

Effect of blood contamination before and after placement on the sealing ability of novel MTA cements as root-end filling materials: An *in vitro* comparative study

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Abstract

Introduction: The success of periradicular surgery depends on the ability of root-end filling materials to provide an effective seal between the root canal system and periapical tissues. Mineral Trioxide Aggregate (MTA) is widely used due to its biocompatibility and sealing ability; however, blood contamination during surgical procedures is often unavoidable and may influence its performance. The timing of contamination, whether before or after placement, may affect the hydration and adaptation of the material.

Objectives: This study aimed to evaluate the effect of blood contamination timing on the sealing ability of MTA cements used as root-end filling materials.

Method: Sixty extracted single-rooted human premolars were prepared, and 3 mm root-end cavities were created following root-end resection. Samples were divided based on the material used and further subdivided into three conditions: dry, blood contamination before placement, and blood contamination after placement. Cavities were filled accordingly, and microleakage was assessed using 2% methylene blue dye penetration under a stereomicroscope. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with a significance level set at $p < 0.05$.

Result: Significant differences in microleakage were observed in one of the tested MTA materials across the three conditions, with greater leakage in dry conditions compared to blood-contaminated groups. No significant difference was found between contamination occurring before and after placement. The other material demonstrated consistent sealing ability across all conditions.

Conclusion: Within the limitations of this *in vitro* study, blood contamination did not adversely affect sealing ability. Contamination timing had a material-dependent influence, with certain MTA cements showing greater tolerance to moisture variability.

Keywords: Mineral Trioxide Aggregate, sealing ability, blood contamination, placement timing, root-end filling materials

Introduction

The goal of endodontic therapy is to hermetically seal all pathways of communication between the pulpal and periradicular tissues. A mandatory requirement of root canal therapy is that the obturation and restoration of the tooth must seal the root canals both apically and coronally to prevent leakage and percolation of oral fluids and to prevent recontamination of infected canals^[1].

The root canal system has the capacity to harbour several species of bacteria as well as their toxins and by-products if exposed to the oral flora. These irritants may escape from the root canal system into the radicular tissues resulting in the formation of radicular lesions^[2].

The first treatment option in failed endodontics is retreatment of the teeth via an orthograde approach. When this has failed or if it cannot be accomplished by an orthograde approach, endodontic surgery is indicated, which is best done by apicectomy followed by root-end restoration^[3,4].

An ideal root filling material should be able to optimally seal the communication paths between the root canal system and periapical tissues. Also, it should be biocompatible, biologically, insoluble in tissue fluids, and radiopaque for easier radiographic detection. Moreover, it should have optimal dimensional stability and easy application, and its

seal should not be compromised by moisture exposure. These characteristics are independent of the purpose of application as an orthograde or retrograde root filling material. Nonetheless, none of the currently available root filling materials possess all of the aforementioned properties^[5].

Among the various root-end filling materials like GIC, Amalgam, Composite resin and others, MTA has shown promising results and because of its desirable features such as biocompatibility and non-toxicity, it is often used as a gold standard for comparing other materials^[6,7].

However, due to its poor handling properties and long-setting time, it is complicated to use which is partially overcome with MTA carrier and other methods^[8].

A calcium silicate-based MTA, Kids -e-Dental MTA (MAARC, India) was introduced and according to the manufacturer, Kids-e-Dental MTA is specifically formulated for rapid setting, making it suitable for clinical situations where fast hardening and reduced procedural time are desired. Its ultrafine particle size and smooth handling properties help reduce washout risk and improve marginal adaptation, even in the presence of moisture contamination such as blood or saliva^[9].

Another new material, MAARC MTA (MAARC Dental, India) was introduced as a powder and liquid system which

claimed to be stain-proof, fast setting, biocompatible, fine grained and, according to the manufacturer, had no adverse reactions reported so far.

Though, there were no studies or literature available proving these characteristics of the two materials.

Clinically, moisture including saliva or blood could contaminate the root end cavity preparations and filling materials as they are placed and the presence of this moisture might affect the sealing ability of the root end filling materials^[10].

Several previous studies have evaluated the effect of different environmental conditions such as dry, moist, or blood-contaminated environments on the sealing ability of Mineral Trioxide Aggregate (MTA). Some of these studies reported that MTA demonstrated a relatively stable sealing ability even when contaminated with blood or saliva, owing to its hydrophilic nature and bioactivity, however focusing only on whether contamination was present or absent, without differentiating the stage at which contamination takes place^[12, 13].

The stage or timing at which blood contamination occurs may play a critical role in how MTA and newer bioceramics interact with the surrounding dentin. Contamination before placement may interfere with the adaptation and penetration of the material into the dentinal tubules, affecting micromechanical retention. In contrast, contamination after placement but during the initial setting period may affect the hydration reaction and crystalline structure formation, especially in slow-setting materials, potentially leading to porosity and microleakage.^[14] There is emerging evidence that fast-setting bioceramic materials like Kids-e-dental MTA, due to their shorter setting times, may be less affected by contamination occurring after placement compared to slow-setting materials like MAARC MTA^[15].

However, in our literature research, no study was found evaluating the sealing properties of the newer bioceramic materials under different blood contamination conditions. Hence, the purpose of this study is to evaluate and compare the influence of blood contamination before and after the placement on the sealing ability of Kids-e-Dental MTA and MAARC MTA as root-end filling materials using 2% methylene blue as tracer.

Aim of The Study

To evaluate and compare the sealing ability of Kids-e-Dental MTA and MAARC MTA when used as root-end filling materials subjected to blood contamination before and after material placement

Materials and Methods

Source of Data

A total of 60 single-rooted human premolars extracted for orthodontic and periodontal reasons were collected from the Department of Oral Surgery, AJ Institute of Dental Science and private clinics in and around Mangalore. They were stored according to the infection control protocol advised by the Centre of Disease Control Global.

Test Specimen Selection

Inclusion Criteria

60 freshly extracted human single rooted premolar teeth which are extracted for orthodontic and periodontal purposes with intact surfaces.

Exclusion Criteria

Teeth with caries, cracks, discolorations, fractured crown, hypoplasia, hypo mineralized teeth and tooth with any developmental anomalies.

Armamentarium and Materials

- Kids-e-Dental MTA ('MAARC' Shiva Products, India)
- MAARC MTA ('MAARC' Shiva Products, India)
- K- Type Files (MANI MEDICAL INDIA PVT LTD)
- 2.5% Sodium hypochlorite (MEDILISE)
- 2% Methelene Blue dye (RANKEM)
- Sterile saline.
- Etchant (37% Phosphoric acid – Total etch by ivoclar)
- Bonding Agent (Prime & bond universal by Dentsply Sirona)
- Composite resin (PREVEST)
- EDTA (Shivam Products)
- Peeso reamer drill
- ProTaper Gutta Percha (DENTSPLY)
- AH Plus (DENTSPLY, KONSTANZ, GERMANY)
- Paper points. (DIADENT GROUP INTERNATIONAL)
- ProTaper Rotary Files (DENTSPLY)
- Lentulospiral (MANI MEDICAL INDIA PVT LTD)
- Diamond Disc
- 23- gauge needle
- Nailpolish Varnish
- Sticky wax (PYREX)
- Stereomicroscope (LAWRENCE & MAYO)

A total of 60 single rooted premolar teeth with intact root canal with completely formed apices was selected. All teeth were examined for cracks, fracture, canal calcification, and external root resorption and excluded. For disinfection, the specimen was stored in 5.25% sodium hypochlorite for an hour and then placed in normal saline.

Teeth were decoronated at the cemento-enamel junction with the diamond disc at high speed with water spray coolant. Real root canal lengths were determined by manually inserting #15 K-files into the canals, until the instrument tips are visible at the apical foramen and then the working length was established 1.0mm shorter than real root canal length. Apical instrumentation of roots was carried out with stainless steel files to a size of 40 K-file as a master apical file and canals flared upto #80 with the step back technique. Canals were irrigated with 1.0mL of 2.5% sodium hypochlorite. After instrumentation was completed, 3.0mL of EDTA was introduced and allowed to remain in the canals for 3 minutes. Next, a final flush with 1.0mL of 2.5% sodium hypochlorite followed by 5.0mL of normal saline was done.

The root canals were dried with paper points and obturated with gutta-percha and AH plus sealer by the cold lateral compaction technique.

The teeth were then incubated at 37 °C temperature and 100% humidity for 5 days

The apical 3 mm of the roots were cut with 90-degree angle relative to the longitudinal axis of the root with a diamond. A cavity with 3 mm depth was created retrogradely at the apex by gutta-percha removal using #4 Peeso reamer drill with internal diameter standardized to 1.3mm.

Then, coronal part of the root at the CEJ level was closed with composite resin restorative material and the external surfaces of the root was rendered impermeable using two

layers of nail varnish and one layer of sticky wax, except for the area corresponding to the resected root end surface. Prior to sample preparation 1 ml of human blood was obtained by phlebotomy procedure using a 23-gauge needle from the observer by trained authorized personnel and then transferred to a glass container containing a clamp and sealed. The container was placed on a laboratory rotator for 6 mins to obtain defibrinated human (DH) blood. Samples were then randomly placed into 2 main experimental groups (n=30), each with 3 subgroups (n=10)

- Group 1a:** Root-end cavity filled with Kids-e-Dental MTA
- Group 1b:** Root-end cavity filled with Kids-e-Dental MTA before blood contamination
- Group 1c:** Root-end cavity filled with Kids-e-Dental MTA after blood contamination
- Group 2a:** Root-end cavity filled with MAARC MTA
- Group 2b:** Root-end cavity filled with MAARC before blood contamination
- Group 2c:** Root-end cavity filled with MAARC after blood contamination

Group 1: Kids-e-Dental MTA

- **Group 1a (Control – Dry):** Kids-e-Dental MTA was prepared according to the manufacturer's instructions and the root end cavity was filled.
- **Group 1b (Blood Contamination Before Placement of MTA):** Kids-e-Dental MTA was prepared according to the manufacturer's instructions. Prior to placing the material, the root ends were filled with the DH blood and excess was removed by aspirating with the syringe to leave a coating of blood on the inner wall of cavity.
- **Group 1c (Blood Contamination After Placement of MTA):** Kids-e-Dental MTA was prepared according to the manufacturer's instructions. Immediately after placement, DH blood was applied to cover the root-end filling material surface using a sterile microbrush and allowed to remain undisturbed during the material's initial setting time.

Group 2: Maarc MTA

- **Group 2a (Control – Dry):** Root-end cavity was filled with MAARC MTA identical to group 1a.
- **Group 2b (Blood Contamination Before Placement of MTA):** Root-end cavity was filled with MAARC before blood contamination. Prepared the same way as group 1b.
- **Group 2c (Blood Contamination After Placement of MTA):** Root-end cavity was filled with MAARC after blood contamination. Prepared the same way as group 1c.

Evaluation

Samples were incubated for 24 hours at 37°C and 100% humidity. The roots then immersed in 2% methylene blue dye for 48 hours.

Afterwards the roots were rinsed in running tap water for 5 minutes and then sectioned vertically in buccolingual direction under water cooling with a slow-speed diamond saw.

Each specimen were examined under a stereomicroscope with a 12-mm ruler placed next to the roots to aid in the evaluation of leakage rate. The degree of leakage were evaluated based on the penetration of the dye stain from apical to coronal of the root, and the dye penetration scores created by modifying the method used in the study of De Moor and Hommez^[12].

Score	Degree of Dye Penetration
0	No dye penetration
1	Dye penetration <1 mm
2	Dye penetration between 1–2 mm
3	Dye penetration >3 mm

Results

Statistical Analysis

All statistical analysis was done using SPSS for Windows Version 24.0. (IBM Corp., Armonk, NY, USA). Descriptive data was represented in mean and standard deviation. Normality of the dye penetration data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests for each MTA material. Comparison between the groups were done by ANOVA with Tukeys test and Chisquare test for qualitative data.

Representative stereomicroscopic images illustrating the dye penetration scoring criteria (scores 0–3) are presented in Figure 1, with descriptive statistics presented in Tables 1 and 2. A total of 60 samples were analyzed to evaluate the apical sealing ability of Kids-e-Dental MTA and MAARC MTA under different blood contamination conditions (Dry, contamination before placement, and contamination after placement). The overall mean dye penetration for all samples was 0.967 ± 0.882 , with scores ranging from 0 to 3. For Kids-e-Dental MTA, the highest mean dye penetration was observed in the Dry group (1.70 ± 1.16), followed by Before contamination (0.60 ± 0.52) and After contamination (0.50 ± 0.53). Similarly, MAARC MTA showed the highest mean dye penetration in the Dry group (1.40 ± 1.07), followed by Before contamination (1.00 ± 0.67) and After contamination (0.60 ± 0.52), indicating a trend toward increased leakage in dry conditions for both materials.

The Kruskal–Wallis test showed a statistically significant difference in dye penetration among the three contamination conditions for Kids-e-Dental MTA ($\chi^2 = 7.606$, $df = 2$, $p = 0.022$). Mean rank values indicated the highest dye penetration in the Dry group (21.30), followed by Before (13.20) and After (12.00) contamination groups. In contrast, MAARC MTA showed no statistically significant difference among the contamination conditions ($\chi^2 = 3.904$, $df = 2$, $p = 0.142$), although a slight trend toward higher penetration in the Dry condition was observed.

Pairwise comparisons using the Mann–Whitney U test for Kids-e-Dental MTA demonstrated significantly greater dye penetration in the Dry group compared with both the Before ($p = 0.026$) and After ($p = 0.017$) contamination groups. However, no significant difference was observed between the Before and After contamination groups ($p = 0.661$). For MAARC MTA, no significant differences were found between any of the contamination conditions ($p > 0.05$).

Discussion

The ultimate objective of periradicular surgery is to eliminate apical pathology and reinfection by establishing a hermetic seal between the root canal system and the periapical tissues. A critical determinant of surgical success is therefore the sealing ability of the root-end filling material, which must prevent the passage of bacteria, toxins, and tissue fluids across the resected apex.^[2] Failure to achieve an adequate apical seal may allow persistent microbial leakage, leading to delayed healing or surgical failure^[1].

In the present study, the effect of blood contamination timing on sealing ability was evaluated. The results demonstrated that Kids-e-Dental MTA showed statistically significant differences in microleakage among the three conditions ($p = 0.022$), with greater leakage observed in the dry group compared to blood-contaminated groups. In contrast, MAARC MTA showed no statistically significant differences ($p = 0.142$), indicating more consistent performance. No significant difference was observed between contamination occurring before and after placement for either material.

Among the various factors influencing the sealing efficacy of retrograde filling materials, contamination of the root-end cavity with blood remains one of the most clinically relevant challenges. During apical surgery, complete isolation of the operative field is often difficult despite the use of suction and hemostatic agents, and blood contamination is frequently unavoidable^[10]. The presence of blood at the time of placement may influence both the marginal adaptation as well as the physicochemical behavior of calcium silicate-based materials^[7, 13]. Therefore, evaluating material performance under simulated contamination conditions is essential for predicting clinical reliability.

Mineral trioxide aggregate (MTA) has long been regarded as a gold standard root-end filling material due to its excellent biocompatibility, bioactivity, and sealing properties^[2]. Several studies have demonstrated that MTA exhibits superior or comparable sealing ability relative to other retrograde materials such as amalgam, IRM, Super-EBA, and glass ionomer cement^[2, 17]. MTAs hydrophilic nature allows it to set in the presence of moisture, which may explain the findings of the present study where dry conditions resulted in greater microleakage. Inadequate moisture may lead to incomplete hydration and increased porosity, thereby compromising the seal^[2, 3].

The present study evaluated two newer calcium silicate-based materials—Kids-e-Dental MTA and MAARC MTA—under three environmental conditions: dry, blood contamination before placement, and blood contamination after placement. While numerous studies have examined dry versus contaminated conditions^[3, 6, 10], very few have specifically investigated the effect of the timing of contamination on sealing performance. This distinction is clinically significant because contamination occurring prior to placement may affect dentin–material interaction differently than contamination occurring after condensation during the initial setting period^[13].

Dye penetration analysis using 2% methylene blue was employed in this study to evaluate apical microleakage. Dye leakage studies remain widely accepted due to their simplicity, cost-effectiveness, and reproducibility^[1, 17]. Although dye molecules are smaller than bacteria and may

overestimate leakage compared to clinical conditions, resistance to penetration by small tracer molecules is generally considered indicative of resistance to microbial infiltration^[2]. Therefore, methylene blue provides a sensitive and stringent method for evaluating sealing ability *in vitro*.

Torabinejad et al. demonstrated that MTA exhibited significantly less dye leakage compared to amalgam, IRM, and Super-EBA when used as a retrograde filling material. Their findings also showed lower dye leakage for MTA in blood-contaminated environments compared with completely dry conditions^[2]. This observation is also consistent with the results of the present study. In a subsequent study evaluating the effect of blood contamination, they reported that the sealing ability of MTA was not significantly compromised by the presence of blood^[10]. These findings suggested that the hydrophilic properties and bioactivity of MTA enable it to tolerate moist conditions during placement.

Thus, the bioactive behavior of calcium silicate cements plays a key role in their sealing mechanism. Upon hydration, MTA releases calcium ions that interact with phosphate ions present in tissue fluids or blood, leading to the precipitation of hydroxyapatite crystals at the dentin–material interface. This interfacial mineralization enhances marginal adaptation and may form tag-like structures that improve micromechanical interlocking. Such biomineralization may compensate for minor gaps created during placement and contribute to long-term sealing stability^[13]. In addition, exposure to blood proteins can also cause slight expansion of the material and partial occlusion of surface porosities. Although this protein adsorption may slow early hydration, the combined effect of porosity blocking, surface expansion, and hydroxyapatite deposition ultimately enhances the seal of the material^[4].

However, blood contamination may also exert adverse effects depending on the stage at which exposure occurs. Islam et al. reported that blood contamination during manipulation adversely affected the marginal adaptation and surface microstructure of MTA. They observed irregular crystalline formation and increased interfacial gaps under scanning electron microscopy^[13]. These findings suggest that blood components—particularly proteins and fibrin—may interfere with intimate adaptation of freshly mixed cement to dentinal walls.

Therefore, when contamination occurs prior to placement, a proteinaceous film may coat the dentin surface and act as a physical barrier between the cement and the canal walls. This layer may reduce wettability, limit penetration of cement particles into dentinal tubules, and impair micromechanical retention^[7]. In addition, early clot formation within cavity irregularities may create a discontinuous material–clot–dentin interface rather than a direct material–dentin interface, potentially increasing susceptibility to leakage^[4].

Conversely, when contamination occurs after placement, the material has already been condensed and adapted to the prepared cavity. In such cases, mechanical adaptation may remain largely intact, and subsequent exposure to blood may primarily influence surface hydration rather than interfacial integrity. Fast-setting calcium silicate cements, in particular, may demonstrate reduced sensitivity to late-stage contamination due to rapid initial hardening. Studies

evaluating newer calcium silicate materials have reported shorter setting times and improved early mechanical properties compared to conventional formulations [14, 15].

The results demonstrated that, the sealing performance of Kids-e-Dental MTA was affected by the timing of blood contamination. Interestingly, the dry group demonstrated comparatively greater dye penetration than contaminated groups. This observation supports the concept that a certain degree of moisture may be beneficial for optimal hydration and setting of hydrophilic calcium silicate cements [2, 3]. In the absence of adequate moisture, incomplete hydration may result in porosity or microstructural defects that predispose to leakage [2].

MAARC MTA demonstrated relatively consistent sealing ability across all experimental conditions in this study. This stability may be attributed to differences in formulation, particle size distribution, or setting kinetics. Fast-setting materials are less susceptible to environmental interference during the vulnerable early hydration phase [15]. Furthermore, improved particle fineness may enhance adaptation to cavity walls and reduce interfacial void formation [14].

Although the blood-before-placement groups demonstrated numerically higher leakage values than the blood-after-placement groups, the difference was not statistically significant. This indicates that both materials may exhibit a degree of tolerance to limited blood contamination under the experimental conditions. Nevertheless, the findings suggest that the timing of contamination could influence sealing performance, particularly in materials with slower hydration kinetics. In contrast, newer fast-setting MTA formulations may offer greater clinical tolerance and stability when exposed to variations in moisture during placement.

Nevertheless, the present study was conducted in vitro, and therefore does not fully replicate the complex biological environment present during periradicular surgery. Factors such as tissue pressure, fluid movement, inflammatory mediators, and long-term degradation cannot be simulated entirely in laboratory conditions. The dye penetration method, although sensitive and widely used, may not directly correlate with microbial leakage in clinical situations. Additionally, only short-term sealing ability was evaluated, and long-term performance under functional and biological stresses was not assessed. Further in vivo studies are required to validate these findings

Conclusion

In the present study, dye penetration values varied among the experimental groups. Kids-e-Dental MTA demonstrated statistically significant differences in microleakage across the three contamination conditions, with the dry group showing comparatively higher mean leakage than the blood-contaminated groups. In contrast, MAARC MTA did not exhibit statistically significant differences between dry, blood-before-placement, and blood-after-placement conditions, indicating uniform performance across environments.

Although the blood-before-placement groups showed numerically higher dye penetration values than the blood-after-placement groups for both materials, this difference was not statistically significant. Overall, MAARC MTA demonstrated more consistent sealing values across all tested conditions, whereas Kids-e-Dental MTA showed variability depending on contamination timing.

Author Contributions

Author 1: Conceptualization, Methodology, Investigation and data collection, Formal analysis, Data curation, Writing – original draft.

Author 2: Conceptualization, Methodology, Formal analysis, Writing – review and editing, Supervision, Project administration.

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AI Use Statement

The authors used ChatGPT (OpenAI) to assist with language editing, grammar refinement, and formatting of the manuscript. All scientific content, interpretation, and conclusions were reviewed, verified, and approved by the authors, who take full responsibility for the final manuscript.

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