structure of restorative materials (Yu et al., 2009). Bleaching agents increase enamel porosities (El-Murr et al., 2011); therefore, acid penetrates deeper into its structure, oxidizing more stain-containing molecules and resulting in an enhanced whitening effect. In contrast, bleaching in composites takes place by disrupting poorly polymerized resin matrix, and oxidation of superficial pigmentation (Monaghan et al., 1992; Villalta et al., 2006). To better understand the clinical situation, another calculation was made utilizing the combined $L^*a^*b^*$ coordinates of both the enamel and the composites in the ΔE equation, at staining and after using our cyclic model, to measure the clinical perceptible color difference between both substrates. The calculations resulted in a color change variation ($\Delta E \geq 3.3$) in the clinically perceptible range.

We also evaluated the effect of bleaching on surface roughness, as roughness may influence the esthetic quality of enamel and composite restorations. All substrates had increased surface roughness after bleaching, which is in agreement with previous studies (Markovic et al., 2014; Polydorou et al., 2018; Yu et al., 2018). The bleaching gel significantly increased roughness as compared to the whitening strips, which can be explained by the exposure time, as mentioned earlier. TS had

significantly higher roughness values compared to HU. This can be explained by the larger size and smaller surface area of the filler content compared to that of HU. The high-energy free radicals in the bleaching agents can easily attack the organic matrix of TS, softening the material and targeting the resin-filler interface (Markovic et al., 2014). Eventually, filler debonding will take place, leaving a larger crater that will result in higher surface roughness (Hafez et al., 2010; Wang et al., 2011; Yu et al., 2018).

The lower enamel surface roughness values in the present study can be attributed to the remineralization role of saliva, which was the storage medium throughout the experiment. The calcium phosphate deposition within the porous enamel compensated for the dissolution of the organic phase (Abouassi et al., 2011). It is worth stating that a roughness value (Ra) of 0.2 μm is critical for plaque retention (Lopes et al., 2018). However, all roughness values in the present study were $\leq 0.08 \mu\text{m}$. Therefore, the change in roughness reported here might not be clinically significant. Moraes et al. (2006) came to a similar conclusion, where bleached (10% and 35% CP) enamel and composite yielded roughness values of 0.17 μm and 0.09 μm (Moraes et al., 2006).

Several studies have found that bleaching agents decrease enamel microhardness (Klaric et al., 2013, 2015). However, their effects on resin composites have been controversial, as some studies have found reduction (Campos et al., 2003; Hatanaka et al., 2013), while others have reported no effect on microhardness (Garcia-Godoy et al., 2002). In the present study, all substrates had significantly reduced microhardness after bleaching. Enamel had a significantly higher reduction in microhardness values compared to HU. This can be attributed to the ability of bleaching agents to penetrate deeper into enamel and may indirectly support our finding of significantly better color improvement in enamel after bleaching compared to that in composite. This finding supports the premise that bleaching does not affect the intrinsic color of composites, only the extrinsic removal of stains. The different responses in the composites might be related to the structural difference between the materials (filler size, matrix type, and volume of resin material) (Celik et al., 2009). Moreover, the oxidation process in the organic matrix may facilitate water absorption and lead to the loss of filler particles, which results in reduced superficial integrity and microhardness (Wang et al., 2011). The minimum change in microhardness values in HU, which is in agreement with that of a previous study (Pinar Yilmaz Atali, 2011), might be justified by the relatively smaller filler size debonded from the resin matrix during the oxidation process.

Within the limitations of this *in vitro* study, coffee consumption during bleaching may produce a clinically perceptible color difference between enamel and different composites, resulting in an unaesthetic color match. Therefore, composite restorations may require polishing or replacement after bleaching. Furthermore, dentists should take into consideration that bleaching agents used for extended periods on micro-hybrid and nano-hybrid composites negatively affect their microhardness and surface roughness, and hence might affect their longevity.

In summary, the effects of the different bleaching systems on the color, surface roughness, and surface microhardness of enamel and the micro-hybrid and nano-hybrid resin composite materials were observed during alternating staining/

bleaching cycles, and these effects were material-dependent. It is worth mentioning that these results may differ to a certain extent from that for other different micro-hybrid and nano-hybrid resin composites. Exposure to coffee during the bleaching treatment using 15% CP and 10% HP affected the bleaching outcome, where it resulted in a color increase to a clinically perceptible range ($\Delta E > 3.3$) except for that in the composites treated with 10% HP. The bleaching gel significantly improved color lightness compared to the whitening strips. However, the gel increased the surface roughness of all substrates. Furthermore, enamel was more susceptible to whitening than the two composites, yet had the highest decrease in hardness.

5. Conclusion

The combined cyclic effect of staining and bleaching using 15% CP bleaching gel and 10% HP whitening strips showed a clinical unacceptable color difference between enamel and the resin composites (nano-hybrid and micro-hybrid).

For optimum bleaching results, clinicians should advise their patients to refrain from colored beverages/foods during the bleaching process and to use remineralizing agents such as topical fluoride immediately after bleaching to minimize its negative effect on surface roughness and hardness.

6. Disclosure statement

The authors do not have any financial interest in the companies whose materials are included in this article.

Ethical statement

This hereby statement declares that all authors confirm that the study was done under full ethical consideration and after the approval of the Indiana University Institutional Review Board (IRB # NSO 911-07).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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