Research Square

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Comparison of Apical Microleakage between CEM Cement and Cold Ceramic as Root-end Filling Materials, an in vitro study

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Abstract

Background

Public attitudes toward oral health have shifted toward preserving natural tooth structure, thereby increasing the importance of conservative treatments, such as root canal therapy. In endodontics, achieving an effective apical seal is critical to prevent bacterial microleakage and subsequent treatment failure. While various root-end filling (REF) materials have been introduced, an ideal material that meets all clinical criteria remains elusive. The purpose of this study was to evaluate apical microleakage between two different root-end filling materials—Calcium-Enriched Mixture (CEM) Cement and Cold Ceramic.

Methods

Sixty-one root- filled teeth that were extracted were prepared, apically resected, and filled with either CEM Cement or Cold Ceramic (n = 25 each). Bacterial leakage was assessed over 90 days using Enterococcus faecalis in a dual-chamber model. Statistical analysis was performed using Fisher's exact test, the Mann–Whitney U test, and Kaplan–Meier survival analysis.

Results

Apical microleakage was detected in 72% of specimens in the CEM Cement group and 92% in the Cold Ceramic group But this difference was not statistically significant. The mean time to leakage was 13.83 days (median 11.5 days) for CEM Cement and 12.70 days (median 10 days) for Cold Ceramic, with no significant difference. Survival analysis using the Kaplan–Meier method demonstrated no statistically significant difference in time to apical leakage .

Conclusion

Our study indicates no statistically significant difference in apical microleakage between CEM Cement and Cold Ceramic. Both materials demonstrated comparable sealing performance, and further long-term clinical studies are warranted to assess their in vivo efficacy and clinical implications.

1. Introduction

Public attitudes toward oral health have shifted toward preserving natural tooth structure, reducing the inclination for extractions. As a result, root canal therapy—which aims to maintain pulp vitality despite damage from caries, infection, trauma, or other causes—has become increasingly important (1, 2). Even in populations with lower caries prevalence, the demand for root canal treatment remains high (3). Additionally, cultural factors, public awareness, hygiene standards, access to care, and socioeconomic

conditions significantly influence decisions to seek dental treatment (1, 2). Procedural factors such as thermal damage, desiccation during cavity preparation, the cytotoxicity of restorative materials, and microleakage can compromise pulp health. When endodontic treatment fails—often due to bacterial infiltration and endotoxin release—pathological lesions may develop, making apical surgery necessary when nonsurgical retreatment is not viable (4). Apical surgery typically involves resecting the diseased root end, preparing a cavity for complete debridement, and placing a root-end filling (REF) material. Although various REF materials have been used over the years, none have met all the ideal criteria (5).

A perfect REF material ought to be harmless, corrosion-resistant, biocompatible, dimensionally stable, radiopaque, moisture-insensitive, and impermeable. It should also stimulate cementum formation, achieve a complete seal, be non-carcinogenic, insoluble in tissue fluids, and easy to handle (6). Clinically, apical plugs are essential for managing apical perforations (often resulting from technical errors or trauma), open apices, and resorptive roots. In these cases, materials like Mineral Trioxide Aggregate (MTA) are used to seal defects, prevent bacterial ingress, and promote tissue healing (7, 8, 9, 10, 11). Despite MTA's favorable biological properties—including an alkaline pH and calcium ion release that stimulate tissue regeneration—it has drawbacks such as a prolonged setting time, challenging handling properties, and high cost (12). Calcium-Enriched Mixture (CEM) Cement is a biocompatible hydraulic cement that converts to hydroxyapatite upon contact with moisture. It has been successfully used in pulp capping, apexogenesis, apexification, and periapical repair, making it a promising alternative to MTA (13, 14, 15, 16, 17, 18). Similarly, Cold Ceramic is a modern bioceramic material with properties akin to MTA and CEM Cement. It offers the advantages of a shorter setting time and minimal inflammatory response, while effectively promoting hard tissue repair (17, 19, 20, 21).

Microleakage—the penetration of microorganisms, toxins, and fluids through microscopic gaps in the REF—continues to be a vital element influencing the enduring effectiveness of endodontic treatment. (22, 23, 24). Therefore, this study aims to compare the apical microleakage between CEM Cement and Cold Ceramic as REF materials.

2. Material & Method

2.1. Study Design

This in vitro investigation utilized extracted human teeth with a single root. Teeth were obtained from extractions performed for orthodontic or periodontal reasons. Inclusion criteria were: single-rooted, single-canaled teeth with closed apices and lengths between 20 and 25 mm. Exclusion criteria included teeth with curvatures greater than 20° (as measured by Schneider's method), internal or external resorption, root fractures, open or immature apices, and significant canal calcification.

The research protocol was sanctioned by the Ethics Committee of Birjand University of Medical Sciences (IR.BUMS.REC.1402.477). Patients gave their informed consent for the utilization of their extracted teeth In this study.

2.2 Sample Size and Sampling Method

Based on a previous study (Ghobadi et al.) (5), considering microleakage proportions of 10% and 45% in two groups with a 95% confidence interval and 80% power, a sample size of 25 teeth per experimental group was calculated (total n = 50). In this study, 60 teeth were initially collected. Five teeth served as positive controls (no apical filling material) and five as negative controls (apex sealed with wax), while the remaining 50 teeth were randomly distributed into two study groups (n = 25 each) to receive either CEM Cement or Cold Ceramic.

2.3 Study Procedures

This laboratory experimental study used extracted human single-rooted teeth with closed apices and lengths of 20–25 mm. Teeth with curvatures > 20° (Schneider's method), resorption, fractures, open apices, severe calcification, or multiple canals were excluded. Sixty teeth were collected; five served as positive controls (no filling) and five as negative controls (apex sealed with wax), while the remaining 50 were randomly assigned to two experimental groups (n = 25) to receive either CEM Cement or Cold Ceramic. To standardize root length to 15 mm, the crowns were decoronated. The working length was determined by subtracting 1 mm from the point at which a #10 K-file became visible at the apical foramen. Root canals were prepared using rotary V Taper files (up to F3 at 300 rpm, 2 N·m torque) with irrigation using 5.25% sodium hypochlorite. The smear layer was eliminated by applying 1 mL of 17% EDTA for 1 minute, followed by a final irrigation with 3 mL of saline. A 3-mm apical resection was performed perpendicular to the root's long axis, and a 3 mm deep retrograde cavity was prepared with an ultrasonic device; these cavities were then filled with the assigned material following manufacturer instructions, excess material was removed, and radiographic confirmation of the filling was obtained. The samples were stored at 37°C with 100% humidity for 24 hours before testing. Microleakage was assessed using a dual-chamber model in which each tooth was mounted in a microtube with the junction sealed, sterilized with ethylene oxide, and placed in Brain Heart Infusion (BHI) broth; every three days, 10^6 CFU of Enterococcus faecalis (ATCC) was inoculated into the upper chamber, and leakage was determined by turbidity over 90 days.

2.4 Statistical Analysis

Statistical analysis was performed using SPSS version 22 and Stata version 11. Non-parametric tests (Mann–Whitney U test and Fisher's exact test) and survival analysis (Kaplan–Meier method, log-rank test, and Breslow–Wilcoxon test) were applied, with the level of significance set at $p < 0.05$.

3. Results

3.1 Descriptive Analysis of Apical Microleakage

Sixty extracted single-rooted human teeth were included in this study, comprising 25 samples in the CEM Cement group, 25 in the Cold Ceramic group, 5 positive controls, and 5 negative controls. In the CEM

Cement group, 18 samples (72%) exhibited apical microleakage while 7 (28%) did not. In the Cold Ceramic group, 23 samples (92%) showed microleakage compared to 2 samples (8%) without leakage. This difference in the frequency of leakage between the two experimental groups was not statistically significant, as determined by Fisher's exact test (p = 0.138).(Table 1)

Table 1
Distribution of samples with apical microleakage in the CEM Cement and Cold Ceramic groups.

Group	Leakage (n, %)	No Leakage (n, %)	Total (n)
CEM Cement	18 (72%)	7 (28%)	25
Cold Ceramic	23 (92%)	2 (8%)	25
Total	41 (82%)	9 (18%)	60

3.1 Analysis of Time to Leakage

For the samples that exhibited leakage, the mean time to leakage in the CEM Cement group was 13.83 days (median = 11.5 days, SD = 10.01, IQR = 18 days). In the Cold Ceramic group, the mean time to leakage was 12.70 days (median = 10 days, SD = 8.86, IQR = 12 days). The difference in leakage time between the two groups did not reach statistical significance (Mann–Whitney U test, p = 0.793)(Table 2). Notably, the negative control group exhibited no leakage throughout the 90-day follow-up, while the positive controls leaked on day 1.

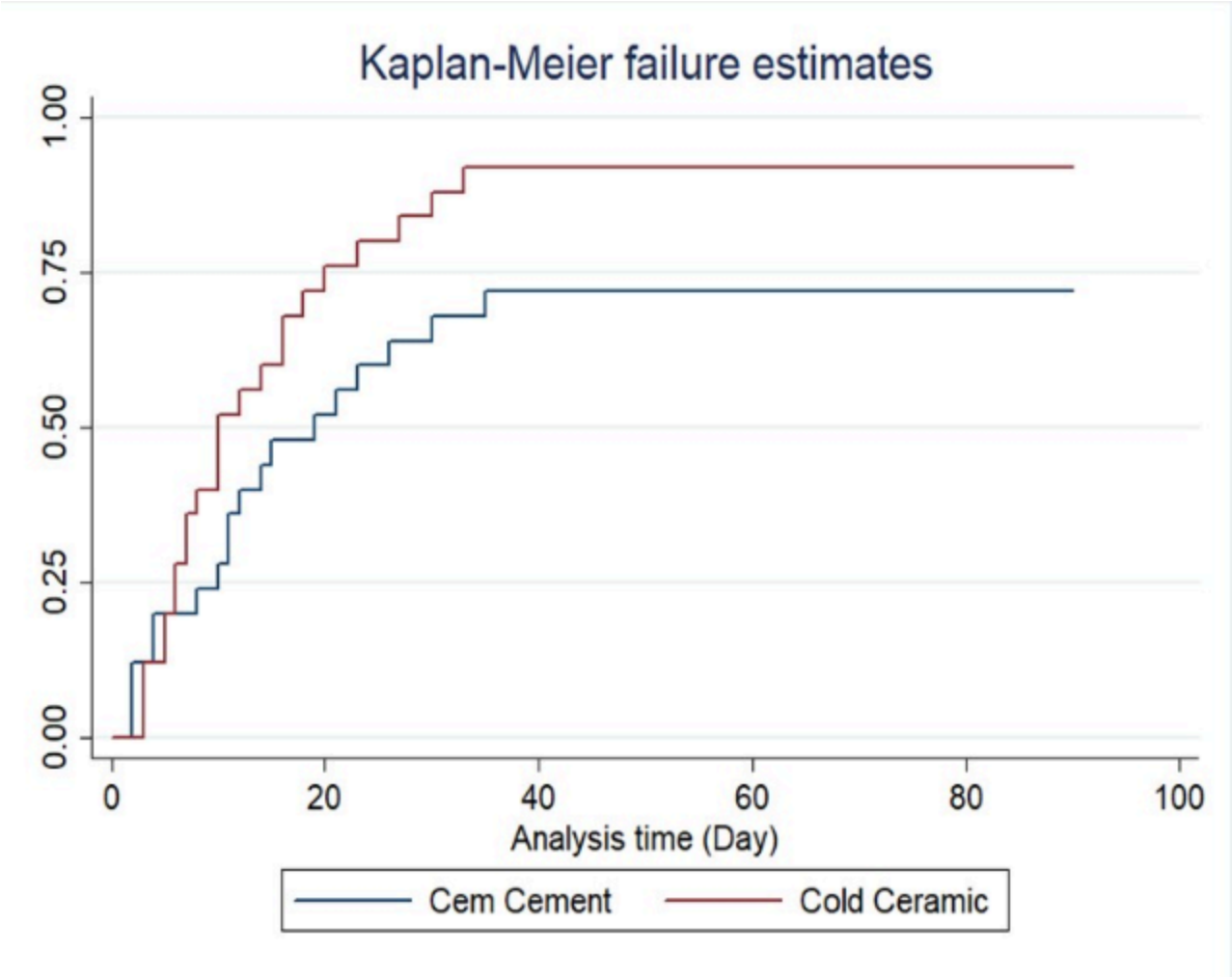
Table 2
Comparison of time to leakage between CEM Cement and Cold Ceramic groups.

Group	Mean Time (days)	Median Time (days)	Standard Deviation	IQR	p value
CEM Cement	13.83	11.5	10.01	18	0.793
Cold Ceramic	12.70	10.0	8.86	12	

4.3. Survival Analysis

The follow-up period was 90 days, during which most samples eventually exhibited microleakage. Kaplan–Meier survival analysis was performed to compare the time to leakage between the CEM Cement and Cold Ceramic groups. Although the Kaplan–Meier plot (Table 3) visually suggested a trend—with a median time to leakage of 19 days for the CEM Cement group and 10 days for the Cold Ceramic group—The differences did not reach statistical significance according to the log-rank test (p = 0.07) and the Wilcoxon (Breslow) test (p = 0.15).

Table 3: Kaplan-Meier survival analysis showing time to apical microleakage between the two groups.



4. Discussion

In this study, we compared the apical microleakage of CEM Cement and Cold Ceramic as root-end filling materials using a bacterial leakage model. Various techniques, such as radioisotopes, electrochemical techniques, dye penetration, scanning electron microscopy, and fluid filtration, have been used for leakage assessment (25); however, due to limitations related to pH, molecular size, and chemical properties, dye penetration cannot precisely evaluate leakage over time (26). In contrast, the bacterial leakage method, which better simulates clinical conditions (27, 28), was employed using Enterococcus faecalis—a bacterium commonly associated with chronic apical periodontitis following unsuccessful root canal treatments (29). Our results showed that 72% of samples in the CEM Cement group and 92% in the Cold Ceramic group exhibited leakage; Nevertheless, there was no statistically significant difference. ($p = 0.138$). Moreover, the interim period for leakage (13.83 days for CEM Cement vs. 12.70 days for Cold Ceramic) and the median leakage times (11.5 vs. 10 days, respectively) did not differ significantly ($p = 0.793$). These findings suggest that both materials provide similar apical sealing capabilities. Our results are consistent with previous studies; for instance, Yavari et al. (30) demonstrated comparable sealing between CEM Cement and MTA, while Ayatollahi et al. (31) reported that both materials function similarly as apical plugs. Likewise, Kazem et al. (32) and Madarsi et al. (33) found no significant differences when comparing CEM Cement with Additional widely used materials for root-end filling. Conversely, Significant variations have been noted in certain studies under specific

conditions, such as the work by Adel et al. (5), which indicated that factors like apical foramen diameter and plug thickness can influence leakage, with CEM Cement showing lower leakage than MTA under certain circumstances. Other investigations, including those by Ghabbadi et al. (5), Ramadanali et al. (34), Ramazani and Sadeghi (35), and Mirhadi et al. (36), have highlighted variations in sealing ability depending on the methodology, environmental pH, and material placement. Recent clinical studies further underscore the potential of these materials; Madarsi et al. (37) reported that Cold Ceramic promotes bone growth, periodontal ligament regeneration, and cementum deposition, while Eghbal et al. (38) and Nasrat et al. (15) demonstrated the favorable clinical outcomes of CEM Cement, including its capacity to promote the formation of hard tissue and its antibacterial qualities. In summary, although slight numerical differences were observed, both CEM Cement and Cold Ceramic exhibited comparable apical sealing abilities based on bacterial microleakage, and further long-term clinical research is needed to validate these findings under various clinical conditions.

5. Conclusion

Overall, the study's findings show that when using CEM Cement and Cold Ceramic as root-end filling materials, there is no statistically significant difference in apical microleakage. However, More research is needed to find out how they work in the long term and in the clinic. Notable strengths of this study include the use of rigorous inclusion and exclusion criteria, proper canal preparation and irrigation with EDTA and sodium hypochlorite, and the application of a bacterial leakage method using Enterococcus faecalis—a bacterium commonly associated with root canal infections. Additionally, the incorporation of both positive and negative control groups helped validate the experimental methodology. Limitations of this study include the lack of true clinical conditions, a relatively short evaluation period, and the use of only a single bacterial species.

Declarations

****Ethics approval and consent to participate:**

This in vitro study was approved by the Research Ethics Committee of Birjand University of Medical Sciences (Approval Code: IR.BUMS.REC.1402.477). The study used extracted human teeth, and informed consent was obtained from the patients for the use of their teeth in research. All procedures were performed in accordance with the ethical standards of the institutional research committee and the Declaration of Helsinki.

****Consent for publication:**

Not applicable.

****Availability of data and materials:**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

****Competing interests:**

The authors declare that they have no competing interests.

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