Effect of QMix irrigant in removal of smear layer in root canal system: a systematic review of in vitro studies

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ABSTRACT

Objectives: To evaluate the outcome of in vitro studies comparing the effectiveness of QMix irrigant in removing the smear layer in the root canal system compared with other irrigants.

Materials and Methods: The research question was developed by using Population, Intervention, Comparison, Outcome and Study design framework. Literature search was performed using 3 electronic databases PubMed, Scopus, and EBSCOhost until October 2019. Two reviewers were independently involved in the selection of the articles and data extraction process. Risk of bias of the studies was independently appraised using revised Cochrane Risk of Bias tool (RoB 2.0) based on 5 domains.

Results: Thirteen studies fulfilled the selection criteria. The overall risk of bias was moderate. QMix was found to have better smear layer removal ability than mixture of tetracycline isonomer, an acid and a detergent (MTAD), sodium hypochlorite (NaOCl), and phytic acid. The efficacy was less effective than 7% maleic acid and 10% citric acid. No conclusive results could be drawn between QMix and 17% ethylenediaminetetraacetic acid due to conflicting results. QMix was more effective when used for 3 minutes than 1 minute.

Conclusions: QMix has better smear layer removal ability compared to MTAD, NaOCl, Tubulicid Plus, and Phytic acid. In order to remove the smear layer more effectively with QMix, it is recommended to use it for a longer duration.

Keywords: QMix; Root canal treatment; Smear layer; Systematic review

INTRODUCTION

Root canal treatment failure comprises a variety of reasons and persistence of microorganisms in the root canal system even after shaping and cleaning is one of them [1]. The primary objective of the root canal therapy revolves around thorough debridement [2]. However, due to the complexity of the root canal system, shaping and cleaning procedure has become a challenging phase for the clinician. The disinfection of the entire root canal system relies on the ability of chemical irrigants in the root canal system [3]. Smear layer forms on the inner root canal wall when it is in contact with the instruments and during filing motion. It comprises 2 parts, the thick superficial layer on the surface of root canal wall (approximately 1 to 2 μm) and a deeper layer (up to 40 μm) into the dentinal tubules which contain organic and inorganic tissues including microorganisms and necrotic debris [4-7]. The smear layer interrupts the
penetration of root canal irrigants and acts as a barrier between the root filling and the canal wall, which is a potential path of leakage for bacteria contamination between the 2 surfaces [4,8,9]. It has been known that removal of the smear layer is essential to achieve thorough disinfection and 3-dimensional filling of the root canal system, thereby affecting the outcome of the endodontic procedure [10-12].

Various types of root canal irrigants such as sodium hypochlorite (NaOCl) [11], chlorhexidine (CHX) [12], and mixture of tetracycline isonomer, an acid and a detergent (MTAD) [13] have been used based on their tissue dissolving and antimicrobial properties, whereas 17% ethylenediaminetetraacetic acid (EDTA) [14] and citric acid [15] have been advocated to remove the smear layer. Thus far, it has yet to be found a single irrigant that is able to fulfill the objectives of root canal irrigation that dissolves the organic and inorganic components in the canal, disruption of biofilms, neutralization of endotoxins and eliminating smear layer [16-20]. Amongst all the materials, the combination of NaOCl and 17% EDTA has been the preferred choice to remove both organic and inorganic parts of the smear layer in the root canal system [21,22].

As an alternative to the recommended irrigation protocol (NaOCl + 17% EDTA as final irrigant), QMix was introduced for the dual effect of smear layer removal [18]. This consists of polyaminocarboxylic acid chelating agent, bisbiguanide antimicrobial agent (2% CHX), surfactant and deionized water. QMix has shown to have higher antimicrobial property against Enterococcus faecalis as compared to CHX and found similar to EDTA in removing the smear layer [23,24]. Many studies have compared the effectiveness of QMix in smear layer removal with other irrigants and have shown varying results. Some studies have shown that QMix removes smear layer more effectively than other irrigants whereas some results were contradictory. Thirteen studies have claimed that higher effectiveness was affected by the duration of QMix irrigation [23-35].

Due to the lack of standardization of methodology in in vitro studies, such investigations can only be appraised individually. To the best of the authors’ knowledge, no systematic review has been done to evaluate the effectiveness of QMix compared to other irrigants in removing the smear layer in a human root canal system, although a number of in vitro studies had been published. Therefore, the aim of this systematic review was to compare the smear layer removal efficacy of QMix with other commonly employed root canal irrigants.

**MATERIALS AND METHODS**

**Review question**

The Population, Intervention, Comparison, Outcome and Study design framework was employed to develop the research question: In the extracted permanent human teeth with smear layer (P), does QMix irrigant (I) show better smear layer removal ability (O) compared to the other irrigants (C) from in vitro studies (S).

**Search strategy**

Literature search was performed comprehensively using 3 electronic databases including PubMed, Scopus, and EBSCOhost (Dentistry; Oral Sciences Source) was used for literature search to identify relevant articles: using the search strategy ((QMix) AND smear layer) AND ((root canal or endod)). Articles published from inception to October 2019 was included in...
this search. Reference list of the eligible studies and the journals publishing content relevant to the topic including Journal of Endodontics, International Endodontic Journal, Journal of Dentistry, Australian Endodontic Journal and Journal of Conservative Dentistry were hand-searched to identify any relevant studies. A flow chart of the search strategy was presented in Figure 1.

**Inclusion criteria**
Inclusion criteria for the selection of articles in this review were; 1) studies published in English, 2) studies performed in the extracted permanent human teeth, 3) studies that compared the smear layer removal ability of QMix with at least one irrigant, and 4) studies that tested the smear layer removal efficacy using scanning electronic microscopy (SEM), polarized light microscopy, and SEM-energy dispersive X-ray spectrometry.

**Exclusion criteria**
Studies conducted in vivo, on animals or in bovine teeth were excluded.

**Study selection and data extraction process**
Firstly, the title and abstract of the selected articles were screened by 2 reviewers (MC, AP) based on the specified inclusion and exclusion criteria. Secondly, the reviewers independently read the articles and extracted the data using the data extraction form based on the items exclusive to this study. The following details were included in the data extraction form: author/s, year of publishing, country of the corresponding author, the total number of samples, type of teeth, interventions, evaluation method, results, irrigation regime including volume and duration,
and scoring system. Any disagreement between the 2 reviewers in the selection of articles and the extraction of data was resolved in consultation with a third reviewer (JJ).

Quality assessment of the included articles
The quality of articles included in this review was assessed using revised Cochrane Risk of Bias tool (RoB 2.0). This tool was specifically modified to incorporate the contents relevant to the methodology of the included in vitro studies. Based on this, the quality of the studies was assessed on the following domains: randomization process, standardization of canal length, protocol for canal size enlargement, protocol for irrigating regimen (volume and duration) and bias in measurement outcome. The quality appraisal was independently performed by 2 authors (AP, MC) based on the above domains. In case of disagreement, a consensus was reached in discussion with a third reviewer (JJ).

RESULTS

Study selection process
A total of 181 studies were identified from 3 electronic bases. 2 studies were retrieved through additional hand searching. After excluding studies based on title and abstract screening, 16 articles were available for full-text assessment. On careful reading, further 3 studies were excluded due to the following reasons: study done on bovine teeth [36], study that evaluated the effect of smear layer in infected dentinal tubules [8] and study that tested the bond strength of glass fiber post [37]. Finally, 13 papers were included in this systematic review. The search process employed to identify included studies was shown in Figure 1. Meta-analysis was not performed due to the presence of heterogeneity in the methodology and reporting outcomes of the included studies.

Eleven studies compared QMix to various irrigants like EDTA [23-31,33,35], 4 studies on MTAD [23,31,34,35], 5 studies on NaOCl [23,27-29,33], 2 studies on 7% EDTA (SmearClear) [30,32], 10% citric acid [29,33], one study on chitosan [32], glyde [32], 1% peracetic acid [33], Tubulicid Plus [34] and 7% maleic acid [28]. The effectiveness of smear layer removal was measured by different scoring systems. Four studies used Hülsmann et al. scoring system [25,26,30,35], 2 studies used Takeda et al. system [27,29], 3 studies used Torabinejad et al. scoring system [28,31,34] and others included Gishi et al. scoring system [33], Dai et al. scoring system [23] and Rome et al. scoring system [32] respectively. One study did not mention the scoring system and adhered to counting the number of open and closed tubules using imaging software, Adobe Photoshop CS3 [24].

Characteristics of included studies
Out of 13 studies, 4 studies included single-rooted teeth [23-25,32] and 5 studies specifically mentioned the type of single-rooted teeth. 2 studies utilized maxillary anterior teeth or incisors [28,35], one study included canine [30], one used central incisors [27] and mandibular incisors [33] respectively. 4 studies included single-rooted mandibular premolar [26,29,31,34]. The apical instrumentation size and taper contribute to the efficacy of smear layer removal [38,39]. The final instrumentation size, taper, standardized length of canal and system used for canal preparation varied among the included studies. It was found that 3 studies prepared canal length at 17 mm [23,27,30] while other studies prepared the canal at 15 mm [25,28,35], 14 mm [31,34], 13 mm [26] and 16 mm [33] respectively. Three studies did not mention the standardized canal length in their studies [24,29,32]. One study employed a combination of passive step-back and rotary 0.06 taper nickel-titanium files [32] and one did not mention the canal preparation system in their study [24]. Apical size of root canal in one study was reported
as ISO #25 [26], 6 studies enlarged up to ISO #30 [27-29,31,32,34], 3 studies enlarged up to the ISO #40 [25,33,35] and 2 studies enlarged up to the ISO #50 [23,30]. One study was done on dentin disc; hence no apical enlargement was mentioned in the study.

Four studies showed the effectiveness of QMix in smear layer removal was comparable to EDTA [23,24,28,30], while 3 studies showed QMix was more effective in removing the smear layer as compared to EDTA [25,27,35]. Only 4 studies showed 17% EDTA was better than QMix in smear layer removal [26,29,31,32]. QMix was found to be more effective when used for 3 minutes as compared to 1 minute [26]. Comparing with other materials, one study showed QMix was more effective than MTAD [31] while another study showed QMix has smear layer removal ability similar to MTAD [35] and SmearClear [30]. 2 studies showed QMix was more effective than NaOCl [29,33], Tubulicid Plus [34] and phytic acid [26]. One study showed QMix less effective as compared to 7% maleic acid [27] and 10% citric acid [28]. QMix and 1% peracetic acid remove the smear layer without alterations in inorganic structures, while 10% citric acid and 17% EDTA remove the smear layer with loss of structure (Table 1) [33].

Quality of included studies

The studies were analyzed using the modified Risk of Bias tool and the overall quality of the included studies was found to be “moderate” (Figure 2). Most of the studies followed the randomization process [26-29,31-35] and all studies stated the protocol for canal size enlargement, respectively. Three studies did not mention the standardization of canal length [24,29,32]. In the protocol for irrigating regimens, all studies reported information on the volume of irrigants and the duration of their use except one study [30]. Four studies reported bias in measurement outcomes [30-32,34].

DISCUSSION

Root canal disinfection can be achieved by mechanical and chemical means and irrigation plays a crucial role [8]. Irrigants can reach areas with anatomical complexities including isthmus, ramifications, dentinal tubules of the root canal system, where instrumentation cannot reach thereby, facilitate a reduction of microbial biofilms [11,12]. Various studies published on QMix comparing with other irrigants on its smear layer removal property [23-35], but the results were inconsistent. Although it would be more applicable if the effectiveness was tested using in vivo settings, no study has been conducted in this manner. Hence, to answer our research question, only in vitro studies were included in the systematic review.

To evaluate the risk of bias of the included studies, Revised Cochrane ROB has been modified based on the characteristics of the included in vitro studies. A total of 5 parameters (randomization process, standardization of canal length, protocol for canal size enlargement, protocol for irrigating regimen [volume, duration], and bias in measurement outcome) have been used to appraise the quality of included studies. The standardization of canal length varied among the published literature. Root canal length and depth of insertion of irrigation needle have an effect on the efficacy of irrigant to remove the debris from the canal [40], and cleaning of the apical third of the canal has always been challenging compared to coronal and middle third [41]. This can be due to the formation of the vapor lock phenomenon at the apical third, as the root is a closed-ended channel being surrounded by periodontium [42-44]. Hence, the importance of the depth of irrigation needle should be at the working length to irrigate the apical third of the canal, which can be implemented
Table 1. Characteristics of included studies in the systematic review

| No. | Author Year Country | Total number of samples | Type of teeth | Interventions (groups) | Evaluation method | Results (main results; group showing significantly higher smear layer removal) | Irrigation regime (volume, duration) | Scoring system |
|-----|---------------------|-------------------------|---------------|------------------------|------------------|-----------------------------------------------------------------------------|--------------------------------------|---------------|
| 1   | Dai et al. [23]     | China                   | 50            | Single-rooted teeth    |                  | 1. 5.25% NaOCl + QMix I (pH 8.0) 2. 5.25% NaOCl + QMix II (pH 7.5) 3. 5.25% NaOCl + sterile distilled water 4. 5.25% NaOCl + 17% EDTA 5. 1.3% NaOCl + BioPure MTAD | Smear layer: 2 versions of the experimental antimicrobial (QMix) are as effective as 17% EDTA in removing canal wall smear layers Debris: BioPure MTAD and EDTA and 2 QMix versions are ineffective in clearing debris completely from the canal spaces | 30-G side-vented needle, 5 mL, 2 min | Dai et al. [23] |
| 2   | Stojicic et al. [24]| Canada                  | 3             | Single-rooted teeth    |                  | 1. QMix 2. 17% EDTA 3. Water | Proportion of open tubules by QMix = 0.88, EDTA = 0.85. No significant difference between these 2 groups | 10 mL, 5 min Experiment carried out by soaking discs in beaker | By counting the number of open and closed tubules in Adobe Photoshop CS3 |
| 3   | Eliot et al. [25]   | USA                     | 80            | Single-rooted teeth    |                  | 1. 3 mL of QMix A for 60 sec 2. 3 mL of QMix A for 90 sec 3. 3 mL of QMix B for 60 sec 4. 3 mL of QMix B for 90 sec 5. 3 mL of QMix C for 60 sec 6. 3 mL of QMix C for 90 sec 7. 3 mL of 17% EDTA for 60 sec 8. 3 mL of 17% EDTA for 90 sec | No significant difference in smear layer removal among the 3 QMix formulations was demonstrated. All 3 of the modified QMix solutions removed significantly more smear layer when compared with EDTA | 30-G side-vented needle, 3 mL,60 sec/90 sec | Hülsmann et al. [14] |
| 4   | Aranda-Garcia et al. [30]| Brazil              | 40            | Canines                |                  | 1. DW 2. 17% EDTA 3. SmearClear 4. QMix | SEM (>1,000 magnification coronal, middle, and apical levels) | 17% EDTA, SmearClear, and QMix promoted higher debris and smear layer removal than control group. No significant difference between the groups | NA | Hülsmann et al. [14] |
| 5   | Banode et al. [29]  | India                   | 20            | Single-rooted mandibular first premolar |                  | 1. 1% NaOCl 2. 10% citric acid 3. 17% EDTA 4. QMix | SEM (>200, >1,000, >2,000 magnification coronal, middle, and apical levels) | 17% EDTA, 10% citric acid, and QMix removes smear layer effectively from cervical and middle parts of canal as compared to apical third (citric acid > EDTA > QMix > NaOCl) | NA | Takeda et al. |
| 6   | Vemuri et al. [31]  | India                   | 40            | Single-rooted mandibular premolar |                  | 1. Saline 2. EDTA 3. BioPure MTAD 4. QMix | SEM (>1,000 magnification in apical 3rd) | Saline > EDTA > MTAD > QMix | 30-G side-vented needle, 5 mL, 3 min | Torabinejad et al. |
| 7   | Ballal et al. [28]  | India                   | 40            | Maxillary anterior teeth |                  | 1. 2.5% NaOCl + QMix 2. 2.5% NaOCl + 7% Maleic acid 3. 17% EDTA 4. 2.5% NaOCl + 0.9% saline | SEM (>500 magnification coronal, middle, and apical levels) | No significant difference between QMix, MA and EDTA in removal of smear layer from the coronal and middle thirds (p > 0.05). MA shows significantly better than EDTA and QMix in apical third (p < 0.001) | 29-G stainless steel needles, 5 mL, 1 min | Torabinejad et al. |
| 8   | Venghat and Hegde [32]| India               | 48            | Single-rooted teeth    |                  | 1. QMix 2. 0.2% Chitosan 3. Smear Clear 4. Glyde | SEM (>1,000 and >2,000 magnification coronal, middle, and apical levels) | EDTA shown maximum removal of smear layer followed by 0.2% chitosan, smear clear and QMix 2 in 1. No statistical significance difference seen in removal of smear layer among all the group | 30-G side-vented needle, 5 mL | Rome et al. |

(continued to the next page)
| No. | Author et al. | Year | Country | Total number of samples | Type of teeth | Interventions (groups) | Evaluation method | Results (main results; group showing significantly higher smear layer removal) | Irrigation regime (volume, duration) | Scoring system |
|-----|---------------|------|---------|-------------------------|---------------|-----------------------|-------------------|------------------------------------------------|-----------------------------|----------------|
| 9   | Jagzap et al. [26] | 2017 | India   | 30                      | Single-rooted mandibular premolar | 1. 17% EDTA 2. QMix 3. Phytic acid | SEM (>1,000 magnification coronal, middle, and apical levels) | Smear layer removing ability in descending order: 17% EDTA > QMix > phytic acid; coronal > middle > apical | 30-G side-vented needle, 1 ml, 1 min | Hülsmann et al. [14] |
| 10  | Aksel and Serper [27] | 2017 | Turkey  | 80                      | Central incisors | 1. 3 min 5% NaOCl + QMix 2. 3 min 5% NaOCl + EDTA 3. 1 min 5% NaOCl + QMix 4. 1 min 5% NaOCl + EDTA 5. 3 min 2.5% NaOCl + QMix 6. 3 min 2.5% NaOCl + EDTA 7. 1 min 2.5% NaOCl + QMix 8. 1 min 2.5% NaOCl + EDTA | SEM (the first scan was made at a magnification of <30, and >2,000 at both middle and apical) | QMix allowed more smear layer removal than EDTA after using 5% initial NaOCl for 3 min. In the apical part of the root canal walls, the smear layer was not completely removed | 30-G side-vented needle, 5 ml, 3 min/1 min | Takeda et al. |
| 11  | Baldasso et al. [33] | 2017 | Brazil  | 60                      | Mandibular incisors | 1. QMix 2. 17% EDTA 3. 10% citric acid 4. 1% peracetic acid 5. 2.5% NaOCl 6. DW | PLM for organic component analysis (>40 to >400 magnifications) SEM for inorganic structure analysis (>1,000 magnifications) | PLM: NaOCl and DW has no effect on collagen, QMix and PA caused little alteration, EDTA shows increased deleterious effect and CA caused severe disorganization in organic component SEM: QMix and PA remove smear layer without alterations. CA and EDTA groups removed the smear layer with a loss of structure. NaOCl and DW group shows dense smear layer | 30-G needle | Ghisi et al. |
| 12  | Kolanu et al. [34] | 2018 | India   | 40                      | Mandibular premolar | 1. Saline 2. Tubulicid Plus 3. BioPure MTAD 4. QMix | SEM (>1,000 magnification, apical third) | Group IV > group III > group II > group I | 30-G side vented needle | Torabinejad et al. |
| 13  | Nogo-Živanović et al. [35] | 2019 | Europe  | 40                      | Maxillary incisors | 1. MTAD 2. QMix 3. 17% EDTA 4. Distilled water | SEM (>2,000 magnification, coronal, middle and apical) | Group 1 = group 2 > group 3 | 30-G syringe needle | Hülsmann et al. [14] |

NA, not available; G, gauge; SEM, scanning electron microscope; DW, distilled water; NaOCl, sodium hypochlorite; EDTA, ethylenediaminetetraacetic acid; MTAD, mixture of tetracycline isonomer, an acid and a detergent; CA, Citric acid; MA, maleic acid; CSI, conventional syringe irrigation; PLM, polarized light microscopy.
in a clinical setting [45]. Moreover, when activation and agitation were added on QMix, it enhanced its smear layer removal ability and showed a significant difference compared to QMix without agitation [46-53].

It has been shown that the protocol for canal size preparation plays a crucial role in the removal of debris in the root canal. There was a significant difference in larger canal preparation which enabled a more effective removal of smear layer than a smaller sized canal [54,55]. It was reported from a study that an increase in the size of ISO #35 to ISO #40 has a significant increase in mean irrigant volume, which was at 44% of the root canal system. It was proposed to enlarge the apical third to ISO #40 with a 0.04 taper to allow a maximum volume of irrigation at the apical third and tooth structure preservation [56]. Hence, the different protocol of canal preparation implied in respective studies has a significant impact on smear layer removal ability. In addition, few studies have shown that the duration of irrigation with QMix affects the smear layer removal effectiveness. This was apparent in this review as QMix showed better effect when used for a longer time (3 minutes) as compared to 1 minute [26].

Substantial differences in the method of scoring were observed in the studies included in this review. The use of different scoring systems can result in variations in the level of scoring based on the definition of each scoring criterion. For example, Torabinejad et al. scoring system consists of 3 scores (score 1: no smear layer; score 2: moderate smear layer; score 3: heavy smear layer) [28,31,34]. In contrast, Hülsmann et al. scoring system has scores 1 to 5 [25,26,30,35], which is similar to Takeda et al. [27,29] that consists of scores 1 to 4. The additional score in Hülsmann et al. [14] is score 4, corresponding to the complete root canal wall covered by a homogenous smear layer, with no open dentinal tubules. It is hard to interpret the gold standard scoring system and no evidence was found to make this recommendation. It is our view that a scoring system with more level of scores is precise and consequently provides accurate analysis of the presence of the smear layer. However, this may compromise the reliability of scoring. Considering the accuracy and reliability, Hülsmann et al. [14] scoring system can be considered more superior to other scoring systems used in the included studies.

Different endodontic file systems produce different amounts of debris within the root canal system [57,58]. However, it is to be noted that studies have reported no significant difference in debris and smear layer formation in the root canal system prepared by ProTaper and MTtwo.
rotary system [59,60] and between ProTaper, K3 nickel-titanium and MTtwo rotary system [41]. Therefore, it can be inferred that the rotary system does not have a huge impact on the evaluation of smear layer removal of irrigants despite various endodontic file systems employed in the included studies.

All the included in vitro studies performed in human teeth and smear layer was produced by using actual endodontic file except for one study [24] which uses dentin disc and formation of smear layer by drilling with long neck burs. One of the limitations of this review was a lack of homogeneity in the study design of the included studies. For example, different scoring systems were used to score the presence of smear layer. This would have led to inaccurate scoring and a possible obstacle to comparing the outcome between the studies. SEM analysis has been used to evaluate the number of blocked canals by the smear layer. Seven studies used ×2,000 magnification [23,24,27,29,30,32,35] whilst 5 studies only used ×1,000 magnification [25,26,31,33,34] and one study used ×500 magnification [28]. Higher magnification influences the accuracy in evaluating the dentinal tubules blocked by the smear layer. Hence, SEM analysis using lower magnification might have impacted the results. Also, it was also found that one study included only 3 teeth as a sample size, which would have contributed to biased results [24].

It is recommended for future in vitro studies to use Hülsmann et al. [14] scoring system to evaluate dentinal tubules blocked by smear layer and SEM analysis with higher magnification as it is more accurate. To simulate the closed environment of the root canal which is surrounded by periodontium, it is recommended to close the apex of the tooth with wax, which provides an environment almost similar in the clinical setting.

CONCLUSIONS

This systematic review showed better smear layer removal using QMix compared to MTAD, NaOCl, Tubulicid Plus, and Phytic acid. However, it was less effective than 7% maleic acid and 10% citric acid. There was no conclusive result between QMix and 17% EDTA due to conflicting results. To improve smear layer removal efficacy of QMix, it is recommended to use it for a longer duration.

REFERENCES

1. Vieira AR, Siqueira JF Jr, Ricucci D, Lopes WS. Dentinal tubule infection as the cause of recurrent disease and late endodontic treatment failure: a case report. J Endod 2012;38:250-254.
2. Nair PN, Sjögren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. J Endod 1990;16:580-588.
3. Gutmann J, Lovdahl P. Problem solving in endodontics. 5th ed. Maryland Heights (MO): Elsevier Mosby; 2011.
4. Sen BH, Wesselink PR,Türkün M. The smear layer: a phenomenon in root canal therapy. Int Endod J 1995;28:141-148.
5. Mader CL, Baumgartner JC, Peters DD. Scanning electron microscopic investigation of the smeared layer on root canal walls. J Endod 1984;10:477-483.
6. Convissar R. Principles and practice of laser dentistry. London: Elsevier Health Sciences; 2010. p223.
7. Yang G, Wu H, Zheng Y, Zhang H, Li H, Zhou X. Scanning electron microscopic evaluation of debris and smear layer remaining following use of ProTaper and Hero Shaper instruments in combination with NaOCl and EDTA irrigation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:e63-e71.
8. Wang Z, Shen Y, Haapasalo M. Effect of smear layer against disinfection protocols on Enterococcus faecalis-infected dentin. J Endod 2013;39:1395-1400.
9. Meryon SD, Brook AM. Penetration of dentine by three oral bacteria in vitro and their associated cytotoxicity. Int Endod J 1990;23:196-202.
10. Pintor AV, Dos Santos MR, Ferreira DM, Barcelos R, Primo LG, Maia LC. Does smear layer removal influence root canal therapy outcome? a systematic review. J Clin Pediatr Dent 2016;40:1-17.
11. Zehnder M. Root canal irrigants. J Endod 2006;32:389-398.
12. Weber CD, McClanahan SB, Miller GA, Diener-West M, Johnson JD. The effect of passive ultrasonic activation of 2% chlorhexidine or 5.25% sodium hypochlorite irrigant on residual antimicrobial activity in root canals. J Endod 2003;29:562-564.
13. Johal S, Baumgartner JC, Marshall JG. Comparison of the antimicrobial efficacy of 1.3% NaOCl/BioPure MTAD to 5.25% NaOCl/15% EDTA for root canal irrigation. J Endod 2007;33:48-51.
14. Hülsmann M, Heckendorf M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J 2003;36:810-830.
15. Vallabhaneni K, Kakarla P, Avula SS, Reddy NV, Gowd MP, Vardhan KR. Comparative analyses of smear layer removal using four different irrigant solutions in the primary root canals - a scanning electron microscopic study. J Clin Diagn Res 2017;11:ZC64-ZC67.
16. Drake DR, Wiermann AH, Rivera EM, Walton RJ. Bacterial retention in canal walls in vitro: effect of smear layer. J Endod 1994;20:78-82.
17. Haapasalo M, Ostavik D. in vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66:1375-1379.
18. Jose J, Krishnamma S, Peedikayil F, Aman S, Tomy N, Mariodan JP. Comparative evaluation of antimicrobial activity of QMix, 2.5% sodium hypochlorite, 2% chlorhexidine, guava leaf extract and aloe vera extract against Entercoccus faecalis and candida albicans - an in-vitro study. J Clin Diagn Res 2016;10:ZC20-ZC23.
19. Siqueira JF Jr, Rôças IN. Optimising single-visit disinfection with supplementary approaches: a quest for predictability. Aust Endod J 2011;37:92-98.
20. Goldman LB, Goldman M, Kronman JH, Lin PS. The efficacy of several irrigating solutions for endodontics: a scanning electron microscopic study. Oral Surg Oral Med Oral Pathol 1981;52:197-204.
21. Yamada RS, Armas A, Goldman M, Lin PS. A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions: part 3. J Endod 1983;9:1374-42.
22. Goldman M, Goldman LB, Cavaleri R, Bogis J, Lin PS. The efficacy of several endodontic irrigating solutions: a scanning electron microscopic study: part 2. J Endod 1982;8:487-492.
23. Dai L, Khechen K, Khan S, Gillen B, Loushine BA, Wimmer CE, Gutmann JH, Pashley D, Tay FR. The effect of QMix, an experimental antibacterial root canal irrigant, on removal of canal wall smear layer and debris. J Endod 2011;37:80-84.
24. Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of a novel irrigant, QMiX. Int Endod J 2012;45:363-371.
25. Eliot C, Hatton JE, Stewart GP, Hildebolt CF, Jane Gillespie M, Gutmann JL. The effect of the irrigant QMix on removal of canal wall smear layer: an ex vivo study. Odontology 2014;102:232-240.

26. Jigzap JB, Patil SS, Gade VJ, Chandhok DJ, Upagade MA, Thakur DA. Effectiveness of three different irrigants - 17% ethylenediaminetetraacetic acid, Q-MIX, and phytic acid - in canal wall smear removal: a comparative scanning electron microscope study. Contemp Clin Dent 2017;8:459-463.

27. Aksel H, Serper A. Concentration and time-dependent effect of initial sodium hypochlorite on the ability of QMix and ethylenediaminetetraacetic acid to remove smear layer. J Conserv Dent 2017;20:185-189.

28. Ballal NV, Jain I, Tay FR. Evaluation of the smear layer removal and decalcification effect of QMix, maleic acid and EDTA on root canal dentine. J Dent 2016;51:62-68.

29. Banode AM, Gade V, Patil S, Gade J, Chandhok D, Sinkar R. Comparative scanning electron microscopy evaluation of smear layer removal with 17% ethylenediaminetetraacetic acid, 10% citric acid and newer irrigant QMix: in vitro study. Indian J Oral Health Res 2015;1:56-61.

30. Aranda-García AF, Kuga MC, Vitorino KR, Chávez-Andrade GM, Duarte MA, Bonetti-Filho I, Faria G, Só MV. Effect of the root canal final rinse protocols on the debris and smear layer removal and on the push-out strength of an epoxy-based sealer. Microsc Res Tech 2013;76:533-537.

31. Vemuri S, Kolano SK, Varri S, Pabbati RK, Polineni S. Scanning electron microscopic evaluation of smear layer removal ability of novel irrigant QMix compared with others at apical third of the root. World J Dent 2018;9:220-224.

32. Baldasso FE, Cardoso LR, Silva KD, Morgental RD, Kopper PM. Evaluation of the effect of four final irrigation protocols on root canal dentin components by polarized light microscopy and scanning electron microscopy. Microsc Res Tech 2017;80:1337-1343.

33. Gulabivala K, Patel B, Evans G, Ng YL. Effects of mechanical and chemical procedures on root canal surfaces. Endod Topics 2005;10:103-122.

34. Ahmad M, Pitt Ford TJ, Crum LA. Ultrasonic debridement of root canals: acoustic streaming and its possible role. J Endod 1987;13:490-499.

35. DiVito E, Peters OA, Olivé G. Effectiveness of the erbium:YAG laser and new design radial and stripped tips in removing the smear layer after root canal instrumentation. Lasers Med Sci 2012;27:273-280.

36. Ahmad M, Pitt Ford TJ, Crum LA. Ultrasonic debridement of root canals: acoustic streaming and its possible role. J Endod 1987;13:490-499.

37. Gulabivala K, Patel B, Evans G, Ng YL. Effects of mechanical and chemical procedures on root canal surfaces. Endod Topics 2005;10:103-122.

38. Raut AW, Mantri V, Palekar A, Gadodia R, Kala S, Raut RA. Comparative analysis of cleaning ability of three nickel-titanium rotary systems: ProTaper universal, K3 and Mtwo: an in vitro scanning electron microscopic study. Niger Postgrad Med J 2016;23:221-226.
42. Senia ES, Marshall FJ, Rosen S. The solvent action of sodium hypochlorite on pulp tissue of extracted teeth. Oral Surg Oral Med Oral Pathol 1971;31:96-103.

43. de Gregorio C, Estevez R, Cisneros R, Heilborn C, Cohenca N. Effect of EDTA, sonic, and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals: an in vitro study. J Endod 2009;35:891-895.

44. de Gregorio C, Estevez R, Cisneros R, Paranjpe A, Cohenca N. Efficacy of different irrigation and activation systems on the penetration of sodium hypochlorite into simulated lateral canals and up to working length: an in vitro study. J Endod 2010;36:1216-1221.

45. Chow TW. Mechanical effectiveness of root canal irrigation. J Endod 1983;9:475-479.

46. Arslan D, Guneser MB, Dincer AN, Kustarci A, Er K, Siso SH. Comparison of smear layer removal ability of QMix with different activation techniques. J Endod 2016;42:1279-1285.

47. Niu LN, Luo XI, Li GH, Bertoluzzi EA, Mao J, Chen JH, Gutmann JL, Pashley DH, Tay FR. Effects of different sonic activation protocols on debridement efficacy in teeth with single-rooted canals. J Dent 2014;42:1001-1009.

48. Monterro-Miralles P, Estévez-Luña R, DeGregorio-González C, Valencia-dePablo O, Jaramillo DE, Cisneros-Cabello R. Effectiveness of Nd:YAG Laser on the elimination of debris and Smear Layer. A comparative study with two different irrigation solution: EDTA and QMix® in addition to NaOCl. J Clin Exp Dent 2018;10:e70-e74.

49. Koçak S, Bağcı N, Çiçek E, Türker SA, Can Sağlık B, Koçak MM. Influence of passive ultrasonic irrigation on the efficiency of various irrigation solutions in removing smear layer: a scanning electron microscope study. Microsc Res Tech 2017;80:537-542.

50. Prado MC, Leal F, Gusman H, Simão RA, Prado M. Effects of auxiliary device use on smear layer removal. J Oral Sci 2016;58:561-567.

51. Prado MC, Leal F, Simão RA, Gusman H, do Prado M. The use of auxiliary devices during irrigation to increase the cleaning ability of a chelating agent. Restor Dent Endod 2017;42:105-110.

52. Souza MA, Hoffmann IP, Menchik VHS, Zandoná J, Dias CT, Palhano HS, Bertol CD, Rossato-Grando LG. Influence of ultrasonic activation using different final irrigants on antimicrobial activity, smear layer removal and bond strength of filling material. Aust Endod J 2019;45:209-215.

53. Peters OA, Barbakow F. Effects of irrigation on debris and smear layer on canal walls prepared by two rotary techniques: a scanning electron microscopic study. J Endod 2000;26:6-10.

54. Marques AC, Aguiar BA, Frota LM, Guimarães BM, Vivaqu-A-Gomes N, Vivan RR, Duarte MA, de Vasconcelos BC. Evaluation of influence of widening apical preparation of root canals on efficiency of ethylenediaminetetraacetic acid agitation protocols: study by scanning electron microscopy. J Contemp Dent Pract 2018;19:1087-1094.

55. Brunson M, Heilborn C, Johnson DJ, Cohenca N. Effect of apical preparation size and preparation taper on irrigant volume delivered by using negative pressure irrigation system. J Endod 2010;36:721-724.

56. Arya A, Bali D, Grewal MS. Histological analysis of cleaning efficacy of hand and rotary instruments in the apical third of the root canal: a comparative study. J Conserv Dent 2011;14:237-240.

57. Poggio C, Dagna A, Chiesa M, Scribante A, Beltrami R, Colombo M. Effects of NiTi rotary and reciprocating instruments on debris and smear layer scores: an SEM evaluation. J Appl Biomater Funct Mater 2014;12:256-262.
59. Baxter S, Beck F, Hülsmann M. Root canal preparation using S5, Mtwo, and ProTaper Universal nickel-titanium systems: a comparative ex-vivo study. Quintessence Int 2019;50:358-368.

60. Wadhwani KK, Tikku AP, Chandra A, Shalva VK. A comparative evaluation of smear layer removal using two rotary instrument systems with ethylenediaminetetraacetic acid in different states: a SEM study. Indian J Dent Res 2011;22:10-15.