Cumhuriyet Dental Journal, 28(1): 99-107, 2025
DOI: https://doi.org/10.7126/cumudj.1610597

Cumhuriyet Dental Journal

| cdj.cumhuriyet.edu.tr | Founded: 1998 Available online, ISSN: 1302-5805 Publisher: Sivas Cumhuriyet Üniversitesi

In-Vitro Comparison of Radicular Penetration of Hydrogen Peroxide Among Different Intracoronal Bleaching Agents

Somayeh Hurmuzlu[1,a,*], Berna Aslan[2,b], Mehmet Olgun Saka[3,c]

[1] *Disvest Polyclinic, Cankaya, Ankara, Turkiye.*
[2] *Ankara University Faculty of Dentistry, Department of Endodontics, Ankara, Turkiye.*
[3] *Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Turkiye.*
**Corresponding author*

Research Article

History

Received: 04/01/2025
Accepted: 15/01/2025

ABSTRACT

Objective: In this study, aimed to compare the penetration of hydrogen peroxide into the cervical region from different whitening agents used in intracoronal bleaching techniques, which is a conservative treatment approach.
Material and Methods: In our study, 60 single-rooted maxillary incisors were used. After root canal treatment procedures, the gutta percha canal filling was removed 4 mm below the enamel-cementum junction. Glass ionomer was placed as a protective barrier cement 2 mm below the enamel-cementum junction.
Results: The difference between the amount of hydrogen peroxide release in the Whiteness SuperEndo group on the 1st day and the 3rd day was not found to be statistically significant. ($p>0.05$), but in the other experimental groups, it was observed that the difference between the amounts of hydrogen peroxide release on the 1st, 3rd and 7th days was statistically significant ($p < 0.05$). While there was no statistically significant difference between the Whitness Super Endo group and the sodium perborate + distilled water group ($p>0.05$), the difference between the other groups was statistically significant ($p<0.05$).
Conclusions: In conclusion, within the limits of this study, sodium perborate + distilled water mixture and carbamide peroxide gels can be shown as a good alternative to bleaching agents with high radicular hydrogen peroxide penetration to minimize the risk of cervical root resorption. In addition, we can say that carbamide peroxide gel is a good alternative to sodium perborate + distilled water mixture due to its ease of use and the fact that it is as effective as hydrogen peroxide in studies.

Keywords: Intracoronal bleaching, radicular penetration, hydrogen peroxide, carbamide peroxide, cemento enamel junction

Farklı İntrakoronal Beyazlatma Ajanlarından Hidrojen Peroksitin Radiküler Penetrasyonunun In-Vitro Karşılaştırılması

Araştırma Makalesi

Süreç

Geliş : 04/01/2025
Kabul: 15/01/2025

ÖZ

Amaç: Çalışmamızda konservatif bir tedavi yaklaşımı olan intrakoronal beyazlatma tekniklerinde kullanılan farklı beyazlatma ajanlarından servikal bölgeye hidrojen peroksit penetrasyonunun karşılaştırılması amaçlanmıştır.
Gereç ve Yöntemler: Çalışmamızda 60 adet, tek köklü, maksiller keser dişler kullanıldı. Kanal tedavisi prosedürlerinden sonra güta perka kanal dolgusu mine-sement birleşiminin 4 mm altından uzaklaştırıldı. Koruyucu bariyer olarak cam iyonomer siman mine-sement birleşiminin 2 mm altına yerleştirildi.
Bulgular: Whiteness SüperEndo grubunun, 1. gün ile 3. gün hidrojen peroksitsalınım miktarı arasındaki fark istatistiksel olarak anlamlı bulunmamıştır ($p>0,05$), ancak diğer deney gruplarında 1., 3. ve 7. günlerde hidrojen peroksitsalınım miktarları arasındaki farkın ise istatistiksel olararak anlamlı olduğu gözlendi ($p <0,05$). Whitness Super Endo grubu ile sodyum perborat + distile su grubu arasında istatistiksel olarak anlamlı fark bulunamazken ($p>0,05$), diğer gruplar arasındaki fark istatistiksel olarak anlamlı bulunmuştur ($p<0,05$).
Sonuç: Sonuç olarak bu çalışmanın sınırları dahilinde servikal kök rezorbsiyon riskini en aza indirmek için yüksek oranda radiküler hidrojen peroksit penetrasyonu gösteren beyazlatma ajanlarına iyi bir alternatif olarak sodyum perborat + distile su karışımı ve karbamid peroksit jeller gösterilebilir. Ayrıca karbamid peroksit jelin kullanım kolaylığı ve yapılan çalışmalarda hidrojen peroksit kadar etkili olmalarından dolayı sodyum perborat + distile su karışımına iyi bir alternatif olduğunu söyleyebiliriz.

Anahtar Kelimeler: İntrakoronal beyazlatma, Hidrojen peroksit, Karbamid peroksit, Sodyum perborat, radiküler penetrasyon

[a] *somaye.faraji88@gmail.com* *https://orcid.org/0000-0001-8756-2134*
[b] *baslan@dentistry.ankara.edu.tr* *https://orcid.org/0000-0002-4673-7791*
[c] *omsaka@gmail.com* *https://orcid.org/0000-0002-8726-6391*

How to Cite: Hurmuzlu S, Aslan B, Saka MO (2025) In-Vitro Comparison of Radicular Penetration of Hydrogen Peroxide Among Different Intracoronal Bleaching Agents, Cumhuriyet Dental Journal, 28(1): 99-107.

Introduction

Aesthetics in dentistry aims to provide a beautiful smile by restoring the teeth in harmony with the gums, lips and face. Tooth discoloration is an important aesthetic problem. Discolored teeth can cause significant aesthetic problems, especially in the anterior region, and can negatively affect the social and emotional behavior of the person. Nowadays, the desire to have beautiful and white teeth is increasing, which leads to an increase in the options of aesthetic applications. These applications include restorative techniques such as mechanical abrasion, composite restorations, porcelain laminates, composite and full crowns and whitening procedures.[1]

Vital and devital bleaching procedures applied in the treatment of discolored teeth are a more conservative approach compared to porcelain crowns and composite lamina restorations. The process of lightening tooth color by oxidation of organic pigments in enamel and dentin tissues using a chemical agent is called bleaching. Vital and devital whitening methods are frequently preferred today for the removal of tooth discoloration. These methods can also be examined under two titles as intracoronal and extracoronal bleaching.[2]

Internal discoloration caused by necrotic pulp tissue, intra-pulp hemorrhage or root canal filling materials left in the pulp chamber can be removed easily, economically and conservatively with intracoronal bleaching techniques used as an alternative to prosthetic approaches.[3-5]

Commonly used bleaching methods for root canal treated teeth are 'thermocatalytic technique' and 'walking bleach' technique. Both techniques give similar results in the removal of discoloration despite their different application methods. The 'walking bleach' technique is more preferred because it is more comfortable and reliable for the patient and consists of shorter sessions.[2]

Commonly used bleaching agents are hydrogen peroxide solutions in different concentrations, sodium perborate and carbamide peroxide. Sodium perborate and carbamide peroxide are chemical components that release low levels of hydrogen peroxide. Sodium perborate is most commonly used in intracoronal bleaching.[2,6]

In one study, the researcher recommended leaving a mixture of sodium perborate and distilled water in the pulp chamber of canal treated teeth for a few days and changing the bleaching agent periodically.[7] In addition, it has been reported many times that the mixture of sodium perborate with hydrogen peroxide is very effective in intracoronal bleaching.[4,8,9] In spite of all these, if the correct methods are chosen for the bleaching procedure and appropriate materials are preferred, the possibility of these and similar complications may be reduced and successful treatments that can respond to the patient's request may be possible.

The most important complication of intracoronal bleaching is cervical root resorption.[10,11] In the literature, many cases of cervical root resorption caused by penetration of hydrogen peroxide into the surrounding tissues through dentin canals in the cervical part of the root have been reported.[8,12,13] In addition, hydrogen peroxide is caustic and may cause chemical burns and gingival peeling.[2,6]

In in vitro studies[10-13] it has been emphasized that hydrogen peroxide applied intracoronally can diffuse from root dentin in the cervical region and this diffusion will occur more in the presence of cemental root defects. In an in vivo study, it has been suggested that there is a relationship between cervical root resorption and intracoronal bleaching.[14] However, when the intracoronal bleaching technique is applied correctly and the right material is selected, the possibility of these undesirable complications can be reduced.[15] Bleaching materials may be in liquid, powder or gel form. After the development of gel and adhesive forms of hydrogen peroxide and carbamide peroxide for home-based vital bleaching, gel forms of high concentration products used for clinical vital bleaching have also been produced.[5,6,11]

Whitening products produced with this new understanding and used both at home and in the clinic are non-flowing, non-foaming, adhesive and easy to apply like liquid preparations during application. Considering both the ease of application and the low probability of irritation by leaking into the surrounding tissues, it is thought that it can also be used in intracoronal whitening.[11,15,19] There are many studies in which bleaching gels were applied to the outer surface of the tooth and the transition to the pulp chamber was evaluated.[11,16-19] However, there are very few studies in the literature on radicular hydrogen peroxide penetration from intracoronal carbamide peroxide and hydrogen peroxide containing gels. In our study, it was aimed to determine and compare the peroxide penetration into the cervical region by intracoronal application of gels containing carbamide peroxide and hydrogen peroxide, which are produced by different companies, which are practical and more up-to-date in terms of use, and which are available in the form of ready-made preparations at certain concentrations, with each other and with sodium perborate, which has been used most frequently in intracoronal bleaching for many years. Thus, the possibility of resorption that may occur depending on the amount of penetration into the cervical region from different bleaching agents can be evaluated. Considering that less penetration may reduce the risk of possible cervical resorption, we believe that the results of the study will contribute to clinical studies.

Materials and Methods

This study was conducted in Ankara University Faculty of Dentistry Research Laboratory and Ankara University Faculty of Pharmacy Pharmaceutical Technology Research Laboratory. Our study was conducted with the approval of Ankara University Faculty of Dentistry Ethics Committee dated 04/12/2019 and numbered 14/04.

Selection and Preparation of Teeth

In our study, 60 freshly extracted, permanent, single-rooted, maxillary incisor human teeth were used. The extracted teeth were soaked in 2.5% sodium hypochlorite

(NaOCl) solution (Sultan Chemists Inc., Englewood, New Jersey, USA) for 24 h, after which the hard and soft tissue residues on the root surfaces were removed with a periodontal curette and then soaked in distilled water containing thymol. In order to ensure standardization as much as possible, straight and single-rooted teeth without caries and restorations and with complete root development were included in the study. The selected teeth were examined under x12 magnification with a stereo microscope (Zeiss Stemi; Carl Zeiss, Jena, Germany) and teeth with fractures and cracks on the root surface were excluded. In addition, patients with cement defects or dentin exposure at the enamel-cement junction were excluded.

Preparation and Filling of Root Canals

The endodontic access cavities of the teeth were prepared with a rond diamond bur #16 (Hager & Meisinger, Neuss, Germany) and a fissure bur #14 (Hager & Meisinger) using a water-cooled high-speed aerator. Pulp tissue was removed with a tirnerf (Dentsply Maillefer, Ballaigues, Switzerland). To determine the working length (WL), a #15 K-type file (Maillefer, Ballaigues) was inserted into the root canal until the apical foramen was seen and this length was measured in mm. The WL was calculated to be 1 mm shorter than this length. WaveOne Gold (Dentsply Maillefer) rotary file system, the only file system with reciprocal motion, was used for root canal preparation. For this purpose, the mode of the endodontic motor (X Smart Plus; Dentsply Maillefer) designed for the use of the WaveOne Gold was selected and used with a pecking motion. Firstly, an entryway was provided with a WaveOne Gold Glider file at the WL. Then, root canal preparation was performed with a single file system, WaveOne Gold 45/0.5 large file, until the working length was reached. After every 3 back and forth movements, the file was removed from the canal, cleaned and the canals were irrigated with 2 mL of 1% NaOCl (Wizard, Rehber Kimya, Istanbul, Turkiye). A total of 10 mL of 1% NaOCl was used for each tooth. To ensure standardized preparation, the teeth were prepared by a single person. The root canals were dried with paper cones (Sure-Endo, Sure Dent, Seoul, Korea) and prepared for filling. The root canals were then filled with WaveOne Gold large gutta-percha (WaveOne Gold Gutta Percha Points; DeTrey Dentsply, Konstanz, Germany), and AH Plus (DeTrey Dentsply) root canal paste using the single cone method.

After the root canal filling was completed, the distance of the enamel-cement border was determined externally with a periodontal probe and the gutta percha was removed with a heated plugger so that the level of the root canal filling was 4 mm below the labial enamel-cement border. The reference point of the enamel-cement junction was taken buccally. The residues of gutta percha and paste were wiped from the access cavity with cotton pellets soaked in alcohol (Riedel de Haen, Seelze, Germany) and removed using a tungsten round carbide bur #21 (Hager & Meisinger) at low speed. The pulp chamber was then washed with distilled water. The teeth were kept in an oven at 37 °C, 100% humidity for 24 h to harden the root canal paste.

Riva Self Cure (SDI, Southern, Australia), a glass ionomer cement in capsule form, was used as a protective barrier material. The material was mixed in the amalgamator for 10 s in accordance with the company's recommendation and then placed on the root canal filling approximately 2 mm thick, 2 mm below the labial enamel-cement junction. The specimens were kept in an oven at 37 °C, 100% humidity for 24 h to completely harden the protective barrier material.

The specimens were randomly divided into 5 different experimental groups of 12 teeth each:

Group 1: Whiteness Super Endo Group (37% carbomide peroxide)

Group 2: Opalescence Endo Group (35% hydrogen peroxide)

Group 3: Sodium Perborate + Distilled Water Group

Group 4: Sodium Perborate + 30% Hydrogen Peroxide Group

Group 5: Control Group (Distilled Water)

Preparation of The Experimental Setup

The outer surfaces of the roots were coated with boxing wax (Kerr, Emeryville, CA, USA) and 2 coats of nail polish (FlorMar, Kosan Kozmetik, Kocaeli, Turkiye), leaving the enamel-cementum junction and the coronal third of the root exposed, including the apical foramen. The specimens were then placed in Eppendorf tubes (Altanlab, Fındıkzade, Istanbul, Turkiye) containing 2 mL of bidistilled water (Altanlab, Fındıkzade, Istanbul, Turkey), positioned 1 mm above the enamel-cementum junction so that the cervical part of the root and the enamel-cementum junction remained in the bidistilled water and tightly packed with the boxing wax.

The areas where the enamel came into contact with the boxing wax were fixed using adhesive wax (Kerr). Before placement of the bleaching material, the teeth were placed in a 37°C oven at 100% humidity for 1 h to simulate the temperature of the oral environment. All procedures were performed by a single person.

Group 1- Whitness Super Endo Group (FGM Dental, Joinville, Brazil) In this group, Whitness Super Endo, a whitening agent in gel form containing 37% carbomide peroxide, produced for whitening devital teeth, was used. The material consists of a ready-to-use syringe containing 3 g of gel and 15 tips. The whitening material was placed into the pulp chamber with the help of special needle tips, approximately 0.03 mL, in accordance with the manufacturer's instructions.

Group 2- Opalescence Endo Group (Ultradent, South Jordan, USA) In this group, Opalescence Endo, a whitening material in gel form containing 35% hydrogen peroxide and produced for whitening devital teeth, was used. The material consists of 2 syringes containing 1.2 mL of gel and 20 tips ready for use. The bleaching material was placed into the pulp chamber with the help of special needle tips,

approximately 0.03 mL, in accordance with the manufacturer's instructions.

Group 3- Sodium Perborate + Distilled Water Sodium perborate (Sultan Healthcare, Hackensack, NJ, USA) in powder form. 2 g of powder and 1 mL of distilled water (2 g: 1 mL) were mixed homogeneously on the glass with a spatula to the consistency of wet sand. The mixture was placed in the pulp chamber with the help of an amalgam carrier.

Group 4- Sodium Perborate + 30% Hydrogen Peroxide Sodium perborate in powder form (Sultan Healthcare), 2 g powder, 1 mL 30% hydrogen peroxide solution (Merck, Darmstadt, Germany), (2 g:1 mL) was mixed homogeneously on the cement glass with a spatula. The mixture was placed in the pulp chamber with the help of an amalgam carrier.

Group 5- Control group (Distilled water) 0.03 mL of distilled water was placed in the pulp chamber of the specimens belonging to this group with the help of a micropipette (Sclavo Diagnostici, Siena, Italia).After placing a small cotton pellet on the bleaching materials placed in the pulp chambers of the specimens in each experimental group, the entrance cavities were measured with a periodontal probe and closed with Cavit (3M-ESPE, Seefeld, Germany), a temporary filling material approximately 3 mm thick.

After the samples were kept in an oven at 37 ^{o}C for 24 h, the teeth were removed from the Eppendorf tubes and the bidistilled water in the tube was removed to measure the amount of hydrogen peroxide in the water, 2 mL of bidistilled water was added back into the Eppendorf tubes and the teeth were placed back into the tubes as previously described. This process was repeated on the 3rd and 7th days and a total of 3 measurements were made for each group.

Measurement of Hydrogen Peroxide Penetrating into Bidistilled Water

In our study, the iron thiocyanate method, which is a colorimetric method, was used to measure the amount of hydrogen peroxide penetrating into bidistilled water. [25] The red color intensity was measured spectrophotometrically by adding the prepared reagent one by one to bidistilled water samples collected from four different experimental groups and the control group. The amount of peroxide was calculated by comparing with the standard calibration curve obtained by diluting 30% hydrogen peroxide with bidistilled water.

Preparation of Reagents

All reagents (markers) to be used in this study were prepared daily in the laboratories of the Department of Pharmaceutical Technology, Faculty of Pharmacy, Ankara University in order to avoid interactions with ambient oxygen.

1 mL of 25% sulfuric acid (Merck, KgaA, Darmstadt, Germany) was added to 200 mL of freshly prepared bidistilled water. To the resulting acidic solution, 1 gram of ammonium thiocyanate (Riedel-De Haen AG, Seelze-Hannover, Germany) was added and stirred on a magnetic stirrer for 5 min. When the dissolution was complete, 0.2 grams of ferro ammonium sulfate (Merck, KgaA, Darmstadt, Germany), which was thoroughly powdered in a mortar and pestle, was added to the medium and stored in tightly sealed bottles wrapped with aluminum foil until use and consumed on the day of preparation. From the Eppendorf tube, 100µL of bidistilled water samples were applied to round-bottomed Eliza plates. Hydrogen peroxide solution of known concentration was applied to the last row of the 96-well Eliza plate each time. Freshly obtained bidistilled water was also added to the designated wells. To all of them, 100 µL of the reagent obtained above was added and shaken for 5 min in the Eliza reader to mix. Finally, readings were taken on a spectrophotometer (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA, USA) at a wavelength of 480 nm to evaluate the red color. In our study, spectrophotometric measurements were completed within 10 min following color formation.

Obtaining The Calibration Curve

To calculate the amount of hydrogen peroxide in the medium, hydrogen peroxide test solutions of known concentrations were prepared from 30% hydrogen peroxide. The prepared samples were sampled at concentrations reflecting the test solutions from which we expected results at the ppm level. Briefly, 100 µL of the 30% hydrogen peroxide stock solution diluted to 103% was transferred to the Eliza plate. Freshly prepared 100 µL bidistilled water was added. The liquids in the wells were mixed by pulling and releasing with a micropipette and 100 µL was transferred to the next well and 100 µL of freshly prepared bidistilled water was added. This process was performed for 20 wells. In other words, hydrogen peroxide, which was initially diluted 1/1000, was diluted 220 times more to obtain the calibration equation. Since 100 µL of sample was taken from 2mL sample to facilitate mathematical operations, the 20-fold dilution factor was reflected in the read concentrations and the amount of hydrogen peroxide in milliliter volume was calculated in micrograms by taking its half value. Samples of known concentration were read at 480 nm wavelength in an Eliza reader. For the calibration equation, the data were taken on 5 different days and the average values were taken into account. When the calculated data were graphed, the equation y=1.7734 x - 0.0002 was obtained. Samples of unknown concentration were calculated using this equation. In addition, the linearity of the equation obtained is desired to be close to 1. The linearity value of the obtained equation was found to be 0.9973. In order to calculate the amount of hydrogen peroxide at the desired scale depending on the experimental conditions and to eliminate mathematical error, a 20-fold dilution factor was reflected in the calibration equation and correction factors were included in the calculation to calculate the amount of hydrogen peroxide per milliliter directly in micrograms by taking its half value.

Evaluation of Results

The statistics of our study were performed at the Department of Analytical Chemistry, Faculty of Pharmacy, Hacettepe University and the Department of Pharmaceutical Technology, Faculty of Pharmacy, Ankara University. One-way ANOVA was used to determine whether there was a difference in the averages for the amounts of hydrogen peroxide (µg/ml) passing into the liquid in the experimental groups.

In addition, Student's t-test was used to compare the groups showing differences in our study and the differences were interpreted. A value of $p<0.05$ was considered statistically significant for both tests.

Results

Results Regarding the Amount of Hydrogen Peroxide Released from the Experimental and Control Groups

All whitening materials used in this study released hydrogen peroxide into the Eppendorf tubes. A spectrophotometer was used to measure the quantity of hydrogen peroxide released into the distilled water in the Eppendorf tubes for both the experimental and control groups on the 1st, 3rd, and 7th days.

Group 1- Whiteness Super Endo Group (37% carbomide peroxide)

In the Whiteness Super Endo group, measurements of hydrogen peroxide levels in the bidistilled water within the Eppendorf tubes were taken on the 1st, 3rd, and 7th days. These measurements were recorded using a spectrophotometer, and the mean values (µg/ mL) were calculated by comparing the absorbance readings with the standard calibration curve, as detailed in Table 1.

Accordingly, while the difference between the amounts of hydrogen peroxide released on Day 1 and Day 3 was not found to be statistically significant ($p> 0.05$), it was determined that the amount of hydrogen peroxide released on Day 7 decreased and the difference between them was statistically significant ($p<0.05$) (Table 6).

Group 2- Opalescence Endo Group (35% hydrogen peroxide)

The amounts of hydrogen peroxide released into the bidistilled water in the Eppendorf tubes of the Opalecense Endo group on the 1st, 3rd and 7th days were measured on the spectrophotometer and the average values (µg/mL) found by comparing the absorbance values obtained with the standard calibration curve are given in Table 2.

According to the results obtained from group 2, while the average amount of hydrogen peroxide released on the 1st day was 0.81 (µg/mL), this value decreased rapidly to 0.06 µg/mL on the 7th day. In addition, it was determined that the difference between the amounts of hydrogen peroxide release for all three days was statistically significant ($p <0.05$) (Table 6).

Group 3- Sodium Perborate + Distilled Water Group

The mean concentrations (µg/mL) of hydrogen peroxide in bidistilled water measured in Eppendorf tubes on days 1, 3, and 7 for the Sodium Perborate + Distilled Water group were compared to a standard calibration curve using spectrophotometry. These values are presented in Table 3. Accordingly, the highest release value was observed on Day 1, while no hydrogen peroxide release was observed on Day 7. When the values between Days 1, 3 and 7 were compared, it was determined that the difference between them was statistically significant ($p<0.05$)

Group 4- Sodium Perborate + 30% Hydrogen Peroxide Group

The amounts of hydrogen peroxide released into the bidistilled water on the 1st, 3rd and 7th days of the Sodium Perborate + 30% Hydrogen Peroxide group were measured in the spectrophotometer and the average values (µg/mL) obtained by comparing the absorbance values with the standard calibration curve are given in Table 4.

Accordingly, while the average hydrogen peroxide release amount on Day 1 was 1.40 (µg/mL), this value gradually decreased on Days 3 and 7. When the hydrogen peroxide release amounts between the days were compared, it was observed that the difference between them was statistically significant ($p<0.05$) (Table 6).

Control Group (Distilled Water)

In the measurements made on the 1st, 3rd and 7th days for the control group (distilled water), it was observed that hydrogen peroxide release was nearly zero (Table 5). When the values between the days were compared, it was determined that the difference between them was not statistically significant ($p>0.05$) (Table 6).

Results Regarding the Comparison of the Amount of Hydrogen Peroxide Released Between the Experimental and Control Groups

The experimental and control groups were compared with each other in terms of the amounts of hydrogen peroxide released into bidistilled water on the same day and the values obtained are shown in Figure 2 and Table 7 ($P<0.05$). As of day 1, the most effective bleaching agent was “Sodium Perborate + 30% Hydrogen Peroxide”. However, bleaching efficiency decreased significantly in all groups as time passed. In the control group, the effectiveness remained at the lowest level at all measurement times.

Discussion

In this study, four different bleaching agents; Whiteness Super Endo (37% carbomide peroxide), Opalescence Endo (35% hydrogen peroxide), Sodium Perborate + Distilled water and Sodium Perborate + 30% Hydrogen Peroxide were used for intracoronal bleaching. Among these bleaching agents, sodium perborate has been used for intracoronal bleaching by mixing with distilled water or hydrogen peroxide for many years, while the gel form preparations Whiteness Super Endo and Opalescence Endo have been introduced to the market relatively recently. In our study, we aimed to investigate and compare the radicular hydrogen peroxide penetration

of these four different bleaching agents by iron thionate method. For some time, the most popular technique for whitening devital teeth was thermocatalytic whitening, in which 30-35% hydrogen peroxide is used intracoronally in combination with heat or light to accelerate the breakdown.

Today, the thermocatalytic technique is not preferred except in very special cases. Walking bleach technique is effective, practical, reliable and more comfortable for the patient because it requires less time in the dental chair.[21-24] In our study, the walking bleach technique was preferred because it is a reliable and widely used technique.

When the hydrogen peroxide release values of the bleaching materials used in our study were compared on different days, it was observed that only the difference between the hydrogen peroxide release amounts of the Whiteness Super Endo group on day 1 and day 3 was not statistically significant ($p>0.05$), but the difference between the hydrogen peroxide amounts released on days 1, 3 and 7 in all other groups was statistically significant ($p<0.05$). According to these results, we can say that a more controlled release was realized in the Whitness super Endo (37% carbamide peroxide) group compared to the other groups since the amounts of hydrogen peroxide released on days 1 and 3 were similar.

In our study, the amounts of hydrogen peroxide released on days 1, 3 and 7 were also compared between the experimental groups. Accordingly, it was observed that the amount of hydrogen peroxide release of the sodium perborate + 30% hydrogen peroxide group on day 1 was higher than the other bleaching materials we used ($p<0.05$). This was followed by Opalecense Endo, sodium perborate + distilled water and Whitness Super Endo group, respectively. While there was no statistical difference between the Whitness Super Endo group and the sodium perborate + distilled water group ($p>0.05$), the difference between the other groups was found to be statistically significant ($p<0.05$). When the hydrogen peroxide release values of day 3 were examined, Whitness Super Endo, Opalecense Endo and sodium perborate + hydrogen peroxide groups showed similar results in terms of hydrogen peroxide released ($p>0.05$).

However, sodium perborate + distilled water group showed a statistically significant lower release value than the other groups ($p<0.05$). On the 7th day, it was determined that the amount of hydrogen peroxide released from all groups was similar and low ($p>0.05$). It was also observed that there was no hydrogen peroxide release from the sodium perborate + distilled water group and the control group and there was no statistical difference between the experimental groups and the control group ($p>0.05$).

Lee *et al.*[16] reported that the radicular hydrogen peroxide penetration rates of 35% carbamide peroxide gel (Opalesence Quick) and sodium perborate + distilled water mixture were similar, but the diffusion of 35% hydrogen peroxide gel (Opalesence Endo) was statistically significantly higher. The researchers showed that the least amount of penetration was in the 35% carbamide peroxide gel (Opalesence Quick) group. Although the carbamide peroxide gel preparation used by the researchers in their studies was different, the results they obtained are in parallel with the results of our study.

Gökay *et al.*[25], and Madhu *et al.*[26], showed that the highest amount of hydrogen peroxide release was found in the sodium perborate + 30% hydrogen peroxide group in their spectrophotometric measurements after 24 hours, as in our study.

Madhu *et al.*[26] aimed to measure extraradicular peroxide release with the iron thiocyanate method by intracoronal application of a mixture of sodium perborate and distilled water, 30% hydrogen peroxide, a mixture of sodium perborate and 30% hydrogen peroxide and 10% carbamide peroxide peroxide gel (Opalescence) in upper incisors.

Therefore, the researchers emphasized that carbamide peroxide is a very reliable alternative for intracoronal bleaching. Similarly, in our study, the amount of hydrogen peroxide at the end of day 1 was lower in the Whitness Super Endo group containing 37% carbamide peroxide than in the other groups.

Rokaya *et al.*[27]. applied Opalescence Endo (35% hydrogen peroxide), 35% carbamide peroxide peroxide (Opalescence PF), sodium perborate + 30% hydrogen peroxide and sodium perborate + distilled water mixture intracoronally in their study to compare the extraradicular hydrogen peroxide release of various bleaching agents used in intracoronal bleaching and according to the results obtained on the 1st day of release, the highest amount of hydrogen peroxide was found in the Opalescence Endo group. According to the results obtained on day 1, the highest hydrogen peroxide release was observed in the Opalescence Endo group and there was no significant difference between the other groups, on day 7 and day 14, the highest hydrogen peroxide release was observed in the Opalescence Endo group, followed by the sodium perborate + 30% hydrogen peroxide group, Opalescence PF and sodium perborate + distilled water groups.Unlike the results of this study, in our study, the highest hydrogen peroxide release was observed in the sodium perborate + 30% hydrogen peroxide group.

In addition, unlike our study, all bleaching materials used in this study showed an increase in radicular hydrogen peroxide amounts after 7 and 14 days. The reason for these differences in the results may be the artificial defects created in the teeth unlike the method used in our study.

Karayil *et al.*[28] compared the extraradicular hydrogen peroxide release amounts of different bleaching materials, applied Opalescence PF (10% carbamide peroxide peroxide gel, 15% carbamide peroxide gel, 35% carbamide peroxide gel) containing different concentrations of carbamide peroxide in gel form and 30% hydrogen peroxide mixture with sodium perborate intracoronally. When the groups were compared, it was observed that the highest hydrogen peroxide release was observed in the sodium perborate + 30% hydrogen

peroxide group, as in our study, and the lowest hydrogen peroxide release was observed in the 10% carbamide peroxide gel group, as expected. The results of this study are similar to the findings of our study.

They reported that the efficacy of 37% carbamide peroxide gel was acceptable for intracoronal bleaching. The reason why hydrogen peroxide was observed in the results obtained from the control group (distilled water) in our study, as in the study of Zoya *et al.*[20], is that hydrogen peroxide is a molecule present in every environment, and it is not possible to completely eliminate it in the measurements. Perrine et al.[29] reported that 10% carbamide peroxide gel has a whitening efficiency equivalent to sodium perborate.

Although hydrogen peroxide has good whitening efficacy when used alone or in combination with sodium perborate, there is a greater risk of cervical root resorption. This has led researchers to search for alternative bleaching materials that are both effective and less likely to cause complications. Carbamide peroxide gels have been proposed as a good alternative to sodium perborate + water mixture. [16,25,30,31]

Within the limits of this study, we can say that carbamide peroxide gel and sodium perborate + distilled water mixture can be safely used in bleaching applications because they show the lowest radicular hydrogen peroxide penetration. In addition, carbamide peroxide gel forms may be more preferred in the clinic because of their ease of application.

This in vitro study cannot fully simulate in vivo conditions where physiologic or pathologic obliteration of the periodontium and dentinal tubules prevents root penetration of peroxide. Moreover, existing inflammation of the periodontium may affect the extraradicular environment, thus affecting the body's ability to cope with the radicals produced. Further studies are needed to confirm the findings of this in vitro study in clinical conditions.

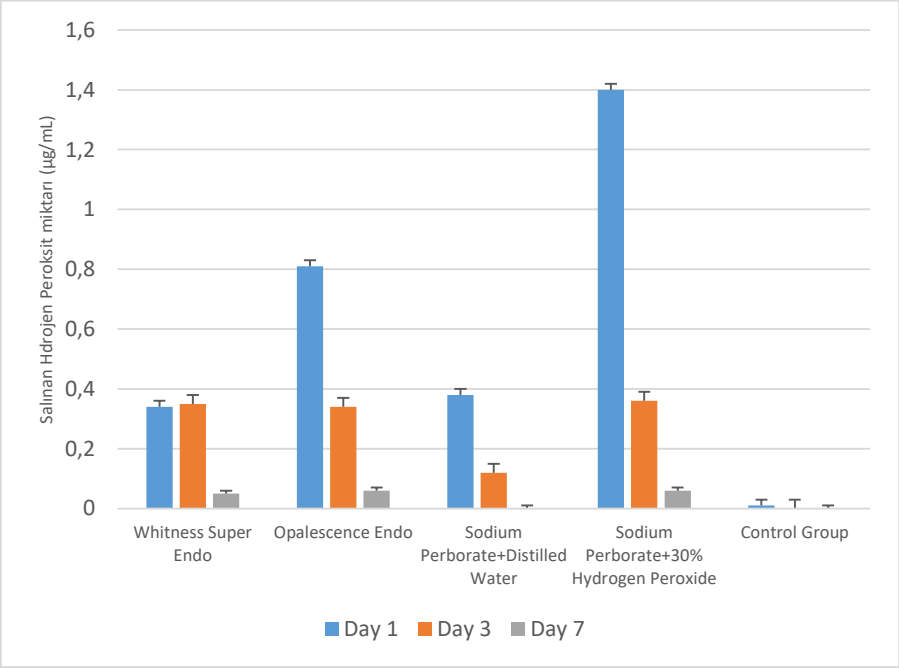

Figure 3.1. displays the mean values (µg/mL) for each group obtained through comparison of absorbance values measured by the spectrophotometer with the standard calibration curve.

Table 1 Amounts of hydrogen peroxide released into bidistilled water on the Whiteness Super Endo group (µg/mL)

Duration (day)	n	Mean (µg/mL)	Standard deviation
1	12	0.34	0.02
3	12	0.35	0.02
7	12	0.05	0.02

Table 2 Amounts of hydrogen peroxide released into bidistilled water on the Opalescence Endo group (µg/mL)

Duration (day)	n	Mean (µg/mL)	Standard deviation
1	12	0.81	0.05
3	12	0.34	0.01
7	12	0.06	0.04

Table 3 Amounts of hydrogen peroxide released into bidistilled water on the Sodium perborate + distilled water group (µg/mL)

Duration (day)	n	Mean (µg/mL)	Standard deviation
1	12	0.38	0.05
3	12	0.12	0.02
7	12	0.00	0.01

Table 4 Amount of hydrogen peroxide released into bidistilled water from Sodium Perborate + 30% Hydrogen Peroxide group (µg/ml)

Duration (day)	n	Mean (µg/mL)	Standard deviation
1	12	1.40	0.07
3	12	0.36	0.04
7	12	0.06	0.03

Table 5 Amounts of hydrogen peroxide released into bidistilled water from the control group (µg/mL)

Duration (day)	n	Mean (µg/mL)	Standard deviation
1	12	0.01	0.01
3	12	0.00	0.01
7	12	0.00	0.01

Table 6 Statistical comparison of the amount of peroxide released in the experimental and control groups on the 1st, 3rd and 7th days. ((p <0.05))*

Groups DAY count	Day 1 and Day 3	Day 1 and Day 7	Day 3 and Day 7
Whiteness Super Endo	0.587914	0.000038*	0.000033*
Opalescence Endo	0.000071*	0.000027*	0.000233*
Sodium Perborate + Distilled Water	0.000697*	0.000189*	0.001305*
Sodium Perborate +30% Hydrogen Peroxide	0.000023*	0.000006*	0.000334*
Control Group: Distilled Water	0.250815	0.416866	1.000000

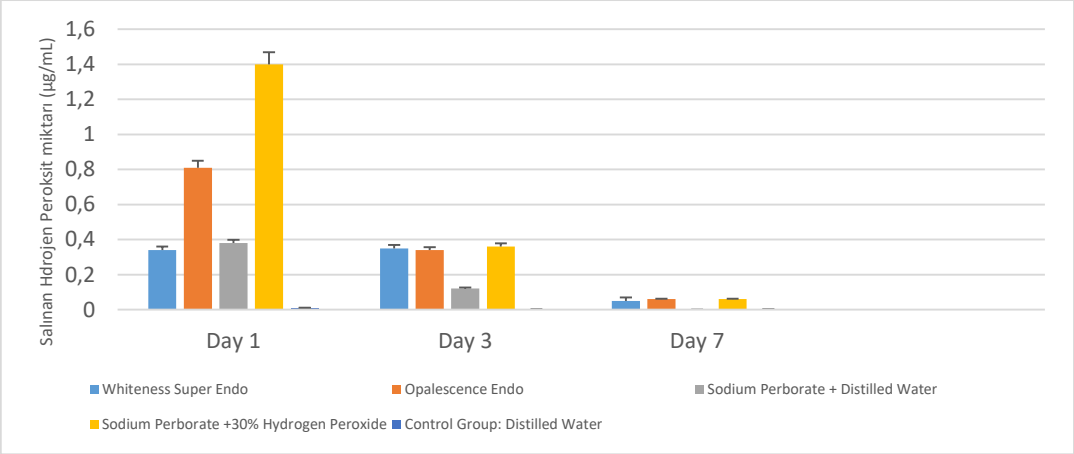

Figure 3.2 The amount of hydrogen peroxide released by the experimental and control groups on the 1st, 3rd and 7th days.

Table 7 Statistical comparison of the average peroxide amount released in the experimental and control groups on the 1st, 3rd and 7th days (mean ± standard deviation). Superior different letters indicate statistical significance (p<0.05), while same superior letters show no statistical significance within groups (p>0.05).

	Whiteness Super Endo	Opalescence Endo	Sodium Perborate + Distilled Water	Sodium Perborate +30% Hydrogen Peroxide	Control Group: Distilled Water
Day 1	$0.34^{a} \pm 0.02$	$0.81^{b} \pm 0.05$	$0.38^{a} \pm 0.05$	$1.40^{c} \pm 0.07$	$0.01^{d} \pm 0.01$
Day 3	$0.35^{a} \pm 0.02$	$0.34^{a} \pm 0.01$	$0.12^{b} \pm 0.02$	$0.36^{a} \pm 0.04$	$0.00^{d} \pm 0.01$
Day 7	$0.05^{d} \pm 0.02$	$0.06^{d} \pm 0.04$	$0.00^{d} \pm 0.01$	$0.06^{d} \pm 0.03$	$0.00^{d} \pm 0.01$

References

1. Arens D. The role of bleaching in esthetics. Dent Clin North Am 1989;33:319-336.
2. Rotstein I. In vitro determination and quantification of 30% hydrogen peroxide penetration through dentin and cementum during bleaching. Oral Surg Oral Med Oral Pathol 1991;72:602-606.
3. Rotstein I, Walton R. Bleaching Discolored Teeth: Internal and External. In: Torabinejad M, Walton JR. Principles and Practice of Endodontics, 4. Ed., Philadelphia: WB Saunders Com., 2009;p391-404.
4. Knezevic N, Obradovic M, Dolic O, Veselinovic V, Kojic Z, Josipovic R, Arapovic-Savic M. Clinical testing of walking bleach, in-office, and combined bleaching of endodontically treated teeth. Medicina (Kaunas) 2022;59:18.
5. Frank Ac, Kanzow P, Rödig T, Wiegand A. Comparison of the bleaching efficacy of different agents used for internal bleaching: a systematic review and meta-analysis. J Endod 2022;48:171-178.
6. Kahler B. Present status and future directions - managing discoloured teeth. Int Endod J 2022;55:922-950.
7. Greenwall L. Bleaching Techniques in Restorative Dentistry: An Illustrated Guide: CRC Press, 2001.
8. Heithersay GS. Invasive Cervical Resorption: An analysis of potential predisposing factors. Quintessence Int 1999;30:83-95.
9. Madison S, Walton R. Cervical root resorption following bleaching of endodontically treated teeth. J Endod 1990;16:570-574.
10. Harrington GW, Natkin E. External resorption associated with bleaching of pulpless teeth. J Endod 1979;5:344-348.
11. Newton R, Hayes J. The Association of external cervical resorption with modern internal bleaching protocols: what is the current evidence? Br Dent J 2020;228:333-337.
12. Mavridou Athina M, Hauben E, Wevers M, Schepers E, Bergmans L, Lambrechts P. Understanding external cervical resorption in vital teeth. J Endod 2016;12:1737-1751.
13. Heithersay GS. Invasive cervical resorption. Endod Topics 2004;7:73-92.
14. Halliwell B, Clement MV, Ramalingam J, Long LH. Hydrogen peroxide. Ubiquitous in cell culture and in vivo? IUBMB Life 2000;50:251-257.
15. Goldberg M, Grootveld M, Lynch E. Undesirable and adverse effects of tooth-whitening products: a review. Clin Oral Investig 2010;14:1-10.
16. Lee GP, Lee MY, Lum SOY, Poh RSC, Kim KC. Extraradicular diffusion of hydrogen peroxide and pH changes associated with intracoronal bleaching of discoloured teeth using different bleaching agents. Int Endod J 2004;37:500-506
17. Rotsteini Li Y. Tooth Discoloration and Bleaching. In: Ingle's Endodontics, Ed.: J. I. Ingle, K. Bakland, J. C. Baumgartner, 6th Ed., Hamilton: Bc Decker Inc., P1383-1399.
18. Yongil Jin, Klenise S. Paranhos, Ann Salamone, William Bongiorno, Melina Brizuela. (2024). Internal Tooth Whitening. In: Statpearls [Internet]. Treasure Island (Fl): Statpearls Publishing.
19. Amer M. Intracoronal tooth bleaching - a review and treatment guidelines. Aust Dent J 2023; 68: S141-S152.
20. Zoya A, Tewari RK, Mishra SK, Faisal SM, Ali S, Kumar A, Moin S. Sodium percarbonate as a novel intracoronal bleaching agent: assessment of the associated risk of cervical root resorption. Int Endod J 2019;52:701-708.
21. Devji T. Walking bleach technique for endodontically treated teeth with 35% hydrogen peroxide and 37% carbamide peroxide may result in similar improvements in tooth color and patient satisfaction. J Am Dent Assoc 2018;149:E113.
22. Oskoee SS, Bahari M, Daneshpooy M, Ajami AA, Rahbar M. Effect of different intraorifice barriers and bleaching agents on the fracture resistance of endodontically treated anterior teeth. J Endod. 2018;44:1731-1735.
23. Sismanoglu S, Yildirim-Bilmez Z, Gurcan AT, Gumustas B, Taysi M, Berkman M. Influence of intracoronal bleaching agents on the bond strength of MTA cements to composite resin and their surface morphology. Odontology 2022;110:148-156.
24. Abduljalil M, Sakalli B, Basmaci F. Impact of different intraorifice barriers on fracture resistance of non-vital bleached teeth. Niger J Clin Pract 2023;26:95-101.
25. Gökay O, Ziraman F, Çali Asal A, Saka OM. Radicular peroxide penetration from carbamide peroxide gels during intracoronal bleaching. Int Endod J 2008;41:556-560.
26. Madhu K, Hegde S, Mathew S, Lata D, Bhandî SH, Shruthi N. Comparison of radicular peroxide leakage from four commonly used bleaching agents following intracoronal bleaching in endodontically treated teeth- an in vitro study. J Int Oral Health 2013;5:49-55.
27. Rokaya ME, Beshr K, Mahram AH, Pedir SS, Baroudi K. Evaluation of extraradicular diffusion of hydrogen peroxide during intracoronal bleaching using different bleaching agents. Int J Dent 2015;2015:493795.
28. Karayil Binu Nathan, Roopa R Nadig, Tisson Varghese Job, Puthan Veetil Nithin, Rajesh Karthik, Sailaja Choudary. (2019) Radicular Peroxide Penetration from Different Concentrations of Carbamide Peroxide Gel during Intracoronal Bleaching-An In vitro Study J Contemp Dent Pract. 1;20(5):587-592.
29. Perrine GA, Reichl RB, Baisden MK, Hondrum SO. Comparison of 10% carbamide peroxide and sodium perborate for intracoronal bleaching. Gen Dent 2000;48:264-270.
30. Lim MY, Lum SOY, Poh RSC, Lee GP, Lim KC. An in vitro comporation of bleaching efficacy of 35% carbamide peroxide with established intracoronal bleaching agents. Int Endod J 2004;37:483-488.
31. Teixeira ECN, Hara AT, Serra MC. Use of 37% carbamide peroxide in the walking bleach tecnique: a case report. Quintessence Int 2004;35:97-102.