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In vitro assessment of sealing performance of C-Root BP as a retrograde root-end filling material: a preliminary study

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Abstract

Background Achieving a predictable apical seal is essential for the long-term success of endodontic microsurgery. Recently, bioceramic cements have been developed to address the shortcomings of traditional retrograde filling materials. C-Root BP, a strontium-silicate–based bioceramic, has been introduced as a potential alternative.

Aim The present study investigated the sealing ability of C-Root BP when used as a retrograde filling material.

Methods Fifty extracted human maxillary premolars were prepared, obturated, and divided into experimental, positive control, and negative control groups. In the experimental group, root-end resection and cavity preparation were followed by retrofilling with C-Root BP. Positive control specimens were resected but left unfilled, whereas negative control specimens were completely coated with nail varnish to prevent external leakage. Dye penetration, glucose leakage, and scanning electron microscopy (SEM) were employed to evaluate microleakage and interfacial adaptation. For each leakage assay, specimens were distributed as follows: C-Root BP group, $n = 15$; positive control, $n = 5$; negative control, $n = 5$.

Results Teeth filled with C-Root BP showed markedly reduced dye penetration compared with positive controls, with values approaching those of negative controls ($p < 0.05$). SEM observations revealed intimate adaptation of the material to dentin, with few interfacial discontinuities. In the glucose leakage model, leakage in the C-Root BP group remained low throughout 120 days, was significantly lower than the positive control group ($p < 0.001$), and did not differ significantly from the negative control group ($p > 0.05$).

Conclusion Within the limitations of this in vitro study, C-Root BP exhibited favorable sealing performance as a retrograde root-end filling material, as demonstrated by reduced dye penetration, minimal glucose leakage, and close interfacial adaptation to apical dentin.

Keywords Apical seal · Bioceramic · C-Root BP · Retrograde filling · Microleakage

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Introduction

Persistent apical periodontitis after primary root canal treatment is generally managed first by orthograde retreatment when feasible. Endodontic microsurgery is typically considered when nonsurgical retreatment is impractical, has failed, or cannot adequately address the apical pathology. With the advent of microsurgical techniques, ultrasonic retropreparation, and modern calcium-silicate cements, EMS now achieves high success rates, and the quality of the apical seal provided by the retrograde root-end filling is a key determinant of healing [1, 2]. Mineral trioxide aggregate (MTA) established the benchmark for retrograde fillings because of its biocompatibility, sealing ability, and long-term outcomes; however, it is hampered by drawbacks such as long setting time, handling difficulty, and potential discoloration [3]. These limitations have catalyzed the development of newer bioceramic materials—principally calcium-silicate–based putties and pastes (e.g., EndoSequence/TotalFill/iRoot BP Plus/BC-RRM)—designed to set in moisture, release calcium hydroxide, form apatite at the dentin interface, and exhibit dimensional stability in clinical conditions [4]. In vitro and ex vivo investigations report favorable physicochemical properties (e.g., interfacial apatite, low porosity) that support their sealing function at the resected apex [4, 5]. Because coronal and apical leakage can sustain intraradicular infection, "sealing ability" has been widely tested using dye penetration, fluid filtration, glucose or protein leakage, and dynamic bacterial or endotoxin models. Bacterial and nutrient-leakage models are widely regarded as more clinically relevant surrogates of microleakage compared with static dye penetration assays, as they simulate dynamic transport of microbial byproducts across the interface [6].

Across several designs, calcium-silicate putties generally perform on par with MTA for apical sealing. In a bacterial-nutrient leakage model, EndoSequence BC-RRM Fast-Set Putty showed sealing comparable to MTA [6]. Similarly, a 2021 bacterial-leakage study found no significant difference among BC-RRM putty, BC-RRM paste+putty, and MTA used as retrofills [5]. These laboratory findings align with clinical evidence: randomized and cohort studies indicate that bioceramic putties yield healing rates comparable to MTA in EMS when evaluated radiographically (including CBCT-based criteria) [1, 7, 8]. C-Root BP is a newer strontium-silicate–based bioceramic root repair putty intended for root-end filling [9, 10]. These attributes justify a preliminary in vitro evaluation of the sealing performance of C-Root BP under standardized laboratory conditions. Because the present study did not include an established retrograde filling material as a comparator, it was designed to characterize the sealing behaviour of C-Root BP rather than to determine its equivalence or superiority relative to other materials [11, 12].

A significant challenge in endodontic success is the prevention of microleakage—the infiltration of microorganisms, toxins, and fluids into the root canal system through gaps or voids in the obturation material or sealer. Incomplete root canal obturation and the degradation of sealing materials can lead to bacterial recontamination, causing persistent periapical pathology. Achieving an effective and durable apical seal is therefore critical to prevent microbial invasion and to ensure the long-term success of root canal therapy [13, 14].

Retrograde root-end filling is performed as part of endodontic microsurgery when orthograde retreatment is not feasible or is unlikely to predictably resolve persistent apical disease. Following apical resection, a 3-mm retropreparation is created ultrasonically to seal the apical terminus. The resected root surface presents open dentinal tubules, apical ramifications, and isthmuses that may harbor residual microorganisms. Therefore, the retrograde filling material must provide dimensional stability, intimate adaptation to cut dentin, and resistance to microleakage under moist surgical conditions.

To evaluate the sealing efficacy of root canal sealers, various laboratory methods have been developed, among which the dye penetration technique remains one of the most widely applied. This method allows direct visualization and measurement of microleakage by tracking the extent of dye infiltration in the interface between the sealer and dentinal walls. Stereomicroscopic evaluation of dye penetration provides a reliable and reproducible means to compare the sealing capabilities of different commercial root canal sealers. Independent evidence concerning the sealing performance of C-Root BP when used as a retrograde root-end filling material remains

limited. Therefore, this preliminary in vitro study evaluated the apical sealing performance of C-Root BP using three complementary methods: linear dye penetration, qualitative scanning electron microscopic assessment of the dentin–material interface, and a 120-day dynamic glucose leakage model. Positive and negative controls were included to verify the functioning of the leakage models and were not intended to serve as established material comparators. The null hypothesis was that no statistically significant differences in leakage would be detected among the C-Root BP, positive-control and negative-control conditions under the applied laboratory models.

Materials and methods

Sample selection and preparation

In total, 50 extracted human permanent maxillary second premolars, free of cracks and showing an apical isthmus, were selected after micro-CT screening. Standardized endodontic access cavities were prepared to establish straight-line access. Mechanical shaping was performed using the ProTaper Universal rotary system (Dentsply Sirona, Ballaigues, Switzerland) up to size F3 (tip size 30, taper 0.09), following the manufacturer's recommended sequence under continuous irrigation. Irrigation was performed using 1% sodium hypochlorite (NaOCl), with a total volume of 10 mL per canal, delivered using a side-vented needle after each instrumentation step. Each irrigation cycle was maintained for approximately 30–60 s to ensure adequate chemical debridement. Following completion of instrumentation, a final rinse with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) was performed for 1 min to remove the smear layer, followed by a final flush with 5 mL of distilled water to eliminate residual irrigants. Obturation was performed using gutta-percha cones in combination with an epoxy resin–based sealer, AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany), using the warm vertical compaction technique. The use of a standardized sealer ensured consistency in obturation quality and minimized variability in apical preparation prior to retrograde cavity formation. The total sample of 50 teeth was selected on the basis of feasibility and previous exploratory in vitro microleakage studies using similar experimental designs [5, 6]. No a priori sample-size calculation was performed. The specimens were divided between two independently analysed leakage assays, with each assay comprising 15 C-Root BP specimens, five positive-control specimens and five negative-control specimens. Consequently, the individual comparisons involving the control subgroups were not formally powered to detect small or moderate differences and were likely to have adequate sensitivity only for large effects. The effect-size assumption of Cohen's ($f = 0.40$), used during exploratory assessment of statistical power, represented a conventional large-effect threshold and was not derived from pilot data or a previously published study of C-Root BP. Accordingly, the analyses should be interpreted as exploratory, and the absence of a statistically significant difference should not be interpreted as evidence of equivalence.

For the experimental groups, root apices were resected 3 mm perpendicular to the long axis. Retrograde cavities were prepared using ultrasonic retrotips (KiS ultrasonic tips, Spartan/Obtura, Fenton, MO, USA) mounted on a piezoelectric ultrasonic unit operating at a frequency of approximately 30 kHz. The retropreparation was performed under continuous sterile saline irrigation to prevent overheating and remove debris. A standardized cavity depth of 3 mm was achieved along the long axis of the root. The 50 specimens were randomly allocated into three groups: C-Root BP group ($n = 30$), positive control group (resected but unfilled; $n = 10$), and negative control group (externally sealed with nail varnish; $n = 10$). Each group was subsequently subdivided for the two leakage assays. For dye penetration testing, 15, 5, and 5 specimens were used, respectively; the remaining 15, 5, and 5 specimens were used for glucose leakage analysis. Although the control subgroup size ($n = 5$) was limited, this allocation was consistent with prior exploratory in vitro leakage studies and was intended to validate the experimental model rather than serve as a primary comparative analysis. In the experimental group, root apices were resected 3 mm perpendicular to the long axis, and a 3 mm deep retrograde cavity was prepared using ultrasonic tips under continuous saline irrigation. The cavity was filled incrementally with C-Root BP, a

strontium-silicate–based bioceramic root repair putty (Meta Biomed Co., Ltd., Chungbuk, South Korea; lot/batch no. CRBP240315), using a micro-applicator and gently condensed with a micro-plugger under magnification.

After retrofilling, all specimens were stored in Hanks' Balanced Salt Solution (HBSS) at 37 °C for seven days to allow complete material setting and simulate physiologic moisture conditions. Following maturation, roots were dried and coated with three layers of nail varnish, leaving only the resected apical surface exposed in the experimental and positive control groups. The larger allocation to the experimental group was planned to allow independent assessment of the material in both dye penetration and glucose leakage models while maintaining corresponding positive and negative control subgroups for each assay.

Dye penetration test

For dye penetration analysis, 15 specimens from the experimental group, 5 from the positive control group, and 5 from the negative control group were used. The specimens were immersed in 1% methylene blue solution for seven days. After rinsing with distilled water, each tooth was sectioned longitudinally along the mesiodistal plane. The maximum depth of linear dye penetration at the resected root surface was measured under a stereomicroscope. Measurements were performed by a blinded examiner to minimize observational bias. Thermocycling, mechanical loading, and bacterial leakage models were not incorporated in this experimental design.

Scanning electron microscopic assessment

Following completion of the dye-penetration assessment, the longitudinally sectioned C-Root BP specimens from the experimental group ($(n = 15)$) were subjected to qualitative scanning electron microscopic assessment. The specimens were thoroughly rinsed with distilled water to remove residual methylene blue and surface debris. They were then dehydrated through a graded ethanol series of 50%, 70%, 80%, 90% and 100%, with each dehydration step performed for 10 min. The specimens were subsequently air-dried at room temperature for 24 h.

After drying, the specimens were mounted on aluminium stubs using conductive carbon adhesive tape, with the longitudinally sectioned dentin–material interface oriented towards the electron beam. The mounted specimens were sputter-coated with a thin layer of gold approximately 10 nm in thickness to improve surface conductivity and minimise charging during imaging. The specimens were examined using a scanning electron microscope at an accelerating voltage of 15 kV. Images were acquired at magnifications ranging from 100 × to 1,000 ×. Multiple fields distributed along the coronal, middle and apical portions of the dentin–C-Root BP interface were examined in each specimen.

The SEM assessment was qualitative and focused on the continuity of the dentin–material interface, the presence of visible interfacial gaps or separation, and the occurrence of microporosities within the material. The predominant interfacial pattern was determined after examination of all included specimens. The micrograph presented in Fig. 2 was selected because it represented the predominant pattern observed across the examined specimens and was not selected as an isolated best-performing region.

Glucose leakage test

A glucose filtration model was used to evaluate dynamic leakage over time. For glucose leakage analysis, the remaining 15 specimens from the experimental group and 5 specimens each from the positive and negative control groups were included. Crowns were sectioned to obtain roots measuring 12 mm. Each root was fixed in a plastic tube, with the apical 6 mm extending through an opening at the base. The interface between root and tube was sealed with cyanoacrylate adhesive and coated with nail varnish. The assembly was suspended in a glass vial containing 15 mL of distilled water with 0.5% sodium benzoate, ensuring that 3 mm of the root apex was immersed. A fine needle allowed communication with the atmosphere. The upper chamber of the tube was filled with 1 mol/L glucose solution containing 0.5% sodium benzoate, maintained at a constant height of 15 cm to

provide hydrostatic pressure. The set-up was stored at 37 °C under humid conditions. At predetermined intervals (days 1, 7, 14, 20, 30, 50, 70, 90, 110, 120), 100 µL of solution was withdrawn from the lower chamber (Table 1). An equal volume of fresh sodium benzoate solution was added to maintain a constant volume, and the glucose solution in the upper chamber was replenished to sustain pressure. The glucose concentration in the lower chamber was measured spectrophotometrically at 505 nm.

Statistical analysis

Data normality was assessed using the Shapiro–Wilk test. One-way ANOVA followed by Tukey post hoc test was used to compare dye penetration among groups. Glucose leakage data across time points were analyzed using repeated-measures ANOVA. A significance level of $p < 0.05$ was established.

Results

Dye penetration analysis

The positive-control specimens exhibited extensive dye infiltration along the resected root surface and canal walls, whereas no dye penetration was detected in the negative-control specimens. In the C-Root BP group, dye penetration was predominantly confined to the superficial apical interface. Mean linear dye penetration was approximately 1.8 ± 0.6 mm in the C-Root BP group, 4.2 ± 1.3 mm in the positive-control group and 0.0 mm in the negative-control group. One-way ANOVA followed by Tukey's post-hoc test demonstrated significantly less dye penetration in the C-Root BP group than in the positive-control group ($p < 0.05$) (Fig. 1).

Interfacial adaptation analysis

Qualitative SEM assessment of the 15 C-Root BP specimens showed that the predominant dentin–material interfacial pattern was characterized by close contact and a largely continuous interface with minimal observable separation. Occasional microporosities were identified within the bulk of the material; however, these generally did not extend to the dentin–material interface. No large interfacial gaps were observed in the examined fields. Figure 2 presents a single micrograph selected to illustrate the predominant interfacial pattern observed across the assessed specimens.

Glucose leakage analysis

The glucose leakage model was employed to simulate long-term permeability of the apical seal under continuous hydrostatic pressure. In the positive control group, glucose concentration in the lower chamber rose sharply

Table 1 Mean glucose concentrations detected in the lower chamber during the 120-day leakage assessment

Assessment day	C-Root BP, ($n = 15$)	Positive control, ($n = 5$)	Negative control, ($n = 5$)
1	0 mmol/L	0 mmol/L	0 mmol/L
7	0 mmol/L	10 mmol/L	0 mmol/L
14	0 mmol/L	31 mmol/L	0 mmol/L
20	0 mmol/L	61 mmol/L	0 mmol/L
30	5 mmol/L	98 mmol/L	0 mmol/L
50	10 mmol/L	152 mmol/L	0 mmol/L
70	20 mmol/L	193 mmol/L	0 mmol/L
90	39 mmol/L	265 mmol/L	10 mmol/L
110	58 mmol/L	318 mmol/L	10 mmol/L
120	79 mmol/L	362 mmol/L	10 mmol/L

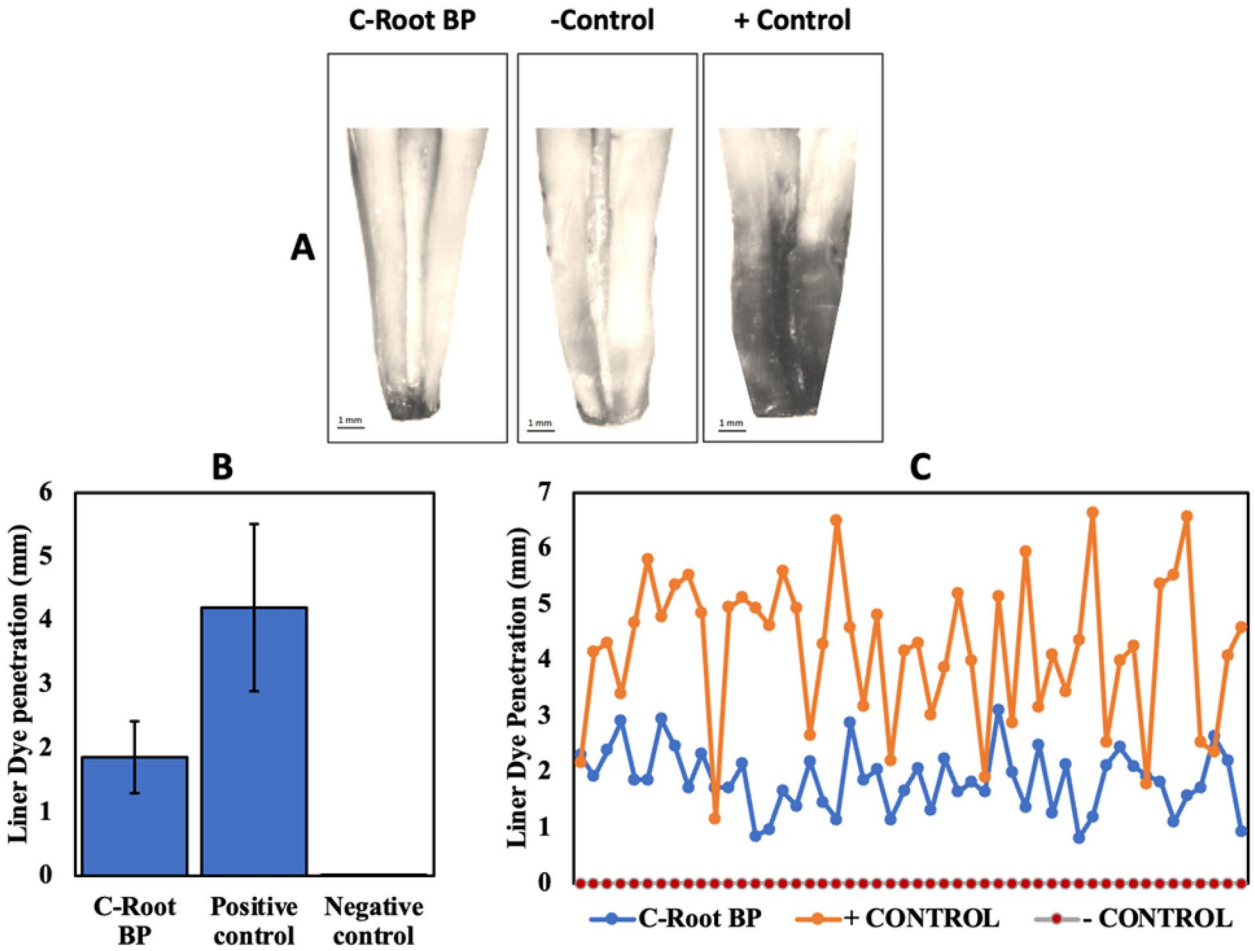


Fig. 1 Dye penetration analysis of apical microleakage. Representative stereomicroscopic images of the experimental (C-Root BP), positive control, and negative control groups are shown (top panel). The bar graph (lower left) depicts mean linear dye penetration depth (mm ± SD). The positive control exhibited extensive leakage, while the C-Root BP group showed significantly reduced penetration. The negative control demonstrated no dye infiltration. The line graph (lower right) illustrates variability in dye penetration depth across individual specimens

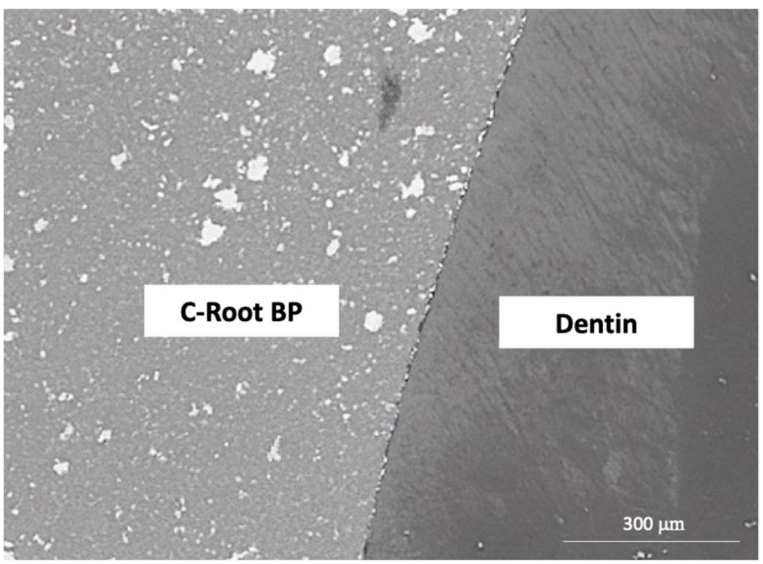


Fig. 2 Representative scanning electron micrograph of the dentin–C-Root BP interface. The image illustrates the predominant interfacial pattern observed across the 15 examined specimens, characterized by a largely continuous dentin–material interface with minimal observable gaps. The assessment was qualitative. Scale bar: 300 µm

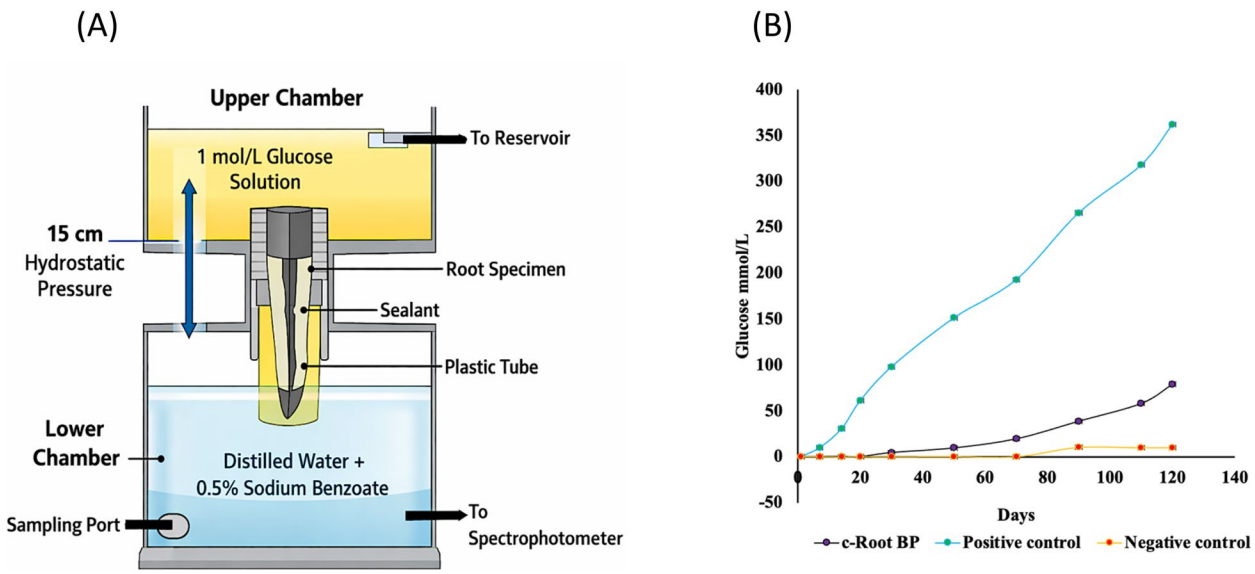


Fig. 3 Glucose leakage during the 120-day observation period. **A** Schematic representation of the glucose leakage apparatus. **B** Mean glucose concentration detected in the lower chamber at the specified assessment points. The positive-control group showed a progressive increase in glucose concentration, whereas the C-Root BP group demonstrated a slower increase. The negative-control group remained at or close to baseline throughout the assessment period. Data are presented as mean ± SD

from the initial days of testing and continued to increase throughout the 120-day observation period (Fig. 3A-B, Table 1). This progressive rise reflected unimpeded passage of the glucose solution across the resected surface and highlighted the absence of a sealing barrier. In contrast, the C-Root BP group showed minimal glucose transmission. Concentrations in the lower chamber remained near baseline for the entire observation period, with no significant upward trend. Statistical analysis confirmed that leakage in the C-Root BP group was not significantly different from that of the negative control ($p > 0.05$) but was highly distinct from the positive control ($p < 0.001$). The negative control group demonstrated complete resistance to glucose passage throughout the study duration, further validating the model. Notably, the stability of the leakage profile in the C-Root BP group across multiple time points suggests that the material was able to maintain its sealing integrity even under sustained hydrostatic challenge. Throughout the 120-day observation period, glucose leakage in the C-Root BP group remained low, differed significantly from the positive control group ($p < 0.001$), and did not differ significantly from the negative control group ($p > 0.05$).

Discussion

The present preliminary in vitro study evaluated the sealing performance of C-Root BP using dye penetration, qualitative SEM-based interfacial assessment and dynamic glucose leakage. Under the applied experimental conditions, the C-Root BP group demonstrated less dye and glucose leakage than the unfilled positive-control group. No statistically significant difference was detected between the C-Root BP and negative-control conditions; however, the limited subgroup sizes and absence of a predefined equivalence margin preclude interpretation of this finding as evidence of equivalent sealing performance. The results therefore provide preliminary information on the barrier behaviour of C-Root BP but do not establish its performance relative to MTA or other established retrograde filling materials.

Comparison with MTA and contemporary bioceramics

Mineral Trioxide Aggregate (MTA) has long been the clinical standard for retrograde fillings due to its sealing ability and biocompatibility. However, well-documented limitations—such as difficult handling, extended setting time, and tooth discoloration—have motivated the development of alternative materials [3].

Modern premixed bioceramics, such as EndoSequence BC RRM, iRoot BP Plus, and TotalFill, were engineered to address these practical concerns. Across multiple studies, including bacterial and nutrient leakage models, these materials perform comparably to MTA regarding sealing efficacy [15, 16]. In the present study, C-Root BP showed reduced dye penetration and low glucose leakage throughout the experimental period. Although these findings are consistent with the favorable sealing behavior reported for other calcium silicate–based root-end materials in previous laboratory studies, no direct material-to-material comparison was performed here; therefore, comparative superiority cannot be inferred.

Interfacial adaptation and material-biological interaction

Apart from sealing leakage, adaptation at the dentin–material interface plays a critical role in long-term success. SEM analysis in this study revealed intimate contact of C-Root BP with dentinal walls, with no notable interfacial gaps. This finding echoes prior results for calcium silicate–based materials that form an interfacial hydroxyapatite-like layer, establishing chemical bonds with dentin and reducing microgaps [17]. The tight adaptation observed may also contribute to resistance against mechanical dislodgement or shrinkage when subjected to clinical stresses such as thermal cycling or occlusal load.

Long-term microleakage resistance

The glucose leakage model, featuring molecules smaller than bacteria, offers a stringent test of sealing integrity. The relatively low glucose concentrations observed in the C-Root BP group over the 120-day laboratory period suggest maintenance of barrier function within the conditions of the glucose leakage model. This observation should not be interpreted as demonstrating long-term clinical stability because thermocycling, mechanical loading, bacterial challenge and biological degradation were not evaluated. This performance mirrors robust outcomes found for BC RRM in similar extended assessments [15]. Notably, sustained low permeability suggests that C-Root BP could effectively block the ingress of microbial byproducts and inflammatory mediators, which are often significantly smaller than glucose in size.

Biological compatibility as a complementary advantage

Sealing performance alone does not guarantee clinical success. Material biocompatibility, especially the ability to support tissue healing and regeneration, is equally crucial [8, 9, 18, 19]. Taken together, the present findings suggest that C-Root BP is capable of establishing a favorable apical barrier under the specific in vitro conditions applied in this study. The present study focused on the sealing performance of C-Root BP under in vitro conditions. No direct conclusions regarding its biological compatibility can be drawn from the current findings, and dedicated biocompatibility studies are required to evaluate its biological behavior in endodontic microsurgery. Alternative methodologies for assessing microleakage have been described in the literature. Kielbassa et al. [19] introduced the high-pressure replica technique, which evaluates leakage by forcing a tracer medium under controlled pressure conditions and subsequently analyzing penetration patterns. This method is considered highly sensitive, as it simulates pressure-driven fluid movement that may better approximate clinical conditions compared to static models. In comparison, the dye penetration method used in the present study provides direct visualization of leakage pathways but lacks dynamic pressure simulation. The glucose leakage model, although more sensitive due to the small molecular size of glucose and its ability to assess leakage over time, does not

replicate the pressure gradients encountered in vivo. While the high-pressure replica technique offers enhanced sensitivity and physiological relevance, it requires specialized equipment and complex specimen handling, which may limit its routine application. Therefore, the combination of dye penetration, glucose leakage, and SEM-based interfacial assessment used in this study represents a practical and widely accepted approach for evaluating sealing performance, although it should be interpreted within the limitations of in vitro methodologies.

Limitations

This study has several important limitations. First, no established retrograde filling material, such as MTA or a contemporary bioceramic putty, was included as a material comparator. Consequently, the findings cannot establish equivalence, non-inferiority or superiority of C-Root BP relative to currently used materials. The positive and negative controls were included primarily to verify the functioning of the leakage models.

Second, no a priori sample-size calculation was performed. Although 50 teeth were included overall, each leakage assay was analysed separately using 15 C-Root BP specimens and only five specimens in each control subgroup. These individual comparisons were therefore not formally powered to detect small or moderate effects. The assumed effect size of ($f=0.40$) represented a conventional large-effect threshold and was not derived from C-Root BP-specific pilot or published data. In particular, failure to detect a difference between C-Root BP and the negative control should not be interpreted as evidence of equivalence.

Third, SEM assessment was qualitative and did not include a predefined scoring system, blinded quantitative gap measurement or image-based morphometric analysis. Although all included specimens were examined and the presented image represents the predominant observed pattern, only one representative micrograph is shown.

Fourth, numerical leakage measurements were generated using laboratory dye-penetration and glucose-filtration models that do not reproduce the full biological and mechanical environment of endodontic microsurgery. Thermocycling, mechanical loading, bacterial penetration, fluid filtration and pressure conditions representative of the clinical environment were not included. Finally, the 120-day glucose leakage findings represent performance under sustained laboratory hydrostatic pressure and should not be directly extrapolated to long-term clinical outcomes.

Conclusion

Within the limitations of this preliminary in vitro study, C-Root BP limited dye and glucose leakage and demonstrated a predominantly continuous interface with apical dentin under the applied laboratory conditions. These findings provide preliminary evidence of its barrier performance but do not establish equivalence or superiority relative to MTA or other established retrograde root-end filling materials. Comparative studies with adequately powered groups, quantitative interfacial assessment and clinically relevant ageing and leakage models are required.

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

Declarations

Competing interests The authors declare no competing interests.

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