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RESEARCH ARTICLE

Use of Resin Infiltrant to Prevent Discoloration after Teeth Whitening

Ji-Hyun Min†

Department of Dental Hygiene, College of Medical and Health Sciences, Cheongju University, Cheongju 28503, Korea

Background: This study attempted to apply resin infiltrant (RI) as a method to maintain the effect of tooth bleaching treatment and compared it with fluoride varnish (FV) or artificial saliva to evaluate the effect.

Methods: Sixty healthy lozenge specimens were classified into five groups. Group 1 was the negative control group, and discoloration was induced after artificial saliva treatment of the tooth specimen ($G1_{S+C}$). Group 2 was a positive control group, in which pigmentation was induced after bleaching treatment and artificial saliva treatment ($G2_{B+S+C}$). Coloration was induced in group 3 (experimental group 1) after bleaching treatment and artificial saliva treatment, followed by application of fluorine varnish ($G3_{B+FV+S+C}$). Coloration was induced in Group 4 (experimental group 2) after applying RI after bleaching treatment and artificial saliva treatment ($G4_{B+RI+S+C}$). Pigmentation was induced in group 5 (experimental group 3) after bleaching treatment and artificial saliva treatment, followed by acid treatment (etching) and treatment with RI ($G5_{B+E+RI+S+C}$). Coffee and wine were used to induce discoloration. The lightness value (L*) of the CIE L*a*b* color system was obtained by image analysis. Kruskal-Wallis H analysis was performed for the mean difference in L* values by group.

Results: When coloration was induced with coffee, there was no significant difference in L* value between artificial saliva ($G2_{B+S+C}$), FV ($G3_{B+FV+S+C}$), and RI ($G4_{B+RI+S+C}$, $G5_{B+E+RI+S+C}$) groups. There was no significant difference in L* values between the artificial saliva ($G2_{B+S+C}$), FV ($G3_{B+FV+S+C}$), and RI ($G4_{B+RI+S+C}$, $G5_{B+E+RI+S+C}$) groups, even in the case of wine induced coloration.

Conclusion: It was confirmed that artificial saliva or RI treatment had similar effects to the FV previously used to maintain the effect of tooth bleaching treatment.

Key Words: Artificial saliva, Fluoride varnish, Penetrating resin, Tooth bleaching, Tooth discoloration

Introduction

1. Background

Most people perceive brightly colored teeth as more beautiful, and tooth color is associated with self-appearance satisfaction[1,2]. Tooth bleaching can be performed to achieve a bright tooth color. Tooth bleaching brightens the color of teeth by chemically decomposing the chromogen that causes tooth coloration. A chromogen source is a generic term for a precursor of this pigment when the color of an organism is formed and it has a double bond in its chemical structure[3]. The materials most used as tooth-whitening agents are hydrogen peroxide (H_2O_2) or carbamide peroxide ($CH_6N_2O_3$)[3]. Hydrogen peroxide decomposes in an aqueous solution to produce hydroxyl radicals such as −OH and −OOH−, which chemically react well and oxidize organic molecules that cause coloration, thereby exhibiting bleaching action. Carbamide peroxide is produced secondary to hydrogen peroxide and it has a tooth-whitening effect through the process described above.

According to a previous study, it was found that enamel permeability increases when hydrogen peroxide is applied to dental enamel[4]. Therefore, the effectiveness of tooth whitening is not permanent and it may decrease. Tooth

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†Correspondence to: Ji-Hyun Min, *https://orcid.org/0000-0001-5177-7600*
Department of Dental Hygiene, College of Medical and Health Sciences, Cheongju University, 298 Daesung-ro, Cheongwon-gu, Cheongju 28503, Korea
Tel: +82-43-229-8375, Fax: +82-43-229-8969, E-mail: jhmin@cju.ac.kr

color returning to the same color as before whitening treatment may be caused by daily activities, such as smoking, drinking coffee, red wine, or drinking dark liquids[5,6]. Therefore, it is necessary to find a way to solve the discoloration that prevents whitening from continuing after tooth bleaching treatment. Previous studies have explored ways to maintain the whitening effect by applying minerals such as fluoride or calcium phosphate after tooth bleaching treatment[7-9]. However, there is still a lack of diversity in the treatment substances, and the number of studies to determine its effectiveness is insufficient.

On the other hand, resin infiltrant (RI) is a resin material with excellent flowability that was developed to prevent the progression of caries by penetrating carious lesions in the early stage. When RI is applied to dental caries lesions in the early stage, the tooth surface with high mineral concentration is removed, and sulfuric acid is applied for a short time so that RI can penetrate the lower part where there is a lot of mineral loss to remove the tooth surface tissue. Thereafter, RI penetrates through the capillary phenomenon into the pores of the early carious lesion formed by mineral loss, and it has acid resistance, slowing the progression of possible early carious lesions and improving esthetics[5,6].

2. Objectives

It was expected that if such an RI was applied to the tooth surface, whose permeability was increased by tooth whitening, the permeability of the tooth would be reduced, and this in turn would prevent tooth discoloration. Therefore, in this study, we tried to apply RI as a method to maintain the effect of tooth whitening treatment, and to evaluate the effect by comparing it with fluoride varnish (FV) or artificial saliva.

Materials and Methods

1. Manufacture of tooth specimens

Sound permanent bovine teeth without dental caries or cracks were cut into 4×3×5 mm pieces using a low-speed handpiece (Lasungmedice, Incheon, Korea) and a diamond disk (NTI-Kahla, Kahla, Germany). They were molded with a self curing resin (Vertex, Zeist, Netherlands) to expose the enamel. The specimens were ground stepwise using fine silicon carbide paper (Allied High Tech Products, Rancho Dominguez, CA, USA) initially and then they were finally polished using 800 grit silicon carbide paper. Half of the surface of the specimen (2×3 mm) was coated with acid-resistant varnish to prevent coloring and other treatments from being applied to preserve the sound tooth area, and the remaining half of the surface of the specimen was exposed. Thus, a total of 60 specimens were prepared in this study.

2. Research materials

Artificial saliva contained gastric mucin (0.22%; Sigma-Aldrich, Saint Louis, MO, USA), KH_2PO_4 (5.42 mM), NaCl (6.51 mM), KCl (14.93 mM), and $CaCl_2 \cdot 2H_2O$ (1.45 mM; Junsei Chemical, Tokyo, Japan) at pH 6.8. The tooth whitening agent was a self-treatment product (Opalescence; Ultradent, USA) containing 15% carbamide peroxide. The solutions for inducing coloration were coffee (5 g coffee powder/200 ml distilled water; Nescafe original, USA) and red wine (Nuevo mundo, Los Lagos, Chile). RI and HCl were used in an Icon® kit (DMG Co., Hamburg, Germany). FV was a product containing 5% sodium fluoride and casein phosphopeptide-amorphous calcium phosphate (MI varnish™; GC Corp., Tokyo, Japan).

3. Classification of experimental groups

This study sample was divided into the following five groups. Group 1 was induced with discoloration after

Table 1. Group Composition of this Study

	Group	Treatment method
1	Negative control group ($G1_{S+C}$)	Artificial saliva+coloration
2	Positive control group ($G2_{B+S+C}$)	Bleaching+artificial saliva+coloration
3	Experimental group 1 ($G3_{B+FV+S+C}$)	Bleaching+FV+artificial saliva+coloration
4	Experimental group 2 ($G4_{B+RI+S+C}$)	Bleaching+RI+artificial saliva+coloration
5	Experimental group 3 ($G5_{B+E+RI+S+C}$)	Bleaching+etching+RI+artificial saliva+coloration

FV: fluoride varnish, RI: resin infiltrant.

artificial saliva treatment of the tooth specimens ($G1_{S+C}$). Pigmentation was induced in Group 2 after whitening treatment and artificial saliva treatment ($G2_{B+S+C}$). Pigmentation was induced in Group 3 after FV application which was done after whitening treatment and artificial saliva treatment ($G3_{B+FV+S+C}$). Coloration was induced in Group 4 after applying RI post whitening treatment and artificial saliva treatment ($G4_{B+RI+S+C}$). Pigmentation was induced in Group 5 after whitening treatment and artificial saliva treatment, followed by acid treatment (etching) and treatment with RI ($G5_{B+E+RI+S+C}$) (Table 1).

4. Study design to confirm the anti-pigmentation effect according to the treatment material

Table 2 lists the treatment methods and order in each group. The tooth specimens in all groups were washed with ultrasonic waves for 60 minutes and then stored in artificial saliva for 3 hours. Then, for 14 days, the dental specimens of Group 1 were immersed in artificial saliva, and the dental specimens of Groups 2～5 were subjected to tooth whitening treatment for 6 hours a day and then immersed in artificial saliva for the rest of the time. On the 15th day, the first image of the specimen surface was obtained accordingly.

Subsequently, the dental specimens of group 1 were continuously immersed in artificial saliva, and those of groups 2～5 were treated with artificial saliva, FV application, RI treatment, and acid erosion after teeth whitening for 6 hours a day, respectively. Secondary images of the tooth specimen surfaces were obtained thereafter. After immersion in artificial saliva for 18 hours, the specimens of each group were divided in half. One half of each of the groups was immersed in coffee solution while the other half was immersed in red wine for 6 hours a day to induce coloration. It was then immersed in artificial saliva, except for the induction time of coloring, and this process was repeated for nine days. At this point in time, the third pictures of the surfaces of the tooth specimens were taken accordingly.

In the case of the above treatment, all treatments except for etching in group 5 were performed in an incubator at 37°C. Whitening treatment was performed for 6 hours a day based on the manufacturer's recommendation of 4～6 hours[10]. A whitening agent was applied such that the height from the tooth surface was 1 mm.

5. Color analysis of images

A digital image of the tooth specimen surface was obtained using a quantitative light-induced fluorescence digital (QLF-Digital2+ Biluminator™; Inspektor Research Systems BV, Amsterdam, Netherlands). The image shooting conditions were a white light on, shutter speed of 1/45 seconds, aperture value of 13.0, and ISO 1600. Digital images were again obtained after treatment with anti-pigmentation materials such as FV and RI.

After immersion in artificial saliva for 18 hours, half of

Table 2. Treatment Schedule and Method by Group

<table>
<tr><th>$G1_{S+C}$</th><th colspan="4">$G2_{B+S+C}$ / $G3_{B+FV+S+C}$ / $G4_{B+RI+S+C}$ / $G5_{B+E+RI+S+C}$</th></tr>
<tr><td colspan="5">Ultrasonic 60-minute cleaning, 3 hours immersion in artificial saliva</td></tr>
<tr><td rowspan="2">Immersion in artificial saliva</td><td colspan="4">Teeth whitening treatment for 6 hours,</td></tr>
<tr><td colspan="4">Immerse in artificial saliva other than teeth whitening treatment time</td></tr>
<tr><td colspan="5">(The above process was repeated for 14 days) Taking pictures of the surface of the tooth specimen (primary color measurement)</td></tr>
<tr><td rowspan="2">Immersion in artificial saliva</td><td colspan="4">Teeth whitening treatment for 6 hours,</td></tr>
<tr><td>Immersion in artificial saliva</td><td>FV application</td><td>RI treatment</td><td>RI treatment after etching</td></tr>
<tr><td colspan="5">Taking pictures of the surface of the tooth specimen (secondary color measurement)</td></tr>
<tr><td colspan="5">Immersion in artificial saliva for 18 hours</td></tr>
<tr><td colspan="5">Half of the specimens in each group were immersed in coffee solution and in red wine for 6 hours, respectively, and colored. Immerse in artificial saliva at times other than coloring. This process was repeated for 9 days. Taking pictures of the surface of the tooth specimen (third color measurement).</td></tr>
<tr><td colspan="5">CIE L*a*b* analysis using image analysis program</td></tr>
</table>

FV: fluoride varnish, RI: resin infiltrant.

the specimens (6 pieces) of each group were immersed in coffee solution and red wine for 3 hours a day, and for the remaining time they were stored in artificial saliva to induce coloration for a total of 9 days. Digital images were then acquired again. RGB values were analyzed from the acquired digital images using an image analysis program (Image-Pro Premier; Media Cybernetics, Media Cybernetics, Warrendale, PA, USA). The RGB values were converted to the CIE L*a*b* color system. The lightness (L*) value indicates lightness and ranges from 0 (black) to 100 (white). a* indicates that negative values are closer to green, and positive values are closer to magenta. b* indicates that negative values are closer to blue and positive values are closer to yellow. The color difference (ΔE) between two objects was calculated using the following equation:

$$\Delta E = \sqrt{\triangle L^{*2} + \triangle a^{*2} + \triangle b^{*2}}$$

6. Scanning electron microscope

Two specimens were randomly selected for each coloration induction group of coffee and wine, the teeth were dried, and the surface was coated with platinum and observed 1,000 times with a field emission scanning electron microscope (CX-200TA; COXEM, Daejeon, Korea).

7. Statistical analysis

The normality of all data was confirmed using the Shapiro-Wilk normality test. Kruskal-Wallis H analysis was performed for the mean difference in L* values by group. When post-hoc analysis was required, the Mann-Whitney U test was performed accordingly. Repeated

Table 3. Comparison of the Mean L* Values or Coffee-Stained Tooth Specimens

Group	n	After 14 days of bleaching treatment	After anti-pigmentation treatment	After coloring treatment	p-value†
$G1_{S+C}$*	6	-	70.97 ± 1.40^{A}	55.97 ± 8.28^{aB}	0.010
$G2_{B+S+C}$	5	71.99 ± 1.80^{A}	70.72 ± 2.60^{A}	66.87 ± 1.90^{bB}	<0.001
$G3_{B+FV+S+C}$	4	72.04 ± 1.37^{A}	69.80 ± 1.63^{A}	63.19 ± 6.10^{bB}	<0.001
$G4_{B+RI+S+C}$	4	71.18 ± 0.24^{A}	70.99 ± 0.83^{A}	64.39 ± 2.70^{bB}	<0.001
$G5_{B+E+RI+S+C}$	5	71.72 ± 1.11^{A}	71.86 ± 0.64^{A}	64.56 ± 3.22^{bB}	<0.001
p-value††		0.629	0.389	0.026	

*In group 1, as a negative control group, whitening treatment was not performed and only artificial saliva immersion and coloring treatment were performed.
†p-value obtained by performing repeated measured ANOVA analysis.
††p-value obtained by performing Kruskal-Wallis H analysis.
a, b shows the differences in L* values etween the groups from 1 to 5.
A, B represent the differences in L values after bleaching, after treatment, and after discoloration.

Table 4. Comparison of the Mean L* Values or Wine-Stained Tooth Specimens

Group	n	After 14 days of bleaching treatment	After anti-pigmentation treatment	After coloring treatment	p-value†
$G1_{S+C}$*	6	-	71.42 ± 1.94^{A}	43.2 ± 6.90^{B}	0.002
$G2_{B+S+C}$	5	72.33 ± 1.07^{A}	70.21 ± 1.90^{B}	41.4 ± 5.60^{C}	<0.001
$G3_{B+FV+S+C}$	4	72.53 ± 1.72^{A}	71.62 ± 1.16^{A}	39.3 ± 7.70^{B}	<0.001
$G4_{B+RI+S+C}$	7	71.84 ± 0.64^{A}	71.41 ± 0.47^{A}	39.4 ± 10.40^{B}	<0.001
$G5_{B+E+RI+S+C}$	7	72.50 ± 1.20^{A}	71.61 ± 0.81^{A}	30.7 ± 10.00^{B}	<0.001
p-value††		0.498	0.635	0.166	

*In group 1, as a negative control group, whitening treatment was notperformed and only artificial saliva immersion and coloring treatment were performed.
†p-value obtained by performing repeated measured ANOVA analysis.
††p-value obtained by performing Kruskal-Wallis H analysis.
A~C represent the difference in L values after bleaching, treatment, and discoloration, respectively.

measures ANOVA analysis was performed to determine whether there was a change in the L* value after 14 days of treatment for each group, after treatment with an anti-pigmentation material, and after coloring. The average difference in the L* values according to the type of colorant in coffee and wine was confirmed using a paired t-test. All statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA).

Results

After 14 days of whitening, there was no significant difference in the L* values between the groups. There was no significant difference between the groups in the L* value after treatment with the anti-pigmentation material. In all groups, significant differences were found between the L* values according to the time of whitening treatment, anti-pigmentation material treatment, and coffee or wine coloration induction for 14 days ($p < 0.05$).

There was no significant difference in the L* values between the groups, even when wine staining was induced ($p > 0.05$, Table 3, 4). However, the L*value of $G1_{S+C}$ was significantly lower than that of the other groups during coffee coloring induction (p=0.026, Table 3). The L* values of the teeth induced with wine were significantly lower than those of the teeth induced with coffee ($p < 0.001$).

When inducing coffee coloration, the ΔE of $G1_{S+C}$, which had never been subjected to whitening treatment, was the most significant (p=0.030). However, in the induction of wine staining, the $G5_{B+E+RI+S+C}$ group, which was treated with acid and then RI, showed the largest ΔE on average, although it was not significant (p=0.087, Table 5).

From the SEM observation, it was confirmed that the tooth structure around the enamel rod was lost in $G5_{B+E+RI+S+C}$ and that the tooth was dented. No loss of tooth structure was observed in any of the other groups (Fig. 1).

Table 5. ΔE Values according to the Coloring Method

Group	Coffee	Wine
$G1_{S+C}$	16.33 ± 9.92^{a}	28.87±5.26
$G2_{B+S+C}$	4.39 ± 2.00^{b}	29.68±6.63
$G3_{B+FV+S+C}$	7.55 ± 5.72^{b}	32.98±8.88
$G4_{B+RI+S+C}$	7.35 ± 3.19^{b}	33.03±10.14
$G5_{B+E+RI+S+C}$	8.52 ± 4.04^{b}	42.49±10.74
p-value*	0.030	0.087

*p-value obtained by performing Kruskal–Wallis H analysis.
[a,b]shows the differences in ΔE values between the groups from 1 to 5.

Discussion

The effect of tooth whitening treatment is not permanent, and the duration of the whitening effect varies from person to person[11,12]. Patients undergoing tooth whitening treatment will, of course, want the tooth whitening effect to last for a long time. In other words, you will want the teeth whitened

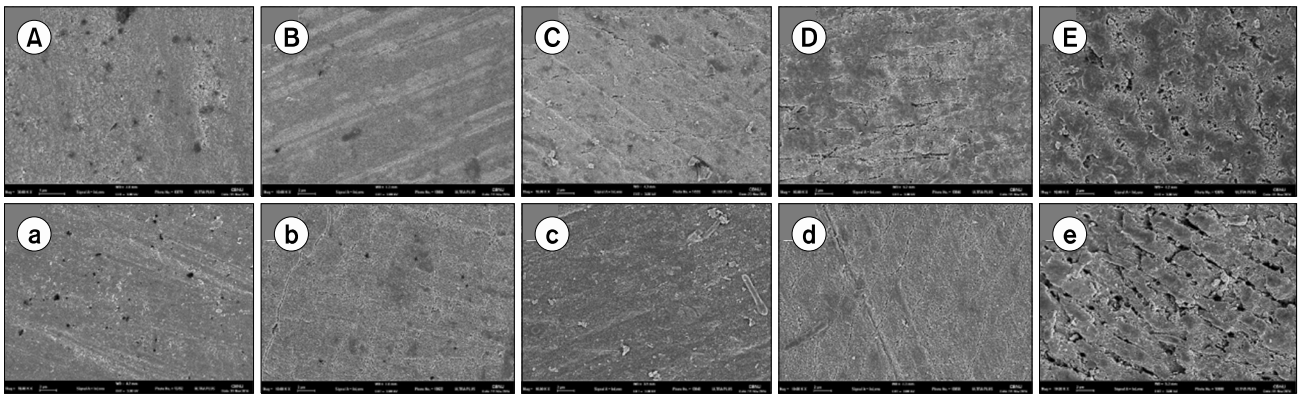

Fig. 1. Scanning electron microscope (SEM) pictures according to each group and discoloration method. (A~E) Images of a SEM of dental specimens of groups 1, 2, 3, 4, and 5 stained with coffee, respectively. (a~e) Images of a scanning electron microscope of dental specimens of groups 1, 2, 3, 4, and 5 stained with wine, respectively. SEM photos were obtained at 1,000 magnification.

by the teeth whitening treatment to remain white, and you will want the teeth not to darken on account of discoloration. Therefore, in this study, RI was applied to maintain the effect of tooth whitening treatment or to prevent tooth discoloration, and the effect was confirmed accordingly.

In this study, there was no significant difference in L* values between the groups when whitening treatment or anti-pigmentation material was administered for 14 days ($p > 0.05$). In the case of coffee coloring, flowable RI treatment ($G4_{B+RI+S+C}$, $G5_{B+E+RI+S+C}$) with FV ($G3_{B+FV+S+C}$) or immersion in a large amount of artificial saliva ($G2_{B+S+C}$) was confirmed to have a similar effect on coffee staining. These results indicate that the whitening treatment can result in brighter teeth than the non-whitening treatment. However, according to a previous study, when whitening with a high concentration bleaching agent was used, a lot of discoloration occurred after whitening[12], and it was reported that enamel and dentin softened due to tooth bleaching[13]. When the tooth becomes soft, the minerals that make up the tooth structure escape sparsely and the porosity increases. This creates an empty space in the tooth structure, which can increase permeability[4]. This may lead to increased susceptibility to pigmentation[12]. In contrast, in this study, the effect of whitening was well maintained in the group that had undergone whitening, and no significant difference was found in the color values between the negative control group 1, positive control group, and the experimental group. The reason for these results is believed to be the tooth remineralization effect due to the minerals contained in artificial saliva, along with the dilution and washing action caused by the immersion of an excessive amount of saliva[14]. In a previous study, the anti-pigmentation effect for 30 days was confirmed when remineralization with artificial saliva or additional whitening treatment was performed during whitening treatment ($p < 0.05$)[15]. In addition, when calcium phosphate or fluoride preparations were applied to the teeth, the whitening effect was maintained for up to four weeks compared to the group without any treatment ($p < 0.05$)[7-9].

The wine used as a colorant caused more coloration than coffee, which is consistent with the results of previous studies[15-17]. According to a previous study, age, sex, coffee/tea consumption, and dental treatment were identified as variables that had significant effects on tooth yellowing (b* value) and brightness (L* value)[18]. There was no significant difference between the L* values of all groups in the case of wine staining, which was more prominent than coffee staining ($p=0.166$). This is thought to be so since the wine coloring was too strong, and it is considered necessary to check whether there is a difference in brightness in the middle of the coloring for 9 days.

RI was originally used to prevent the progression of early caries. Since there is a significant loss of minerals below the surface of early caries, and the surface layer has a structure that is strengthened by remineralization due to saliva, acid treatment was performed to blow off the surface layer with strong hardness. However, since the teeth whitening treatment in this study had a different structure from the initial caries, it was considered that acid treatment may not be necessary, and hence the group without acid treatment before RI treatment was included in the experiment. In the case of wine staining, group 4, treated with flowable resin without acid treatment, had a slightly brighter tooth surface color than group 5, which was treated with acid treatment and flowable resin, however there was no statistically significant difference. Electron microscopy revealed that the tooth surface of group 5 had many cracks, unlike other groups, which was considered to be the result of acid etching during the treatment process.

1. Conclusion

In this study, artificial saliva, fluorine varnish, or RI were applied as a method to prevent discoloration after teeth whitening, and the effects were compared. When coloration was induced with coffee, there was no significant difference in L* value between artificial saliva, fluoride varnish, and RI groups. There was no significant difference in L* value between artificial saliva, fluoride varnish, and RI groups even in the case of inducing coloration with wine. Therefore, it was confirmed that artificial saliva or RI treatment had similar effects to the previously used fluoride varnish to maintain the effect of tooth bleaching treatment.

2. Limitation

In this study, in order to reproduce the actual oral situation in the laboratory, the material was not simply applied once, but repeated several times, and storedin artificial saliva except for the treatment time. In addition, most of the treatments were performed in a 37°C incubator to reproduce the temperature in the oral cavity. However, in the actual oral cavity, the teeth are not completely immersed in saliva, and various microorganisms exist in the oral cavity[19,20]. Therefore, it is difficult to interpret the results of this study as results that can be confirmed in the oral cavity. In addition, when RI was applied to teeth for the purpose of preventing staining, it was confirmed that staining was not evenly prevented for each part, so repeated experiments with more subdivided experimental conditions are needed in the future.

3. Generalizability

Fluoride varnish has been used in clinical practice as a method toprevent discoloration after teeth whitening. As a result of this study, there was no significant difference in the anti-discoloration effect of fluoride varnish, artificial saliva, or penetrating resin after teeth whitening. Therefore, artificial saliva and penetrating resin can be considered as measures to prevent discoloration after teeth whitening.

4. Suggestion

Teeth whitening treatment is not permanent, and the prognosis for each patient may be different. In this study, artificial saliva, fluoride varnish, and penetrating resin were selected as measures to prevent discoloration after teeth whitening, and it was confirmed that there was no difference in effect between them. In the future, it is considered necessary to verify the anti-pigmentation effect after teeth whitening for more diverse materials.

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

Because this study was not conducted in humans, IRB approval is not required.

ORCID

Min Ji-Hyun, *https://orcid.org/0000-0001-5177-7600*

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Data availability

Raw data is provided at the request of the corresponding author for reasonable reason.

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