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# Comparative evaluation of calcium ion release from three bioceramic cements in simulated immature teeth with open apices

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## Abstract

**Objective:** This study aimed to evaluate the calcium ion ($Ca^{2+}$) release from apical plugs formed by three different bioceramic cements in simulated immature teeth with open apices.

**Methods:** In this in-vitro study, 66 single-rooted lower premolar teeth were divided into three groups, each containing 22 samples, as follows: Group A: ProRoot MTA, Group B: Bio-C Repair, and Group C: Dia-Root Bio MTA. Each sample was prepared to simulate immature teeth with open apices. A 4 mm apical plug was inserted at the open apex area. Calcium ion release was measured on the 7$^{th}$, 15$^{th}$, and 30$^{th}$ days using an atomic absorption spectrophotometer. Data were analyzed using Kruskal–Wallis, and Mann-Whitney U tests at the significance level of $P < 0.05$.

**Results:** The apical plugs formed by different groups showed significant differences in $Ca^{2+}$ release over 30 days ($P<0.001$). Group C had the highest release on days 7 (18.08 ± 0.57 ppm) and 15 (16.39 ± 0.75 ppm), whereas Group B showed the lowest levels ($P < 0.05$). On day 30, Group A had the highest $Ca^{2+}$ release (2.36 ± 0.25 ppm), which was significantly greater than that of Groups B and C ($P < 0.05$).

**Conclusions:** Dia-Root Bio MTA showed the highest calcium ion release on days 7 and 15, whereas ProRoot MTA exhibited the most significant release on day 30. Both Dia-Root Bio MTA and ProRoot MTA are more favorable options than Bio-C Repair for root repair in immature, non-vital teeth.

**Keywords:** Apexification, Calcium release, Dental cement, Mineral trioxide aggregate, Regeneration, spectrophotometry

## Introduction

During tooth eruption, teeth are highly susceptible to mechanical, physical, or microbiological insults that can lead to incomplete root maturation and an open root apex (1). Endodontic management in these cases is quite challenging due to large open apices and thin, divergent dentinal walls that are prone to fracture (2). In such cases, creating an apical barrier is crucial to prevent the extrusion of obturating materials beyond the apex.

Traditionally, long-term calcium hydroxide [$Ca(OH)_2$] therapy has been used to induce an apical barrier in non-vital immature teeth (3). However, recent studies have shown that prolonged use of $Ca(OH)_2$ can reduce the fracture resistance of teeth, challenging its effectiveness as a treatment approach (4). An alternative to this conventional technique suggests placing an apical barrier using materials such as dentin chips, freeze-dried cortical bone/dentin, or calcium phosphate (5). This approach allows for the efficient creation of an apical barrier and enables the completion of obturation in a single appointment. However, these materials do not always provide a well-sealed apical environment. The introduction of mineral trioxide aggregate (MTA) as an apical plug by Torabinejad and Chivian in 1996 addressed some of these limitations (6). However, MTA still has disadvantages, including long setting time, handling difficulties, and the potential for dentin discoloration (7).

Bioceramic-based materials have revolutionized root repair, providing several advantages such as antibacterial activity, increased biocompatibility, reduced solubility, appropriate film thickness, induction of an alkaline environment, and enhanced calcium ion ($Ca^{2+}$) release. These properties are critical for their

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clinical performance and healing of periapical tissues (8). Research suggests that bioceramic-based materials promote tissue repair by depositing mineralized tissues. The effectiveness of bioceramics depends on their pH level and $Ca^{2+}$ release capacity (9,10).

Despite the availability of various bioceramic root repair materials (RRMs), more high-quality evidence is needed to guide clinicians in selecting the most effective option. Calcium ion release, a key determinant of clinical success, remains an essential factor in this decision (11). Therefore, this study aimed to evaluate and compare the $Ca^{2+}$ release from apical plugs formed by three different bioceramic RRMs, including ProRoot MTA, Bio-C Repair, and Dia-Root Bio MTA, in simulated immature teeth with open apices.

## Materials and methods

### *Study design and sample selection*

This in-vitro study was approved by the Scientific Review Board (SRB) of Yenepoya University (Protocol No. YEC2/1095). A total of 66 anonymized extracted human single-rooted mandibular premolar teeth were selected for the study. The inclusion criteria were teeth with intact enamel surfaces, no caries, and closed apices that were extracted for orthodontic reasons. Teeth with root fractures, preexisting external defects on the root surface, and anatomical irregularities were excluded from the study.

### *Teeth preparation and simulation of open apices*

The collected teeth were immersed in 0.5% chloramine T solution, cleaned with a scaler tip to remove debris, and polished with the prophylaxis paste (PSP Prophy Paste, Belvedere, Kent, UK). Access openings were made using an endo access bur (EA 10, Mani INC., Tokyo, Japan). Canal patency was established using a 25 mm long No.10 Kerr file (K file, Mani INC., Tokyo, Japan), and the working length was determined.

Biomechanical preparation of the root canals was carried out to simulate immature teeth with open apices, using F3 Protaper gold rotary files (Dentsply Tulsa Dental, Johnson City, TN, USA). Between the steps, canals were irrigated with 2 ml of 3% sodium hypochlorite (NaOCl) solution. Then, roots were separated from crowns by sectioning transversely through the cementoenamel junction (CEJ) with a diamond disc. Three mm of the apical root was also removed transversely with a diamond disc on a rapidly rotating handpiece to simulate an open apex. Samples were standardized to a root length of 10 mm, and canal width was standardized using Gates Glidden drills #1-6 (GG Drill, Mani INC.). The canals were irrigated with 17% ethylenediaminetetraacetic acid (EDTA) gel (Canal+, Septodont, France) and 3% NaOCl to remove the smear layer, dried with absorbent paper points, autoclaved, and stored in sterilized jars in an incubator at 37°C. Figure 1 illustrates the preparation steps of the specimens.

The prepared teeth were then randomly divided into three experimental groups (n = 22) according to the root repair material used:

- Group A: ProRoot MTA (WMTA, Dentsply Tulsa Dental)
- Group B: Bio-C Repair (Angelus, Londrina, PR, Brazil)
- Group C: Dia-Root Bio MTA (Diadent, Cheongju, South Korea)

### *Formation of apical plugs and calcium ion release evaluation*

A 4 mm thick apical plug was formed in each sample using the respective RRMs for Groups A, B, and C. This procedure followed the manufacturer's instructions and was performed using endodontic pluggers. At the final setting of the apical plugs, each specimen was immersed in a test tube containing 10 ml of deionized water. The tubes were sealed and incubated at 37°C. The $Ca^{2+}$

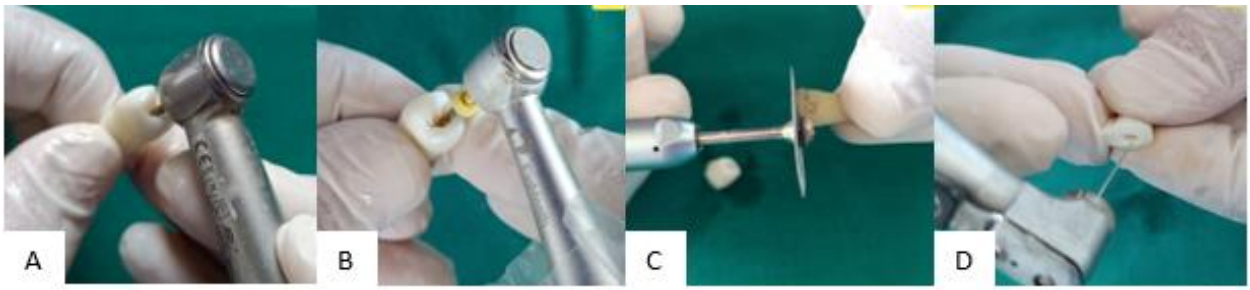


**Figure 1.** Preparation steps of samples prior to apical plug placement. A) Access opening using an Endo Access bur, B) Cleaning and shaping the canal with Protaper Gold up to size F3, C) Decoronation using a diamond disc, and D) Standardization of canal width using Gates Glidden drills #1-6.

**Figure 2.** Calcium ion release evaluation using an atomic absorption spectrophotometer (AAS)

release was measured on the 7th, 15th, and 30th days using an atomic absorption spectrophotometer (AA6800, Shimadzu, Tokyo, Japan) (Figure 2). Device conditions were set per the manufacturer's instructions, with a wavelength of 422.70 nm, a gap of 0.2 nm, a lamp current of 10 mA, and an acetylene flow of 2.0 L per minute supported by air. A lanthanum chloride solution (10 g/L) was used to prevent interference from phosphates and sulfates and avoid forming refractory oxides. A standard 10 mg/dL stock solution was diluted to achieve final concentrations ranging from 0.025 mg/dL to 1.0 mg/dL.

After each $Ca^{2+}$ release measurement on days 7, 15, and 30, the distilled water was replaced, and the samples were returned to the tubes for further evaluation. The amount of $Ca^{2+}$ release was measured in parts per million (ppm).

### *Statistical analysis*

Descriptive statistics, including mean and standard deviation, were used for continuous data. The Kolmogorov-Smirnov test was used to check the normality of the data. Given that the data did not follow a normal distribution ($P < 0.05$), the Kruskal-Wallis test was used to compare the three groups. Additionally, the Mann-Whitney U test was used for pairwise comparisons. The significance was set at $P < 0.05$. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (version 21.0; IBM Corp., Armonk, NY, USA).

## Results

Table 1 presents calcium ion ($Ca^{2+}$) release levels over the 30-day study period in ProRoot MTA (Group A), Bio-C Repair (Group B), and Dia-Root Bio MTA (Group C). All groups demonstrated the highest levels of $Ca^{2+}$ release

**Table 1.** Descriptive statistics and comparison of the groups' $Ca2^{+}$ release (ppm) profiles

| Assessment days | Groups | Mean ± SD | Median | IQR |
|---|---|---|---|---|
| 7th day | A (ProRoot MTA) | $16.13 \pm 0.56^{b}$ | 16.02 | 0.79 |
| | B (Bio-C Repair) | $13.12 \pm 0.58^{c}$ | 13.18 | 0.88 |
| | C (Dia-Root Bio MTA) | $18.08 \pm 0.57^{a}$ | 18.07 | 0.75 |
| | P-value | <0.001 | | |
| 15th day | A (ProRoot MTA) | $14.84 \pm 0.52^{b}$ | 14.81 | 0.70 |
| | B (Bio-C Repair) | $8.68 \pm 0.52^{c}$ | 8.67 | 1.64 |
| | C (Dia-Root Bio MTA) | $16.39 \pm 0.75^{a}$ | 16.47 | 1.17 |
| | P-value | <0.001 | | |
| 30th day | A (ProRoot MTA) | $2.36 \pm 0.25^{a}$ | 2.31 | 0.26 |
| | B (Bio-C Repair) | $2.02 \pm 0.35^{b}$ | 1.97 | 0.50 |
| | C (Dia-Root Bio MTA) | $1.87 \pm 0.29^{b}$ | 1.85 | 0.37 |
| | P-value | <0.001 | | |

SD: Standard deviation, IQR: Interquartile range
The groups that have been marked by different superscript letters indicate significant differences at P<0.05.

on the seventh day, which gradually decreased throughout 30 days.

On the seventh day, Group C (Dia-Root Bio MTA) released the highest amount of Ca2+ (18.08 ± 0.57 ppm), while Group B (Bio-C Repair) exhibited the lowest (13.12 ± 0.58 ppm). Similarly, on the 15th day, Group C released the highest amount of Ca2+, with a mean value of 16.39 ± 0.75 ppm, while Group B exhibited the lowest, with a mean value of 8.68 ± 0.52 ppm.

The results of the Kruskal-Wallis test showed significant differences between groups in all three assessment points ($P < 0.001$). Pairwise comparisons using the Mann-Whitney U test revealed significant differences between the three groups on days 7 and 15 ($P < 0.05$). However, on day 30, Group A exhibited significantly higher $Ca^{2+}$ release than Groups B and C ($P < 0.05$), which were statistically comparable ($P > 0.05$; Table 1).

Figure 3 illustrates the changes in calcium ion release over the study period.

## Discussion

The success of endodontic treatment in a tooth with an open apex depends on creating a periapical barrier that seals the space between the periodontium and the root canal, preventing microleakage and secondary infections (12). Apexification using biocompatible materials is essential for achieving a "closed apex" by prompting the formation of mineralized tissues such as bone or osteodentin (13,14). This study evaluated the calcium ion release from different materials used in apexification for immature teeth, including ProRoot MTA, Bio-C Repair, and Dia-Root Bio MTA.

In this study, we assessed the effectiveness of root repair materials (RRMs) by measuring the release of calcium ions ($Ca^{2+}$). The calcium release profile is critical for successful apexification or apexogenesis. Calcium aids in cell differentiation, hard tissue mineralization, and the regulation of osteopontin and bone morphogenetic protein 2 (BMP-2) levels, thus enhancing dental pulp cell development (15). $Ca^{2+}$ also enhances dentin mineralization, forms a dentin bridge, supports cellular attachment, and creates an antibacterial environment through high pH levels (16,17). In addition, it stimulates hard tissue-producing cells and promotes hydroxyapatite formation on root-end materials, providing a biological seal (18,19).

In this study, $Ca^{2+}$ release was confirmed in deionized water surrounding the root-end filling materials. ProRoot MTA, Bio-C Repair, and Dia-Root Bio MTA contain calcium oxide, which converts to calcium hydroxide upon contact with moisture. The $Ca(OH)_2$ dissociates into $Ca^{2+}$ and $OH^-$ ions, raising the pH level and releasing $Ca^{2+}$ into the surrounding environment(20). $Ca^{2+}$ also induces BMP-2 expression, which stimulates mineralization and triggers mineralized tissue deposition (21, 22).

Dia-Root Bio MTA exhibited the highest $Ca^{2+}$ release in this study on the 7th and 15th days among the three groups. Dia-Root Bio MTA contains calcium silicate, amorphous fumed silica, and zirconium dioxide, which produce a strong antibacterial effect and a highly alkaline pH.

The findings of this study align with those of Kang et al. (23), who reported that Dia-Root Bio MTA had a higher calcium ion release than ProRoot MTA. Another in-vitro study reported that MTA reacted with the

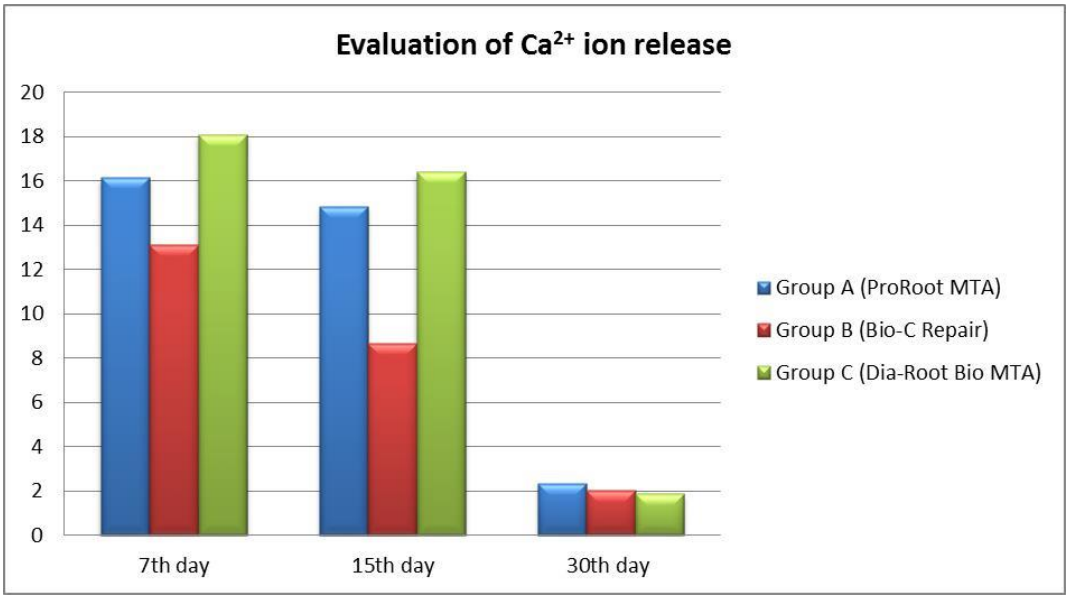


**Figure 3.** A graphical representation of mean $Ca^{2+}$ release trend across the study groups over the experiment

medium to form $Ca(OH)_2$ through a hydration process. This reaction caused the dissociation of $Ca(OH)_2$ into $Ca^{2+}$ and $OH^-$ ions, resulting in an increased pH and a higher calcium concentration in the medium (18).

In this study, Bio-C Repair exhibited the least $Ca^{2+}$ release capacity on the 7th and 15th days, which can be attributed to its composition (24). Bio-C Repair consists of iron oxide, silicon dioxide, calcium aluminate, zirconium oxide, and tricalcium silicate, containing comparatively lower amounts of calcium than other biomaterials. Due to this limitation, Bio-C Repair is not an ideal choice for root repair. Rodríguez-Lozano et al. (25) reported that Bio-C Repair had the lowest Ca2+ release, whereas the release of strontium (Sr) and silicon (Si) ions was significantly higher compared to MTA.

Pro Root MTA exhibited higher Ca2+ release on the 7th, 15th, and 30th days than Bio-C Repair. Pro Root MTA comprises Portland cement, bismuth oxide, calcium sulfate dihydrate, tetra calcium aluminoferrite, gypsum, and calcium oxide. Research by Guimarães et al. (26) also reported an increased Ca2+ release in the first week of MTA application. This increase may be due to the rapid release of calcium and hydroxyl ions upon contact with fluids, creating an alkaline pH on the material's surface, which leads to the nucleation and crystallization of apatite(27).

Several studies have reported that MTA forms calcium phosphate apatite crystals on its surface after contact with phosphate-containing simulated body fluid. The subsequent deposition of $Ca^{2+}$ and phosphate apatite between the root canal dentin and filling material enhances the regeneration and remineralization of adjacent hard tissues and improves the sealing capacity (28). However, interstitial fluid introduced through the crown or apex, necrotic tissue, and normal cellular respiration can partially dissolve $Ca(OH)_2$ (29). A recent study found that the average time for barrier formation following $Ca(OH)_2$ placement was approximately 5 to 20 months (30). Kim and Kim (31) observed that $Ca(OH)_2$ is rapidly absorbed during the initial stages of treatment, with a decreased absorption rate coinciding with barrier formation. Takita et al. (32). demonstrated that the sustained release of $Ca^{2+}$ is essential for the proliferation of human dental pulp cells in calcium silicate-based cement.

This study was limited by its in-vitro design, which might not fully replicate clinical conditions. Future research should focus on in-vivo studies to confirm these findings and explore the long-term effects of these materials in clinical settings. Additionally, investigating the interaction between these materials and different types of fluids in the root canal system could provide deeper insights into their performance and efficacy in clinical scenarios.

## Conclusions

In this in-vitro study, Dia-Root Bio MTA exhibited the highest Ca2+ release on days 7 and 15, whereas ProRoot MTA demonstrated the highest release profile on day 30. Bio-C Repair showed the lowest Ca2+ release on days 7 and 15. Given their higher calcium ion release, Dia-Root Bio MTA and ProRoot MTA appear more suitable than Bio-C Repair for root repair in immature, non-vital teeth.

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## Conflict of interest

The authors have no conflict of interest to declare.

## Authors' contributions

US and SG contributed to the manuscript's research inception, study management, and supervision. AA and MA contributed to data collection, chemical analysis, and interpretation. US, SG, MSP, AA, and MA contributed to the manuscript's data gathering, writing, and editing. All the authors read and approved the final manuscript.

## Ethical approval

All experimental procedures were performed in accordance with the study's ethical guidelines and were approved by the Institutional Ethical Committee (YEC2/1095), Yenepoya Dental College, Yenepoya University, Deralakatte, Mangalore, India.

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