

Bleaching Efficacy and Re-Staining Susceptibility of Stained Arrested Caries Lesions In-Vitro

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Abstract

To investigate the efficacy of two dental bleaching systems on stained-arrested caries lesions (s-ACLs) and assess the bleached lesions' susceptibility to re-staining.

Sixty human molars with s-ACLs were selected. Baseline color was measured spectrophotometrically (CIE/L*a*b*), then specimens were randomized into three groups (n=20) based on the bleaching protocol used. G1- no bleaching (negative control); G2- simulated at-home bleaching (15% carbamide peroxide, 4h/d×7); G3- simulated in-office bleaching (40% hydrogen peroxide; 20 min×3), after which specimens were stained (coffee and tea solution; 8h/d×5). The study outcome was color change (ΔE), measured at baseline, after bleaching and after staining. Data were analyzed using ANOVA and Dunnett T3 tests ($\alpha=0.05$).

G2 significantly ($p<0.001$) improved the color lightness (ΔE 7.6) compared to G3 and G1 (ΔE 3.7, 3.2) respectively. After staining, G2 and G3 (ΔE 8, 7.3) had a significant increase ($p\leq 0.009$) in stains absorption (darker) compared to G1 (ΔE 3.1). However, G3 had a significant difference ($p<0.003$) after bleaching and after staining compared to G1 and G2.

Bleaching s-ACLs using at-home bleaching systems (15% CP) demonstrated significant color improvement compared to in-office bleaching systems (40% HP). However, both bleaching protocols resulted in a more susceptible lesion surface to re-staining.

To maintain the optimum bleaching outcome of s-ACLs, clinicians should consider using remineralizing agents and instruct their patients to avoid coffee and tea consumption after the bleaching treatment.

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Introduction

In recent years, esthetic dental care has escalated as one of the most common treatments in dental practice. As patients nowadays have a greater understanding of esthetic dentistry, they demand procedures with optimum health, function, and esthetics.¹ Consequently, this has directed clinicians to newly implemented dental preventive strategies and diagnostic tools.² These measures improve the quality of dental care, as it focuses on minimum invasive dentistry and limits the use of surgical intervention along with an improved esthetic outcome.³

The use of different remineralizing materials such as fluoride,⁴ would minimize or halt the active caries process, resulting in a stained arrested caries lesion (s-ACL). These lesions are considered non-active and are presented with highly mineralized surfaces,^{2, 5} due to the mineral deposition in the inter-crystal and inter-rod dental spaces.² However, they are associated with an unesthetic dark discoloration.⁴ Discoloration is related to dietary pigments, amino acids from the proteolytic processes, metallic ions, organic debris, and chromogenic bacteria incorporated within the lesion during the remineralization process.^{6, 7} In such clinical situations, some patients would psychologically refuse the dark discolorations and demand esthetic measures to mask the stains,⁸ which commonly would result in surgical intervention.⁹

Based on the concept of minimum invasive dentistry, bleaching natural teeth may present a safe, effective and economical option to improve teeth discoloration without surgical

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intervention.^{10,11} Natural teeth have shown good bleaching results using different bleaching protocols,¹² yet they tend to re-stain after bleaching.¹³¹⁴ Studies have reported that bleached teeth may undergo morphological alterations such as increased surface roughness, depressions, porosities and formation of shallow erosions areas, which leads to an increased susceptibility to staining after bleaching.^{11,13,15}

We have previously reported the success of bleaching s-ACLs, as it has shown clinical and laboratory notable color improvements, it reduced surgical intervention and limited iatrogeny.^{4,16,17}

These satisfactory results might be compromised if susceptible to re-staining by extrinsic organic stains such as; tea, coffee and other pigmented dietary food types. The concern of re-staining after bleaching is known to affect natural sound teeth,¹⁴ but its effect on bleached ACLs is not yet explored in literature. This study aimed to investigate the efficacy of at-home and in-office bleaching systems on s-ACLs and determine the susceptibility of the bleached lesions to re-staining.

Materials and methods

Experimental design

In this study, s-ACLs were subjected to two different bleaching systems, then subjected to a staining protocol to assess their susceptibility to re-staining. The study investigated two factors: bleaching treatment at three levels (negative control, at-home and in-office bleaching protocols) and the susceptibility of the bleached s-ACLs to re-staining at two levels (yes, $\Delta E > 3.3$, and no $\Delta E < 3.3$). The experimental units were extracted human molars with stained ACLs (pit and fissure surface) embedded in acrylic blocks (n=20/group). The study outcome was color change (ΔE) measured at three-time points; at baseline, after bleaching, and after staining. Color measurements were performed by a spectrophotometry.

Specimen preparation

Sixty freshly extracted permanent human molars with darkly stained ACLs (pit and fissure surface) were selected after the protocol was approved by the King Saud University Institutional Review Board (IRB # E-18-3162). Teeth were decoronized using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) then embedded in acrylic resin (Techno Sin Standard Kit, Protechno, Girona, Spain). Each

specimen was cleaned, washed and rinsed then kept moist at 4 °C.

Color evaluation

The color values represented in the L*a*b* coordinates (Commision Internationale de l'Eclairage) were measured for each tooth at three different time points: baseline, after bleaching and after staining. The spectrophotometer (ColorEye 7000A, GretMacBeth, New Windso, NY, USA) was calibrated with a ceramic plate provided by the manufacturer. All color values were taken three times using a light beam diameter of 0.3 mm.

To measure the difference in the color value (ΔE), the color means (L*a*b*) were taken and calculated in the given equation in order to measure the change after bleaching ($\Delta E_{\text{bleaching}}$: bleaching-baseline) and staining ($\Delta E_{\text{staining}}$: staining-bleaching):

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

Where ΔE represents the amount of color difference, ΔL^* coordinate represents an increase (positive direction) or decrease (negative direction) in lightness, Δa^* coordinate represents redness (positive direction) or greenness (negative direction), and Δb^* coordinate represents yellowness (positive direction) or blueness (negative direction) of a material.

Dental bleaching efficacy test

Based on the baseline color measurements, specimens were randomly assigned into three groups (n=20) according to the bleaching protocol used. Group 1 was the control group, it received no bleaching treatment and was kept moist in an incubator for 8 h, then stored moist at 4°C. Group 2 was subjected to at-home bleaching gel, 15% carbamide peroxide (CP; pH 6.5), (Opalescence PF, Ultradent Products, South Jordan, UT, USA). An amount of 0.5-1.0 mm of the bleaching agent was applied on the occlusal aspect of each tooth and incubated (~37°C) for 4 h per day¹⁸ for seven days. Specimens were rinsed thoroughly after each bleaching cycle with deionized water then dried and kept moist at 4°C until the next bleaching cycle.

Group 3 was treated with an in-office bleaching gel, using 40% hydrogen peroxide (HP; pH 6.0 to 8.5), (Opalescence Boost, Ultradent Products, South Jordan, UT, USA). The bleaching gel was applied (~0.5–1.0 mm thick

layer) on the occlusal aspect of each sample, for 20 min, removed then reapplied twice resulting in a one-hour treatment, which was based on the instructions of the manufacturer. At the end of the bleaching treatment, specimens were washed with deionized water for one min, then dried and stored moist at 4°C.

Post-bleaching staining test

All groups were subjected to the same staining protocol. The teeth were incubated in a staining medium which consisted of coffee (Folger Coffee Company, Orrville, OH, USA) and tea (Nestea, Nestle, Glendale, CA, USA), prepared according to each company package instructions. The coffee/tea solution was used directly after preparation and changed daily. Each group was kept in an incubator (~37°C) stirring for 8 h/ per day for five consecutive days.

After staining, all groups were thoroughly washed with distilled water for one min in order to eliminate any staining residue, allowed to air dry then kept moist in an incubator overnight, until the next staining cycle after which, the final color was measured.

Statistical analysis

Color change (ΔE) was calculated after the bleaching and staining protocols for each specimen. Multiple groups comparison was analyzed using one-way ANOVA ($\alpha=0.05$), followed by multiple comparison tests, Dunnett T3 with a controlled alpha level of 0.016. Statistical analysis was performed using SPSS version 21.0 (IBM SPSS Statistics, IBM, Armonk, NY, USA). A sample size calculation indicated that a sample size of 20 specimens per group was designed to have 89% power to detect a ΔE difference (0.8) among the tested groups, assuming a 5% significance level and standard deviation of 0.7.

Results

Means of color change (ΔE) were significantly different after at-home and in-office bleaching treatments and after staining ($p \leq 0.003$).

The at-home bleaching protocol (G2), was significantly more effective ($p < 0.001$) in improving the color lightness of s-ACLs (ΔE 7.6) compared to the in-office bleaching protocol and control groups (G3 and G1), (ΔE 3.7 and ΔE 3.2) respectively.

After staining the bleached specimens, both bleaching groups (G2, G3) presented

significantly ($p \leq 0.009$) darker stains (ΔE 8, and ΔE 7.3) respectively, indicating more surface stain absorption, compared to the control group (ΔE 3.1).

Within each tested group, in-office bleaching group (G3), had significant difference ($p < 0.003$) after bleaching treatment and after staining, while at-home bleaching protocol and control (G2, G1) had no significant difference. The color values (mean ΔE) after bleaching treatment and staining are found in Table 1.

Groups	ΔE Bleaching *	Sign.	ΔE Staining *	Sign.
G1 (control)	3.2 (1.9)	A/a	3.1 (1.7)	A/a
G2 (At-home bleaching)	7.6 (4.1)	A/b	8.0 (3.4)	A/b
G3 (In-office bleaching)	3.7 (2.3)	A/a	7.3 (5.1)	B/b

Table 1. Color change (ΔE) mean values (standard-deviation) after bleaching and staining. Uppercase letters indicate significant difference within treatment (row, $p < 0.05$); while lower case among treatments (column, $p < 0.05$). Sign.: Statistical Significance. * ΔE Bleaching: Bleaching-baseline, ΔE Staining: Staining-bleaching.

Discussion

To our knowledge, this study is the first attempt to evaluate the susceptibility of the bleached s-ACLs to re-staining. It is clinically significant because re-staining is critical for the long-term esthetic outcome of the bleached lesions. This study gathered s-ACLs teeth from different patients, this included teeth with different stain types, depth, and history. To standardize the specimens and minimize the influence of color differences among them, the baseline color measurement (L^* value), was used for stratified randomization of specimens into the three tested groups. Color was evaluated objectively, using the CIE Lab color system, which is a standard method to characterize colors based on human perception and to eliminate subjective errors.⁸ It verified the bleaching efficacy and the creation of stains of the tested systems/techniques, as the change in color value (ΔE) is considered clinically perceptible when it equals or exceeds 3.3.⁸

Stained ACLs treated with both bleaching protocols had a clinically notable color improvement ($\Delta E > 3.3$), as it gradually blended with the surrounding tooth color, improving its esthetics, and indicating that our bleaching treatment

developed a distinct visual improvement in all the specimens. The lighter color improvement was based on the color difference value (ΔE) coordinates as color lightness was associated with an increase in the lightness (L^*) and a decrease in the greenness (a^*) and blueness (b^*) values, this directed the ΔE change in the lighter color scale indicating color improvement.¹⁹

Although the at-home bleaching protocol had a lower peroxide concentration compared to the in-office bleaching protocol (15% CP versus 40% HP), it was significantly more effective in color improvement (ΔE 7.6) and in minimizing or eliminating stains in all substrate. This might be associated with the longer exposure time of the at-home bleaching protocol than the in-office bleaching protocol (24 hours versus one hour), which was done based on the manufacturers' instructions. The more time the bleaching gel is in contact with the tooth, the more it diffuses into the dental tissue, increasing the enamel micro-porosities, allowing the acid penetrates deeper into its structure, oxidizing more stain-containing molecules and resulting in an enhanced whitening effect.²⁰ This is in line with several studies, which suggested that an improved color outcome is associated with longer bleaching time regardless of the concentration of the bleaching agent.^{17, 21-23}

Our study observed differences in the color improvement within each bleaching group. All of the bleached specimens had a clinically significant color improvement, yet, some stains were completely eliminated while a few improved to a lesser extent. This can be explained by the stain history of the ACLs (chemical composition, type and depth) and by the mechanism of action of peroxides which affected the extent of the bleaching outcome. Non-metallic stains (orange/brown) consist of organic chromogens that have a small molecular weight and are water-soluble, which are easily oxidized by the peroxides. On the contrary, metallic compounds (grey/black) have a larger molecular weight and are less water-soluble than organic stains,²⁴ which is difficult to oxidize and degrade by the bleaching agent. Our study and previously reported studies have shown that stains of organic origin (orange/brown) had better color improvement, whereas stains of metallic origin (grey/black) were bleached to a lesser extent. Therefore, the optimum bleaching outcome may not be as efficacious in some s-ACLs.^{4, 16, 17, 24} It is noteworthy to mention that

even if the bleached ACLs did not achieve the desired esthetic result, it would need less amount of tissue removal to mask the stains, resulting in a minimized restoration.

In such a clinical scenario, careful consideration should be taken when restoring the bleached surface using composite resin, as immediate bonding may decrease the shear bonding strength of the restoration due to residual oxygen from the bleaching agent that interferes with resin polymerization and may affect the restoration's long-term clinical outcome.^{25,26}

Coffee and tea were selected as the staining medium for this study, as they are the most consumed colored beverages world-wide.²⁷⁻²⁹

They are rich in pigments (yellow chromophores) that have different polarities, hence they have been reported to have an intense stain effect on natural teeth.²⁷⁻²⁹ The staining period (8h/5days) represented a 40-hour storage time, which simulated more than five months of coffee/tea staining in the oral cavity. This calculation was based on the coffee manufacturer, as the regular time consumption of a coffee cup is around 15 minutes, and the average daily intake of a person is 3.2 cups.³⁰

Re-staining was significantly associated with both bleaching protocols ($\Delta E \leq 8$) compared to the control group (ΔE 3.1) which was not in the clinically perceptible range. The visible changes in the color parameters (dark discoloration) was directed by the decrease in lightness value (ΔL^*) and increase in redness (a^*) and yellowness (b^*) values, which confirmed the validity of this staining protocol. This supports our findings of clinically perceptible color lightness related to both bleaching agents ($E > 3.3$).

The at-home bleaching system had better color improvement than the in-office bleaching system, yet, they stained almost the same. The increased absorption of stains can be explained by the high concentration of the peroxides in the in-office bleaching gel (40% HP) and the long-time exposure of the at-home bleaching agent (15% CP). This would generate more reactive oxygen radicals and consequently, improved the color outcome yet induce morphological alteration within the dental surface.^{11,17} These morphological alterations result from a shift in the composition of the bleached tooth and would

lead to surface roughness, demineralization, decreased protein concentration, increased porosity and surface depression.¹¹ Consequently, these alterations would create channels for stains to penetrate deeper within the tooth surface, thus resulting in a relapse of the bleaching treatment. Furthermore, the specimens' anatomical feature of the specimens (pit and fissure surface) would promote the stagnation of stains within the pits and fissures, favoring greater retention of coloring agents.

Studies have reported that the use of saliva during the bleaching process would reduce the enamel susceptibility to demineralization, hence, minimize the discoloration.^{7, 16, 31, 32}

However, the reason saliva was not used in our study, was to focus on the effect of the bleaching systems' effect solely without the influence of other variables. This in-vitro study would help understand the bleaching aspects related to s-ACLs as it is possible to control different variables.

However, in-vitro studies are hard to mimic the biological complexity of the oral cavity. Therefore, clinical extrapolation of the results should be done carefully.

Clinicians should choose suitable cases of s-ACLs to achieve the effectiveness and the safety of the bleaching protocols used. Bleaching improved the color lightness of s-ACLs to a clinically perceptible range, yet, it was susceptible to re-staining. This process might be minimized in the patient's mouth by using remineralizing agents such as topical fluoride applications immediately after bleaching. Moreover, clinicians should advise patients to avoid consuming any colored beverages/food directly after bleaching their teeth, and to brush their teeth right after that.

Conclusions

Stained ACLs treated with at-home bleaching systems (15% carbamide peroxide) had a significant color improvement than the in-office bleaching systems (40% hydrogen peroxide). However, both bleaching protocols were susceptible to re-staining to a clinically perceptible range ($\Delta E > 3.3$). Therefore, clinicians should use preventive measures such as fluoride immediately after the bleaching procedure and instruct their patients to avoid coffee and tea consumption after the bleaching treatment to maintain the optimum bleaching outcome.

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Declaration of Interest

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

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