Dental Research Journal

Original Article

Evaluation of the discoloration of cold ceramic, ortho mineral trioxide aggregate, and retro mineral trioxide aggregate in the presence of blood and normal saline: An *in vitro* study

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Received: 05-Nov-2024
Revised: 11-Dec-2024
Accepted: 12-Jan-2025
Published: 22-May-2025

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ABSTRACT

Background: Coronal discoloration is a common complication when using calcium silicate-based cements in esthetic zones. An ideal endodontic cement should provide favorable esthetic results alongside optimal biological and mechanical properties. This study aims to evaluate the discoloration of three calcium silicate-based cements—Cold Ceramic, Ortho MTA, and Retro MTA—in the presence of blood and normal saline.
Materials and Methods: In this experimental study, 48 human anterior teeth were prepared and randomly divided into six groups (n = 8) based on the type of cement (Cold Ceramic, OrthoMTA, RetroMTA) and environment (blood or normal saline). Color analysis of tooth crowns was performed using a spectrophotometer before applying the cements and at 30 and 60 days after application. Repeated measures analysis of variance was used to evaluate the effects of blood, material type, and time on discoloration (ΔE). As the data showed a non-normal distribution, the Kruskal-Wallis test was used for intergroup comparisons, and the Wilcoxon test was applied for intragroup analyses over time (P-value < 0.05).
Results: After one month, specimens exposed to blood exhibited greater discoloration than those exposed to normal saline. All groups showed noticeable discoloration at two months, with blood exposure exacerbating the effect. Across different times and environments, OrthoMTA caused more discoloration than the other materials. However, the differences were not significant. (P-value > 0.05).
Conclusion: In all three groups, blood-exposed and normal saline environments caused clinically noticeable discoloration over time. These materials are, therefore, not recommended for use in esthetic zones.

Key Words: Blood, mineral trioxide aggregate, saline solution, tooth discoloration

INTRODUCTION

The esthetic nature of dental structures is significantly influenced by the materials used, the final restorative filling, and the overall health, hygiene, and condition of soft and hard tissues.[1,2] Coronal discoloration is a common complication associated with calcium

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Website: www.drj.ir
https://journals.lww.com/DERJ
www.ncbi.nlm.nih.gov/pmc/journals/1480
DOI: 10.4103/drj.drj_513_24

How to cite this article: Hemati H, Honarmand F, Abolhasani M, Mollahasani H, Iranmanesh P, Kashani MS, *et al*. Evaluation of the discoloration of cold ceramic, ortho mineral trioxide aggregate, and retro mineral trioxide aggregate in the presence of blood and normal saline: An *in vitro* study. Dent Res J 2025;22:21.

silicate-based cement used in vital pulp therapy (VPT), regenerative endodontics, and perforation repair, posing a challenge for dental clinicians. An ideal endodontic cement should offer excellent esthetic outcomes alongside optimal biological and mechanical properties.[3,4]

Since the introduction of gray mineral trioxide aggregate (MTA) by Dr. Torabinejad *et al*. in 1991, white MTA was developed to address discoloration concerns. However, white MTA also presented limitations in clinical application.[5,6] In response, alternative calcium silicate-based cement has been developed to address these challenges.[7]

OrthoMTA is a novel orthograde root canal material that maintains its properties in the presence of moisture and blood. It exhibits excellent sealing ability, biocompatibility, radiopacity, and antibacterial properties. In addition, it contains no heavy metals, does not expand, is easy to handle and remove, and sets within 3 min.[8]

RetroMTA, designed for VPT, comprises fine hydrophilic particles and achieves a compressive strength of 105 MPa. Calcium zirconia is used as its radiopacifier. This material is resistant to washing, has a rapid setting time of 150 s (extendable to 10 min by the dentist), and offers minimal discoloration and superior sealing ability.[9]

Cold ceramic is another calcium silicate-based cement widely used for root-end filling, apical plug formation in teeth with open apices, root perforation repairs, and pulp capping procedures.[10,11] It demonstrates comparable biocompatibility to MTA and superior sealing ability in the presence of blood compared to glass ionomer cement, calcium hydroxide, and MTA. Its initial setting time is approximately 15 min.[12]

Despite the availability of multiple materials for perforation repair, VPT, and regenerative endodontics, none currently provides minimal discoloration suitable for use in the esthetic zone. This study aims to determine the discoloration caused by three calcium silicate-based cement – Cold Ceramic, RetroMTA, and OrthoMTA – in the presence of blood and normal saline for 1–2 months.

MATERIALS AND METHODS

This study was approved by the local Ethics Committee (IR.MUI.RESEARCH.REC.1400.361) and adheres to the PRILE 2021 checklist [Supplementary Table 1].

Using the formula $n = (z_1 + z_2)^2 \times S^2/d^2$ and based on a 95% confidence level with a Type I error, the values are set as follows: $z_1 = 1.96$, $z_2 = 0.84$, $P = 0.5$, $d = 0.98$, and $S = 0.98$.

Accordingly, the minimum number of teeth required for each group in the study is 8. This experimental study included 48 extracted human maxillary anterior teeth, extracted due to periodontal disease and free of cracks and attrition. Exclusion criteria included teeth with discoloration (due to smoking, alcohol, or aging), crowns shorter than 8 mm, visible cracks, anatomical variations, or internal resorption. Debris pigments and periodontal ligament remnants

were removed using an ultrasonic scaler (NSK, Japan). The teeth were then polished with prophylaxis paste and disinfected in a 5.25% sodium hypochlorite solution (Chlora, Cerkamed, Poland) for 1 h before being stored in normal saline until further preparation and testing.

To standardize the samples, tooth roots were sectioned perpendicularly to the axial axis below the cementoenamel junction (CEJ) using a diamond fissure bur (Teeskavan, Iran), leaving 5 mm of root length. Standard access cavities were prepared, and the canals were shaped using Gates Glidden drills (#1 to #6, Dentsply Maillefer, Switzerland) with 2.5% sodium hypochlorite irrigation between each step. A final rinse with normal saline was performed following the completion of debridement.

Open root apices were sealed with adhesive wax, and the teeth were mounted and numbered by placing them in floral foam sponge cylinders, extending from the root to the CEJ. Glass ionomer restorative cement (GC Fuji II, Tokyo, Japan) was used to fill the root canal up to the CEJ as an apical restoration. On the labial surface of each tooth, a 2 mm × 2 mm window was created at the mid-cervical area using a thin taper bur (Teeskavan, Iran) to ensure consistent color measurement during the process.

The specimens were divided into six groups using simple randomization (n = 8 per group), based on the type of calcium silicate-based cement and the environment:

- Cotton saturated with blood and OrthoMTA
- Cotton saturated with blood and Cold Ceramic
- Cotton saturated with blood and RetroMTA
- Cotton saturated with normal saline and OrthoMTA
- Cotton saturated with normal saline and Cold Ceramic

- Cotton saturated with normal saline and RetroMTA.

Fresh venous blood was collected from the researcher by a trained professional. A 1-mm thick cotton pellet was placed on the apical restoration and saturated with either blood or normal saline using an insulin syringe. Each group was further subdivided for application of one of the three calcium silicate-based materials: OrthoMTA (BioMTA, Seoul, Korea), Cold Ceramic (SGM, Iran), or RetroMTA (BioMTA, Seoul, Korea). Each material was prepared according to the manufacturer's instructions and applied in a 3-mm thick layer onto cotton pellets saturated with either blood or normal saline, using an MTA carrier (Juya, Tehran, Iran). The compatibility of the cement with the cotton pellet was ensured by gently contacting it with a 35# plugger (Densply, Mani, USA). A 1-mm thick cotton pellet moistened with distilled water was then placed on the material to facilitate setting, and the access cavity was sealed with a temporary restoration.

After placing the cement, the color at the mid-cervical labial surface of each specimen was measured three times with a spectrophotometer (Shade Pilot, DeguDent GmbH) to determine the initial enamel color. Specimens were incubated at 37°C and 100% humidity. After 24 h, temporary restorations were removed, and the setting of the cement was confirmed with a probe. Cavities were then etched, bonded, and sealed with an A3-colored resin composite (3M™ ESPE™, USA).

Color measurements were repeated at 30 and 60 days. Discoloration (ΔE) was calculated using the formula:

In this formula: L = Light level, a = Green-to-red axis, and b = Blue-to-yellow axis.

The experiment was conducted by a researcher who was blinded to the experimental conditions.

A ΔE value of <3.3 was considered clinically acceptable discoloration.[13]

Data were analyzed using SPSS version 22 (IBM Corp., Armonk, NY). The Kruskal–Wallis test was used to compare the mean discoloration among the three materials across different environments and time points, and the Wilcoxon test was applied to evaluate changes over time within each group. A significance level of 0.05 was used.

RESULTS

According to the results in Table 1, the highest discoloration was observed in OrthoMTA at both 30 and 60 days. However, there were no significant differences in discoloration at either time point ($P > 0.05$, Wilcoxon test). In addition, the mean (standard deviation [SD]) discoloration did not change significantly over time (30 days vs. 60 days) for all calcium silicate-based cement, except for RetroMTA, where a significant difference was observed ($P = 0.025$, Wilcoxon test).

According to the results in Table 2, when in contact with saline, the highest discoloration was observed in Cold Ceramic at 30 days and OrthoMTA at 60 days. However, no significant differences were found at either time point ($P > 0.05$, Kruskal–Wallis test). Similarly, the mean (SD) discoloration did not differ significantly over time (30 vs. 60 days) for all calcium silicate-based cement ($P > 0.05$, Wilcoxon test).

DISCUSSION

Tooth discoloration is a significant disadvantage, particularly in VPT for anterior teeth.[5] Clinicians should consider the potential impact of calcium silicate-based cement on tooth discoloration.[14] This study showed that within 30 and 60 days, the mean discoloration of all three cement in a blood-contaminated environment

Table 1: Comparison of the mean and standard deviation of discoloration in contact with blood at 30 and 60 days

Materials Time	Discoloration 30 days, mean (SD)	Discoloration 60 days, mean (SD)	P^{+}
Ortho MTA	3.81 (1.89)	5.56 (4.2)	0.093
Cold ceramic	3.67 (1.71)	4.65 (2.55)	0.161
Retro MTA	3.39 (2.24)	5.32 (3.05)	0.025
P^{++}	0.877	0.793	

[+]Wilcoxon test; [++]Kruskal–Wallis test. SD: Standard deviation; MTA: Mineral trioxide aggregate

Table 2: Comparison of the mean discoloration in contact with normal saline at 30 and 60 days

Materials Time	Discoloration 30 days, mean (SD)	Discoloration 60 days, mean (SD)	P^{+}
Ortho MTA	3.17 (1.46)	5.31 (3.80)	0.123
Cold ceramic	3.45 (1.75)	4.37 (2.3)	0.484
Retro MTA	2.74 (1.84)	4.37 (2.1)	0.093
P^{++}	0.623	0.968	

[+]Wilcoxon test; [++]Kruskal–Wallis test. SD: Standard deviation; MTA: Mineral trioxide aggregate

exceeded the clinically detectable threshold (ΔE >3.3). Although OrthoMTA exhibited the highest average discoloration, this difference was not statistically significant among the three materials, indicating comparable discoloration.

According to the manufacturer, OrthoMTA has a composition similar to ProRoot MTA but contains lower levels of heavy metals. RetroMTA, on the other hand, uses zirconium oxide as a radiopacifier.[15] Zirconium oxide is used as a radiopacifier in RetroMTA and Biodentine.[15-18] In the present study, RetroMTA demonstrated minimal potential for staining.

Our results align with Kang *et al*., who reported the highest discoloration in materials containing bismuth oxide and minimal discoloration in those using zirconium oxide.[9] Similarly, Możyńska *et al*.'s systematic review categorized OrthoMTA as having a high discoloration potential, while RetroMTA was among the materials with the lowest potential.[5] Metlerska *et al*. further observed that RetroMTA and MTA repair HP caused less discoloration than ProRoot MTA, Biodentine, and Ortho MTA, supporting their suitability for esthetic zones. Based on clinical findings, Biodentine can be considered for use due to its absence of gray discoloration.[14]

The presence of blood significantly exacerbates discoloration, as supported by previous research. This is related to material porosity and the presence or absence of the smear layer, both of which influence dentin permeability. Materials with longer setting times, such as MTA, remain porous for extended periods, absorbing more blood and undergoing hemolysis, leading to greater discoloration than faster-setting alternatives such as Biodentine.[19] Within 24 h, blood can discolor teeth by more than 15%, depending on the type of material and the duration of exposure.[5,20]

Shokouhinejad *et al*. reported similar findings, stating that blood-contaminated samples exhibited the highest discoloration, while samples without blood contamination showed significantly less discoloration.[21]

Adl *et al*. concluded that time significantly impacts discoloration regardless of the material. While our study observed discoloration over 1 and 2 months, the study by Adl *et al*. included 11-week, 11-month, and 3-month intervals. These extended time intervals and other factors, such as a larger sample size in their study, likely account for differences in findings.[22]

Moazzami *et al*. also noted that discoloration increases over time. Differences in experimental durations (1 and 2 months in our study vs. 1 and 3 months in theirs) and the materials used (RetroMTA, OrthoMTA, and Cold Ceramic in our study vs. ProRoot MTA and Nano Fast Cement in theirs) could explain variations in results.[23]

Shokouhinejad *et al*. highlighted that both the presence of blood and the passage of time significantly affect discoloration, regardless of the type of material. Differences in experimental durations (1 and 2 months in our study vs. 1 and 6 months in theirs), materials tested (RetroMTA, OrthoMTA, and Cold Ceramic in our study vs. ProRoot MTA, Endosequence Root Repair Material, and Biodentine in theirs), and sample size (8 per group in our study vs. 12 per group in theirs) may contribute to discrepancies between the findings.[21]

This study faced various limitations, including challenges in sourcing anterior teeth without cracks and crowns without wear, due to the stringent and time-consuming selection criteria, which reduced the sample size. Future studies should explore additional calcium silicate-based cement with varied compositions and radiopacifiers. Moreover, experiments with longer durations should be designed to more accurately investigate the impact of time on discoloration.

CONCLUSION

Discoloration among RetroMTA, OrthoMTA, and Cold Ceramic showed no significant differences in blood-contaminated and saline environments. However, over time, all materials exhibited clinically detectable discoloration (ΔE > 3.3).

Financial support and sponsorship
Nil.

Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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Supplementary Table 1: PRILE 2021 - checklist of items to be included when reporting laboratory studies in endodontology*

Section/ topic	Item number	Checklist items	Reported on page number
Title	1a	The title must identify the study as being laboratory-based, e.g., "laboratory investigation" or "*in vitro*," or "*ex vivo*" or another appropriate term	1
	1b	The area/field of interest must be provided (briefly) in the title	
Keywords	2a	At least two keywords related to the subject and content of the investigation must be provided	1
Abstract	3a	The rationale/justification of what the investigation contributes to the literature and/or addresses a gap in knowledge must be provided	1
	3b	The aim/objectives of the investigation must be provided	1
	3c	The body of the abstract must describe the materials and methods used in the investigation and include information on data management and statistical analysis	1
	3d	The body of the abstract must describe the most significant scientific results for all experimental and control groups	1
	3e	The main conclusion(s) of the study must be provided	1
Introduction	4a	A background summary of the scientific investigation with relevant information must be provided	2
	4b	The aim(s), purpose(s) or hypothesis(es) of an investigation must be provided ensuring they align with the methods and results	2
Materials and methods	5a	A clear ethics statement and the ethical approval granted by an ethics board, such as an Institutional Review Board or Institutional Animal Care and Use Committee, must be described	2
	5b	When harvesting cells and tissues for research, all the legal, ethical, and welfare rights of human subjects and animal donors must be respected and applicable procedures described	
	5c	The use of reference samples must be included, as well as negative and positive control samples, and the adequacy of the sample size justified	2
	5d	Sufficient information about the methods/materials/supplies/samples/specimens/instruments used in the study must be provided to enable it to be replicated	3
	5e	The use of categories must be defined, reliable and be described in detail	3
	5f	The numbers of replicated identical samples must be described within each test group. The number of times each test was repeated must be described	3
	5g	The details of all the sterilization, disinfection, and handling conditions must be provided, if relevant	3
	5h	The process of randomization and allocation concealment, including who generated the random allocation sequence, who decided on which specimens to be included and who assigned specimens to the intervention must be provided (if applicable)	3
	5i	The process of blinding the operator who is conducting the experiment (if applicable) and the examiners when assessing the results must be provided	4
	5j	Information on data management and analysis including the statistical tests and software used must be provided	4
Results	6a	The estimated effect size and its precision for all the objectives (primary and secondary) for each group including controls must be provided	
	6b	Information on the loss of samples during experimentation and the reasons must be provided, if relevant	
	6c	All the statistical results, including all comparisons between groups, must be provided	4,5
Discussion	7a	The relevant literature and status of the hypothesis must be described	5,6
	7b	The true significance of the investigation must be described	5,6
	7c	The strength(s) of the study must be described	5,6
	7d	The limitations of the study must be described	7
	7e	The implications for future research must be described	7
Conclusion(s)	8a	The rationale for the conclusion(s) must be provided	7
	8b	Explicit conclusion(s) must be provided, i.e., the main "take-away" lessons	7
Funding and support	9a	Sources of funding and other support (such as supply of drugs and equipment) as well as the role of funders must be acknowledged and described	
Conflicts of interest	10a	An explicit statement on conflicts of interest must be provided	7
Quality of images	11a	Details of the relevant equipment, software, and settings used to acquire the image(s) must be described in the text or legend	
	11b	If an image(s) is included in the manuscript, the reason why the image(s) was acquired and why it is included must be provided in the text	
	11c	The circumstances (conditions) under which the image(s) were viewed and evaluated must be provided in the text	

Contd...

Supplementary Table 1: Contd...

Section/ topic	Item number	Checklist items	Reported on page number
	11d	The resolution and any magnification of the image(s) or any modifications/ enhancements (e.g., brightness, image smoothing, staining, etc.) that were carried out must be described in the text or legend	
	11e	An interpretation of the findings (meaning and implications) from the image(s) must be provided in the text	
	11f	The legend associated with each image must describe clearly what the subject is and what specific feature(s) it illustrates	
	11g	Markers/labels must be used to identify the key information in the image(s) and defined in the legend	
	11h	If relevant, the legend of each image must include an explanation whether it is preexperiment, intra-experiment or postexperiment and, if relevant, how images over time were standardized	

*Nagendrababu V, Murray PE, Ordinola-Zapata R, Peters OA, Rôças IN, Siqueira JF Jr., *et al.* PRILE 2021 guidelines for reporting laboratory studies in endodontology: A consensus-based development. Int Endod J 2021;. [doi:. Nagendrababu V, Murray PE, Ordinola-Zapata R, Peters OA, Rôças IN, Siqueira Jr JF, Priya E, Jayaraman J, J Pulikkotil S, Camilleri J, Boutsioukis C. PRILE 2021 guidelines for reporting laboratory studies in Endodontology: a consensus-based development. International Endodontic Journal. 2021 Sep;54(9):1482-90.10.1111/iej.13542]. For further details visit: http://pride-endodonticguidelines.org/prile