

A Comparative Evaluation of Antimicrobial Efficacy of Novel Surfactant-Based Endodontic Irrigant Regimen's on *Enterococcus faecalis*

Abstract

Introduction: Irrigants play an important role in the eradication of microorganisms in the complex root canal system. Sodium hypochlorite (NaOCl), chlorhexidine (CHX), and iodine potassium iodide (IKI) have certain limitations despite their routine clinical use. Surfactant irrigants with antimicrobial properties can be a milestone in endodontics to overcome the drawbacks of conventional irrigants. The aim of this study was to evaluate the antimicrobial efficacy of surfactants in comparison with routine endodontic irrigants on *Enterococcus faecalis*.

Materials and Methods: Primary irrigants NaOCl, CHX, IKI were prepared at concentrations of 5%, 2.5%, 2%, and 1%; while mixture of tetracycline acid and detergent (MTAD) (100%) served as control group. Surfactants such as cetrimide (CTR) and sodium dodecyl sulfate (SDS) were prepared at concentrations of 0.5%, 1%, and 2%. The direct contact assay was used to evaluate the antimicrobial efficacy after 5 min. Statistical analysis was performed using one-way ANOVA and Mann-Whitney *U*-test. **Results:** In the primary group, only 5% CHX was significant over MTAD ($P < 0.05$). In the surfactant group, all concentrations of CTR and SDS (except 0.5%) were significant ($P < 0.05$) in killing *E. faecalis* over MTAD. Two percent CHX in combination with 0.5% CTR and 1% SDS had an effective kill percentage over 2% CHX and MTAD alone.

Conclusion: Surfactant irrigant regimens can be used as adjuncts with CHX to overcome its clinical limitations and potentiate its substantivity, thereby enhancing clinical success in endodontics.

Keywords: Cetrimide, mixture of tetracycline acid and detergent, sodium dodecyl sulfate, surfactants

Introduction

The primary goal of endodontics is complete debridement and disinfection of the root canal system. On the contrary, residual pulpal tissue, bacteria, and dentin debris persist even after the use of commercial endodontic irrigants.^[1] *Enterococcus faecalis* has been found associated with primary apical periodontitis and failed root canal treatments involving chronic apical lesions thus has gained endodontic significance.^[2]

E. faecalis isolated from periapical pathosis were found to be refractory to endodontic treatment.^[3] Being an opportunistic pathogen, it was found to be associated with high prevalence rates in cases with posttreatment disease due to specific virulence factors, adherence to host cells and extracellular matrix, tissue invasions, immunomodulation effect, and causes toxin-mediated damage.^[4]

It has been assumed that microorganisms associated with the root canal space are derived from those colonizing the oral cavity.^[5] *E. faecalis* were present in oral rinse samples from patients who had undergone endodontic treatment; and were rarely detected in healthy mouths.^[6] The ability to form biofilm has been implicated to its virulence and resistance to most irrigants and medicaments.^[7] The initial planktonic bacteria colonize various sites of the oral cavity including root canals to mature adhered *E. faecalis* biofilms.^[8]

Disinfection of the root canal is primarily achieved with the use of irrigants such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), ethylene diamine tetra acetic acid (EDTA), and iodine potassium iodide (IKI). The antibacterial and tissue dissolving property of 5.25% NaOCl in combination with 17% EDTA for the management of inorganic components has been the gold standard in endodontics.

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This protocol has serious limitations that include marked reduction in mechanical properties of dentin and erosion of dentinal tubular microstructure. NaOCl caused a concentration dependent reduction of elastic modulus and flexural strength on root dentin.^[9]

Biopure mixture of tetracycline acid and detergent (MTAD) (Tulsa, Dentsply) contains 3% doxycycline (tetracycline isomer) 150 mg/5 ml, 4.25% citric acid, 0.5% polysorbate 80 (surfactant). It is used only as a final rinse as an adjunct to 1.3% NaOCl. Thus, the combination of NaOCl and MTAD has been claimed to be effective on *E. faecalis*.^[10] Kho and Baumgartner found no difference between NaOCl/EDTA and NaOCl/MTAD regimens on *E. faecalis*.^[11] The nonavailability of MTAD and other commercial products have made researchers to formulate an alternative by developing indigenous irrigants; that would be cost-effective and readily available for endodontic application.

Surfactants sodium dodecyl sulfate (SDS) and cetrinide (CTR) are surface-active agents that primarily exhibit their antibacterial activity by reducing the surface tension in proximity to microorganisms.^[12] Although we hypothesize, there will be no significant difference; the purpose of this study was to evaluate the antimicrobial efficacy of surfactant irrigant regimens on *E. faecalis* by direct contact assay.

Materials and Methods

Bacterial strain and growth conditions

Isolated 24 h colonies of pure cultures of *E. faecalis* (ATCC 29212) grown on 10% sheep blood plus brain heart infusion (BHI, Himedia, India) agar plates were suspended in sterile 0.9% NaCl solution. The cell suspension was adjusted spectrophotometrically to match the turbidity of 0.5 McFarland scale (1.5×10^8 cfu/ml).

Standardization of microorganisms

BHI broth was inoculated with the test microorganism and incubated for 24 h to get a mean optical density of 0.5 McFarland Standards (negative control). One milliliter of each suspension culture was transferred to the required number of sterile screw-cap tubes (Himedia, India). All procedures were performed using sterilized instruments and materials.

Irrigants and surfactants used

Primary irrigants NaOCl (PrevestDenpro Limited), CHX (Sigma) and IKI (Merck) were prepared initially. Subsequently, concentrations of 1%, 2%, 2.5%, and 5% of these irrigants were prepared by serial dilution with distilled water. Surfactant irrigants CTR (Himedia) and SDS (Merck) were prepared by serial dilution to obtain concentrations of 2%, 1%, and 0.5%. Biopure MTAD (Tulsa, Dentsply) which was prepared as per the

manufacturer's instructions served as a positive control. All prepared irrigants were stored in sterile bottles at room temperature.

Evaluation of antimicrobial efficacy

One milliliter (ml) of suspension culture of *E. faecalis* was treated with 1 ml of each of the test irrigant (primary and surfactant irrigants) and then placed in 96 μ l plates (Himedia). Mean optical density was recorded in a spectrophotometer (Lisa Plus) at 630 nm after 5 min.^[13,14] Simultaneously, streaking was performed on already prepared Mueller Hinton agar plates for bacterial colony count. These plates were incubated overnight in an incubator at 37°C. The same was repeated for surfactant group CTR and SDS (0.5%–2%) and Biopure MTAD.

The number of colony-forming unit (CFU) per ml of culture was determined. Statistical analysis was performed using a one-way ANOVA and Mann–Whitney *U*-test. The effect of each test agent on microbes was determined by calculating the percentage kill of viable bacteria with the test agent. The percentage of kill for each test agent was calculated by the formula;

$$1 - (\text{Average CFU [test agent]} / \text{Average CFU [negative control]}) \times 100^{[15]}$$

Results

The CFU values were recorded for the control group MTAD (G0) with experimental primary groups NaOCl (G1), CHX (G2), IKI (G3) and surfactant groups CTR (G4), SDS (G5) are shown in Table 1. When the intergroup comparison was made among the concentrations in the primary group; only 5% CHX was found to be statistically significant ($P < 0.05$) over MTAD [Table 2]. Since CHX showed promising results over NaOCl and IKI; it was chosen. Two percent CHX which is commonly used as a final rinse was thus selected for the combination regimen (CR).

On the contrary in the surfactant group, all test concentrations (0.5%–2%) of CTR were statistically significant; while SDS tested significantly with 1% and 2% only over MTAD. Thus 0.5% CTR and 1% SDS were chosen as effective surfactant concentrations [Table 2]. The CR of this 2% CHX + 0.5% CTR (G6 and 2% CHX+1% SDS (G7) CFU values are shown in Table 1. Intergroup comparison showed no significant differences between the CR groups; however, they were found to be significant over MTAD [Table 2].

The effective kill percentage among primary irrigants in the eradication of *E. faecalis* shows CHX is generally more effective than MTAD at all concentrations [Figure 1]. In the surfactant groups, CTR generally shows greater efficacy than SDS (except 2% SDS) as depicted in Figure 2. Among the CRs G7 achieved higher eradication rates (99.9%); followed by G6 (99.5%) in contrast to 2% CHX (97%) and MTAD (95%) as shown in Figure 3.

Table 1: Mean colony forming unit values of primary, surfactant and combination regimens with mixture of doxycycline

Parameter	Irrigant groups	Concentration (%)	Mean CFU values	ANOVA			
Primary	NaOCl (G1)	1.00	3.000	HS*			
		2.00	3.000				
		2.50	2.750				
		5.00	1.750				
		CHX (G2)	1.00		2.500		
	2.00		2.000				
	2.50		1.500				
	5.00		0.000				
	IKI (G3)		1.00		3.000		
		2.00	2.750				
		2.50	2.500				
		5.00	1.750				
		Control	MTAD (G0)		100	2.750	
	Surfactants				CTR (G4)	0.50	1.500
						1.00	0.500
2.00		0.250					
Combination regimens	SDS (G5)	0.50	2.000				
		1.00	1.000				
		2.00	0.000				
	CHX + CTR (G6)	2 CHX + 0.5 CTR	0.750	NS			
		CHX + SDS (G7)	2 CHX + 1 SDS		0.600		

*Significant at the 0.05 level. CFU: Colony forming unit; NaOCl: Sodium hypochlorite; CHX: Chlorhexidine; IKI: Iodine potassium iodide; CTR: Cetrimide; SDS: Sodium dodecyl sulfate; MTAD: Mixture of doxycycline; HS: Highly significant

Table 2: Intergroup comparison of experimental irrigants with mixture of doxycycline

Control irrigant (%)	Experimental irrigant concentration (%)	MannWhitney test P value						
		Primary irrigants			Surfactant irrigants		Combination regimens	
MTAD (G0) 100	Primary irrigants	NaOCl (G1)	CHX (G2)	IKI (G3)	-	-	-	-
		1.00	1.000	1.000	-	-	-	-
		2.00	1.000	0.874	1.000	-	-	-
		2.50	1.000	0.166	1.000	-	-	-
		5.00	0.147	0.006*	0.624	-	-	-
MTAD (G0) 100	Surfactant irrigants	-	-	-	CTR (G4)	SDS (G5)	-	-
		2.00	-	-	0.001*	0.000*	-	-
		1.00	-	-	0.002*	0.001*	-	-
		0.50	-	-	0.025*	0.051	-	-
MTAD (G0) 100	CR	-	-	-	-	-	CHX + CTR (G6)	CHX + SDS (G7)
		2 CHX+0.5 CTR	-	-	-	-	0.005*	-
		2 CHX+1 SDS	-	-	-	-	-	0.002*

*Significant at the 0.05 level. MTAD: Mixture of doxycycline; NaOCl: Sodium hypochlorite; CHX: Chlorhexidine; IKI: Iodine potassium iodide; CR: Combination regimens; CTR: Cetrimide; SDS: Sodium dodecyl sulfate

Discussion

Studies by direct contact test evaluate their findings by the time taken (contact time) to produce negative cultures, which signify growth inhibition percentage (%) of certain microorganisms in the presence of certain irrigants.^[16] Although the nature of test microorganisms plays a critical role in the assay;^[17] Distel *et al.* stated *E. faecalis* resists intracanal medication with calcium hydroxide and was found to form biofilm *in vivo*.^[18] On the contrary, Duggan and Sedgley stated *E. faecalis* strains from oral and

endodontic sources have a lower inherent capacity to form biofilms.^[19]

Estrela *et al.* assessed the efficacy of NaOCl and CHX on *E. faecalis* and concluded NaOCl or CHX showed low ability to eliminate *E. faecalis* when evaluated by either polymerase chain reaction or culture techniques.^[20] Commercial irrigants such as MTAD, Chlor-Xtra (6% NaOCl + surface modifiers), and cetrexedin (0.2% CTR + 0.2% CHX) claim to have superior antibacterial activity and mixed results have been implicated.^[21] As an alternative to commercial

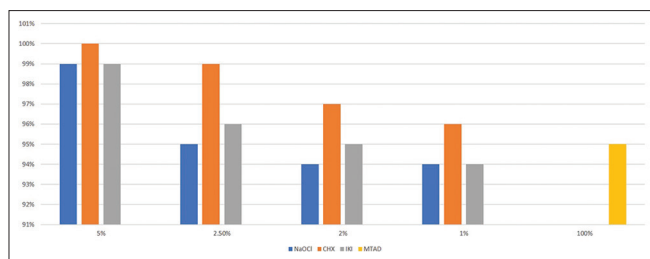


Figure 1: Percentage kill of primary irrigants with mixture of tetracycline acid and detergent

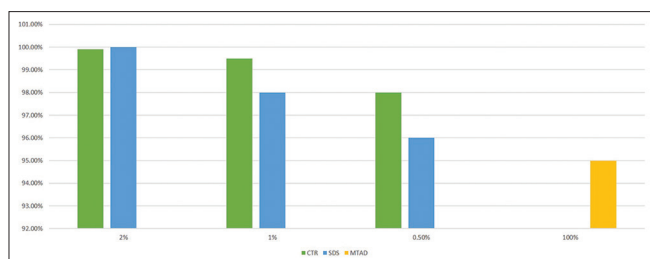


Figure 2: Percentage kill of surfactants with mixture of tetracycline acid and detergent

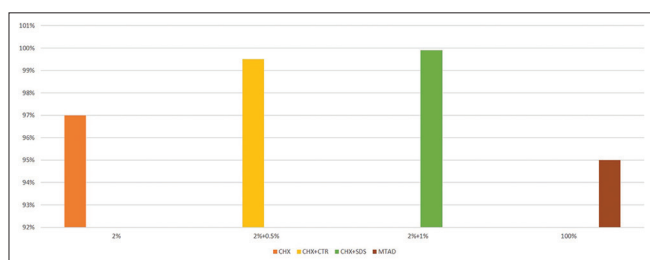


Figure 3: Percentage kill of combination regimens with chlorhexidine and mixture of tetracycline acid and detergent

irrigants; the current study focused to find a cost-effective combination surfactant regimen that will be highly effective in eradicating *E. faecalis*.

E. faecalis ATCC 29212 (Van A negative, Vancomycin sensitive) was chosen as a quality control strain since numerous studies have evaluated its susceptibility to various endodontic irrigants.

MTAD as a final rinse requires a contact time of 5 min as per manufacturer's instructions. The antimicrobial efficacy of primary irrigants, surfactants and MTAD were assessed after 5 min by direct contact assay to standardize the antibacterial efficacy. Further culturing was done to evaluate the effective kill percentage of the test irrigants.^[22]

The results of our study indicated that CHX was effective even at 2%. Although 0.5% CTR and 1% SDS had significant antibacterial activity; experimental results of combination surfactant regimen achieved higher eradication rates than 2% CHX alone.

Similar results have been implicated by Vianna *et al.*; who reported that antibacterial activity of NaOCl was

effective at higher concentrations only.^[23] Jungbluth stated chlor-xtra (6% NaOCl + surface modifiers) has no unique features other than its price; and reduced surface tension with surfactants did not result in greater soft-tissue dissolution by NaOCl.^[24] Radcliffe *et al.* and Retamozo *et al.* also found higher concentrations of NaOCl and longer exposure times are required to eliminate *E. faecalis*.^[25,26] IKI with surfactant combinations yielded a lesser antimicrobial activity as compared with IKI alone. This could be due to the interaction of IKI with the organic surfactants and resulting in decreased antibacterial activity.^[27]

Also in favor of our study, Giardino *et al.* also stated that cetrexedon (0.2% CTR + 0.2% CHX) has the lowest surface tension value; thus increasing the intimate contact of irrigant solution with the dentinal walls, thus permitting deeper penetration of the irrigant.^[21] Baca *et al.* stated that 2% CHX + 0.2% CTR would be an effective alternative as final irrigation regimen given its antimicrobial action over time.^[28]

On the contrary to our hypothesis stated earlier; the results of our study were not in accordance with Estrela *et al.*, who reported 2% CHX was ineffective.^[29] Portenier *et al.* who compared the antimicrobial activity of MTAD to that of CHX digluconate found MTAD and CHX to be equally effective in killing *E. faecalis*.^[30]

CHX gluconate is a cationic bisguanide that seems to act by adsorbing onto the cell wall of the microorganism and causing leakage of intracellular components. At higher concentrations, CHX has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein cross-linking. The possible explanation for significant antibacterial activity could be related to the nature of CHX liquid that mixes well with the bacterial suspension, thus immediately exerting its bactericidal action.^[31]

CTR is a cationic quaternary ammonium compound with antimicrobial ability, stability and solubility in water. The cationic environment of the molecule encourages linking with anionic compound at the bacterial surface and is capable of altering the cytoplasmic membrane integrity, protein denaturation, and resulting in cell death.^[32] CTR is noncytotoxic and has been used as endodontic irrigant.^[33] Our previous study results suggested; 0.5% CTR alone showed the same antimicrobial effect as primary irrigants (2.5% NaOCl, 2% CHX and 2% IKI).^[34]

SDS is an anionic alkyl sulfate; has the properties of low surface tension, can solubilize proteins, increase lipopolysaccharides disaggregation and inhibit bacterial coaggregation; which could account for its antimicrobial activity. SDS is not carcinogenic and is a potentially effective topical microbicide, which can also inhibit and possibly prevent infection by various enveloped and nonenveloped viruses such as the Herpes simplex viruses,

HIV, and the Semliki Forest Virus.^[35] Barbosa stated the association of calcium hydroxide and sodium lauryl sulfate combines the beneficial properties of these solutions and was not harmful to the fibroblast cell line, seeming to be a suitable endodontic irrigating solution.^[36]

The antimicrobial effect of Biopure MTAD may be primarily attributed to the doxycycline component of the irrigant; however, CHX has greater substantivity compared to MTAD.^[37] Handal reported that Gram-positive microorganisms are more susceptible to lower concentrations of tetracycline (doxycycline in MTAD) than the Gram-negative ones.^[38] Fujii *et al.* stated that the efficacy of doxycycline in the endodontic application needs to be limited since Gram-negative species dominate established infections.^[39]

Conclusion

Surfactants 0.5% CTR and 1% SDS being antimicrobial and organic solvent in nature, have played a vital role in enhancing the efficacy of 2% CHX (which lacks tissue dissolving property) in increasing negative cultures. This experimental surfactant regimen has clinical significance in reducing the toxicity of irrigants at higher concentrations employed in routine endodontics. Further studies on biofilm, cytotoxicity, and tooth models are required to evaluate the experimental results of this study.

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Conflicts of interest

There are no conflicts of interest.

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