

IN VITRO EVALUATION OF DISCOLORATION CAUSED BY ROOT CANAL SEALERS AND COLOR CHANGES AFTER BLEACHING

Emre Bodrumlu¹, Esma Dinger^{2*}

¹Department of Endodontics, Faculty of Dentistry, Zonguldak Bulent Ecevit University, Esenköy, Kozlu, Zonguldak, Turkey 67600.

²Department of Endodontics, Faculty of Dentistry, Bolu Abant İzzet Baysal University, Gököy Campus, 14030 Bolu, Turkey. esmadingerr@gmail.com

<https://doi.org/10.51847/S3rok2RpH4>

ABSTRACT

The aim of this study is to evaluate tooth discoloration caused by different root canal sealers and the effect of subsequent bleaching treatment. In the present study, the coronal areas of the teeth whose crowns were cut and filled with the bioceramic root canal sealer and the resin-based root canal sealer, and before filling, after waiting for 1 week, 1 month and applying bleaching treatment. Color changes were observed. Resin-based root canal sealer group showed more coloration in the t2 measurement compared to the first measurement, which showed a statistical difference compared to the control group values. After the bleaching treatment, more bleaching was observed in the teeth with root canal sealer and also, the control group showed a statistically significant difference due to the small change in the control group. It has been shown that color changes can achieve the desired appearance by applying bleaching treatment after root canal treatment.

Key words: Discoloration, Root canal sealers, Bleaching, Bioceramic.

Introduction

Understanding the etiology of tooth discoloration is very important for accurate diagnosis and treatment planning [1, 2]. Coloration types, which can be classified as external and internal, are externally caused by oral hygiene, habits, nutrition, and environmental factors; Internally, it is affected by genetic and metabolic diseases, medications used, trauma, and dental treatments [3].

In addition to pulp-related discoloration in endodontic treatments, intracanal medications, antibiotics, and root canal sealers can also cause internal discoloration in teeth [4, 5]. When endodontic materials are left in the canal orifices and pulpal walls without being adequately cleaned during root canal treatment, they penetrate the dentinal tubules and cause discoloration of the teeth [6]. Another view is that some endodontic treatment materials, especially root canal sealers or medicaments, cause a chemical reaction in the presence of moisture and cause color change in the teeth over time [7].

As a solution to the discoloration of the teeth, internal bleaching treatment is applied [8, 9]. Bleaching is a conservative treatment option that can be an alternative to most restorative and prosthetic procedures [10]. Bleaching procedures on traumatized devital teeth that have undergone root canal treatment can be applied with various options such as the thermocatalytic technique activated by heat, bleaching with visible light, and the "walking bleach" technique without the use of heat or light, and bleaching agents such as hydrogen peroxide, carbamide peroxide, and sodium perborate [11-13].

In our study, the walking bleach technique and hydrogen peroxide, which are frequently used today, were preferred [14, 15]. The bleaching that occurs on the enamel and dentin surface is based on the principle that hydrogen peroxide easily penetrates and transforms color pigments into more colorless hydroxyl groups [16].

The success and prognosis of bleaching treatment may also vary depending on the materials used during root canal treatment [17, 18]. Bleaching may be insufficient for coloring caused by metal-containing compounds [19].

For this reason, aesthetic problems that may occur after the treatment should be taken into consideration, and materials that will not cause color change should be preferred [20]. In our study, we used Well Root-ST (Vericom, Gangwon-Do, Korea), a bioceramic root canal sealer, and resin-based MM-Seal (Micro Mega, Besançon, France) root canal sealer. Well, Root-ST (Vericom, Gangwon-Do, Korea) is an easily injectable root canal paste containing calcium silicate, zirconium oxide, and unspecified thickening filling agents [21]. MM Seal (Micro Mega, Besançon, France) is a resin-based root canal sealer containing epoxy polymer resin, ethylene glycol salicylate, calcium phosphate, bismuth subcarbonate, and zirconium oxide components [22]. According to our research, it is reported in the literature that the in vitro color change of Well Root-ST (Vericom, Gangwon-Do, Korea) and MM-Seal (Micro Mega, Besançon, France) root canal sealers is no studies have been found regarding color change after bleaching.

This study aims to evaluate tooth discoloration caused by different root canal sealers and the effect of subsequent bleaching treatment. Hypotheses tested; it is stated that the

root canal sealer used does not cause color change on the teeth and that the bleaching treatment creates a color tone change.

Materials and Methods

In the present study, 28 maxillary incisors with no caries, restorations, or discoloration were used. The teeth were cleaned with polishing paste and rubber bur. The crowns were cut 2 mm below the cemento-enamel border with the help of a diamond bur. The pulp was removed with the help of an excavator, and the pulp chamber was washed with 5% NaOCl (Wizard Rehber Kimya, Istanbul, Türkiye) and distilled water. The air was then dried with water spray and paper points. The teeth were divided into three groups: two experimental and one control group. Experimental groups consisting of 12 teeth each and a control group consisting of 4 teeth were created. Before placing root canal sealer into the pulp cavities, the first color measurements of the teeth were made on a white background in a constant light environment using the Vita EasyShare digital spectrophotometer device. Then, the pulp chambers of the teeth in the first experimental group were coated with Well Root-ST (Vericom, Gangwon-Do, Korea), and the pulp chambers of the teeth in the second experimental group were coated with MM-Seal (Micro Mega, Besançon, France). The root canal sealers' contents are shown in **Table 1**. The samples were temporarily sealed with glass ionomer cement and stored in a 37 °C incubator with 100% humidity. The color measurement was repeated a week and a month later. Then, for the bleaching process, the teeth were cleaned of temporary restorations and pasted with a bur. 16% hydrogen peroxide was used as a bleaching agent and placed in the pulp chambers. The cavities were temporarily closed with glass ionomer cement. The teeth were stored in a 37°C incubator with 100% humidity for one week. Calculate the color change using a spectrophotometer after one week. Afterward, the color values were recorded according to the CIEDE 2000 system formula by determining the first L* a* and b* color values and the last L* a* and b* color values between the groups whose differences were to be measured with a digital spectrophotometer device and the color differences (ΔE^*) were analyzed. Microsoft Office Excel was used to apply the findings to each sample (Microsoft Corporation, Redmond, WA, USA) using the following formulation [23].

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

Measurement times and values are indicated symbolically in the **Table 2**.

Table 1. The root canal sealers' contents.

Groups	Material	Manufacturer	Contents
Group 1	Well Root-ST (Vericom, Gangwon-Do, Korea)	Vericom Dental, Korea	Calcium silicate, zirconium oxide, fillers
Group 2	MM Seal (Micro Mega, Besançon, France)	Micro-Mega, Besançon, France	Epoxy resin, ethylene glycol salicylate, calcium phosphate, bismuth carbonate, color pigment, poly aminobenzoate, zirconium dioxide, calcium oxide

Table 2. Symbolic display of measurement times and values.

Values obtained at the first measurement (without applying any treatment to the teeth)	t0
Values obtained in the second measurement one week later (after the canal sealer was placed and waited for 1 week)	t1
Values obtained at the third measurement time one month later (after waiting with canal sealer for 1 month)	t2
*Bleaching agent placement (placed after the third measurement)	-
Values were obtained in the fourth measurement, one week after applying the bleaching agent. (after waiting 1 week with the bleaching agent)	t3

$\Delta E1$ (t0-t1); $\Delta E2$ (t0-t2); $\Delta E3$ (t1-t2); $\Delta E4$ (t1-t3); $\Delta E5$ (t2-t3); $\Delta E6$ (t0-t3)

Results and Discussion

No statistically significant difference was observed between the control group and the tooth groups with root canal sealer between the second measurement 1 week later (t1) and the first measurement (t0). Between the initial measurement (t0) and the 3rd measurement (t2), the MM Seal (Micro Mega, Besançon, France) group showed more coloration compared to the first measurement, which showed a statistical difference compared to the control group values. (A statistically significant difference was obtained between $\Delta E2$ mean values according to groups ($p = 0.046$). The mean value of $\Delta E2$ was 2.51 in the Well Root-ST (Vericom, Gangwon-Do, Korea) group, 2.80 in the MM-Seal (Micro Mega, Besançon, France) group, and 2.16 in the control group. MM-Seal and control groups differed from each other.)

No statistically significant difference was observed in the coloration changes of the groups between the 2nd and 3rd measurements (t1-t2). With the 4th measurement (t3) after one week of bleaching agent application, all other measurement values (t0, t1, t2) showed a statistical

difference in the control group compared to the root canal sealer groups. More bleaching was observed in teeth with root canal seals, and the control group showed a statistically significant difference due to the small change in the control group. (A statistically significant difference was obtained between ΔE4 mean values according to groups (p<0.001). The mean value of Δ E4 was 8.09 in the Well Root-ST (Vericom, Gangwon-Do, Korea) group, 8.27 in the MM-Seal (Micro Mega, Besançon, France) group, and 2.34 in the control group. The control group differed from other groups. A statistically significant difference was obtained between ΔE5 mean values according to groups (p <0.001). The mean value of ΔE5 was obtained as 8.36 in the Well Root-ST

(Vericom, Gangwon-Do, Korea) group, 8.59 in the MM-Seal (Micro Mega, Besançon, France) group, and 2.45 in the control group. The control group differed from other groups. A statistically significant difference was obtained between ΔE6 mean values according to groups (p<0.001). The mean value of Δ E6 was obtained as 6.46 in the Well Root-ST (Vericom, Gangwon-Do, Korea) group, 6.41 in the MM-Seal (Micro Mega, Besançon, France) group, and 1.41 in the control group. The control group differed from other groups. No statistically significant difference was found between the distributions of other ΔE values according to groups (p>0.05).

Table 3. The average results of color changes (ΔE₀₀) of the samples.

	Groups						Test Statistics	p
	Well Root-ST		MM-Seal		Control			
	Average±SD	Middle (Min-Max)	Average±SD	Middle (Min-Max)	Average±SD	Middle (Min-Max)		
ΔE1	2,25 ± 0,42	2,22 (1,58 - 3,16)	2,45 ± 0,52	2,39 (1,48 - 3,19)	2,01 ± 0,84	2,08 (1,06 - 2,8)	1,14	0,335*
ΔE2	2,51±0,46 ^{ab}	2,51 (1,65- 3,11)	2,80± 0,45 ^b	2,89 (2,02 - 3,37)	2,16± 0,31 ^a	2,18 (1,82- 2,45)	3,50	0,046*
ΔE3	1,68 ± 0,64	1,59 (1 - 2,83)	1,67 ± 0,59	1,40 (1,05 - 2,56)	1,52 ± 0,48	1,44 (1,03- 2,16)	0,24	0,884**
ΔE4	8,09± 0,94 ^b	7,97 (6,74 - 9,55)	8,27± 0,72 ^b	8,40 (7,17 - 9,51)	2,34± 0,29 ^a	2,33 (2,03 - 2,67)	345,5	<0,001*
ΔE5	8,36± 0,98 ^b	8,25 (6,33 - 9,91)	8,59± 0,83 ^b	8,40 (7,33- 9,86)	2,45± 0,33 ^a	2,45 (2,05 - 2,85)	84,60	<0,001*
ΔE6	6,46± 0,89 ^b	6,30 (5,1 - 8,21)	6,41± 0,58 ^b	6,22 (5,81 - 7,6)	1,41± 0,39 ^a	1,32 (1,06 - 1,94)	82,77	<0,001*

*One-Way Analysis of Variance; **Kruskal Willis H test; a-b There is no difference between groups with the same letter. ΔE1 (t0-t1); ΔE2 (t0-t2); ΔE3 (t1-t2); ΔE4 (t1-t3) ; ΔE5 (t2-t3); ΔE6 (t0-t3)

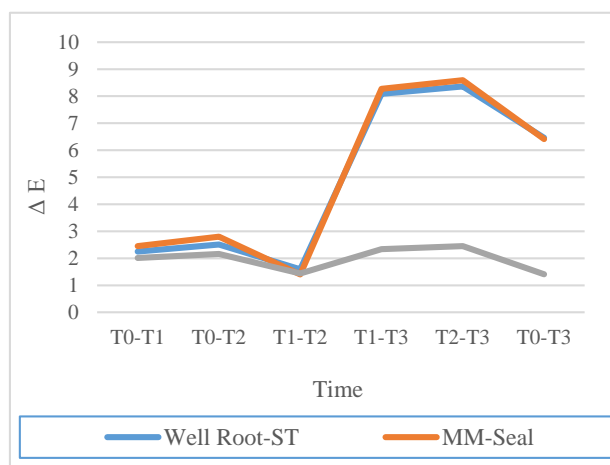


Figure 1. Change graph of ΔE values in groups over time.

Data were analyzed in IBM SPSS V23. The suitability of the data for normal distribution was examined with the Shapiro-Wilk test. The One-Way Analysis of Variance test was used

to compare normally distributed data according to three or more groups, and multiple comparisons were examined with Duncan and Tamhane test. Kruskal Wallis H test was used to compare data that did not comply with normal distribution in groups of three or more. The significance level was taken as p<0.05.

The color change seen in endodontically treated teeth is a type of internal discoloration that occurs over time. Clinically, it creates undesirable aesthetic problems for both physicians and patients. As a result of clinical observations, it is stated that the color change after endodontic treatment is caused by residual pulp residues in the canal and the chemical materials used. Studies have found that endodontic materials such as Walkhoff paste, Grossman paste, zinc oxide eugenol, endometasone, and N2 cause discoloration in teeth [24, 25]. Lena *et al.* stated that all endodontic pastes cause some color change [26].

Although there are many studies in the literature that include discoloration caused by root canal sealers, there is no study evaluating the effect of Well Root-ST and MM-Seal root

canal sealers on discoloration and bleaching treatment [24-27]. Well, Root-ST and MM Seal are structurally different from root canal sealers, which contain discoloration-causing substances such as tricalcium phosphate and bismuth oxide [28]. In the present study, although there was no statistically significant difference between the Well Root-ST and MM Seal groups in terms of coloration and bleachability, a significant difference was observed between the MM Seal and the control group in terms of coloration. This situation made us think that MM Seal root canal sealer components may also cause discoloration. In addition, factors such as non-standard tubule penetration of the teeth used and the measurement sensitivity of the device also affect the results. Further studies on this subject will help in evaluation.

In the present study, while the effect of bioceramic and resin-based root canal sealer on the discoloration of teeth was evaluated, during the application phase, the occlusal entry cavity was not opened for the placement of root canal sealer as recommended in the studies, and EDTA was not used for irrigation. The materials were placed in the chole area. Thus, problems such as discoloration and leakage of the temporary restorative material were eliminated without affecting the tubule permeability of the dentin and the optical properties of the enamel [29].

At the end of the first 1-month color change process, internal bleaching treatment was applied to provide aesthetic treatment of the tooth. For internal bleaching treatment, minimally invasive 16% Hydrogen peroxide was used [30]. Nathoo stated that hydrogen peroxide oxidizes the long, double-chain chromogens that cause coloration and turns them into lighter-colored small chromogenic substances [26]. Coelho *et al.* stated that high concentrations of hydrogen peroxide may spread through the dentinal tubules to the periodontal tissues and root surface, causing inflammation and root resorption [31]. Through the etching process, it is known to ensure increased penetration of the bleaching agent into the dentin, resulting in a quick and efficient bleaching process [5]. However, acid etching was not performed in this study, because this application may cause cervical resorption.

In the present study, both tooth groups with root canal sealer showed more bleaching than the control group, showing that the color changes can reach the desired appearance by applying bleaching treatment after root canal treatment.

A spectrophotometer device, which is the most commonly used device, was used for color measurement. The spectrophotometer device measures the amount of light reflected from the measured object in the visible spectrum range, at 1-25 nm intervals [23]. The accuracy rate of the values measured by spectrophotometers is quite successful compared to other traditional methods [23, 32]. In the present study, the CIEDE 2000 color evaluation system was used as the evaluation system for color differences. Although the CDE Lab system is generally available in the

literature, the CIEDE 2000 color evaluation system was preferred in our study because some studies reported that the CIEDE 2000 color evaluation system was more successful in detecting color differences and that it was a system that we could use within our means [23, 32].

Conclusion

No visible color change that would create a clinically aesthetic problem was observed. As a result of our study, although a statistically significant difference was observed between the root canal sealers used in the MM-Seal group compared to the control group, there was no statistical difference between them and the Well Root ST group regarding their effects on tooth discoloration. It has been shown that the desired bleaching can be achieved by creating a statistical difference with the control group after the bleaching process.

Acknowledgments: All authors approved the final version of this paper.

Conflict of interest: None

Financial support: None

Ethics statement: This study was approved by Research Ethics Committee of Bolu Abant İzzet Baysal University Ethic Committee. (Registration number: 2023/317)

References

1. Delcea C, Siserman C. The emotional impact of covid-19 on forensic staff. *Rom J Leg Med.* 2021;29(1):142-6.
2. Delcea C, Bululoi AS, Gyorgy M, Siserman CV. Medico-Legal approach to incestuous sexual orientation in men. *Arch Pharm Pract.* 2023;14(4):69-74.
3. Wang J, Zou D, Li Y, Liu P, Guo C. Drug-induced tooth discoloration: An analysis of the US food and drug administration adverse event reporting system. *Front Pharmacol.* 2023;14:1161728.
4. Krastl G, Algayer N, Lenherr P, Filippi A, Taneja P, Weiger R. Tooth discoloration induced by endodontic materials: A literature review. *Dent Traumatol.* 2013;29(1):2-7.
5. Kansal S, Jindal L, Garg K, Thakur K, Mehta S, Pachori H. Discoloration of teeth: A literature review. *Int J Health Clin Res.* 2020;3(2):58-62.
6. Afkhami F, Elahy S, Nahavandi AM, Kharazifard MJ, Sooratgar A. Discoloration of teeth due to different intracanal medicaments. *Restor Dent Endod.* 2019;44(1):e10.
7. Santos LGPD, Chisini LA, Springmann CG, Souza BDM, Pappen FG, Demarco FF, et al. Alternative to avoid tooth discoloration after regenerative endodontic

- procedure: Asystematic review. *Braz Dent J.* 2018;29(5):409-18.
8. Radu CC, Delcea C, Plesa A, Rad D. Transforming perceptions of drug consumption among youth through a cognitive-social-medico-legal educational approach. *Pharmacophore.* 2023;14(4):50-6.
 9. Delcea C, Bululoi AS, Gyorgy M, Rad D. Psychological distress prediction based on maladaptive cognitive schemas and anxiety with random forest regression algorithm. *Pharmacophore.* 2023;14(5):62-9.
 10. Amer M. Intracoronary tooth bleaching—A review and treatment guidelines. *Aust Dent J.* 2023;68:(1 Suppl):S141-52.
 11. Lim MY, Lum SO, Poh RS, Lee GP, Lim KC. An *in vitro* comparison of the bleaching efficacy of 35% carbamide peroxide with established intracoronary bleaching agents. *Int Endod J.* 2004;37(7):483-8.
 12. Gür G. non-vital discolored teeth bleaching using the walking bleach technique: A case report. *Cumhuriyet Dent J.* 2023;26(1):101-4.
 13. Attin T, Paque F, Ajam F, Lennon AM. Review of the current status of tooth whitening with the walking bleach technique. *Int Endod J.* 2003;36(5):313-29.
 14. Sood A, Mishra D, Yadav R, Bhatt K, Priya H. The application of the Bethesda system for reporting cervical cytology to oral cytology: An institutional study. *Clin Cancer Investig J.* 2022;11(4):25-32.
 15. Sohal KS, Owibingire SS, Moshy JR, Deoglas DK, Laizer PJ, Kalyanyama BM, et al. Oropharyngeal squamous cell carcinoma: Analysis of histopathological reports of 465 patients in Tanzania. *Clin Cancer Investig J.* 2022;11(3):9-14.
 16. Prising MD, Malinda MY. Aesthetic rehabilitation of discoloured anterior tooth with intra-coronal bleaching. *Int Dent J.* 2023;73(Supplement 1):S44.
 17. Poleyov GG, Sablin AB. Development of coordination abilities with the help of the burpee exercise. *Int J Pharm Res Allied Sci.* 2022;11(1):108-12.
 18. Suragimath G, Ashwinirani SR, Shetgaonkar KA. Assessment of knowledge, awareness and practices about periodontal disease among secondary school teachers. *Int J Pharm Res Allied Sci.* 2022;11(3):60-5.
 19. Kwon SR, Wertz PW. Review of the mechanism of tooth whitening. *J Esthetic and Resto Dent.* 2015;27(5):240-57.
 20. Nabavi SS, Gholizadeh B. Evaluation of the quality of life of the patients with heart failure in Ahvaz teaching hospitals. *Entomol Appl Sci Lett.* 2022;9(1):26-30.
 21. Boyadzhieva E, Dimitrova S, Filipov I, Zagorchev P. Setting time and solubility of premixed bioceramic root canal sealer when applied with warm gutta percha obturation techniques. *IOSR J Dent Med Sci.* 2017;16(03):125-9.
 22. Camargo RVD, Silva-Sousa YTC, Rosa RPF, Mazzi-Chaves JF, Lopes FC, Steier L, et al. Evaluation of the physicochemical properties of silicone-and epoxy resin-based root canal sealers. *Braz Oral Res.* 2017;31:e72.
 23. Llena C, Herrero A, Lloret S, Barraza M, Sanz JL. Effect of calcium silicate-based endodontic sealers on tooth color: A 3-year *in vitro* experimental study. *Heliyon.* 2023;9(2):e13237.
 24. Sharma D, Worlikar N, Shah K, Sharma Y. Recent advancements in root canal sealers—An overview. *J Adv Med Dent Sci Res.* 2023;11(4):82-91.
 25. Bosenbecker J, Barbon FJ, de Souza Ferreira N, Morgental RD, Boscato N. Tooth discoloration caused by endodontic treatment: A cross-sectional study. *J Esthet Restor Dent.* 2020;32(6):569-74.
 26. Lena IM, Bagnara CDR, Visentini JE, Ribeiro CEVDC, May LG, Morgental RD. Tooth discoloration induced by endodontic sealers and cervical limit: 1-year *in vitro* evaluation. *Braz Dent J.* 2023;34(6):30-9.
 27. Demiral M, Keskin C, Uzun I. *In Vitro*, assessment of the tooth discoloration induced by well root ST, diaproseal and MTA fill apex root canal sealers. *Sch J Dent Sci.* 2017;4(1):27-30.
 28. Simsek N, Keles A, Ahmetoglu F, Ocak MS, Yologlu S. Comparison of different retreatment techniques and root canal sealers: A scanning electron microscopic study. *Braz Oral Res.* 2014;28(1):1-7.
 29. Forghani M, Gharechahi M, Karimpour S. *In vitro* evaluation of tooth discoloration induced by mineral trioxide aggregate fillapex and iroot sp endodontic sealers. *Aust Endod J.* 2016;42(3):99-103.
 30. Abou Basha B, Alagamawy E. Non-Vital Internal tooth bleaching using 35% hydrogen peroxide gel: A case report. *J Bahrain Med Soc.* 2022;34(3):57-61.
 31. Coelho AS, Garrido L, Mota M, Marto CM, Amaro I, Carrilho E, et al. Non-Vital tooth bleaching techniques: A systematic review. *Coatings.* 2020;10(1):61.
 32. Dias S, Dias J, Pereira R, Silveira J, Mata A, Marques D. Different methods for assessing tooth colour—*In vitro* study. *Biomimetics.* 2023;8(5):384.