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Superalkaline reductive solution versus sodium hypochlorite for root canal irrigation: A comparative in vitro study

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Abstract

Objective: To evaluate the effectiveness of a Superalkaline reductive solution (SRS) as a root canal irrigant in terms of smear layer removal, antibacterial activity, cytotoxicity, and dentinal tubule permeability.

Methods: Forty-five single-rooted teeth were instrumented and irrigated with 3% NaOCl, SRS, and 0.9% NaCl, each combined with EDTA and PUI. Smear layer removal was assessed using Scanning electron microscopy (SEM) with scoring and ImageJ. Antibacterial efficacy against *Enterococcus faecalis* was evaluated in sixty single-rooted teeth using colony-forming units (CFU) analysis and SEM. Cytotoxicity was tested on L929 cells. Dentinal tubule permeability was assessed in thirty-six single-rooted teeth using confocal laser scanning microscopy (CLSM)and ImageJ.

Results: Based on scoring system evaluation, SRS group was superior to 3% NaOCl and 0.9% NaCl groups in smear layer removal across all root thirds ($P < 0.05$). In the middle and apical thirds, the SRS group exhibited the highest dentinal tubule opening rate among the three groups, followed by the 3% NaOCl group($P < 0.05$).For dentinal tubule area percentage, the SRS group showed superiority in the apical third($P < 0.05$). SRS showed slightly lower efficacy against *Enterococcus faecalis* than 3% NaOCl. 3% NaOCl exhibited severe cytotoxicity, whereas SRS showed no toxicity. For dentinal tubule permeability at 3 mm, the SRS group showed superior ($P < 0.05$).

Conclusion: SRS showed superior smear layer removal, dentinal tubule permeability, and biocompatibility.

Clinical significance: Evaluating the smear layer removal, antibacterial activity, cytotoxicity, and dentinal tubule permeability of SRS is essential to ensure its efficacy and safety for clinical application.

1. Introduction

Root canal treatment constitutes the primary therapeutic strategy for pulpal and periapical diseases, with root canal preparation representing a critical determinant of treatment outcome. [1, 2]. Root canal preparation includes both mechanical and chemical components. Mechanical preparation alone is insufficient to achieve ideal cleaning [3, 4]. After biomechanical preparation, residual pulp tissue, bacteria, and dentin debris may persist in irregular areas of the root canal system. Even after meticulous mechanical preparation, these remnants may cover the canal walls or become lodged in dentinal tubules, and may remain in regions such as isthmuses and apical ramifications [5, 6]; moreover, the smear layer often remains unresolved. In addition,the presence of bacteria and their byproducts, such as *Enterococcus faecalis* (*E. faecalis*), is well recognized in the development of pulpal and periapical lesions [7]. *E. faecalis* is associated with persistent infection [8], and inadequate elimination of this microorganism may compromise treatment outcomes. Studies have shown that bacteria can penetrate dentinal tubules to depths of 500–2000 μm [9]. One of the goals of root canal treatment is to eliminate microorganisms from the root canal system. However, due to anatomical complexities such as lateral canals, apical ramifications, and dentinal tubules, no instrumentation technique or irrigating solution can completely eliminate all microorganisms or their toxins. Current research aims to maximize the removal of these microorganisms and their toxins. Root canal irrigation, also known as chemical preparation, can clean areas that instruments cannot reach (35–50% of the root canal system) [10, 11], playing a crucial role in root canal cleaning and disinfection, and creating favorable conditions for subsequent three-dimensional obturation.

Therefore, root canal irrigants is essential in endodontic treatment.In recent years, several studies have focused on developing multifunctional irrigants. For instance, Ballal et al. evaluated Triton, which still exhibited cytotoxicity, whereas EndoJuice™ required NaOCl pretreatment. Neither irrigant achieved an optimal balance between safety and efficacy [12]. Therefore, in clinical practice, two types of irrigating solutions are often used alternately. The most frequently used combination is sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA). NaOCl has high-efficiency bactericidal and tissue-dissolving abilities [13, 14], while EDTA is effective in removing the inorganic portion of the smear layer [15]. The combination achieves favorable outcomes. Nevertheless, the drawbacks of NaOCl—including strong redox activity, high bleaching capacity, high corrosiveness, significant irritancy, and poor biosafety—cannot be ignored. Accidental extrusion of NaOCl beyond the apical foramen may lead to severe complications, such as nerve paralysis and jaw necrosis [16]. Studies have demonstrated that mixing NaOCl and EDTA produces toxic gases, posing a risk to human health. Furthermore, this interaction results in the loss of available chlorine from NaOCl, thereby compromising its tissue-dissolving ability and partially diminishing its antimicrobial activity. Consequently, the two solutions must be separated by normal saline and used alternately, which reduces clinical efficiency [17, 18].

Therefore, existing disinfectants currently face challenges in balancing effectiveness and safety, while the development and application of highly safe disinfectants has made limited progress in terms of efficacy. The present study introduced SRS, a superalkaline reductive solution, to evaluate its efficacy and safety as a root canal irrigant.SRS is a superalkaline, highly reductive stock solution prepared from ionized water through deoxygenation and electrolysis at the cathode of an electrolytic device. Composed of a high concentration of hydroxide ions (OH^-), SRS is colorless, odorless, and non-irritating. Previous studies have demonstrated that SRS can inactivate various enveloped and non-enveloped viruses, as well as Gram-positive and Gram-negative bacteria [19–22]. SRS exhibits extremely high bactericidal efficiency. Against *Porphyromonas gingivalis* and commensal *Actinomyces* species, SRS achieves a 99.99% kill rate within 15 seconds and complete inactivation within 30 seconds [23]. However, no detailed reports have been published regarding the application of SRS in root canal irrigation. Therefore, this study aims to evaluate the potential of SRS as a root canal irrigant by assessing its smear layer removal efficacy, antibacterial activity, and cytotoxicity when combined with EDTA and passive ultrasonic irrigation (PUI).

2. Materials and methods

2.1 Ethical clearance

The collection of human extracted teeth in this study was approved by the Ethics Committee of Hangzhou Stomatology Hospital (approval No. 2025HKLLSL15), and informed consent was obtained from all patients.

2.2 Human teeth collection

Mandibular single-rooted premolars extracted for orthodontic purposes were collected from Hangzhou Stomatology Hospital. The inclusion criteria were: intact teeth without cracks or caries, well-developed and intact apical foramen, no prior endodontic treatment, absence of internal resorption or calcification, patent root canals, and root curvature <10°. The selected teeth were stored in 0.1% thymol solution at 4°C for a maximum of 15 days.

2.3 Smear layer evaluation

2.3.1 Specimen preparation

Forty-five teeth were randomly divided into three groups (n = 15 per group). After standardizing the root length to 14 ± 1 mm, the crowns were removed using a high-speed handpiece under water cooling, and the pulps were extirpated. The root canals were negotiated with a #10 K-file. The working length was established, and the master apical file was determined. The apical foramen was sealed with 3M flowable resin. Root canal preparation was performed using M3 Pro files. Each time a file was withdrawn, the root canal was irrigated with the corresponding irrigating solution (0.9% NaCl, SRS, or 3% NaOCl). EDTA was used in conjunction with all irrigants. In the 3% NaOCl group, 3% NaOCl and EDTA were applied alternately, with sterile normal saline used as an intermediate rinse between them. Root canal preparation was carried out to the master apical file size, followed by final irrigation with passive ultrasonic irrigation (PUI) for 60 seconds. After longitudinal splitting, the specimens were fixed, dehydrated, dried, and gold-sputtered for SEM examination.

2.3.2 Scanning electron microscopy (SEM)

Images of the canal wall surface morphology were captured from three regions of each root: the coronal third (10–12 mm from the apex), the middle third (6–7 mm from the apex), and the apical third (1–2 mm from the apex). Three images were taken per region at a magnification of ×2000. Smear layer removal efficacy was evaluated by two independent observers blinded to the experimental groups, using the scoring criteria described by Hülsmann et al [24]. The score for each region was calculated as the mean value of the three images. The Hülsmann scoring criteria are defined as follows :

Score 1: Complete visibility of dentinal tubules with no smear layer.

Score 2: Partial visibility of dentinal tubules with minimal smear layer.

Score 3: Majority of dentinal tubules obscured by moderate smear layer.

Score 4: All dentinal tubules obscured by a homogeneous smear layer covering the entire canal surface.

Score 5: All dentinal tubules obscured by a heterogeneous, thick, irregular smear layer covering the entire canal surface.

ImageJ software was used to quantify dentinal tubule opening rates and the percentage of exposed dentinal tubule area.

2.4 Antimicrobial activity

2.4.1 Sample preparation

Sixty extracted single-rooted teeth were selected. After removing the crowns with a high-speed handpiece, the roots were standardized to a length of 14 ± 1 mm. Any residual calculus, alveolar bone, and soft tissue on the root surface were removed. Root canal preparation of the 60 teeth was performed using M3 Pro files up to a master apical file size of 2506 taper. During the entire preparation process, the root canals were irrigated with NaOCl alternately with ethEDTA. After preparation, two layers of nail varnish were applied to the surface of each tooth. The roots were then autoclaved at 121°C for 20 minutes and stored for subsequent use.

2.4.2 Inoculation with *Enterococcus faecalis*

Frozen *E. faecalis* (ATCC 29212, American Type Culture Collection) was thawed and inoculated into brain–heart infusion (BHI) broth, then cultured under microaerophilic conditions at 37°C for 24 hours. The cell suspension was adjusted to match the turbidity of 1.5 × 108 CFUs /ml. The root canals were then inoculated with the bacterial suspension. Each tooth was placed into a centrifuge tube containing the bacterial suspension and BHI broth, followed by incubation for six weeks. To maintain continuous bacterial growth, the culture medium was replaced every two days.

2.4.3 Biofilm evaluation

After six weeks, two teeth were randomly selected. Each tooth was split longitudinally. The specimens were then fixed in 2.5% glutaraldehyde, dehydrated in graded concentrations of ethanol, dried, sputter-coated with gold, and observed under SEM.

2.4.4 Pre-irrigation and post-irrigation sampling

Before root canal irrigation, 500 μL of sterile water was injected into each root canal. A #25 K-file was then gently used to scrape the root canal wall for 15 seconds. Sterile absorbent paper points were inserted into the root canal to collect the bacteria. The collected bacteria were transferred into a 1.5 mL Eppendorf tube containing 1 mL of sterile water, vortexed, diluted, and plated on agar plates for colony counting. This value was recorded as the bacterial count before irrigation (E). And post-irrigation bacterial counts were recorded as F.

2.4.5 Irrigation according to group assignment

Teeth inoculated with *E. faecalis* for six weeks were randomly divided into three groups: 3% NaOCl, SRS, and 0.9% NaCl. Each root canal was first irrigated with a side-vented needle for 1 minute at a flow rate of 5 mL/min, followed by passive ultrasonic irrigation (PUI) for 1 minute and 3 minutes. Sodium thiosulfate was then used to neutralize NaOCl. Bacterial samples were collected after 1 minute and 3 minutes of PUI for colony counting. And this value was recorded as the bacterial count after irrigation (F).

2.4.6 Calculation of bacterial reduction rate

The bacterial reduction rate was calculated as follows: (E − F) / E × 100%. Additionally, six teeth from each group were randomly selected for SEM observation.

2.5 Cell viability assessment

2.5.1 Cell culture

L929 cells (Cat. No. FH0534, Shanghai Fuheng Biotechnology Co., Ltd., China) were used for in vitro cytotoxicity evaluation. The cells were rapidly removed from liquid nitrogen storage and thawed in a 37°C water bath. The thawed cell suspension from the cryovial was transferred to a 15 mL centrifuge tube containing 5 mL of complete culture medium and centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded, and 10 mL of DMEM high-glucose medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin was added. A cell suspension was gently prepared using a pipette. The cell suspension was aliquoted into culture flasks and incubated at 37°C in a 5% CO_2 incubator. After 48 hours, the medium was replaced, and the cells were further cultured. When the cells reached 80% confluence at the bottom of the flask, they were detached using trypsin, subcultured into new flasks, and maintained for subsequent experiments.

2.5.2 Assessment of cell viability

Cell viability was assessed using the CCK-8 assay. The cell suspension was adjusted to a density of 3×10^4 cells/mL and seeded into 96-well plates. The cells were divided into 0.9% NaCl, SRS, 3% NaOCl, control, and blank groups. After treatment for 1, 3, 5, 15, 30, 60, and 120 minutes, the medium was replaced, and CCK-8 reagent was added. After 4 h of incubation at 37°C, the optical density (OD) was measured at 450 nm. All experiments were performed three times, with five replicate wells per group. Cytotoxicity evaluation was conducted in accordance with ISO 10993-5 standard. The cell relative growth rate (RGR) was calculated as follows:

$$\mathrm{RGR} = \frac{\mathrm{OD}_{\mathrm{experimental}}}{\mathrm{OD}_{\mathrm{control}}} \times 100\%$$

2.6 Analysis of dentinal tubule permeability

2.6.1 Sample preparation

A total of 36 extracted teeth were randomly divided into three groups (n = 12 per group). The root length was standardized to 14 ± 1 mm. The crowns were removed, and the pulps were extirpated. The root canals were negotiated with a #10 K-file. Working length and master apical file were determined.The apical foramen was sealed with 3M flowable resin. Root canal preparation was performed in each group using M3 Pro files. During preparation, EDTA was used in conjunction. The canals were prepared to the master apical file size, followed by final irrigation with PUI for 60 seconds. The root canals were then dried with absorbent paper points, and the external surface of each tooth was coated with a layer of nail varnish.

Rhodamine B dye (0.1% w/v) was introduced into the root canals and ultrasonically activated for 30 seconds, after which the canals were dried with absorbent paper points. Under water cooling, transverse sections perpendicular to the long axis of the tooth were prepared using a diamond cutting disc at distances of 3 mm, 6 mm, and 9 mm from the apex. Each section was sequentially polished with 800-, 1000-, and 1200-grit waterproof abrasive paper, and the thickness of each section was controlled to 1.0 ± 0.1 mm. The obtained sections were then observed and photographed under confocal laser scanning microscopy (CLSM).

2.6.2 CLSM analysis

All sections were observed under a confocal laser scanning microscope (Nikon A1 (HD25)) using the specific excitation wavelength for Rhodamine B, and images were acquired at 1× magnification. For each section at 3 mm, 6 mm, and 9 mm from the apex, the mean penetration depth and penetration range were measured using ImageJ software. The mean penetration depth was calculated as the average of measurements taken at eight standardized points from the inner root canal wall. The penetration range was defined as the ratio of the penetrated area to the total cross-sectional area of the root canal.

2.7 Statistical analyses

For the assessment of smear layer removal, the Kappa coefficient was used to evaluate inter-observer reliability. All statistical analyses were performed using SPSS (version 24). For data that conformed to both a normal distribution and homogeneity of variance, one-way analysis of variance (ANOVA) was applied. For data that did not meet the assumptions of normality or homogeneity of variance, the Kruskal–Wallis (KW) test was used. The significance level was set at $\alpha = 0.05$.

3. Results

3.1 Smear layer evaluation

The Kappa coefficients for inter-observer reliability in smear layer scoring were 0.755 for the SRS group, 0.684 for the 3% NaOCl group, and 0.732 for the 0.9% NaCl group. These numbers indicate reasonable inter-examiner agreement on interpretation of smear layer scores.

Table 1 summarizes the mean ± standard deviation of Hülsmann scores for smear layer removal in the three groups. In the coronal, middle, and apical root thirds, the SRS group exhibited significantly lower scores than the 3% NaOCl group ($P < 0.05$), and both the SRS and 3% NaOCl groups had significantly lower scores than the 0.9% NaCl group ($P < 0.05$). Representative SEM images are presented in Fig. 1, and the corresponding statistical analysis is shown in Supplementary Figure S1.

Table 2 presents the mean ± standard deviation of dentinal tubule opening rates. In the coronal third, no significant difference was observed between the 3% NaOCl and SRS groups ($P > 0.05$), while both groups showed significantly higher opening rates than the 0.9% NaCl group ($P < 0.01$). In the middle third, the SRS group demonstrated a significantly higher dentinal tubule opening rate than both the 3% NaOCl and 0.9% NaCl groups ($P < 0.05$), and the 3% NaOCl group also showed a significantly higher rate than the 0.9% NaCl group ($P < 0.05$). In the apical third, the SRS group exhibited a significantly higher opening rate than the 3% NaOCl group ($P < 0.01$), and the 3% NaOCl group in turn showed a significantly higher rate than the 0.9% NaCl group ($P < 0.001$). The corresponding statistical analysis is shown in Supplementary Figure S1.

Table 3 presents the mean ± standard deviation of the percentage of exposed dentinal tubule area. In the coronal and middle thirds, no significant difference was observed between the SRS and 3% NaOCl groups, while both groups showed a significantly larger area percentage than the 0.9% NaCl group ($P < 0.05$). In the apical third, the SRS group exhibited a significantly larger area percentage than both the 3% NaOCl group ($P < 0.05$) and the 0.9% NaCl group ($P < 0.0001$). Additionally, the 3% NaOCl group demonstrated a significantly larger area percentage than the 0.9% NaCl group ($P < 0.05$). The corresponding statistical analysis is shown in Supplementary Figure S1.

Table 1 Mean ± standard deviation of Hülsmann scores for smear layer removal in the coronal, middle, and apical thirds of root canals after irrigation with three different irrigating solutions combined with EDTA and PUI.

Groups	Coronal-third	Middle-third	Apical-third
SRS + EDTA + PUI	1.47 ± 0.43^{Aa}	1.75 ± 0.50^{ABa}	2.36 ± 0.67^{Ba}
3% NaOCl + EDTA + PUI	2.02 ± 0.51^{Ab}	2.49 ± 0.62^{Ab}	3.18 ± 0.69^{Bb}
0.9% NaCl + EDTA + PUI	2.51 ± 0.63^{Ac}	3.09 ± 0.46^{Ac}	3.98 ± 0.57^{Bc}

Table 2 Mean ± standard deviation of dentinal tubule opening rates (%) in the coronal, middle, and apical thirds of root canals after irrigation with the three test solutions combined with EDTA and PUI.

Groups	Coronal-third	Middle-third	Apical-third
SRS + EDTA + PUI	86.70 ± 9.44^{Aa}	82.31 ± 6.89^{Aa}	66.36 ± 15.70^{Ba}
3% NaOCl + EDTA + PUI	78.99 ± 12.34^{Aa}	69.81 ± 8.07^{Bb}	47.95 ± 15.92^{Cb}
0.9% NaCl + EDTA + PUI	58.35 ± 8.37^{Ac}	48.71 ± 15.25^{Ac}	24.82 ± 11.82^{Bc}

Table 3 Mean ± standard deviation of the percentage of exposed dentinal tubule area in the coronal, middle, and apical thirds of root canals after irrigation with three different irrigating solutions combined with EDTA and PUI.

Groups	Coronal-third	Middle-third	Apical-third
SRS + EDTA + PUI	4.15 ± 1.31^{Aa}	3.04 ± 1.54^{ABb}	2.07 ± 1.22^{Be}
3% NaOCl + EDTA + PUI	3.32 ± 1.26^{Aa}	1.89 ± 0.56^{Ab}	1.02 ± 0.42^{Bf}
0.9% NaCl + EDTA + PUI	2.03 ± 0.95^{Ac}	1.22 ± 0.70^{Ad}	0.47 ± 0.24^{Bg}

Notes: Different superscript letters indicate statistically significant differences between groups ($P < 0.05$), whereas the same letters indicate no statistically significant difference ($P > 0.05$). In the same row, different uppercase letters denote significant differences among different root thirds within the same group ($P < 0.05$). In the same column, different lowercase letters denote significant differences among different groups within the same root third ($P < 0.05$).

3.2 Antibacterial activity

Representative SEM images of *E.faecalis* colonization on the root canal wall six weeks after inoculation are shown in Fig. 3. Table 4 presents the mean ± standard deviation of bacterial reduction rates after irrigation with the three test solutions combined with PUI. In the 3% NaOCl group, the bacterial reduction rate was 99.9975% ± 0.0015% at 1 minute and 99.9998% ± 0.0001% at 3 minutes. In the SRS group, the bacterial reduction rate against *E. faecalis* was 98.4164% ± 0.7972% at 1 minute and 99.8390% ± 0.1298% at 3 minutes. In the 0.9% NaCl group, the bacterial reduction rate was 68.3079% ± 11.752% at 1

minute and 83.5437% ± 3.4314% at 3 minutes. The bacterial reduction rates in both the NaOCl and SRS groups were significantly higher than that in the NaCl group ($P < 0.05$). With prolonged irrigation time, the bacterial reduction rate increased in all groups.

Fig. 4 shows representative SEM images (×5000 magnification) of split root canals after irrigation with the three test solutions combined with PUI. After irrigation, the number of *E. faecalis* on the root canal wall was reduced in all groups. The bacteria appeared shriveled, ruptured, and lysed, with visible cellular debris. The degree of bacterial destruction increased with prolonged irrigation time.

Table 4 Mean ± standard deviation of bacterial reduction rates (%) in root canals after irrigation with 3% NaOCl, SRS, and 0.9% NaCl combined with PUI for 1 and 3 minutes.

Groups	Bacterial reduction rate(%，Mean ± S.D.)	
	1min	3min
3% NaOCl + PUI	99.9975 ± 0.0015^{a}	99.9998 ± 0.0001^{b}
SRS + PUI	98.4164 ± 0.7972^{c}	99.8390 ± 0.1298^{d}
0.9% NaCl + PUI	68.3079 ± 11.752^{e}	83.5437 ± 3.4314^{f}

Notes: Different superscript lowercase letters indicate statistically significant differences ($P < 0.05$).

3.3 Cell viability

Table 5 presents the RGR and grade of L929 cells following exposure to 3% NaOCl, SRS, and 0.9% NaCl, as well as a control group, for 1, 3, 5, 15, 30, 60, and 120 minutes, along with the cytotoxicity scores according to the ISO 10993-5 standard.In the 3% NaOCl group, the RGR at all time points was well below 25%, corresponding to a cytotoxicity score of 4 (severe toxicity), and the RGR showed a continuous decreasing trend over time.In the SRS group, the RGR was below 100% only at 1 minute (score 1), while at all other time points, the RGR exceeded 100% (score 0).In the 0.9% NaCl group, the RGR was below 100% at 1, 3, and 5 minutes (score 1), but reached or exceeded 100% at 15, 30, 60, and 120 minutes (score 0).

In the control group, the RGR remained around 100% at all time points, with a sustained toxicity score of 0, indicating stable experimental baseline conditions. Supplementary Figure S2 presents the statistical graph of optical density (OD) values for the 3% NaOCl, SRS, 0.9% NaCl, and control groups at different time points.

Table 5. RGR and cytotoxicity scores of L929 cells after exposure to 3% NaOCl, SRS, and 0.9% NaCl at different time points.

Sample	1min		3min		5min		15min		30min		60min
	RGR(%)	Grade	RGR(%)	Grade	RGR(%)	Grade	RGR(%)	Grade	RGR(%)	Grade	RGR(%)
3% NaOCl	3.74 ± 1.97	4	2.61 ± 1.14	4	1.96 ± 0.28	4	1.22 ± 0.35	4	1.19 ± 0.24	4	0.35 ± 0.2
SRS	90.03 ± 5.14	1	100.07 ± 4.85	0	100.05 ± 2.21	0	101.16 ± 0.97	0	100.58 ± 2.81	0	100.50 ± 1
0.9%NaCl	91.10 ± 3.89	1	96.53 ± 4.04	1	97.29 ± 3.58	1	100.42 ± 2.52	0	100.04 ± 3.21	0	101.37 ± 1
Control	100.00 ± 1.40	0	100.00 ± 5.13	0	100.00 ± 2.29	0	100.00 ± 3.49	0	100.00 ± 4.51	0	100.00 ± 2

3.4 Root canal permeability

Table 6 presents the mean ± standard deviation of the average penetration depth into dentinal tubules following irrigation with the three test solutions (3% NaOCl, SRS, and 0.9% NaCl) combined with EDTA and PUI. At 9 mm and 6 mm from the apical foramen, no significant difference was observed between the SRS and 3% NaOCl groups ($P > 0.05$), while the SRS group demonstrated significantly greater penetration depth than the 0.9% NaCl group ($P < 0.05$). At 3 mm from the apical foramen, the SRS group was significantly superior to both the 3% NaOCl and 0.9% NaCl groups ($P < 0.05$). Representative CLSM images are shown in Fig. 6. Figure S3 shows the statistical analysis of the mean penetration depth.

Table 7 presents the mean ± standard deviation of the penetration range into dentinal tubules following irrigation with the three test solutions combined with EDTA and PUI. At 9 mm and 6 mm from the apical foramen, no significant difference was observed between the SRS and 3% NaOCl groups ($P > 0.05$), while the SRS group demonstrated a significantly larger penetration range than the 0.9% NaCl group ($P < 0.05$). At 3 mm from the apical foramen, the SRS group was significantly superior to both the 3% NaOCl and 0.9% NaCl groups ($P < 0.05$). Figure S3. shows the statistical analysis of the penetration range.

These findings indicate that SRS combined with EDTA and PUI enhances dentinal tubule permeability, particularly in the apical third, compared with 3% NaOCl and 0.9% NaCl.

Table 6 Mean penetration depth into dentinal tubules after irrigation with 3% NaOCl, SRS, and 0.9% NaCl combined with EDTA and PUI at different distances from the apex (μm, mean ± s.d.).

Groups	9 mm from apex	6 mm from apex	3 mm from apex
SRS + EDTA + PUI	660.33 ± 118.35 ^{Aa}	439.56 ± 125.03 ^{Ba}	292.75 ± 96.64 ^{Ca}
3% NaOCl + EDTA + PUI	511.39 ± 120.85 ^{Aab}	364.83 ± 87.33 ^{Aab}	168.97 ± 95.69 ^{Bb}
0.9% NaCl + EDTA+ PUI	419.63 ± 141.49 ^{Ab}	309.85 ± 147.71 ^{Ab}	165.79 ± 101.07 ^{Bb}

Table 7 Penetration range into dentinal tubules after irrigation with 3% NaOCl, SRS, and 0.9% NaCl combined with EDTA and PUI at different distances from the apex (%, mean ± s.d.).

Groups	9 mm from apex	6 mm from apex	3 mm from apex
SRS + EDTA + PUI	67.48 ± 6.41^{Aa}	59.08 ± 11.65^{ABa}	56.34 ± 11.54^{Ba}
3% NaOCl + EDTA + PUI	60.50 ± 7.12^{Aab}	53.25 ± 6.53^{ABab}	45.15 ± 11.63^{Bb}
0.9% NaCl + EDTA+ PUI	58.99 ± 11.08^{Ab}	47.50 ± 10.15^{Bb}	39.00 ± 9.55^{Bb}

Notes: Different superscript letters indicate statistically significant differences between groups ($P < 0.05$), while the same letters indicate no statistically significant difference ($P > 0.05$). In the same row, different uppercase letters denote significant differences among different root levels within the same group ($P < 0.05$). In the same column, different lowercase letters denote significant differences among different groups at the same root level ($P < 0.05$).

4. Discussion

This in vitro study evaluated the effects of SRS as a root canal irrigant on smear layer removal, *E. faecalis* elimination, cytotoxicity, and root canal penetration, aiming to explore its potential as an alternative irrigation solution.The results demonstrated that, when combined with EDTA and PUI, SRS achieved superior smear layer removal compared with alternating irrigation using NaOCl and EDTA under identical ultrasonic conditions. Moreover, SRS exhibited remarkable penetration efficacy in the apical root segment. Notably, NaOCl showed severe cytotoxicity, whereas SRS was non-cytotoxic and possessed better biocompatibility.

The smear layer removal efficacy was evaluated and validated using three indicators: Hülsmann scores, dentinal tubule opening rate, and the area ratio of exposed dentinal tubules. SRS demonstrated superior smear layer removal, with particularly prominent advantages in the apical third, which may be attributed to its physicochemical properties [25].Unlike NaOCl, SRS has favorable chemical compatibility with EDTA and can be applied either sequentially or simultaneously. Their synergistic action efficiently decomposes the organic and inorganic components of the smear layer, with more pronounced performance in the apical region where irrigant flow is limited. In addition, ultrasonic activation generates acoustic streaming and cavitation effects, which facilitate the delivery of irrigants into the complex anatomical areas of the root canal system and consequently improve smear layer removal [26, 27].Moreover, SRS imparts abundant negative charges to the root canal wall. The resulting electrostatic repulsion strips negatively charged debris from the canal surface and improves the wettability of the irrigant on intricate anatomical surfaces [28–30]. Consequently, SRS possesses a greater smear layer removal capacity than NaOCl, particularly in the anatomically complex apical segment.

To evaluate antimicrobial performance, *E. faecalis* was used to infect extracted teeth, followed by eradication assessment. The results showed that SRS exhibited excellent antibacterial activity against *E. faecalis*, with bacterial reduction rates significantly higher than those of the 0.9% NaCl group at both 1 minute and 3 minutes, reaching over 98% at 1 minute and over 99% at 3 minutes. This is largely attributed to the strong alkalinity and strong reducing potential of SRS. The high pH of SRS plays a critical role in destroying bacterial biofilms and exerting potent penetrating effects, which collectively contribute to microbial eradication [19]. Additionally, the strong reducing potential of SRS disrupts bacterial metabolism and compromises bacterial defense mechanisms, further augmenting its antimicrobial activity [28, 31]. Furthermore, the combination of ultrasound and SRS generates electric field repulsion, producing a synergistic effect that enhances penetration into bacterial biofilms, leading to more effective bacterial killing and detachment [32, 33].

To evaluate the biosafety of the irrigating solutions, cytotoxicity was assessed using the CCK-8 assay. The results demonstrated that SRS exhibited excellent biosafety. SRS showed no significant cytotoxicity to L929 cells at any of the tested time points, with cell relative growth rates close to or above 100%, comparable to those of the 0.9% NaCl group, indicating that SRS possesses satisfactory biosafety characteristics. In contrast, 3% NaOCl exhibited extremely strong cytotoxicity at all time points, with toxicity increasing over time. This finding is consistent with previous reports describing the strong irritancy and corrosiveness of NaOCl to living tissues [16]. This marked difference in biosafety represents the most prominent potential clinical advantage of SRS.

In the root canal permeability experiment, the mean penetration depth and penetration range were evaluated. SRS combined with EDTA and PUI achieved greater penetration depth and broader penetration range than other irrigation protocols. EDTA removes the smear layer, while the debris-detachment action of SRS during root canal flushing helps open the dentinal tubules, facilitating deeper penetration of the irrigant and thereby enhancing subsequent root canal disinfection. Furthermore, the synergistic effects of cavitation and acoustic streaming generated by ultrasonic activation further propel the irrigant into the complex anatomical regions of the root canal system [27], improving overall penetration capability.This also overcomes the limitation of NaOCl, whose high surface tension hinders its penetration into deep root canal areas [34].The superior permeability observed in the SRS group in the apical region suggests that SRS has advantages in improving irrigant penetration into the apical third and complex anatomical structures, thereby facilitating cleaning and disinfection of the root canal system.

In summary, SRS combined with PUI achieves antibacterial effects that meet clinical requirements. Given the high toxicity, strong irritancy, and poor biosafety of NaOCl, SRS has the potential to serve as an alternative to NaOCl, particularly in patients with extensive apical foramen destruction, periapical tissue sensitivity, or allergy to NaOCl, as well as in pediatric patients. Future in vivo studies are needed to simulate the oral environment with polymicrobial infections and to evaluate periapical tissue healing and the effects of root canal filling materials, thereby further validating its clinical efficacy.

5. Conclusion

SRS combined with EDTA and PUI demonstrated superior smear layer removal and root canal permeability compared with 3% NaOCl and 0.9% NaCl, while exhibiting no cytotoxicity and better biosafety. Thus, SRS may be a preferable alternative for patients with apical foramen destruction, periapical sensitivity, NaOCl allergy, or for pediatric patients.

Abbreviations

CFU: Colony forming units; NaOCl: Sodium hypochlorite; EDTA: Ethylenediaminetetraacetic acid ; SRS: Superalkaline reductive solution; *E. faecalis*: *Enterococcus faecalis* ; SEM: Scanning electron microscopy; CLSM: Confocal laser scanning microscopy;BHI: Brain–heart infusion;FBS: Fetal bovine serum ;OD: Optical density;RGR: Relative growth rate ;PUI: Passive ultrasonic irrigation.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Hangzhou Stomatological Hospital (approval No. 2025HKLLSL15) and written consent was obtained from patients to participate in this study.

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Authors' contributions

RW designed and performed the experiment ,and wrote the manuscript. YS, QL,ZH and JZ performed data curation. BP,HZ, GW and HL conceived the original idea, designed the experiment and reviewed the manuscript.All authors have read, reviewed and approved the manuscript.

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Availability of data and materials

The data analyzed during this present study are available from corresponding author on request.

Declaration of competing interest

The authors declare that they have no competing interests

Consent to publish

Consent to publish is not applicable to this manuscript.

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Figures

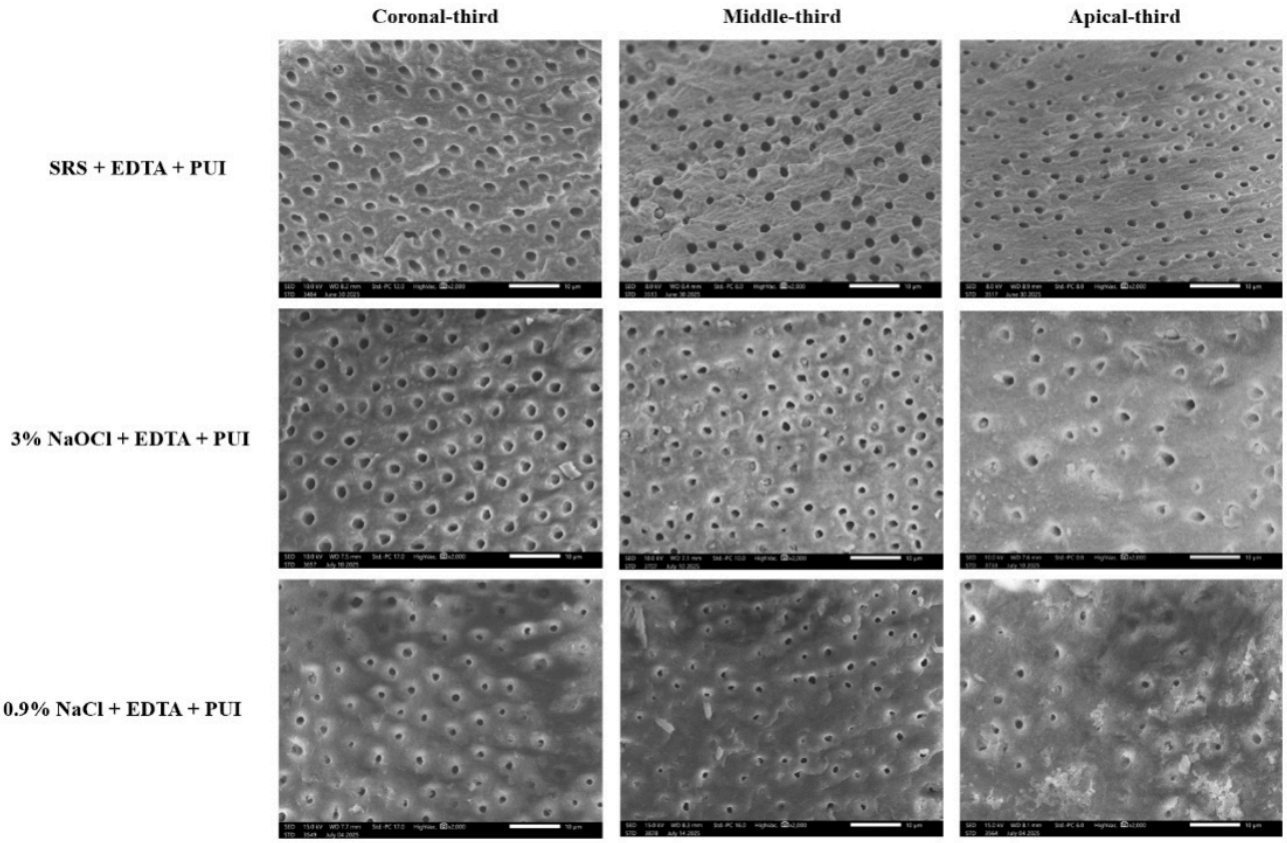


Figure 1

Representative SEM images of the coronal, middle, and apical thirds of root canals after irrigation with 0.9% NaCl, 3% NaOCl, or SRS combined with EDTA and PUI.

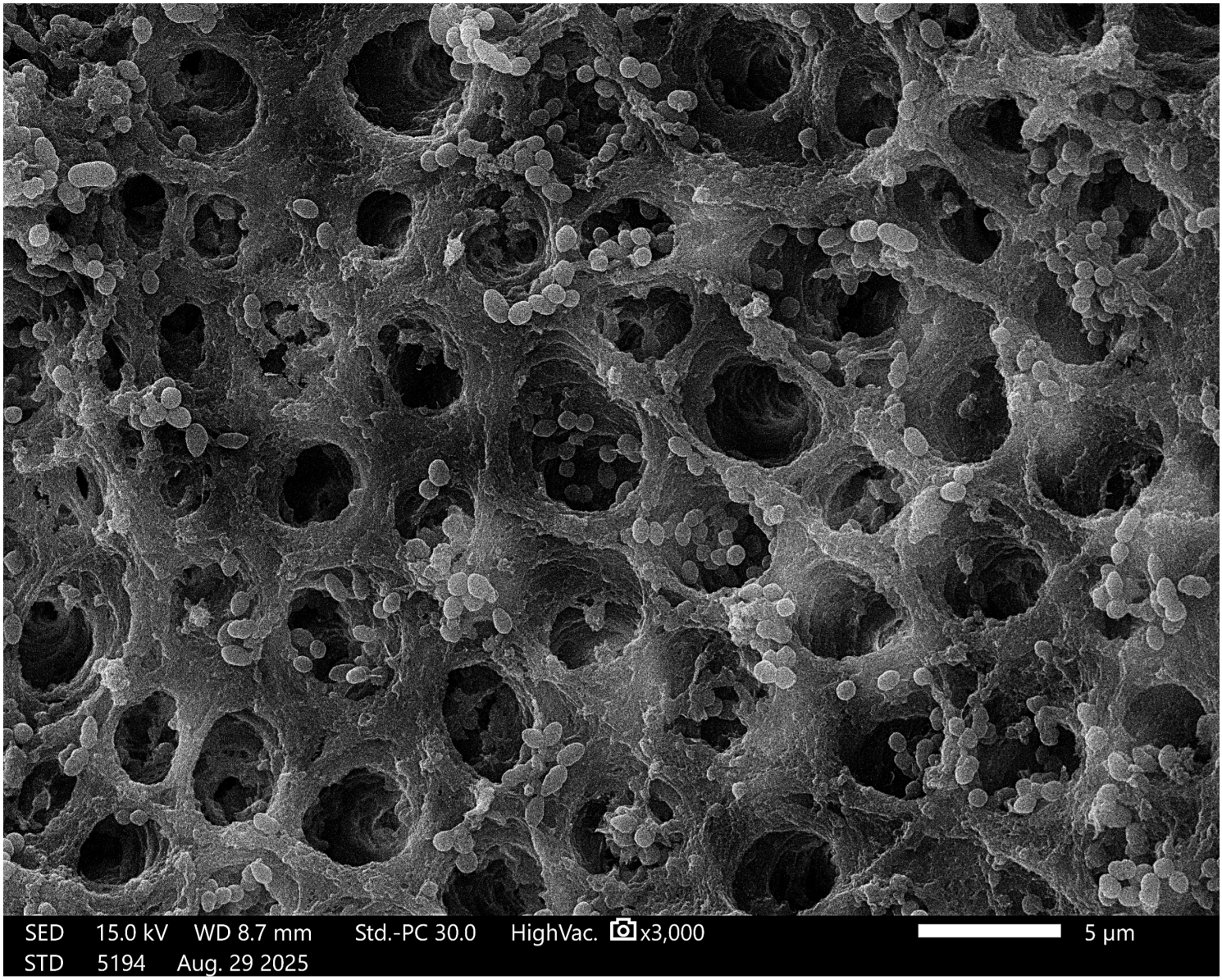


Figure 2

Fig. 3. Representative SEM images of *E. faecalis* colonization on the root canal wall of extracted teeth six weeks after inoculation.

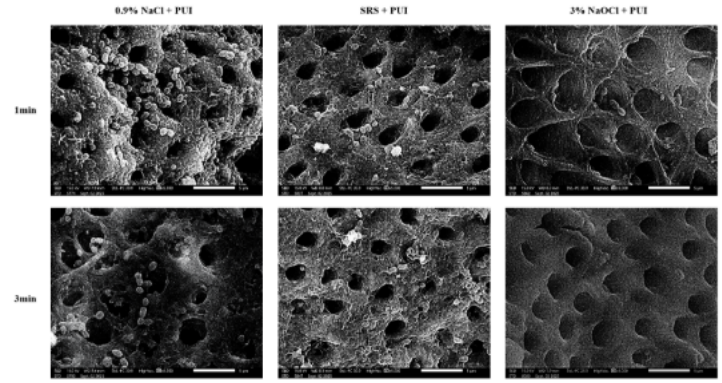


Figure 3

Fig. 4. Representative SEM images (×5000 magnification) of root canal walls after irrigation with 0.9% NaCl, 3% NaOCl, or SRS combined with PUI.

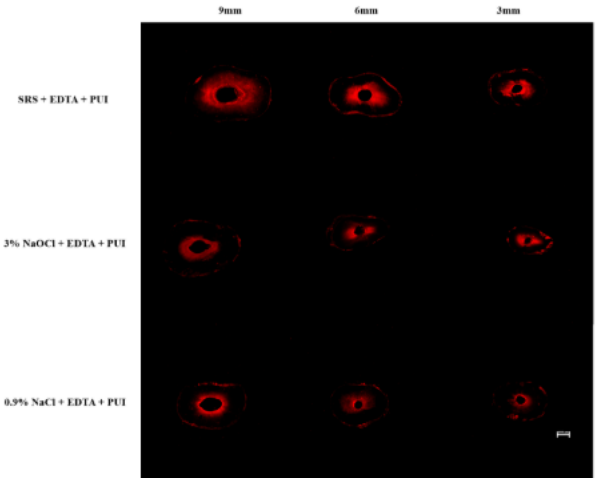


Figure 4

Fig. 6. Representative CLSM images of transverse root sections at 3 mm, 6 mm, and 9 mm from the apex after irrigation with the three test solutions (3% NaOCl, SRS, and 0.9% NaCl) combined with EDTA and PUI. Scale bar = 1000 μm (applies to all images).

Supplementary Files

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- SupplementaryMaterials.docx