Value addition property of a cationic surfactant on endodontic irrigant: A confocal laser scanning microscope study

Sembagalakshmi Thirunarayanan, Mithra N. Hegde
Department of Conservative Dentistry and Endodontics, AB Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore, Karnataka India

Abstract
Introduction: Chlorhexidine (CHX) is a commonly used root canal irrigant with high surface tension which prevents its penetration into the isthmus and fins of the root canal. Surfactants have known to reduce the surface tension of the liquid. Cetylpyridinium chloride (CPC) is a cationic surfactant. The aim of this study is to assess the contact angle formed and depth of penetration of the endodontic irrigant with and without the addition of surfactant.

Methods: Contact angle analysis – Ninety-nine freshly extracted and cleaned single-rooted mandibular premolars were obtained. Thirty-three teeth were sectioned longitudinally and then transversely. The sections were mounted on an acrylic block, polished to obtain a flat dentin surface. Five microliter of Group 1: 2% CHX as control, Group 2: 2% CHX + 0.1% CPC, and Group 3: 2% CHX + 0.2% CPC were placed on the dentin surface and subjected to contact angle analysis.

Depth of penetration – Access cavity was prepared in sixty-six teeth and instrumented to working length till Protaper Gold F2. The samples were assigned to Groups 1, 2, and 3 and irrigated accordingly, and subjected to a confocal laser scanning Microscopy. Data were analyzed using one-way ANOVA and Tukey multiple comparison test. Karl Pearson’s correlation test was performed to analyze the correlation between the two.

Results: Group 3 had the lowest contact angle (12.82°) and the highest depth of penetration (222.74 µm) when compared to Group 1 and Group 2. Karl Pearson’s correlation showed no correlation between the contact angle and the depth of penetration.

Conclusion: Higher the concentration of CPC, lower is the contact angle formed with the dentin and higher is the depth of penetration into the dentinal tubule.

Keywords: Cetylpyridinium chloride; confocal laser scanning microscopy; irrigants; rhodamine B

INTRODUCTION
The objective of cleaning and shaping of the root canal system is to eliminate bacteria causing apical periodontitis as well as to create space in which gutta percha can be placed to seal the root canal system. The bacterial presence has been shown in recess, diverticula isthmus of the main as well as the lateral canal. Enterococcus faecalis, Gram-positive facultative anaerobe, has been shown to penetrate into the dentinal tubule up to depths of 460 µm. To disinfect the areas that are inaccessible for the instrument, the irrigants which have antibacterial effects must flow. Chlorhexidine (CHX) is one of the most commonly used root canal irrigating solutions. It is a synthetic cationic biguanide consisting of 4-chlorophenyl rings and 2 biguanide connected by a central hexamethylene chain. CHX is widely popular for its substantivity, where the irrigant releases positively charged ions which bind to the...
dentin and prevent further colonization of the bacteria for a long period of time. The irrigant must wet the dentinal tubule for this action to take place.[3]

Wettability is measured with the help of contact angle the liquid forms over the solid surface. Various studies have determined the contact angle formed by a root canal sealer; however, there are very few assessing the contact angle formed by the irrigant.[4] Lower the contact angle of the solution, higher is its wettability, and the average contact angle of CHX was measured to be 20.83°.[5] The mean depth of penetration of CHX from the previous study was noted to be 138 µm, 80 µm, and 44 µm in the coronal, middle, and the apical third, with the use of conventional syringe irrigation.[6] Surface tension of the liquid inhibits the penetration of liquid to remote areas, which can be overcome by the addition of surface-active agents such as a surfactant.[7] Previous studies have only assessed the depth of penetration of sealers into root canal dentin, whereas the irrigants have not been assessed.[8,9]

The addition of surfactant promotes the contact of the liquid to the surface, thereby increasing the wettability of the liquid. Surfactants exist in four categories: anionic, cationic, amphoteric, or nonionic. Cetylpyridinium chloride (CPC), which is a cationic surfactant, is the chloride salt form of cetylpyridinium, quaternary ammonium with broad-spectrum antimicrobial activity. CPC is also a pyridinium salt that has N-hexadecylpyridinium as the cation and chloride as the anion. It has antiseptic properties and is used in mouthwash solutions or lozenges for the treatment of minor infections of the mouth and throat.[10] Cationic surfactants have self-assembling properties and are also used in modern drug delivery systems. There has not been any study conducted with the use of CPC, which is a cationic surfactant along with CHX cationic bisbiguanide. This study aims to evaluate the value addition property of the surfactant at varying concentrations of 2% CHX.

**METHODS**

This study was conducted in the Department of Conservative Dentistry and Endodontics, AB Shetty Memorial Institute of Dental Sciences, after obtaining ethical clearance from the Institutional Ethics Committee (certificate no: ABSM/EC 65/2019).

**Groups for testing**
- Group 1: 2% CHX (Control)
- Group 2: 2% CHX and 0.1% CPC
- Group 3: 2% CHX and 0.2% CPC.

**Contact angle analysis**

Thirty-three freshly extracted single-rooted mandibular premolars were cleaned, sectioned longitudinally, and then transversely with a diamond disc to obtain four blocks of dentin from each tooth (two coronal and two radicular dentin blocks). This gave rise to a total of 132 dentin blocks samples. Self-cure acrylic was used to mount the dentin blocks with the dentin surface facing upward. The specimens were polished with 80, 100, 120, and 150 grit abrasive papers, distributed randomly among three groups (n = 22), and 5 µl from each group were pipetted out and placed on coronal as well as radicular dentin blocks. Tangent was drawn on the liquid drop and the angle it formed with the flat dentin surface was measured using SCA software.

**Depth of penetration**

Sixty-six freshly extracted mandibular single-rooted premolars were cleaned, coated with nail varnish on the
root surface, and access cavities were prepared with Endo Access Bur (Dentsply Tulsa Dental Specialties). Working length was estimated with a 10-K file radiographically and was kept 1-mm short of the apex. Teeth were divided into three Groups (n = 22). Cleaning and shaping was done with ProTaper Gold (Dentsply Tulsa Dental Specialties) rotary files in the order of Sx, S1, S2, F1, and F2 under irrigation of 15 ml of the specific group assigned. The samples were rinsed with 0.9% normal saline and stained using rhodamine B (RB).

### 2% rhodamine B preparation

Two milligrams of RB powder was weighed and dissolved in 100 ml of water. It is waited until complete dissolution occurs. The dye was rinsed with 0.9% normal saline. The samples were sectioned at 2 mm, 5 mm, and 8 mm above the apex to check for the depth of penetration in the coronal, middle, and the apical third of the root canal. The slices were mounted on a slide and observed under a confocal laser scanning microscope (CLSM). The length of the RB dye was measured from the center to the end at 12, 3, 6, and 9 o’clock positions for apical, middle, and cervical thirds, and the average of all four was taken per section.

Statistical analysis was performed using SPSS software (SPSS v20, IBM, New York, USA). Data were analyzed with one-way ANOVA and Tukey post hoc test. Karl Pearson’s correlation was performed to test for correlation between the contact angle formed and the depth of penetration of the irrigant.

## RESULTS

Group 1 had the highest contact angle for coronal as well as apical samples with 21.54° ± 1.85° and 20.94° ± 1.63°, respectively, and least with Group 3 measuring 12.82° ± 1.04° in coronal and 12.77° ± 1.11° in the apical third, as seen in Table 1. The difference in the angle between Group 1 and 2 was 3.48° in coronal and 3.12° in the apical third, which were all statistically significant with the $P < 0.001$, as shown Table 2.

Group 3 had the highest depth of penetration in the coronal, middle, and the apical third with 222.74 ± 9.04 µm, 156.57 ± 7.9 µm, and 84.29 ± 3.48 µm, respectively, and the least depth of penetration was observed in Group 1 with 120.88 ± 6.53 µm in the coronal, 67.52 ± 1.45 µm in the middle, and 38.72 ± 2.79 µm in the apical third as seen in Figure 2. When the depth of penetration was compared between Group 2 and Group 3, there was statistically significant improvement by −29.68 µm in the coronal

### Table 1: Mean contact angle and depth of penetration of the irrigant and one-way ANOVA

|                      | Group 1 (n=22) | Group 2 (n=22) | Group 3 (n=22) | One-way ANOVA |
|----------------------|----------------|----------------|----------------|---------------|
| Coronal contact angle (°) | 21.54±1.85     | 18.05±1.08     | 12.82±1.04     | F = 236.806* | P < 0.001 |
| Apical contact angle (°) | 20.94±1.63     | 17.81±0.94     | 12.77±1.11     | F = 222.858* | P < 0.001 |
| Depth of penetration coronal | 120.88±6.53   | 193.07±9.64    | 222.74±9.04    | F = 833.573  | P < 0.001 |
| Depth of penetration middle | 67.52±1.45   | 134.68±8.23    | 156.57±7.9     | F = 1959.836* | P < 0.001 |
| Depth of penetration apical | 38.72±2.79    | 57.93±3.52     | 84.29±3.48     | F = 1069.951 | P < 0.001 |

*Welch test

### Table 2: Post hoc Tukey comparison among three groups for both contact angle and depth of penetration

|                          | Group 1 versus Group 2 difference (P) | Group 1 versus Group 3 difference (P) | Group 2 versus Group 3 difference (P) |
|--------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| Coronal contact angle (°) | 3.48 (<0.001)                        | 8.72 (<0.001)                        | 5.24 (<0.001)                        |
| Apical contact angle (°)  | 3.12 (<0.001)                        | 8.17 (<0.001)                        | 5.08 (<0.001)                        |
| Depth of penetration coronal (µm) | −72.19 (<0.001) | −101.86 (<0.001) | −29.68 (<0.001) |
| Depth of penetration middle (µm) | −67.16 (<0.001) | −89.05 (<0.001) | −21.9 (<0.001) |
| Depth of penetration apical (µm) | −19.21 (<0.001) | −45.57 (<0.001) | −26.36 (<0.001) |

---

2% CHX: Chlorhexidine, CPC: Cetylpyridinium chloride

---

Figure 2: Confocal images of the depth of penetration in coronal, middle, and apical third for irrigation with 2% CHX, 2% CHX, and 0.1% CPC, and 2% CHX and 0.2% CPC. CHX: Chlorhexidine, CPC: Cetylpyridinium chloride
third, −21.9 µm in the middle and −26.36 µm in the apical third, as seen in Table 2. Karl Pearson correlation shows that depth of penetration of irrigant and the contact angle formed are independent of each other [Fig. 1].

DISCUSSION

Contact angle determines the wettability of the irrigant. Direct calculation of the tangent of the angle between the liquid droplet and the solid surface of the substrate yields experimental contact angle values. It has been determined that if the contact angle is <90°, the surface will be wet; if it is >90°, there will be no wetting; and if the contact angle is zero, the surface will be completely wet. The surface of the dentin was polished with abrasive grit to obtain a flat wide dentin surface. Studies have emphasized the importance of polishing the dentinal surface before the analysis of the contact angle. It alters the surface granulation, thereby affecting the contact angle formed. In the present study, all the three irrigant groups had contact angles <90°, showing there is wetting of the surface. The contact angle formed by 2% CHX is higher than the 2% CHX with surfactant. There was no significant difference in the contact angle formed between the coronal and the apical dentin block, as they were treated the same way during sample preparation. BioPure MTAD showed the highest wettability, followed by 2.5% sodium hypochlorite (NaOCl) and 2% CHX because of the presence of surfactant in MTAD. The addition of surfactant reduces the surface tension of some irrigants up to 50%, thereby increasing the wettability of the irrigant. Tasman, in his study, demonstrated that Cetredixine has the lowest average surface tension value because of the surfactant present in the solution in comparison with 2.5% NaOCl, 17% ethylenediaminetetraacetic acid, Ringer’s solution, hydrogen peroxide, saline solution, and distilled water.

Penetration of irrigant into the dentinal tubule is essential to disinfect the areas that are inaccessible for the rotary systems. In this study, RB dye was used to assess the depth of penetration along with the use of CLSM. RB dye solution at the concentration of 0.1 m. mol is used as a marker to evaluate the depth of penetration of irrigants viewed under CLSM. Although there was no visible chemical reaction observed when dissolving RB powder with CHX or with the surfactants, 0.9% saline was used as a final rinse to avoid direct contact between the irrigating solution and the dye. There was no other method of analysis done to check for the reaction product. According to Vogt et al., the penetrability of RB dye is more than methylene blue and silver nitrate dye.

In the present study, we found a statistically significant increase in the depth of penetration of the irrigant in the coronal third, followed by the middle and the apical third. This could be attributed due to various reasons such as a lower number of patent tubules in the apical region because of dentinal sclerosis and inefficient access to this region for irrigants to flush out the debris and remove the smear layer to keep the tubules patent. 2% CHX + 0.2% CPC, which had a higher concentration of surfactant, also showed the highest depth of penetration. This result is in agreement with the previous study conducted by Mukhlif and Al-Hashimi in 2021, where irrigant with surfactant showed higher penetration into the dentinal tubules than the irrigant without the surfactant, indicating that surfactant in the composition led to the higher penetration. Conversely, Faria et al. showed that the addition of surfactant did not influence the penetration of irrigant into dentin. However, there is a difference in the duration of irrigation between the two studies could attribute to the varying results. The present study is the first to report the use of CPC as a surfactant in combination with the endodontic irrigant for the cleaning of root canals.

The study has its limitation as it was conducted in vitro, no method of activation was attempted to improve the depth of penetration of the irrigant, and also did not assess the antimicrobial activity that could be modified because of the addition of the surfactant. Further research needs to be emphasized on this area.

CONCLUSION

Within the limitations of our study, the contact angle formed by the irrigant was reduced with the addition as well as an increase in the concentration of the CPC which is a cationic surfactant. The depth of penetration improved with the addition of CPC, and it was the highest in the coronal, followed by the middle and apical third.

Acknowledgment

The authors would like to acknowledge by Dr. Shamprasad Varja Raghu, Mangalore University for assisting with the use of confocal laser scanning microscopy.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Siqueira JF Jr., Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008;34:1291-301.e3.
2. Kakoli P, Nandakumar R, Romberg E, Arola D, Fouad AF. The effect of age on bacterial penetration of radicular dentin. J Endod 2009;35:78-81.
3. Athanassiadis B, Abbott PV, Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. Aust Dent J 2007;52:68-74.
4. Nikhil V, Jaiswal S, Bejpai G. In vitro evaluation of the contact angle.
formed between AH Plus, Hybrid Root Seal and mineral trioxide aggregate Plus sealer with dentin and gutta-percha. J Conserv Dent 2018;21:180-3.
5. Lopes HP, De Faria AR, Alves FR, Elias CN. Wettability of irrigants used in root canal treatment. Dentistry 2015;5:1.
6. Vadhana S, Latha J, Velmurugan N. Evaluation of penetration depth of 2% chlorhexidine digluconate into root dentinal tubules using confocal laser scanning microscope. Restor Dent Endod 2015;40:149-54.
7. Cameron JA. The effect of a fluorocarbon surfactant on the surface tension of the endodontic irrigant, sodium hypochlorite. A preliminary report. Aust Dent J 1986;31:364-8.
8. Kamin R, Vikram R, Meena N, Kumari RA, Adarsha MS, Murthy CS. Effect of final irrigating solutions on penetration depth of resin-based sealers into dentinal tubules. J Conserv Dent 2021;24:374-8.
9. Bharti R, Tikku AP, Chandra A, Shakya VK, Yadav S. Depth and percentage of resin-based sealer penetration inside the dentinal tubules using EndoVac, EndoActivator, Navi tip FX irrigation system: A confocal laser scanning microscope study. J Conserv Dent 2018;21:216-20.
10. Hoang TPN, Ghori MU, Conway BR. Topical antiseptic formulations for skin and soft tissue infections. Pharmaceutics 2021;13:558.
11. Kumar G, Prabhu KN. Review of non-reactive and reactive wetting of liquids on surfaces. Adv Colloid Interface Sci 2007;133:61-89.
12. Kontakiots EG, Tzanetakis GN, Loizides AL. A comparative study of contact angles of four different root canal sealers. J Endod 2007;33:299-302.
13. Mukhil TA, Al-Hashimi RA. The Effect of Adding Poloxamer Surfactant on Cleaning Efficiency of NaOCl and NaOH (SEM Study). International Medical Journal. 2021 Jun 2;28.
14. Iglesias JE, Pinheiro LS, Weibel DE, Montagner F, Grecca FS. Influence of surfactants addition on the properties of calcium hypochlorite solutions. J Appl Oral Sci 2019;27:e20180157.
15. Milosevic A. The influence of surface finish and in-vitro pellicle on contact-angle measurement and surface morphology of three commercially available composite restoratives. J Oral Rehabil 1992;19:85-97.
16. Taşman F, Çağreli ZC, Oğan C, Etikan I. Surface tension of root canal irrigants. J Endod 2000;26:586-7.
17. Nakashima K, Terata R. Effect of pH modified EDTA solution to the properties of dentin. J Endod 2005;31:47-9.
18. Xie Q, Bedran-Russo AK, Wu CD. In vitro remineralization effects of grape seed extract on artificial root caries. J Dent 2008;36:900-6.
19. Vogt BF, Xavier CB, Demarco FF, Padiha MS. Dentin penetrability evaluation of three different dyes in root-end cavities filled with mineral trioxide aggregate (MTA). Braz Oral Res 2006;20:132-6.
20. Faria G, Viola KS, Coaguila-Llerena H, Oliveira LR, Leonardo RT, Aranda-Garcia AJ, et al. Penetration of sodium hypochlorite into root canal dentine: Effect of surfactants, gel form and passive ultrasonic irrigation. Int Endod J 2019;52:385-92.