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Research Article

Comparative Evaluation of Antimicrobial Efficacy of Oxum Solution and Other Endodontic Irrigants Against Enterococcus Faecalis - An *In vitro* Study

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Abstract

This study was done to test the antimicrobial efficiency of four irrigating solutions such as chlorhexidine (CHX), sodium hypochlorite (NaOCl), Bio pure MTAD, Oxum, against the *E. Faecalis* microorganisms in root canals. Fifty freshly extracted single- rooted human teeth were selected. They were enlarged to a size 45 k - File (Maillefer). Instrumentation was followed by irrigation with 5 ml of above irrigating solutions for each file used. 20µl of the suspension was inoculated into each of root canals. After 48 hr of incubation at 37°C, microbial growth was verified, confirming the contamination of the root canals. After the instrumentation, the sampling was done. The specimens were incubated at 37°C for 7 days. Within the limitations of this study it was concluded that MTAD significantly reduced the number of *E. Faecalis* followed by chlorhexidine and sodium hypochlorite and least reduction was observed in oxum.

Keywords: Antimicrobial Irrigating Solutions; Chlorhexidine; *E. Faecalis*; MTAD; Oxum

Introduction

Endodontics is a clinical discipline concerned with the elimination of micro-organisms and control of the root canal infection. In 1890, when miller first observed microbial growth associated with pulp tissue since then the contribution of microorganisms to the development of pulpal and periapical disease has been well documented [1-3].

Microbes in the root canals can grow not only as planktonic cells or in aggregates, but they can also form biofilms consisting of a complex network of different microorganisms [4]. The bacteria associated with primary endodontic infections are mixed but are predominantly gram-negative anaerobic rods, whereas the bacteria associated with secondary infection comprise only one or a few bacterial species—the most important of which is *Enterococcus faecalis* [5]. It has the ability to penetrate dentinal tubules, and the smear layer enhances the adhesion of *E. faecalis* and provides a matrix for deeper bacterial infection, thus influencing the effect of root canal irrigant on bacterial removal [6]. Therefore, *E. faecalis* biofilm has strong viability and drug resistance.

Due to the complexities of root canal system the bacteria located in areas such as isthmuses, ramifications, deltas, irregularities, and dentinal tubules will not be eliminated by mechanical means alone [7]. Therefore the use of an antimicrobial irrigant is absolutely indicated.

One of the important requirements of an ideal irrigant is its ability to eliminate microorganisms from the root canal system [8]. This antimicrobial effect can be a direct chemical effect or indirectly by facilitating the mechanical disinfection through lubrication, tissue dissolving, and flushing of contaminated debris accumulated during root canal preparation.

Different antimicrobial agents have been introduced for disinfecting the root canal system, all these antimicrobials have certain disadvantages such limited antimicrobial activity, non selectivity for host cells, inability to penetrate into dentinal tubules, and risk of toxicity and allergy to the patients. Therefore no ideal antimicrobial is available.

BioPure MTAD (Dentsply International, York, PA) is one of the rinses. It has the ability to remove the smear layer and also exert a potent antimicrobial activity. Although citric acid and Tween-80 have an impact on the action of BioPure MTAD, doxycycline is the primary ingredient contributing to its antimicrobial activity.

Chlorhexidine gluconate (CHX) is a potent antimicrobial agent [9], holds substantivity and has a low grade toxicity [10]. CHX is bacteriostatic at low concentrations (0.2%), bactericidal at high concentrations (2%), and adsorbs to dental tissue resulting in its prolonged gradual release at therapeutic levels [11]. However, chlorhexidine is unable to dissolve pulp tissue and debris may remain on canal walls, obstructing the dentinal tubules.

Microcyn (oxum) is a super-oxidized solution with a neutral Ph and a powerful anti-microbial agent against bacteria, fungi, protozoa and viruses. It is rich in reactive oxygen with a neutral pH. The main advantage of this super-oxidized water is that it is stable and has a longer shelf life. This is a hypotonic solution with an osmolarity of 13 mOsm/KG It mainly contains, sodium hypochlorite, hypochlorous acid, hydrogen peroxide, ozone, chlorine dioxide, sodium hydroxide, sodium carbonate and sodium chloride [12].

Aim of the study

To determine the antimicrobial efficacy of chlorhexidine, sodium hypochlorite, MTAD, oxum, in root canals infected by Enterococcus faecalis.

Materials and Methods

This in-vitro study was performed in the Department of Conservative Dentistry and Endodontics, M.R Ambedkar Dental College and Hospitals, Bangalore in the year 2017. This study was approved by an institutional ethics committee. The methodology used in the present study was modified slightly from that described previously by Srikumar and varma [13].

50 intact single rooted human teeth, extracted for orthodontic and periodontics reasons were collected and carefully cleaned with curette to remove the soft tissue remnants. The teeth were stored in 0.9% physiologic saline to avoid dehydration. The samples were radiographically analyzed to ensure a single patent canal. The coronal parts of all the teeth were sectioned with a high speed diamond disk and the root lengths were standardized to 14 mm. The working length of each root canal was performed by passing a 10 k file till its tip was visible at the apex. The cleaning and shaping of the root canal was done using K- files from number 10 to number 45 upto the working length and to number 6o in a step-back method. The canal was irrigated with EDTA and NaOCl during the shaping and cleaning procedure. A final intracanal rinse was done with saline.

Sterilization

Once the samples were prepared they were thoroughly rinsed with saline and sterilized by an autoclave at 121 °C at 15 lbs for 1 5 minutes. The teeth were stored in a BHI broth to ensure that there is no previous microbial contamination. The customized tooth root holder was cold sterilized using 2% glutaraldehyde

The samples were divided into five groups (n = 10) as follows:

- Group A: Chlorhexidine 2%
- Group B: Sodium hypochlorite (NaOCl) 5.0%
- Group C: Biopure MTAD
- Group D: Super oxidized water (Oxum™)
- Group E: Isotonic saline as negative control Experimental Setup.

Ten sockets were made on the tooth root holder according to the five groups. The apical end of all the samples was closed using a gutta percha stick so as to avoid leakage of any irrigant during the procedure. The apically sealed samples were then placed in the sockets of the tooth root holder according to the pre-determined groups.

Preparation of the inoculums

Microbial specimen of *E. faecalis* (ATCC 2921 2) was obtained from a supplier (Hi Media) and was cultivated in BHI broth. The cultured specimen was then diluted in BHI medium to achieve a concentration of 1 McFarland unit (3*108 colonies per ml).

20 microlitres of this inoculum was delivered into the root samples with the help of a micropipette. The coronal orifice of the samples was sealed with guttapercha stick. The inoculated samples were incubated at 37°C for 48 hours for the growth of *E. faecalis* so as to allow its penetration into the dentinal tubules.

Irrigation regimen

The roots in each group were irrigated using a syringe (26 gauge, Unilock ™) in an up and down motion, with 5 ml of their corresponding irrigants, while taking care to keep the irrigation needle passive in the canal. The canals were flushed with saline (5 ml) using a syringe (26 gauge, Unilock ™) to flush out the previous irrigants from the samples. The apical seal of gutta percha was removed and the saline was allowed to drain.

Evaluation of microbial status post irrigation

In order to evaluate the microbial status of the canal it was filled with 20 ml of BHI broth. The broth in the canal was sonicated for 5sec at 10,000 hertz to disengage the adhered bacteria. The post irrigant microbial sample was collected from the canal using sterilized paper points of 35 size. The paper points were left in the canal for 30 seconds and then retrieved and immersed in 5ml of BHI broth. The micro-organisms were suspended using a vortex, and the inoculum was serially diluted 10-fold. 100 microlitres of this suspension was streaked on to agar plate and the plates were incubated at 37°C for 48 hrs. The CFU were counted for each plate in each group and the results were statistically analysed using one way ANOVA and Post Hoc Tukey test.

Groups

Experimental Group	Irrigation Solution	No of roots
A	Chlorhexidine 2%	10
B	Sodium Hypochlorite (NaOCl) 5.0%	10
C	Biopure MTAD	10
D	Oxum (superoxidized water)	10
E	Isotonic Saline (control)	10

Table A

Results

This study was aimed to evaluate the antibacterial efficacy of oxum and MTAD when compared against the established endodontic irrigants such as chlorhexidine and sodium hypochlorite. One way ANOVA suggests that all the four test irrigants in groups A,B,C,D showed reduction in CFUs when compared to saline On comparison between groups, no significant difference was reported between chlorhexidine and sodium hypochlorite group. A significant difference was seen between the MTAD group when compared with chlorhexidine, sodium hypochlorite and oxum group.

In this study bacterial sampling was used to indicate the presence of infection in the canal. All the teeth treated, with superoxidised water and MTAD, showed a positive reduction in bacterial growth when compared with saline, although the reduction in colony count was inferior in other groups when compared to the MTAD group. This study showed that there was no statistically significant difference in reduction of CFUs in between the chlorhexidine and 5% sodium hypochlorite group. Although there was a reduction in the CFU counts in the groups irrigated with with oxum, the results weren't satisfactory. Between MTAD and oxum, MTAD proved to be better than oxum. Oxum on the other hand was more effective than saline but not as effective as Chx or NaOCl. One of the suggested advantages of superoxidized water, when compared to sodium hypochlorite, is its lower level of toxicity. It is worth noting, that the mechanism of action of super-oxidized water involves oxidative damage which might cause ageing and irreversible dysfunctions that eventually produce cellular death (Table 1).

One way ANOVA shows there is significant difference between the root canal irrigants. To find exactly which root canal irrigant differs from the other, LSD (Least Significant Difference) Post Hoc test was done (Figure 1).

Irrigants	N	Mean	Standard Deviation±	Range	Anova	Significance At 5% Level	Non Significant Group
Group A	10	4.80	7.269	0-22	P<0.005	A vs E	A vs B
Group B	10	307.80	243.134	68-640		B vs E	
Group C	10	1.30	1.494	0-5		C vs A, B,D,E	
Group D	10	414.60	235.881	96-800		D vs A, B,C, E	
Group E	10	1253.40	222.561	790-1500		E vs A,B,C,D	

Table 1: (p<0.001- Highly significant*)

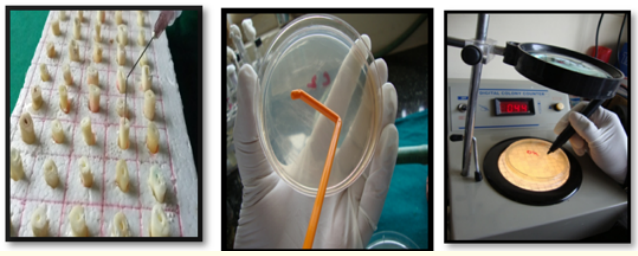

Figure 1: Irrigation Samples FIG2 Spreading the suspension on culture plate using an 'l spreader FIG3 Digital colony counter.

Discussion and Conclusion

The primary endodontic treatment goal must be to optimize root canal disinfection and to prevent reinfection. Microorganisms and their by products are the main cause of pulpal and periapical infections, and their elimination is primary endodontic treatment goal for promoting a favourable environment for periapical lesion healing. Mechanical enlargement of canals must be accompanied by copious irrigation in order to facilitate maximum removal of microorganisms so that the prepared canal becomes as bacteria-free as possible [14]. Chemical debridement is especially needed for teeth with complex internal anatomy such as fins, anastomosis, and other irregularities that is usually missed by instrumentation. An ideal intra-canal irrigating solution should be able to disinfect the dentin and dentinal tubules in the first treatment session and maintain its antimicrobial potential for some time after being used [15].

E. faecalis was chosen as the test organism because it has been associated with persistent apical inflammation in clinical situations and is one of the intra-canal bacteria which are most resistant to elimination by disinfecting agent [16]. The prevalence of Enterococci, has been a conspicuous finding in a high percentage of root-canal failures which has been attributed to its high resistance and its ability to survive as a single organism in monocultures The significant characteristics of Enterococci include their ability to grow in the range of 10°C-45°C and to survive around 30 min at 60°C, and at high salt concentrations of 6.5% saline as well as at extremely alkaline pH of up to 11 [17]. *E. faecalis* endures prolonged period of nutritional deprivation. It binds to dentin and proficiently invades dentinal tubules. It alters the host response and suppresses the action of lymphocytes. It possesses lytic enzymes, cytolysin, aggregation substance, pheromones and lipoteichoic acid and utilizes serum as the nutritional source. It also resists intracanal medicaments i.e. calcium hydroxide by maintaining pH haemostasis [18].

Sodium hypochlorite and EDTA were used during the cleaning and shaping procedure. This was done to eliminate the variable effects of mechanical instrumentation and smear layer removal in reducing bacterial count. It was accomplished before sterilization of samples was carried out.

Regardless of the type of irrigant used, the bacterial population inside the root canal is significantly reduced by the mechanical effects of irrigation. In this study normal saline was used as control as it was devoid of antibacterial action when compared to other test solutions which had some amount of known antibacterial activity. It was also interesting to note that all the cultures obtained following normal saline irrigation remained positive for *E. faecalis*. The significant bacterial reduction in the normal saline treated group may also be attributed to the volume of the solution used in each sample (5 ml). However there was a highly significant difference in colony counts after irrigation between the normal saline group and the other irrigant groups, thereby stressing the need for an antimicrobial irrigant during biomechanical preparation of the root canal [19].

We choose several irrigants to evaluate their efficacy in eliminating *E. faecalis* from the root canal system. NaOCl, has excellent anti-bacterial properties, our results demonstrate that irrigation with NaOCl, even at the high concentration, eliminated *E. faecalis* in only half of the samples. This lack of efficacy of NaOCl in consistently disinfecting root canals is in agreement with results from previous investigations. A clinical study by Sjorgren., *et al.* [20] concluded that 40% of root canal systems remain infected following irrigation with 0.5% NaOCl. In another study, Siqueira Jr., *et al.* [21] investigated the ability of 4% NaOCl solution, used in various irrigation protocols, to eliminate *E. faecalis* from the root canal system and found that after irrigation with 4% NaOCl 30 - 40% of root canal systems remained infected with *E. faecalis.*

CHX digluconate is a bisguanide disinfectant that has broad antimicrobial substantive activity against some resistant bacteria such as *E. faecalis* because it has the ability to be adsorbed and released gradually from the hydroxyapatite surfaces. It is also effective mainly against Gram positive and also Gram negative bacteria

as well as yeasts. Despite its usefulness as a root canal irrigant, it cannot be advocated as the main irrigant in standard endodontic procedures as it lacks the property to dissolve necrotic tissue remnants and fails to remove smear layer. Thus it has been widely used as an auxiliary canal irrigant or a canal soaking agent against *E. faecalis.*

In recent years, several studies have focused on evaluating the effectiveness of Bio Pure MTAD as a root canal irrigant against *E. faecalis*. Newberry., *et al* [22]. determined the antimicrobial effect of Bio Pure MTAD as a final irrigant on eight strains of *E. faecalis.* After irrigating with 1.3% NaOCl, the root canal and external surfaces were exposed to Bio Pure MTAD for 5 min. Roots or dentin shavings were cultured to determine the growth of E. faecalis, and results showed that this treatment regimen was completely effective at eliminating the growth of *E. faecalis* strains. Furthermore, Davis., *et al* [23]. used in vitro experiments to show that 2% chlorhexidine and 5.25% NaOCl both exhibited less antimicrobial efficacy against *E. faecalis* than Bio Pure MTAD, demonstrating that Bio Pure MTAD is a viable medicament against *E. faecalis*. In another study, Mohammadi and Shahriari [24] compared the antimicrobial effect of Biopure MTAD, 2% chlorhexidine and 2.6% NaOCl on *E. faecalis* in human root dentin. Their findings showed the Bio-Pure MTAD was more effective than the other solutions, and was retained in the root canal dentin for at least 28 days. These findings are consistent with our own results (Group III) and those of other researchers [25,26] who have reported the superior efficacy of Bio Pure MTAD against *E. faecalis*. MTAD contains 4.25% citric acid and 0.5% polysorbate 80 detergent (Tween 80). Its low PH 2.15 contributes to its role as a calcium chelator, thereby causing root surface demineralization. Tween 80 (polyoxyethylene sorbitan monooleate), is a detergent present in MTAD and is a nonionic surfactant, helps in reducing the surface tension of distilled water, NaOCl and EDTA, thereby enhancing the flow and penetration of irrigating solutions like MTAD deeper into the dentinal tubules. It has a pH of 7.0 and is a biologically acceptable material. Powder contains Doxycycline hyclate which is a broad spectrum antibiotic effective against a wide range of microorganisms. It is bacteriostatic and shows the property. MTAD preserves its antibacterial properties even after being diluted 200 times, whereas antibacterial activity of NaOCl is maintained up to 32 times of being diluted [24]. Doxycycline present in MTAD can remove organic and non- organic material from the root surface and preserves its effects for a long time since it is chelated to calcium ions. Doxycycline has extensive activity in the presence of citric acid and Tween80 (polysorbate), which is a detergent and lowers surface tension. Low pH (lower than 3), anti-collagenase activity and a dentin- binding ability, resulting in its slow release, are prominent properties of doxycycline.

In a study done by Nagayoshi and Kitamura [27] they concluded super-oxidized water had nearly the same antimicrobial activity as 2.5% NaOCl during irrigation, especially when combined with sonication, and showed a low level of toxicity against cultured cells. This difference in resuts between this study and study done by Nagayoshi., *et al.* [27] can be attributed the different method of activation of irrigant. Sonication improved the antimicrobial activity of super-oxidized water. Super-oxidized water is a powerful antimicrobial agent against bacteria, fungi, protozoa and viruses. It is rich in reactive oxygen with a neutral pH. The main advantage of this super-oxidized water is that it is stable and has a longer shelf life. It mainly contains oxidized solution (H_2O), sodium hypochlorite, hypochlorous acid, hydrogen peroxide, ozone, chlorine dioxide, sodium hydroxide, sodium carbonate and sodium chloride. The molecules are broken into ions and free radicals, which rapidly react and denature protein of bacterial cell wall. It produces an environment of unbalanced osmolarity that damages the cell wall of single cell organisms. Oxum is a stable, non-flammable and noncorrosive bactericidal, virucidal, fungicidal and sporicidal solution that is ready to use with no further dilution or mixing. The low pH in oxum may sensitize the outer membrane of bacterial cell, thereby enabling oxygen anion radicals to attack the bacterial cell more efficiently [28]. The damage is due to the difference in osmolarity between the concentrations of ions in solution vs the concentration of same ions in the cell [29]. Multicellular organisms are not prone to such changes so host tissues are spared.

Limitation

This study was based on antibacterial activity of the irrigants against E faecalis only. There are chances that the irrigants might perform differently in a polymicrobial model or *In vivo*, where there is a wide range and complexity of the microbes in a biofilm. More researches are required to find a better irrigant which can satisfy the ideal requirements of an irrigant and is yet biocompatible with the human tissues. Much more research is needed to use ozonated water in clinical or endodontic therapy. However, our results suggested that the application of ozonated water might be useful for the root canal irrigation

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