REVIEW

Turk Endod J 2022;7(1):28–42
doi: 10.14744/TEJ.2021.41736

# Antimicrobial efficacy of calcium hypochlorite in endodontics: A systematic review of in vitro studies

**Reshma Rajasekhar,[1] Sooraj Soman,[2] Varsha Maria Sebastian[1]**

*[1]Department of Conservative Dentistry and Endodontics, Mes Dental College, Perinthalmanna, India*
*[2]Department of Oral and Maxillofacial Surgery, Mes Dental College, Perinthalmanna, India*

**Purpose:** This systematic review analyzes the antimicrobial effectiveness of calcium hypochlorite ($Ca(OCl)_2$) compared to other disinfection strategies used in endodontics.

**Methods:** *In vitro* studies on human teeth were included, whereas in vivo studies on animals, bovine teeth, and artificial or immature teeth and review articles were excluded. A search on PubMed, Scopus, Trip, ScienceDirect, and Wiley Online Library until June 2021 for research published in English language was conducted, and additional manual searching using references from eligible studies was also performed. Articles were then transferred to a reference management software, from which titles and abstracts were screened. Selected articles were then retrieved in full text, and data extraction was done using Microsoft Excel. Risk of bias assessment was performed using the methods adapted from previous systematic reviews on in vitro studies.

**Results:** In total, 11 articles were included in this study, wherein a high to moderate overall quality was observed. Ten studies showed moderate risk of bias, whereas only one study exhibited a low risk of bias. Based on the available evidence, $Ca(OCl)_2$ demonstrated good antimicrobial efficacy, which is comparable to that of sodium hypochlorite as an irrigant.

**Conclusion:** $Ca(OCl)_2$ can be a potential irrigant in endodontics, and its potency depends on its concentration and duration of action, which needs further analysis.

**Keywords:** Calcium hypochlorite, root canal treatment, sodium hypochlorite, systematic review.

## Introduction

Microorganisms and their biofilms have been identified as some of the main etiological agents responsible for pulpal and periapical pathologies (1,2). Consequently, eradication of microorganisms and their toxic byproducts is deemed crucial to eliminate infection within the root canal system, and this is achieved by chemomechanical preparation (3). However, studies have shown that complete cleaning and shaping of the root canal system might not be achieved as there are non-instrumented areas within the root canal system, along with its complex anatomies, such as isthmuses, fins, deltas, and dentinal tubules (4,5). Mechanical instrumentation with an effective antibacterial irrigant is essential to improve clinical outcomes.

There are several antimicrobial irrigants in the market that possess good disinfection capability. However, the only ir-

Cite this article as: Rajasekhar R, Soman S, Sebastian VM. Antimicrobial efficacy of calcium hypochlorite in endodontics: A systematic review of in vitro studies. Turk Endod J 2022;7:28-42.
Correspondence: Reshma Rajasekhar. Department Of Conservative Dentistry And Endodontics, Mes Dental College, Perinthalmanna, India.
Tel: 8606008164 e-mail: reshmarajasekhar@gmail.com
Submitted: July 17, 2021 Accepted: October 25, 2021

rigant that is considered the gold standard is sodium hypochlorite (NaOCl). It has been used at 0.5%–6% concentration during endodontic procedures (6). It is known to possess excellent antibacterial and tissue dissolving property (7). However, the limitations of this irrigant are: its cytotoxicity (8), interfering with the bonding of restorative material (9), and availability free active chlorine due to chemical instability (10). For this reason, alternative irrigation solutions have been investigated to overcome the limitations.

For the past few years, application of calcium hypochlorite ($Ca(OCl)_2$) as an irrigant has been studied in the field of endodontics. It is available as an alkaline white powder, which was used initially for water treatment and industrial sterilization as a bleaching agent (11). It is relatively more stable and has considerable available chlorine compared to NaOCl. $Ca(OCl)_2$ is available as powder; it is prepared by mixing the powder with distilled water at required concentration. The following reaction occurs during the aqueous preparation of $Ca(OCl)_2$ (12):

$$Ca(OCl)_2 + 2\ H_2O \rightarrow 2HOCl + Ca(OH)_2.$$

Dutta and Saunders were the first to evaluate the application of $Ca(OCl)_2$ as an irrigant in endodontics. As per their findings, it was determined that $Ca(OCl)_2$ was capable of tissue dissolution (12). Several studies have shown that necrotic pulp tissue pretreated with calcium hydroxide medicament enhanced the tissue dissolving effect of NaOCl (13,14). The release of calcium hydroxide in an aqueous environment can enhance the action of $Ca(OCl)_2$. Subsequent studies in the following years found that $Ca(OCl)_2$ has good antibacterial (15) and tissue dissolving property (16)without interfering the mechanical properties of dentin (17). It is also chemically stabile during storage (18) and biocompatible (19). However, the amount of information is still limited; hence, further studies are required to provide knowledge for its use in endodontics.

This systematic review aims at assessing the antimicrobial efficacy of $Ca(OCl)_2$ by evaluating with other endodontic disinfection protocols from *in vitro* studies.

## Materials and Methods

### Protocol Registration

This systematic review was conducted by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and registered in Open Science Framework (10.17605/OSF.IO/KVYN5).

### Review Question

Population (P), intervention (I), comparison (C), outcome (O), and study framework (S) criteria were used to develop the research question as follows: "In extracted human permanent teeth (P), does $Ca(OCl)_2$ (I) when compared to other endodontic disinfection protocol (C) possess better antimicrobial efficacy (O) from *in vitro* studies (S)?"

### Search Strategy

A comprehensive literature search was conducted in the following databases: PubMed, Scopus, Trip, ScienceDirect, and Wiley Online Library. To identify relevant articles, Medical Subject Heading (MeSH) terms such as "calcium hypochlorite," "root canal," "dentin," and "endodontics" and text words such as "bactericidal," "antimicrobial," and "antibacterial" were utilized, along with Boolean Operators (AND, OR) in between the keywords; the search was conducted till June 2021. The keyword selection was based on previous works within this framework and their most

**Table 1.** Database

| Database | Search strategy | Result |
|---|---|---|
| PubMed | "Calcium hypochlorite"[All Fields] AND ("anti bacterial agents"[Pharmacological Action] OR "anti bacterial agents"[MeSH Terms] OR ("anti bacterial"[All Fields] AND "agents"[All Fields]) OR "anti bacterial agents"[All Fields] OR "antibacterial"[All Fields] OR "antibacterials"[All Fields] OR "antibacterially"[All Fields] OR ("anti infective agents"[Pharmacological Action] OR "anti infective agents"[MeSH Terms] OR ("anti infective"[All Fields] AND "agents"[All Fields]) OR "anti infective agents"[All Fields] OR "antimicrobial"[All Fields] OR "antimicrobials"[All Fields] OR "antimicrobially"[All Fields]) OR ("bactericidal"[All Fields] OR "bactericidality"[All Fields] OR "bactericidally"[All Fields] OR "bactericidals"[All Fields] OR "bactericide"[All Fields] OR "bactericides"[All Fields] OR "bactericidic"[All Fields] OR "bactericidity"[All Fields])) AND ("root canal"[All Fields] OR ("dentin"[MeSH Terms] OR "dentin"[All Fields] OR "dentine"[All Fields] OR "dentines"[All Fields] OR "dentins"[All Fields] OR "dentin s"[All Fields] OR "dentinal"[All Fields]) OR ("endodontal"[All Fields] OR "endodontic"[All Fields] OR "endodontical"[All Fields] OR "endodontically"[All Fields] OR "endodontics"[MeSH Terms] OR "endodontics"[All Fields])) | PubMed=25 |
| Scopus | TITLE-ABS-KEY("calcium hypochlorite" AND (antibacterial OR antimicrobial OR bactericidal) AND ("root canal" OR dentin OR endodontics)) | Scopus=15 |

cited descriptors. An example for search strategy in databases was given in Table 1. Additional manual search for articles was done using the references of eligible studies and Google Scholar.

### Inclusion Criteria

1. *In vitro* studies performed on extracted fully formed permanent human teeth.
2. Studies evaluating the antimicrobial efficacy of $Ca(OCl)_2$.
3. Studies comparing $Ca(OCl)_2$ with other endodontic disinfection protocols.
4. Studies published in English.

### Exclusion Criteria

Studies conducted *in vivo*, in animals, bovine teeth, artificial, or immature teeth and review articles.

### Study Selection Process

Articles from databases were transferred to Zotero reference management software. After merging the duplicates in Zotero, the title and abstract of the remaining articles were screened by two reviewers (R.R and S.S) independently, based on the inclusion and exclusion criteria. Studies that fulfilled the criteria were then further assessed for eligibility for qualitative synthesis by screening the full text of the article. The references of all eligible studies were also examined. Disagreements if any between the reviewers were discussed with a third reviewer (V.M.S).

### Data Extraction

Selected articles were read independently by two reviewers (R.R and S.S), and the data extraction was done on a customized data extraction sheet created in Microsoft Excel. Data extraction form comprises the following details such as author name, year, country, sample size, tooth type, microorganism evaluated, intervention, disinfection protocol, evaluation, and results. Any disagreements between the two reviewers (R.R and S.S) that occurred during the process were discussed with a third reviewer (V.M.S).

### Quality Assessment of the Included Studies

Quality of the included studies was assessed using the methods adapted from previous systematic reviews on *in vitro* studies (18,19). Two reviewers (R.R and S.S) independently judged the quality of the studies based on following parameters: (1) randomization, (2) sample size calculation, (3) standardization of samples, (4) cleaning and shaping before inoculation of samples, (5) sterilization of samples before inoculation, (6) smear layer removal before inoculation of samples, (7) verification of the inoculum, (8) explanation of the disinfection protocol, (9) complete outcome assessment, and (10) blinding. Two authors (R.R, S.S) independently assessed the quality of studies, In case of disagreement, it was discussed with a third author (V.M.S). Moreover, the articles were scored based on the following criteria: high risk of bias, if only 1–3 criteria were reported; moderate risk of bias, if 4–7 criteria were reported; and low risk of bias, if 8–10 criteria were reported.

## Results

### Study Selection

Initially, 45 studies were identified from the electronic databases. After screening the title and abstract and removal of duplicates, in total, 15 articles were sought for full text retrieval. After full text assessment of articles, only 11 articles were included into this systematic review based on eligibility criteria (15,20–29). However, four studies were excluded as the procedures were not performed on human permanent teeth (30–33). These studies are summarized in Table 2, and PRISMA flowchart is shown in Figure 1. A meta-analysis was not performed because of variations in the methodology and the differences in the concentration of $Ca(OCl)_2$ and other irrigants used in the included studies.

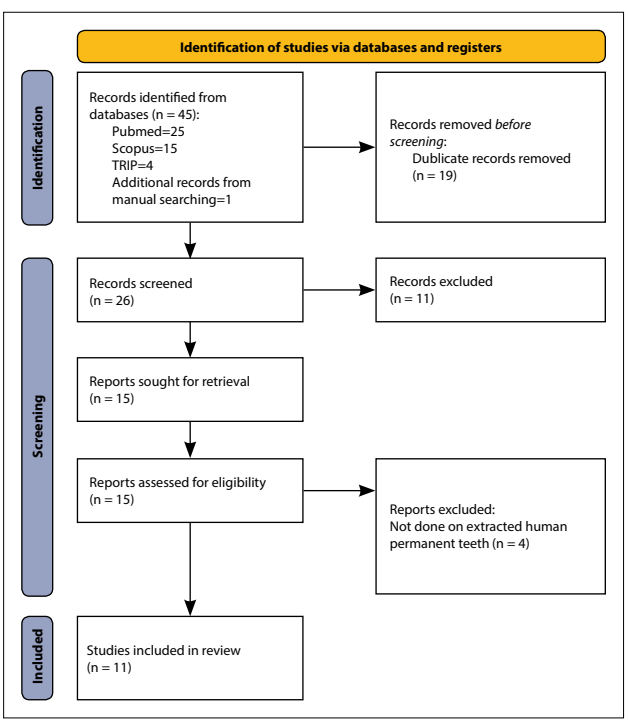


**Fig. 1.** PRISMA Flowchart.

**Table 2.** Summary of studies

| Number | Author, year | Country | Sample | Type of tooth | Microorganism evaluated | Intervention | Disinfection regimen | Evaluation | Results |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Dumani et al., 2016 (15) | Turkey | 84 | Single-rooted premolars | *E. faecalis* (ATCC 29212) | Group 0: no treatment; Group 1: distilled water; Group 2: 2.5% NaOCl; Group 3: 2.5% $Ca(OCl)_2$; Group 4: distilled water + sonic activation; Group 5: 2.5% NaOCl + sonic activation; Group 6: 2.5% $Ca(OCl)_2$ + sonic activation | Syringe irrigation group: a side-vented needle tip was placed at 12 mm, and 5 ml of irrigant was delivered for a period of 2 min. Sonic irrigation groups: 5 ml irrigation solution was delivered and sonically activated for 2 min at 1 mm from the working length. | Counting CFU | There was no statistically significant difference between syringe and sonic irrigation systems with $Ca(OCl)_2$ and NaOCl. |
| 2 | Sedigh-shams et al., 2016 (24) | Iran | 50 | Mandibular premolars | *E. faecalis* (ATCC 29212) | Group 1: 0.5%NaOCl; Group 2: 5% $Ca(OCl)_2$; Group 3: Control | Irrigation was performed using disposable syringes with a 30-gauge needles. 10 ml of irrigant was used for each canal. | Quantitative real-time PCR | 5% $Ca(OCl)_2$ was more effective than 0.5% NaOCl in eliminating *E. faecalis* with significant difference. |
| 3 | Dalbello et al., 2018 (23) | Brazil | 60 | Single-rooted teeth | *E. faecalis* (ATCC 19433) | Group 1: no treatment; Group 2: WaveOne (25.08) + distilled water; Group 3: WaveOne (25.08) + 2.5% NaOCl; Group 4: WaveOne (25.08) + 2.5% $Ca(OCl)_2$; Group 5: WaveOne (25.08) + 5.25% NaOCl; Group 6: WaveOne (25.08) + 5.25% $Ca(OCl)_2$ | Root canals filled with tested irrigant using 5 ml syringe and 19-gauge needle before progression into each third of instrumentation. | Counting CFU | Groups 3, 4, 5, and 6 showed the lowest contamination means with no significant difference between them. |
| 4 | Souza et al., 2018 (21) | Brazil | 132 | Single-rooted teeth | *E. faecalis* (ATCC 19433) | Group 1: Distilled water + Reciproc R40 (control); Group 2: 1% NaOCl + Reciproc R40; Group 3: 2.5% NaOCl + Reciproc R40; Group 4: 1% $Ca(OCl)_2$ + Reciproc R40; Group 5: 2.5% $Ca(OCl)_2$ + | Irrigation done with 5 ml irrigant using 5 ml syringe with 19-gauge needle, which were renewed during instrumentation. | Counting CFU | Highest bacterial reduction was observed in groups 9, 10, and 11 with no significant difference between them. |

**Table 2.** Summary of studies *(continue)*

| Number | Author, year | Country | Sample | Type of tooth | Microorganism evaluated | Intervention | Disinfection regimen | Evaluation | Results |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Reciproc R40;<br>Group 6: PDT;<br>Group 7: Distilled water + Reciproc R40 + PDT;<br>Group 8: 1% NaOCl + Reciproc R40 + PDT;<br>Group 9: 2.5% NaOCl + Reciproc R40 + PDT;<br>Group 10: 1% $Ca(OCl)_2$ + Reciproc R40 + PDT;<br>Group 11: 2.5% $Ca(OCl)_2$ + Reciproc R40 + PDT | | | |
| 5 | Soligo et al., 2018 (29) | Brazil | 96 | Mesiobuccal canals of mandibular molars | *E. faecalis* (ATCC 19433) | Group 1 (ProTaper Next)<br>a) PTN + sodium chloride (control);<br>b) PTN + 6% NaOCl;<br>c) PTN + 6% $Ca(OCl)_2$;<br>d) PTN + 50% GSE<br>Group 2 (Reciproc R25)<br>a) Reciproc + sodium chloride (control);<br>b) Reciproc+ 6% NaOCl;<br>c) 6% $Ca(OCl)_2$;<br>d) Reciproc + 50% GSE | 5 ml of the respective solution using a syringe and a 30-gauge needle NaviTip | Counting CFU | No significant differences were observed in bacterial reduction between the ProTaper Next and Reciproc R25 systems regardless of the irrigant solution used. |
| 6 | Shadmehr et al., 2019 (25) | Iran | 135 | Maxillary central incisors | *E. faecalis* (ATCC 29212), *F. nucleatum* (ATCC 10953), *and* *P. intermedia* (ATCC 25611) | Group 1: 2% CHX;<br>Group 2: 5.25% NaOCl;<br>Group 3: 5% Ca(OCl)2;<br>Group 4: Positive control (normal saline) and one negative group (no infection) | Irrigation done for a duration of 10 min with 5 ml irrigant using a 27-gauge syringe. | Spectrophotometer | 2% CHX and 5% $Ca(OCl)_2$ had significantly lower medium turbidity at both S2 and S3, in comparison with 5.25% NaOCl. But there were no significant differences between 2% CHX and 5% $Ca(OCl)_2$ at both S2 and S3. |

**Table 2.** Summary of studies *(continue)*

| Number | Author, year | Country | Sample | Type of tooth | Microorganism evaluated | Intervention | Disinfection regimen | Evaluation | Results |
|---|---|---|---|---|---|---|---|---|---|
| 7 | Dumani et al., 2019 (20) | Turkey | 105 | Single rooted premolars | *E. faecalis* (ATCC 29212) | Group 1: Syringe irrigation + distilled water;<br>Group 2: Syringe irrigation + 2.5% NaOCl;<br>Group 3: Syringe irrigation + 2.5% $Ca(OCl)_2$;<br>Group 4: LAI + distilled water;<br>Group 5: LAI + 2.5% NaOCl;<br>Group 6: LAI + 2.5% $Ca(OCl)_2$;<br>Group 7: LAI was performed with no solution | Groups 1, 2, and 3: Irrigation was performed with 5 ml of irrigation solution using a side vented needle.<br>Groups 4, 5, and 6: LAI was used for root canal irrigation with 1.25 ml of irrigation solution for 15 s. The total volume of irrigant was 5 ml, and the total time of treatment was 2 minutes.<br>Group 6: LAI was performed, but no irrigation solution was used. | Counting CFU | High bactericidal reduction was observed in groups 2, 3, 4, 5, 6, and 7 with no statistically significant difference. |
| 8 | Souza et al., 2020 (22) | Brazil | 110 | Single-rooted teeth | *E. faecalis* (ATCC 19433) | Group 1: Reciproc + distilled water (control);<br>Group 2: Reciproc + 1% NaOCl;<br>Group 3: Reciproc + 5.25% NaOCl;<br>Group 4: Reciproc + 1% $Ca(OCl)_2$;<br>Group 5: Reciproc + 5.25% $Ca(OCl)_2$;<br>Group 6: PDT;<br>Group 7: Reciproc + distilled water + PDT;<br>Group 8: Reciproc + 1% NaOCl + PDT;<br>Group 9: Reciproc + 5.25% NaOCl + PDT;<br>Group 10: Reciproc + 1% $Ca(OCl)_2$+PDT;<br>Group 11: Reciproc + 5.25% $Ca(OCl)_2$ + PDT | Irrigation done with 5 ml irrigant using 5 ml syringe with 19-gauge needle, which were renewed during chemomechanical preparation for a duration of 5 min. | Counting CFU | Highest bacterial reduction observed in groups 3, 4, 5, 8, 9, 10, and 11, with no statistical difference between them. |

**Table 2.** Summary of studies *(continue)*

| Number | Author, year | Country | Sample | Type of tooth | Microorganism evaluated | Intervention | Disinfection regimen | Evaluation | Results |
|---|---|---|---|---|---|---|---|---|---|
| 9 | Subbiya et al., 2020 (26) | India | 63 | Mandibular incisor | *E. faecalis* (ATCC 29212) | Group A: 3% NaOCl; Group B: $Ca(OCl)_2$; Group C: CHX; Group D: Control Groups A, B, and C were further divided into two subgroups based on two medicaments ($Ca(OH)_2$ and metronidazole + CHX combination) | Irrigation procedure not mentioned. Intracanal medicaments were placed for a duration of 7 days. | Counting CFU | No remarkable reduction in bacteria seen post irrigation of samples. Statistically no significant difference was found between the irrigants. Both $Ca(OH)_2$ and metronidazole +CHX medicament have effectively destroyed the remaining bacterial culture. |
| 10 | Kaur et al., 2020 (27) | India | 70 | Single-rooted mandibular premolars | *E. faecalis* (ATCC 29212) | Group I: Control Group; Group II: 5% NaOCl + 17% EDTA; Group III: 5% $Ca(OCl)_2$ + 17% EDTA; Group IV: 5% $Ca(OCl)_2$ + 1% chitosan irrigant | Irrigated with 2 mL of each irrigant for 5 min. | Counting CFU | Group IV showed the highest bacterial reduction and the highest amount of smear layer removal with a statistically significant difference when compared with the other three groups. |
| 11 | Alfadda et al., 2021 (28) | USA | 40 | Mandibular premolars with single canal | *E. faecalis* (ATCC 47077) | Group 1: Ca(OH)2; Group 2: TAP; Group 3: Ca(OCl)2 Infected but untreated canals were used as a positive control, and uninfected root canals were used as a negative control | Filled with intracanal medicaments using 1 ml Luer Lock syringe with 20-gauge dispensing needle and incubated for 7 days in the incubator. | Counting CFU, CLSM analysis | All medicaments decreased the initial bacterial load. The highest bacterial reduction in the main canal and dentinal tubules was observed in the $Ca(OCl)_2$ group with significant difference. |

$Ca(OCl)_2$: Calcium hypochlorite; $Ca(OH)_2$: Calcium hydroxide; CFU: Colony-forming units; CHX: Chlorhexidine; EDTA: Ethylenediaminetetraacetic acid; GSE: Grape seed extract; LAI: Laser-activated irrigation; NaOCl: Sodium hypochlorite; PCR: Polymerase chain reaction; PDT: Photodynamic therapy; PTN: ProTaper Next; S1: Sample after inoculation; S2: Sample after decontamination procedure; S3: Sample collected from deep dentin after decontamination procedure; TAP: Triple antibiotic paste.

**Table 3.** Characteristics of studies

| Number | Author, year | Sample | Root length | Root canal preparation before contamination | Sterilization | Biofilm formation time | Samples obtained | Inoculation procedure |
|---|---|---|---|---|---|---|---|---|
| 1 | Dumani et al., 2016 (15) | Single-rooted premolars | 14 mm | GG drill was used to flare the coronal aspect of each canal, and the root canal preparations were performed with a WaveOne rotary system to #40 file size. | Samples were autoclaved for 30 minutes at 121°C. Sterilization was checked with an indicator placed in the sachets. | | S1 was obtained by sterile absorbent point; S2 was obtained by 35 H-file; if root canals were dry, distilled water was introduced into the canals, and absorbent points are used. | 20 µl of *E. faecalis* suspension was introduced into the canals using sterile automatic micropipettes. #15 K-files were used to carry the bacterial suspension to the working length. |
| 2 | Sedig-shams et al., 2016 (24) | Mandibular premolars | Not mentioned | Each root canal was enlarged with a #20 K-file. | Autoclaved at 121°C for 30 min and filled with BHI broth to check for turbidity. | | S1 and S2 obtained by sterile paper point. | Each root canal was contaminated with 10 µl of the bacterial suspension by inserting sampler microtip into the access cavities, and suspension was introduced into the whole length of the canal using a #20 k-file. |
| 3 | Dal bello et al., 2019 (23) | Single-rooted teeth | 15 mm | Cervical third was prepared using Largo drill, and serial instrumentation was done using K-file following sequences #10, #15, and #20. | Sterilization was done using autoclave at 120°C for 30 min. Sterile paper point was used for checking sterility. | | Sterile absorbent paper points. | 100 µl of the bacterial suspension inoculated into root canal. |
| 4 | Souza et al., 2018 (21) | Single-rooted teeth | 15 mm | Cervical third was prepared using a Largo drill and manual K-files and serial instrumentation following the sequence #15, #20, #25, #30, and #35. | Sterilization of samples done at 120°C in autoclave for 30 min. Sterile paper points were used to check sterility. | | Sterile absorbent paper. | 100 µl of the bacterial suspension inoculated into root canal. |
| 5 | Soligo et al, 2018 (29) | Mesiobuccal canals of extracted mandibular molars | 15 mm | All specimens were instrumented with #10 and #15 K-files. | Samples were autoclaved for 30 min at 120°C. Sterile paper point placed in root canal used for assessing the sterility. | | Sterile absorbent paper. | 100 µl of the bacterial suspension inoculated into the root canal. |

**Table 3.** Characteristics of studies *(continue)*

| Number | Author, year | Sample | Root length | Root canal preparation before contamination | Sterilization | Biofilm formation time | Samples obtained | Inoculation procedure |
|---|---|---|---|---|---|---|---|---|
| 6 | Shadmehr et al., 2019 (25) | Maxillary central incisors | 8 mm cervical and apical portion removed with double-faced cylindrical saw | GG drill was used to enlarge each canal. | Samples were sterilized in autoclave at 121°C for 30 min at 20 psi pressure. All teeth were cultured in Schaedler broth and incubated to confirm sterility. | | Sterile absorbent paper used for collecting S1 Dentine chips from within the lumen of all infected and noninfected specimens were collected using the GG drills to test for bacterial survival. | 5 ml of mixed bacterial suspension used for inoculation. |
| 7 | Dumani et al., 2019 (20) | Single-rooted premolars | 14 mm | Size 4 and 3 GG drills were used to flare the coronal aspect of each canal, and the root canal preparations were performed with a WaveOne system #40 file. | Sterilization done with autoclave for 30 minutes at 121° C. Sterility was checked with an indicator placed in the sachets. | | S1 obtained by sterile absorbent point. S2 obtained by 35 H-file; if root canals were dry, distilled water was introduced into the canals, and absorbent points are used. | 20 µl of *E. faecalis* suspension introduced using sterile automatic micropipettes. 15 size k files were used to carry the bacterial suspension to the working length. |
| 8 | Souza et al., 2020 (22) | Single-rooted teeth | 15 mm | Serial instrumentation done up to #45 file. | Sample sterilization done at 120°C in autoclave for 30 minutes. Sterile paper points were used for checking sterility. | | Sterile absorbent paper. | 100 µl culture aliquot was inoculated into the root canal of each sample. |
| 9 | Subbiya et al., 2021 (26) | Mandibular incisor | 16 mm | Procedure not mentioned. | Autoclaved at 121 ° C at 15 lbs pressure for 20 min. Samples were transferred into glass test tubes containing MHB, and autoclaving was repeated. The tubes were checked for sterility at 48 hours. | | Sterile paper points and peeso reamer used for obtaining sample collection. | Procedure not explained. |

**Table 3.** Characteristics of studies *(continue)*

| Number | Author, year | Sample | Root length | Root canal preparation before contamination | Sterilization | Biofilm formation time | Samples obtained | Inoculation procedure |
|---|---|---|---|---|---|---|---|---|
| 10 | Kaur et al., 2020 (27) | Single-rooted mandibular premolars | 17 mm | Step back technique till apical enlargement of # 35 K-file. | Samples were sterilized in an autoclave at a temperature of 121°C at 15 lbs pressure for 20 min. Sterile tooth samples were immersed in 5 mL of MHB; sterilization was repeated by plugging the test tubes with cotton. | | Sterile absorbent paper points. | Procedure not explained. |
| 11 | Alfadda et al., 2021 (28) | Mandibular premolar with single canal | 12 mm | Root canals were enlarged to size ProTaper F5. | The root samples were placed in Eppendorf tubes containing BHI broth medium and incubated and observed for 2 days at 37°C to ensure sterility before the infection with bacteria. | | H-file used for sample collection. | 10 µl of the bacterial solution introduced in to canal and #15 K-file were used to carry the solution up to canal length. |

BHI: Brain Heart Infusion; GG: Gates Glidden; MHB: Mueller Hinton Broth; S1: Sample after inoculation of bacteria; S2: Sample after decontamination procedure.

### *Characteristics of the Included Studies (Table 3)*

Among the 11 included articles, 3 articles mentioned only single-rooted teeth (21–23), whereas the remaining studies mentioned the type of teeth obtained. One study included mesiobuccal canals of mandibular molars (29), one study mentioned maxillary central incisors (25), one study selected mandibular incisors (26), three studies incorporated mandibular premolars (24,27,28), and two studies included single-rooted premolars without mentioning either maxillary or mandibular (15,20).

Root length after sectioning the samples and canal preparation protocol before bacterial inoculation were noted to vary among studies. Manual filing with K-files and serial instrumentation were used in the studies up to #45 size (22), #35 size (21), #15 size (29), #20 size (23,24) respectively . No canal preparation was mentioned in one study (25); meanwhile, step-back technique till enlargement of apical portion to #35 size was mentioned in one study (27), two studies employed WaveOne rotary system to #40 file size (15,20), whereas one study used ProTaper rotary system to F5 (28); however, instrumentation technique was not mentioned in one study (26).

Moreover, six studies employed *Enterococcus faecalis (E.faecalis)* ATCC 29212 (15,20,24–27), four studies utilized *E. faecalis* ATCC 19433 (21–23,29), and one study utilized *E. faecalis* ATCC 47044 as a test microorganism (28). Along with *E. faecalis* ATCC 29212, both *F. nucleatum* ATCC 10953 and *P. intermedia* ATCC 25611 were used in one study (25).

Regarding the irrigation volume, duration, and type of equipment used, duration of irrigation was not mentioned in five studies (21,23,24,26,29). Meanwhile, one study has failed to report the irrigation time, volume, and equipment used for delivery (26). Irrigation was done using a 5-ml syringe with 19-gauge

**Table 4.** Bias assessment

| Number | Author, Year | Has randomization of samples done? | Does sample size calculation reported? | Does the standardization of all samples done? | Does the samples undergo cleaning and shaping procedure before bacterial inoculation? | Has all the samples sterilized before inoculation of microorganism? | Does the smear layer removal done in samples before inoculation? | Does the verification of inoculum done? | Has complete disinfection regimen explained in study? | Complete outcome assessment done? | Is there blinding reported? | Overall score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Dumani et al., 2016 (15) | Not reported | Not reported | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Not reported | 7 |
| 2 | Sedighshams et al., 2016 (24) | Yes | Not reported | Yes | Yes | Yes | Not reported | Yes | Not reported | Yes | Not reported | 6 |
| 3 | Souza et al., 2018 (21) | Yes | Not reported | Yes | Yes | Yes | Yes | Yes | Not reported | Yes | Not reported | 7 |
| 4 | Soligo et al., 2018 (29) | Yes | Not reported | Yes | Yes | Yes | Yes | Yes | Not reported | Yes | Not reported | 7 |
| 5 | Dalbello et al., 2018 (23) | Yes | Not reported | Yes | Yes | Yes | Yes | Not reported | Not reported | Not reported | Not reported | 5 |
| 6 | Shadmehr et al., 2019 (25) | Yes | Not reported | Yes | Not reported | Yes | Yes | Yes | Not reported | Yes | Not reported | 6 |
| 7 | Dumani et al., 2019 (20) | Yes | Not reported | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Not reported | 8 |
| 8 | Souza et al., 2020 (22) | Yes | Not reported | Yes | Yes | Yes | Yes | Yes | Not reported | Yes | Not reported | 7 |
| 9 | Subbiya et al., 2020 (26) | Not reported | Not reported | Yes | Yes | Yes | Not reported | Not reported | Not reported | Yes | Not reported | 4 |
| 10 | Kaur et al., 2020 (27) | Yes | Not reported | Yes | Yes | Yes | Not reported | Not reported | Not reported | Not reported | Not reported | 4 |
| 11 | Alfadda et al., 2021 (28) | Not reported | Not reported | Yes | Yes | Not reported | Yes | Not reported | Yes | Yes | Not reported | 5 |

needle using a 5-ml irrigant, which was replenished during chemo-mechanical preparation for 5 min in one study (22), while the duration was not mentioned in another study by the same authors (21); 5 ml of the solution using a syringe and a 30-gauge needle NaviTip were used in one study (29); in another study, irrigation was done for 10 min with 5 ml irrigant using a 27-gauge syringe (25). Irrigation was performed using disposable syringes having 30-gauge needle with 10 ml for each canal done in one study (24). In another study, the irrigation was done using 2 ml of each irrigant for 5 min (27). In two studies, syringe irrigation was performed using a side-vented needle tip, which was placed at 12 mm, and 5 ml of irrigant was delivered for approximately 2 min (15,20). One study has used $Ca(OCl)_2$ as intracanal medicament that was delivered into the canal using 1 ml of Luer Lock syringe with 20-gauge dispensing needle and incubated for 7 days in the incubator (28).

Evaluation was performed using percentage reduction in colony-forming units (CFU) between S1 and S2 in five studies (15,20–22,29), spectrophotometer was used in one study (25), and quantitative polymerase chain reaction (qPCR) was used in another study (24). Nine studies did evaluation by counting CFU (15,20–23,26–29). One study also used confocal laser scanning microscopic (CLSM) analysis for assessing the percentage of live bacteria (28).

### *Quality of the Included Studies*

Quality of the included studies was assessed using the methods adapted from previous systematic reviews on *in vitro* studies (18,19). One study reported having low risk of bias (20), whereas ten studies have moderate risk of bias (15,21–29) (Table 4). Randomization of the samples before undergoing the disinfection protocol were not given in three studies (15,26,28); however, the rest of the studies have only mentioned the randomization, and method has not been provided.

Sample size calculation was not provided in any of the studies, all included studies have standardized the sample before undergoing treatment procedure. Initial cleaning and shaping of the samples before sterilization and inoculation were not mentioned in one study (25).

Smear layer removal before inoculation of the samples was not reported in three studies (24,26,27). Sample sterilization before inoculation using autoclave was not mentioned in one study (28). Verification of the samples for the presence of inoculated microorganism has not been explained in four studies (23,26–28).Verification of microbial growth during biofilm growth period were done by randomly selecting a sample and checking for its presence in the respective culture media used in the studies. Complete disinfection protocol regarding the irrigation volume and duration as well as placement of irrigation needle within the root canal were explained only in three studies (15,20,28). Complete outcome assessment were not done in two studies (23,27). Both operator and evaluator blinding were not reported in any of the included studies.

## Discussion

Achieving successful disinfection of the root canal system is essential for a favorable endodontic treatment outcome. To reach this goal, instrumentation alone cannot reach the complexities within the root canal system; hence, a good antimicrobial irrigant is indispensable. $Ca(OCl)_2$ as a possible alternative irrigant to other established irrigants especially NaOCl has been studied for the past few years (15,20,24,25). Considering no *in vivo* studies have been reported for this irrigant, the current systematic review included the possible *in vitro* studies on antimicrobial efficacy of $Ca(OCl)_2$.

From the studies obtained, $Ca(OCl)_2$ was compared to NaOCl (15,20–27,29), chlorhexidine (25,26), grape seed extract (29), triple antibiotic paste, as well as calcium hydroxide in the form of medicament (28) and along with other adjunctive aids, such as photodynamic therapy (PDT) (21,22), sonic activation (15), and laser-activated irrigation (LAI) (20).

### *$Ca(OCl)_2$ vs. NaOCl as Irrigant*

Six studies reported a comparable antimicrobial efficacy of $Ca(OCl)_2$ with NaOCl (15,20–23,29); three studies reported better bactericidal efficacy (24,25,27), whereas one study did not find any remarkable antibacterial effect post irrigation of samples when compared with NaOCl as irrigant (26).

### *$Ca(OCl)_2$ vs. Chlorhexidine as Irrigant*

Chlorhexidine was used as an irrigant for comparison in two studies. One study reported similar antimicrobial efficacy compared to $Ca(OCl)_2$ (25), whereas one study did not find a remarkable antibacterial reduction post irrigation of samples (26).

### *$Ca(OCl)_2$ vs. Grape Seed Extract*

Soligo et al. (29) evaluated the antimicrobial efficacy of 50% grapeseed extract and 6% $Ca(OCl)_2$ irrigants used with both rotary and reciprocation systems. They found

good antibacterial efficacy in all groups regardless of the instrumentation techniques used with no significant difference among them.

### *Ca(OCl)$_2$ as Medicament*

Alfadda et al. (28) used Ca(OCl)$_2$ as medicament and compared against calcium hydroxide and triple antibiotic paste for evaluating its application in regenerative endodontic procedure. They used human mandibular premolars, which were instrumented till F5 to simulate immature root apex, which is different from the tooth models from the rest of the included studies (15,20–27,29). They found that Ca(OCl)$_2$ medication improved root canal disinfection from the main canal and dentinal tubules but had lower cellular viability compared to triple antibiotic paste and calcium hydroxide medicaments. However, by using 10% ascorbic acid as final rinse, it could neutralize the cytotoxic effects of Ca(OCl)$_2$.

### *Other Adjunctive Aids*

Ca(OCl)$_2$ irrigant has been used along with other adjunctive aids such as PDT (21,22), sonic activation (15), and LAI (20), and results showed that this combination with aids helped in improving the overall microbial reduction in root canal system.

Apart from the antimicrobial efficacy, few studies in literature have evaluated other properties of Ca(OCl)$_2$ such as its tissue dissolving ability (12,34), smear layer removal efficacy (35), pH, and available chlorine content (10). Dutta and Saunders reported the potential of Ca(OCl)$_2$ solutions to dissolve bovine muscle tissue. According to their study, there is no difference in tissue dissolution among 5.25% NaOCl and 5% or 10% Ca(OCl)$_2$ solutions after 35–60 minutes of testing (12). Taneja et al. (34) demonstrated that Ca(OCl)$_2$ solutions can dissolve pulp tissue, and their efficiency gradually increased with the time of exposure and concentration. According to Cardoso et al. (35), Ca(OCl)$_2$, when used alone, was not able to remove smear layer, and the effect is similar to NaOCl. Thus, for efficient smear layer removal, EDTA has to be used along with them. Carlotto et al. (10) further evaluated the available chlorine content, pH, and surface tension of Ca(OCl)$_2$ and NaOCl. It was demonstrated that more chlorine content were liberated along with higher surface tension property due to the high alkalinity of Ca(OCl)$_2$ as compared to NaOCl.

### *Limitations of This Review*

Studies included in this review have compared the irrigants against *E. faecalis* (15,20–29). *E. faecalis* has been found to be associated with persistent endodontic infection and treatment failures; they are known to survive tough environmental conditions (36). Studies in this review have shown an overall positive antimicrobial efficacy of Ca(OCl)$_2$ against *E. faecalis.* However, endodontic infection has been associated with different types of microorganisms; a polymicrobial biofilm evaluation has been determined to have a more clinical relevance.

The disinfection protocol was not completely mentioned in majority of the studies; volume, duration, gauge and type of needle, depth of insertion of needle within the root canal, and the root canal preparation were noted to vary between the studies (21–27,29). Thus, standardization in the disinfection protocol is essential to obtain an unbiased outcome. Some studies performed only irrigation and did not instrument the root canal after inoculation of bacteria, which might possibly affect the results obtained from these studies, since both instrumentation and irrigation are essential for bacterial reduction (15,20,25,28).

During chemomechanical preparation, large volume of irrigant with frequent irrigant replacement is essential for optimum antimicrobial effect (34). In clinical scenario, presence of organic matter, inflammatory exudates, and microbial biomass can weaken the effect of irrigants. The chlorine ion in NaOCl is rapidly consumed within 2 min during tissue dissolution; thus, frequent irrigant replacement is required, and an increased time of contact of at least 30 minutes within root canal is essential for increased efficacy throughout the treatment procedure (37,38). During syringe irrigation, syringe with a capacity of <5 ml and 30 gauge side-vented needle are ideal to avoid excess pressure on canal walls and prevent accidental spills (38). Root canal should be enlarged to a minimum 30/35 size, with increased taper required to ensure irrigant flow till working length at a flow rate of approximately 0.25 ml/s. The needle must be placed within 1 mm from working length for close-ended and 2–3 mm from working length for open-ended needle to ensure optimum irrigant delivery (38,39).

Smear layer removal protocol before bacterial inoculation was not reported in three studies (24,26,27). Its removal has been identified to be essential because the presence of smear layer could prevent the penetration of microorganisms into the root canal and dentinal tubules, which could affect the efficacy of the disinfection protocol.

Complete outcome assessment was not conducted in two studies (23,27) because they only evaluated the CFU of samples after disinfection protocol and compared it with control groups. Comparison between CFU after biofilm formation and after disinfection protocol in the same group helps in assessing the degree of bacterial reduction and effectiveness of disinfection protocol. These studies

were not excluded as they have compared the CFU of study groups with control groups.

The concentration of $Ca(OCl)_2$ varied between studies, but two studies have reported the minimum inhibitory concentration (MIC) of $Ca(OCl)_2$ and assessed bactericidal efficacy based on the MIC values (24,25). Seddigshams et al. found the MIC values of NaOCl and $Ca(OCl)_2$ against *E. faecalis* were 0.5% and 5%, respectively. Based on their findings, 5% $Ca(OCl)_2$ had better antibacterial efficacy and least cytotoxicity compared with 0.5% NaOCl. Meanwhile, Shadmehr et al. evaluated the MIC of $Ca(OCl)_2$ against *E. faecalis*, *F. nucleatum*, and *P. intermedia* to be 25, 8, and 7.5 µg/mL, respectively. They reported that 5% $Ca(OCl)_2$ had better bactericidal efficacy than 5.25% NaOCl.

Most of the evaluations were done by counting CFU. However, advanced microscopic techniques like CLSM have to be utilized as biofilm structure and cell viability are examined using appropriate stains, composition, and metabolism in several different microorganisms. One of the biggest advantages of CLSM is that it allows in-depth analysis of biological structures, without killing or damaging it (40).

Main limitations of this review are as follows: all the studies obtained were *in vitro*, methodologies were varied, and no operator/evaluator blinding was reported in all studies. Even though random allocation of samples was mentioned, the method was not described (20–25,27,29). Randomization and allocation concealment helps in preventing selection bias, and blinding of operator/evaluator is essential to avoid bias in outcome assessment, thus improving the quality of evidence synthesized.

More future *in vitro* and clinical studies should be conducted regarding the antibacterial property, pulp tissue dissolving efficacy, effect on dentin structure, concentration, biocompatibility of $Ca(OCl)_2$ following a standardized protocol to ensure reliability in outcomes obtained.

Within the limitations of this systematic review, the studies are mostly of high to moderate quality, and the evidence obtained from the included literature indicates that $Ca(OCl)_2$ has good antimicrobial efficacy, which is almost comparable to the antimicrobial property of NaOCl in irrigant form. Further *in vivo* and *in vitro* studies are required to assess the applicability of $Ca(OCl)_2$ in routine endodontic practice.

**Authorship Contributions:** Concept: R.R., S.S., V.M.S.; Design: R.R., S.S., V.M.S.; Supervision: R.R., S.S., V.M.S.; Data: R.R., S.S., V.M.S.; Analysis: R.R., S.S., V.M.S.; Literature search: R.R., S.S., V.M.S.; Writing: R.R., S.S.; Critical revision: S.S., V.M.S.

**Source of Funding:** None declared.

**Conflict of Interest:** None declared.

## References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965; 20: 340–9. [CrossRef]
2. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod 2010; 36: 1277–88. [CrossRef]
3. Siqueira JF Jr, Lima KC, Magalhães FA, Lopes HP, de Uzeda M. Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. J Endod 1999; 25: 332–5. [CrossRef]
4. Peters OA, Arias A, Paqué F. A micro-computed tomographic assessment of root canal preparation with a novel instrument, TRUShape, in mesial roots of mandibular molars. J Endod 2015; 41: 1545–50. [CrossRef]
5. Siqueira JF Jr, Alves FR, Versiani MA, *et al*. Correlative bacteriologic and micro-computed tomographic analysis of mandibular molar mesial canals prepared by self-adjusting file, reciproc, and twisted file systems. J Endod 2013; 39: 1044–50. [CrossRef]
6. Arias-Moliz MT, Ordinola-Zapata R, Baca P, Ruiz-Linares M, Ferrer-Luque CM. Antimicrobial activity of a sodium hypochlorite/etidronic acid irrigant solution. J Endod 2014; 40: 1999–2002. [CrossRef]
7. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dent Clin North Am 2010; 54: 291–312. [CrossRef]
8. Tanomaru Filho M, Leonardo MR, Silva LA, Aníbal FF, Faccioli LH. Inflammatory response to different endodontic irrigating solutions. Int Endod J 2002; 35: 735–9. [CrossRef]
9. Santos JN, Carrilho MR, De Goes MF, *et al*. Effect of chemical irrigants on the bond strength of a self-etching adhesive to pulp chamber dentin. J Endod 2006; 32: 1088–90.
10. Leonardo NG, Carlotto IB, Luisi SB, Kopper PM, Grecca FS, Montagner F. Calcium hypochlorite solutions: evaluation of surface tension and effect of different storage conditions and time periods over pH and available chlorine content. J Endod 2016; 42: 641–5. [CrossRef]
11. Whittaker HA, Mohler BM. The sterilization of milk bottles with calcium hypochlorite. Am J Public Health (N Y) 1912; 2: 282–7. [CrossRef]
12. Dutta A, Saunders WP. Comparative evaluation of calcium hypochlorite and sodium hypochlorite on soft-tissue dissolution. J Endod 2012; 38: 1395–8. [CrossRef]
13. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. J Endod 2004; 30: 785–7. [CrossRef]
14. Cobankara FK, Ozkan HB, Terlemez A. Comparison of organic tissue dissolution capacities of sodium hypochlorite and chlorine dioxide. J Endod 2010; 36: 272–4. [CrossRef]
15. Dumani A, Guvenmez HK, Yilmaz S, Yoldas O, Kurklu

ZG. Antibacterial Efficacy of Calcium Hypochlorite with Vibringe Sonic Irrigation System on Enterococcus faecalis: An In Vitro Study. Biomed Res Int 2016; 2016: 8076131.
16. Cecchin D, Soares Giaretta V, Granella Cadorin B, Albino Souza M, Vidal CMP, Paula Farina A. Effect of synthetic and natural-derived novel endodontic irrigant solutions on mechanical properties of human dentin. J Mater Sci Mater Med 2017; 28: 141. [CrossRef]
17. Blattes GB, Mestieri LB, Böttcher DE, Fossati AC, Montagner F, Grecca FS. Cell migration, viability and tissue reaction of calcium hypochlorite based-solutions irrigants: An in vitro and in vivo study. Arch Oral Biol 2017; 73: 34–9. [CrossRef]
18. Lim BSH, Parolia A, Chia MSY, Jayaraman J, Nagendrababu V. Antimicrobial efficacy of QMix on Enterococcus faecalis infected root canals: a systematic review of in vitro studies. Restor Dent Endod 2020; 45: e23. [CrossRef]
19. Bhandi S, Mehta D, Mashyakhy M, *et al*. Antimicrobial efficacy of silver nanoparticles as root canal irrigant's: a systematic review. J Clin Med 2021; 10: 1152. [CrossRef]
20. Dumani A, Tanrisever D, Sihay D, Kuzu SB, Yilmaz S, Guvenmez HK. Efficacy of calcium hypoclorite with and without Er,Cr: Yttrium, scandium, gallium, garnet laser activation on Enterococcus faecalis in experimentally infected root canals. Niger J Clin Pract 2019; 22: 215–20.
21. Souza MA, Tumelero Dias C, Zandoná J, *et al*. Antimicrobial activity of hypochlorite solutions and reciprocating instrumentation associated with photodynamic therapy on root canals infected with Enterococcus faecalis - An in vitro study. Photodiagnosis Photodyn Ther. 2018; 23: 347–52.
22. Souza MA, Lago BLT, Pletsch A, *et al*. Association of calcium hypochlorite, reciprocating instrumentation and photodynamic therapy: Antimicrobial analysis and effects on root dentin structure. Photodiagnosis Photodyn Ther 2020; 29: 101625. [CrossRef]
23. Dal Bello Y, Mezzalira GI, Jaguszewski LA, *et al*. Effectiveness of calcium and sodium hypochlorite in association with reciprocating instrumentation on decontamination of root canals infected with Enterococcus faecalis. Aust Endod J 2019; 45: 92–7. [CrossRef]
24. Sedigh-Shams M, Gholami A, Abbaszadegan A, *et al*. Antimicrobial efficacy and cytocompatibility of calcium hypochlorite solution as a root canal irrigant: an in vitro investigation. Iran Endod J 2016; 11: 169–74.
25. Shadmehr E, Davoudi A, Sarmast ND, Saatchi M. Evaluation of antimicrobial efficacy of calcium hypochlorite as an endodontic irrigant on a mixed-culture biofilm: an ex vivo study. Iran Endod J 2019; 14: 178–84.
26. Padmavathy K, Subbiya A, Madan N, *et al*. Evaluation of irrigant-intracanal medicament regimen against Enterococcus Faecalis biofilm count. Eur J Mol Clin Med 2020; 7: 1219–24.
27. Kaur G, Kumar Reddy TV, Venkatesh KV, Mahalakshmi K. Effects of chitosan oligosaccharide and calcium hypochlorite on E. Faecali dentinal biofilm and smear layer removal - SEM analysis. Indian J Dent Res 2020; 31: 550–6.
28. Alfadda S, Alquria T, Karaismailoglu E, Aksel H, Azim AA. Antibacterial effect and bioactivity of innovative and currently used intracanal medicaments in regenerative endodontics. J Endod 2021; 47: 1294–300. [CrossRef]
29. Soligo LT, Lodi E, Farina AP, Souza MA, Vidal CMP, Cecchin D. Antibacterial efficacy of synthetic and natural-derived novel endodontic irrigant solutions. Braz Dent J 2018; 29: 459–64. [CrossRef]
30. de Almeida J, Cechella BC, Bernardi AV, de Lima Pimenta A, Felippe WT. Effectiveness of nanoparticles solutions and conventional endodontic irrigants against Enterococcus faecalis biofilm. Indian J Dent Res 2018; 29: 347–51.
31. Gómez C, Salcedo-Moncada D, Ayala G, *et al*. Antimicrobial efficacy of calcium and sodium hypochlorite at different concentrations on a biofilm of Enterococcus faecalis and Candida albicans: an in vitro comparative study. J Contemp Dent Pract 2020; 21: 178–82. [CrossRef]
32. Paula KB, Carlotto IB, Marconi DF, Ferreira MBC, Grecca FS, Montagner F. calcium hypochlorite solutions - an in vitro evaluation of antimicrobial action and pulp dissolution. Eur Endod J 2019; 4: 15–20. [CrossRef]
33. Soligo LT, Cecchin D, Souza MA, Gabrielli ES, Palhano HS, Farina AP. Assessment of antimicrobial activity of sodium hypochlorite, calcium hypochlorite and grape seed extract against Enterococcus faecalis. Rev Odonto Ciênc 2017; 32: 136–9. [CrossRef]
34. Taneja S, Mishra N, Malik S. Comparative evaluation of human pulp tissue dissolution by different concentrations of chlorine dioxide, calcium hypochlorite and sodium hypochlorite: An in vitro study. J Conserv Dent 2014; 17: 541–5.
35. Cardoso LR, Baldasso FER, Delai D, Montagner F, Kopper PMP. Effect of EDTA, sodium, and calcium hypochlorite on the inorganic component of root canal dentin: A SEM analysis. Microsc Res Tech 2019; 82: 128–33. [CrossRef]
36. Rôças IN, Siqueira JF Jr, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004; 30: 315–20. [CrossRef]
37. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. Int Endod J 1982;15:187–96. [CrossRef]
38. Basrani B. Endodontic irrigation. Chemical Disinfection of the Root Canal System. Switzerland: Springer; 2015.
39. Boutsioukis C, Kastrinakis E, Lambrianidis T, Verhaagen B, Versluis M, van der Sluis LW. Formation and removal of apical vapor lock during syringe irrigation: a combined experimental and Computational Fluid Dynamics approach. Int Endod J 2014; 47: 191–201. [CrossRef]
40. Mallya L, Mala K, Jathanna V. Confocal LASER scanning microscopy (CLSM) for evaluation of endodontic microflora-a review. Indian J Public Health Res Dev 2019; 10.