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Effect of Final Irrigants on Tooth Discoloration in Regeneration Treatment

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ABSTRACT

This study evaluated the effect of different final irrigation solutions used with blood or PRF scaffolds on crown discoloration during regenerative treatment.

The apical portion of eighty single-rooted teeth was resected. Root canal preparation and irrigation procedures were done, and calcium hydroxide paste was placed into the canals. The teeth were incubated for three weeks at 37 °C. After removing the dressings, specimens were randomly divided into four groups (n = 20) according to the final irrigants used: group 1; NaOCl, group 2; EDTA, group 3; MTAD, and group 4; Qmix. Then, each group was split into two subgroups according to the scaffold (blood or PRF) applied, and teeth were left for incubation at 37 °C. The color measurements were performed at one and three months. Statistical analyses were performed using Two-way ANOVA and Duncan's test.

At one and three months, all irrigant groups showed crown discoloration. The differences among the irrigation groups were not statistically significant in the blood group either in the first or third months. There were no significant differences between NaOCl and EDTA in the PRF-used groups, whereas MTAD and Qmix caused significantly higher discolorations than the other irrigants at both periods.

Under the limitations of this study, from the esthetic point of view, the use of Qmix and MTAD in RET seems unsuitable. When the minimal discoloration effects are considered, the NaOCl may be the first choice to use either with blood or PRF.

Keywords: Regeneration, Tooth Discoloration, Root canal irrigants, Blood, PRF

Introduction

Currently, available treatment choices for immature necrotic teeth are apexification with calcium hydroxide ($CaOH_2$), placement of an apical plug, and regenerative endodontic treatment (1). Endodontic management of these teeth is challenging and poses various difficulties in routine cleaning, shaping, and obturation procedures. The conventional apexification with $CaOH_2$ prolongs the treatment period and cannot increase the thickness of the dentine, which could negatively affect the long-term survival of the teeth. Although the apical barrier (plug) method shortens the treatment period to days and achieves an apical seal, it does not stimulate physiologic root development. Due to these disadvantages, these methods have been largely phased out in favor of regenerative treatment (2). This option is promising for young permanent teeth because it promotes physiologic root development, increases healing capacity, and keeps teeth functional longer (2,3). However, coronal discoloration of teeth that have undergone RETs is a negative result of the protocol.

Since RET consists of a multi-stage procedure, such as minimum canal shaping, irrigation, medicament application, stimulation of stem cells, and hermetical sealing of the root canal, the discoloration can be associated with any of these phases (4). Irrigation solutions, intracanal medicaments, blood product dispersion, and barrier materials used in RETs are all possible sources of discoloration. Therefore, attention should be paid to choosing the material used and its coloring effects.

To the best of our knowledge, no scientific paper has been published that discusses the discoloration ability of various final irrigants in combination with different scaffolds. This study aimed to assess the influence of various final irrigation solutions used with blood or PRF scaffolds on crown discoloration during regenerative treatment.

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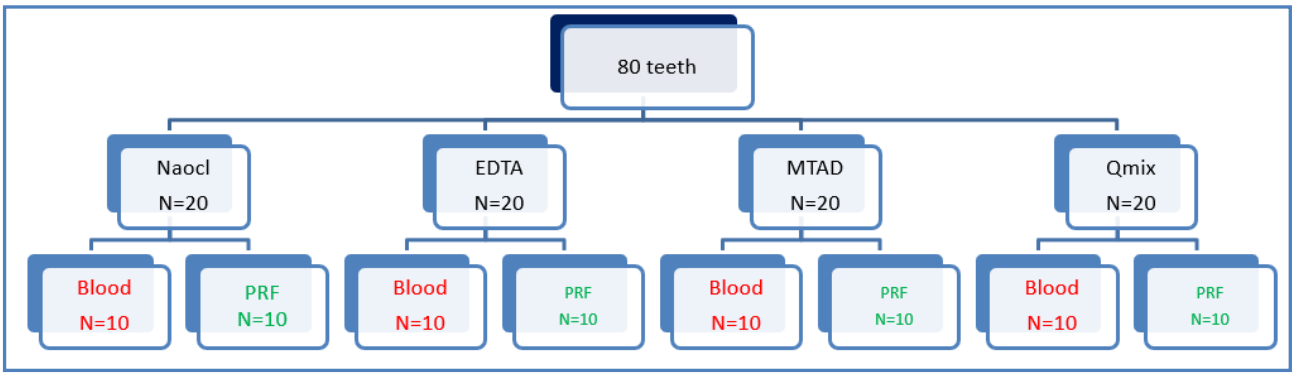

Fig. 1. Schematic Representation of Group Distributions

Materials and Methods

The protocol of this study was approved by the Non-Invasive Clinical Research Presidency of Van Yüzüncü Yil University (date: 12/02/2021, number 2021/02- 07).

Specimen Preparation: In this study, eighty caries-free single-rooted teeth were used. Soft and hard tissue residues covering the crown and root were removed. Teeth were polished through the periodontal pumice powder using a brush on a low-speed handpiece and stored in physiological saline until the experiment. The apical portion of each tooth was resected using a low-speed precision device (Isomed 1000, Buehler, USA) with a diamond disc (Diamond disk DIMOS Ø125 mm, Metkon, Turkey) to obtain a standardized root length (apex to CEJ = 8 mm).

Straight-line endodontic access cavities were prepared and the root canals were instrumented using nickel-titanium rotary files to a size of 30 and 06 taper. Size 4# and 5# Gates Glidden burs were used for extra enlargement (ENDOART, Inci Dental, Turkey) to imitate immature permanent teeth and to facilitate a convenient space for blood or PRF application inside the canals. The apical end of the root was incrementally sealed with a composite resin material (G-aenial composite restorative, GC Dental products Corp, Japan).

Each of the teeth was embedded in silicone impression putty (Zeta Plus, Zhermack, Italy) and placed in a cylindrical plastic mold. At this point, the colors of the teeth was determined using a spectrophotometer (SpectroShade, MHT Optic Research AG, Verona Italy) and the data were recorded as 'original color (t_0)'.

Root Canal Irrigation and Disinfection Procedures: A 5% NaOCl (Sodium Hypochlorite, Microvem, Turkey) was diluted to a 1.5 % concentration. Each root canal was flushed with 20 mL 1.5 % NaOCl and 20 mL 17% EDTA respectively for 5 minutes each. (17% EDTA, IMICRYL, Turkey). The canals were dried using paper points (Absorbent paper points, Pearl Dent Co., Ltd., Vietnam). To minimize the risk of crown discoloration, a bonding agent (G-Premio BOND, GC Corporation, Japan) was applied to the internal walls of the pulp chamber according to the manufacturer's instructions. Calcium hydroxide (Ultracal XS calcium hydroxide paste, Ultradent Products, Inc., USA) was delivered into the canal below the cementoenamel junction (CEJ) using a syringe. Then, the access cavities were sealed with 3-4 mm of a temporary restoration (Caviton, GC ASAHI CORP., Japan). The specimens were then incubated for three weeks at 37 °C in 100% humidity.

Group Distribution and Final Irrigations: The temporary restorations were removed, the canals were irrigated using 20 ml/canal of 17% EDTA to remove calcium hydroxide from the canal, and dried with paper points. The specimens were randomly divided into four groups (n=20) according to the final irrigant used (Figure 1):

NaOCl Group (n=20): 20 ml of 1.5 % NaOCl was used for irrigation. EDTA Group (n=20): 20 ml of 17% EDTA was used for irrigation. MTAD Group (n=20): 20 ml of MTAD was used for irrigation.

Qmix Group (n=20): Before using Qmix, 20 ml of physiological saline irrigation was performed to rinse out the residual NaOCl, then the canals were irrigated with 20 mL of Qmix.

Following the irrigations, each group was separated into two groups (n=10) according to the scaffold type. Half of the specimens in each irrigation group received blood, while the other half received PRF scaffolds (Figure 1).

Scaffold Application and Restoration Procedures: For this purpose, sheep blood samples were provided from Van Yüzüncü Yil University, Veterinary Faculty. Blood scaffolds were prepared and placed as following:

The blood was collected in a blood tube containing 18.0 mg K2E (EDTA) (BD Vacutainer K2E Plus Blood collection Tubes, UK.) and delivered to the canal using a 27 -gauge needle. The entire canal was filled with blood to the cementoenamel junction level and it was allowed to form a blood clot for 15 minutes. A resorbable matrix (Clinisponge, Yüncel Med., Turkey) was placed over the blood clot and biodentine (Biodentine®, Septodont, France) barrier was placed.

PRF scaffolds were prepared and placed as following: The blood was placed in tubes, and PRF was utilized in single centrifugation cycles of 12 minutes at 2,700 rpm (750 g) without using anticoagulation factors. As a result, the blood was split into three layers: the bottom layer was red blood cells, the middle layer was PRF, and the serum was at the top. The platelet-rich fibrin, which appears as a yellow-white extract, was removed from the centrifugation tube with a sterile tweezer and inserted inside the canal. After that, a layer of a resorbable matrix (Clinisponge, Yüncel Med., Turkey) was placed over the PRF matrix; then, biodentine (Biodentine®, Septodont, France) was placed.

The access cavities were restored using a composite resin material (G-aenial, GC Dental products Corp, Japan) according to the manufacturer's instructions.

Discoloration measurements: Color measurement was carried out at one (t_1), and three months (t_2) after regeneration treatment. All measurements were made in the same room on a gray background when the light came at the same angle. During the color measurements, the spectrophotometer device was calibrated at specific intervals. Measurements were performed three times for each sample, and the average L*, a*, and b* values were obtained and the data were recorded.

Statistical Analysis: The sample size was determined according to the data obtained from a pilot study. The value of standard deviation was taken as 3.3. The calculation indicated that ten samples would be sufficient per group (power=80%; type-1α error = 0.05; β = 0.20; effect size=2, and Z=1.96).

Descriptive statistics for the continuous variables were presented as mean, standard deviation, minimum, and maximum values, while count and percentages were used for categorical variables. The normality assumption of the continuous variables was tested with the Kolmogorov-Simirnov test. As the normality was approved, Two-way Factorial Analysis of Variance (Two-way Factorial ANOVA) was performed to compare scaffold and solutions' discoloration levels (2x4). Since the interaction was statistically insignificant, comparisons were performed with main effects. Duncan's multiple comparison test was also used to identify different groups. In addition, Paired t-test was used to compare means of 1 vs 3 months.

The statistical significance level was considered 5%, and the SPSS (ver: 21) statistical program was used for all statistical computations.

Results

Table 1 shows the mean values different groups at one and three months. Table 2 shows the p values between one and three months.

Discoloration Comparisons between Two Time Periods: When the discoloration of the same irrigant at one and three months compared: In blood groups;

MTAD group showed significantly higher discoloration at three months compared to one month (p= 0.005). The difference among the discolorations caused by NaOCl, EDTA, and Qmix irrigants were not statistically significant when two time periods compared (p= 0.878; p= 0.172; p= 0.305).

In PRF groups;

EDTA was the only irrigant not to show a significant difference in discolorations between one and three months (p= 0.558). Other irrigants (NaOCl, Qmix and MTAD) showed significantly higher discolorations at third-month compared to first month (p= 0.08; p= 0.002; p= 0.020).

Discoloration Comparisons at One Month: In blood groups;

There was no statistically significant difference among the irrigant groups in terms of discoloration after one month (p= 0.315). The discoloration intensities caused by irrigants at one month were in the following order: Qmix >EDTA>NaOCl>MTAD

In PRF groups; NaOCl and EDTA caused significantly lower discoloration compared to Qmix and MTAD. Qmix and MTAD groups showed statistically similar discoloration values. No statistically significant difference was detected among NaOCl and EDTA groups.

The discoloration intensities caused by irrigants at one month were in the following order: Qmix>MTAD>EDTA>NaOCl.

Discoloration Comparisons at the Third Month: In blood groups;

There was no statistically significant difference among the irrigant groups in terms of discoloration at the third month (p= 0.484).

The discoloration intensities caused by the irrigants at three months were in the following order: Qmix>MTAD>EDTA> >NaOCl

In PRF groups; Qmix and MTAD caused significantly higher discolorations compared to NaOCl and EDTA. The difference between Qmix and MTAD was not statistically significant. Similarly, there was no statistically significant difference between NaOCl and EDTA.

The discoloration intensities caused by the irrigants at the third month were in the following order: Qmix>MTAD> NaOCl>EDTA.

Discussion

Discoloration of the tooth has been documented as a negative side effect of the materials and processes utilized during RET. Discoloration can occur as a result of irrigation solutions, intracanal medicaments, blood product dispersion, and barrier materials used in RETs (4). Therefore, the staining effect of the materials should be taken in consideration when treating the teeth especially in esthetic zone. In this study, the possible role of different irrigants and their interactions either with blood or PRF and their impact on the esthetic results was assessed.

It was attempted to exclude as much as possible the effect of other potential coloring elements such as intracanal medicaments and barrier materials in this study in order to appropriately analyze the coloring effect of irrigants and scaffolds. In recent years, the European Society of Endodontics (ESE) has issued a guideline recommending the use of calcium hydroxide over TAP, based on TAP's susceptibility to cause discoloration and a lack of enough evidence for antibiotic usage in RET (5,6). For this reason, in this study, calcium hydroxide paste was preferred for intracanal medication. Some calcium hydroxide pastes have also been demonstrated to cause discoloration of tooth. Lehnherr et al (7) compared the staining effects of three Ca (OH)2 prepares (Pure calcium hydroxide, Ultracal XS, and ApexCal) and reported that the capacity of calcium hydroxide dressings to discolor teeth varied according on their ingredients. They stated that Ultracal XS and pure calcium hydroxide caused no discoloration, whereas, Apex-Cal did. The researchers attributed this staining effect to bismuth carbonate, a component of ApexCal's chemical composition (7). Therefore, in our study, Ultracal XS, a non-bismuth preparate, was chosen as the intracanal medicament.

Calcium silicate materials were used previously as barriers to provide a hermetic seal of root canal in RET. Some of these calcium silicate-based cements have a metallic component that can cause discoloration of teeth, like bismuth oxide (8,9). Therefore, a biodentine barrier was used in our study, containing zirconium oxide as an opacifier instead of bismuth oxide to avoid discoloration.

To date, a variety of scaffolds have been used in RET, including blood clots, PRP, and PRF. The blood clot is the most popular approach; unfortunately, it is associated with the discoloration due to hemoglobin metabolism (10,11). For that reason, PRF was recommended as an alternative to the blood clot technique (12). In this study, PRF and blood was used to compare their effects on tooth discoloration and their interactions with different solutions.

Shokouhinejad et al. (10) evaluated discoloration of teeth following RETs using blood clot or PRF. These reseachers revealed that tooth discoloration was significantly greater in blood clot groups than in PRF groups one month later whereas no significant difference was found at 6-month follow-up. In contrast to Shokouhinejad's findings, in this thesis study, no significant difference was found between PRF and blood groups in discoloration after one and three months of RET. This difference between the findings of these two studies could be attributed to the pulp chambers being entirely isolated by bonding, which could have minimized the tubule penetration of blood products in our study.

In RET protocol; the elimination of microorganisms from the canal system is performed widely by NaOCl and EDTA irrigation (13). In our study, NaOCl and EDTA showed similar discoloration with other irrigants (MTAD and Qmix), in blood group, whereas they caused significantly lower discoloration compared to MTAD and Qmix, in PRF group at both time periods. While sodium hypochlorite is a bleaching chemical and is not normally associated with tooth discoloration, it has been shown to cause dentine discoloration when it gets in contact with erythrocytes (14,15). In this thesis study, the statistically insignificant difference among irrigant groups can be attributed to the dominant effect of blood as well as the reactions between irrigants and blood. Besides, in PRF groups, the significantly higher discoloration caused by MTAD and Qmix compared to NaOCl and EDTA can be associated with MTAD and Qmix's ingredients, such as doxycycline and CHX.

In regenerative endodontics, the use of EDTA is recommended as it modulates the biological activities of cells taken from periradicular tissues by releasing bioactive molecules from dentin. There is no evidence in the scientific literature that EDTA alone causes coloration; however, it has been reported that EDTA can disrupt the structure of Biodentine, allow blood to flow through the cement and cause firstly

discoloration of the cement and indirectly the entire tooth (16). In our study EDTA caused discoloration, which is statistically similar to NaOCl at both time periods either with blood or PRF. The mechanism of the discoloration by EDTA in our study could be explained by the aforementioned reaction of the solution with Biodentine.

MTAD is an irrigant composed of a mixture of doxycycline, citric acid, and polysorbate 80 detergent, introduced in 2003 by Mahmoud Torabinejad (17). There is only one study that investigated the usage of MTAD in RET (18). In that study, the reseachers had not attributed the tooth discoloration to MTAD, but to the placement of barrier material (grey MTA) just above the CEJ level. In our study, although Biodentine was used instead of grey MTA, MTAD caused a significant discoloration demonstrating the second highest value among the irrigants used. In contrast to Hung and Torabinejad's consideration, according to our findings, MTAD might be the main cause of staining, and this discoloration could be related to the reaction of MTAD with remaining NaOCl in the dentinal tubules. Similarly, in a much earlier study of Torabinejad et al., a chemical interaction was reported between NaOCl in the root canal and the remaining MTAD in dentinal tubules, which resulted in brown staining (17).

Irrigation duration and volume of the solutions were reported to have a crucial effect on the penetration of irrigants to dentin tubules (19). Although the manufacturer of MTAD recommended 5mL solution per canal in this thesis study, 20 ml of MTAD solution was used, like all other solutions used. It raises the question whether using MTAD at higher than recommended volume caused the significantly higher discoloration in our study. However, similar to our finding, Tay et al. reported a remarkable dark discoloration of dentin after the usage of 5 mL MTAD (20). The presence of discoloration in both studies indicates that the discoloration arising by MTAD could be more closely related to solution's ingredients than volume.

This study indicated that Qmix was the irrigant that induced the highest discoloration among all groups with a statistically significant difference compared to NaOCl and EDTA in PRF groups. We think that the intense coloring caused by Qmix could be a result of the interaction between the biodentin barrier and the product's CHX component. This finding is in line with the findings of Sobhnamayan and Ghanbaran (21), who reported that Biodentine exhibited severe discoloration when immersed in CHX. Shah and Banga similarly reported the discoloration of Biodentin when exposed to CHX. In addition, Keskin et al. confirmed that all calcium silicate-based compounds showed clinically detectable color changes when exposed to sodium hypochlorite or chlorhexidine (22). It is also known that the mixture of NaOCl and CHX results in an orange-brown precipitate due to NaOCl's chlorination of the guanidine nitrogens (23). The interaction between the product's CHX component and the remaining NaOCl in dentinal tubules could have contributed to the intense coloring caused by Qmix in this study.

Under the limitations of this study, all irrigants in this study caused a crown discoloration to some degree, either used with blood or PRF. The discoloration caused by any of the irrigant groups did not show statistically significant different when they took blood or PRF scaffolds. This result highlights the importance of applying a bonding agent to pulp chamber walls.

Qmix and MTAD caused the highest degree of discoloration in both time periods; therefore, our recommendation is to avoid using Qmix and MTAD in the esthetic zone.

When blood was used as a scaffold, statistically similar values were obtained at three or one-month evaluations in all groups. However, NaOCl showed the lowest coloring effect in three months. From this point of view, NaOCl can be the first choice as the irrigant to be used with a blood scaffold.

When PRF was used as a scaffold, EDTA caused the lowest discoloration at three months with no significant difference from NaOCl. Therefore, EDTA and NaOCl, respectively, can be recommended as irrigants when the scaffold is PRF.

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Data availability statement: The data that support the findings of this study are available on request from the corresponding author.

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