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Assessing Sealing Ability of C-Root SP Strontium Silicate Sealer With Different Obturation Techniques: An in vitro Study

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ABSTRACT

Objectives: To evaluate sealing ability of C-Root SP strontium silicate sealer combined with 3 different obturation techniques in the root canal treatment of single-canal teeth.
Methods: 192 human single-canal permanent teeth were decoronated at the cementoenamel junction to standardize 10 mm length, which were then randomly divided into 4 groups (n = 43/group): Group A (C-Root SP+single-cone technique, SC), Group B (C-Root SP +warm vertical condensation, WVC), Group C (C-Root SP+cold lateral condensation, CLC) and Group D (iRoot SP+SC, as control), with additional positive/negative controls (n = 10 each). Following root canal preparation and technique-specific obturation with bioceramic sealers and gutta-percha, the samples were incubated (7 days, 37°C, 100% humidity) for sealer setting. Assessment techniques included micro-CT based void quantification in the apical (0-3.5 mm), middle (3.5-7 mm) and coronal (7-10.5 mm) regions (n = 5/group); SEM of material–dentin interfaces (n = 3/group); dye penetration via 7-day 1% methylene blue immersion with stereomicroscopic leakage measurement (n = 20/group); and 28-day glucose leakage monitoring with spectrophotometric analysis (n = 15/group).
Results: Micro-CT revealed no significant differences in void volume between groups across all regions ($P > .05$). SEM showed minimal gaps only in Group C (CLC), with good adaptation in other groups. Dye leakage was similarly low in all experimental and control groups (A: 0.58 ± 0.10 mm; B: 0.62 ± 0.11 mm; C: 0.62 ± 0.10 mm; D: 0.60 ± 0.13 mm), significantly less than positive controls (5.99 ± 0.32 mm; $P < .05$). Glucose leakage increased over time but remained significantly lower than in positive controls, with no statistically significant differences observed among the experimental and control groups.
Conclusion: C-Root SP demonstrated a sealing ability equivalent to that of iRoot SP across all the tested obturation techniques (SC, WVC and CLC), indicating that the substitution of components (strontium silicate for calcium silicate) does not compromise performance. However, these in vitro findings do not reflect long-term sealing performance, further in vivo studies and prolonged evaluations are still warranted.

Introduction

The goal of nonsurgical root canal treatment (NSRCT) is to completely clear the root canal space and achieve complete and hermetic 3-dimensional obturation[1] to prevent microbial reinfection. At present, the combined application of gutta-percha and different types of endodontic sealers to seal the

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whole root canal system, including the accessory canals, canal irregularities and minor discrepancies,[2] and obturation techniques such as the cold lateral condensation (CLC) technique, warm vertical compaction (WVC) technique and single-cone (SC) technique have been reported.[3] However, microleakage is still among the main reasons for the failure of nonsurgical root canal treatment (NSRCT),[4] and none of the techniques or materials have had a perfect effect on the production of this type of sealant.[1,5]

In recent years, bioceramic-based sealers, which are tricalcium silicate sealers, have been developed and introduced into clinical root canal filling operations.[6] Some in vitro studies have shown that they have a suitable setting time and good sealing ability, antibacterial activity, bioactivity and biocompatibility.[2,7-9] This kind of material was designed as a premixed single injection for clinical use, and its excellent characteristics have made its clinical application quite satisfactory.

C-Root SP was recently developed by Beijing C-Root Dental Medical Devices Co. Ltd. (Beijing, China) and is a type of bioceramic-based sealer that uses a strontium silicate-based setting system. Our preliminary clinical study confirmed the efficacy of C-Root SP when it is combined with different obturation techniques in root canal treatment for elderly patients.[10] Only 2 in vitro studies have reported that compared with AH-Plus (Dentsply International Inc., York, PA, USA), C-Root SP has quite fair marginal adaptation with the WVC or SC technique,[11] is highly biocompatible in terms of cell proliferation and exhibits superior osteogenic potential.[12] Additionally, C-Root SP is less cytotoxic than iRoot SP (Innovative BioCeramix Inc., Vancouver, Canada) is, and after setting, the lower concentration of C-Root SP promotes the cell migration ability of stem cells derived from rat apical papilla (rSCAPs).[13] These factors make it a promising alternative for root canal obturation. Given the lack of relevant research reports, we evaluated the sealing ability of C-Root SP in combination with 3 kinds of obturation techniques in the root canal treatment of single-canal teeth by using micro-computed tomography (micro-CT) evaluation, scanning electron microscopy (SEM) analysis, dye penetrant examination and glucose leakage tests, and iRoot SP, a calcium silicate-based sealer combined with SC, was used as a control. The composition of these 2 premixed bioceramic-based sealers is shown in Table 1. The null hypothesis of this study was that there would be no significant difference in the sealing ability between C-Root SP and iRoot SP sealers when used with different obturation techniques. The aim of this study is to provide evidence for the clinical application of C-Root SP combined with different obturation techniques.

Materials and methods

Tooth collection

The primary objective of this study was to address a clinical question formulated using the PICOT framework: In extracted human single-canal permanent teeth (P), how does obturation using C-Root SP combined with different techniques (SC, WVC and CLC) (I) compare to iRoot SP with the SC technique (C) in terms of sealing ability (O) when evaluated over a 7- to 28-day period (T)? This study was conducted according to the principles of the Declaration of Helsinki (10th version, October 2013) (www.wma.net, accessed on 18 March 2023) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and the modified CONSORT checklist.[14] The protocol was approved by the Ethics Committee of Chinese PLA General Hospital (No. S2023-141-01).

Human single-rooted permanent teeth, extracted for orthodontic and periodontal reasons, were collected from from the Department of Stomatology of the First Medical Center of Chinese PLA General Hospital. The initial inclusion criteria were teeth with fully developed apices and a single root canal. Exclusion criteria comprised teeth with: (1) previous root canal treatment; (2) root canal curvature greater than 25°; (3) visible fractures or cracks; (4) internal or external root resorption; (5) calcified canals; or (6) root caries.[7,15-17] To confirm anatomical homogeneity and adherence to the criteria, all potential samples underwent a standardized radiological screening protocol. This included periapical radiographs taken from both bucco-lingual and mesio-distal angles to verify the absence of any anomalies about the root canal morphology and curvature. Furthermore, cone beam computed tomography (CBCT) with a voxel size of 0.3 mm (110 kV; 5.0 mA; NewTom VGi, Verona, Italy) was utilized to 3-dimensionally assess the root canal morphology, curvature, and rule out fine cracks or resorptions that might not be visible on 2D radiographs. Only teeth that passed this rigorous screening process were included in the final study cohort.[18] After the tissue residue and calculus of all sample surfaces were removed, the samples were immersed in 5.25% sodium hypochlorite solution and disinfected for 1 h and then rinsed with water and stored in sterile 0.9% saline at room temperature until the experiment.[19]

Sample size determination

The minimum sample size required for each test per group was determined using G*Power 3.1 software (Heinrich Heine University, Düsseldorf, Germany) based on parameters from

Table 1 – Composition of the premixed bioceramic-based sealers.

Sealers	Manufacturer	Lot number	Composition
C-Root SP	C-Root Dental Medical Devices Co. Ltd. Beijing, China	20230403	Strontium silicate, calcium phosphate, calcium hydroxide, zirconium dioxide, fillers and thickening agents
iRoot SP	Innovative BioCeramix Inc., Vancouver, Canada	23001SP	Tricalcium silicates, dicalcium silicates, calcium phosphate monobasic, calcium hydroxide, zirconium oxide, fillers, and thickening agents

previous research (α = 0.05, power = 80%). The final allocation was as follows: micro-CT evaluation for void quantification (n = 5/group), SEM analysis of material-dentin interfaces (n = 3/group), dye penetrant examination using 1% methylene blue (n = 20/group), and glucose leakage monitoring via spectrophotometric analysis (n = 15/group).[4,7,20,21] With additional positive and negative controls (n = 10 each) for dye penetrant examination and glucose leakage test.[22]

Sample preparation and randomization

The crowns of all the tooth samples were removed perpendicular to their longitudinal axis at the cementum–enamel junction by using a thin diamond disc and a high-speed handpiece with a water coolant to retain at least 10 mm of root length.[19] The random allocation sequence was generated by an independent researcher using a computerized random number generator with a randomized block design. This researcher was not involved in the subsequent allocation or experimental procedures. The assignment of the prepared teeth to the 4 experimental groups was then carried out by a second investigator according to this predetermined list, which was implemented using sequentially numbered, opaque, sealed envelopes to conceal allocation. Due to the in vitro interventional nature of this study, the operators who performed the root canal obturation could not be blinded to the group allocation after the assignment was made. Through this method, all samples were randomly selected and evenly allocated into 4 groups (n = 43/group): Group A (C-Root SP combined with the SC technique), Group B (C-Root SP combined with the WVC technique), Group C (C-Root SP combined with the CLC technique) and Group D (iRoot SP combined with the SC technique; the control group). The remaining 20 samples were further randomly and equally divided into positive and negative control groups (n = 10 each). Due to the in vitro interventional nature of this study, the operators could not be blinded to the group allocation during the experimental procedures.

Root canal preparation

All samples underwent root canal preparation under a microscope (OPMI Sensera; Carl Zeiss, Germany). Working length (WL) was determined by subtracting 0.5 mm from the length of a #15 ISO stainless steel hand file (K-reamers, MANI, Japan) until the tip could barely be seen at the apex.[23]

For each group, apical preparation was performed using ISO stainless steel hand files (#10–15, K-reamers, MANI, Japan), starting with the selection of the first file to bind at the WL. Afterwards, rotary files (ProTaper Next, Dentsply Maillefer, Switzerland) with an endodontic motor (X-Smart Plus, Dentsply Maillefer, Switzerland) were used for mechanical preparation according to the manufacturer's recommendations (speed of 200 rpm and torque of 2 N·cm). In the presence of EDTA gel, the rotary files were inserted into the canal up to the full WL in the following sequence: X1 (#17, taper of 0.04), X2 (#25, taper of 0.06), X3 (#30, taper of 0.07), X4 (#40, taper of 0.06) and X5 (#50, taper of 0.06). All the root canals were shaped with at least 3 files and at most 5 files according to the size of the canal, which was determined by the operator.[5]

The canals were extensively irrigated using 5.25% NaOCl (5 mL for 1 min), and an equal volume of irrigant was administered to each tooth (5 mL applied prior to instrumentation, 5 mL between files and a final 5 mL flush following root canal instrumentation). The canals were subsequently dried using sterile paper points (Dayading Medical Appliance Co., China).

Root canal obturation

The gutta-percha (GP) master cone was selected and trimmed using a blade to fit snugly in the apical root canal and elicit a 'tug back' phenomenon before obturation.

The teeth in Groups A and D were filled with bioceramic-based sealers, namely, C-Root SP and iRoot SP, respectively, by injecting the sealer into the coronal third of each canal. A Lentulo spiral (Paste Carriers, MANI, Japan) with a size of 25 that was coated with an additional sealer was introduced 3 mm short of the WL at 300 rpm. Afterwards, a 0.06-taper GP (Dayading Medical Appliance Co., China) was dipped in the corresponding bioceramic-based sealer and introduced into the canal to the WL. A heated plugger size of 30/.04, 35/.04 or 40/.04 (Alpha unit, B&L Biotech USA Inc., USA) was used to sear the GP point at each orifice.

The samples in Group B were filled with 0.06-taper GP (Dayading Medical Appliance Co., China) and C-Root SP was introduced using the master cone. A heated plugger (Alpha unit, B&L Biotech, USA, Inc., USA) was inserted 3 to 5 mm short of the WL, and the remaining canal space was backfilled with additional sealer and thermoplasticized GP using a beta unit (B&L Biotech, USA, Inc., USA).

For Group C, the root canals were obturated using a 0.06-taper GP cone (Dayading Medical Appliance Co., China), which was selected according to the master's apical file. The cone was lightly coated with C-Root SP and placed into the canal up to the WL. Lateral compaction was performed using finger spreaders (MANI, Japan), which were 25 in size. The excess filling material was then removed using a heating instrument.

In the positive control group, the canals were filled with a GP master cone alone, whereas the sample teeth remained empty in the negative control group.

The coronal access cavities of all the samples were filled with glass ionomer cement (Fuji IX, GC America, USA) as a temporary restoration after obturation, after which they were incubated at 37°C and 100% humidity for 7 days until all the sealers were completely set. After all the samples were dried, the entire surface of the teeth of the experimental group, control group and positive control group, except the resection surface, were coated with 3 layers of nail polish, whereas the entire surface of the samples of the negative control group were coated similarly. Each layer of nail polish was applied at intervals of 1 h and then left at room temperature for 1 day to dry.[22]

All operations for root canal preparation and obturation were performed by the same operator with 10 years of experience in endodontics, who is proficient in the clinical application of various bioceramic-based sealers and different obturation techniques.

Micro-CT evaluation

Micro-CT evaluation of root canal filling density was immediately performed on 5 randomly selected samples per group (experimental and control). All the samples were fixed on a positioning stage and scanned using a micro-CT scanner (Xradia 515 Versa, ZEISS, Germany) with settings of 100 kVp, 100 mA, a 11 μm resolution, and a 0.5° rotation increment. CT-Analyser (CTAn) software (v1.18.4.0; Bruker micro-CT, Belgium) was used for image reconstruction and subsequent analysis (Figure 1A). Teeth were segmented for morphometric analysis into apical (0-3.5 mm), middle (3.5-7 mm) and coronal (7-10.5 mm) sections. Void volumes were quantified to calculate percentage void areas and classified as internal (within the sealer), external (at the sealer−dentin interface) or combined (incorporating both internal and external spaces), as shown in Figure 1B.[24]

SEM analysis

For SEM analysis, 3 samples were randomly chosen from both the experimental and control groups. All 12 teeth were immediately sectioned longitudinally along the root axis, sputter-coated with gold and then examined using a scanning electron microscope (Sigma 360, ZEISS, Oberkochen, Germany). The junction between the filling material and the inner dentinal wall was observed at magnifications ranging from 200 × to 400 × to evaluate the adaptation and penetration of the sealers along the root canal walls from the apical region to the coronal region.[25]

Dye penetrant examination

Twenty samples were randomly selected from each experimental group and control group, and 5 samples were randomly selected from the positive control group and negative control group separately. All the samples were processed in accordance with previously reported methods:[4,22] the samples were immersed in 1% methylene blue solution (Sigma–Aldrich, StLouis, MO) and incubated at 37°C and 100% humidity for 7 days, and the dye on the root surface was subsequently washed with distilled water. Afterwards, the sample teeth were cut longitudinally along the long axis of the root to observe the degree of dye penetration under a stereomicroscope (KEYENCE VHX-7000, Japan). Images of each root were taken with a millimetre ruler, and image processing software for Windows (ImageJ 1.52a; National Institutes of Health, USA) was used to measure the maximum linear depth of dye leakage along the apical boundaries of the teeth between the filling materials and the dentin wall.

Glucose leakage test

The glucose leakage model was constructed according to methods described previously.[22,26] The glass ionomer cements of all the remaining sample crowns of each group were removed to expose the filling sections, and each root was placed in a 5 ml disposable plastic tube (21 cm long) with a hole on the top and an appropriate hole on the lower side so that approximately 5 to 6 mm of the apical part of the root extended out; then, the joint was sealed with cyanoacrylate and sticky wax. After the sealants were fully cured, the plastic tube was inserted into a glass bottle containing 5 mL of distilled water (containing 0.5% sodium benzoate to inhibit the growth of decomposing glucose bacteria) to keep the root tips 2 to 3 mm below the liquid level and fixed with sticky wax and parafilm. A 27# syringe needle was inserted into the bottle stopper to connect the inside to the atmosphere. The plastic tube was injected with 1 mol/L glucose solution (containing 0.5% sodium benzoate) to maintain the liquid level at 15 cm and placed in an incubator at 37°C and 100% humidity. At 1, 7, 14, 21 and 28 days, 100 μL of solution was extracted from the glass bottle with a micropipette, and an equal amount of fresh 0.5% sodium benzoate was added to maintain a constant volume of 5 mL. Moreover, a certain amount of fresh glucose solution (containing 0.5% sodium benzoate) was added to the plastic tube to maintain a solution height of 15 cm. The glucose concentration of each sample (calculated as mmol/L) was determined using a glucose assay kit (Solarbio, Beijing, China) and a spectrophotometer at a wavelength of 505 nm.

Statistical analysis

All calculations were performed using IBM SPSS Statistics for Windows (version 26.0, IBM Corp. Released 2019). The Levene test and the Shapiro−Wilk test were used to test the variance homogeneity and distribution normality, respectively, of all the collected data. Since the data were not normally distributed, analysis by the Kruskal–Wallis test was adopted to determine whether there were significant differences in leakage among the groups. Pairwise comparisons were conducted using the Mann–Whitney test with Bonferroni correction to identify which group was significantly different. All levels of statistical significance were set at $\alpha = 0.05$.

Results

The porosities of representative 3D tooth model segments in each group are summarized in Table 2. Statistical analysis revealed minimal total void volumes and no significant differences between the experimental and control groups (Figure 1C, $P > .05$). Measurements and calculations were performed for the volume and proportion of internal, external, and connecting voids located in the apical, middle, and coronal thirds, respectively (Figure 1D-F). The findings indicate equivalent and high sealing efficacy across all groups ($P > .05$).

Longitudinal SEM revealed both the SC technique (Groups A and D) and the WVC method (Group B) demonstrated tight interfaces between the filling materials and root canal walls (Figure 2A-D, 2G and 2H). In contrast, minimal gaps among C-Root SP, gutta-percha, and root canal walls exclusively in the CLC method (Group C) (Figure 2E and 2F).

After 7 days of soaking and dyeing, the degree of dye leakage was observed in the root canals for all 3 experimental groups, the control group, and the positive control group (Figure 3A-E); the linear depths of dye leakage were 0.58 ± 0.10, 0.62 ± 0.11, 0.62 ± 0.10, 0.60 ± 0.13 and 5.99 ± 0.32 mm,

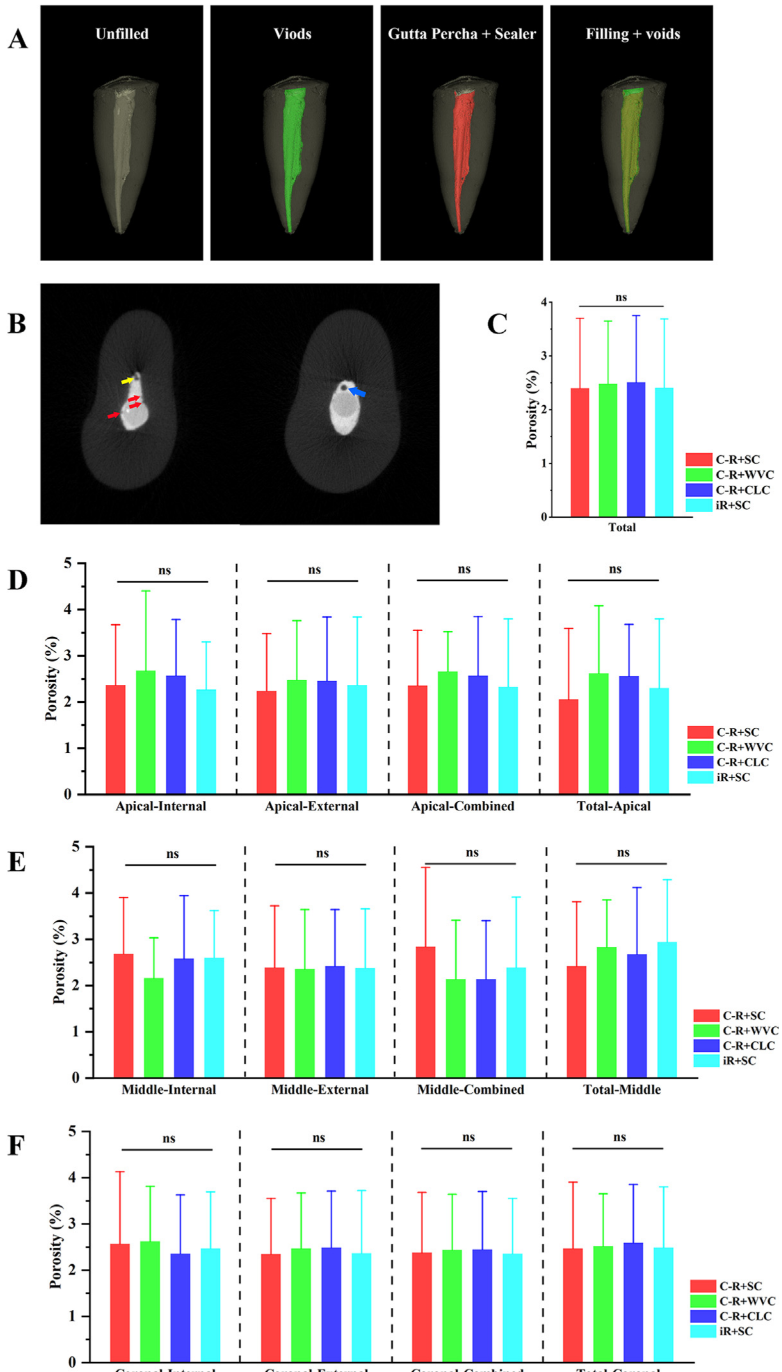

Fig. 1 – Micro-CT evaluation of porosity within representative sample segments from each group. A, 3D reconstructions of isolated teeth; B, sagittal cross-sections with void characterization, where the blue arrow indicates internal voids (within the sealer), the red arrow indicates external voids (at the sealer–dentin interface) and the yellow arrow indicates combined voids (incorporating both internal and external spaces); C, total porosity quantification across groups; and D-F, quantitative assessment of the sealing capacity in the apical, middle, and coronal regions. Values are expressed as the mean ± standard deviation; ns: nonsignificant ($P > .05$).

respectively (Figure 3G), and there was no dye leakage in the root canal of the negative control group (Figure 3F). The comparison of multiple groups revealed statistical significance ($F = 1707.85$; $P < .001$), and the results of the pairwise comparison of groups revealed that there was no statistically significant difference in the linear depth of dye leakage between the 3 experimental groups and the control group ($F = 0.515$; $P > .05$). However, the linear depth of dye leakage in these 4 groups was significantly less than that in the positive control group ($P < .05$).

During the experimental period, the amount of glucose that leaked from the coronal aspect in each glucose leakage

Table 2 – Micro-CT analysis of porosity in each tooth model segment across different groups (mean ± SD, %).

Group	Apical third			Middle third			Coronal third			Total		
	Internal	External	Combined	Internal	External	Combined	Internal	External	Combined	Apical	Middle	Coronal
A	2.37 ± 1.30	2.24 ± 1.24	2.36 ± 1.19	2.06 ± 1.53	2.69 ± 1.21	2.39 ± 1.33	2.84 ± 1.71	2.42 ± 1.39	2.57 ± 1.56	2.35 ± 1.20	2.38 ± 1.30	2.47 ± 1.43
B	2.68 ± 1.72	2.48 ± 1.28	2.66 ± 0.86	2.62 ± 1.46	2.16 ± 0.87	2.36 ± 1.28	2.14 ± 1.27	2.83 ± 1.02	2.62 ± 1.19	2.47 ± 1.20	2.44 ± 1.20	2.52 ± 1.13
C	2.57 ± 1.21	2.46 ± 1.38	2.57 ± 1.28	2.56 ± 1.12	2.58 ± 1.36	2.42 ± 1.22	2.14 ± 1.26	2.68 ± 1.44	2.36 ± 1.27	2.49 ± 1.22	2.45 ± 1.25	2.59 ± 1.26
D	2.27 ± 1.03	2.37 ± 1.47	2.33 ± 1.47	2.30 ± 1.50	2.60 ± 1.02	2.38 ± 1.28	2.39 ± 1.52	2.94 ± 1.35	2.47 ± 1.22	2.37 ± 1.35	2.36 ± 1.19	2.49 ± 1.31

Note: A: C-R combined with SC; B: C-R combined with WVC; C: C-R combined with CLC; and D: iR combined with SC.
C-R, C-Root SP; iR, iRoot SP; SC, single-cone; WVC, warm vertical compaction; and CLC, cold lateral condensation.

model of the 3 experimental groups, control group, and positive control group increased with time (Figure 4A-B). The positive control group exhibited the greatest increase in glucose leakage at all time intervals, whereas no glucose leakage was detected in the negative control group throughout the experiment, indicating the excellent sealing integrity of the device. While glucose leakage increased over time in all groups, no statistically significant differences in the sealing ability of the root canal filling materials were observed among the applied pulp cavity filling methods ($P > .05$), as shown in Figure 4C.

Discussion

Based on the comprehensive results from micro-CT, SEM, dye leakage, and glucose leakage tests, this study accepts the null hypothesis. No statistically significant differences were found in the sealing ability between C-Root SP and iRoot SP sealers when used with different obturation techniques. Several studies using micro-CT to compare different obturation techniques with respect to void occurrence in canals filled with the same bioceramic sealers found that their voids were minimal and prevalently external.[27-29] Especially when using high-flowability sealers, the void volume was lower at different canal depths,[20] which aligns with the conclusions drawn from our study. Meanwhile, our SEM results showed that C-Root SP combined with the SC and WVC technique exhibited good interfacial adaptability, which is similar to previous test results on other bioceramic sealers.[30-32] Similarly, our tests on the coronal and apical penetration of different obturation techniques with C-Root SP also corresponded to the findings reported in studies using dye penetration examination[15,18,32] and glucose leakage tests.[21,33]

Strontium-containing compounds are used in dentin desensitization and caries prevention in stomatology[34-36] and exert their analgesic effects by inhibiting the release of inflammatory cytokines.[37] In addition, studies have shown that strontium can replace calcium in bioactive materials without adverse local or systemic effects[38] and that it enhances the differentiation, physicochemical properties, and bioactivity of stem cells.[39] Therefore, C-Root SP, which uses strontium silicate instead of calcium silicate, is considered a kind of bioceramic-based sealer with potential application advantages. The results of our study revealed that the SC techniques with C-Root SP and iRoot SP were similar in terms of sealing ability and achieved sealing high on both the crown and root sides, indicating that the replacement of calcium silicate does not affect the final sealing ability of C-Root SP.

The proposed modified list of criteria contains 12 items for endodontic sealers summarized by Takashi et al., among which certain adhesion, appropriate flowability, dimensional stability, suitable setting time and insolubility were the direct factors that affected the final sealing properties of the sealers.[2] The design of premixed and injectable moulds, such as C-Root SP and iRoot SP, not only is convenient for clinical application but also improves the sealing effect of the materials on difficult-to-access areas, such as the isthmus and accessory canals, because of its good flowability.[40] Root canal sealer adhesion or bonding is another influencing factor for flow,[41] and the solidification mechanism of C-Root SP is

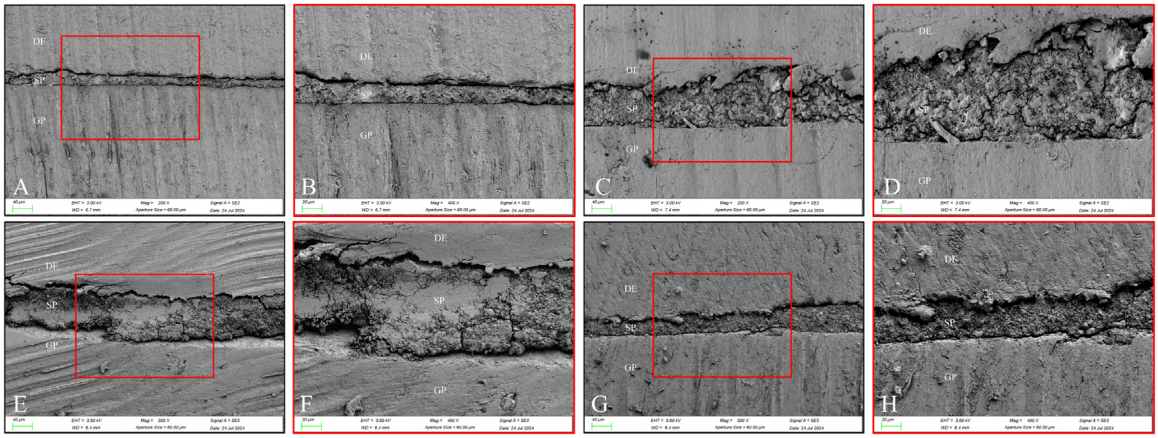

Fig. 2 – Representative SEM images of longitudinal sections. A, C-R combined with SC; C, C-R combined with WVC; E. C-R combined with CLC; and G. iR combined with SC. B, D, F, are H are high-magnification images of the red-framed areas in A, C, E and G, respectively. C-R, C-Root SP; iR, iRoot SP; SC, single-cone; WVC, warm vertical compaction; CLC, cold lateral condensation; DE, dentin; and GP, gutta-percha. SP, C-Root SP in A–F and iRoot SP in G–H.

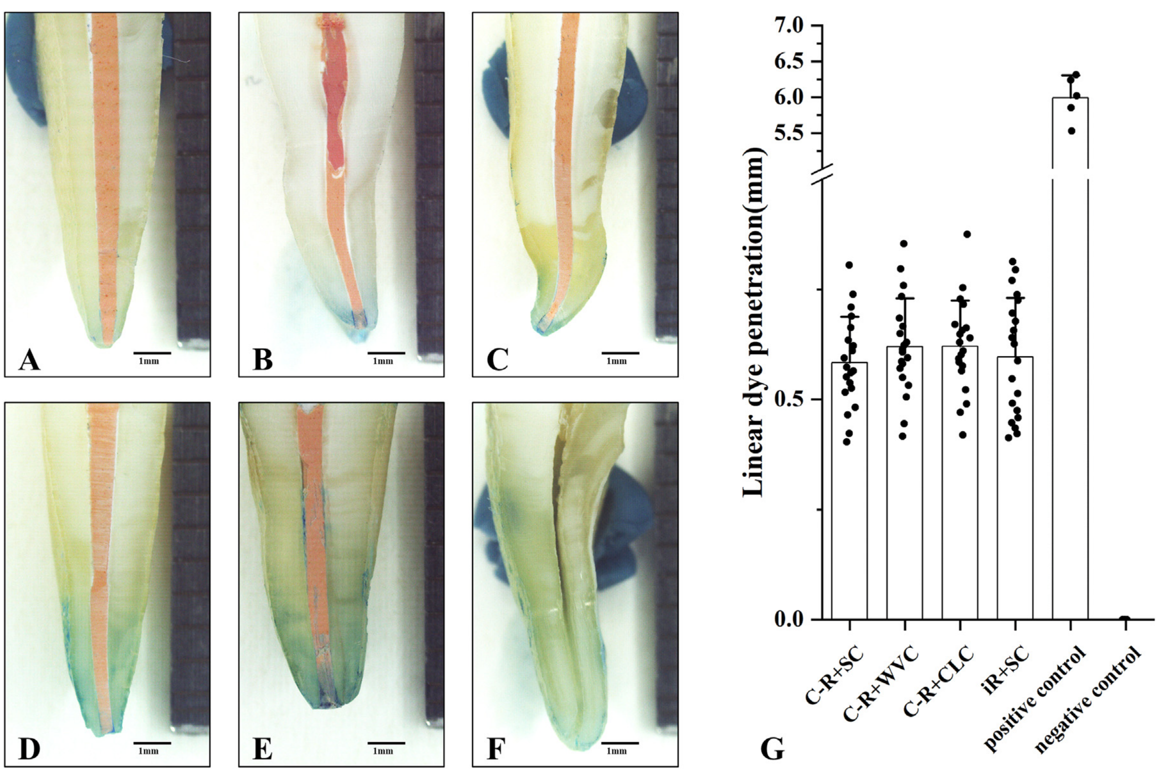

Fig. 3 – Representative images of dye penetration observed under a stereomicroscope. A, C-R combined with SC; B, C-R combined with WVC; C, C-R combined with CLC; D, iR combined with SC; E, positive control; F, negative control; and G, comparison of the maximum linear depth of dye leakage in each group. The data are presented as the mean and standard deviation. C-R, C-Root SP; iR, iRoot SP; SC, single-cone; WVC, warm vertical compaction; and CLC, cold lateral condensation.

similar to that of iRoot SP, which absorbs water from dentinal tubules, releases calcium, strontium and hydroxide ions, and then forms hydroxyapatite (HA) on the inner surface of root canals in an alkaline environment, resulting in bonding.[42,43] Additionally, the slight expansion during the process of setting[44] and the dimensional stability and insolubility after setting further ensure the sealing effect of the materials.[11,45]

The obturation technique could be another factor for achieving complete sealing, which may affect the long-term effects of the root canal system and each obturation technique has its own operational characteristics and technical advantages; thus, each technique should be proposed and advocated.[46] Among them, the CLC and SC techniques have a relatively low cost, are easy to perform and have a short

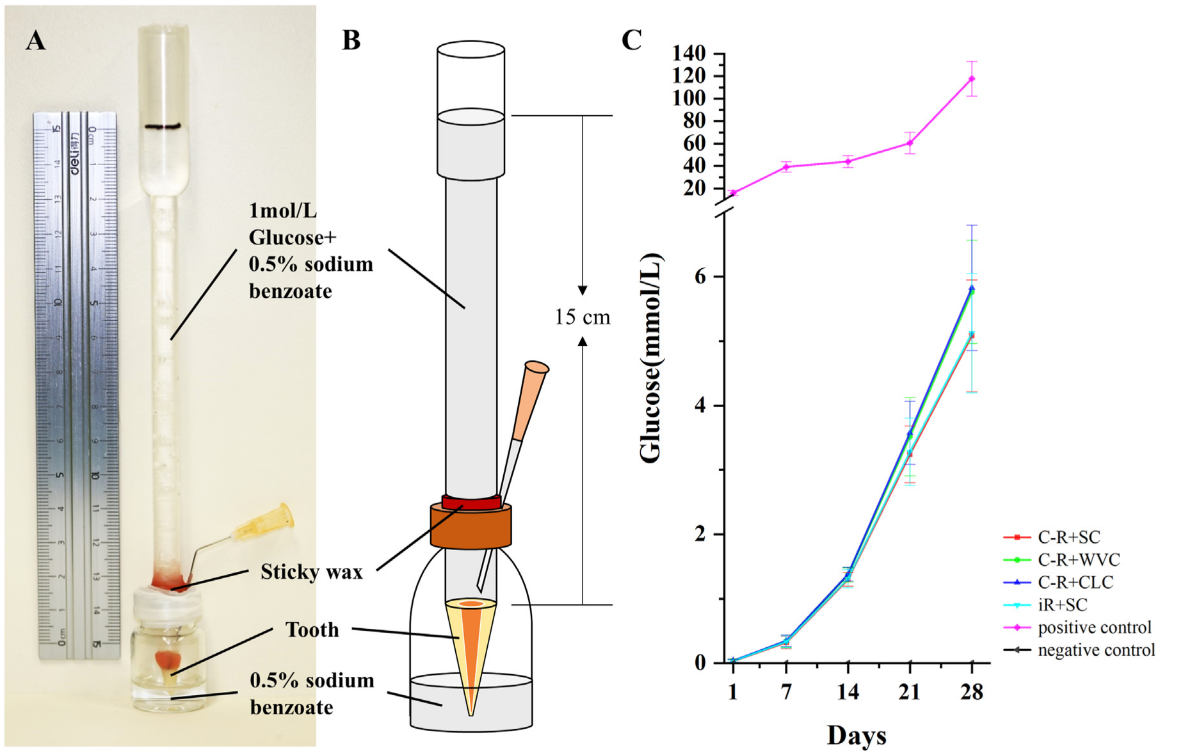

Fig. 4 – A-B, Physical diagram and schematic diagram of the glucose leakage model. C, Glucose leakage concentrations for 28 days. The data are presented as the mean and standard deviation. C-R, C-Root SP; iR, iRoot SP; SC, single-cone; WVC, warm vertical compaction; and CLC, cold lateral condensation.

working time, but gaps and voids may appear when they are applied in irregular root canal systems;[47] however, although the filling effect of the WVC technique could be more adequate and uniform, the technical sensitivity might be greater, and thermal damage and extrusion could occur.[48,49] Some clinical control studies, systematic reviews and meta-analyses have shown that the clinical and radiographic success rates of the CLC, WVC and SC techniques with bioceramic-based sealers are similar.[5,50,51] However, the corresponding in vitro studies suggest that compared with the CLC and SC techniques, the WVC technique can result in a greater percentage of obturation volume (POV) and less leakage.[3,52] In the present study, high sealing ability with no significant differences was observed among the combinations of C-Root SP with SC, CLC and WVC techniques, suggesting compatibility of this sealer across various obturation techniques. Among these 3 commonly used obturation techniques, the SC technique has the least technical sensitivity; thus, the use of root canal sealers with good physical and chemical properties is very important for fully filling the root canal system.[47] The operational goal of the CLC technique is to create an apical space for insertion of the accessory GP cones after the master GP cone reaches the WL until the canal is filled; if the preparation is poor, inadequate pressure is applied to the spreaders or a mismatch of tapers occurs between the spreaders or the GP cone and the canal walls and gaps and voids may appear and affect the final sealing process.[53,54] However, the good flowability and dimensional stability of the sealing material could partly compensate for the technical deficiency to achieve a good filling effect.[55] In addition, the lubrication of root canal sealers is helpful for the positioning of the filling materials, but owing to the larger particles of bioceramic-based sealers, which is described in the literature,[1] greater vertical friction resistance was felt by the operator when master and accessory GP cones or spreaders entered the root canal, which is not conducive to obtaining the ideal depth for GP cones and instruments and may lead to more microleakages and microgaps; however, the WVC and SC techniques were almost unaffected by this vertical friction resistance. Moreover, some studies have reported that heating operations involving obturation techniques may affect the physical and chemical properties of sealers (such as flowability and setting time) and thus may impact the final sealing effect;[56-59] however, other studies have reported that the chemical composition and bonding strength of some high-flowability bioceramic-based sealers are not affected by heat-based obturation techniques.[44,60,61] Although our study did not directly test the changes in flowability and setting time of C-Root SP after the heating operation, the lack of a significant difference in the sealing test results may suggest that the sealing ability of this new strontium silicate-based sealer, which has greater flowability because of its lower viscosity,[62] was not affected by the heating operation; however, the effect of heating on relevant performance indicators should still be tested to provide a reliable basis. This equivalence affirms the application flexibility of C-Root SP when combined with different obturation techniques. It provides a basis for the clinical use of the less technically sensitive and faster single-cone technique. This approach holds the potential to reduce chairside time and improve patient acceptance.

Several experimental approaches, such as dye penetrant examination, glucose leakage tests, bacterial leakage tests, SEM, CBCT and micro-CT evaluation, have been commonly applied to investigate and evaluate the sealing ability of different types of sealers and obturation techniques, while radioactive isotope ^{99}mTc penetrant examination, electrochemical techniques and fluid filtration have been applied at the same time.[3,16,22] Dye penetration examination is the most commonly used and simple technique, and methylene blue staining is often used for clinical root examination. However, the test results may be affected by the type of dye, dyeing time and residual voids in the sample,[63] and the staining of a single section in a single direction cannot fully represent the whole boundary of dye leakage.[64] Glucose is a commonly used clinical detection index and leakage tracer because of its small molecular size and high detection sensitivity, and glucose penetration could be a simple method for obtaining reliable quantitative leakage measurements.[65] Additionally, compared with other tracers, glucose could be more clinically relevant as a bacterial nutrient, which may lead to periapical inflammation if microleakage and bacteria are present in the root canal system of patients.[33] Nevertheless, this approach has several limitations, such as potential reactions with filling materials, difficulties in aseptic maintenance and the risk of water loss.[66] Directly determining the leakage of bacteria and their metabolites, which are the main pathogenic factors of periapical diseases, is a method used to evaluate the sealing effect of root canals, and *E. faecalis* is commonly used for detection in bacterial leakage models because it is the major pathogen in refractory apical periodontitis.[67,68] However, the antibacterial properties of endodontic sealing materials may be confounders that affect the final results.[69] SEM enables high-resolution imaging of samples and visualization of material adaptation to dentinal walls but remains limited by its requirement for irreversible sample sections.[70] CBCT and micro-CT have been used to evaluate root canal filling voidage and can provide 3-dimensional imaging and quantitative analyses;[3,22,71] however, quantitative analyses depend on the scanning parameters and threshold selection, and the artefacts of the obturation materials may also affect the accuracy of the results.[72] Therefore, the conclusions obtained from the studies designed with a single method are not necessarily reliable. In the present study, the limitations of these technologies must be evaluated and compensated for through different models and methods to obtain reliable conclusions.[73] The synergistic application of different testing methods in this study provides a clinically relevant, multidimensional evaluation of the root canal seal. They simulate various clinical challenges, from bulk void formation to resistance against bacterial nutrient penetration, thereby reducing the risks associated with clinical translation. Furthermore, the consistent final results across all methods provide reliable evidence for the clinical application of C-Root SP and justify the rationale for advancing to clinical trials.

It is important to acknowledge the limitations of this in vitro study. Firstly, these conclusions do not represent the long-term sealing ability of C-Root SP, and there are few reports on the long-term sealing effect of bioceramic-based sealers at present.[74] Secondly, in vitro studies might differ from the actual clinical situation; for example, leakage tracers with smaller molecular sizes may lead to excessive sensitivity.[16,19] Therefore, the findings should be interpreted with caution, and more studies focusing on the long-term sealing effects are warranted. Future research should prioritize well-designed clinical trials to validate these promising in vitro results under real-world clinical conditions. Additionally, further investigations into the long-term chemical stability, antibacterial efficacy, and biocompatibility of C-Root SP in vivo are essential to comprehensively assess its clinical value. Exploring its performance in more complex root canal anatomies and its interaction with other restorative materials would also be of significant clinical relevance.

Conclusion

To the best of our knowledge, this is the first in vitro examination performed to evaluate the sealing ability of C-Root SP combined with 3 commonly used obturation techniques. Despite the limitations of this study, the substitution of strontium silicate with calcium silicate in C-Root SP did not affect its sealing ability, so it can be used as a bioceramic-based sealer with potential clinical application value. C-Root SP combined with different obturation techniques presented a similar sealing ability and sealed both the crown and root side of extracted human tooth samples well, similar to iRoot SP combined with the SC technique.

Informed consent

Informed consent was obtained from the patients who had teeth extracted for orthodontic reasons or severe periodontal disease.

Author contributions

F.C.: conceptualization, methodology, project administration, funding acquisition. S.H., J.L. and M.X.: performed all the experimental procedures, data curation and drafting and writing of the manuscript. L.X., Y.Y. and P.X.: sample testing and data collection. L.D., L.W. and H.H.: data analysis. Q.L. and Y.L.: writing, review & editing and supervision. All the authors contributed to the study conception and design and read and approved the final manuscript.

Ethics approval

Ethical approval for this study was obtained from the Ethics Committee of the First Medical Center of PLA General Hospital (No. S2023-141-01).

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Declaration of competing interest

The authors declare that they have no competing interests.

Availability of trial protocol

The full study protocol is available from the corresponding authors upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.identj.2025.109283.

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