Journal of
Babol University of Medical Sciences
JBUMS
e-ISSN: 2251-7170 p-ISSN: 1561-4107

Laboratory Evaluation of Color Change and Surface Roughness of White Spot Lesions Treated with Resin Infiltration and Fluoride Therapy

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Article Type

Research Paper

Received:
Jul 28th 2021
Revised:
Nov 24th 2021
Accepted:
Jan 16th 2022

ABSTRACT

Background and Objective: Two non-invasive treatment methods for treating white spot lesions (WSLs) include resin infiltration and fluoride therapy. Contradictions have been raised regarding the color change and surface roughness of the lesions based on these methods. Therefore, this study was conducted to investigate the color change and surface roughness of white spot lesions after treatment with resin infiltration and fluoride therapy.

Methods: In this laboratory study, 40 buccal and lingual sections were prepared from 20 extracted healthy premolar teeth. 10 samples were considered as the control group, and in the other 30 samples, decayed lesions were created artificially. White spot lesions were randomly prepared in three groups without treatment, 0.05% sodium fluoride solution and resin infiltration (n=10). Then, the rate of color change and surface roughness of the samples after being placed in black tea and also after brushing were measured and compared using spectrophotometer and profilometer.

Findings: The surface roughness of samples in resin infiltration, intact enamel and fluoride groups were 163.46±64.67, 259.6±43.12 and 293.92±41.36 micrometers, respectively ($p<0.001$). Before placing in tea and after brushing, no significant difference was observed in the color of the samples, but after staining, the color change in WSL (9.14±5.85), fluoride (17.40±4.13) and resin infiltration (12.13±4.88) groups was significant ($p=0.004$); the fluoride group showed significantly more color change compared to the WSL group ($p=0.003$), but the difference between the other groups was not significant.

Conclusion: The results of this study show that if the resin infiltration method is used in the treatment of white spot lesions, less surface roughness and color change is observed compared to fluoride therapy.

Keywords: *White Spot Lesions, Icon Infiltrant, Sodium Fluoride, Tooth Color Change*.

Cite this article: Esmaeilnia Shirvani M, Gharehkhani S, Esmaeili B, Hagh Haghighi A, Gholinia Ahangar H. Laboratory Evaluation of Color Change and Surface Roughness of White Spot Lesions Treated with Resin Infiltration and Fluoride Therapy. *Journal of Babol University of Medical Sciences*. 2022; 24(1): 338-46.

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Publisher: Babol University of Medical Sciences

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[DOR: 20.1001.1.15614107.1401.24.1.41.7]
[DOI: 10.22088/jbums.24.1.338]

Introduction

White Spot Lesions (WSLs), which are known as primary caries, are frequently seen in some patients, including those undergoing orthodontic treatment and those with poor hygiene. These lesions are caused by the subsurface demineralization of enamel and can turn into cavities. Despite these lesions, the beauty of the teeth can be compromised (1-3).

Fluoride therapy and resin infiltration can be mentioned among the treatment methods used for white spot lesions. Infiltration of fluoride into the enamel by producing fluorapatite crystals reduces tooth decay. In addition, by exchanging the hydroxyl group and reducing the carbonate component, it reduces the solubility of tooth minerals, which can lead to remineralization (4, 5).

Recently, a non-invasive treatment method involving infiltration of low-viscosity resin into smooth and proximal surface lesions has been introduced. Resin infiltration can stop the progression of primary enamel lesions up to the first third of the dentin without unnecessary destruction of healthy tooth structure. Lesions treated by Icon resin have a similar appearance to the surrounding healthy enamel. Therefore, it is an alternative method in terms of aesthetics compared to enamel microabrasion methods and invasive restorative methods. In this method, the outer surface of the enamel is transformed into a more permeable layer with the help of etching (15% HCL) and the underlying porous structure is infiltrated with triethylene glycol dimethacrylate resin. This resin has the same refractive index as tooth enamel and is the same color as the tooth, which improves the appearance of the lesion in addition to strengthening the weakened enamel prism (6, 7).

There are various tool-based methods to evaluate tooth color, including calorimetry, spectrophotometry, and digital image analysis. Since the spectrophotometer device evaluates the color objectively and numerically and accurately, this device is used in most studies. By means of this device, the color of the studied samples is registered in the CIE system and based on three parameters L^*, a^* and b^*. L^* indicates the degree of brightness and has a range of 0 (dark) to 100 (bright). a^* indicates the greenness/redness of the sample, and the increase in this parameter indicates that the redness of the sample has increased. b^* indicates the blueness/yellowness of the sample, and the increase in this parameter indicates that the yellowness of the sample has increased. When a comparison is made from the beginning to the end of a color change according to L, a and b parameters, it is defined as ΔE, and if this difference is more than 3.3, it is clinically observed (5, 6).

Aswani et al. and Hammad et al. introduced resin infiltration as an effective method that improves the surface characteristics and the opaque appearance of primary enamel lesions (8, 9). On the other hand, Zhao et al. state that the surface characteristics and color stability of enamel lesion in resin infiltration is inferior to healthy enamel (10). Neres et al. showed that infiltration resin is not able to restore the properties of healthy enamel, including surface roughness (11). Due to these contradictions regarding the resin infiltration method, this study was conducted with the aim of investigating the color change and surface roughness of primary caries lesions treated with resin infiltration and low dose fluoride.

Methods

This laboratory study was approved by the ethics committee of Babol University of Medical Sciences with code MUBABOL.REC.1396.34. According to similar studies (10, 11), the samples included 20 healthy human premolar teeth that were extracted for orthodontic reasons, at most 6 months before the beginning of the study. The teeth were observed under a stereomicroscope (Olympus Bx41, Olympus Optical Co,

[DOR: 20.1001.1.15614107.1401.24.1.41.7]
[DOI: 10.22088/jbums.24.1.338]

Tokyo, Japan) and the teeth that were free of white lesions, caries, restoration and cracks were included in the study.

Preparation of samples: The samples were kept in physiological saline solution from the time they were extracted until the beginning of the study, and the solution was changed weekly. 24 hours before the start of the experiment, the teeth were immersed in 0.5% thymol solution. The roots of all teeth were separated from the CEJ area and the crowns of the teeth were cut into two buccal and lingual sections by a microtome (Delta precision sectioning machine, Mashhad, Iran). Samples were cut into approximately 5×5 mm sections by a D&Z disc (Drendel + Zweiling, Berlin, Germany). Then, each of these sections was mounted on acrylic (Acropars, Marlic, Tehran, Iran) in such a way that the outer enamel of the tooth was facing outward and parallel to the horizon. Then, these sections were flattened with 400, 800 and 1200 Grit silicon carbide abrasive paper (Softflex Matador, Wasserfest, Germany) and polished with Cement paste (FGM, Joinville, Santa Catarina, Brazil). Of the 40 samples prepared for the study, 10 healthy enamel samples were set aside as a control group to check stain absorption and surface roughness, and white spot lesions were created on the other 30 samples. In order to artificially create white spot lesions, the samples were placed in demineralization solution (12 mM CaCl2, 10 mM KH2PO4, 50 mM Lactic Acid, 100 mM NaCl) with pH=4.5 at 37ºC for 6 hours and then placed in remineralization solution (1.5 mM CaCl2, 5 mM KH2PO4, 100 mM Lactic Acid, 100 mM NaCl) with pH=6.5 at 37ºC for 18 hours and this process was repeated for 14 days (12, 13). Then these 30 samples were divided into three groups of 10: group 1: no treatment, group 2: treatment with fluoride, group 3: treatment with resin infiltration.

Group 1 (no treatment): The samples were stored in artificial saliva (Kin hydrate spray, Kin, Spain) at room temperature in a sealed container. Artificial saliva was changed every two days.

Group 2 (treatment with sodium fluoride): The samples were immersed in 2 ml of 0.05% sodium fluoride solution (Sodium Fluoride Mouthwash, Behsa Pharmaceutical, Tehran, Iran) for one minute daily for 30 days. Between these steps, the samples were kept in artificial saliva.

Group 3 (resin infiltration with icon resin [Icon, DMG, Hamburg, Germany]): first, the samples were etched by HCL 15% (Icon Etch) for 120 seconds, washed with water for 30 seconds and dried with air compressor. Then, the surface of the samples was soaked in 99% ethanol (Icon Dry) for 30 seconds and dried with air compressor. The first layer of infiltrant was placed on the surface by a micro brush. This layer remained on the tooth for 180 seconds and then cured for 60 seconds. The second layer was left on the tooth for 60 seconds and then cured for 40 seconds (12-14). After the treatment, the samples of group 3 were kept in artificial saliva solution that was changed every two days for 30 days in order to compare with groups 1 and 2 in terms of the effect of artificial saliva and time.

Staining stage: groups 1 to 3 were placed in black tea solution (Yellow Label Tea, Lipton, London, England) for 24 hours. Considering the consumption of 3 cups of tea per day and one minute for each cup, three minutes were considered for daily tea consumption. Thus, 24 hours of immersion of samples in tea is equivalent to 16 months. To prepare black tea solution, 5 tea bags were placed in one liter of boiling distilled water (100ºC) for 10 minutes. After cooling the tea to 55°C, each sample was immersed in the tea in a vertical position with a piece of thread. The samples were placed inside the tea in such a way that they do not contact each other and the wall of the container. In order to keep the temperature constant, the samples were kept in an incubator.

Brushing: Brushing was done with an electric toothbrush (Oral-B D12.513 Vitality Precision Clean Electric Toothbrush, P&G Oral Care, USA) and toothpaste (Crest 3D White Deluxe Anti-Tobacco, P&G Oral Care, USA). The distance between the electric toothbrush and the tooth was measured with a ruler to be the same in all samples. For this purpose, the electric toothbrush was placed in a fixed place to apply constant force and pressure to all samples. Each sample was brushed for 5 seconds.

[DOR: 20.1001.1.15614107.1401.24.1.41.7]
[DOI: 10.22088/jbums.24.1.338]

Measurement of surface roughness: The surface roughness of all samples was measured separately at three points on the surface of each sample by a profilometer (tr2000, Time-Group, USA) before being placed in the staining solution, and their average value was recorded. For this purpose, the mounted samples were placed in a special place for measurement, then by moving the diamond needle of the profilometer device on three points of the sample surface randomly, the average surface roughness for each sample was shown numerically in the device based on microns.

Color measurement: Color measurement was done in the control group. In groups 1 to 3, color measurement was done in three stages before staining, after staining and after brushing. The indicators related to the color of the samples were measured using a spectrophotometer (Vident, VITA Easyshade, Brea, California, USA) which was calibrated in a special room and in a fixed place according to the manufacturer's instructions. By means of this device, the color of the studied samples was recorded in the CIE system and in three parameters L^* (brightness), a^* (red-green) and b^* (blue-yellow). The total color change of the samples (ΔE) was calculated according to the following formula (6):

$$\Delta E = [(L^*_1 - L^*_2)^2 + (a^*_1 - a^*_2)^2 + (b^*_1 - b^*_2)^2]^{0.5}$$

The calculation of ΔE in each group was obtained by comparing the color evaluation data (b^*, a^*, L^*) of each step compared to the previous step in the above formula. ΔE_1= color difference of WSL and primary samples, ΔE_2= color difference of WSL and tea samples, ΔE_3= color difference of tea and brushing samples. The information obtained in this study was assessed using SPSS 22 software and ANOVA tests and Tukey's post hoc test to analyze the data of surface roughness and ΔE, as well as Bonferroni post hoc test to analyze and evaluate the data of a, L and b. $p<0.05$ was considered significant.

Results

In this study, the highest surface roughness was obtained in the WSL group (368.80±93.06) and the lowest in the resin infiltration group (163.46±64.67) (Figure 1). In general, a significant difference was observed in the mean surface roughness of the groups under study ($p<0.01$). The mean surface roughness of resin infiltration group was significantly lower than other groups ($p=0.00$).

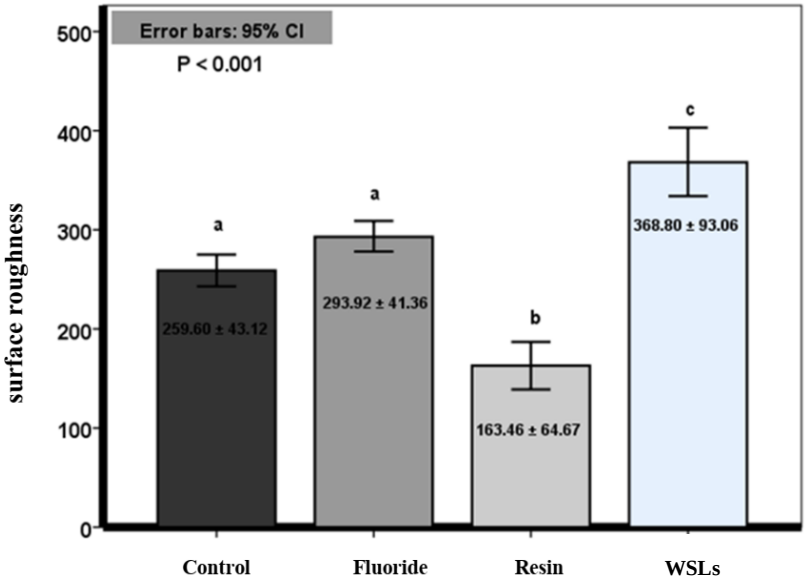

Figure 1. Comparison of surface roughness of samples in different groups (micrometers).

Dissimilar letters (a, b and c) indicate significant differences.

[DOR: 20.1001.1.15614107.1401.24.1.41.7]
[DOI: 10.22088/jbums.24.1.338]

In the investigation of the effect of fluoride therapy and resin infiltration on staining, a significant difference was seen in the ΔE values of the groups in the second stage of staining (p=0.004). The color change of the fluoride group was significantly higher than the WSL group (p=0.003), but no significant difference was found in the two by two comparison of the other groups (Table 1).

Table 1. Average ΔE of groups in three stages

Color change Groups	Before staining (ΔE1) Mean±SD	After staining (ΔE2) Mean±SD	After brushing (ΔE3) Mean±SD
WSLs	7.21±3.43[a]	9.14±5.85[a]	7.58±5.3[a]
Fluoride	6.59±4.09[a]	17.40±4.13[b]	7.65±3.06[a]
resin infiltration	5.57±3.92[a]	12.13±4.88[ab]	11.53±5.71
p-value	0.59	0.004	0.13

ΔE1= color difference of WSL and primary samples, ΔE2= color difference of WSL and tea samples, ΔE3= color difference of tea and brushing samples. Non-similar letters indicate significant differences.

The results of this comparison indicate that there was a significant difference in the mean L in the groups of WSLs, fluoride and resin infiltration during three stages (from left to right, p=0.001, p<0.001, p=0.004, respectively). The amount of L decreased significantly in all three mentioned groups after exposure to tea (from left to right, p<0.001, p<0.05, p=0.003). In the group of WSLs and resin infiltration, after brushing, the amount of L was close to the estimated value before staining and had no significant difference with it (Table 2). In the group of WSLs and infiltration resin, there was no significant difference in the mean value of a in the three stages (p=0.056, p=0.22, respectively). In the fluoride group, the value of a increased significantly after being placed in tea, but decreased slightly after brushing and was still significantly higher than the value estimated in the pre-staining stage (p=0.001). There was no significant difference between the values of b in the groups in the three stages.

Table 2. Average value of L, a and b in groups in three stages

Color parameters Groups	Step 3 (after brushing) Mean±SD			Step 2 (after staining) Mean±SD			Step 1 (before staining) Mean±SD		
	L	a	b	L	a	b	L	a	b
WSLs	78.31±4.59	1.56±0.78	27.26±1.50	74.95±3.17	2.65±2.12	29.07±4.52	8.14±3.70	1.31±1.51	30.09±3.73
Fluoride	77.68±4.99	3.85±2.37	30.06±3.42	73.45±3.25	5.40±2.60	31.74±3.05	89.06±3.06	0.99±0.90	28.36±2.99
Resin infiltration	80.21±5.68	0.49±0.89	25.60±2.51	73.37±4.38	2.36±2.49	27.37±2.96	84.22±3.81	0.75±1.22	27.35±2.99
p-value	0.001	0.03	0.03	<0.001	0.03	0.025	<0.001	0.03	0.03

L (brightness), a (red-green) and b (blue-yellow)

Discussion

The results of this study showed that both the use of fluoride and the use of resin infiltration reduce the surface roughness of WSLs samples. This study showed that resin infiltration reduced the surface roughness of WSLs samples, which is in line with the results of some studies (15, 16). In addition, Taher et al. also concluded that enamel treated with resin infiltration reduces surface roughness (17). This result can occur due to the high permeability, low viscosity and deep infiltration of this substance in primary caries lesions,

[DOR: 20.1001.1.15614107.1401.24.1.41.7]
[DOI: 10.22088/jbums.24.1.338]

which makes the surface smooth by filling the surface roughness (8, 18, 19). In contrast, El Meligy et al. and Gurdogan et al. reported a significant increase in surface roughness in the resin infiltration group compared to the primary caries group. The difference in the measurement tool of surface roughness can be the cause of this difference. They used AFM to measure surface roughness. In AFM, three-dimensional images of the surface showed a non-uniform layer with small granules scattered on the surface of the enamel (14, 20). Fluoride also increases enamel resistance to demineralization due to its combination with surface hydroxyapatite and the formation of fluoroapatite and calcium fluoride. When salivary pH decreases, bound calcium ions can be released and stimulate remineralization (5). In addition, the application of fluoride on WSL lesions can reduce surface roughness (21, 22). The presence of surface roughness causes the accumulation of plaque and as a result can disrupt the remineralization process. Along with the surface roughness, it seems necessary to evaluate the change and stability of the tooth color after performing any of the treatment methods. WSL lesions have an opaque white appearance, in fact when light strikes these decalcified lesions that have subsurface porosity, they scatter differently compared to healthy enamel. In this study, samples treated with resin infiltration and fluoride had lower ΔE1 than WSLs samples, which shows that their color is closer to healthy enamel and improves aesthetics, but because the amount of color change is still higher than the clinically acceptable threshold (ΔE1>3.7), the opaque appearance of the lesion is not completely eliminated (6). These results are somewhat consistent with the findings about surface roughness. Yektinar et al. showed that resin infiltration was more effective than fluoride therapy in covering the opaque appearance of early caries lesions. Of course, in that study, the conditions of demineralization and remineralization of the samples were different and the samples were polished after resin infiltration (6).

After the staining stage, the lowest amount of color change (ΔE2) was observed in the WSLs group despite the surface roughness results, which was in agreement with the results of a study by Yetkiner et al. (6). On the other hand, the ΔE2 of the resin group was lower than that of the fluoride group, despite its inherent tendency to color change, which could probably be due to the lower surface roughness of the resin infiltration group. On the other hand, Borges et al. concluded that the demineralized enamel treated with resin infiltrate showed more color change compared to other groups (control group, artificial saliva group, sodium fluoride 0.05 group), which is not consistent with the results of the present study (18). This difference can be due to the longer storage time of the samples (8 days) in the staining solution compared to the present study (one day), during which the resin has more opportunity to absorb the stain. Also, in the study of Borges et al., samples treated with fluoride were washed before being placed in the stain solution (18).

After brushing, the color changes (ΔE3) in the resin infiltration group were higher than the other groups, and a clear increase in "L" and a decrease in "a" and "b" were observed, which can be attributed to the smoothness of the surface of the samples in resin infiltration group compared to fluoride and WSLs groups. Borges et al. also showed that polishing the samples reduced the color change, which is in line with the results obtained in this research (18).

In this study, the changes of L, a, and b after immersing dental samples of WSLs, infiltration resin, and fluoride in tea indicated a decrease in brightness and an increase in redness and yellowness of the samples. However, changes of L, a and b of the samples after brushing in fluoride and resin infiltration groups indicated the relative removal of surface pigments, increase in brightness and decrease in yellowness of the samples. Of course, in clinical conditions, diluting tea with saliva is effective in the staining of restorative materials. It is suggested that in future studies, the stain absorption, roughness and hardness of the surface should be investigated in the same conditions as in the clinic and compared with other demineralizing materials.

[DOR: 20.1001.1.15614107.1401.24.1.41.7]
[DOI: 10.22088/jbums.24.1.338]

The results of this study suggest the resin infiltration method in the treatment of WSLs as a non-invasive treatment with less surface roughness and stain absorption compared to fluoride therapy.

Acknowledgment

The Deputy of Research and Technology of Babol University of Medical Sciences is hereby thanked for the financial support of this research.

[DOR: 20.1001.1.15614107.1401.24.1.41.7]
[DOI: 10.22088/jbums.24.1.338]

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[DOI: 10.22088/jbums.24.1.338]