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Preventive Effects of Laser Irradiation and Dentin Bonding Agent Application on Tooth Discoloration Induced by Mineral Trioxide Aggregate

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Abstract: The aim of the present study was to evaluate the effect of the pre-application of dentin bonding agent and laser application on the prevention of tooth discoloration caused by (MTA) in the presence of blood. Fifty extracted human anterior teeth were prepared by standardizing root lengths to 10 mm and shaping root canals. Specimens were divided into five groups (n = 10) based on the treatment applied to the pulp chamber dentin wall: Group 1/no surface treatment; Group 2/Optibond FL; Group 3/Clearfil SE; Group 4/Optibond Universal adhesive application; Group 5/Nd:YAG laser application. Root canals were filled with fresh human blood below the cemento-enamel junction (CEJ), followed by ProRoot MTA, and collagen barrier placement. Color changes were monitored using a spectrophotometer at 0, 7, 30, 90, and 180 days post MTA placement. Color differences (ΔE) were calculated and analyzed using two-factor mixed-design ANOVA with Sidak adjustment at $p = 0.05$. The degree of coloration increased with time within each group ($p < 0.05$). No significant differences were observed between Optibond FL and Optibond Universal within each time interval, or between Clearfill SE Bond and the control group ($p > 0.05$). When compared to the control group, the Nd YAG group exhibited the least degree of discoloration in all time intervals ($p < 0.05$). Although the Nd:YAG laser had promising results, none of the methods can guarantee a 100% prevention of discoloration resulting from tooth discoloration caused by MTA-blood contact.

Keywords: calcium silicate materials; dentin bonding; dentin tubule occlusion; Nd:YAG laser; tooth discoloration

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1. Introduction

Regenerative endodontic treatment (RET) represents a biologically oriented therapy employed for necrotic teeth with an immature apex, aimed at promoting ongoing root development, increasing dentinal wall thickness, and achieving apical closure [1,2]. The treatment protocol is based on inducing a blood clot or protein scaffold formation in the root canal, followed by the placement of a biomaterial to support this process [1,3]. Mineral Trioxide Aggregate (MTA) has been widely utilized for this purpose due to its excellent biocompatibility and low cytotoxicity [4,5]. However, one of the undesirable consequences associated with this material is its potential to cause tooth discoloration, for which the exact mechanism is currently uncertain [4,6,7].

Tooth discoloration induced by MTA can be attributed to several factors. The presence of metal oxides within MTA, such as bismuth oxide, ferrous oxide, aluminum oxide, and magnesium oxide, contributes to the discoloration [7]. Moreover, undesired tooth discoloration may be linked to MTA contamination with blood [8,9], interactions between bismuth oxide and irrigation solutions [10,11], and chromogenic alterations resulting from

light irradiation in an oxygen-free environment [12]. Furthermore, some studies suggest that the color change resulting from a blood clot formation could be linked to the infiltration of blood products like hemoglobin, hematin, and erythrocytes into the dentin tubules [2].

The mechanisms underlying the blood-associated discoloration of Mineral Trioxide Aggregate (MTA) remain unclear. One potential explanation for discoloration resulting from contact between blood and MTA may involve the interaction between erythrocytes and unset MTA. The gradual hydration process of MTA might facilitate the absorption and subsequent hemolysis of erythrocytes from the blood, leading to both material and subsequent tooth discoloration [13]. Notably, discoloration linked to collagen bismuth oxide has been documented as well [14]. Nevertheless, it is important to highlight that MTA without bismuth oxide can also induce discoloration in the presence of blood, potentially due to the porosity of the cement or the hypothesis of heme absorption from hemoglobin [15]. Research indicates that the heme group is a prosthetic group composed of a ferrous (Fe^{2+}) ion located at the center of a large heterocyclic organic ring called a porphyrin. This structure is formed by four pyrrolic groups interconnected by methine bridges [16]. Over time, blood in contact with the cement undergoes a natural redox reaction leading to the conversion of Fe^{2+} (with a red color) to ferric (Fe^{3+}), which imparts a dark brown color. This transformation may contribute to the darkening of the cement and, subsequently, tooth discoloration [13,17]. Hence, it is advisable to consider alternative treatment approaches, focusing on the pre-treatment occlusion of dentin tubules, to prevent discoloration [7,18].

The application of one or more layers of dentin bonding agent (DBA) prior to MTA application has demonstrated effectiveness in preventing tooth discoloration [19,20]. However, this approach may not entirely prevent MTA penetration into the dentin tubules [18,20]. An alternative strategy for dentin tubule occlusion could be the application of a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser, utilizing a technique known as 'melting and re-solidification' [18]. This laser procedure effectively obliterates and narrows dentinal tubules, resulting in a glazed [21] surface without causing any dentinal cracks or craters [22,23].

Nevertheless, there is a gap in the existing literature as few studies have evaluated the impact of the pre-application of laser irradiation in preventing tooth discoloration. Upon reviewing the existing literature, it was determined that research on this topic is highly scarce. Hence, the objective of this study is to assess the efficacy of the pre-application of Nd:YAG laser irradiation and DBA on preventing tooth discoloration induced by MTA in a simulated regenerative endodontic procedure (REP) scenario. The null hypothesis of this study was that there will be no difference between the different tubule occlusion methods in terms of effectiveness on the prevention of RET-induced tooth discoloration.

2. Materials and Methods

The local ethics committee granted approval for the study protocol (Approval no: 2023-05/2). Conducting a power analysis with G*Power 3.1 software (Heinrich Heine University, Dusseldorf, Germany), and drawing from a comparable study [6], the sample size for each experimental group in this study was determined as a minimum of 10 (alpha probability of error = 0.05, power = 0.95) for this study.

2.1. Sample Selection

Fifty human permanent anterior teeth, which were extracted due to periodontal reasons, were selected. The teeth were inspected under a dental operation microscope (OMS2380, Zumax, Suzhou, China) to eliminate teeth with cracks, fractures, and caries. Initial bucco-lingual and mesio-distal periapical radiographs were taken. The teeth with single root, single canal morphology were included. The teeth with decay, restoration, old root canal filling, any anatomical variations, resorption, calcification, or initial tooth discoloration were excluded.

2.2. Preparation of Specimens

A standardized length of 10 mm from the buccal cementoenamel junction (CEJ) was achieved by resecting the root end using high-speed burs. Subsequently, an endodontic access cavity was created with a round bur, and the root canals were instrumented using Gates-Glidden drills #3 to #5, spanning the entire length from crown to root end. The root canals underwent irrigation with 5 mL of 2.5% sodium hypochlorite, followed by 5 mL of 17% EDTA, and finally, 5 mL of distilled water. Following irrigation, the root canals were dried using sterile paper points, and a sterile cotton pellet was inserted from the access cavity, reaching down to the CEJ. The closures of the root ends were accomplished using Z250 universal micro-hybrid resin composite (3M ESPE, St. Paul, MN, USA), while the access cavities were sealed with a temporary restorative material (Cavit; 3M ESPE, St. Paul, MN, USA). All procedures were conducted under a dental operating microscope (OMS2380, Zumax, Suzhou, China). Subsequently, the teeth were stored in a saline solution until the commencement of the experiment.

2.3. Experimental Groups

The teeth were removed, the cotton pellet was taken out, and the same irrigation process was performed. Afterward, the canals were dried with sterile paper points. The samples were randomly divided into 5 main groups according to the prevention of discoloration strategies:

- Group 1: (No laser, no bonding application) The internal walls of the pulp chamber were not sealed with any method, and this group was used as the control group.
- Group 2: (Optibond FL, Kerr Corporation, Orange, CA, USA) After 15 s of acid application to the pulp chamber, the acid was washed away for 15 s. After gentle air drying, prime was applied by rubbing for 15 s. After air drying, adhesive was applied by rubbing for 15 s and polymerized for 20 s after air drying.
- Group 3: (Clearfill SE Bond, Kuraray Medical Inc., Tokyo, Japan) It was applied to the pulp chamber by rubbing primer for 20 s. After 5 s of gentle air drying, the bond was applied to the specimens. After air drying, it was polymerized for 10 s.
- Group 4: (Optibond Universal, Kerr Corporation, Orange, CA, USA) With a self-etch approach, it was applied to the pulp chamber by rubbing for 20 s. After 5 s of gentle air drying, it was polymerized for 10 s.
- Group 5: (Laser irradiation) The internal walls of the pulp chamber in this group were irradiated using an Nd:YAG laser (Fotona, Lightwalker, San Clemente, CA, USA). Lasers were used with 1 W power, at an energy level of 100 mJ per pulse, and a repetition rate of 10 Hz, for 10 s, firing interval: 250 msn. A 300 mm quartz fiber was used with pre-established movements in the occluso-apical and mesio-distal directions and vice versa.

After applying sealing strategies, a volume of 0.2 cc of fresh human blood was obtained from one of the researchers (T.Ö). This blood was injected directly into the root canal using an insulin syringe, filling it up to approximately 4 mm below the CEJ. Subsequently, a blood clot was allowed to form over a 15 min period, and spongostan (Cutanplast, Milan, Italy) was placed on the clot. A 3 mm thickness of ProRoot MTA in the coronal third of the root canal was positioned 1 mm below the CEJ. The endodontic access cavity was sealed with temporary restorative material, and the thickness and depth of the MTA were verified by capturing a periapical radiograph. Throughout the entire experiment, the samples were maintained at 37 °C with 100% humidity (Figure 1).

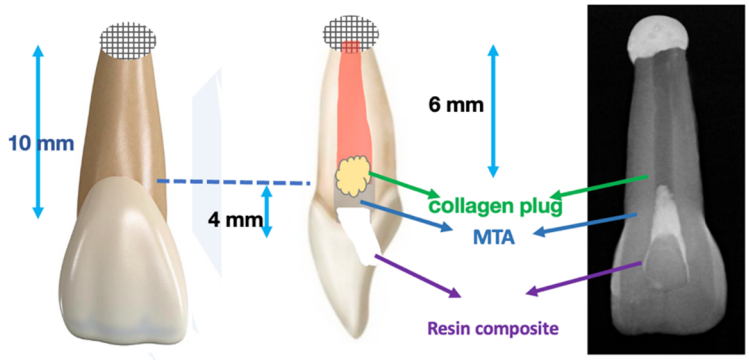

Figure 1. Schematic representation of the experimental setup.

2.4. Tooth Color Assessment

To ensure an equitable distribution of teeth for each experimental group, a spectrophotometer device (VITA Easyshade Advance 4.0, VITA Zahnfabrik, Germany) was used to measure initial color values before the experiment, thus calibrating the groups. An equal number of color tones were distributed to each group (three teeth, color A2; two teeth, color B1; two teeth, color B2; and three teeth, color A3 in the same experimental group) [24]. Teeth were placed against an absolute white background (standard calibration tile according to Commission Internationale de l'Éclairage [CIE] L*: 93.84, a*: 1.48, b*: 3.76), and the central area of the buccal surface was subjected to color measurement.

Care was taken to ensure the device's tip made full contact with the flattest area of the tooth's buccal surface, limiting the measurement to the central area only. The same researcher performed all measurements, verifying the spectrophotometer's tip contact with the tooth before each measurement. The device was specially calibrated according to the manufacturer's instructions before measuring each new sample group, and the measurements were subsequently repeated three times for each tooth by the same operator (YSU).

The color evaluation was conducted following the CIE Lab* color space parameters. The lightness of the object is indicated by the L* coordinate, which scales from white (0) to absolute black (100). The a* coordinate signifies the chromaticity on a green (−) to red (+) axis, with values typically ranging from −70 for green to +70 for red. The b* coordinate measures chromaticity on a blue (−) to yellow (+) axis, where values range from −80, indicating blue, up to +100, signifying yellow.

Following the baseline assessment (T0), the teeth were immersed in artificial saliva and placed in an incubator set at 37 °C to mimic the oral environment and aging procedures. This saliva was refreshed every seven days. Subsequent color evaluations were conducted after (T1) 7, (T2) 30, (T3) 90, and (T4) 180 days to benchmark against the initial L*a*b* values (baseline), enabling comparisons over time and assessments of color stability or change. The measurements were performed in accordance with the CIEDE2000 (ΔE00) system. ΔE00 was calculated using the following formula [25,26]:

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

2.5. Statistical Analysis

Statistical analyses were performed using SPSS version 21. Descriptive statistics for each variable were computed and expressed as "Mean ± Standard Error of Mean (SEM)". Subsequently, a two-factor mixed-design ANOVA (analysis of variance) was employed using the General Linear Model procedure for repeated measurements. The model incorporated "Group" and "Time" as the primary effects, along with their interaction term (Group*Time). In instances where Mauchley's test indicated a violation of the assumption of sphericity, the Greenhouse–Geisser adjustment was implemented. To further dissect any significant interaction effect terms as a post hoc analysis, a simple effect analysis with Sidak

adjustment was conducted. A probability value less than 0.05 was considered indicative of significance, unless stated otherwise.

3. Results

The means and standard deviations of the color changes (ΔE) are shown in Table 1 and Figure 2, and the luminosity (ΔL*) at each time period are shown in Figure 3 and Table 2. Discoloration was observed in all groups (Figure 4). The ΔE values for experimental groups from T0 to T180 ranged from 7.11 to 12.83 (Table 1).

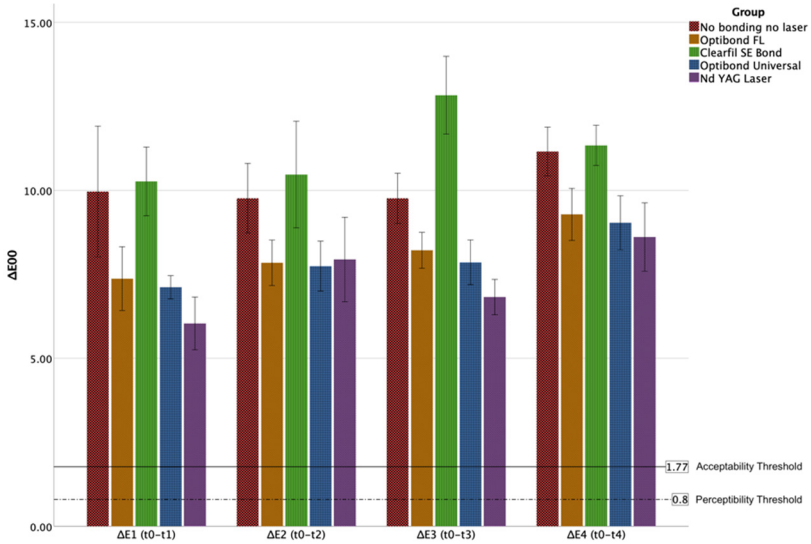

Figure 2. Comparison of the mean ΔE values of the groups for each treatment step with the graph. (The dotted lines represent: clinically acceptability threshold AT = 1.77, perceptibility threshold PT = 0.8).

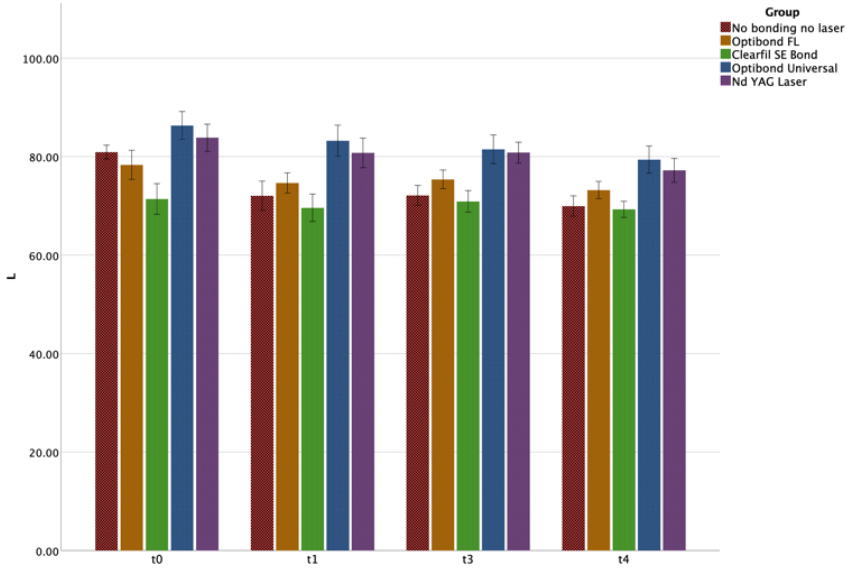

Figure 3. Comparison of the mean L (lightness) values of the groups for each treatment step with the graph.

Table 1. Means and standard deviations of color change values (ΔE00) in all time periods. ΔE values express color variation from baseline (T0) to each evaluation timepoint (T1—7 days, T2—30 days, T3—90 days, and T4—180 days).

Group	*n*	ΔE1 (T0-T1) *Mean* ± *Sd*	ΔE 2 (T0-T2) *Mean* ± *Sd*	ΔE 3 (T0-T3) *Mean* ± *Sd*	ΔE 4 (T0-T4) *Mean* ± *Sd*	*p* *Group*	*Time*	*Group*Time*
No bonding, no laser	10	$9.97 \pm 1.95^{ab,B}$	$9.77 \pm 1.04^{ab,AB}$	$9.76 \pm 0.75^{ab,AB}$	$11.16 \pm 0.72^{ab,A}$	<0.001	0.029	0.777
Optibond FL	10	$7.37 \pm 0.95^{bc,B}$	$7.84 \pm 0.68^{bc,AB}$	$8.21 \pm 0.53^{bc,AB}$	$9.28 \pm 0.77^{bc,A}$			
Clearfil SE Bond	10	$10.27 \pm 1.02^{a,B}$	$10.47 \pm 1.59^{a,AB}$	$12.83 \pm 1.16^{a,AB}$	$11.34 \pm 0.6^{a,A}$			
Optibond Universal	10	$7.11 \pm 0.35^{bc,B}$	$7.74 \pm 0.74^{bc,AB}$	$7.86 \pm 0.66^{bc,AB}$	$9.03 \pm 0.8^{bc,A}$			
Nd YAG Laser	10	$6.04 \pm 0.78^{c,B}$	$7.94 \pm 1.26^{c,AB}$	$6.82 \pm 0.53^{c,AB}$	$8.61 \pm 1.02^{c,A}$			

T0: Day 0, T1: 1st Week, T2: 1st Month, T3: 3rd Month, T4: 6th Month. ^{a,b,c}: different letters in the same column show statistical significance ($p < 0.05$). ^{A,B,C}: different letters in the same row show statistical significance ($p < 0.05$).

Table 2. Mean and standard deviation values of each of the L*a*b* coordinates of each experimental group for all periods of evaluation.

	Time:	**T0**	**T1**	**T2**	**T3**	**T4**	***p***		
				ΔL*					
Group	***n***	***Mean* ± *Sd***	***Mean* ± *Sd***	***Mean* ± *Sd***	***Mean* ± *Sd***	***Mean* ± *Sd***	***Group***	***Time***	***Group*Time***
No bonding, no laser	10	$80.9 \pm 1.41^{ab,A}$	$72.05 \pm 2.97^{ab,B}$	$73.21 \pm 2.42^{ab,B}$	$72.13 \pm 2.01^{ab,B}$	$69.97 \pm 2.07^{ab,B}$	0.002	<0.001	0.219
Optibond FL	10	$78.34 \pm 2.96^{ab,A}$	$74.63 \pm 2.06^{ab,B}$	$72.74 \pm 1.88^{ab,B}$	$75.38 \pm 1.88^{ab,B}$	$73.21 \pm 1.75^{ab,B}$			
Clearfil SE Bond	10	$71.4 \pm 3.18^{b,A}$	$69.6 \pm 2.79^{b,B}$	$69.31 \pm 2.71^{b,B}$	$70.91 \pm 2.18^{b,B}$	$69.09 \pm 1.64^{b,B}$			
Optibond Universal	10	$86.32 \pm 2.83^{a,A}$	$83.21 \pm 3.18^{a,B}$	$82.54 \pm 3.16^{a,B}$	$81.49 \pm 2.92^{a,B}$	$79.39 \pm 2.77^{a,B}$			
Nd YAG Laser	10	$83.82 \pm 2.76^{a,A}$	$80.75 \pm 3^{a,B}$	$78.09 \pm 3.15^{a,B}$	$80.8 \pm 2.12^{a,B}$	$77.19 \pm 2.42^{a,B}$			
a*									
No bonding, no laser	10	$5.77 \pm 0.69^{b,A}$	$5.12 \pm 0.97^{a,A}$	$2.38 \pm 0.4^{a,B}$	$1.83 \pm 0.32^{a,B}$	$1.74 \pm 0.31^{a,B}$	0.014	<0.001	<0.001
Optibond FL	10	$7.49 \pm 1.2^{b,A}$	$3.43 \pm 1.09^{a,BC}$	$2.57 \pm 0.49^{a,B}$	$1.6 \pm 0.45^{a,BC}$	$1.64 \pm 0.52^{a,C}$			
Clearfil SE Bond	10	$12.91 \pm 1.47^{a,A}$	$4.72 \pm 0.68^{a,B}$	$3.28 \pm 0.48^{a,B}$	$1.33 \pm 0.29^{a,C}$	$2.07 \pm 0.41^{a,C}$			
Optibond Universal	10	$5.29 \pm 0.87^{b,A}$	$1.82 \pm 0.66^{a,B}$	$1.61 \pm 0.45^{a,B}$	$1.26 \pm 0.37^{a,B}$	$1.01 \pm 0.36^{a,B}$			
Nd YAG Laser	10	$5.56 \pm 0.92^{b,A}$	$2.99 \pm 1.02^{a,AB}$	$1.93 \pm 0.51^{a,B}$	$1.5 \pm 0.34^{a,B}$	$1.32 \pm 0.28^{a,B}$			

Table 2. *Cont.*

	Time:	**T0**	**T1**	**T2**	**T3**	**T4**	***p***		
				ΔL*					
Group	***n***	***Mean* ± *Sd***	***Mean* ± *Sd***	***Mean* ± *Sd***	***Mean* ± *Sd***	***Mean* ± *Sd***	***Group***	***Time***	***Group*Time***
b*									
No bonding, no laser	10	41.86 ± 1.36 ^{a,A}	29.83 ± 2.28 ^{a,B}	27.98 ± 1.94 ^{a,B}	25.56 ± 1.12 ^{a,B}	26.79 ± 0.98 ^{a,B}			
Optibond FL	10	44.81 ± 1.76 ^{a,A}	30.56 ± 2.51 ^{a,B}	30.18 ± 1.07 ^{a,B}	29.61 ± 1.06 ^{a,B}	28.17 ± 1.23 ^{a,B}			
Clearfil SE Bond	10	40.06 ± 1.72 ^{a,A}	27.36 ± 1.29 ^{a,B}	27.34 ± 1.45 ^{a,B}	26.14 ± 1.37 ^{a,B}	25.99 ± 1.28 ^{a,B}	0.315	<0.001	0.644
Optibond Universal	10	45.51 ± 1.41 ^{a,A}	29.4 ± 1.56 ^{a,B}	28.12 ± 1.74 ^{a,B}	29.56 ± 1.43 ^{a,B}	27.89 ± 1.74 ^{a,B}			
Nd YAG Laser	10	41.68 ± 1.65 ^{a,A}	30.53 ± 1.94 ^{a,B}	28.43 ± 1.41 ^{a,B}	29.34 ± 1.58 ^{a,B}	26.49 ± 1.8 ^{a,B}			

T0: Day 0, T1: 1st Week, T2: 1st Month, T3: 3rd Month, T4: 6th Month. ^{a,b,c}: different letters in the same column show statistical significance ($p < 0.05$). ^{A,B,C}: different letters in the same row show statistical significance ($p < 0.05$).

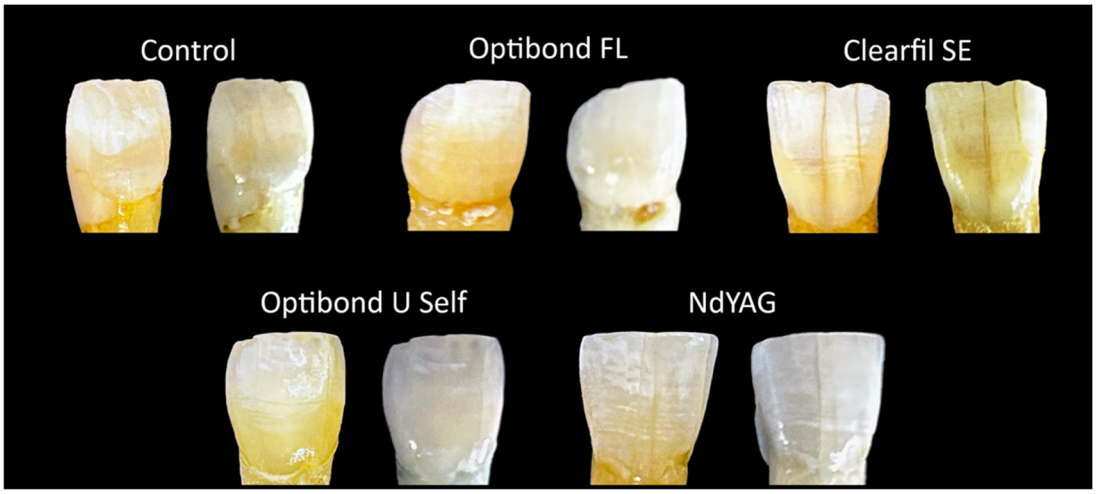

Figure 4. Photographs of a randomly selected tooth from all groups at initial T0 and 6 months (T18) time intervals.

The results indicate that the degree of coloration increased with time within each group, with statistically significant differences observed among the groups in terms of the extent of discoloration ($p < 0.05$). The highest degree of discoloration in each experimental group was observed at the end of the 6th month, as compared to the 1st month, except the Clearfil SE Bond group ($p < 0.05$). On the other hand, no significant differences were observed between Optibond FL and Optibond Universal within each time interval, or between Clearfill SE Bond and the control group ($p > 0.05$). When compared to the control group, the Nd YAG group exhibited the least degree of discoloration in all time intervals ($p < 0.05$).

4. Discussion

Color change is a significant aesthetic concern encountered following regenerative endodontic treatment. Although the pre-application of an adhesive has been shown to potentially prevent discoloration caused by MTA [19,20], there is currently no definitive treatment protocol for preventing such discoloration [27,28]. In the present study, the efficacy of different pre-treatment applications for preventing discoloration were evaluated, and no significant differences were observed among Optibond FL, Optibond Universal, and Nd:YAG laser treatments across all time intervals. The Nd YAG laser group exhibited the least color change across all time intervals compared to the control group. Accordingly, the null hypothesis stating that there would be no significant difference in the effectiveness of various tubule occlusion methods in preventing tooth discoloration was rejected.

In all experimental groups, a significant reduction in enamel lightness was observed over time compared to the initial values. When each experimental group was evaluated individually, no differences were noted between the time intervals, but there was a reduction when compared to the baseline. The decrease in enamel lightness across all groups indicates a darkening of the samples over time, demonstrating a perceptible change in tooth color or staining [29].

The CIEDE2000 is the latest formula for calculating color differences, enhancing the number of parameters used and presenting a more complex model than the CIELAB (ΔEab) formula. It is advised to employ the CIEDE2000 (ΔE00) for its superior performance and closer correlation with visual perception in detecting color discrepancies. In dentistry, the primary clinical importance of comparing color differences lies not only in detecting statistical differences but also in understanding their relevance to perceptibility and acceptability thresholds [26,30]. In this study, these factors have been taken into account when assessing the impact of strategies to prevent color changes induced by the application of

MTA following regenerative endodontic treatment (RET), with the CIEDE 2000 system utilized for measurement.

For the results to be interpretable in real-life terms, it is necessary to compare them with color difference thresholds, preferably known as perceptibility thresholds (PTs) and acceptability thresholds (ATs). A PT represents a situation where observers can discern a color difference between two items, whereas an AT indicates the level at which the degree of color difference detected is still considered acceptable [30]. Paravina et al. revealed an approach to evaluating color discrepancies using 50:50% perceptibility and acceptability thresholds, which was an extension of their prior research [26]. They proposed a new classification for the degree of color mismatch: moderately unacceptable (more than 1.8 but up to 3.6 ΔE00), distinctly unacceptable (more than 3.6 but up to 5.4 ΔE00), and highly unacceptable (exceeding 5.4 ΔE00) [30]. Based on this classification, the present study found that all the values obtained, across all the tested groups, were within the range deemed highly unacceptable.

The literature reports that in regenerative endodontic procedures, tissue scaffolds such as blood, platelet-rich fibrin (PRF), and platelet-rich plasma (PRP) have been utilized to support cell growth, differentiation, and organization [31]. Although each method has some advantages and limitations, the use of a blood clot, platelet-rich plasma (PRP), and platelet-rich fibrin (PRF) as scaffolds has been shown to not have a statistically significant difference in supporting root development in immature teeth undergoing RET [32]. For these reasons, the blood clot method, which offers the advantages of low cost and ease of clinical application, was used in the present study. However, there are several benefits; when a blood clot interacts with the coronal tooth structures, red blood cells that enter the dentinal tubules undergo hemolysis. The accumulation of released hemoglobin and hematin molecules in the dentinal tubules leads to tooth discoloration [33]. Another cause of color change associated with regenerative endodontic treatment (RET) is the contact between blood and calcium silicate cement and dentin. The tendency for color change upon contact with blood is reported in all calcium silicate cements, even in those known to have a lower potential for color change. Given the fact that a blood clot is the most commonly used matrix in RET [34], the significance of procedures to prevent discoloration becomes apparent.

In order to prevent tooth discoloration induced by RET procedures or triple antibiotic paste TAP applications, Teethmate desensitizer, a bonding agent, Nd:YAG laser, and Er:YAG laser have been used in the literature [18,20,27]. Shokouhinejad et al. [20] evaluated for the first time, using an ex vivo model, the role of sealing the dentin walls of the pulp chamber with a dentin bonding agent (DBA) in preventing crown discoloration prior to a regenerative endodontic procedure (REP). While the application of the DBA did not completely prevent clinically perceptible coronal color changes, it was observed that there was significantly less crown discoloration in each stage of the regenerative treatment. This outcome directed the researchers to find another method.

The Nd:YAG laser is frequently preferred in the treatment of dentine sensitivity due to its highly effective dentinal tubule occlusion and high penetration depth effects [35–37]. Additionally, the Nd:YAG laser, when applied with appropriate parameters, reduces unwanted side effects such as cracks and pulp damage in teeth [23,36]. Within this aspect, Küçükekenci et al. [18] used an Nd:YAG laser to prevent tooth discoloration induced by triple antibiotic paste and compared its effectiveness with those of a bonding agent and Teethmate desensitizer. They reported no significant difference between these three experimental groups. Ateş and Aydın performed a similar study with an additional experimental group, Er:YAG laser, and they applied Biodentin directly over the blood clot [27]. Their results were also in accordance with those of Küçükekenci et al. In the present study, no significant differences were observed among Optibond FL, Optibond Universal, and Nd:YAG laser treatments across all time intervals. Surprisingly, the effect of Clearfill SE Bond on the prevention of the tooth discoloration was lesser than those of the other bond systems. The possible reason could be the yellowish color of this bond, which may induce coloration.

Another reason for this result could be the application of the primer on the unetched surface, which may cause discoloration as reported by the manufacturer (Figure 5) [38].

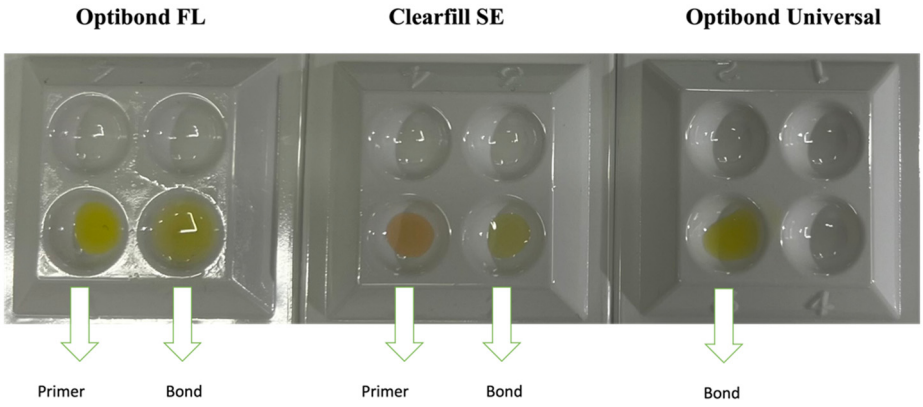

Figure 5. The dentin bonding agents used in this study.

In using extracted teeth that were kept in saliva, an attempt was made to standardize the clinical conditions. The groups were also divided by taking into account the initial color of the teeth. On the other hand, the color analysis was conducted from the same region each time.

The alteration of certain key parameters in the study could have led to variations in the results, which is the limitation of the present study. The teeth allocations of each group were conducted based on the previous studies [24,39]. According to this, an equal number of color tones were distributed to each group based on colors A2, B1, B2, and A3. The initial luminosity values were recorded but not considered for the allocation of the groups. For this reason, in the result part, luminosity change was evaluated within each group, rather than between the groups. Accordingly, the luminosity value of each group decreased compared to that on T0. In this research, various methods were employed to prevent tooth discoloration caused by MTA–blood contact. Different outcomes might have been obtained using another calcium silicate-based material. Additionally, changes in laser usage parameters could also have influenced the result. Lan et al. [40] reported the critical power for the same laser as 1.5 W; exceeding this may result in fractures and damage on the tooth surface. On the other hand, White et al. [41] highlighted that the Nd:YAG laser power should be equal to or less then 1 W in order not to damage pulp. This result is also in accordance with previous studies [23,36]. In this study, due to its effective tubule occlusion and high penetration depth [23,36], the Nd:YAG laser was utilized for tubule occlusion with a parameter setting of 1 W. Using the Nd:YAG laser at a power of 1.5 W may yield more effective results. Further in vitro and in vivo studies are needed.

5. Conclusions

Within the limitations of this study, although dentin tubule occlusion methods have a preventive effect against staining of the tooth, none of the methods can completely prevent this coloration. The use of the Nd:YAG laser is a promising feature in preventing tooth discoloration, and further studies with different usage parameters are needed.

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Informed Consent Statement: This study involved the collection and placement of human blood into the tooth root. The procedure was carried out with informed consent from the participant, who is also the researcher named T.Ö., and received approval from the Ethics Committee of Bahçeşehir University, ensuring compliance with all applicable ethical standards.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available to prevent the potential for unauthorized duplication and use of the data.

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References

1. American Association of Endodontics. *AAE Guide for Regenerative Endodontic Procedure (REP)*; American Association of Endodontics: Chicago, IL, USA.
2. Fagogeni, I.; Metlerska, J.; Lipski, M.; Falgowski, T.; Maciej, G.; Nowicka, A. Materials used in regenerative endodontic procedures and their impact on tooth discoloration. *J. Oral Sci.* **2019**, *61*, 379–385. [CrossRef]
3. Saoud, T.M.A.; Ricucci, D.; Lin, L.M.; Gaengler, P. Regeneration and Repair in Endodontics-A Special Issue of the Regenerative Endodontics-A New Era in Clinical Endodontics. *Dent. J.* **2016**, *4*, 3. [CrossRef] [PubMed]
4. Akbulut, M.B.; Terlemez, A.; Akman, M.; Buyukerkmen, B.; Guneser, M.B.; Eldeniz, A.U. Tooth discoloration effects of calcium silicate based barrier materials used in revascularization and treatment with internal bleaching. *J. Dent. Sci.* **2017**, *12*, 347–353. [CrossRef] [PubMed]
5. Torabinejad, M.; Parirokh, M. Mineral trioxide aggregate: A comprehensive literature review—Part II: Leakage and biocompatibility investigations. *J. Endod.* **2010**, *36*, 190–202. [CrossRef] [PubMed]
6. Chen, S.J.; Karabucak, B.; Steffen, J.J.; Yu, Y.H.; Kohli, M.R. Spectrophotometric Analysis of Coronal Tooth Discoloration Induced by Tricalcium Silicate Cements in the Presence of Blood. *J. Endod.* **2020**, *46*, 1913–1919. [CrossRef] [PubMed]
7. Shokouhinejad, N.; Nekoofar, M.H.; Pirmoazen, S.; Shamshiri, A.R.; Dummer, P.M. Evaluation and Comparison of Occurrence of Tooth Discoloration after the Application of Various Calcium Silicate-based Cements: An Ex Vivo Study. *J. Endod.* **2016**, *42*, 140–144. [CrossRef]
8. Lenherr, P.; Allgayer, N.; Weiger, R.; Filippi, A.; Attin, T.; Krastl, G. Tooth discoloration induced by endodontic materials: A laboratory study. *Int. Endod. J.* **2012**, *45*, 942–949. [CrossRef]
9. Parirokh, M.; Torabinejad, M. Mineral trioxide aggregate: A comprehensive literature review—Part III: Clinical applications, drawbacks, and mechanism of action. *J. Endod.* **2010**, *36*, 400–413. [CrossRef]
10. Camilleri, J. Color stability of white mineral trioxide aggregate in contact with hypochlorite solution. *J. Endod.* **2014**, *40*, 436–440. [CrossRef]
11. Marciano, M.A.; Duarte, M.A.; Camilleri, J. Dental discoloration caused by bismuth oxide in MTA in the presence of sodium hypochlorite. *Clin. Oral Investig.* **2015**, *19*, 2201–2209. [CrossRef]
12. Valles, M.; Mercade, M.; Duran-Sindreu, F.; Bourdelande, J.L.; Roig, M. Influence of light and oxygen on the color stability of five calcium silicate-based materials. *J. Endod.* **2013**, *39*, 525–528. [CrossRef] [PubMed]
13. Felman, D.; Parashos, P. Coronal tooth discoloration and white mineral trioxide aggregate. *J. Endod.* **2013**, *39*, 484–487. [CrossRef] [PubMed]
14. Mozynska, J.; Metlerski, M.; Lipski, M.; Nowicka, A. Tooth Discoloration Induced by Different Calcium Silicate-based Cements: A Systematic Review of In Vitro Studies. *J. Endod.* **2017**, *43*, 1593–1601. [CrossRef] [PubMed]
15. Torabinejad, M.; Parirokh, M.; Dummer, P.M.H. Mineral trioxide aggregate and other bioactive endodontic cements: An updated overview—Part II: Other clinical applications and complications. *Int. Endod. J.* **2018**, *51*, 284–317. [CrossRef]
16. Dean, L. *Blood Groups and Red Cell Antigens*; NCBI: Bethesda, MD, USA, 2005.
17. Guimaraes, B.M.; Tartari, T.; Marciano, M.A.; Vivan, R.R.; Mondeli, R.F.; Camilleri, J.; Duarte, M.A. Color stability, radiopacity, and chemical characteristics of white mineral trioxide aggregate associated with 2 different vehicles in contact with blood. *J. Endod.* **2015**, *41*, 947–952. [CrossRef]
18. Fundaoglu Kucukekenci, F.; Kucukekenci, A.S.; Cakici, F. Evaluation of the preventive efficacy of three dentin tubule occlusion methods against discoloration caused by triple-antibiotic paste. *Odontology* **2019**, *107*, 186–189. [CrossRef]
19. Choi, Y.L.; Jang, Y.E.; Kim, B.S.; Kim, J.W.; Kim, Y. Pre-application of dentin bonding agent prevents discoloration caused by mineral trioxide aggregate. *BMC Oral Health* **2020**, *20*, 163. [CrossRef]
20. Shokouhinejad, N.; Khoshkhounejad, M.; Alikhasi, M.; Bagheri, P.; Camilleri, J. Prevention of coronal discoloration induced by regenerative endodontic treatment in an ex vivo model. *Clin. Oral Investig.* **2018**, *22*, 1725–1731. [CrossRef]

21. Liu, H.C.; Lin, C.P.; Lan, W.H. Sealing depth of Nd:YAG laser on human dentinal tubules. *J. Endod.* **1997**, *23*, 691–693. [CrossRef]
22. Dilsiz, A.; Aydin, T.; Canakci, V.; Gungormus, M. Clinical evaluation of Er:YAG, Nd:YAG, and diode laser therapy for desensitization of teeth with gingival recession. *Photomed. Laser Surg.* **2010**, *28* (Suppl. S2), S11–S17. [CrossRef]
23. Solati, M.; Fekrazad, R.; Vahdatinia, F.; Farmany, A.; Farhadian, M.; Hakimiha, N. Dentinal tubule blockage using nanobioglass in the presence of diode (980 nm) and Nd:YAG lasers: An in vitro study. *Clin. Oral Investig.* **2022**, *26*, 2975–2981. [CrossRef]
24. Jesus, L.S.; Reis, T.; Machado, B.; Costa, A.; Savaris, J.M.; Volpato, C.A.M.; Bortoluzzi, E.A.; Teixeira, C.D.S.; Fernandes, P.; Garcia, L. Dental and composite resin discoloration induced by different hydraulic calcium silicate-based cements: Two-year in vitro assessment. *J. Appl. Oral Sci.* **2023**, *31*, e20220444. [CrossRef]
25. Da Silva, V.A.; Da Silva, S.A.; Pecho, O.E.; Bacchi, A. Influence of composite type and light irradiance on color stability after immersion in different beverages. *J. Esthet. Restor. Dent.* **2018**, *30*, 390–396. [CrossRef]
26. Paravina, R.D.; Ghinea, R.; Herrera, L.J.; Bona, A.D.; Igiel, C.; Linninger, M.; Sakai, M.; Takahashi, H.; Tashkandi, E.; Perez Mdel, M. Color difference thresholds in dentistry. *J. Esthet. Restor. Dent.* **2015**, *27* (Suppl. S1), S1–S9. [CrossRef] [PubMed]
27. Ates, M.O.; Ugur Aydin, Z. Evaluation of the effectiveness of different treatment approaches in preventing coronal discoloration caused by regenerative endodontic treatment. *Clin. Oral Investig.* **2023**, *27*, 4595–4603. [CrossRef] [PubMed]
28. Kahler, B.; Rossi-Fedele, G. A Review of Tooth Discoloration after Regenerative Endodontic Therapy. *J. Endod.* **2016**, *42*, 563–569. [CrossRef] [PubMed]
29. Garcia Lda, F.; Aguilar, F.G.; Rossetto, H.L.; Sabino, M.G.; Pires-de-Souza Fde, C. Staining susceptibility of new calcium aluminate cement (EndoBinder) in teeth: A 1-year in vitro study. *Dent. Traumatol.* **2013**, *29*, 383–388. [CrossRef] [PubMed]
30. Paravina, R.D.; Perez, M.M.; Ghinea, R. Acceptability and perceptibility thresholds in dentistry: A comprehensive review of clinical and research applications. *J. Esthet. Restor. Dent.* **2019**, *31*, 103–112. [CrossRef] [PubMed]
31. Glynis, A.; Foschi, F.; Kefalou, I.; Koletsi, D.; Tzanetakis, G.N. Regenerative Endodontic Procedures for the Treatment of Necrotic Mature Teeth with Apical Periodontitis: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *J. Endod.* **2021**, *47*, 873–882. [CrossRef] [PubMed]
32. Del Fabbro, M.; Lolato, A.; Bucchi, C.; Taschieri, S.; Weinstein, R.L. Autologous Platelet Concentrates for Pulp and Dentin Regeneration: A Literature Review of Animal Studies. *J. Endod.* **2016**, *42*, 250–257. [CrossRef]
33. Zizka, R.; Sedy, J.; Gregor, L.; Voborna, I. Discoloration after Regenerative Endodontic Procedures: A Critical Review. *Iran. Endod. J.* **2018**, *13*, 278–284. [PubMed]
34. Kontakiotis, E.G.; Filippatos, C.G.; Tzanetakis, G.N.; Agrafioti, A. Regenerative endodontic therapy: A data analysis of clinical protocols. *J. Endod.* **2015**, *41*, 146–154. [CrossRef] [PubMed]
35. de Magalhaes, M.F.; Matson, E.; de Rossi, W.; Alves, J.B. A morphological in vitro study of the effects of Nd:YAG laser on irradiated cervical dentin. *Photomed. Laser Surg.* **2004**, *22*, 527–532. [CrossRef] [PubMed]
36. Sesen Uslu, Y.; Donmez, N. The effects on dentin tubules of two desensitising agents in combination with Nd:YAG laser: An in vitro analysis (CLSM and SEM). *Opt. Laser Technol.* **2020**, *129*, 106225. [CrossRef]
37. Xiao, S.; Liang, K.; Liu, H.; Zhang, M.; Yang, H.; Guo, S.; Ding, Y. Effect of Water-Cooled Nd:YAG Laser on Dentinal Tubule Occlusion In Vitro. *Photomed. Laser Surg.* **2017**, *35*, 98–104. [CrossRef]
38. Clearfil SE Bond İnstructions for Use. Available online: https://www.kuraraynoritake.eu/pub/media/pdfs/clearfil-se-bond-instructions-for-use-en-fr-es-it-nl-de.pdf (accessed on 23 January 2024).
39. de Jesus, L.S.; Volpato, C.A.M.; Bortoluzzi, E.A.; da Silveira Teixeira, C.; Rossetto, H.L.; de Carvalho Panzeri Pires-de-Souza, F.; da Fonseca Roberti Garcia, L. Tooth discoloration induced by the different phases of a calcium aluminate cement: One-year assessment. *J. Esthet. Restor. Dent.* **2021**, *33*, 999–1009. [CrossRef]
40. Lan, W.H.; Liu, H.C. Treatment of dentin hypersensitivity by Nd:YAG laser. *J. Clin. Laser Med. Surg.* **1996**, *14*, 89–92. [CrossRef]
41. White, J.M.; Goodis, H.E.; Setcos, J.C.; Eakle, S.; Hulscher, B.E.; Rose, C.L. Effects of pulsed Nd:YAG laser energy on human teeth: A three-year follow-up study. *J. Am. Dent. Assoc.* **1993**, *124*, 45–51. [CrossRef]