Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2074-2079 | ISSN:2251-6727

Comparative Evaluation of Cervical Penetration of Hydrogen Peroxide Using 35% Hydrogen Peroxide and Sodium Perborate Bleaching Agents with Different Intraorifice Barriers-An in Vitro Study

Dr Piyush Mantri,[1] Dr Aparna Palekar,[2] Dr Basawaraj Biradar,[3] Dr Mukund Singh,[4] Dr Vaidehi Joshi,[5] Dr Pushpal Ingle,[6]

[1] *PG Student, Department of Conservative Dentistry and Endodontics, Rural Dental College, Loni, Maharashtra*

[2] HOD and Professor, Department of Conservative Dentistry and Endodontics, Rural Dental College, Loni, Maharashtra

[3,4] Professor, Department of Conservative Dentistry and Endodontics, Rural Dental College, Loni, Maharashtra

[5] PG student, Department of Conservative Dentistry and Endodontics, Bapuji Dental College and Hospital, Davangere, Karnataka

[6] PG Student, Department of Conservative Dentistry and Endodontics, Dr. D.Y. Patil Dental College, Pimpri, Maharashtra

(Received: 16 August 2025 Revised: 20 September 2025 Accepted: 01 October 2025)

KEYWORDS

Intracoronal bleaching, hydrogen peroxide, sodium perborate, intraorifice barrier, Biodentine, MTA

ABSTRACT:

Introduction: Tooth discoloration following trauma or endodontic treatment is commonly managed using intracoronal bleaching. However, bleaching agents can diffuse through dentinal tubules and cause external cervical resorption. Placement of an intraorifice barrier is therefore recommended to limit peroxide penetration and protect surrounding tissues.

Objectives: The present study aimed to evaluate the effectiveness of three different intraorifice barrier materials named GIC, ProRoot MT, and Biodentine against cervical penetration of commonly used bleaching.

Methods: A total of 120 extracted single-rooted permanent teeth were endodontically treated and randomly allocated to three barrier groups (GIC, MTA, Biodentine) and one control group (no barrier). Each group was further subdivided based on bleaching agent HP, SP, and HP+SP. Peroxide penetration was quantified after 7 days using a titration-based hydrogen peroxide testing kit.

Results: Data were analyzed using one-way ANOVA with Tukey's post hoc test. All barriers significantly reduced peroxide penetration compared to controls *($p < 0.05$)*. Biodentine consistently showed lowest penetration across all bleaching agents and least with SP (HP: 0.241 ± 0.0107 ppm; SP: 0.199 ± 0.0491 ppm; HP+SP: 0.210 ± 0.0272 ppm). MTA demonstrated similar efficacy to Biodentine *($p > 0.05$)*, while GIC exhibited the highest permeability.

Conclusions: Intraorifice barriers play a critical role in reducing peroxide penetration. Among which Biodentine provided most effective sealing, followed by MTA, while GIC showed inferior performance. Biodentine combined with sodium perborate was the safest option, minimizing peroxide leakage.

1. Introduction

Tooth discoloration may be caused by a variety of local, systemic, intrinsic or extrinsic factors, which can lead to intrapulpal hemorrhage and subsequent breakdown of blood components within dentinal tubules, causing a darkened appearance. Similarly, insufficient tissue pulp removal during endodontic procedures or root resorption may also alter dentin's translucency and color, further exacerbating discoloration,[1] impacting self-esteem and quality of life of individuals. Thus, with increasing cosmetic demands, intracoronal bleaching has become a conservative and effective treatment compared to crowns, veneers, or composites.[2]

This technique utilizes chemical agents to oxidize and break down the pigmented molecules within the dentinal tubules. There are two minimally invasive bleaching techniques, thermocatalytic method, which

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 2074-2079 | ISSN:2251-6727

requires heat to activate hydrogen peroxide in pulp chamber for faster bleaching but increases the risk of external cervical resorption and walking bleach technique requiring sealing agent in pulp chamber for several days, allowing gradual whitening with fewer complications, making it ideal for patients who can allow a longer treatment period.[3]

A strong oxidizing agent, HP (30%–35%) is frequently used. Its mechanism of action involves the release of oxygen radicals, which interact with and break down pigmented molecules within the tooth structure,[4] while SP is a milder and more stable bleaching agent, commonly mixed with distilled water or hydrogen peroxide to enhance its efficacy. SP undergoes a chemical reaction in the presence of water or an acid, releasing hydrogen peroxide as a byproduct. This gradual release of peroxide allows for a controlled bleaching effect, making SP a safer alternative for patients with higher risks of adverse reactions and when HP is combined with SP, its effectiveness is further amplified.[5]

Despite its conservative nature, intracoronal bleaching may cause invasive cervical resorption due to acidic pH and osteoclastic activation.[6]To mitigate this penetration, use of protective barrier is essential to safeguard root canal during procedures.[7] Barriers like amalgam, calcium-enriched mixture (CEM), composite resin, glass-ionomer cement (GIC), cement, mineral trioxide aggregate (MTA), Cavit, intermediate restorative material (IRM), Super-EBA, and Biodentine are used, out of which GIC has been favored material for the coronal barrier in order to stop bleaching agents from leaking coronally,[8,9]whereas MTA has a high marginal adaptability, high calcium hydroxide concentrations, and resistance to microleakage, but its lack of solubility, lengthy setting time, difficulty in handling, tooth staining, and struggle in removing are some of the disadvantages.[10-12]However, Biodentine's non-toxicity, dentin-like mechanical characteristics, simplicity of usage and handling, rapid setting time, and leakage resistance also make it a suitable barrier.[13-16]

The proper selection and application of these barriers and understanding the comparative performance of bleaching agents will not only enhance safety profile of intracoronal bleaching but also optimize the overall treatment outcome. Thus, the aim of the study is to assess how commonly used bleaching agents affect the sealing properties of different intraorifice barriers which will ultimately aid clinicians in making evidence-based decisions and will contribute to safer and more effective bleaching protocols, enhancing patient care and satisfaction.

2. Objectives

Intracoronal bleaching is widely used for aesthetic improvement of endodontically treated teeth, but hydrogen peroxide penetration can cause external cervical root resorption. Intraorifice barriers are recommended to limit peroxide diffusion and protect surrounding tissues. This in-vitro study aimed to evaluate and compare the effectiveness of three barrier materials, glass ionomer cement (GIC), mineral trioxide aggregate (MTA), and Biodentine. The goal was to identify the material that provides the most efficient seal against cervical peroxide penetration during intracoronal bleaching.

3. Methods

Teeth Selection: Sample size was calculated using OpenEpi ($\alpha = 0.05$, 80% power), yielding 10 per group. Approval was granted by Institutional Ethics Committee (PIMS/IEC/RDC/UG-PG/04-2023). A total of 120 extracted, intact, permanent single rooted teeth were included. Teeth with any caries, cracks, restorations, anatomical variations, resorption, calcification, apical curvatures or previously endodontically treated teeth were excluded. Samples were randomized (simple random sampling) and kept in saline at room temperature after removal of soft and hard tissue remnants with ultrasonic tips.

Sample Preparation: Standard access cavities were prepared with endo access burs (Mani, Japan) with high speed, water-cooled airotor (Waldent, India). Using a #10 K-file (Mani, Japan), root canal lengths were calculated by deducting 1 mm from the root apex. Hero Gold (MicroMega, France) rotary files powered by an endomotor (Endo Gold, China) were used to instrument the canals up to 30 6%. Following each filing, irrigation was performed with 3% NaOCl (Prime Dental, India). The final irrigation protocol included sequential application of 3% NaOCl, 17% EDTA (Prime Dental, India), and saline solution. The canals were filled using a single cone obturation technique utilizing gutta-percha (Diadent, Korea) and AH Plus® sealer (Dentsply, Switzerland) after having been dried using paper points (Diadent, Korea). Cavit-G (3M ESPE, Germany) was used as a temporary sealant to close the access cavities. For a whole day, the samples were kept in an incubator (Bio-Technics, India) at 37°C and 100% humidity. Following obturation, a slow speed handpiece (NSK Ltd. Tokyo, Japan) and Peeso Reamer #3 (Mani, Japan) was used to remove root fillings up to 3 mm short of the cementoenamel junction (CEJ) to prepare space for intraorifice barrier materials. The depth was confirmed with a William's periodontal probe.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 2074-2079 | ISSN:2251-6727

Grouping: Teeth were assigned to three intraorifice barrier groups (n = 30 each):

- GIC (Ketac™ Molar, Germany)
- ProRoot MTA (Dentsply, USA)
- Biodentine (Septodont, France)
- Each group was further divided into subgroups (n = 10) based on bleaching agent:
- 35% hydrogen peroxide (HP) (Prevest DenPro, India)
- Sodium perborate (SP) (SD Fine-Chem, India)
- HP + SP combination

Positive control groups (n = 10 per agent) received bleaching without a barrier.

Intraorifice barrier were prepared according to the manufacturer's guidelines and were placed into respective prepared spaces. It was then shaped by using a round diamond bur at a low-speed setting to gradually contour the material to mimic the natural contour of the CEJ. An inclined contour formed by the ski-sloped design on the mesial and distal aspects allowed for a seamless transition from the access cavity to the canal region. On the labial aspect, the bobsled tunnel configuration was shaped, characterized by a concave, tunnel-like structure. A radiograph was taken to check the location of the barrier after placement (Fig. 1). After covering the barrier with Teflon, Cavit was used to fill the tooth.

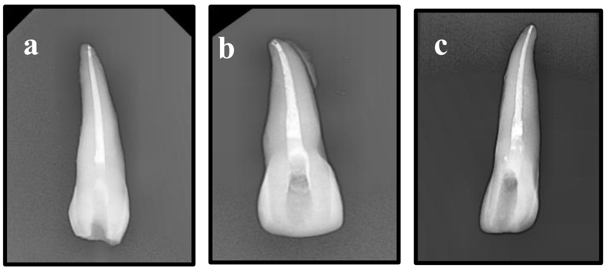


Fig. 1. Tooth Sample Radiograph after placing the Intraorifice Barrier

a) GIC b) ProRoot MTA c) Biodentine

Peroxide Penetration Analysis: All surfaces except 3 mm cervical zone below CEJ were coated with nail varnish and modelling wax (Maarc, India) (Fig. 2). Specimens were immersed in 10 mL distilled water and incubated for seven days at 37ºC and 100% humidity. Peroxide penetration was determined using a titration-based Hydrogen Peroxide Testing Kit from Aquasol (Rakiro Biotech, India). The test jar was filled with water sample to be tested up to the 10ml mark, 20 drops of HP1 solution were added to this jar and mixed well then 2 spoonsful of HP2 were added, mixed well until a dark blue-black colour appears. To this, dropwise HP3 (diluted 1 drop in 100ml of distilled water) solution was added. The number of drops were counted of this HP3 till the solution became colourless. The concentration of hydroxyl ions in ppm was calculated according to the following formula:

Calculations: Hydrogen Peroxide (in ppm) = .0025 x No. of drops of HP3

Fig. 2. Tooth Sample Covered with Nail Varnish and Modelling Wax with 3mm wax-free space below CEJ

4. Results

Analysis of data was performed using SPSS Version 20.0 (IBM Inc., USA). One-way ANOVA was applied to compare mean peroxide penetration among groups, followed by Tukey's post hoc test for pairwise comparisons. For statistical analysis, value of $p < 0.05$ was considered significant.

Peroxide penetration varied significantly among bleaching agents and intraorifice barriers (Table 1) In HP group, Biodentine showed the least peroxide penetration (0.241 ± 0.0107 ppm), followed by MTA (0.253 ± 0.059 ppm), while GIC exhibited the highest (0.337 ± 0.0619 ppm). The control group recorded maximum penetration (12.317 ± 0.2366 ppm). One-way ANOVA revealed significant differences (F = 10.953, p = 0.0003). Post hoc analysis confirmed Biodentine and MTA were significantly superior to GIC ($p < 0.05$), with no significant difference between them ($p > 0.05$).

In SP group, Biodentine again showed the lowest penetration (0.199 ± 0.0491 ppm), followed by MTA (0.261 ± 0.0321 ppm) and GIC (0.288 ± 0.0463 ppm). Control values were highest (8.893 ± 0.0218 ppm). ANOVA showed significant group differences (F = 11.277, p = 0.0002). Biodentine significantly

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 2074-2079 | ISSN:2251-6727

outperformed GIC *($p < 0.05$)*, while its difference with MTA was not significant *($p > 0.05$).*

In HP + SP group, Biodentine (0.210 ± 0.0272 ppm) showed least penetration, followed by MTA (0.270 ± 0.0798 ppm), and GIC (0.344 ± 0.0509 ppm). The control group again had the highest penetration (10.297 ± 0.7157 ppm). Statistical analysis confirmed significant differences (F = 13.877, p = 0.0001). Both Biodentine and MTA were significantly more effective than GIC *($p < 0.05$).*

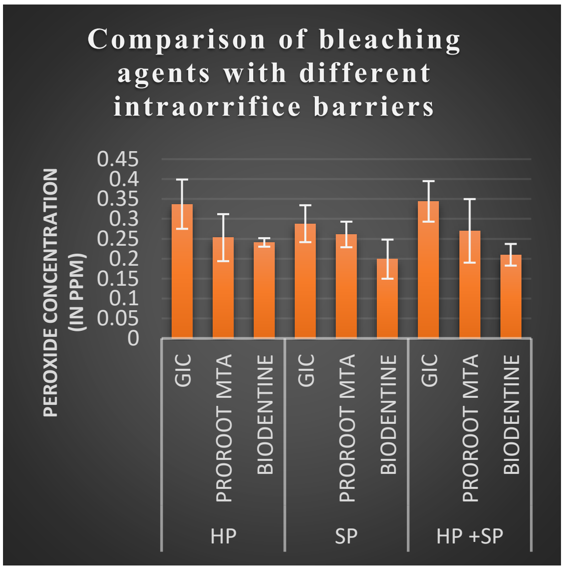
Across all control groups, highest penetration was seen in the HP group (12.355 ± 0.2543 ppm), followed by HP + SP (10.513 ± 0.8736 ppm), and SP group (8.889 ± 0.0568 ppm). One-way ANOVA revealed highly significant differences (F = 108.543, $p < 0.001$). Post hoc comparisons confirmed significantly lower peroxide diffusion in SP compared to HP and HP + SP ($p < 0.05$).

Use of intraorifice barriers significantly reduced peroxide penetration compared to controls ($p < 0.05$), with Biodentine consistently demonstrating the most effective sealing ability.

Table 1: Comparison of bleaching agents with different intraorifice barriers

GROUPS		MEAN	SD
BLEACHING AGENT	**BARRIER**		
Hydrogen peroxide (HP)	GIC	0.337	0.0619
	PROROOT MTA	0.253	0.059
	BIODENTINE	0.241	0.0107
	CONTROL	12.355	0.254
Sodium perborate (SP)	GIC	0.288	0.0463
	PROROOT MTA	0.261	0.0321
	BIODENTINE	0.199	0.0491
	CONTROL	8.889	0.057
Hydrogen peroxide (HP) + Sodium perborate (SP)	GIC	0.344	0.0509
	PROROOT MTA	0.270	0.0798
	BIODENTINE	0.210	0.0272
	CONTROL	10.513	0.874

Graph 1: Comparison of bleaching agents with different intraorifice barriers



5. Discussion

Trauma-induced anterior tooth discoloration is often treated with intracoronal bleaching after placing an intraorifice barrier, but reports of external cervical resorption have been cited, with its cause still uncertain.[17]

Hydrogen peroxide (HP) is a potent oxidizing agent that effectively breaks down stains, while sodium perborate (SP) with water shows good results but needs multiple applications. Combining HP with SP achieves desired bleaching outcomes in fewer sessions. Since dentinal tubules can allow cervical penetration of bleaching agents, an effective intraorifice barrier is essential to prevent microleakage and minimize adverse effects. Commonly used barrier materials include glass-ionomer cement, CEM cement, composite resin, IRM, Super-EBA, and calcium silicate-based cements such as Biodentine and MTA.[18]

Single rooted permanent teeth were included in this study as they are most commonly associated with trauma which often leads to discoloration.[19]Gutta-percha and AH plus sealer were used to obturate the canals as they have exceptional sealing capacity by closely adapting to dentin. In order to avoid the peroxide penetration, the barrier was applied at CEJ prior bleaching procedure. Teeth specimens were coated with nail varnish, leaving 3 mm around the cervical margin exposed to control hydrogen peroxide penetration, and incubated at 37 °C with humidity to mimic oral conditions, maintain a controlled environment, and prevent dehydration during testing.

The results demonstrated cervical penetration of hydrogen peroxide through GIC was highest with HP, moderate with combination of HP+SP, and lowest with SP. Likely due to its inherent porosity and weaker bonding properties, GIC tends to be porous and is susceptible to hydrolytic degradation in moist oral conditions, potentially resulting in compromised integrity over time increasing the permeability of peroxide. Similar findings were seen from studies done by Sakalli et al. (2022).[20]

Lesser penetration of H_2O_2 was found with ProRoot MTA barrier when compared to GIC across all subgroups. Highest penetration was seen in HP+SP followed by SP and least in HP. The excellent performance of MTA is by its superior physicochemical properties, including minimal solubility, high biocompatibility, and the ability to create long-lasting hermetic seal with dentinal walls. Its calcium silicate-based composition ensures dimensional stability and strong adhesion through hydroxyapatite formation, which effectively blocks the diffusion pathways for hydrogen peroxide. These results are consistent with prior research by Bolbolian et al. (2020) underscoring MTA's robust barrier qualities.[21]

Biodentine showed the least peroxide penetration as compared to GIC and MTA across all subgroups, with lowest in SP, intermediate values (0.21±0.0272 ppm) in combination (HP+SP) and highest penetration in HP, suggesting it provides the safest profile with Biodentine. It is likely attributed to its superior adaptability, bioactivity, and ability to form hydroxyapatite at the dentin interface. Biodentine's advantageous sealing properties arise from its calcium silicate composition, rapid setting time, and favourable dimensional stability, making it an ideal material to reduce microleakage and chemical diffusion.[22]These findings align with those of earlier studies, such as those by Koubi et al. (2013).[23]However, contradicting findings were seen in a study done by Shah JR et al. (2016) in which MTA outperformed Biodentine in terms of sealing ability when utilized as a barrier.[24]

The control groups without intraorifice barriers demonstrate statistically significant peroxide penetration. The highest was seen with HP, followed by the HP + SP combination, and lowest with SP. These findings underscore the critical role of barrier materials in clinical practice, emphasizing the importance of their selection to prevent chemical injury to periodontal and peri-radicular tissues.[25]

Thus, this study demonstrated that cervical hydrogen peroxide penetration was greatest in control groups without intraorifice barriers, confirming their critical protective role. Among the tested materials, GIC exhibited the highest permeability (*$p < 0.05$*) except when compared to MTA with SP, where differences were not significant *($p > 0.05$)*. Both MTA and Biodentine showed superior sealing, with Biodentine achieving the lowest penetration – particularly with SP (0.199 ± 0.049 ppm). These findings suggest that calcium silicate-based materials – especially Biodentine combined with sodium perborate – are the safest clinical choice to minimize peroxide leakage and safeguard periodontal tissues during intracoronal bleaching.

Despite these promising findings, it should be acknowledged that the study was conducted under in vitro conditions, which cannot fully replicate in vivo settings because of numerous intrinsic limitations. Therefore, further long-term clinical trials are necessary to validate these results and establish definitive clinical recommendations.

Conclusion: Within limitations of this in vitro study, Biodentine and MTA demonstrated superior sealing ability compared to GIC in limiting cervical hydrogen peroxide penetration. Sodium perborate alone showed the lowest penetration, while 35% hydrogen peroxide alone resulted in the highest. The combination of Biodentine with SP produced the least overall penetration. Control groups without barriers exhibited the greatest diffusion, emphasizing the critical role of intraorifice barriers in reducing peroxide leakage and enhancing the safety of intracoronal bleaching. Future research should focus on long-term clinical studies to evaluate these findings in real-world scenarios and explore alternative barrier materials that further enhance peroxide containment.

Funding and sponsorship: Nil.

Conflicts of interest: None

References:

1. Attin T, Paqué 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.
2. de Souza-Zaroni WC, Lopes EB, Ciccone-Nogueira JC, Silva RC. Clinical comparison between the bleaching efficacy of 37% peroxide carbamide gel mixed with sodium perborate with established intracoronal bleaching agent. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;107(2):e43-e47.
3. Roy D, Kataki R, Gogoi S, Seal M. Evaluation of peroxide release during nonvital bleaching using

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 2074-2079 | ISSN:2251-6727

three different coronal barriers: An *in vitro* study. *J Conserv Dent Endod*. 2024;27(9):920-24.
4. Barakah R, Alwakeel R. Non-vital Endo Treated Tooth Bleaching with Sodium Perborate. *Curr Health Sci J*. 2019;45(3):329-32.
5. Mehrotra A, Singh S, Podar RS, Shetty R, Salgar A, Kumar M. An *in vitro* comparative evaluation of intraorifice barriers and bleaching agents on the fracture resistance of the endodontically treated anterior teeth. *J Conserv Dent Endod*. 2023;26(6):646-50.
6. Patel S, Kanagasingam S, Pitt Ford T. External cervical resorption: a review. *J Endod*. 2009;35(5):616-625.
7. Mehta S, Ramugade M, Abrar S, Sapkale K, Giuliani V, Burbano Balseca MJ. Evaluation of coronal microleakage of intra-orifice barrier materials in endodontically treated teeth: A systematic review. *J Conserv Dent*. 2022;25(6):588-595.
8. Zarean P, Zarean P, Ravaghi A, Zare Jahromi M, Sadrameli M. Comparison of MTA, CEM Cement, and Biodentine as Coronal Plug during Internal Bleaching: An In Vitro Study. *Int J Dent*. 2020;2020:8896740. Published 2020 Nov 12.
9. Aboobaker S, Nair BG, Gopal R, Jituri S, Veetil FR. Effect of intra-orifice barriers on the fracture resistance of endodontically treated teeth - an ex-vivo study. *J Clin Diagn Res*. 2015;9(2):ZC17-ZC20.
10. McCabe P. Revascularization of an immature tooth with apical periodontitis using a single visit protocol: a case report. *Int Endod J*. 2015;48(5):484-497.
11. Nagas E, Ertan A, Eymirli A, Uyanik O, Cehreli ZC. Tooth Discoloration Induced by Different Calcium Silicate-Based Cements: A Two-Year Spectrophotometric and Photographic Evaluation in Vitro. *J Clin Pediatr Dent*. 2021;45(2):112-116.
12. Choi YL, Jang YE, Kim BS, Kim JW, Kim Y. Pre-application of dentin bonding agent prevents discoloration caused by mineral trioxide aggregate. *BMC Oral Health*. 2020;20(1):163. Published 2020 Jun 3.
13. Torabinejad M, Parirokh M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part II: other clinical applications and complications. *Int Endod J*. 2018;51(3):284-317.
14. Han L, Okiji T. Bioactivity evaluation of three calcium silicate-based endodontic materials. *Int Endod J*. 2013;46(9):808-14.
15. Shokouhinejad N, Nekoofar MH, Razmi H, et al. Bioactivity of EndoSequence root repair material and bioaggregate. *Int Endod J*. 2012;45(12):1127-34.
16. Zhou HM, Du TF, Shen Y, Wang ZJ, Zheng YF, Haapasalo M. In vitro cytotoxicity of calcium silicate-containing endodontic sealers. *J Endod*. 2015;41(1):56-61.
17. Fernandes M, de Ataide I, Wagle R. Tooth resorption part II - external resorption: Case series. *J Conserv Dent*. 2013;16(2):180-185.
18. Madhu K, Hegde S, Mathew S, Lata D, Bhandi SH, N S. 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(4):49-55.
19. Mittal N, Baranwal HC, Kharat SM, Ayubi A, Samad S, Aggarwal H. Comparative evaluation of quantitative color changes in discolored anterior teeth by bleaching, resin infiltration, and microabrasion: A randomized clinical trial. *J Conserv Dent Endod*. 2025;28(6):537-42.
20. Sakalli B, Basmaci F, Dalmizrak O. Evaluation of the penetration of intracoronal bleaching agents into the cervical region using different intraorifice barriers. *BMC Oral Health*. 2022;22(1):266. Published 2022 Jun 30.
21. Bolbolian M, Ghorbani F, Ghandi M, Ghashami M, Omidi BR, Mirzadeh M. Comparative investigation of microleakage of Biodentine and mineral trioxide aggregate as coronal barrier in nonvital bleaching. *J Mazandaran Univ Med Sci*. 2020;30(186):80-9.
22. Butt N, Talwar S, Chaudhry S, Nawal RR, Yadav S, Bali A. Comparison of physical and mechanical properties of mineral trioxide aggregate and Biodentine. *Indian J Dent Res*. 2014;25(6):692-97.
23. Koubi G, Colon P, Franquin JC, et al. Clinical evaluation of the performance and safety of a new dentine substitute, Biodentine, in the restoration of posterior teeth - a prospective study. *Clin Oral Investig*. 2013;17(1):243-249.
24. Shah JR, Raghavendra SS. Efficacy of barrier materials in Walking Bleach technique – A pH diffusion study. *Br J Pharm Med Res*. 2016;1(3):199-206.
25. Madison S, Walton R. Cervical root resorption following bleaching of endodontically treated teeth. *J Endod*. 1990;16(12):570-4.