Original Article

Potential Crown Discoloration Induced by the Combination of Various Intracanal Medicaments and Scaffolds Applied in Regenerative Endodontic Therapy

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Abstract

Background: Regenerative endodontics involves the use of various root canal medicaments and scaffolds, which may cause crown discoloration. **Aim:** This study aimed to investigate the combined crown discoloration of scaffolds [platelet-rich fibrin (PRF) and blood clot] applied after administration of different medicaments [modified triple antibiotic paste including doxycycline (mTAPd), modified double antibiotic paste (mDAP), calcium hydroxide (CH), and propolis]. **Methods:** In total, 100 human mandibular premolar teeth were selected and prepared. The teeth were apically resected to simulate immature teeth. The positive and negative control groups (n = 10) consisted solely of blood-only and serum-only samples. The remaining 80 teeth were used for the experimental groups with four different medicaments. Three weeks later, either blood or PRF was applied as a scaffold after removing the medicaments (n = 10). Color changes were assessed before medication placement and at the end of the first, second, and third weeks, as well as on days 0, 1, 30, 60, and 90 after scaffold application. Analysis was carried out using repeated measures of variance, Friedman, one-way ANOVA, Kruskal-Wallis, the dependent paired *t*-test, and Wilcoxon test. **Results:** Statistical significance was determined at $P = 0.05$. All groups including blood and the group including propolis and PRF combination, resulted in a significant increase in discoloration ($P < 0.05$) and discoloration exceeding clinically acceptable thresholds. **Conclusions:** CH and the modified versions of TAP (mTAPd) and DAP (mDAP) demonstrated an acceptable level of discoloration when used with a combination of PRF at day 90.

Keywords: *Double antibiotic paste, platelet-rich fibrin, propolis, tooth discoloration, triple antibiotic paste*

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Introduction

Pulp necrosis caused by caries, trauma, periodontal disease, or inadequate dental treatment is prevalent worldwide.[1] The consequences of pulp necrosis include the loss of vital odontoblasts in teeth and the prevention of dentin formation and root development at the root tip, resulting in thin dentin walls.[2]

With the advancement of tissue engineering, there has been a proposal for regenerative endodontic treatment (RET) as an alternative to conventional apexification, which was previously suggested for permanent teeth with necrotic pulp that possess an open apex.[3] RET refers to clinical procedures that aim to substitute the damaged structure of the root and dentin with the cells of the pulp–dentin complex.[4]

Root canal disinfection and stem cell delivery are the main components of RET.[5] The use of intracanal antibacterial medicaments to achieve a sterile biological

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environment without microorganisms is crucial for the long-term success of RET.[6] However, crown discoloration has been identified as the primary treatment complication in 44%–62% of studies on RET due to the use of intracanal medicaments, scaffolds, or calcium silicate materials.[7]

Triple antibiotic paste (TAP) is often administered in high concentrations (600–1000 mg/mL) to achieve the paste-like consistency that remains in the root canals during the disinfection process. However, the clinical use of TAP in high concentrations has been linked to minocycline-associated tooth discoloration.[7] Various alternatives, such as doxycycline, amoxicillin, cefaclor, clindamycin, and double antibiotic paste (DAP), have been proposed instead of minocycline because they cause less discoloration.[8] The American Association of Endodontists has recently advocated for the use of TAP at a concentration of 1–5 mg/mL.[9] In accordance with the provided guidelines, recent research has shown that modified TAP (mTAP), a mixture of methylcellulose gel and 1 mg/mL TAP, is an effective modification.[7] To further decrease the coloration in the mTAP paste, it is possible to prepare mTAP pastes with doxycycline (mTAPd) rather than minocycline.

Calcium hydroxide is a highly recommended intracanal medicament for both multi-visit root canal treatment and RET. Its antimicrobial activity is weaker than that of TAP or DAP.[10] However, it has been demonstrated to stimulate stem cell proliferation[11] and lead to less discoloration.[12]

With the recognition of the efficacy of natural products and their extensive application in medicine, it has been considered that they may also be used in dentistry.[13] Propolis, which is employed by bees to disinfect their hives, is one such product. A study has revealed that propolis is effective against inflammation, bacteria, tumors, and fungi.[14] In light of recent studies, it appears feasible to use propolis as an intracanal medication for regenerative endodontic procedures.[15,16]

According to the regenerative endodontic protocol, after disinfection, various natural or artificial scaffolds are inserted into the canal to place the stem cells and growth factors. Blood clots or blood concentrates are often used as scaffolds for this purpose. Studies have indicated that platelet-rich fibrin (PRF), a type of platelet-rich blood concentrate, does not lead to tooth discoloration because it does not contain eritrosit.[17] However, there is an information gap regarding whether the combination of modified antibiotic pastes and scaffolds, such as PRF and blood, worsens crown discoloration. It is important to assess the potential interactions between these components to improve the understanding of their combined effects and to optimize clinical outcomes. Thus, the aim of this study was to investigate the level of crown discoloration caused by the application of blood and PRF scaffolds after mTAPd, mDAP, CH, and propolis medications.

Subjects and Methods

The study report was prepared in accordance with the Preferred Reporting Items for Laboratory Studies in Endodontology 2021 guidelines.[18,19] Furthermore, the Clinical Research Ethics Committee of Kirikkale University approved the study with certificate number 2021.10.07, dated October 7, 2021. The Animal Hospital of the Faculty of Veterinary Medicine at Kirikkale University received ethical approval on June 26, 2021 (reference number: 2021/06-26). The sample size was calculated using G*Power v3.1 (Heinrich Heine, University of Düsseldorf, Germany) at a significance level of 0.05, an effect size of 0.40, and a power of 0.95. In total, 100 mandibular premolar teeth with fully formed apices, extracted for orthodontic reasons from patients aged between 18 and 25 years, were collected. Only teeth with single root canals and without anatomical malformations or caries were included. The teeth were rinsed with phosphate-buffered saline (Gunduz Chemistry, Istanbul, Turkey) immediately after extraction. Then, the periodontal tissues were removed from the root surface by using a periodontal curette. The teeth were polished with pumice and water. The samples were immersed in a 0.1% thymol solution at 4°C for 48 hours and stored in distilled water at 4°C until required.

Preparation of root canals

The teeth were numbered from 1 to 100. A random number generator (http://www.randomizer.org) was then used to generate 10 sets with 10 numbers in each set. Each number appeared only once in all sets. The teeth were distributed to the groups so that the predetermined tooth numbers matched the numbers in the set.

The samples were apically resected by measuring a 10-mm section from the buccal cementoenamel junction (CEJ) by using water-cooled sterile diamond burs to simulate the formation of immature roots and obtain a standardized root length. The access cavity was prepared using a round diamond bur on the occlusal surface. The root canals were enlarged using gates-glidden burs numbered 1–4 following the method of Chen *et al*.[20] Subsequently, 0.9% saline (sodium chloride solution; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used to irrigate the canals between the gates-glidden burs, and #40 paper points were used to dry them. Finally, the root-end was sealed

using a self-etch adhesive (Gluma Self Etch, Kulzer, Germany) and a composite (Z250, 3M ESPE, St. Paul, MN, USA).

Twenty teeth were allocated to the positive and negative control groups (n = 10). The remaining 80 teeth were distributed into four groups based on the intracanal medications to be administered (n = 20): group 1 (mTAPd), group 2 (mDAP), group 3 (calcium hydroxide [CH]), and group 4 (propolis). Color measurements were taken at four different intervals: prior to intracanal medicament placement (T0), at week 1 (T1), at week 2 (T2), and at week 3 (T3). They were stored in an incubator at 100% humidity and 37°C between each measurement until the scaffold was applied. The teeth were re-accessed, and the Teflon tape was removed. The irrigation protocol of RET was performed by applying passive ultrasonic irrigation activation (IF43727, K 25/25, Satelek, Acteon, Merignac Celdex-France) with 20 mL of 1.5% NaOCl, 20 mL of 17% EDTA, and 20 mL of distilled water for 5 minutes each using a 25-gauge disposable plastic injector (Berika Technology Medical, Konya, Turkey). After the medicaments were completely removed, a color measurement was taken before the scaffolds were placed on day 0 (T4). Then, each group was divided into two subgroups based on scaffold type (blood or PRF) (n = 10). The color measurements were taken at four different intervals, namely day 1 (T5), day 30 (T6), day 60 (T7), and day 90 (T8), following scaffold placement [Figure 1]. In the control groups, no application was performed. Blood was kept in the positive control group and serum in the negative control group. The teeth were stored in plastic containers filled with distilled water in an incubator at 37°C and 100% humidity throughout the experiment.

Preparation of medicaments and scaffolds

The antibiotic mixture was prepared using powdered metronidazole (Flagyl, Sanofi, Kırklareli, Turkey), ciprofloxacin (Cipro, Biofarma, Istanbul, Turkey), and doxycycline (Monodoks, Deva, Tekirdag, Turkey). In group 1, mTAP was mixed at a ratio of 1:1:1. In group 2, DAP was mixed only with metronidazole and ciprofloxacin at a ratio of 1:1. The dose was adjusted to 1 mg/mL by adding 1 mL of water to 1 mg of the mixture. To allow for the application of the antibiotic paste to the canal, a gel was formed by adding 2% (weight/volume) methyl cellulose (MC, MW 88,000 g/mol, Acros Organics, New Jersey, NJ, USA) to the mixture and using a magnetic stirrer (300 rpm) for 24 hours to ensure proper mixing. Medicaments were injected into the canal using an injector. In group 3, UltraCal XS (Ultradent, USA) was injected into the canal up to 4 mm below the CEJ using its own injector tip. In group 4, pure poplar propolis dissolved in ethanol (Nutral Therapy, Kayseri, Turkey) with one drop of glycerin per 150 mg, as described by El-Tayeb *et al*.,[21] was added and brought to a paste consistency and applied into the canal with an injector.

In the first subgroup, blood was applied as a scaffold similar to the positive control group, but the access cavity was sealed with glass ionomer cement (SDI Riva Self Cure, Australia). In the second subgroup, 10 mL of blood was centrifuged at 2700 rpm for 12 minutes to obtain PRF and placed as a scaffold in the root canal. A sterile scalpel was used to cut a 5-mm PRF, which was placed up to 4 mm below the CEJ with a plugger. Finally, a collagen membrane (TutoPatch Tissue Matrix, Germany) was covered on the PRF. The access cavity was sealed with glass ionomer cement (SDI Riva Self Cure, Australia).

In the positive control group, non-anticoagulated blood from healthy sheep was used. A volume of 0.2 mL of blood was injected into each tooth 4 mm below the CEJ with care taken to prevent the blood from reaching the access cavity. Following a 15-minute coagulation period, a collagen membrane (TutoPatch Tissue Matrix, Germany) was placed on the clot. In the negative control group, serum alone was injected into the canal instead of the blood. Small pieces of Teflon tape were placed on the CEJ of all teeth, and the access cavities were sealed with temporary filling material (Cavit G, 3M ESPE, Germany). The specimens were assigned numbers and put into small plastic containers filled with distilled water; the water was changed once a week.

Color measurements

Two different methods were used to determine the color change of the samples. The first method used a spectrophotometer (Vita Easy Shade Compact, VITA, Germany). Measurements were taken in a neutral gray 70 cm × 50 cm × 50 cm cabinet under D65 daylight conditions. The buccal surface of the crown 2 mm above the CEJ was consistently subjected to a standardized circular strip diameter of 6 mm to ensure that measurements were taken from the same area at every time interval and at a vertical position.

Three measurements were taken for each tooth, and the average of the L*a*b* color values under the Commission International de I'Eclairage's (CIE) system was recorded. The spectrophotometer was calibrated according to the manufacturer's instructions for each measurement. For digital camera measurements, a single-lens reflex camera (D5300, Nikon Corp), 105 mm macro lens (Nikon Corp), external twin flash system (model R1C1; Nikon Corp), and cross-polarized

filter were used. Photographs were taken in RAW format from a distance of 35 cm with a setting of 1/160 f 25, ISO 200, and in a neutral gray color cabinet under D65 daylight illumination. A gray card (WhiBal G7, Michael Tapes Design, USA) was used to standardize the white balance of the photographs.

Photographs were processed using the Adobe Photoshop program (Adobe Systems Inc., 2020, USA). L*a*b* values were measured at five different points on the template applied to the tooth using the Digital Colorimeter program (v. 1.5.0. Pro version, Luca Lindholm, MS Apps, USA) and recorded by taking the average of the data.

The data were converted into three parametric values of the CIE L*a*b* color system. The resulting ΔE values were calculated using the following formula:

$$\Delta E = [(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2]^{1/2}$$

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Statistical analysis

The data were analyzed using SPSS (version 23.0; IBM, Armonk, NY, USA). The data were assessed for normal distribution using Kolmogorov–Smirnov and Shapiro–Wilk tests. Repeated analysis of variance (ANOVA) was used to compare the normally distributed data by time within groups, and the Friedman test was used to compare the non-normally distributed data. The Kruskal–Wallis test was used to compare the groups, and multiple comparisons were analyzed by means of the Dunn test. One way ANOVA was used to compare normally distributed data between groups, and multiple comparisons were analyzed using Tukey's HSD and Tamhane's T2 tests. The dependent two-sample *t*-test was used to compare the methods, and the Wilcoxon test was used to compare non-normally distributed data. The significance level was set at $P < 0.05$. Spearman's correlation coefficient was used to examine the consistency between the methods.

Results

Similar results were obtained in the color measurements between the spectrophotometer and digital camera at day 90 ($P > 0.05$). A positive, moderate, and significant correlation was found between the methods ($r = 0.521$, $P < 0.001$). The use of propolis in the medication protocol resulted in significant discoloration (ΔE values exceeded 3.7), while no statistically significant differences were observed between the other medicaments ($P > 0.05$). The blood-only positive control group exhibited the highest discoloration values, while the serum-only negative control group recorded the lowest discoloration values [Figure 2a].

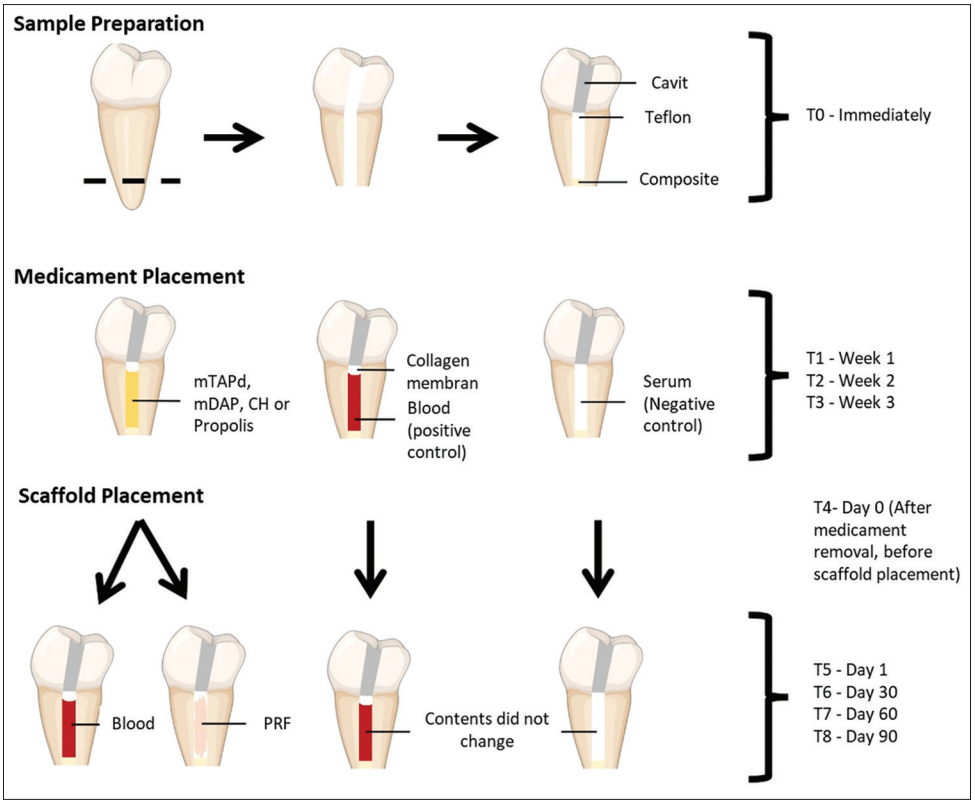

Figure 1: Sample preparation, medicament and scaffold placement, and measurement periods of discoloration in the study. mTAPd: modified triple antibiotic paste containing doxycycline, mDAP: modified double antibiotic paste, CH: Calcium hydroxide, PRF: Platelet-rich fibrin

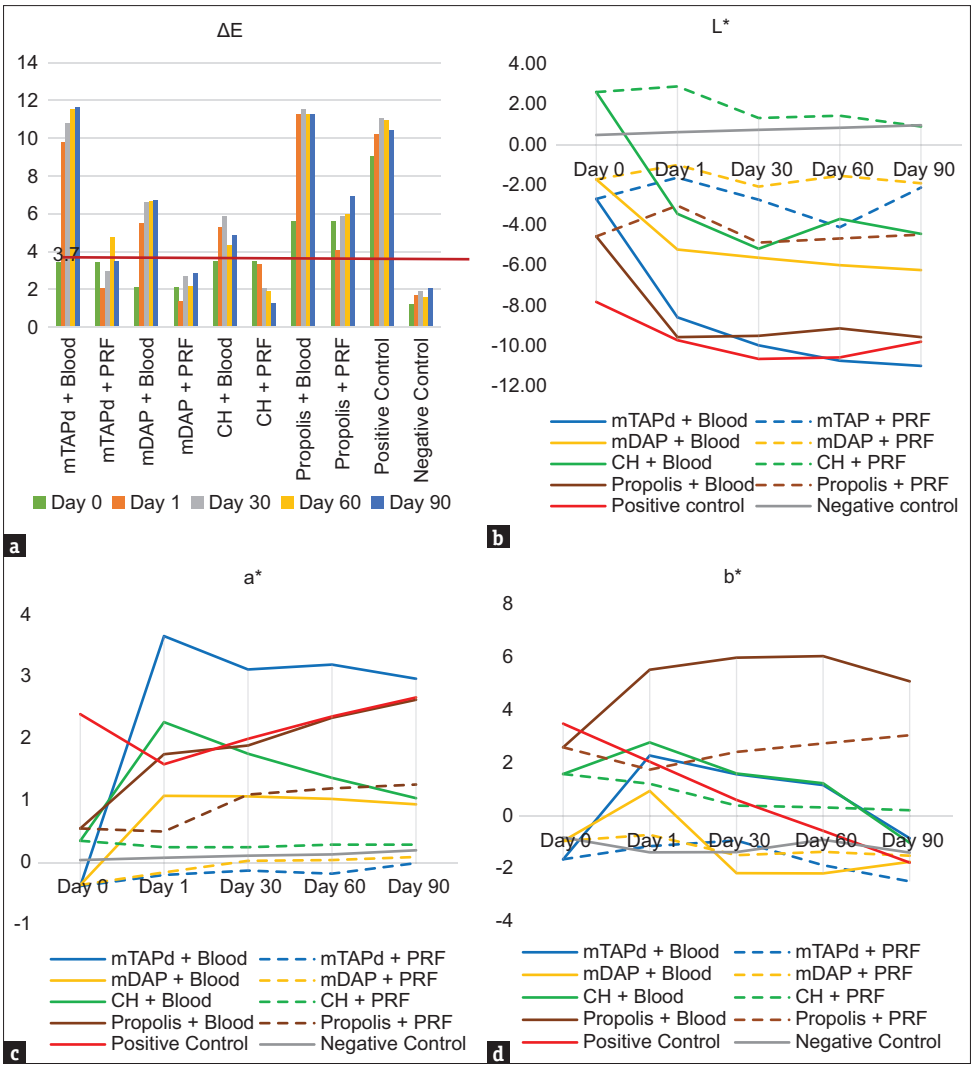

Figure 2: (a) CIE parameter - ΔE value: change in the ΔE value indicates the difference in color, which was calculated using the CIE L*a*b* values. (b) CIE parameter - L* value (luminosity), (c) CIE parameter - a* value (red-green parameter), (d) CIE parameter - b* value (yellow-blue parameter) after scaffold application over time. Red line in Figure 2a shows the clinically acceptable discoloration limit (ΔE = 3,7)

After the application of the scaffold, a visual color change was observed in all groups when blood was present. In addition, statistical significance was observed when comparing the different materials in the presence of blood ($P < 0.05$). Furthermore, color changes were seen solely in the presence of PRF when propolis was combined with this scaffold only [Figure 2]. A significant difference was noticed between the medicaments in the PRF group ($P < 0.05$). ΔE values among all blood groups from T4–T8 ranged from 4.85–11 at the end of day 90. It was 1.26–6.91 for the PRF groups. The mTAPd or propolis applied with the blood scaffold caused more discoloration than the positive control group but had no significant difference ($P > 0.05$). Color changes in all groups are shown in Figure 3.

The lightness values (L*) in the CIE color system represent the lightness or darkness of the surface studied. In this study, all groups became darker over time in the presence of blood. Statistical significance was also determined for both a* and b* values ($P < 0.05$).

Discussion

A spectrophotometric evaluation is a reliable technique for detecting changes in color. It applies standard constants to provide a numerical value for color. Investigator bias is minimal for this device.[20] Digital photography, achieved using cross-polarizing filters, provides optimal, objective information on the color, texture, and anatomy of teeth. This is critical for clear communication to achieve ideal restorative outcomes.[22] When using a spectrophotometer and a digital camera in combination, researchers have found that the results are reliable.[22,23]

CIE is an ISO-recognized organization as the international standardization body for light, vision, and color.[24] The CIE L*a*b* system is a three-dimensional, uniform color space designed to approximate the

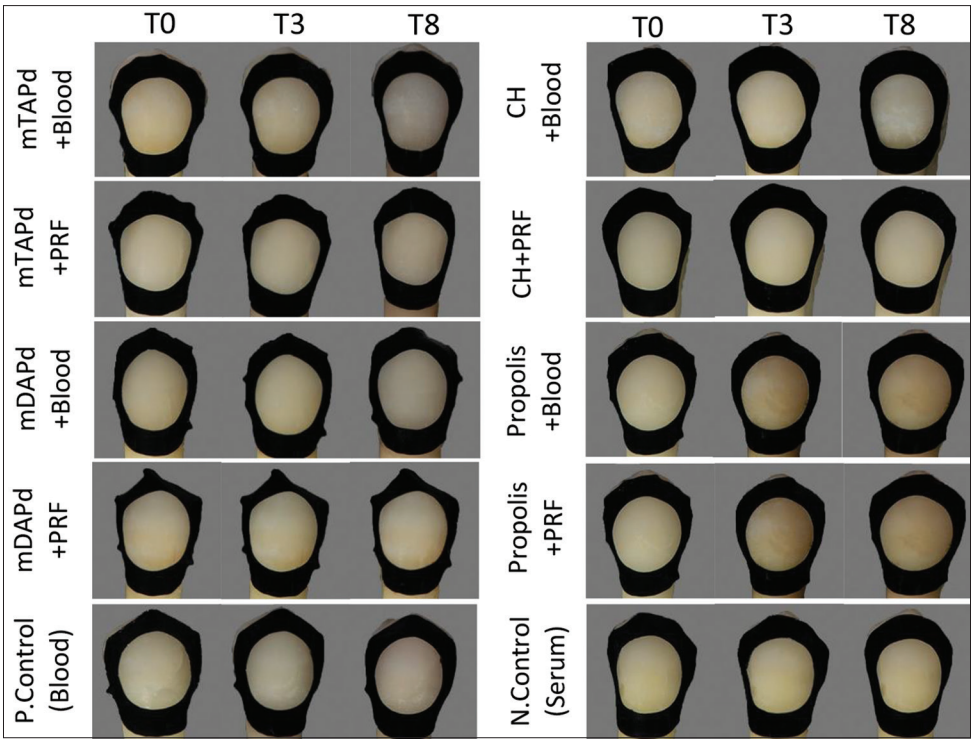

Figure 3: The color of teeth before medication (T0), after 3 weeks (T3) with medication, and after 90 days (T8) with scaffold application showing the pattern of discoloration

perceptual response of the human eye to all perceivable colors. The position of a color in the CIE L*a*b* color space is defined by three chromatic parameters. The value of L* indicates lightness (L* = 0 [black] and L* = 100 [white]). The a* and b* values indicate the position on the green (-a) to red (+a) and blue (-b) to yellow (+b) gradients.[24] The changes ΔL, Δa, and Δb are determined by calculating the difference between the L*, a*, and b* values obtained at different time intervals. The magnitude of the color difference between two objects can be measured quantitatively using Euclidean distance in ΔE values. A difference greater than 3.7 ΔE values is perceivable as a change in color.[25]

Methylcellulose is one of the materials recommended as a thickening agent for the preparation of low-concentration antibiotic pastes and a synthetic cellulose polymer used as a carrier for the application of intracanal medication.[26] The aim of these materials is to achieve injectable consistency while providing a slow and prolonged release of antibiotics through the hydrogel.[27] Previous research has demonstrated that high concentrations of antibiotics used in root canal treatment may be harmful to stem cells in the apical papilla.[28,29] In addition, lower concentrations of antibiotics have been proposed as a means to prevent tooth discoloration.

AAE recommended a concentration of 1–5 mg/mL for mTAP based on its non cytotoxic effect on stem cells and beneficial impact on regeneration.[9] The use of low concentrations of mTAP has been reported to significantly reduce discoloration but not completely prevent it.[7,30] Modified TAPs containing antibiotics, such as doxycycline, cefaclor, amoxicillin, amoxicillin, and clindamycin, have been proposed as a means of preventing coloring and maintaining antibacterial properties without using minocycline.[30] Sabrah *et al*.[7] reported that TAP caused more discoloration than mTAP.

This study investigated the combined effects of different medicaments and scaffolds on crown discoloration because of the limited information in the literature. The findings indicated that the mTAPd and propolis groups showed the highest degree of discoloration when blood was used as the scaffold at the end of day 90. When the PRF scaffold was used, both mTAPd and mDAP resulted in the same degree of coloration, while CH displayed less coloration. Furthermore, the level of discoloration caused by propolis exceeded the clinically approved threshold, even when used with PRF. In a study by Yaghmoor *et al*.,[12] mDAP prepared with methylcellulose at a concentration of 1 mg/mL and mTAP containing minocycline were compared with calcium hydroxide, and all medicaments caused coloration below the clinically acceptable limit (ΔE = 3.7) at the end of week 4, but the CH group had significantly more coloration than the mTAP and mDAP groups. Betancourt *et al*.[30] conducted a study using CH and mTAPd containing doxycycline at

a concentration of 0.1 mg/mL. The results indicated that although the highest ΔE value was observed in mTAPd, no significant difference was found between the groups. In one study, the ΔE color changes resulting from mTAPd and mDAP medications were evaluated at a concentration of 0.1 mg/mL. The mTAPd group exceeded the clinically acceptable limit, while the mDAP group remained below it at the end of the third week.[8] Sabrah *et al*.[9] reported that the mTAP group caused more coloration than the mDAP group and significantly decreased the L* value according to ΔE values in teeth in which mTAP and mDAP were applied to root canals at a rate of 1 mg/mL.

After irrigation procedures to remove the medication from the dentinal tubules, bleeding is clinically initiated from the apical side to coagulate within the canal and form a scaffold. In cases where bleeding cannot be attained, PRF is applied.[31] However, it is acknowledged that blood within the root canal may affect the color of the crown.[32] In necrotic teeth with an open apex, PRP or PRF has been shown to promote thickening of the root canal walls and continued growth of the root, with no significant difference compared to a blood clot.[33] Unfortunately, very few studies have explored how the application of blood or PRF as a scaffold after intracanal medicament application affects coloration. Shokouhinejad *et al*.[17] applied 1 mg/mL of blood and PRF to the canal after a concentration of mDAP medication. It was observed that blood exhibited more coloration than PRF, similar to our results.

Recent research has demonstrated the effectiveness of propolis as an intracanal medicament.[34] The potential tooth discoloration of propolis consists of a few studies that provide limited information.[35] In this study, the impact of propolis on discoloration when used as an intracanal medicament was investigated. As a medicament, propolis caused more discoloration than mTAPd, mDAP, and CH. This may be due to its low pH (4.2) and the natural components and pigments that accumulate in the tubules after demineralization of the dentinal tubules, such as the TAP medicament.

The limitations of this study include the possibility of insufficient removal of medication before scaffold placement, the inability to standardize the initial crown color in extracted human teeth due to teeth belonging to different individuals, the evaluation of extracted teeth only according to devital pulp conditions, and the inability to adequately compare the results as there is no study in the literature in which both medication and scaffold were used consecutively in the root canal.

In conclusion, mTAPd, mDAP, and CH applications exhibited acceptable levels of discoloration when used with PRF. Propolis surpassed the clinically approved threshold, even when used with the PRF combination. This suggests that propolis may not be an appropriate substitute when combined with scaffolds or alone. Further research is necessary to examine the different concentrations of modified root canal medicaments and scaffolds that retain microbial efficacy and stem cell viability while preventing discoloration in endodontic regeneration applications.

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Conflicts of interest

There are no conflicts of interest.

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