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Comparative evaluation of antibacterial efficacy of SDF and NaOCl as Root canal irrigants against the bacterial strain of enterococcus faecalis – An *In vitro* study

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Abstract
Background: For successful root canal treatment, elimination of microorganisms during endodontic treatment is the key feature. This is achieved through mechanical cleaning and shaping in conjunction with irrigating agent. Irrigation of the root canal is essential for effective elimination of bacteria. The aim of this study was to evaluate the antimicrobial efficacy of Silver Diamine Fluoride (SDF) and Sodium Hypochlorite (NaOCl) against E. faecalis.
Methods: The access opening was done on extracted 50 human teeth and canal orifices was enlarged with the help of orifice opener. The root canal was inoculated with E. faecalis using a sterile micropipette and incubated at 37°C for 24 h. Biomechanical preparation was done using Universal hand K files 20 no. and irrigation was done with saline. The contaminated teeth were divided into two groups: in a Group A, 25 sample were irrigated with 3.8% SDF and in Group B, 25 sample were irrigated with 3% NaOCl. The canals were irrigated for 10 minutes with 2 ml of respective irrigation solutions. A final flush with saline to avoid long-term action of the respective irrigating solutions. The paper points were left in the wet canal and then transferred to the tubes containing the peptone water. The specimens were kept at 37°C for 48 h. In order to check the growth for E. faecalis in the tubes, samples were inoculated onto chocolate agar plates. Colonies were counted using colony counter.
Results: For E. faecalis, the greatest bacterial reduction was reported in group SDF, which showed significantly greater reduction in comparison to the other group NaOCl (p value < 0.05).
Conclusion: The endodontic irrigating solutions that we tested showed significant differences in their effectiveness against E. faecalis. The present study confirmed the *in vitro* antimicrobial efficacy SDF, which was higher than NaOCl.

Keywords: Silver diamine fluoride (SDF), sodium hypochlorite (NaOCl), enterococcus faecalis, antimicrobial, irrigation

Introduction

Effective root canal treatment (RCT) depends on appropriate instrumentation, irrigation, and obturation. The elimination of microorganisms in the root canal during endodontic treatment is the key feature for successful treatment. This is achieved through mechanical cleaning and shaping in conjunction with irrigating agent. [1]

Endodontic infection is a polymicrobial infection and obligate anaerobic bacteria are the main cause of pulpal and periapical inflammation in primary infections. Anaerobic bacteria, especially Gram-negatives, have been linked to the endodontic disease but Gram-positive facultative anaerobic microorganism, such as Enterococcus faecalis (E.faecalis), have also been isolated from pathologically involved root canals and a possible cause of chronic recurrent periapical infection and failed endodontic therapy. E.faecalis can survive for a long time, attach to dentin, attack on dentinal tubules and develop a biofilm under various environmental conditions and survive difficult conditions within endodontically treated teeth. However, conventional disinfectants and medicaments have not been able to achieve and maintain sterilization of root canals. [2] Complete bacterial elimination does not appear to be possible with traditional hand instrumentation or with newer rotary NiTi-systems [3].

Irrigation of the root canal is essential for effective elimination of bacteria. An irrigant serves to flush out debris from within the instrumented root canals, dissolve organic tissue remnants, disinfect the root canal space and provide lubrication during instrumentation, without causing irritation to biological tissues. The use of irrigating solutions is an important part of effective chemo-mechanical preparation. Therefore, several irrigating solutions have been recommended in combination with canal preparation. [4] Based on mechanism of action the irrigating solutions are Sodium Hypochlorite (NaOCl 5.25%), Chlorhexidine (CHX, 2%), Iodine, Hydrogen peroxide, Ethylene Diamine Tetra Acetic Acid (EDTA, 17%), Citric Acid (10-50%), Mixture of Tetracycline Acid and Detergent (MTAD), Tetraclean. The American Endodontics Association (AEA) has defined NaOCl as yellowish-green, pale and extremely alkaline liquid. NaOCl is most effective 'gold standard irrigant'. NaOCl has been accepted as the endodontic irrigant because of its antimicrobial and tissue dissolving properties. [5]

In the present study, Silver Diamine Fluoride (SDF) is used as irrigating material because of its anticariogenic and high fluoride releasing property; it has been proposed to be a very effective agent, especially in Paediatric dentistry. It has also been used in endodontic medicaments by Mathew, Madhusudhana, Sivakumar, Venugopal, & Reddy (2012). SDF as an endodontic irrigant can effectively remove the microbes present in the canal and circumpulpal dentin. [2] SDF contains fluoride and silver ions; both of which have ability to inhibit the formation of cariogenic biofilms. Silver ions have antibacterial action which penetrates and destroys the bacteria cell wall, inhibits enzymatic activity thus

influences metabolic processes, and inhibit the replication of bacterial DNA. [1]

In the present study, the antibacterial effect of the 3.8% SDF solution was used to check the elimination of single-species E.faecalis model using colony forming units (CFU) in the microbiology lab.

METHODS

In the present study, Data was collected and analyzed statistically with Chi square test. The study was carried out in the Department of Pedodontics and Preventive dentistry of Ahmedabad Dental College and Hospital and in the department of microbiology, Sola civil hospital, Gujrat, India from August 2021 to September 2022. After obtaining the ethical clearance from the institutional review board. The ethical clearance has been given by the ethical committee from the institutional review board. A total of 50 extracted permanent single rooted teeth was selected for the study based on inclusion and exclusion criteria. The antimicrobial activity of E.faecalis in root canal was analyzed by comparing the presence and absence of CFU of bacteria.

a. The criteria for selection of teeth to perform the study was done by no prior endodontic treatment, with no fracture lines, extracted due to orthodontic purpose or periodontal reasons, no deep carious lesion with furcation involvement, no calcified canals, no anatomical irregularities or curvatures. The present study was carried out in the department of microbiology, Sola civil hospital using incubator. Airotor, large round bur, universal hand K files (No.15, No. 20), 27-gauge needle & 2 ml disposable syringe, disposable glass, tweezer, paper point, micropipette, inoculating loop & holder, incubator were used for study. Material used for study were 3.8% silver diamine fluoride (Kids E), 3% sodium hypochlorite, peptone water, chocolate agar and saline.

The apex of the teeth was sealed with wax to prevent leakage of irrigant through apical opening. Conventional access opening was done with high speed airotor and no.6 round bur (Fig 1). Canal orifices were enlarged with the help of orifice opener. Pure culture of E.faecalis ATCC 29212, grown in Brain Heart Infusion broth (BHI-Difco1) was used to contaminate the root canals. The root canals were inoculated with 10 ml of a 1 x $10^{[8]}$ CFU ml $^{[-1]}$ suspension of E.faecalis using a sterile micropipette (Fig 2). Teeth were incubated inside the incubator at 37.8°C for 24 h.

After 24 h, initial filing was done using Universal hand K files no.15 & 20 and canals were irrigated with saline (Fig 3). After that, the samples were randomly divided into two groups according to the irrigation regimen used.

GROUP A- Twenty-five root canals were irrigated with 2 ml of 3.8% SDF.

GROUP B- Twenty-five root canals were irrigated with 2 ml of 3% NaOCl.

A 3.8% SDF solution was formulated for intracanal irrigation. This solution was a 1:10 dilution of the initial 38% SDF solution that had been utilized for root canal disinfection. A 27-gauge needle was attached to sterile 2 ml disposable syringe. Needle was inserted into the root canal until it binds, and was then withdrawn approximately 1mm so that it was no longer in contact with the root canal walls. 2 ml of irrigant was delivered which remained in the canal for 3 minutes. A final flush with 2 ml of saline was done to avoid long-term action of the respective irrigating solutions. The paper point was left in the wet canal for 1 minute and then transferred to the test tubes containing 5 ml of the peptone water (Fig 4). The specimens were then incubated at 37°C for 48 h in the incubator. In order to check the growth of E.faecalis in the tubes, samples were inoculated onto chocolate agar plates (Fig 5). Colonies were counted using colony counter after 24 h.

Statistical analysis

Chi-square test was used for statistical analysis; p values ≤ 0.05 were considered statistically significant. All collected data were at the graph and table.

Fig 1: Access opening

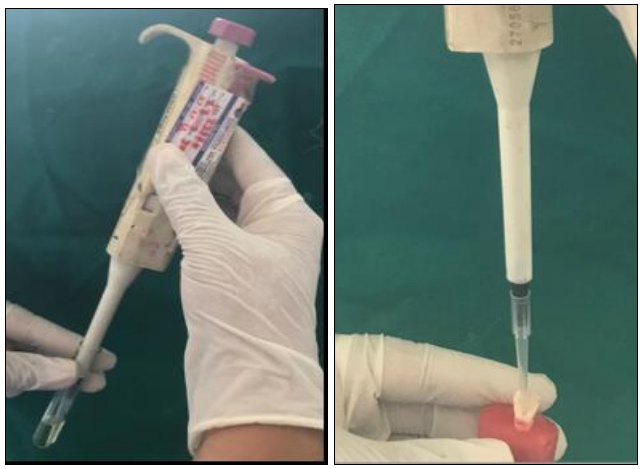


Fig 2: Inoculation of E.faecalis in root canals using a sterile micropipette

Fig 3: Initial filing done and irrigation with respective irrigating solution

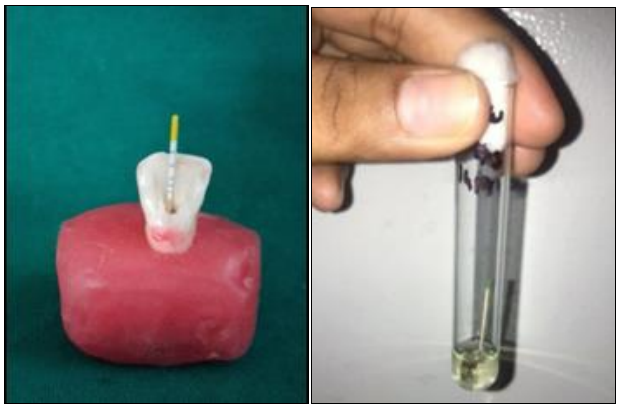
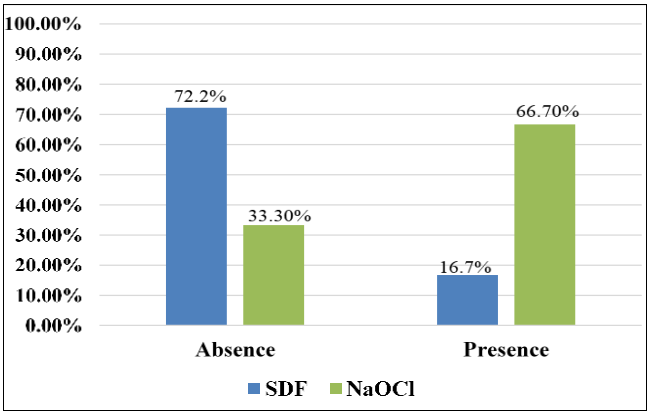


Fig 4: The paper points were left in the wet canal and then transferred to peptone water

Fig 5: Incubator and E.faecalis CFU on chocolate agar plate

Graph and Table



Graph 1: The effects of different antimicrobial solutions against E.faecalis after the 15- and 60-minute exposure times

Results

The main purpose of this investigation was to check antimicrobial efficacy of irrigants used in root canal. The antimicrobial activity of E.faecalis in root canal was analyzed by comparing the presence and absence of CFU of bacteria. Data was collected and analyzed statistically with Chi square test; with a level of significance set at 5%.

In Group A (3.8% SDF), most samples have shown absence of E.faecalis CFU in the canal (72.2%). While some samples have shown presence of E.faecalis CFU in the canal (16.7%). In Group B (3% NaOCl), 33.3% samples have shown absence of E.faecalis CFU. While majority samples (66.7%) have shown presence of E.faecalis CFU in the canal (Graph 1).

On comparing the two groups, a significant difference was observed (p value < 0.05). The results showed that reduction in bacterial count was seen more in SDF group as compared to NaOCl group (Graph 1) (Table 1).

Table 1: Result of microbial count reduction

Groups	E. faecalis CFU		*p* value
	Absence	Presence	
Group A (3.8 %SDF)	72.2%	16.7%	0.00*
Group B (3% NaOCl)	33.3%	66.7%	
* Significant (p value ≤0.05; Chi square test)			

Discussion

Irrigation of root canals is imperative for debridement of infected root canals to remove necrotic tissue remnants, microorganisms and smear layer, which is created during mechanical canal instrumentation. Irrigating solutions are considered to be essential for a successful endodontic treatment. [7] The present study compared the effect of various irrigants 3.8% SDF and 3% NaOCl for antimicrobial efficacy when used as root canal irrigant. Nowadays, use of SDF as an irrigant has also been proposed. But there are not enough studies, that conducted to evaluate the SDF as irrigating solution.

SDF is a promising material having useful applications in clinical dentistry. It is a colourless, alkaline (pH ~10) agent which has been used for 50 years in dentistry. SDF is considered as a safe and effective antimicrobial with a potential to prevent dental caries, has a role in the inhibition of oral biofilm formation and treatment of hypersensitivity. [8] In the 1960s, Japan approved the use of SDF as a therapeutic agent to arrest dental caries in children. [9] In 2014, Food and Drug Administration (FDA) cleared the first use of SDF to manage early childhood caries, prevent pits and fissure caries in erupting permanent molars, prevent root caries in the elder patient, sterilize infected root canals, and treat dentinal hypersensitivity. Being a painless modality, it is particularly indicated in uncooperative patients and children. [8]

In the present study a 3.8% SDF solution was prepared for intracanal irrigation. This solution was a 1:10 dilution of the initial 38% SDF solution that has been utilized for root canal disinfection. Similar formulation was used by Hiraishi, Yiu, King, Tagami, Tay *et al* (2010) [10] and they reported that SDF discolours radicular dentin but in the present study it showed no discoloration in root canal.

Walker *et al* [11] was the first researcher to think of NaOCl as an irrigant and it is the most widely used irrigation solution in endodontics. In endodontic therapy, NaOCl solutions are used in concentrations varying from 0.5% to 5.25%. [4] Nowadays, NaOCl is the most preferred irrigating solution, because of its properties such as excellent antibacterial action, a long shelf life, easy availability and low cost. [2] The tissue-dissolution capability and the antimicrobial efficiency as well as the toxicity of NaOCl is dependent on the concentration of the solution. Berber VB *et al* (2007), [12] Giardino L (2007) [13] suggested that 5.25% NaOCl have greater antibacterial activity inside the infected root canal with E.faecalis. The higher the concentration (5.25% NaOCl) of solution, greater the cytotoxicity (Mehdipour *et al*;2007, Silva *et al*;2009) [14, 15] therefore, lower concentration of NaOCl (3%) has been used in the present study.

E.faecalis was used in the present study because it shows a low prevalence in primary endodontic infections (4–40%) and a high prevalence in persistent infections (24–77%) (Baca P, Junco P, Arias-Moliz, González-Rodríguez, Ferrer-Luque;2011). [16] E.faecalis has the capability to survive and resist treatment with the commonly used irrigating solutions. It has a dentinophilic nature that allows it to bind to the dentin surface and permeate up to 400 µm, proliferate, and recolonize in the root canal system (Mathew *et al*;2012, Bukhary S, Balto H;2017). [6, 17] E.faecalis is a major pathogen associated with chronic recurrent periapical infection and failed endodontic therapy. [6] In the present study we used ATCC strain of E.faecalis because it was also utilized in previous *in vitro* studies investigating the antibacterial effects of endodontic irrigants by Siqueira *et al* [1997, 1998], [18, 19] Gomes *et al* (2001). [20] Persistence of E.faecalis into the endodontic environment is known to be one of the main reasons for pulp therapy failure.

In the present study, efficacy of 3.8% SDF and 3% NaOCl was compared and evaluated for reduction of E.faecalis count. Pure culture of E.faecalis ATCC 29212, grown in Brain Heart Infusion broth (BHI-Difco1), was used to contaminate the root canals using a sterile micropipette, similar criteria was used by Hiraishi, Yiu, King, Tagami, Tay (2010). [10] The antimicrobial activity of irrigating material on extracted teeth infected with E.faecalis was analysed by comparing the count of bacteria. The results showed that irrigation with 3.8% SDF was more efficient than 3% NaOCl for reduction of E.faecalis from the root canal. Gomes *et al* (2001) [20] conducted a study of antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of E.faecalis. All irrigants were effective in killing E.faecalis, but at different times. Al-Madi *et al* (2019) [2] evaluated the antibacterial efficacy of 3.8% SDF in comparison with 2% CHX against E.faecalis biofilm. SDF possessed higher antimicrobial activity than 2% CHX against E. faecalis biofilms. Nara A *et al* (2010) [21] concluded that MTAD was more effective than 3% NaOCl and Propolis against E. faecalis. Results of these studies are in accordance to the present study.

This study supports the hypothesis that thorough use of root canal irrigants can efficiently remove bacteria from the root canals which is the key for successful root canal treatment. Based on CFU count, the present study helps in choosing an appropriate irrigant that can ensure complete removal of bacteria from the root canals. The conclusion drawn from the present study is that 3.8% SDF has greater antimicrobial property as an intracanal irrigant than NaOCl in remaining E.faecalis from root canals.

Declarations

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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