

SPECTROPHOTOMETRIC EVALUATION OF COLOR CHANGE OF WHITE SPOT LESIONS TREATED WITH THREE DIFFERENT APPROACHES (AN IN VITRO STUDY)

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ABSTRACT

Background: White spot lesions (WSLs) are characterized by mineral loss beneath an intact surface layer of enamel giving a whitish appearance. They constitute a common problem that may impair the esthetics.

Aim of the study: Spectrophotometric evaluation of color change of induced artificial WSLs treated with three different approaches.

Materials and Methods: Artificial WSLs were created in the enamel of thirty sound human premolars using demineralizing solution, then, premolars were randomly assigned to three groups; group (A); treated with bleaching followed by resin infiltration (RI), group (B); treated with micro-abrasion followed by RI; and group (C); treated with RI alone. After treatment, thermocycling was executed with 50000 cycles. Specimens were evaluated for color changes using spectrophotometer at baseline, after demineralization, after treatment and after thermocycling. Analysis of the results was executed by one way ANOVA followed by Tukey's post hoc tests.

Results: Group (A) exhibited the highest esthetic improvement, immediately after treatment ($\Delta E = 1.55$), followed by group (B) ($\Delta E = 1.93$), while the highest color change values were recorded in group (C) ($\Delta E = 2.46$). Similar results were obtained after thermocycling with $\Delta E = 1.87$, 2.48 and 3.24 respectively. The differences between the three groups, after treatment and after thermocycling, were statistically significant ($p < 0.0001$)

Conclusion: WSLs treated with bleaching followed by resin infiltration exhibited superior improvement in color matching immediately and after ageing procedure.

KEY WORDS: Resin infiltration, Microabrasion, Bleaching material, White spot lesion, Spectrophotometer, Thermocycling.

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INTRODUCTION

WSLs are characterized by mineral loss beneath an intact surface layer of enamel giving a whitish appearance. They are thought to be a common problem that may impair aesthetics. These lesions can be the initial signs of enamel caries ⁽¹⁾. They can also result from developmental defects of the enamel, such as molar incisor hypomineralization and dental fluorosis. Post-orthodontic treatment constitutes a major etiological factor ^(2,3).

Treatment of WSLs is challenging. It comprises non-invasive techniques as remineralizing agents, minimally invasive techniques such as RI, invasive approaches such as microabrasion and resin restorations, or even the invasive veneers or crowns ⁽⁴⁾.

The current study aimed to assess the color change of WSLs in extracted human premolars treated with RI alone, bleaching followed by RI, or microabrasion followed by RI using a spectrophotometer.

MATERIALS AND METHODS

Ethical Regulations

The current study was approved by the Ethics Committee, Faculty of Dentistry, Minia University, [approval number (522 / 2021)]. A permission from parents to use their children's extracted teeth for scientific purpose was obtained.

Sample size

The sample size was calculated using **G-power 3.1.9.4 software**. Considering a **one-way (ANOVA)** test to compare means of independent groups at a 0.05 alpha level of significance and 0.08 study power; 10 teeth in each group were selected and 30 teeth were estimated for the study.

Teeth collection

Thirty sound human premolars, extracted due to orthodontic reasons, were collected from patients of the Pediatric and Community Dentistry Department at Minia University Dental Hospital (MUDH). They were free from caries, microcracks, white spots, or enamel structural defects. The selected premolars went through washing, cleaning with distilled deionized water, for soft tissue debris or blood removal, and then kept in physiologic saline in a closed container for a maximum of one month.

Teeth preparation

An adhesive tape (3 x 3 mm) was applied on the center of the middle third of the buccal surfaces of the crowns. Nail varnish was applied onto the surface and the adhesive tape was then removed resulting in an enamel window for demineralization to standardize treatment and evaluation area. WSLs were induced by immersing the specimens in a demineralizing solution, prepared in the Faculty of Pharmacy, Minia University, consisting of 2.2 mmol/L Ca²⁺, 2.2 mmol/L PO₄⁻³, and 50 mmol/L acetic acid at a pH of 4.4 (measured by pH-Metre daily) for ten days ^(5,6). After inducing WSLs, specimens were imbedded into an acrylic resin mould. Then, they were stored in a dark box to prevent reflection of light from distorting color assessment.

Grouping

Random allocation of the samples equally into three groups (n = 10) was performed; group (A), treated with bleaching followed by RI; group (B), treated with microabrasion followed by RI; and group (C), treated with RI alone. **For RI, ICON (DMG) kit** (DMG, Hamburg, Germany) was used. **White Smile Kit** (GmbH, Germany), containing 40% hydrogen peroxide, was used for bleaching with 3 applications performed in one session; and each application lasted 20 minutes. While for

microabrasion, equal amounts of **Opalustre** (Ultra-dent, Utah, USA), consisting of 6.6% hydrochloric acid slurry and micro-particles of silicon carbide, were used. The WSLs were microabraded only once for each specimen by using Opalustre microabrasion paste with a low speed contra-angle hand piece for 60 seconds. For each specimen, a new Prophy Cup was used. After treatment, thermocycling was executed with 50000 cycles, equivalent to thermal ageing for 6 months.

Color measurement

For each specimen, measuring the color was performed, at baseline, after demineralization, after treatment and after thermocycling, using Vita Easyshade Spectrophotometer (Cary 5000-Agelant, USA). The change of color was assessed at each stage.

Diffuse light reflectance measurements were performed in the 380 to 780nm wavelength range using a UV-Vis (ultraviolet-visible) spectrophotometer. The color of each sample was measured against black background. CIE-Lab color values (D 65/10) for each sample were then calculated from the diffuse reflectance data, using the color software application, which was available through Cary Win UV instrument.

Color Analysis

According to the (Commission Internationale de l'Eclairage) CIELAB color space model, the following color measurements were taken; L^* for degree of lightness, a^* referring to red-green color and b^* referring to yellow-blue color components. The visible color change (ΔE) was calculated as $\Delta E^*_{Lab} = [(\Delta L^2) + (\Delta a^2) + (\Delta b^2)]^{1/2}$.

Statistical analysis

Statistical Package for Social Sciences (IBM SPSS Statistics for windows, version 21.0. Armonk, NY: IBM Corp) was used. Mean and standard deviation of color measurements values (L , a , and b) and color change (ΔE) in the three groups were calculated after testing the normality of data.

Normally distributed data were compared to different groups using One-way Analysis of Variance (ANOVA) test followed by Tukey's post hoc test for comparison between more than two groups in related and non-related samples respectively. For all tests, p-value of (0.05) or less was considered statistically significant and the confidence interval was 95%.

RESULTS

In each group, there was no statistically significant difference in the means of the color measurements values (L , a , and b axes) at the 4 color measurements. (**Table: 1**).

TABLE (1) Mean values of ΔE in three groups.

ΔE / Mean \pm SD	Demineralization	After treatment	After thermocycling
Group (A)	5.22 \pm 0.35	1.55 \pm 0.16	1.87 \pm 0.15
Group (B)	5.20 \pm 0.21	1.93 \pm 0.17	2.48 \pm 0.18
Group (C)	5.19 \pm 0.24	2.46 \pm 0.15	3.24 \pm 0.14
p-value	0.96	<0.0001*	<0.0001*

Regarding relation between groups, no statistically significant difference was found in ΔE before and after demineralization ($p > 0.05$). However, after treatment and after thermocycling, a significant difference was found in ΔE between the three groups ($p < 0.0001$) (Table: 2).

TABLE (2) Mean difference of color measurement values of ΔE in the three groups.

ΔE	Mean difference	SE	95% CI	p-value
After demineralization				
A-B	0.02	0.12	-0.28; 0.32	0.98
A-C	0.04	0.12	-0.27; 0.34	0.96
B-C	0.01	0.12	-0.29; 0.32	0.99
After treatment				
A-B	-0.38	0.07	-0.55; -0.20	<0.0001*
A-C	-0.90	0.07	-1.08; -0.73	<0.0001*
B-C	-0.53	0.07	-0.70; -0.35	<0.0001*
After thermocycling				
A-B	-0.62	0.07	-0.79; -0.44	<0.0001*
A-C	-0.41	0.07	-1.58; -1.23	<0.0001*
B-C	-0.79	0.07	-0.97; -0.62	<0.0001*

DISCUSSION

Resin infiltration is a minimally invasive treatment modality that was introduced to arrest the progression of initial caries, and to improve the aesthetic appearance of WSLs restoring the natural color of enamel⁽⁷⁾. Resin infiltration provides remarkable esthetic improvement of WSLs over microabrasion and bleaching⁽⁸⁾, however, some WSLs exhibit partial or no response to resin infiltration⁽⁴⁾. The current study aimed to identify the best practice in managing WSLs; either RI alone or in combination with microabrasion or bleaching, in addition to assessment of the color stability for these treatment approaches.

The study was designed as an in vitro study since it allows comparing various treatment approaches under standardized conditions. Also, it is faster and less complicated than clinical trials⁽⁹⁾. Although, **Yetkiner et al. (2014)**⁽¹⁰⁾ and **de Freitas et al. (2012)**⁽¹¹⁾ used bovine enamel to assess management techniques of WSLs, thirty sound human premolars were selected for the current study to avoid

differences in morphology, chemical composition and physical characteristics between human and bovine teeth. Moreover, the specimens comprised both enamel and dentin since the perceived color results from the reflectance from dentin through the enamel^(10,12).

In order to simulate WSLs, samples were immersed in a demineralizing solution for 10 days. This resulted in ΔE equivalent to 5.19 – 5.22, that was within the clinically perceptible range. This complies with **Montasser et al. (2015)**⁽⁵⁾ and **Abd Elkader et al. (2019)**⁽⁶⁾ who used the same demineralizing solution for the same period of time.

Specimens were randomly allocated into three groups with three different treatment approaches. In group (A), bleaching was performed before RI since the existence of cured resin within the enamel impairs the effectiveness of bleaching agents⁽¹¹⁾ while bleaching by peroxide does not induce significant changes in tooth enamel organic and inorganic contents⁽¹²⁾. In Group (B), microabrasion was performed for 60 seconds to remove less than 10% of the enamel thickness⁽²⁰⁾.

Color stability was evaluated since long-term effectiveness of resin infiltration is still under investigation⁽⁷⁾. Specimens were subjected to thermocycling to simulate the oral environment, where the samples experienced 50000 cycles that are equivalent to thermal aging for 6 months. Similar procedures were performed by **Abd Elkader et al. (2019)**⁽⁶⁾. Thermal aging for 6 months was selected since it is a median follow-up time that ranged, in the majority of studies, between 1 day and 12 months to allow for comparisons. In addition, long term studies revealed no significant difference between 6 months and 2 years of follow up after RI⁽¹³⁾. Nonetheless, long-term studies with 3 years follow up period, recommended for direct restoration, are still required⁽¹⁴⁾.

In the present study, spectrophotometer was used to quantify color change since it is a preferred color measuring tool with high precision and

reliability. It enables objective evaluation of color providing precise data ⁽¹⁵⁾. This tool supersedes visual assessment that is subjective and lacks reliability, and digital cameras that require uniform illuminating environment ⁽¹⁶⁾. Color was assessed according to CIE L*a*b* color analysis. This frequently used color space has the ability to present the results in a way that is much more close to the human perception ⁽¹⁷⁾.

Results revealed a statistically significant decrease in ΔE values in samples treated with bleaching followed by resin infiltration compared to other groups both immediately and after aging process. This goes in accordance with **Horuztepe and Baseren, (2017)**⁽¹⁸⁾, who proved that combining bleaching and RI resulted in better color change of WSLs compared to bleaching alone. Similarly, **Gugnani et al. (2017)**⁽¹⁹⁾, found that such combination yielded superior esthetic improvement of mild and moderate fluorosis. Additionally, **Alverson et al. (2021)**⁽²⁰⁾, stated that patients with WSLs, treated with both bleaching and RI, were more satisfied immediately after treatment and after 8 months follow up period.

This improvement of the color of artificially formed WSLs after bleaching and RI is attributed to that bleaching removes the pigments and then the infiltrating resin occludes the porosities in the WSLs⁽¹⁸⁾. Also, WSLs treated with microabrasion followed by RI showed better color improvement than RI alone since microabrasion removes approximately 40 μm of defective enamel and precondition the surface allowing for better penetration of the resin ^(6, 10, 21). This goes in accordance with **Yetkiner et al. (2014)**⁽¹⁰⁾ and **Abd Elkader et al. (2019)**⁽⁶⁾, who stated that microabrasion combined with RI result in immediate color improvement.

Within the limitations of this laboratory study, bleaching followed by RI can be recommended as an effective treatment approach in masking WSLs with adequate color stability. However, further studies are required to assess this treatment approach in

the more challenging oral environment and to assess color stability over long follow up periods. Further studies are also recommended to evaluate combining the three treatment approaches; microabrasion, bleaching and resin infiltration.

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