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Dentistry Section

Comparative Evaluation of Radiopacity and Dentin Penetration Depth of Calcium Silicate-based Materials used for Apexification Procedure: An In-vitro Study

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ABSTRACT

Introduction: Successful apexification requires materials that provide reliable sealing ability, favourable biological properties and adequate radiographic visibility. Newer calcium silicate–based bioceramic materials have been introduced to overcome the limitations of traditional Mineral Trioxide Aggregate (MTA).

Aim: To assess and compare the dentinal tubule penetration depth and radiopacity of four commercially available calcium silicate-based materials used for apexification.

Materials and Methods: This in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics at GITAM Dental College and Hospital, Visakhapatnam, Andhra Pradesh, India, from October 2024 to April 2025. A total of 80 single-rooted human mandibular premolars were prepared to simulate open apices and randomly allocated into four groups (n=20 each): Group 1-Control- ProRoot MTA®, Group 2- Biodentine® , Group 3- Biostructure MTA Putty® and Group 4-Bio-C Repair®. In each group, a 6 mm apical plug was prepared using the respective material, mixed with 0.1% Rhodamine B dye to facilitate microscopic evaluation. Radiopacity was measured using standardised digital radiographs with an aluminium step wedge, and grey scale values were obtained using ImageJ analysis. Dentinal tubule penetration at 4 mm and 2 mm from the apex was evaluated with Confocal Laser Scanning Microscopy (CLSM). Data were analysed using One-way Analysis of Variance (ANOVA), independent t-tests, and Tukey's post-hoc test, with the level of significance set at p-value ≤0.05.

Results: ProRoot MTA demonstrated the highest radiopacity measured in Grey Scale Value (242.93±2.84 GSV), followed by Bio-C Repair (241.12±2.61 GSV), Biostructure MTA Putty (233.17±6.01 GSV), and Biodentine (186.91±3.99 GSV). Biodentine showed significantly lower radiopacity than all other materials (p<0.001), while no significant difference was observed between ProRoot MTA and Bio-C Repair (p=0.5014).

For penetration depth, Biodentine demonstrated the highest mean dentinal tubule penetration depth (641.40±126.89 µm), followed by ProRoot MTA (608.35±203.18 µm), Bio-C Repair (577.32±138.02 µm), and Biostructure MTA Putty (453.16±135.64 µm). Biostructure MTA Putty showed significantly lower penetration depth compared to the other materials (p<0.001). However, no statistically significant differences were found among ProRoot MTA, Biodentine, and Bio-C repair (p>0.05).

Conclusion: Biodentine exhibited the greatest dentinal tubule penetration, whereas ProRoot MTA showed the highest radiopacity. Bio-C Repair showed a favourable balance between penetration and radiographic detectability. Selection of apexification materials should consider both sealing potential and radiographic visibility. Further clinical studies are required to validate these findings.

Keywords: Confocal laser scanning microscopy, Dentinal tubules, Radiography, Root canal filling materials

INTRODUCTION

Pulpal and periapical diseases are predominantly initiated by microorganisms and their metabolic by-products within the root canal system, usually following microbial ingress through caries, fractures, or developmental defects. The primary objective of endodontic therapy is the elimination of intraradicular microorganisms and the prevention of reinfection. Chemo-mechanical preparation followed by three-dimensional obturation using a biocompatible material to prevent reinfection of the shaped and disinfected root canal is considered the most critical step for successful treatment outcomes [1].

Immature teeth with incomplete root formation, often due to trauma, caries, or developmental anomalies, present additional clinical challenges. These teeth commonly exhibit open apices, thin dentinal walls, and blunderbuss canals, making it difficult to establish an apical stop, adequately debride, and obturate the canal. The conventional management of such teeth has been apexification, aimed at inducing apical closure via calcific barrier formation or creating an artificial apical stop using a bioinert material. Traditionally, calcium hydroxide has been used in multiple-visit apexification due to its ability to stimulate calcific barrier formation. However, the prolonged treatment duration (5-20 months), multiple visits, risk of coronal leakage, reinfection, and persistent root fragility limit its effectiveness. Furthermore, the calcific barrier formed is often porous with soft tissue inclusions [2].

Mineral Trioxide Aggregate (MTA), introduced in 1993 and approved for clinical use by the US Food and Drug Administration in 1998, has emerged as a predictable alternative for apexification. Comprising mainly Portland cement derivatives with bismuth oxide, MTA exhibits bioactivity, biocompatibility, low solubility, radiopacity, hydrophilicity, and superior sealing ability. It sets in moist conditions, releases calcium ions, creates an alkaline microenvironment with antibacterial properties, and promotes cementum deposition and calcific barrier formation. These features allow for one-visit apexification procedures. However, MTA has limitations, including prolonged setting time (3-4 h),

difficult handling, high cost, risk of tooth discolouration, and trace impurities such as arsenic [3,4].

In response to these drawbacks, newer calcium silicate–based bioceramics have been introduced. Bio-C Repair (Angelus, Brazil) is a ready-to-use putty with favourable handling, bioactivity, and cytocompatibility comparable to MTA and Biodentine [5]. Biodentine (Septodont), introduced in 2010, offers dentin-like properties, improved handling, shorter setting time (~12 min), and reduced discolouration potential [6]. Biostructure MTA Putty exhibits prolonged working time, good dimensional stability, and adequate radiopacity [7].

Radiopacity is an essential property that allows filling materials to be distinguished from the surrounding anatomical structures. The ISO 6876:2001 standard specifies that endodontic cements should exhibit a radiopacity of at least 3 mm Aluminium (Al), higher than dentin or bone. From previous studies, the method, involving radiographic comparison with an aluminium step wedge, is widely used for radiopacity assessment [8,9]. Previous research has reported variations in radiopacity among materials such as MTA and Biodentine due to differences in radiopacifying agents, as demonstrated by Gandolfi MG et al., [10].

Marginal adaptation is equally critical for preventing bacterial penetration and periapical pathology. Penetration of materials into dentinal tubules enhances adaptation, mechanical retention, and microbial entombment. Factors influencing penetration include tubule size and density, material particle size, and flow properties. CLSM offers accurate, artefact-free evaluation of dentinal penetration depth [11]. Viapiana R et al., reported that particle size and flow properties significantly influence dentinal tubule penetration [12].

Despite the growing body of literature evaluating traditional materials such as MTA and Biodentine, limited data are available regarding newer ready-to-use bioceramic putties such as Bio-C Repair and Biostructure MTA Putty, particularly with respect to their radiopacity and dentinal tubule penetration when used for apexification procedures. Existing research has focused primarily on biocompatibility, sealing ability, or physical properties, while comparative investigations assessing both radiopacity and dentinal penetration depth using CLSM remain scarce.

In light of these considerations, the present in-vitro study was aimed to compare the radiopacity and root dentin penetration depth of calcium silicate–based materials used for apexification.

MATERIALS AND METHODS

The study was carried out in the Department of Conservative Dentistry and Endodontics at GITAM Dental College and Hospital, Visakhapatnam, from October 2024 to April 2025. Before commencement, consent was procured and clearance pertaining to ethical issues was obtained from the clearance pertaining to ethical issues was obtained from the Institutional Ethics Committee and Dr NTR University of Health Sciences (IEC No: D210050124). The present in-vitro study followed the Preferred Reporting Items for Laboratory studies in Endodontology

(PRILE) 2021 guidelines [13].

Inclusion criteria: The present study included premolars that were extracted due to mobility or as part of orthodontic treatment. Teeth with similar mesiodistal and buccolingual dimensions (±0.2 mm), which were non carious, free from cracks, with completely formed roots, without restorations or fractures, were also included in the study.

Exclusion criteria: Teeth were excluded if caries, prior fillings, crack lines, or fractures were present on the crown or root surfaces.

Sample size calculation: A total sample size of 80 specimens was calculated using G*Power software (version 3.1.9.7, Heinrich Heine University, Düsseldorf, Germany), assuming an effect size derived from previous studies [14,15], a statistical power of 95%, and an alpha error probability of 0.05.

Sample selection: The collected samples were inspected under 2.5x magnification (ORO clip-on loupes, Reach Global India, Pune) and radiographed in both mesiodistal and buccolingual directions to verify the presence of a single canal.

External debris was removed using an ultrasonic scaler, and the teeth were disinfected in 3% sodium hypochlorite for five minutes. Thereafter, the specimens were stored in 0.1% thymol solution at room temperature to preserve hydration until further use.

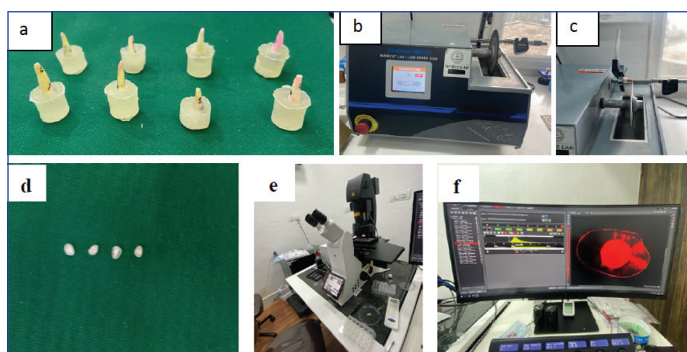
The specimens were randomly allocated into four groups (n=20 each) using a computer-generated randomisation method. The composition and manufacturers of the materials used in the study are presented in [Table/Fig-1].

Groups	Composition	Manufacturer
Control-ProRoot MTA® (Group-I)	55% - Tricalcium silicate, 19% - Dicalcium silicate, 10% - Tricalcium aluminate, 7% - Tetracalciumaluminoferrite, 2.8% - MgO, 2.9% - SO3, Bismuth oxide (for radiopacity)	Dentsply, Johnson City, TN, USA
Biodentine® (Group-II)	80.1%- Tricalcium silicate (3CaO.SiO2) 14.9%- Dicalcium silicate (2CaO.SiO2) Calcium carbonate (CaCO3) 5%- Zirconium Oxide (ZrO2) (Radiopacifier) Iron oxide (Colouring agent)	Septodont, Saint-Maur-des-Fossés, France
Biostructure MTA Putty® (Group-III)	55%- Tricalcium silicate, 16%- Dicalcium silicate, 6%- Tricalcium aluminate, 5%- Zirconium oxide, Paste forming agents	Itena Clinical, Paris, France
Bio-C Repair® (Group-IV)	40%- Calcium silicate, 15%- Calcium oxide, 10%- Zirconium oxide, 5%- Silicon dioxide, <1%- Iron oxide, Dispersing agent	Angelus, Londrina, Brazil

[Table/Fig-1]: Composition of the materials used in the study.

- Group 1: Control- ProRoot MTA®
- Group 2: Biodentine®
- Group 3: Biostructure MTA Putty®
- Group 4: Bio-C Repair®

Representative images showing specimen preparation, microtome sectioning, and Confocal Laser Scanning Microscopy (CLSM) evaluation are presented in [Table/Fig-2].



[Table/Fig-2]: Composite images: a) Tooth samples mounted on acrylic resin; b,c) Microtome with tooth attached for sectioning; d) Sectioned root samples; e,f) Confocal Laser Scanning Microscope (CLSM) equipment with image processing.

Study Procedure

Specimen preparation: Access opening and canal preparation: Standardised access cavities were prepared using a BR-45 spherical diamond bur (Mani Inc., Japan) and an Endo Access Bur (Dentsply, Switzerland) mounted on a high-speed handpiece (NSK, Japan) with continuous water cooling. Canal patency was verified with a DG-16 endodontic explorer, and the working length was

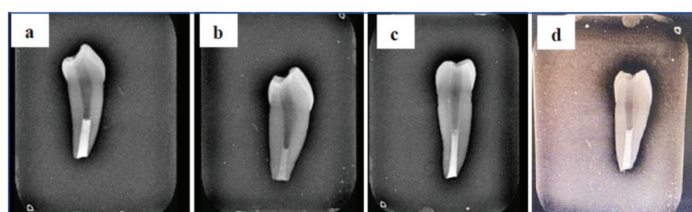
established with a #15 K-file (Mani Inc.), set at 1 mm short of the apical foramen.

Biomechanical preparation was performed using ProTaper Rotary NiTi files (Dentsply), progressing to size F3 (#30/06) at 300 rpm. Between successive instruments, the canals were irrigated with 2 mL of 3% sodium hypochlorite delivered through a 27-gauge side-vented needle.

Simulation of open apices: The apical region was enlarged with Peeso reamers (#1–5), and the #5 reamer extended beyond the apex to obtain a 1.5-mm diameter opening. To remove the smear layer, canals were treated with 5 mL of 17% Ethylenediaminetetraacetic Acid (EDTA) for one minute, then flushed with saline and dried with sterile paper points.

Placement of apical plug: For fluorescence tracing, 0.1% Rhodamine B dye was incorporated into the tested materials. An apical plug of 6 mm thickness was delivered using an MTA carrier and compacted vertically with endodontic pluggers. A moistened cotton pellet was placed over the set material, and the access cavity was sealed with Cavit-G (3M ESPE, MN, USA).

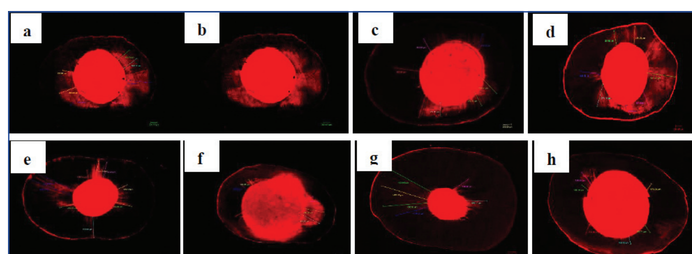
Radiopacity assessment: Representative radiographic images of the four groups used for radiopacity assessment, obtained immediately after placement of the apical plug, are shown in [Table/Fig-3].



[Table/Fig-3]: Radiopacity images of four experimental groups: a) Group 1: Control-ProRoot MTA; b) Group 2: Biodentine; c) Group 3: Biostructure MTA Putty; d) Group 4: Bio-C Repair.

Images were acquired using the Vista Scan Phosphor Storage Plate (PSP) system (Dürr Dental, Germany) with a size 2 PSP plate. A PSP plate was used with an exposure time of 0.14 seconds and a focus-to-film distance of 30 cm. The radiographs were analysed using ImageJ software (National Institutes of Health, USA) to determine the grey scale values (0 = black; 255 = white).

Penetration depth evaluation using Confocal Laser Scanning Microscopy (CLSM): Representative CLSM images demonstrating dentinal tubule penetration of the tested materials at 2 mm and 4 mm levels are presented in [Table/Fig-4]. After mixing the test materials with Rhodamine B dye, the specimens were stored at 37°C and 100% humidity for seven days to allow complete setting. Transverse root sections were then prepared at 4 mm and 2 mm from the apex using a hard tissue microtome.



[Table/Fig-4]: Confocal images showing the four experimental groups penetration depths at intervals of 4 mm and 2 mm: a,b) Group 1: Control-ProRoot MTA; c,d) Group 2- Biodentine; e,f) Group 3- Biostructure MTA Putty; g,h) Group 4- Bio-C Repair.

Penetration depth was evaluated and quantified separately for the sections obtained at the 4 mm and 2 mm levels to assess material penetration in the middle and apical regions of the root canal. The sections were examined under a CLSM (Leica DMi8, Leica Microsystems GmbH, Germany) with excitation at 540 nm and emission at 590 nm to visualise the Rhodamine B-labelled material. Images (1024 × 1024 pixels) were acquired at a depth of 10 µm beneath the cut surface at 10x magnification. The penetration of the material into dentinal tubules was quantified using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

STATISTICAL ANALYSIS

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) software version 20.0 for Windows (IBM Corp., Armonk, NY, USA). Intergroup comparisons of radiopacity and dentinal tubule penetration depth were performed using One-way Analysis of Variance (ANOVA). Pairwise comparisons between groups were conducted using Tukey's post-hoc test. A p-value <0.05 was considered statistically significant.

RESULTS

Radiopacity: The mean radiopacity values and pairwise comparisons among the four experimental groups are presented in [Table/Fig-5]. The mean radiopacity values were highest for ProRoot MTA (242.93±2.84), followed by Bio-C Repair (241.12±2.61), Biostructure MTA Putty (233.17±6.01), and Biodentine (186.91±3.99). Tukey's post-hoc analysis revealed statistically significant differences between most groups (p<0.05). Biodentine demonstrated significantly lower radiopacity compared to ProRoot MTA, Biostructure MTA Putty, and Bio-C Repair (p <0.001). Biostructure MTA Putty also showed significantly lower radiopacity than ProRoot MTA (p<0.001). However, no statistically significant difference was observed between ProRoot MTA and Bio-C Repair (p=0.5014).

Groups	ProRoot MTA (Group 1)	Biodentine (Group2)	Biostructure MTA Putty (Group 3)	Bio-C repair (Group 4)
Mean (Grey scale values (GSV))± SD	242.93±2.84	186.91±3.99	233.17±6.01	241.12±2.61
ProRoot MTA	-			
Biodentine	p<0.001**	-		
Biostructure MTA Putty	p<0.001**	p<0.001**	-	
Bio-C Repair	p=0.5014	p<0.001**	p<0.001**	-

[Table/Fig-5]: Pair-wise comparisons of mean radiopacity scores of four groups using Tukey's multiple post hoc procedure.
*p<0.05 indicates a statistically significant difference. p>0.05 non-significant. SD: Standard deviation

Penetration depth: The mean dentinal tubule penetration depth values and pairwise comparisons among the four groups are presented in [Table/Fig-6]. The Highest for Biodentine (641.40±126.89 µm), followed by ProRoot MTA (608.35±203.18 µm), Bio-C Repair (577.32±138.02 µm), and Biostructure MTA Putty (453.16±135.64 µm). Tukey's post-hoc analysis showed that Biostructure MTA Putty had significantly lower penetration depth compared to ProRoot MTA, Biodentine, and Bio-C Repair (p<0.001). No statistically significant differences were observed between ProRoot MTA and Biodentine (p=0.6568), ProRoot MTA and Bio-C Repair (p=0.7004), or Biodentine and Bio-C Repair (p=0.1139).

Groups	ProRoot MTA (Group 1)	Biodentine (Group 2)	Biostructure MTA Putty (Group 3)	Bio-C Repair (Group 4)
Mean±SD (µm)	608.35±203.18	641.40±126.89	453.16±135.64	577.32±138.02
ProRoot MTA	-			
Biodentine	p=0.6568	-		
Biostructure MTA Putty	p<0.001**	p<0.001**	-	
Bio-C Repair	p=0.7004	p=0.1139	p<0.001**	-

[Table/Fig-6]: Pair-wise comparisons of mean dentinal tubule penetration depth among four groups using Tukey's multiple post-hoc procedure..*p <0.05 indicate a statistically significant difference. p>0.05 non-significant.

Pairwise comparisons of dentinal tubule penetration depth among the tested materials at 2 mm and 4 mm levels are presented in [Table/Fig-7]. Interaction analysis showed higher penetration depth at 4 mm compared to 2 mm for most materials ($p<0.05$).

Comparison	ProRoot MTA (2 mm)	ProRoot MTA (4 mm)	Biodentine (2 mm)	Biodentine (4 mm)	Biostructure MTA Putty (2 mm)	Biostructure MTA Putty (4 mm)	Bio-C Repair (2 mm)	Bio-C Repair (4 mm)
Mean±SD (μm)	481.27±181.93	735.44±132.79	553.53±55.15	729.27±117.29	383.70±110.70	522.62±123.91	541.49±145.24	613.14±123.73
ProRoot MTA (2 mm)	-							
ProRoot MTA (4mm)	p<0.001**	-						
Biodentine (2 mm)	p=0.6323	p<0.001**	-					
Biodentine (4 mm)	p<0.001**	P=1.0000	p<0.001**	-				
Biostructure MTA Putty (2 mm)	p=0.2382	p<0.001**	p<0.001**	p<0.001**	-			
Biostructure MTA Putty (4 mm)	p=0.9716	p<0.001**	p=0.9949	p<0.001**	p<0.001**	-		
Bio-C Repair (2 mm)	p=0.8156	p<0.001**	p=0.9999	p<0.001**	p<0.001**	p=0.9998	-	
Bio-C Repair (4 mm)	p<0.001**	p<0.001**	p=0.8236	p=0.0801	p<0.001**	p=0.3320	p=0.6427	-

[Table/Fig-7]: Tukey's Post-hoc pairwise comparison of interaction among materials at two levels (2 mm and 4 mm).
*p<0.05 indicates a statistically significant difference. p>0.05 non significant.

DISCUSSION

The penetration and adaptation of sealers increase surface contact between the material and dentin [16] and enhance the antimicrobial effect by entombing residual microorganisms within the dentinal tubules (Siqueira JF et al.,) [17]. However, orthograde application of materials during endodontic treatment of necrotic immature permanent teeth is challenging because open apices provide minimal tissue resistance and are associated with thin dentinal walls, which limit condensation. Consequently, gaps may form between the dentin and the material, or the material may fail to penetrate sufficiently into the dentinal tubules, compromising the quality of the seal and increasing the risk of leakage [18].

Across all materials compared, the dentinal tubule penetration and radiopacity of four calcium silicate-based apexification materials: ProRoot MTA, Biodentine, Bio-C Repair, and Biostructure MTA Putty, penetration into dentinal tubules was consistently higher at 4 mm from the apex than at 2 mm ($p<0.05$). This pattern reflects well-established anatomical characteristics of root dentin, in which tubules are larger in diameter and more numerous coronally, while the apical region often contains fewer or even tubule-free areas, sometimes replaced by cementum-like tissue [11]. These microstructural variations create more favourable pathways for sealer and biomaterial infiltration in the middle and coronal thirds compared with the apical third.

When individual materials were compared, Biodentine demonstrated the greatest mean dentine tubule penetration depth. This finding corroborates earlier reports that attribute Biodentine's performance to its fine particle size, low porosity, and the formation of a mineral infiltration zone with tag-like structures that extend into dentinal tubules [19,20]. These features enhance micromechanical interlocking with dentin, thereby improving sealing potential.

In the present study, ProRoot MTA achieved the second-highest mean penetration depth. The hydration reaction of MTA leads to the release of calcium hydroxide, creating a highly alkaline environment that facilitates intratubular mineralisation and deposition of apatite-like crystals [21]. This property supports its well-established clinical success in apexification and perforation repair. However, compared with Biodentine, the larger particle size and slower setting reaction may partly explain the relatively reduced depth of penetration [6,22].

Bio-C Repair exhibited intermediate results, with deeper penetration than Biostructure MTA Putty ($p<0.001$) but less than Biodentine ($p=0.1139$) and ProRoot MTA ($p=0.7004$). The premixed formulation of Bio-C Repair, based on calcium silicate technology, provides ease of handling and hydration stability [23,24]. Nevertheless, its particle characteristics and rheological behaviour may limit the extent of tubular infiltration compared with Biodentine's finer microstructure.

Biostructure MTA Putty demonstrated the lowest penetration depth. Differences in material consistency, particle distribution, and setting kinetics may have restricted its adaptability to the dentinal walls. Previous studies have noted that dense or putty-like formulations can compromise penetration into narrower or more irregular tubular spaces, which could account for the present findings [25,26].

Radiopacity evaluation revealed a different ranking of the tested groups. ProRoot MTA demonstrated the highest radiopacity, followed by Bio-C Repair, Biostructure MTA Putty, and Biodentine. The superior radiographic contrast of ProRoot MTA is explained by its high content of bismuth oxide, a radiopacifier with a high atomic number [27]. While this property facilitates easy radiographic detection, it has been associated with adverse outcomes such as tooth discolouration and potential cytotoxic effects [28].

In contrast, Biodentine exhibited the lowest radiopacity ($p<0.001$) among the tested materials. This is due to its use of zirconium oxide as a radiopacifier, which, although avoiding discolouration, has a lower atomic number than bismuth oxide [28]. Despite this, Biodentine's radiopacity remains clinically acceptable, though less pronounced than ProRoot MTA. Interestingly, this contrast highlights a trade-off: Biodentine excels in dentinal penetration and handling properties but has reduced radiographic visibility, whereas ProRoot MTA offers superior radiopacity but relatively lower penetration [29-31].

Bio-C Repair presented radiopacity values greater than Biodentine ($p<0.0001$) but below ProRoot MTA ($p=0.5014$). Its composition includes zirconium oxide, which contributes to adequate radiographic detectability without the discolouration risk linked to bismuth oxide. Biostructure MTA Putty, meanwhile, demonstrated radiopacity slightly higher than Biodentine but lower than Bio-C Repair, indicating material-specific differences in radiopacifier content and distribution [31].

From a comparative perspective, Biodentine appears superior in dentin interaction, correlating with its particle size and ability to form tag-like mineral extensions [32]. ProRoot MTA, though less penetrative, remains advantageous in radiographic verification due to its high bismuth oxide content. Bio-C Repair provides a balance between radiopacity and penetration, while Biostructure MTA Putty

performs lower in both aspects, possibly due to its formulation characteristics.

Together, the present study findings suggest that the clinical choice of apexification material should be guided not only by sealing ability or radiographic detectability alone but also by the correlation between material composition, physicochemical behaviour, and biological interaction.

Limitation(s)

The present study's main limitation is its in-vitro nature, which does not accurately reproduce the biological conditions and fluid dynamics found in-vivo. Moreover, repeated use of PSP plates and differences in radiographic settings may have affected radiopacity results, emphasising the importance of future in-vivo investigations on long-term sealing, colour stability, and clinical outcomes.

CONCLUSION(S)

Biodentine demonstrated the greatest dentinal tubule penetration depth among the evaluated apexification materials; however, this difference was not statistically significant when compared with ProRoot MTA and Bio-C Repair. Biostructure MTA Putty exhibited the lowest dentin penetration depth, with a statistically significant difference relative to the other materials tested. ProRoot MTA demonstrated the highest radiopacity among the tested materials. The difference was statistically significant when compared with Biodentine and Biostructure MTA Putty ($p<0.001$), whereas no statistically significant difference was observed between ProRoot MTA and Bio-C Repair ($p=0.5014$). Thus, while Biodentine exhibited superior dentin penetration, its radiopacity was lower than that of ProRoot MTA. Bio-C Repair demonstrated dentin penetration depth and radiopacity comparable to ProRoot MTA, indicating its potential as an effective alternative apexification material. Nevertheless, further in-vitro and clinical studies are required to validate and support the findings of the present investigation.

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