



A Comparative Evaluation of Depth of Penetration of Sodium Hypochlorite with Various Surfactants in the Dentinal Tubules: An In Vitro Study.

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The present study aimed to evaluate and compare the depth of penetration of sodium hypochlorite (NaOCl) solution combined with various surfactants—cetrimide and benzalkonium chloride—into dentinal tubules using a confocal laser scanning microscope (CLSM).

Introduction: Effective irrigation is critical for successful endodontic therapy. Sodium hypochlorite (NaOCl) remains the most widely used irrigant due to its antimicrobial and tissue-dissolving properties. However, its high surface tension restricts its penetration into dentinal tubules. Incorporating surfactants like cetrimide and benzalkonium chloride may enhance its wettability and effectiveness.

Aim: To evaluate and compare the depth of penetration of 2.5% sodium hypochlorite alone and in combination with 0.2% cetrimide and 0.1% benzalkonium chloride into dentinal tubules using confocal laser scanning microscopy (CLSM)

Materials and Methods: Thirty extracted single-rooted human premolars were decoronated and instrumented with ProTaper Gold rotary files. The specimens were divided into three groups (n=10): Group A - 2.5% NaOCl; Group B - 2.5% NaOCl + 0.1% Benzalkonium chloride; Group C - 2.5% NaOCl+ 0.2% Cetrimide. Rhodamine B dye was added for visualization under CLSM. Data were analyzed using one-way ANOVA and Tukey's post hoc test ($p < 0.05$).

Results: Group B (NaOCl + Benzalkonium Chloride) demonstrated the highest mean depth of penetration in the coronal, middle and apical third, while Group A (NaOCl alone) showed the lowest depth of penetration. Inter-group comparisons revealed statistically significant differences between NaOCl alone and surfactant-modified NaOCl in all thirds.

Conclusion: Addition of benzalkonium chloride to NaOCl improved dentinal tubule penetration compared to NaOCl alone or NaOCl with cetrimide. However, apical penetration remained the lowest in all the groups.



INTRODUCTION

Successful endodontic therapy depends largely on the effective elimination of microorganisms and dissolution of organic tissue from the root canal system. Mechanical instrumentation alone is insufficient to clean the complex anatomy of the canal, especially in lateral canals, fins, and dentinal tubules; therefore, chemical irrigation becomes indispensable.^{1,2}

Sodium hypochlorite (NaOCl) is widely accepted as the primary irrigant due to its potent antimicrobial activity, organic tissue-dissolving capability, and ability to neutralize endotoxins.^{2,4} Despite these advantages, one of the major limitations of NaOCl is its inherently high surface tension, which restricts its penetration into deeper layers of dentin and reduces its ability to disinfect the apical third, where microbial persistence is most commonly encountered.^{5,6}

To overcome this limitation, research has focused on modifying NaOCl with surface-active agents (surfactants) to reduce its surface tension and improve its wettability. Surfactants lower the contact angle of irrigants, promoting deeper penetration into dentinal tubules and enhancing interaction with intratubular microorganisms.⁷⁻⁹ Among various surfactants, cetrimide and benzalkonium chloride (BAK) have gained attention due to their cationic nature, broad-spectrum antimicrobial activity, and ability to improve the physicochemical behavior of irrigating solutions. Previous studies have demonstrated enhanced smear layer removal, improved substantivity, and increased dentinal tubule penetration when surfactants are combined with conventional irrigants.¹⁰⁻¹²

Confocal laser scanning microscopy (CLSM) has emerged as a reliable tool for evaluating irrigant penetration because it allows precise visualization and quantification of dye-labeled irrigants within dentinal tubules without causing significant sectioning artifacts. By combining CLSM with fluorescent dyes such as Rhodamine B, the depth of irrigant penetration can be accurately assessed across the coronal, middle, and apical thirds of root dentin.^{13,14}

Given the clinical significance of achieving optimal penetration of irrigating solutions, especially in the apical region, it becomes essential to evaluate whether modifying NaOCl with surfactants results in meaningful

improvements. Although surfactant-modified NaOCl has shown promising physicochemical properties, evidence regarding its penetration depth compared to unmodified NaOCl remains limited and inconclusive.

Therefore, this *in vitro* study aims to compare the depth of penetration of 2.5% sodium hypochlorite, 2.5% sodium hypochlorite with 0.1% benzalkonium chloride, and 2.5% sodium hypochlorite with 0.2% cetrimide using CLSM. Understanding the differences in penetration efficacy among these irrigants may contribute to optimizing irrigation protocols and improving overall treatment outcomes.

MATERIALS AND METHODS

Thirty freshly extracted human single-rooted premolars were selected and stored in 0.9% saline until use. Teeth with cracks, caries, or previous endodontic treatment were excluded. The crowns were sectioned to obtain standardized root lengths of 15 mm. Root canal preparation was performed using ProTaper Gold rotary instruments (Dentsply Tulsa Dental, USA) up to F3, with irrigation between each file using the respective group irrigants.

In the current study 2.5% NaOCl was utilized for 15 minutes according to multiple primary studies measuring pulp/tissue dissolution at room temperature (Almeida 2013; Stojicic 2010; Iandolo 2019) use 5, 15 and 30-minute intervals; 15 minutes consistently yields substantially greater dissolution than 5 minutes and is therefore commonly used as a standardized laboratory exposure time for comparative.¹⁵⁻¹⁷

Among commonly used concentrations, 2.5% NaOCl provides an optimal balance between antimicrobial efficacy, tissue dissolution, and reduced cytotoxicity, making it suitable for *in vitro* evaluation.^{3,18}

Each canal was irrigated with 15 mL of the assigned solution, as supported by earlier studies demonstrating effective antimicrobial and tissue-dissolving activity with this volume.¹⁹⁻²¹ Irrigation was performed using side vented needles to minimize apical extrusion.

Groups:

Group-A: 2.5% Sodium hypochlorite

Group-B: 2.5% Sodium hypochlorite + 0.1% Benzalkonium-chloride



Group-C: 2.5% Sodium hypochlorite + 0.2% Cetrimide
 Each irrigant was mixed with Rhodamine B dye to allow fluorescence visualization under CLSM. After irrigation, specimens were longitudinally sectioned and examined under confocal laser scanning microscopy (CLSM) to measure the depth of dye penetration at coronal, middle, and apical-third.

STATISTICAL ANALYSIS

Data were analyzed using SPSS version 20 (IBM, New York, USA). One-way ANO and Tukey’s post hoc test were applied to compare mean penetration depths among groups, with significance set at $p < 0.05$.

RESULTS

Mean	N	Mean	Std. Deviation
Coronal	Group A(2.5% NaOCl)	6	510.85 ± 17.23
	Group B(2.5%NaOCl+BAK)	6	714.33 ± 162.72
	Group C(2.5%NaOCl+Cetrimide)	6	682.95 ± 40.73
Middle	Group A(2.5% NaOCl)	6	438.74 ± 80.67
	Group B(2.5% NaOCl+BAK)	6	622.02 ± 151.34
	Group C(2.5% NaOCl+ Cetrimide)	6	535.35 ± 78.41
Apical	Group A(2.5% NaOCl)	6	374.77 ± 43.92
	Group B (2.5% NaOCl+BAK)	6	462.00 ± 86.35
	Group C (2.5% NaOCl+ Cetrimide)	6	438.67 ± 23.09

Table 1. The mean depth of penetration (µm) of the irrigants at different root levels is presented

Inter-Group Comparison	Mean Difference	p value	95% CI Lower Bound	95% CI Upper Bound	Significance
Coronal Third					
Group A(2.5%NaOCl) vs Group B(2.5%NaOCl+BAK)	-203.48	<0.05	-365.20	-41.76	Significant
Group A(2.5%NaOCl) vs Group C(2.5%NaOCl+Cetrimide)	-172.10	<0.05	-249.80	-94.40	Significant
Group B(2.5%NaOCl+BAK) vs Group C (2.5% NaOCl+ Cetrimide)	31.38	>0.05	-145.60	208.40	Non-Significant

Table 2. Tukey's Post-Hoc Inter-Group Comparison – Coronal Third

Inter-Group Comparison	Mean Difference	p value	95% CI Lower Bound	95% CI Upper Bound	Significance
Middle Third					
Group A(2.5%NaOCl) vs Group B(2.5%NaOCl+BAK)	-183.29	<0.05	-345.80	-20.78	Significant
Group A(2.5%NaOCl) vs Group C(2.5%NaOCl+Cetrimide)	-96.62	<0.05	-259.10	-4.13	Significant
Group B(2.5%NaOCl+BAK) vs Group C (2.5% NaOCl+ Cetrimide)	86.67	>0.05	-79.60	252.90	Non-Significant

Table 3. Tukey's Post-Hoc Inter-Group Comparison – Middle Third



Inter-Group Comparison Apical Third	Mean Difference	p value	95% CI Lower Bound	95% CI Upper Bound	Significance
Group A(2.5%NaOCl) vs Group B(2.5%NaOCl+BAK)	-87.23	<0.05	-170.40	-4.06	Significant
Group A(2.5%NaOCl) vs GroupC(2.5%NaOCl+Cetrimide)	-63.90	<0.05	-125.70	-2.10	Significant
GroupB(2.5%NaOCl+BAK)vs Group C (2.5% NaOCl+Cetrimide)	23.33	>0.05	-48.60	95.20	Non-significant

Table 4. Tukey's Post-Hoc Inter-Group Comparison –Apical Third

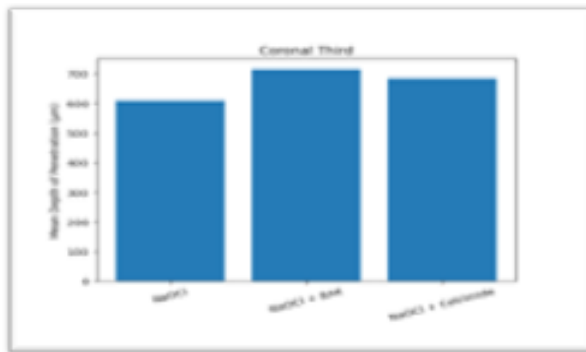


Figure - 1

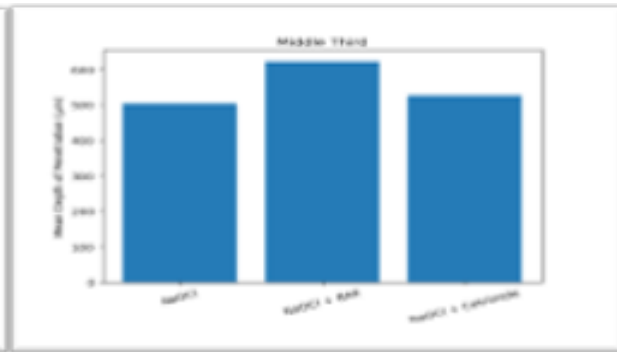


Figure - 2

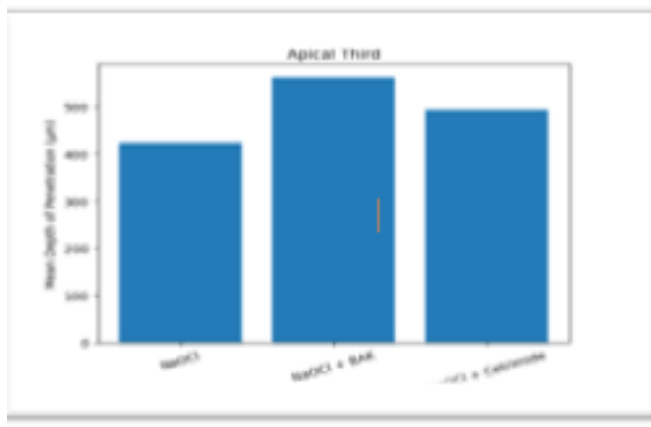
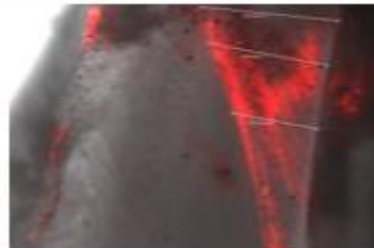
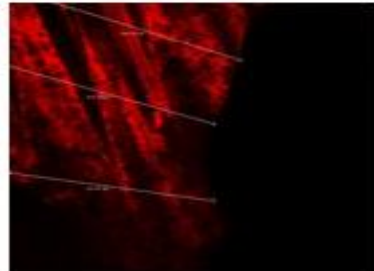


Figure - 3

Confocal microscopic images



Confocal microscopic images





The mean depth of penetration (in μm) of the irrigating solutions was evaluated at the coronal, middle, and apical thirds using confocal laser scanning microscopy (Table 1).

The mean penetration depth values (mean \pm standard deviation) for the coronal, middle, and apical thirds are presented in Table 1. At all root levels, the highest mean penetration depth was observed in the 2.5% NaOCl + BAK group, followed by the 2.5% NaOCl + Cetrimide group, while the 2.5% NaOCl group demonstrated the lowest values.

Coronal Third

At the coronal third, the mean penetration depth was lowest in Group A (2.5% NaOCl: 510.85 ± 17.23), while higher values were observed in Group B (2.5% NaOCl + BAK: 714.33 ± 162.72) and Group C (2.5% NaOCl + Cetrimide: 682.95 ± 40.73). Tukey's post-hoc analysis revealed a statistically significant difference between Group A and Group B as well as between Group A and Group C ($p < 0.05$). However, no statistically significant difference was observed between Group B and Group C ($p > 0.05$) (Table 2).

Middle Third

In the middle third, Group A (2.5% NaOCl) showed the lowest mean penetration depth (438.74 ± 80.67), whereas Group B (622.02 ± 151.34) and Group C (535.35 ± 78.41) demonstrated higher values. Tukey's post-hoc test indicated statistically significant differences between Group A and Group B and between Group A and Group C ($p < 0.05$). The difference between Group B and Group C was not statistically significant ($p > 0.05$) (Table 3).

Apical Third

At the apical third, the lowest mean penetration depth was again observed in Group A (374.77 ± 43.92), followed by Group C (438.67 ± 23.09), while Group B exhibited the highest mean value (462.00 ± 86.35). Tukey's post-hoc comparison showed statistically significant differences between Group A and Group B and between Group A and Group C ($p < 0.05$). No statistically significant difference was noted between Group B and Group C ($p > 0.05$) (Table 4).

Overall, although NaOCl combined with benzalkonium chloride showed higher mean penetration values across

all root levels, statistically significant inter-group difference.

DISCUSSION

Irrigation is a crucial step in root canal treatment that complements mechanical instrumentation by removing debris, necrotic tissue, microorganisms, and smear layer from the root canal system. Effective irrigation ensures disinfection as the mechanical preparation alone cannot adequately clean lateral canals, fins, isthmuses, and dentinal tubules, the chemical action of irrigants is indispensable for successful endodontic treatment.^{1,2}

NaOCl is the most widely used irrigating solution. In water, NaOCl ionizes to produce Na^+ and the hypochlorite ion, OCl^- , which establishes an equilibrium with hypochlorous acid, HOCl . Hypochlorous acid has long been considered the active moiety responsible for bacterial inactivation by chlorine-releasing agents, the OCl^- ion having a minute effect compared with undissolved HOCl .^{2,22}

Despite these advantages, NaOCl has inherent limitations, most notably its high surface tension, which restricts its penetration into dentinal tubules, particularly in the apical third of the root canal.^{5,6} To overcome this limitation, the addition of surfactants has been proposed to reduce surface tension, improve wettability, and enhance irrigant penetration.^{7,9}

In the current study 2.5% NaOCl was utilized for 15 minutes. According to multiple primary studies measuring pulp/tissue dissolution (Almeida 2013; Stojicic 2010; Iandolo 2019) use 5, 15 and 30-minute intervals; 15 minutes consistently yields substantially greater dissolution than 5 minutes and is therefore commonly used as a standardized laboratory exposure time for comparative.¹⁵⁻¹⁷

Among the various standardized concentrations of NaOCl used, 2.5% makes an optimal irrigating solution by not posing a significant risk of toxicity and, at the same time, having good pulpal dissolution and antibacterial properties. Thus, in the current investigation, 2.5% NaOCl was utilized.^{3,18}

According to Subhashini N et al Pinheiro SL et al. Zehnder M al. Zhou X et al Irrigation was performed with 15 mL of the assigned solution for each group. 15 ml of 2.5% NaOCl was efficient. So in our study we used 15



ml of 2.5% NaOCl at room temperature for 15 minutes for each group with vented irrigation needles.¹⁹⁻²¹

Surfactants act as detergents, emulsifiers, or wetting or foaming agent. When added to the irrigants, these agents reduce their surface tension, enhance microbial effectiveness, and improve clinical performance. The present study used 0.2% cetrimide and 0.1% Benzalkonium Chloride as surfactants.^{7,10,11}

In our study following groups were made

Group 1 (2.5%NaOCl as control group)

Group 2 (2.5%NaOCl+0.1%Benzalkonium Chloride as experimental groups) and

Group 3 (2.5%NaOCl+0.2%Cetrimide as experimental groups)

The CLSM used in the current study is better suited than other microscopy by producing fewer artifacts and enables a comprehensive analysis of the sample surface at a smaller magnification.^{13,14}

RB dye is an ideal dye because of its easier visibility, enhanced infusibility into dentinal tubules, and smaller particle size. DOP was analyzed to determine the efficiency of irrigants in reaching inaccessible areas of the root canal system.

In our study Benzalkonium Chloride and Cetrimide were used as surfactant for increasing dentinal tubule penetration. Surface-active agents (surfactants) have two regions in their molecular structures, one a hydrocarbon, water-repellent (hydrophobic) group and the other a water-attracting (hydrophilic polar) group.

The surfactants used in this study have antimicrobial activity, agreeing with the results from previous studies. Their molecules are positively charged and bind strongly to bacterial cell walls and membranes because of their opposite negative charge, leading to progressive leakage of cytoplasmic materials, causing cell disruption.

The present study when 2.5%NaOCl+Benzalkonium Chloride(experimental group) and 2.5% NaOCl+Cetrimide(experimental group)compared with 2.5% NaOCl result was significant.with p value(>0.05) and (>0.05) respectively.

This indicates that a surfactant when added to 2.5% NaOCl it increases the depth of penetration agreeing

with Gupta R et al (2019),Abdelaziz AM et al.(2020),Sirtes G et al. (2005).

When coronal middle and apical section 2.5% NaOCl+0.1%Benzalkonium Chloride(Group B) section compared with coronal middle and apical section2.5% NaOCl+0.2%Cetrimide(Group C) of the result is significant with p value . Our findings are in accordance with findings given by Iglesias JE et al.(2019) and Coaguila Llerena H et al.(2022).They compared the surface tension and penetration ability of sodium hypochlorite when combined with different surfactants and they concluded that benzalkonium chloride+ NaOCl showed lower surface tension than Cetrimide+NaOCl .

Lower surface tension means better wettability and greater depth of penetration.^{10,12}

Even though both surfactants are cationic quaternary ammonium compound,but differences in molecular structure like alkyl chain length and substituents may lead to different inter molecular interaction at the interface (electrostatic repulsion,hydration of head groups),influencing how tightly they pack and thus how much they reduce surface tension.NaOCl+Benzalkonium Chloride(Group B) showed better dentinal penetration might be due to its mixed chain length distribution(contains mixture of C12 and C14 etc) and higher hydrophilicity.^{10,23}

This mixture may allow more efficient packing/adsorption at the air/water (or liquid/solid) interface, reducing surface tension more effectively.

Because of its chain length distribution and perhaps somewhat higher hydrophilicity than a “pure long chain” surfactant, BAC may reach and saturate the interface quicker. That is the reason Benzalkonium Chlorite showed lower surface tension than Cetrimide

The results align with studies by N. S. et al. (2024), Faria et al. (2019), and Zou et al. (2010), which demonstrated that surfactant-modified NaOCl exhibited superior wettability and deeper penetration. However, even with surfactants, complete penetration into apical regions remains a challenge.

CONCLUSION

Within the limitations of this in vitro study, it can be concluded that the addition of 0.1% benzalkonium chloride to 2.5% sodium hypochlorite improves its



penetration into dentinal tubules compared to NaOCl alone or when mixed with 0.2% cetrimide. The apical third remains a challenging region for irrigant penetration, warranting further research into advanced delivery system

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