

Comparison of Crown Discoloration with Triple Antibiotic Paste and Minocycline Paste Using Spectrophotometric Analysis



Medical Science

KEYWORDS : Discoloration, intracanal medicament, minocycline, spectrophotometric analysis, triple antibiotic paste.

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ABSTRACT

Aim: We evaluated the degree of crown discoloration induced by two different antibiotic pastes. Methodology: Ninety-five extracted human incisors were prepared to size #30. After pulp tissue removal, shaping and irrigation procedures, the specimens were randomly divided into two groups (n=45), which received either triple antibiotic paste or minocycline paste as intracanal medicament. In five teeth calcium hydroxide was used as a control group. Spectrophotometric readings were obtained on the buccal surfaces of the crowns on day 0 to week 4 after dressing, and the differences in color were recorded. Data were analysed with the Mann-Whitney U and Wilcoxon sign tests ($P < 0.05$). Results: Both groups showed statistically significant coronal discoloration from day 0 to weeks 1, 2, 3 and 4 ($P < 0.01$), but their final shades did not significantly differ between the groups ($P > 0.05$). Conclusions: Both antibiotic pastes induced crown discoloration, and neither was superior.

INTRODUCTION

The most important success factor in endodontic treatment is to obtain a bacteria-free root canal system (1). To disinfect an infected root canal system, the first step is to eliminate the bacteria using a chemomechanical preparation. However, this limits but does not totally prevent regrowth of endodontic bacteria (2). Therefore, the use of intracanal medicaments may also be necessary (1).

Calcium hydroxide ($\text{Ca}(\text{OH})_2$) has antibacterial activity and the potential to stimulate the formation of hard tissue, and is considered the best material among intracanal medicaments (3). It can also dissolve organic tissue and shows anti-inflammatory effects (4). Its antimicrobial activity, due to its high pH, is determined by the release of hydroxyl ions. Direct or indirect contact in dentinal tubules for an optimal amount of time is required for effective destruction of microorganisms (5).

However, clinical studies have shown that it is not possible to sterilise root canals in necrotic teeth, even with $\text{Ca}(\text{OH})_2$ (6, 7). Therefore, new therapeutic agents, such as chlorhexidine and antibiotics, and natural products have been suggested as alternative intracanal medicaments (8).

The traditional triple antibiotic paste (TAP) consisting of ciprofloxacin, metronidazole, and minocycline was developed by Hoshino *et al.* (9). Several reports have confirmed the antimicrobial properties of this mixture in infected root canals (10-12). Furthermore, Gomes-Filho *et al.* (13) investigated TAP over various experimental periods and found it to be biocompatible. Despite these positive features, several case reports have shown that minocycline causes visible crown discoloration (14, 15).

Spectrophotometers are commonly used in dental studies to quantify tooth discoloration (staining or bleaching effects) (16, 17) and color changes in dental materials such as ceramics (18, 19), but they have not been used to evaluate color changes possibly caused by intracanal medicaments containing antibiotic pastes.

To the best of our knowledge, no studies have compared the ef-

fects of different antibiotic combinations used as intracanal medicaments on crown discoloration. Therefore, we used spectrophotometric techniques to assess the coronal discoloration potential of traditional TAP and minocycline paste (MP) prepared with saline solution.

MATERIALS AND METHODS

Ninety-five single-rooted human maxillary central incisors with single canals were selected, and root surfaces were cleaned with curettes to remove remnants of periodontal tissue. They were disinfected by immersion in 0.5% Chloramine-T solution (Sigma-Aldrich, Taufkirchen, Germany) for 48 h. Standard access preparation was carried out using high-speed #2 diamond burs (Acurata, Thurmansbang, Germany) and a water spray. The pulp tissue was removed, and the working length was estimated to be being 1 mm short of the radiological apex. The root canals were enlarged to size #30 using ProTaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) rotary instruments in a crown-down technique in combination with a torque-controlled engine (Anthogyr; Anthogyr Inc. Sallanches, France) at 250 rpm according to the manufacturer's instructions. The apical portions of the teeth were sealed with composite resin, and the outer surfaces of the roots were coated with fingernail varnish. The specimens were embedded in an acrylic resin block. The root canals were irrigated using 2 mL 5.25% NaOCl between all instrument changes. The smear layer was removed by irrigations with 10 mL 5.25% NaOCl, 17% EDTA, and 5.25% NaOCl. Then a final rinse was performed with 10 mL distilled water. Following this procedure, the canals were dried with sterile paper points and the teeth were divided randomly into two groups of 45 teeth each. Two different intracanal medicaments were used. In Group 1 (TAP group), the specimens received equal portions of metronidazole (Eczacıbasi, İstanbul, Turkey), ciprofloxacin (Biofarma, İstanbul, Turkey), and minocycline (Ratiopharm, Ulm, Germany) (1:1:1) mixed with saline solution at a powder/liquid ratio of 1:2 to obtain a creamy paste. In Group 2 (MP group), the specimens received equal portions of minocycline mixed with saline solution at a powder/liquid ratio of 1:2. Five teeth received calcium hydroxide as a control group.

The intracanal medicaments were placed into the root canals

using a #25 lentulo spiral (MANI, Inc. Tokyo, Japan) at slow speed, just below the cemento-enamel junction (CEJ). Then the teeth were coronally sealed with a cotton pellet and temporary filling material (Cavit, 3M ESPE, Neuss, Germany). All samples were stored at 100% humidity in an incubator at 37°C for 4 weeks in a dark environment.

Color measurements were performed immediately after root canal preparation (on day 0) and at 1, 2, 3 and 4 weeks after treatment. Thus, each specimen had its own control at each time point. A standardised circular strip with a diameter of 5 mm was bonded to the buccal surface of the crown 2 mm above the CEJ to ensure that color measurement was performed on the same region at every turn. Measurements were carried out in the same room and same lighting conditions each time to achieve standardisation. The color of each specimen was assessed using a spectrophotometer (VITA Easyshade, Bad Säckingen, Germany) on the buccal surfaces of the crown. Before each measurement, the spectrophotometer was calibrated according to the manufacturer's instructions. The color measurements were performed three times at each time point on a white background, and the mean was calculated. We analysed the discoloration measured on day 0 and at weeks 1, 2, 3 and 4 for each tooth. Differences in shade were determined by the VITA Toothguide 3D-Master scale.

Statistical analyses were performed using the IBM SPSS Statistics 22 (IBM SPSS, Istanbul, Turkey) software. Intergroup comparisons were performed using the Mann-Whitney *U* test, and the Wilcoxon sign test was performed to identify significant differences between the groups. The level of significance was set at $P < 0.05$.

RESULTS

Coronal discoloration was observed in each group over time; however, color shade did not significantly differ ($P > 0.05$) between the groups at any time point (Table 1). Within each group, there was statistically significant ($P < 0.01$) discoloration between day 0 and weeks 1, 2, 3 and 4; between week 1 and weeks 2, 3 and 4; between week 2 and weeks 3 and 4; and between weeks 3 and 4. The TAP group showed slightly more (but non-significant) discoloration on weeks 1, 3 and 4 than the MP group. The MP group had slightly more discoloration on week 2. In the control group, teeth filled with calcium hydroxide, no discoloration was observed.

Table 1. Evaluation of discoloration in each study group over time (VITA Toothguide 3D-Master scale).

	TAP Mean \pm SD (median)	MP Mean \pm SD (median)	P
Week 1	1.84 \pm 1.02 (2)	1.58 \pm 0.54 (2)	0.529
Week 2	2.98 \pm 1.64 (2)	3.24 \pm 1.54 (2)	0.344
Week 3	4.47 \pm 1.53 (5)	4.33 \pm 1.49 (5)	0.797
Week 4	5.36 \pm 1.49 (6)	5.29 \pm 1.25 (6)	0.760
Weeks 1–2	1.13 \pm 1.18 (1)	1.67 \pm 1.71 (1)	0.338
Weeks 1–3	2.62 \pm 1.42 (3)	2.76 \pm 1.65 (3)	0.669
Weeks 1–4	3.51 \pm 1.32 (4)	3.71 \pm 1.44 (4)	0.355
Weeks 2–3	1.49 \pm 1.34 (1)	1.09 \pm 1.4 (1)	0.136
Weeks 2–4	2.38 \pm 1.39 (2)	2.04 \pm 1.76 (2)	0.316
Weeks 3–4	0.89 \pm 0.96 (1)	0.96 \pm 1.3 (0)	0.705

Mann-Whitney *U* Test

DISCUSSION

Bacteria play an important role in pulpal and periapical infections. They can exist both within the root canal and in other regions such as the dentinal tubules, accessory canals, canal ramifications, apical deltas, fins, and transverse anastomoses

(20). However, during mechanical instrumentation and irrigation procedures, it is impossible to access these areas. Therefore, medicaments should be used to eliminate the bacteria in these inaccessible areas. Ca(OH)₂ is the most commonly used and most popular endodontic medicament in the world (8). However, its low solubility and diffusibility could limit its ability to rapidly increase the pH to the level necessary to eliminate bacteria within the dentinal tubules (8, 21). Hence, new-generation intracanal medicaments have been investigated. One of the most promising agents is TAP.

TAP has been widely used in routine endodontic therapy, regeneration cases, persistent cases with periradicular lesions, and root resorptions since it was developed by Sato *et al.* (1996) (10, 22). However, it also has some disadvantages; for example, it is difficult to remove from the root canal (23, 24), there is uncertainty about the optimal mixing ratio, and it can lead to tooth discoloration (25).

Crown discoloration during endodontic therapy is a major aesthetic problem, especially for anterior teeth. Root canal obturation materials and intracanal dressing materials are the major causes of discoloration. Lenherr *et al.* (25) investigated the discoloration potential of endodontic materials such as white MTA, Portland cement, Ca(OH)₂, TAP, Ledermix paste, AH Plus and blood using a bovine tooth model for a 1-year time period. Discoloration was observed mostly in the TAP and Ledermix groups. It is unknown whether the cause of this discoloration was the combination of antibiotics used or one of the antibiotics individually. Akçay *et al.* (12) observed the crown discoloration of bovine incisors that was induced by TAP and TAP derivatives including doxycycline, amoxicillin, and cefaclor instead of minocycline. TAP with minocycline, doxycycline, and cefaclor induced more discoloration compared to a control group that received no dressing. However, all of the procedures, including the removal of pulp tissue, irrigation, placement of antibiotic pastes, and placement of the cotton pellet and temporary filling material, were performed from the apical aspect to avoid disruption of the intact crown and to prevent coronal microleakage. Because an intact crown will affect the chroma and the color of the tooth, this method does not reflect routine endodontic treatment.

In light of the previous studies, tetracycline is proven to be the mostly responsible antibiotic for the discoloration of the dentin structure (14, 26). These materials were used in the present study which was aimed to investigate if minocycline caused more discoloration than TAP when used alone in the root canal. However our results were not statistically significant.

In studies that have compared tooth discoloration caused by endodontic materials, removal of the smear layer has created a paradox (17, 27): it facilitates the penetration of the material into the dentin tubules but causes more severe tooth discoloration. However, in routine root canal therapy, the smear layer should be removed to disinfect the root canal and prevent reinfection. For this reason, we removed the smear layer using both EDTA and NaOCl together in the present study.

The best time to observe tooth coloration depends on many factors such as the thickness of the remaining dentin, presence of the smear layer and the quality and quantity of the sealer (16). In the present study, crown discoloration was measured immediately after placement and at weeks 1, 2, 3 and 4, similar to a previous study (12). Hence, each specimen had its own control. In addition, the coloration process could be observed at each time point.

We attempted to minimise potential errors by performing each measurement three times. In addition, measurements were carried out by the same operator. All specimens were stored in the

same dark environment to mimic the human mouth. A standardised circular strip was bonded to the buccal surface of the crown 2 mm above the CEJ to ensure that the spectrophotometric readings were performed on the same region at all times.

Tooth color can be measured in several ways including via visual assessment (e.g. digital image analysis) and using instruments such as colorimeters, chromameters and spectrophotometers (28). Kim *et al.* (14) used a colorimeter method to measure color changes and reported that only minocycline, among the components of TAP, caused discoloration of teeth. Our results support that claim. Spectrophotometers have been used in dental studies not only to measure tooth coloration but also to measure metamorphism and spectral reflectance at each wavelength (25, 28, 29, 30). Thus, we preferred to use a spectrophotometer rather than a

colorimeter to measure the color changes in our specimens.

Our results indicate that both antibiotic pastes cause tooth discoloration and this discoloration advances over time.

CONCLUSION

Antibiotic pastes used as intracanal medicaments can stain teeth. In the present study, both antibiotic pastes tested TAP and MP, induced crown discoloration. When considering the use of these medicaments, biological, functional aspects and aesthetic considerations should be taken into account.

REFERENCE

- Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endod Dent Traumatol* 1990; 6: 142-9. | 2. Üstün Y, Arslan S, Aslan T. Effects of calcium hydroxide and propolis intracanal medicaments on bond strength of resin-based endodontic sealer as assessed by push-out test. *Dent Mater J* 2013; 32(6): 913-9. | 3. Poorni S, Miglani R, Srinivasan MR, Indira R. Comparative evaluation of the surface tension and pH of calcium hydroxide mixed with five different vehicles: An in vitro study. *Indian J Dent Res* 2009; 20: 17-20. | 4. Zehnder M, Grawehr M, Hasselgren G, Waltimo T. Tissue dissolution capacity and dentin-disinfecting potential of calcium hydroxide mixed with irrigating solutions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003; 96: 608-13. | 5. Türkaydn D, Tarçın B, riboz E *et al.* Influence of Different Vehicles on the pH and Surface Tension of Calcium Hydroxide Pastes. *Marmara Dent J* 2013; 2: 57-60. | 6. Chavez de Paz LE, Dahlen G, Molander A, Moller A, Bergenholtz G. Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment. *Int Endod J* 2003; 36: 500-8. | 7. Kvist T, Molander A, Dahlen G, Reit C. Microbiological evaluation of one- and two-visit endodontic treatment of teeth with apical periodontitis: a randomized, clinical trial. *J Endod* 2004; 30: 572-6. | 8. Athanassiadis B, Abbott PV, Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. *Aust Dent J Suppl* 2007; 52(1): 64-82. | 9. Hoshino E, Kurihara-Ando N, Sato I *et al.* In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. *Int Endod J* 1996; 29: 125-30. | 10. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E. Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline *insitu*. *Int Endod J* 1996; 29(2): 118-24. | 11. Thibodeau B, Teixeira F, Yamauchi M, Caplan DJ, Trope M. Pulp revascularization of immature dog teeth with apical periodontitis. *J Endod* 2007; 33: 680-9. | 12. Akcay M, Arslan H, Yasa B, Kavrak F, Yasa E. Spectrophotometric Analysis of Crown Discoloration Induced by Various Antibiotic Pastes Used in Revascularization. *J Endod* 2014; 40(6): 845-8. | 13. Gomes-Filho JE, Duarte PC, de Oliveira CB *et al.* Tissue reaction to a triantibiotic paste used for endodontic tissue self-regeneration of nonvital immature permanent teeth. *J Endod* 2012; 38: 91-4. | 14. Kim JH, Kim Y, Shin SJ, Park JW, Jung IY. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. *J Endod* 2010; 36: 1086-91. | 15. Miller EK, Lee JY, Tawil PZ, Teixeira FB, Vann WF Jr. Emerging therapies for the management of traumatized immature permanent incisors. *Pediatr Dent* 2012; 34: 66-9. | 16. Scaminaci Russo D, Viano M, Bambi C, Nieri M, Giachetti L. Color stability of bleached teeth over time: an in vitro study. *Eur J Esthet Dent* 2010; 5: 300-10. | 17. Meincke DK, Prado M, Gomes BPF, Della Bona A, Sousa ELR. Effect of endodontic sealers on tooth color. *J Dent* 2013; 41: 93-6. | 18. Della Bona A, Barrett AA, Rosa V, Pinzetta C. Visual and instrumental agreement in dental shade selection: three distinct observer populations and shade matching protocols. *Dent Mater* 2009; 25: 276-81. | 19. Corciolani G, Vichi A, Louca C, Ferrari M. Influence of layering thickness on the color parameters of a ceramic system. *Dent Mater* 2010; 26: 737-42. | 20. Abbott PV. Medicaments: aids to success in endodontics. Part 1. A review of literature. *Aust Dent J* 1990; 35: 438-48. | 21. Ta demir T, Çelik D, Er K, Yildirim T, Ceyhanlı KT, Ye ilyurt C. Efficacy of several techniques for the removal of calcium hydroxide medicament from root canals. *Int Endod J* 2011; 44: 505-9. | 22. Yassen GH, Vail MM, Chu TG, Platt JA. The effect of medicaments used in endodontic regeneration on root fracture and microhardness of radicular dentine. *Int Endod J* 2013; 46(7): 688-95. | 23. Arslan H, Akcay M, Capar ID, Ertas H, Ok E, Uysal B. Efficacy of Needle Irrigation, EndoActivator, and Photon-initiated Photoacoustic Streaming Technique on Removal of Double and Triple Antibiotic Pastes. *J Endod* 2014; 40(9): 1439-42. | 24. Berkhoff JA, Chen PB, Teixeira FB, Diogenes A. Evaluation of triple antibiotic paste removal by different irrigation procedures. *J Endod* 2014; 40(8): 1172-7. | 25. 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(10): 942-9. | 26. Rosen T, Hoffmann TJ. Minocycline-induced discoloration of the permanent teeth. *J Am Acad Dermatol* 1989; 21(3): 569. | 27. Shahrami F, Zaree M, Mir APB, Abdollahi-Armani M, Mesgarani A. Comparison of tooth crown discoloration with Epiphany and AH26 sealer in terms of chroma and value: an in vitro study. *Braz J Oral Sci* 2011; 10(3): 171-4. | 28. El Sayed MAA, Etemadi H. Coronal discoloration effect of three endodontic sealers: An in vitro spectrophotometric analysis. *J Conserv Dent* 2013; 16(4): 347-51. | 29. Kwon YH, Huo MS, Kim KH, Kim SK, Kim YJ. Effects of hydrogen peroxide on the light reflectance and morphology of bovine enamel. *J Oral Rehabil* 2002; 29: 473-7. | 30. Wetter NU, Barroso MC, Pelino JEP. Dental bleaching efficacy with diode laser and LED irradiation: an in vitro study. *Lasers Surg Med* 2004; 35: 254-8. |