

Evaluation of Color Changes in Resin Infiltrated White Spot Lesion after Exposure to Different Colored Oral Rinses

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Received May 02, 2023; Revised June 04, 2023; Accepted June 13, 2023

Abstract This study aimed to compare the colour changes of resin-infiltrated white spot lesions after exposure to artificial saliva and oral rinses (Oral-B Pro-Expert, Listerine and Colgate Plax) at different time intervals (24 h, 72 h and 7 d). The premolar teeth were selected and sectioned into two halves. The artificial white spot lesions were created and resin-infiltration procedure was performed to seal the pores. The specimens immersed in the control subgroup showed an insignificant colour change (ΔE) after 72 h. While significant differences in colour change among the experimental groups across time intervals ($p < 0.001$), oral rinse groups ($p < 0.001$), and the interaction between timeline and groups ($p < 0.001$). All resin-infiltrated specimens showed colour differences after immersion in oral rinses with visually perceptible change. Although, Colgate Plax and Oral B oral rinses demonstrated the highest and lowest colour change, respectively in the initial 24 h. However, at 72 h and 7 d time-points, Listerine oral rinse demonstrated the highest colour change, while the lowest values were observed in Oral-B oral rinse. The use of Listerine should therefore be cautiously advised in resin-infiltrated cavities.

Keywords: color change, oral rinses, resin-infiltration, white spot lesion

Cite This Article: Mohammed AlGhamdi, Nada Shokair, Saleh Al Qahtani, Abdulrahman Abusaq, and Roula Albounni, "Evaluation of Color Changes in Resin Infiltrated White Spot Lesion after Exposure to Different Colored Oral Rinses." *International Journal of Dental Sciences and Research*, vol. 11, no. 1 (2023): 8-14. doi: 10.12691/ijdsr-11-1-3.

1. Introduction

Subsurface enamel porosity caused by carious demineralization on the tooth's flat surfaces is known as a white spot lesion (WSL). It emerges as an opaque milky white area. [1] Simplistically, WSL is the loss of enamel minerals while the lesion's surface is still mostly intact. [2]

The WSLs develop swiftly however the onset of clinical symptoms might not be noticeable up to two weeks following the start of biofilm production. [3,4,5] In WSL, the surface appears undamaged however beneath the affected surface porous lesion is present. [5] In WSL, light is mostly diffused inside the lesion, hence the active lesion appears chalky and opaque. [6] Dispersion at the interfaces of materials with various refractive indices (RIs) such as enamel/apatite (RI 1.62-1.65), water (1.33), apatite (RI 1.62-1.65), or air (1.00), is what causes scattering. [6,7] As the RI of water is more similar to that of enamel than

that of air, an early lesion must be dried before it can be seen visually. [8] Apart from drying, WSL scattering is largely hooked on the enamel's mineral content. [9]

Historically, the treatment options for these lesions included prevention, remineralization to preserve the lesion surface and tooth structure, or restoration and replacement of the affected lesion. [10] However, few investigators believed that the former preventive option is very subjective and requires high patient compliance, while others believe that the surgical intervention will compromise the tooth's strength and hence require more frequent replacement. [11]

Resin infiltration has recently been promoted as a noninvasive technique that might successfully inhibit caries from progressing. Such an approach requires the resin to penetrate and fill the WSL pore. The main goal is to fill the lesion body's pores with a low-viscosity light-curing resin by capillary action. Thereby preventing the further spread of bacteria and, consequently, the progression of the caries lesion. By placing resin infiltrate

on WSLs, a barrier can be established within the caries lesion that reinforces the enamel structure and prevents or delays cavitation and surface disruption. [12,13] The best part is that resin infiltration does not require any anaesthesia or removal of anatomical tooth structure. [14]

While resin infiltration was initially created to halt proximal caries lesions, it also had a positive impact on the aesthetics of anterior teeth when treating white spot lesions (WSLs) on these teeth. The low-viscosity resin infiltrated the enamel porosities, which allowed for a change in the light's refractive index and an improved final appearance of the tooth. It has been demonstrated that the infiltrant resin can conceal WSLs because its refractive index (1.51) is comparable to that of hydroxyapatite. [8,15,16]

Oral rinses are commonly used during the interim periods involving oral surgical treatments to reduce plaque formation and improve gingival and periodontal health. This is because of their anti-inflammatory, antiseptic, and analgesic properties. [17] Additionally, home care products with chemical antimicrobials can reduce gingivitis. Clinical studies have shown that mouthwashes containing triclosan/copolymer dentifrices and essential oils have advantages in terms of antiplaque and anti-gingivitis properties. [18]

To the greatest of our understanding, no study has ever examined the impact of marketed mouthwashes on resin-infiltrating substances (ICON). Extrinsic colourants from oral rinses can adversely impact the infiltrant's resin matrix's colour sensitivity, making it more susceptible to colour change. Due to these concerns, resin infiltrant properties are of great relevance to avoid future esthetic problems. Therefore, the purpose of this in-vitro study was to compare the colour changes of resin-infiltrated white spot lesions after exposure to artificial saliva and commercially available coloured oral rinses such as Oral B, Listerine and Colgate Plax at different time intervals (24 h, 72 h and 7 d). It was predicted that no change in colour of resin-infiltrated WSLs of the teeth exposed to artificial saliva and coloured oral rinses would be observed at different time intervals.

2. Methodology

The study proposal was submitted (Registration no. FPGRP/43830009/328) to the research centre of Riyadh Elm University and, the Institutional review board issued the formal approval for conducting the study RC/IRB/2019/256. Table 1 displays the materials employed in this investigation.

2.1. Development of Artificial WSL

In this experimental study, 30 extracted human sound premolar teeth extracted for orthodontic reasons were selected. The teeth were sectioned longitudinally into two halves to obtain 60 specimens using a cutting machine (Isomet 4000 micro saw, Buehler, USA). The specimens were subjected to a demineralizing solution listed in Table 1 for four days to generate white spot lesions. Following this, all 60 specimens were implanted using an auto-polymerizing acrylic resin.

Following demineralization, the specimens were assigned to three categories depending on the mouthwash used, and each group was further subdivided into two subgroups, namely the control group, which included 10 specimens immersed in artificial saliva and 10 specimens immersed in the corresponding mouthwash.

2.2. ICON Infiltration Treatment

Firstly, the demineralized enamel surface underwent a 2 min treatment with a 15% hydrochloric acid gel called Icon-Etch, followed by rinsing with water and air-drying for 30 s. Next, Icon-Dry was applied for 30 s, followed by additional air-drying. The demineralized surface was then treated twice with a low-viscosity resin infiltrant known as Icon-Infiltrant, applied for 3 min and 1 min respectively, to fill the enamel pores. Both applications were subjected to light curing for 40 s using the apparatus specified in Table 1. To remove any excess resin, the resin-infiltrated teeth were polished with aluminum oxide abrasive sheets (4000 grit, FEPA-P; Struers) for 20 s.

Table 1. Materials employed in this investigation

Material	Details
Demineralizing solution	The solution contains 2.2 mM of calcium chloride, 2.2 mM of monopotassium phosphate, and 0.05 mM of acetic acid. The pH of the solution has been adjusted to 4.4 using potassium hydroxide, which has a concentration of 1M.
Resin infiltrant ICON	Syringe Icon-Etch 0.45 ml (Hydrochloric Acid, Pyrogenic Silicic Acid, and Surface-active substances) Syringe Icon-Dry 0.45 ml (99% Ethanol) Syringe Icon-Infiltrant 0.45ml (TEGDMA-based resin matrix and initiators) (DMG, Hamburg, Germany),
Light cure device	Demetron, Dan-bury, CT, USA
(Oral-B PRO-EXPERT)	Aqua, Glycerin, Aroma, Cetylpyridinium Chloride, Poloxamer 407, Methylparaben, Sodium Saccharin, Cinnamal, Propylparaben, Eugenol, CI 42090
Listerine	Eucalyptol, Zinc Chloride, Sodium Benzoate Methyl Salicylate, Thymol, Menthol, Sodium fluoride (220 ppm F)
Colgate Plax	Water, Glycerin, Propylene Glycol, Sorbitol, Poloxamer 407, Flavour, Cetylpyridinium Chloride, Potassium Sorbate, Sodium Saccharin, Menthol, Citric Acid, CI 42053, CI 15985.
Polishing	Rubber cup (DENSICO® Prophy Cups, soft, blue, ribbed, Water Pik, Inc., Fort Collins, CO)
Spectrophotometer	LabScan XE
Spectramagic software	Konica Minolta, INC.
Cutting machine	Isomet 4000 microsaw, Buehler, USA
Teeth	Human premolar teeth

2.3. Colorimetric Analysis

After polishing all the teeth, the baseline colour shade (T_0) was recorded by Labscan XE scanning spectrophotometer (Hunter Associates Laboratory, Reston, VA, USA) attached with a universal software V4.10 (Hunter Associates Laboratory). Following that, teeth with resin-infiltrated WSLs were subjected to different coloured mouth rinses, as shown in Table 1, and color alteration was documented after 1 d, 3 d, and 7 d.

2.4. Statistical Analysis

The normality of the data was checked initially. At various intervals, descriptive statistics of mean and standard deviation values were obtained for the color coordinates L^* , a^* , and b^* . Colour differences at time points were obtained by using $\Delta E^* = [(L^*1-L^*2)^2 + (a^*1-a^*2)^2 + (b^*1-b^*2)^2]^{1/2}$. Color differences across groups were analyzed using one-way and two-way ANOVA testing, followed by Tukey's multiple comparison tests. A paired t-test was used to compare the two variables. A p-value less than 0.05 was regarded as significant. SPSS ver. 25 (IBM Corp., New York, NY) was used for all analyses.

3. Results

Table 2 presents the mean and standard deviation color coordinates values of the control groups at baseline and 72 h. The changes in color coordinates (L^* , a^* , and b^*). None of the study group showed statistically significant difference in the ΔE value at 72 h.

Table 3 displays the descriptive statistics of mean, standard deviation, minimum, and maximum values of L , a , and b coordinates among the study groups at different time intervals. The L , a , and b values for samples soaked in Oral-B mouthwash at baseline (70.16±3.74, -2.08±0.78, 4.13±2.32), 24 h (60.46±1.54, -3.05±0.76, 2.26±2.67), 72 h (62.95±2.59, -2.27±0.92, 3.14±2.52), and 7 d (65.72±3.03, -2.39±0.83, 3.33±1.98) were reported. Samples immersed in Listerine mouthwash showed L , a , and b values at baseline (72.15±4.34, -2.11±.69, 3.67±4.19), 24 h (61.03±2.33, -3.83±.58, 0.01±3.72), 72 h (62.11±2.23, -4.32 ±1.42, -2.72±3.87), and 7 d (80.52±3.12, -4.73±2.19, 1.04± 5.15). Similarly, samples soaked in Colgate Plax mouthwash showed L , a , and b values at baseline (73.53±3.41, -2.56±0.75, 2.89±5.15), 24 h (60.47±1.78, -3.72±0.52, -1.17± 2.68), 72 h (61.72±2.02, -2.83±.64, 0.79±2.14), and 7 d (66.10±3.14, -2.79±0.80, 1.85±2.76).

Table 2. Descriptive statistics of L , a , b , and ΔE coordinates at baseline and 72 h in the control group

Mouthwash		Baseline			72 hours			ΔE
		L1	a1	b1	L2	a2	b2	
(Oral-B)	Mean	68.28	-2.09	2.93	64.15	-2.45	1.48	5.07
	SD	4.81	.79	2.25	3.24	.61	2.24	4.35
	Min	62.00	-3.32	-2.45	58.97	-3.29	-2.44	.72
	Max	78.53	-1.22	5.74	70.34	-1.59	5.43	14.45
(Listerine)	Mean	69.40	-2.22	2.52	64.87	-2.55	.81	5.44
	SD	4.86	.53	2.10	3.90	.47	3.42	4.76
	Min	60.10	-2.98	-1.52	58.62	-3.22	-4.40	.81
	Max	77.42	-1.59	5.34	73.70	-2.02	6.62	15.01
(Colgate Plax)	Mean	69.23	-2.45	1.08	66.19	-2.72	.54	5.51
	SD	2.58	.82	2.46	3.94	.58	4.51	2.67
	Min	65.50	-3.80	-2.20	60.20	-3.80	-4.20	1.80
	Max	73.50	-1.10	4.80	74.10	-1.90	8.70	10.18

Table 3. Descriptive statistics of L , a , b coordinates at baseline, 24 hours, 72 hours, and 1 week in mouthwash groups

Mouth wash		Baseline			24 hours			72 hours			7 day		
		L1	a1	b1	L2	a2	b2	L3	a3	b3	L4	a4	b4
(Oral-B)	Mean	70.16	-2.08	4.13	60.46	-3.05	2.26	62.95	-2.27	3.14	65.72	-2.39	3.33
	SD	3.74	.78	2.32	1.54	.76	2.67	2.59	.92	2.52	3.03	.83	1.98
	Min	64.08	-3.09	.88	58.66	-4.37	-2.43	59.07	-3.75	.45	62.59	-3.85	.72
	Max	74.60	-1.13	7.62	63.67	-1.97	6.56	67.03	-1.25	7.63	72.17	-1.20	6.81
(Listerine)	Mean	72.15	-2.11	3.67	61.03	-3.83	.01	62.11	-4.32	-2.72	80.52	-4.73	1.04
	SD	4.34	.69	4.19	2.33	.58	3.72	2.23	1.42	3.87	3.12	2.19	5.15
	Min	65.55	-3.12	-2.42	58.27	-5.14	-4.58	59.13	-8.26	-10.91	75.56	-10.85	-12.18
	Max	80.27	-1.19	12.22	64.94	-3.22	7.57	66.73	-3.12	2.65	85.79	-3.28	5.41
(Colgate Plax)	Mean	73.53	-2.56	2.89	60.47	-3.72	-.17	61.72	-2.83	.79	66.10	-2.79	1.85
	SD	3.41	.75	5.15	1.78	.52	2.68	2.02	.64	2.14	3.14	.80	2.76
	Min	67.72	-3.91	-2.24	57.13	-4.65	-3.18	58.23	-3.61	-1.64	61.76	-3.59	-1.34
	Max	78.12	-1.27	11.70	62.54	-3.10	3.57	64.41	-1.96	4.21	71.56	-1.26	6.69

The mean and standard deviation colour change (ΔE) of 5.07 \pm 4.35, 5.44 \pm 4.76, and 5.51 \pm 2.67 were observed for Oral-B, Listerine, and Colgate Plax, respectively when samples were immersed in artificial saliva. An evaluation of mean colour change by One-Way ANOVA did not show any significant differences ($F=0.034$, $p=0.967$). While samples immersed in the test group showed a

statistically significant difference in colour change (ΔE) ($p=0.048$). The details are presented in Figure 1.

Table 4 demonstrates univariate analysis indicated significant differences in color change values across different time intervals ($p<0.001$), oral rinse groups ($p<0.001$), and the interaction between timeline and groups ($p<0.001$).

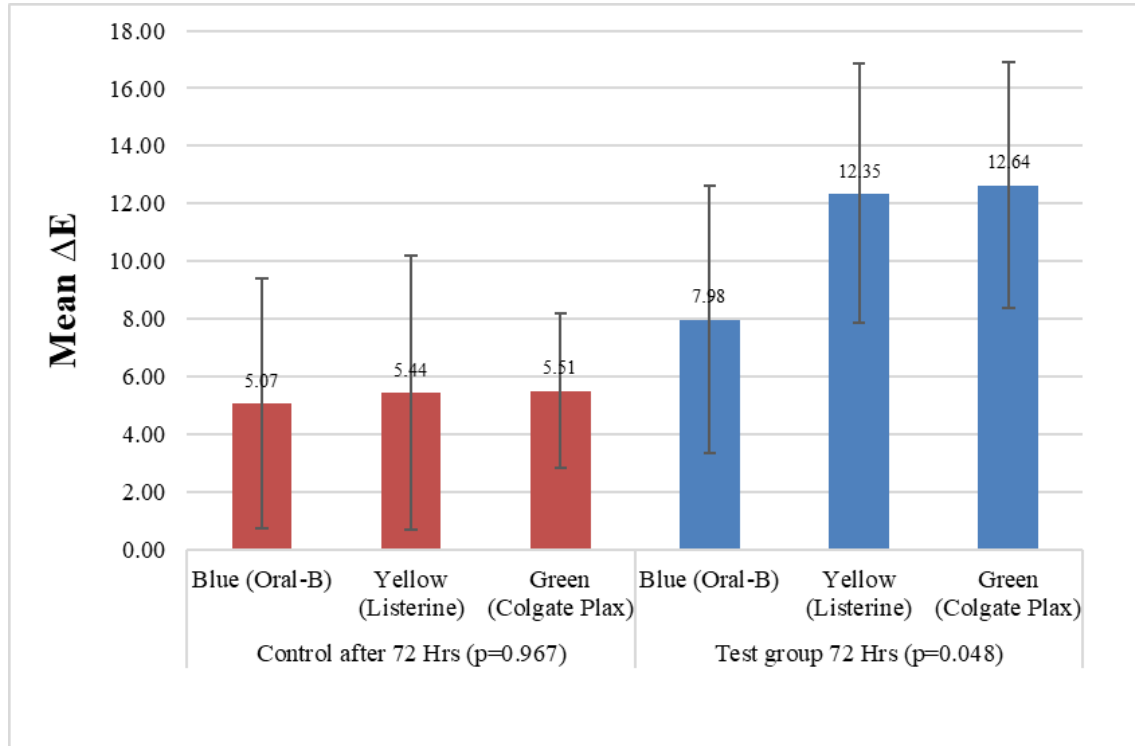


Figure 1. Comparison of mean colour change value (ΔE) in control and experimental groups after 72 h

Table 4. Model of univariate analysis

Source	Type III Sum of Squares	df	Mean Square	F	p
Corrected Model	4734.164	17	278.480	23.031	<0.001
Intercept	14566.143	1	14566.143	1204.648	<0.001
Time interval	1524.706	5	304.941	25.219	<0.001
Groups	1578.052	2	789.026	65.254	<0.001
Time interval* Groups	1631.407	10	163.141	13.492	<0.001
Error	1958.843	162	12.092		
Total	21259.151	180			
Corrected Total	6693.007	179			

The mean colour change of samples at 24 h (12.08 \pm 4.52) and 72 h (10.99 \pm 4.83) did not show any significant difference ($t=2.024$, $p=0.052$). While the mean colour change of samples at 24 h (12.08 \pm 4.52) and 7 d (8.34 \pm 4.36) showed a significant difference ($t=3.876$, $p=0.001$). Similarly, a mean colour change at 72 h (10.99 \pm 4.83) and 7 d (8.34 \pm 4.36) demonstrated a significant difference ($t=3.150$, $p=0.004$). The details are in Table 5.

Table 5. Pairwise comparison of colour change (ΔE) values at different time intervals

Color change at different intervals	Mean	N	SD	SEM	t	p
Pair 1	ΔE After 24 h	12.08	30.00	4.52	2.024	0.052
	ΔE After 72 h	10.99	30.00	4.83		
Pair 2	ΔE After 24 h	12.08	30.00	4.52	3.876	0.001
	ΔE After 7 d	8.34	30.00	4.36		
Pair 3	ΔE After 72 h	10.99	30.00	4.83	3.150	0.004
	ΔE After 7 d	8.34	30.00	4.36		

The paired sample t-test comparison of the study groups at different time points suggests that no significant difference was observed among the study groups in control at 72 h and among the study groups at 24 h. However, a significant difference was observed among the study groups at 72 h ($F=3.405$, $p=0.048$). Similarly, a significant difference was observed at 7 d ($p<0.05$). The details are in Figure 2.

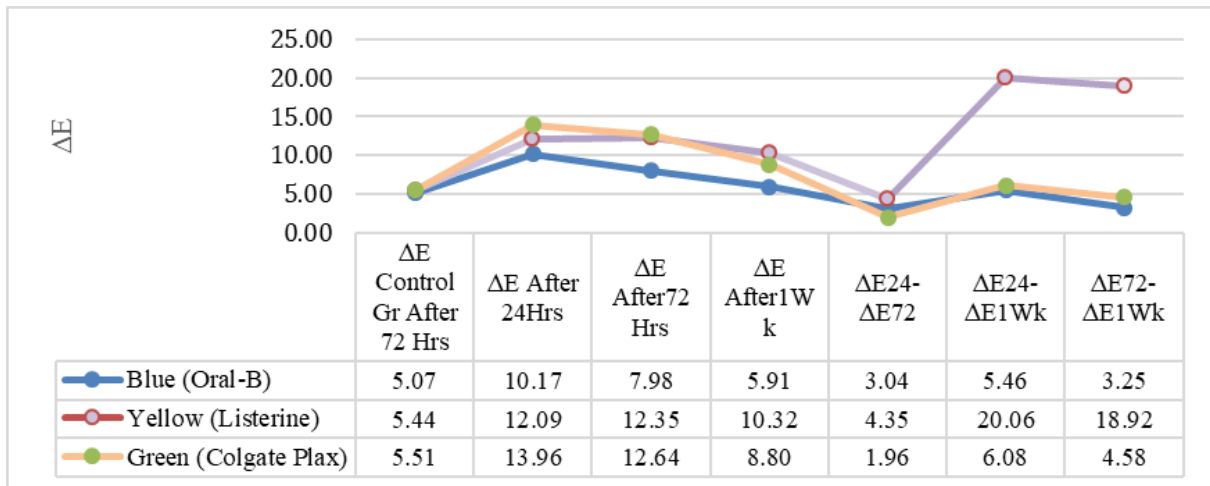


Figure 2. Mean colour change (ΔE) of samples exposed to various types of mouthwash at different time intervals

4. Discussion

The present study demonstrated that white spot lesions infiltrated with ICON are susceptible to staining when exposed to various colored mouth rinses considered in this study. Moreover, the study also found variations in ΔE values in different treatment subgroups over some time. Hence the null hypothesis of no change in color has been rejected.

The absence of studies on resin infiltrant's color stability in the presence of oral rinses has made direct comparison difficult. Hence, the resin infiltrant's color stability is compared with the composite resins after soaking in different colored oral rinses. This color shift seen in the resin-infiltrated specimen might be attributed to the reality that acrylic materials are susceptible to pigmentation even after acceptable polymerization and polishing. The resin formulation's composite structure, including the filler size and the type of photo-initiator directly impacts its staining susceptibility. Moreover, the chemical formulation of individual mouth rinses could have played an essential role in the specimen's staining. [19]

The resins infiltrated control (range 5.07 – 5.51) subgroups showed ΔE value higher than 3.3 units. These findings are similar to the previous studies conducted on the nano-hybrid composite in which ($\Delta E > 3.3$) clinically unacceptable color change was observed. [19,20] In this study, specimens in the control subgroup (artificial saliva) were measured at baseline and after 72 hours of immersion in artificial saliva. A color change value (ΔE) was recorded with no significant difference across all the control subgroups. It could be due to the infiltrant's post-polymerization reaction. [21]

Resins infiltrated treatment (range 7.98 – 12.64) subgroups on exposure to blue (Oral-B), yellow (Listerine), and green (Colgate Plax) colored oral rinses showed ΔE value well above the 3.3 units, representing an unacceptable color change in the treatment subgroups. This finding is in line with the study on the nano-hybrid composite. [22,23,24]

Treatment subgroups of blue (Oral-B), Listerine, and Colgate Plax resulted in clinically perceivable color changes after 24 h, 72 h, and 7 d. However, a statistically significant difference in color change value of the specimen was observed only after 72 hours after exposure to various oral rinses. After 24 h and 72 h immersion in green color (Colgate Plax) resulted in the highest color change values. However, at 7 d highest color change value was observed with yellow (Listerine) mouthwash. While color change value (ΔE) remained lowest with Oral B (Blue). The length of time a specimen is immersed in a test solution can affect its level of color change. [25] This study examined immersion periods of 24 h, 72 h, and 7 d. Therefore, this study's results cannot be used to speculate on color changes in longer or shorter periods. Additionally, comparing results from different immersion durations would be inaccurate, as color change levels vary over time.

Previous research has indicated that low pH and alcohol concentration in solutions may harm the surface integrity of resins, leading to discoloration. [26] This study found a statistically significant difference in color change values among the blue (Oral-B), yellow (Listerine), and green (Colgate Plax) alcohol-free mouth rinses, with a ΔE value above 3.3 units, which is visually noticeable.

Although the alcohol percentage (21.6%) and pH value (3.5) of Listerine have been reported to be relatively high, the color stability of resin materials was not influenced by this factor, and there was no substantial difference among the mouth rinses evaluated. Mouth rinses containing a high concentration of alcohol may soften the composite resin substance. Ethanol, in particular, softens BIS-GMA-based polymers. Others have shown that both alcohol-containing and alcohol-free mouth rinses might affect resin-restorative materials. [27]

The consequence of staining solutions on color changes of resins may be material-dependent, and a restorative material's staining sensitivity may be related to its resin matrix or filler type. [27] According to the current study results, although only a single resin infiltrant brand was used, statistically significant differences in color change

values were observed after immersion in various types of oral rinses after 72 h only. However, no such difference was evident at 24 h and 7 d of immersion.

Here it is notable that the degree of conversion after polymerization also plays a substantial role in the physical attributes of resins. It is proven that a low degree of conversion may result in higher solubility [28] and more water sorption, [29] resulting in higher staining potential. However, it was made sure that the infiltrant material used in this study was polymerized by following the manufacturer's recommendation with a calibrated high-power curing device to overcome this problem.

Moreover, the inclusion of TEGDMA (Triethylene glycol dimethacrylate) in the ICON composition can be ascribed to the alterations seen in the color resin penetrated specimens. Since it may go deep into the lesion, TEGDMA is an essential component of resin icon infiltration. [30] Because of its greater lesion penetration coefficient, resin mostly composed of TEGDMA is the preferred choice. [8] However, TEGDMA has the highest water sorption rate, resulting in resin discoloration. [31] Consequently, immersion in mouthwashes, caused Icon to become more discolored by imbibing the additional colorant from the mouthwash.

Moreover, discoloration may be associated with the amount of water sorption, since water serves as a carrier for colors to permeate deeply into the acrylic matrix. As a result, staining sensitivity correlated with water sorption rate [24]. Some research has advocated polishing the specimens to lessen the coloring impact to offset this effect [8]. However, the polishing could cause needless enamel wear instigated by abrasion. [32]

The staining potentials of several oral rinses for dental hard and soft tissues have been previously documented. [33] The staining capabilities of the oral rinses have also been examined on a variety of resin composites. Gürdal et al. showed that the consequences of oral rinses on colour stability are identical to those of distilled water. [34] Similarly, Lee et al. discovered that, while being visually imperceptible, oral rinses alter color stability. [35] The specimens examined in the present study demonstrated noticeable discoloration following immersion for varying periods of time. It is possible that the aqueous element present in the oral rinses could have influenced both the alteration in color and the changes in microhardness. [36] There were no significant differences between the oral rinses and distilled water except for Oral-B.

The limitations of this study include the selection of teeth having different mineral content, which may affect white spot lesions. Oral rinses' influence may vary on infiltrant materials in clinical scenarios due to a variety of characteristics that cannot be mimicked in vitro. The color stability of resin composites may be affected by saliva, salivary pellicle, meals, and drinks. Further, in vivo research is needed to establish the staining capability of various oral rinses. The staining potential of resin-infiltrated teeth inside the oral environment may be influenced by oral temperature and duration of exposure to colored oral rinses.

The present study demonstrated that white spot lesions infiltrated with ICON are susceptible to staining when exposed to various colored mouth rinses. Moreover, the study also found variations in ΔE values in different

treatment subgroups over some time. Hence the null hypothesis of no change in color has been rejected.

5. Conclusions

At 24 h time interval, Colgate Plax demonstrated the highest color change while the lowest was observed in Oral B Pro-Expert. Whereas, after 24 hours, 72 hours and 1 week Yellow (Listerine) oral rinse showed the highest color change, and the lowest values were observed with the Blue (Oral B) oral rinse. Therefore, the immersion of resin-infiltrated specimens in different oral rinses over different time intervals may exert detrimental effects on the color stability of the material. Future research should look at the impact of saliva, food colours, water, dental structure, or ions from the oral cavity, as well as the influence of infiltrator penetration depth on the color stability of resin infiltration during extended periods of immersion in oral rinses.

Funding Resources

This research did not receive any external fund.

Ethical Compliance

Research experiments conducted in this article with animals or humans were approved by the Ethical Committee and responsible authorities of our research organization(s) following all guidelines, regulations, legal, and ethical standards as required for humans or animals.

Conflicts of Interest

There are no conflicts to declare.

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