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Original Article

Investigation of the Color Change on Nano-Hybrid Composite Resin Caused by Non-Hydrogen Peroxide Whitening Mouthwashes

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CLINICAL SIGNIFICANCE

Non-hydrogen peroxide whitening mouthrinses can cause perceptible color changes on nano-hybrid composite restorations; clinicians should consider these effects when recommending them to patients with anterior restorations.

ABSTRACT

Objectives: This study aimed to examine the color change on nano-hybrid composite resin caused by non-peroxide whitening mouthrinses.

Materials and Methods: A nano-hybrid composite resin (shade A3) was used to prepare fifty disc-shaped specimens (8 mm in diameter and 2 mm in thickness) using a custom-made PTFE mold. Polymerization was performed with an LED light-curing unit according to the manufacturer's instructions, applying light for 20 seconds on each side. The specimens were then stored in distilled water at 37 °C for 24 hours. Subsequently, they were randomly assigned into five subgroups (n = 10): four experimental groups treated with whitening mouthrinses (Listerine Advanced White [LAW], Colgate Optic White [COW], Colgate Plax White Charcoal [CPWC], Splat White Plus [SWP]) and one control group (distilled water). Color measurements were performed at baseline (T0) and after a 4-week mouthrinse regimen (T1) using a spectrophotometer (VITA Easyshade Compact, VITA Zahnfabrik, Bad Säckingen, Germany) based on the CIELAB system. Color difference (ΔE00) and whiteness index difference (ΔWID) values were calculated.

Results: Significant differences were found among groups ($p<0.05$). All experimental groups exhibited perceptible color changes (Δ E 00>0.8), while LAW, COW, CPWC, and SWP exceeded the acceptability threshold (Δ E 00>1.8). SWP demonstrated the highest color and whiteness change, showing statistically significant differences from other groups ($p<0.05$).

Conclusion: Non-peroxide whitening mouthrinses caused clinically acceptable color changes on nano-hybrid composite resin. SWP, which contains bromelain, exhibited the highest whitening effect.

1. Introduction

Aesthetic teeth and a flawless smile have become one of the most common reasons for patients to visit dentists today. In response to this need, patients interest in tooth whitening has been increasing. Tooth whitening treatments have become a focus of interest for researchers, and as a result of studies, new whitening products are being introduced to the market.[1] Moreover, access to over-the-counter (OTC) whitening products has become widespread. There are various whitening products available on the market, including mouth rinses, toothpastes, dental floss, whitening strips, and chewing gums. Nowadays, patients can easily purchase these products from supermarkets, pharmacies, and online. Most of these products are inexpensive alternatives to tooth whitening treatments performed by dentists.[2]

One of the OTC products recommended by dentists is mouth rinses. These products are generally recommended for plaque control and gum health. Recently, whitening mouth rinses containing different ratios of hydrogen peroxide or various chemicals have been introduced to the market.[3] Whitening mouth rinses are produced to prevent tooth stains and quickly whiten teeth. They have become one of the most popular OTC products due to their ease of use by patients and low cost.[4] Some whitening mouth rinses do not contain hydrogen or carbamide peroxide as active ingredients.[5] Although most studies have focused on whitening mouth rinses containing hydrogen peroxide, their whitening effects are significantly lower compared to professional whitening treatments.[6] Mouth rinses containing hydrogen peroxide pose potential risks due to their low pH and uncontrolled application in the mouth. Cytogenetic damage, chemical burns on the gums, and dentin erosion have been considered potential side effects of hydrogen peroxide-containing mouth rinses.[7-10]

Alternatively, whitening mouth rinses without hydrogen peroxide have been developed using ingredients such as sodium hexametaphosphate, tetrasodium pyrophosphate, and phthalimidoperoxycaproic acid.[9,11,12] Additionally, there are whitening mouth rinses on the market containing bromelain, a natural component derived from pineapple, and activated charcoal, although research on these products remains scarce.

The agents used during whitening can affect not only the teeth but also existing restorations, causing color changes.[13] Due to their organic matrix, resin composites are more susceptible to chemical changes caused by the acidic content of whitening agents compared to other restorative materials.[14] Over time, intrinsic and extrinsic discoloration can occur in composite restorations. Intrinsic discoloration includes changes that can occur in the resin structure, resin matrix, and the interface of filler particles.[15] Extrinsic discoloration, on the other hand, occurs due to adsorption or absorption of exogenous colorants such as coffee, tea, nicotine, and beverages.[16,17] This discoloration in composite restorations may cause color mismatches and compromise aesthetics, leading to the replacement of time-consuming and costly restorations.[18] Surface stains in composite resin restorations can be removed by techniques such as polishing, brushing, and whitening.[19] For these reasons, the effects of whitening agents on resin composites need to be fully understood. Among whitening techniques, mouth rinses are among the most popular oral hygiene and tooth whitening products due to their ease of use, low cost, and accessibility.[18] Most studies have tested the color change on stained composite restorations using hydrogen peroxide-containing whitening mouth rinses. However, the literature reveals insufficient information regarding the effects of hydrogen peroxide-free mouth rinses on unstained composite resins. Therefore, this study aimed to investigate the color change on nano-hybrid composite

resin caused by hydrogen peroxide-free whitening mouth rinses developed to avoid the harmful effects of hydrogen peroxide used in whitening.

2. Materials and Methods

2.1. Sample Preparation

A nano-hybrid composite resin (Clearfil Performance Pro, Kuraray Noritake Dental, Okayama, Japan) in shade A3 was used. Fifty disc-shaped specimens were prepared using a custom-made polytetrafluoroethylene (PTFE) mold with a diameter of 8 mm and a thickness of 2 mm. For the polymerization of the composite specimens, an LED light-curing device (VALO Cordless LED, Ultradent, South Jordan, UT, USA) was applied perpendicularly to both the top and bottom surfaces for 20 seconds, following the manufacturer's instructions. The prepared specimens were stored in distilled water at 37°C for the first 24 hours. The composite resin specimens were divided into five subgroups (n=10): four groups for different whitening mouth rinses and one control group using distilled water. The mouth rinses used in the study were as follows: Listerine Advanced White (LAW), Colgate Optic White (COW), Colgate Plax White Charcoal (CPWC), and Splat White Plus (SWP). The ingredients and details of the mouth rinses and restorative materials used in the study are presented in Table 1. After the composite resin specimens were divided into groups, each specimen in all groups was numbered from 1 to 10.

2.2. Color Measurement

The color values of each specimen were measured using a spectrophotometer (VITA Easyshade Compact, VITA Zahnfabrik, Bad Säckingen, Germany) with the Commission Internationale de l'Eclairage (CIE) L*a*b* system. The spectrophotometer was calibrated before each measurement according to the manufacturer's instructions. CIE L*a*b* values were recorded, where L* indicates lightness from white to black (100–0), a* represents the red-green chromatic coordinates, and b* represents the yellow-blue chromatic coordinates. The color and whiteness changes of the specimens were calculated according to CIEDE2000 (ΔE_{00}) [20] and the Whiteness Index for Dentistry (WI_D) [21], respectively. Three readings were obtained for each specimen, and the average was calculated. For the ΔE_{00} index, the clinically acceptable value is 1.8, while the perceptible value is 0.8. For the WI_D index, these values are 2.6 and 0.7, respectively.[22]

$$\Delta E_{00} = [(\Delta L'/K_L S_L)^2 + (\Delta C'/K_C S_C)^2 + (\Delta H'/K_H S_H)^2 + R_T * (\Delta C'/K_C S_C) * (\Delta H'/K_H S_H)]^{1/2}$$

$$WI_D = 0.511\ L^* - 2.324\ a^* - 1.100\ b^*$$

$$\Delta WI_D: WI_D T1 - WI_D T0$$

In the CIEDE2000 (ΔE_{00}) equation, ΔL′, ΔC′, and ΔH′ refer to the differences in lightness, chroma, and hue measurements before and after the procedure, respectively. SL, SC, and SH are weighting functions added to the formula to correct irregularities observed in the CIELAB system. kL, kC, and kH are parametric factors correcting for experimental conditions. RT (rotation function) determines the interaction between chroma and hue changes in the blue region. In the whiteness index (WI_D) equation for dentistry, higher WI_D values indicate whiter specimens, while lower values (including negative) indicate darker specimens. Measurements were taken at baseline (T0) and after the 4-week mouth rinse cycle procedure (T1).

2.3. Mouthwashes Cycle Procedure

After the initial measurements (T0) were completed, each composite group was immersed in 25 mL of the respective mouth rinse. The specimens were shaken continuously for 60 seconds and then rinsed under running water for 30 seconds. Subsequently, they were placed in a refreshed mouth rinse solution for another 60 seconds. This procedure represented the twice-daily use of mouth rinses for 60 seconds.23 The procedure was repeated seven times to simulate one week of use. Afterwards, the specimens were stored in distilled water for one day. The same procedure was repeated four times to simulate four weeks of use. Specimens were always kept in distilled water until the next experimental cycle, and upon completion of this stage, color measurements were repeated (T1).

2.4. Statistical Analysis

Descriptive statistics (number, percentage, mean, standard deviation, median, minimum, and maximum) were presented. The assumption of normal distribution was checked with the Shapiro-Wilk test, and the homogeneity of variances was tested with the Levene test. For the comparison of three or more independent groups with a normal distribution, the ANOVA test was used. Post Hoc Bonferroni tests were performed to determine which group or groups caused the differences. Analyses were conducted using IBM SPSS 27 software. The level of statistical significance was set at $p < 0.05$.

Table 1. Chemical compositions and manufacturers of the mouth rinses and restorative materials used in the study

Product	Manufacturer	Components
Listerine Advanced White Mouth Rinse	Johnson & Johnson, Skillman, NJ, USA	Water, Alcohol, Sorbitol, Tetrapotassium Pyrophosphate, Pentasodium Triphosphate, Citric Acid, Poloxamer 407, Flavors, Sodium Saccharin, Sucralose, Sodium Fluoride, Sodium Benzoate, Tetrasodium Pyrophosphate, Menthol, Eucalyptol, Thymol, Flavor, Propylene Glycol, Disodium Phosphate
Colgate Plax White And Charcoal Mouth Rinse	Colgate-Palmolive, Guildford, GU2 8JZ	Water, Glycerin, Propylene Glycol, Sorbitol, Tetrapotassium Pyrophosphate, Polysorbate 20, Tetrasodium Pyrophosphate, Zinc Citrate, PVM/MA Copolymer, Flavor, Benzyl Alcohol, Sodium Fluoride, Sodium Saccharin, Bambusa Vulgaris Shoot Extract, Charcoal Powder, CI 15510, CI 17200, CI 19140, CI 42051
Colgate Optic White Mouth Rinse	GABA International AG, Therwil, Switzerland	Water, Glycerin, Sorbitol, Propylene Glycol, PVM/MA Copolymer, Tetrapotassium Pyrophosphate, Polysorbate 20, Sodium Fluoride, Sodium Saccharin, CI 42051
Splat White Plus Mouth Rinse	Splat Global, Russia	Aqua, Hydrogenated Starch Hydrolysate, PVP, Polyglyceryl-4 Laurate/Sebacate, Polyglyceryl-6 Caprylate/Caprate, Sodium Coco-Sulfate, Flavor, Cyclodextrin, Zinc Gluconate, Citrus Limon Peel Oil, Ananas Sativus Fruit Extract, Maltodextrin, Thymus Serpillum Oil, Glycyrrhiza Glabra Root Extract, Stevia Rebaudiana Leaf Extract, Glycerin, Pentylene Glycol, Bifida Ferment Lysate, Phthalimidoperoxycaproic Acid, Potassium Thiocyanate, Lactoferrin, Lactoperoxidase, Glucose Oxidase, Glucose Pentaacetate, Sodium Benzoate, Potassium Sorbate, Benzyl Alcohol, Citric Acid, Limonene, Citral, Linalool
Clearfil Performance Pro Composite	Kuraray Noritake Dental, Okayama, Japan	Silanated barium glass (30–80%), Pre-polymerized organic filler (≤40%), Bisphenol A diglycidyl methacrylate (5–15%), Hydrophobic aromatic dimethacrylate (5–15%), Silanated colloidal silica (1–5%), Hydrophobic aliphatic dimethacrylate (1–5%), dl-Camphorquinone (≤0.1%)

Table 2. Distribution and comparison of Δ WI $_D$ and Δ E_{00} measurements according to study groups

		Min.-Max.	Mean±S.D.	Median	Test Statistic	p
ΔWI_D	Distilled Water	-4.64/3.29	-0.98±2.57	-0.41	11.736	**<0.001***
	CPWC	-9.38/-0.05	-4.43±3.28	-3.40		
	LAW	-9.53/11.86	2.28±6.34	1.93		
	COW	-1.85/8.72	2.05±3.35	1.12		
	SWP	1.65/13.11	7.14±3.10	6.58		
ΔE_{00}	Distilled Water	0.50/2.11	1.31±0.50	1.28	3.535	**0.014***
	CPWC	1.06/4.27	2.23±0.96	1.99		
	LAW	0.83/3.99	2.15±1.09	2.08		
	COW	0.70/4.16	2.20±1.06	1.95		
	SWP	1.76/5.48	2.94±1.12	2.72		

*p<0.05

3. Results

The mean ΔE_{00} and ΔWI_D distributions and their comparisons obtained as a result of this study are shown in Table 2. The distributions of ΔWI_D and ΔE_{00} measurements according to the study groups are presented in the table, and ANOVA tests were used for comparisons. As a result of the analyses, statistically significant differences were found among the groups regarding ΔWI_D and ΔE_{00} measurements (p<0.05).

According to the Bonferroni tests conducted for ΔWI_D, statistically significant differences were observed between Distilled Water and SWP (p<0.001) and between CPWC and LAW, COW, and SWP (p=0.004, p=0.007, and p<0.001, respectively). The SWP measurements were higher than CPWC measurements. LAW, COW, and SWP measurements were higher than distilled water measurements.

According to the Bonferroni tests conducted for ΔE_{00}, statistically significant differences were determined between Distilled Water and SWP (p=0.005). The SWP measurements were higher than the distilled water measurements.

4. Discussion

Various color systems, such as CIE L*a*b* (ΔEab) and CIEDE2000 (ΔE_{00}), are commonly used in the analysis of color changes. However, studies have indicated that the CIEDE2000 color system is superior to the CIE Lab* system in determining perceptibility and acceptability.[24,22] Therefore, in our study, the CIEDE2000 (ΔE_{00}) color system and formula were used for analyzing color changes.

When the literature is reviewed, various opinions are found regarding the perceptibility and acceptability of color changes in restorations.[25] A recent study defined the perceptibility threshold of the ΔE 00 index as 0.8 and the acceptability threshold as 1.8.[22] When the ΔE 00 values obtained from our study were evaluated, it was observed that all groups were above the perceptibility threshold (ΔE 00>0.8), and effective whitening was achieved in all groups except the control group (ΔE 00>1.8). Therefore, hydrogen peroxide-free whitening mouth rinses provided acceptable color changes on nano-hybrid composite resin.

Formulas such as ΔEab and ΔE_{00} used to analyze color differences after tooth whitening do not provide sufficient information about changes in tooth whiteness.[26,21,27] In this study, ΔWI_D, based on CIE Lab* coordinates, was used to evaluate changes in whiteness. A previous study defined the perceptibility threshold of the ΔWI_D index as 0.7 and the acceptability threshold as 2.6.[22] According to our findings, all groups except the control group met the perceptibility threshold, while only the CPWC and SWP groups met the acceptability threshold. Statistically, the SWP group was superior to the CPWC group. This may be attributed to the whitening effect of bromelain, a proteolytic enzyme derived from from pineapple. This enzyme breaks down organic compounds responsible for extrinsic stains,[28] thereby allowing light to reflect more easily from the surface and producing a brighter appearance.[29]

Hydrogen peroxide is the most commonly used whitening agent both in clinical and at-home treatments.[30,31] This agent is a strong oxidizer that facilitates whitening by breaking long-chain organic pigment molecules into shorter-chain compounds.[32] However, due to its short shelf life and safety limitations, its use in mouth rinses is problematic. The whitening effects of the mouth rinses used in our study may be attributed to the active charcoal in CPWC, tetrasodium pyrophosphate in LAW, Patent Blue V (color index 42,051) providing optical properties in COW, and bromelain in SWP. Acceptable color change was observed in all groups. The nanoporous structure of activated charcoal provides it with adsorptive properties, allowing it to absorb extrinsic stains on the composite surface and alter tooth color.[33] A previous study observed that toothpastes containing activated charcoal had higher whitening effects,[34] which is consistent with our findings. However, studies on charcoal-containing whitening mouth rinses remain limited. The whiteness change in the LAW group may be attributed to tetrasodium pyrophosphate, which removes extrinsic stains from the surface.[35] The superiority of the SWP group in terms of color change may result from bromelain, which breaks down oxidized protein molecules forming extrinsic stains. In addition, the COW group may have had an acceptable effect in whitening because it contained pyrophosphate like the LAW group.

Chemical agents used during whitening not only affect teeth but may also influence existing restorations, potentially causing color changes due to their organic matrix content. Whitening mouth rinses have become increasingly popular because of their low cost and easy accessibility. Therefore, it is necessary to evaluate their effects not only on teeth but also on composite restorations. Most studies have examined whitening mouth rinses on artificially stained composites, leading to controversial results.[36,37] However, such studies can only evaluate the ability of whitening mouth rinses to remove surface stains. In our study, artificial staining was not performed in order to accurately evaluate the real whitening effects of whitening mouth rinses. In studies examining the color changes of composites, the ΔE_{00} index was generally found to be below the acceptable threshold.[38,39] In contrast, in our study, all groups except the control group were above the acceptable threshold. This may be due to the amount of composite matrix, the degree of polymerization of the resin matrix, and the different active ingredients in the whitening products.

A recent study reported that hydrogen peroxide- and carbamide peroxide-containing whitening mouth rinses caused color changes in composite resins.[40] In contrast to this result, our study showed that even though our groups did not contain peroxide, they

produced acceptable color differences. This suggests that active charcoal, bromelain derived from pineapple, and pyrophosphate derivatives may provide whitening effects on composites comparable to those of peroxides.

In our study, the mouth rinse cycle procedure was applied according to the manufacturers' recommendations. To simulate daily mouth rinse use, specimens were continuously shaken in 25 mL of mouth rinse for 60 seconds, rinsed under running water for 30 seconds, and then shaken again for 60 seconds. Manufacturers' usage instructions should inform consumers about the importance of adhering to the recommended frequency and application duration of products, as consumers may tend to use whitening products more frequently and for longer durations. Due to the harmful effects of long-term use of mouthwashes, dentists should prescribe teeth whitening products.

As with every in vitro study, our study also has limitations. The current study has limitations such as not being able to exactly mimic the real oral environment and the lack of a cleansing effect of saliva. To better analyze the effects of whitening mouth rinses, more studies should be conducted under clinical conditions. Furthermore, with the increasing interest in whitening, new whitening methods will be developed, and future research should address these factors.

5. Conclusion

The results obtained within the limits of this in vitro study are as follows: Non-hydrogen peroxide whitening mouthrinses caused perceptible and, in most cases, clinically acceptable color changes on nano-hybrid composite resin after a 4-week simulation. Four products (LAW, COW, CPWC, SWP) exceeded the acceptability threshold, with SWP producing the greatest change in both ΔE_{00} and ΔWI_D. Clinicians should consider these potential effects when recommending such products to patients with anterior composite restorations, particularly when long-term esthetic stability is important.

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CRediT Author Statement

Elif Varlı Tekingür contributed to the study design, analysis, and interpretation and drafted, critically revised the manuscript, and approved the final version of the manuscript. Emin Alper Özer contributed to the study design.

Conflict of Interest

The author has stated explicitly that there are no conflict of interests in connection with this article.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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