Original Research Article

Antibiofilm efficacy of 0.1% Octenidine, SmearOFF, 1% Alexidine and 5.25% Sodium Hypochlorite against E. faecalis biofilm formed on tooth substrate

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Abstract

Context: The present study was aimed to explore new irrigating solutions, which would probably be as effective as sodium hypochlorite.

Aims: The aim of this in vitro study was to evaluate the antibacterial effectiveness of 0.1% octenidine, SmearOFF, 1% alexidine and 5.25% sodium hypochlorite against E. faecalis biofilm formed on tooth substrate.

Materials and methods: Eighty extracted human teeth were decoronated, biomechanically prepared, vertically sectioned, placed in the tissue culture wells exposing the root canal surface to E. faecalis strain (ATCC 29212) to establish 3-week-old biofilm. At the end of the 3rd week all groups were treated for 10 minutes with 3 ml of test solutions. Samples exposed to 5.25% sodium hypochlorite (NaOCl) were used as control. All the samples were evaluated for E. faecalis growth and number of colony forming units. Statistical analysis was performed by using one-way analysis of variance (ANOVA) to measure the mean values. The intergroup comparison was done by Tukey HSD post hoc test ($p<0.05$).

Results: Maximum inhibition was observed with 0.1% OCT (group 1) followed by NaOCl (group 4) and ALX (group 3). SmearOFF (group 2) showed the least inhibitory effect. NaOCl (group 4) and ALX (group 3) showed no statistically significant difference ($p>0.05$), whereas all the other inter-group differences were statistically significant ($p<0.05$).

Conclusions: 0.1% OCT showed significantly higher antibiofilm effects. No significant differences

were observed between 1% ALX and 5.25% NaOCl. SmearOFF showed significantly lower antibiofilm effects ($p<0.05$).

Key words

Alexidine, Chlorhexidine, Sodium Hypochlorite, Disinfection, Guttapercha, Scanning electron microscopy, *E.faecalis.*

Introduction

Evidence points out that microbial infection of the root canal system are the primary etiologic factor in pulpal and periapical pathology. Free-floating microorganisms in root canal system can attach to one another and form mature biofilms which has inherent resistance to antimicrobial agents and makes it difficult to eradicate from the root canal system [1].

E. faecalis is the most common species cultured from non-healing endodontic cases. Its mode of growth is through biofilm formation, where it can endure severely harsh conditions like obturated root canals. It invades the dentinal tubules and can survive chemo-mechanical instrumentation, intracanal medication and re-infect the obturated root canal [2].

The major aim of endodontic treatment is to remove these microorganisms and their by-products from the root canal system. Therefore, the use of various antimicrobial irrigants, sequentially or in combination, is needed to enhance their antimicrobial effect during mechanical instrumentation [3].

Sodium hypochlorite (NaOCl) is the gold standard in endodontic therapy for the root canal irrigation. It has solvent activity for both necrotic and vital tissues. Recently, however, concerns have been raised that NaOCl cannot predictably eradicate biofilm or diffuse completely into biofilm even at a 2% concentration and that the potential exists for sub-antimicrobial concentrations of NaOCl to actually increase biofilm formation [4]. This may be of clinical significance, considering variations in the use of NaOCl worldwide.

Several antimicrobial disinfectants used in the medical field have been evaluated as irrigants in endodontics, such as Octenisept (OCT) and Alexidine (ALX). OCT was introduced in 1990 as a mucous membrane antiseptic. OCT is a positively charged bis-pyridinamine with a broad spectrum of antibacterial, antifungal, and some antiviral properties [5]. It contains 0.1% octenidine hydrochloride and 2% phenoxyethanol. It is particularly capable of inhibiting the formation of biofilm and disrupting fully formed biofilm even in the presence or absence of serum protein [6]. A recent study reported that no apparent deleterious products are formed when OCT and NaOCl solutions are mixed together [7].

ALX, a bis-biguanide similar to chlorhexidine (CHX) which has broad antimicrobial activity and helps to inhibit the immune response of major virulence factors, such as bacterial lipopolysaccharide and lipoteichoic acid, more effectively than CHX. Also, when ALX is used along with NaOCl, it does not form any reaction precipitate that would block the dentinal tubules, suggesting that ALX could be used as an irrigant with NaOCl [8].

SmearOFF (Vista Dental Products, Racine, WI) is a new irrigant in market containing, among other things, CHX gluconate <1% weight and tetrasodium ethylenediaminetetraacetate dihydrate 18% weight as claimed by the manufacturer. It is marketed as an irrigant that does not produce a precipitate with NaOCl [9].

To date, no study has been done to compare the efficacy of these new test solutions against E.faecalis. Therefore, the aim of this in vitro study was to evaluate the antimicrobial

effectiveness of 0.1% OCT, SmearOFF, 1% ALX and 5.25% NaOCl against E. faecalis biofilm formed on tooth substrate.

Materials and methods

E. faecalis culture preparation

A pure culture of E. faecalis (ATCC 29212) (Himedia, Mumbai, India) was inoculated on Mueller-Hinton agar (Himedia, Mumbai, India) and incubated at 37° C overnight and adjusted to an optical density (OD_{600}) of 1 with sterile Mueller-Hinton broth.

Tooth samples preparation

80 single-rooted human mandibular premolars were taken. The inclusion criteria were fully formed apices, single canal and mature apex. The exclusion criteria were open apices, root resorption, calcifications, root canal treatment and developmental disorders. The teeth were cleaned of superficial debris, calculus, and tissue tags and stored in normal saline to prevent dehydration before use. The tooth specimens were sectioned below the cementoenamel junction with a diamond disc to obtain a standardized tooth length of 8 mm for uniform specimen.

Biofilm Formation on Tooth Substrate

The root canals were then instrumented using the crown-down technique and rotary instruments (ProTaper, Dentsply Maillefer, Ballaigues, Switzerland), and the canals were enlarged to an apical size F3. 5.25% NaOCl (2 ml) was used between each instrument during the cleaning and shaping procedure. All the teeth were then vertically sectioned along the midsagittal plane into two halves. The concave tooth surface was minimally grounded to achieve a flat surface to enable placement in the tissue culture wells, exposing the root canal surface to E. faecalis to form a biofilm. The bacterium was cultured as described previously, and the wells containing tooth samples were inoculated with 2 ml of bacterial solution and incubated at 37° C. The culture medium (Mueller-Hinton broth) was replaced every alternate day to avoid nutrient depletion and accumulation of toxic end products. The samples were taken from each well with a sterile paper point, inoculated on Mueller-Hinton agar plates, and incubated at 37° C for 24 hours to check for cell viability and purity of culture.

At the end of the third week, the samples were divided into 4 groups (n=20) and were treated for 10 minutes with 3 ml of following test solutions respectively:

- Group 1- 0.1% Octenidine (Octenisept, Schülke & Mayr GmBH, Norderstedt, Germany)
- Group 2- SmearOFF (Vista Dental Products, Racine, WI), a proprietary mixture of CHX and tetrasodium salt of EDTA
- Group 3- 1% Alexidine (A525000; Gentaur, Kampenhout, Belgium)
- Group 4- 5.25% NaOCl- (Control)

Then, the biofilm on the root canal portion was taken with a sterile paper point and inoculated on Mueller-Hinton agar plates and incubated for 24 hours at 37°C. The plates were then analyzed for colony forming units by a digital colony counter and the readings were recorded.

Statistical analysis was performed by using one-way analysis of variance (ANOVA) to measure the mean values. The intergroup comparison was done by Tukey HSD post hoc test using SPSS software. The criterion for statistical significance was set as $p < 0.05$.

Results

The mean zone of inhibition of the experimental and the control groups showed statistically significant values (**Table - 1**). Maximum inhibition was observed with 0.1% OCT (group 1) followed by NaOCl (group 4) and ALX (group 3). SmearOFF (group 2) showed the minimum zone of inhibition (**Figure - 1**).

The intergroup comparison was done by using Tukey HSD post hoc test. No statistical difference was observed between NaOCl (group

4) and ALX (group 3) (p>0.001). The intergroup comparison of mean zone of inhibition values proved to be statistically significant (p < 0.05) for all the other groups (**Table - 2**).

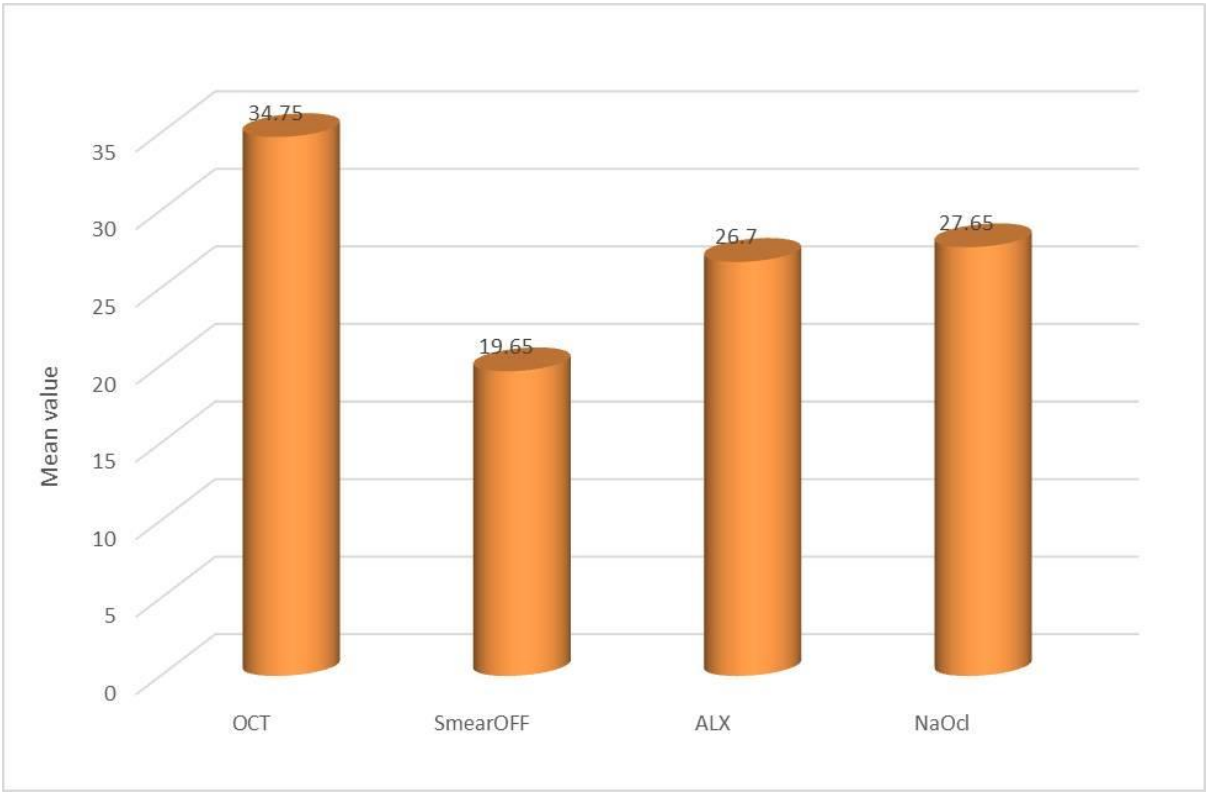
Table - 1: Mean zone of inhibition along with standard deviation of the experimental and the control groups (n=20).

	n	Mean zone of inhibition	Standard Deviation	Minimum	Maximum
Group 1 (OCT)	20	34.75	1.52	32.00	38.00
Group 2 (SmearOFF)	20	19.65	2.11	16.00	23.00
Group 3 (ALX)	20	26.70	1.56	24.00	29.00
Group 4 (NaOCl) (Control)	20	27.65	1.42	25.00	30.00

Table - 2: Intergroup comparison of mean zone of inhibition of all groups with significance value set at p< 0.05.

		Mean Difference	Statistical significance "p"
Group 1	Group 2	15.1	<0.01
	Group 3	8.05	<0.01
	Group 4	7.1	<0.01
Group 2	Group 3	-7.05	<0.01
	Group 4	-8.00	<0.01
Group 3	Group 4	-0.95	0.28

Figure - 1: Comparison of mean zone of inhibition of all groups.



Discussion

It has been well established that endodontic disease is a biofilm mediated infection [10]. E. faecalis was selected in this study because it is commonly detected in the root canals of teeth

associated with persistent periradicular lesions. It has a high binding ability to the dentin surface and the ability to grow in a biofilm style [11]. Therefore, the elimination of bacterial biofilms is an essential element for the successful outcome of endodontic treatment.

The results of this study showed that OCT had the highest mean zone of inhibition. The results of our findings are in line with some previous studies [12-14]. OCT exerts its antimicrobial effect by binding to the negatively charged bacterial cell envelope, thereby disrupting the vital functions of the cell membrane and killing the cell. It has a high affinity towards cardiolipin, a prominent lipid in bacterial cell membranes, making it selectively lethal to bacterial cells without adversely affecting eukaryotic cells [15]. Also, OCT has shown good in vivo tissue tolerability [16], mitigating the issue of tissue damage from inadvertent apical extrusion.

When group 4 (5.25% NaOCl) was compared with group 1 (0.1%OCT), it was found that group 4 had significantly less antimicrobial property than group 1. Previous investigations have shown that OCT can disinfect dentinal tubules and reduce the colony-forming units at a depth of 400 μm in the dentinal tubules [12, 17]. However, researches have shown that at room temperature, 3% NaOCl is ineffective in achieving dentin tubule disinfection, with 60% of live bacteria remaining within the tubules at 300 μm [18]. Even if 6% NaOCl is used at 45 degrees C for 20 minutes, the intratubular penetration of NaOCl is limited to 300 μm in dentin slab models in vitro [19]. This could be the reason of lower antibiofilm activity seen with Group 4. Our findings are not in accordance with Bukhary, et al. (2017) who showed that 5.25% NaOCl demonstrated almost complete removal of E. faecalis biofilm. The low antimicrobial effect of 5.25% NaOCl in this study could be because of the buffering effects of the dentin and the organic matter of the biofilm [20].

When group 3 (1% ALX) was compared with group 4 (5.25% NaOCl), no significant differences were observed. We tested 1% concentration of ALX solution because ALX with a concentration higher than 1% has been showed to cause moderate cytotoxicity against human gingival fibroblasts [21]. It is a cationic bisguanide that exerts its antibacterial effects by inducing lipid phase separation and domain formation at bacterial membranes [22]. Gram-positive bacteria like E faecalis are more sensitive to cations because they are more negatively charged. It has a great affinity for the major virulence factors such as bacterial lipopolysaccharide and LTA of bacteria [23]. This characteristic might result in the formation of many ruptured (damaged) or antiseptic-attached bacteria in the 10-min-soaked ALX group. Our results are in accordance Arias-Moliz [24] and Baca, et al. [25] Sodium hypochlorite kills E. faecalis by the high alkaline pH [26] but the ALX attaches to the bacterial membrane surface, cause leakage of intracellular components and rupture bacterial membranes. This might be the reason for nearly equal efficacy of both ALX and NaOCl.

When group 3 (1%ALX) was compared with group 1 (0.1%OCT), significant differences were observed. This could be explained by the anti-adhesive property of OCT as compared to biguanides. It has been shown by Cherian, et al. [27] that the shear viscosity and surface tension of OCT is less as compared to cationic bisguanides like ALX and hence better flow characteristics in the canal. Previous studies have shown an increase in penetration of the irrigant with a decrease in viscosity and surface tension [28]. Thus, OCT effectively prevents bacterial co-aggregation, which is critical for biofilm formation.

SmearOFF showed limited antibiofilm effects in this study when compared with other groups. Krishnan U, et al. [29] found that when SmearOFF is mixed with NaOCl, it does not retain free active chlorine, which is responsible for protein breakdown and inhibition of bacterial enzymes. Its lower antibacterial activity could be

attributed to its low pH of only 8 to 9. They concluded that even though it does not produce a precipitate when mixed with NaOCl, SmearOFF should not be used concurrently with NaOCl because of the rapid displacement of available chlorine. It should only be used alone as a final irrigant. Our results are in corroboration with this study.

Earlier investigations have shown that 10 minutes of exposure to an irrigant solution is the maximum effective time to kill 3-week-old E. faecalis biofilms [30]. Therefore, in the present study, the samples were exposed to the irrigant solutions for 10 minutes.

It has been shown that in young biofilm, the bacteria are in the active and exponential growth phase, and neither the structural development of the biofilm nor the production of the extracellular polymeric matrix has been completed. Three-week-old E. faecalis biofilm has been shown to be mature and more resistant to disinfecting solutions than young biofilm [30]. Therefore, a 3-week incubation period was used in this study to ensure biofilm maturation.

Octenidine hydrochloride (OCT) is a better choice in root canal irrigation because of its faster ability to produce intratubular disinfection when compared with NaOCl, ALX and SmearOFF, its specific effectiveness against endodontic pathogens. However, its potential as a stand-alone irrigant is limited because of its poor tissue-dissolving properties [31].

Removal of persistent mature biofilm cannot be achieved by sodium hypochlorite alone. Combined use of OCT along with NaOCl is suggested. The white precipitate called phenoxyethanol formed by mixing the two irrigants is easily removable by passive ultrasonic irrigation from canal.

Future research analyzing various activation techniques and temperature of the irrigants under conditions closer to clinical reality should be carried out to corroborate the findings of this study and to determine its scope and usefulness in clinical set-up.

Conclusion

Under the conditions of the present study, it can be concluded that

- 0.1% OCT demonstrated maximum antibiofilm effects against E. faecalis.
- 1% ALX and 5.25% NaOCl were equally effective against E. faecalis.
- SmearOFF showed limited antibiofilm effects against E. faecalis.

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