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LITERATURE REVIEW

Optimising single-visit disinfection with supplementary approaches: A quest for predictability

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Abstract

Reduction of the bacterial populations to levels compatible with periradicular tissue healing is the primary microbiological goal of the endodontic treatment of teeth with apical periodontitis. New systems and substances have been proposed to improve root canal disinfection either by replacing conventional chemomechanical procedures or by supplementing their effects. This review focuses on supplementary approaches for optimised single-visit disinfection (OSD). The main OSD strategies reviewed include: final rinse with chlorhexidine, MTAD or iodine potassium iodide; sonic or ultrasonic activation of NaOCl; and photodynamic therapy. It is concluded that some OSD approaches may have the potential to improve disinfection in a single visit, but most of the results so far represent low-level evidence. There is no consistent clinical evidence showing that any of these OSD approaches can predictably reduce the bacterial bioburden in infected root canals to levels significantly below that achieved by chemomechanical procedures and to the point of eliminating the need for an antimicrobial inter-appointment medication. Therefore, while expediting disinfection is a goal that has been pursued, there is a need for the currently proposed protocols to be consistently evaluated for clinical effectiveness or new ones to be devised and tested before OSD is considered a predictable approach.

Introduction

The number of visits required to treat the root canals of teeth with apical periodontitis represents one of the most controversial issues in endodontics (1–3). The establishment of treatment protocols that can predictably control the root canal infection in a single visit has the potential to help smooth this controversy. In this regard, the idea of speeding up root canal disinfection while maintaining the same efficacy observed in two-visit treatment sounds interesting and has been fuelled by some clinicians and researchers. The ultimate question to be answered is how quickly one can predictably disinfect root canals.

Chemomechanical preparation is certainly the most important step to control the root canal infection. Instruments and antimicrobial irrigants, especially NaOCl, are highly effective in reducing bacterial populations. However, about 40% to 60% of the previously infected root canals still contain detectable bacteria after chemomechanical procedures (4–13). Because the presence of persistent bacteria may result in a poor outcome (14–16), efforts should be directed towards finding treatment protocols that predictably eliminate or at least reduce bacterial populations to levels undetectable by microbiological culture techniques (17). In this regard, protocols to improve disinfection may rely on alternative methods or substances for chemomechanical preparation or approaches to supplement the disinfecting effects of conventional chemomechanical procedures.

Strategies that have been recommended to enhance disinfection after chemomechanical preparation include the use of an inter-appointment intracanal medication or an optimised single-visit disinfection (OSD) approach (18). The rationale is to reduce still more the bacterial bioburden in the canal before placing a filling. Numerous studies have demonstrated the efficacy of inter-appointment medications in improving disinfection after chemomechanical procedures (4,5,8,11,13,19). The OSD approach in turn is intended to supplement the disinfecting effects of the chemomechanical preparation by either applying a short-term (minutes) intracanal medication or making use of an additional procedure at the same visit of preparation. OSD holds the potential to eliminate the need for inter-appointment medication, by expediting disinfection.

Early studies on potential OSD approaches included short-term intracanal medication with either calcium hydroxide for 10 min (4) or 5.25% NaOCl for 30 min with changes every 5 min (5), both applied immediately following preparation. Both intra-appointment medications failed to significantly enhance elimination of intracanal bacterial populations (4,5). Other approaches have been proposed, some with promising *in vitro* and *ex vivo* results and others with only preliminary clinical results. This article reviews the literature for the antimicrobial effectiveness of OSD approaches.

Approaches for OSD

The main OSD strategies reviewed in this paper include: final rinse with chlorhexidine (CHX), MTAD (Biopure, Dentsply, Tulsa, OK) or iodine potassium iodide (IPI); sonic or ultrasonic activation of NaOCl; and photodynamic therapy (PDT). All these OSD approaches are recommended for application following chemomechanical preparation, preferably after smear layer removal.

Final rinse with CHX

CHX is a cationic bis-biguanide widely used as an antiseptic, disinfectant and preservative (20). It is highly effective against several oral bacterial and fungal species (21–23). Because CHX has substantivity in dentin (24,25), the antibacterial effects of this substance are expected to remain active for some period after irrigation (24–31). The rationale behind the use of a final rinse with CHX is mostly to take advantage of this residual antibacterial effect. Some authors have recommended that the root canal be eventually rinsed with 2% CHX, which is then allowed to act for up to 5 min (32).

In a clinical study, Zamany *et al.* (33) evaluated the supplemental antibacterial effects of a 2% CHX rinse after chemomechanical preparation using 1% NaOCl as the irrigant. Culture analysis revealed that while after chemomechanical preparation, 8 of 12 (67%) root canals yielded positive cultures, a final rinse with CHX reduced this number to only 1 of 12 (8%). In a recent *in vitro* study using root canals of extracted teeth experimentally infected with *Enterococcus faecalis*, Alves *et al.* (34) showed that the cumulative antibacterial effects of passive ultrasonic irrigation (PUI) of 2.5% NaOCl and a CHX final rinse significantly reduced the bacterial counts to levels below those achieved after preparation of long oval canals. The authors concluded that there may be a benefit of using the PUI for activation of NaOCl followed by a final rinse with CHX as supplementary steps in the treatment of infected root canals.

Although the final rinse with CHX has shown promising *in vitro* and *in vivo* results in terms of antibacterial effectiveness, more studies are required on the subject to confirm the benefits of this OSD approach.

Final rinse with MTAD

MTAD is a commercial product for root canal irrigation and consists of a **m**ixture of a **t**etracycline isomer (doxycycline), citric **a**cid and a **d**etergent (Tween 80). MTAD has a low pH (2.15) due to the presence of citric acid and is recommended for smear layer removal after chemomechanical preparation with NaOCl as irrigant. In addition to participating in smear layer removal by chelating calcium ions, tetracycline also has antibacterial effects against several endodontic pathogens. Moreover, this antibiotic exhibits substantivity in calcified tissues, generating a residual antibacterial effect (35). As with CHX, this residual effect might enhance disinfection after preparation and potentially eliminate the need for inter-appointment medication. A study (36) demonstrated that the antibacterial substantivity of MTAD to dentin was superior to 2% CHX, and lasted 28 days.

The proposed protocol consists of using NaOCl as the irrigant during preparation and then proceed to a final rinse of the canal with MTAD, which is left to act in the canal for 5 min (37). In a clinical study, Malkhassian *et al.* (38) reported that a final rinse with MTAD and medication with 2% CHX did not reduce the bacterial bioburden to levels below those achieved by root canal preparation with 1.3% NaOCl. Therefore, while there are inconclusive *in vitro* results for MTAD as used for OSD (39–41), the only available clinical study showed no antibacterial benefits of using this substance (38). Further clinical studies are warranted to check for the purported efficacy of MTAD in maximising single-visit disinfection.

Final rinse with IPI

In IPI, potassium iodide is used to dissolve iodine in water, but it is iodine that is responsible for the antibacterial activity. A few clinical studies have evaluated the effectiveness of IPI for OSD. Peciuliene *et al.* (42) left a 2% IPI solution for 5 min after preparation of root canals under retreatment and reported a decrease in the number of positive cultures. In cases of primary endodontic infections, Kvist *et al*. (43) demonstrated that 5% IPI left in the canal for 10 min after chemomechanical preparation with NaOCl as irrigant reduced the incidence of positive cultures from 64% to 36%. The long-term outcome of teeth treated with this protocol was not significantly different when compared with teeth treated with inter-appointment calcium hydroxide medication, even though the latter approach presented 10% more success (16). Thus, the clinical results with IPI are promising, but more studies are also required, especially comparing with NaOCl left in the canal for the same time period following preparation. IPI must not be used in patients reporting hypersensitivity reactions to iodine.

Sonic or ultrasonic activation of NaOCl

Ultrasound is a sound energy with frequency greater than 20 kHz, which is beyond the limit of human hearing. Sonic frequencies range from 20 Hz to 20 kHz. Several sonic endodontic devices operate at 1–6 kHz, but some can be far lower (e.g. EndoActivator (Dentsply Tulsa Dental, Tulsa, OK) = 167 Hz or 10 000 cpm). Some authors recommend filling the root canal with NaOCl after preparation and then sonically or ultrasonically activating this substance.

Although ultrasound has been widely used and tested for root canal instrumentation, a few consistent clinical studies have been published on its antibacterial efficacy. Sjögren and Sundqvist (44) performed ultrasonic instrumentation with 0.5% NaOCl as the irrigant and reported better antibacterial effects than hand instrumentation alone. However, the use of ultrasonic instrumentation significantly declined over the years because of the increased risks of procedural accidents (45–47).

Endodontic instruments are employed in ultrasonic units oscillating at frequencies ranging from 25 to 30 kHz (47,48). Ultrasonic irrigation of the root canal can be performed with or without simultaneous ultrasonic instrumentation. The term ‘passive ultrasonic irrigation’ (PUI) has been proposed to describe the latter approach and in fact ‘passive’ relates to the ‘noncutting’ action of the ultrasonically activated instrument (47,49). PUI can refer to either intracanal placement of the irrigant by a syringe with subsequent ultrasonic activation or continuous delivery of the irrigant through the ultrasonic handpiece (48).

Data from *in vitro* studies evaluating the effectiveness of PUI of NaOCl in reducing bacterial populations have been somewhat inconclusive, with studies showing superiority over syringe irrigation (50) or no significant differences (34,51). PUI was not superior than syringe irrigation or passive sonic activation, all using 5.25% NaOCl, in eliminating *E. faecalis* from root canals of extracted teeth (52). Alves *et al*. (34) reported that although PUI alone was not significantly effective in reducing bacterial populations after chemomechanical preparation, excellent results were observed for the sequential use of PUI and CHX final rinse.

A variation in PUI with the irrigant being pumped under a high flow rate through a needle attached to an ultrasonic handpiece has been proposed (53,54) and shown to improve cleaning (53) and disinfection (54,55). A clinical study in mesial canals of lower molars revealed that post-instrumentation ultrasonic irrigation for 1 min using 15 mL of 6% NaOCl reduced the incidence of positive cultures from 73% after preparation with NaOCl as irrigant to 20% (54).

Ultrasound seems to exert its antibacterial and cleaning effects (along with NaOCl) through acoustic streaming and cavitation, in addition to warming and moving the irrigating substance to areas of complex anatomy (47,56–58). Acoustic streaming and cavitation may disturb and cause de-agglomeration of endodontic biofilms, flushing out the detached bacterial cells from the canal or rendering the resultant planktonic bacteria more susceptible to the possibly enhanced antibacterial effects of warmed NaOCl (48,59,60). Cavitation may produce a transient weakening of the bacterial cell wall and cytoplasmic membrane, making the bacteria more susceptible to NaOCl (48,54). Both acoustic streaming and cavitation depend on the free displacement amplitude of the ultrasonic instrument (57,58,61). Therefore, it has been recommended that, for optimal effects, root canals be enlarged for example at least to a #40 instrument to permit enough clearance for the free oscillation of a #15 instrument and consequently acoustic streaming and cavitation formation (57,58,61).

Although data from studies are still uncertain, it seems that PUI has the potential to contribute to reducing the bacterial populations. Adding a final rinse with CHX after PUI may also be of some benefit, but clinical studies are required to support these assumptions. What remains to be illuminated is whether or not this OSD approach is predictable enough in eliminating bacteria to the point of making an additional inter-appointment medication unnecessary.

As for sonic devices, the EndoActivator system consists of a sonic handpiece (167 Hz or 10 000 cpm) and plastic tips of variable size developed to energise substances in the canal, such as NaOCl, so as to generate a hydrodynamic phenomenon that could theoretically maximise the biological effects of the substance (62). An *in vitro* study failed to show a significant advantage of using this sonic system to increase NaOCl antibacterial effectiveness (63). In a clinical study, Huffaker *et al*. (13) evaluated the ability of a sonic activation of NaOCl with the EndoActivator system to eliminate cultivable bacteria from root canals and compared it with that of standard syringe irrigation. Activation of 0.5% NaOCl with EndoActivator resulted in 60% of positive cultures compared with 52% for the standard syringe irrigation, with no significant difference between the groups. A second session and inter-appointment calcium hydroxide medication eliminated cultivable bacteria from significantly more teeth than a single session of treatment.

PDT

PDT, or photoactivated disinfection, utilises light of a specific wavelength to activate a nontoxic photoactive dye (photosensitiser) in the presence of oxygen. The transfer of energy from the activated photosensitiser to available oxygen gives rise to formation of highly reactive oxygen species that kill microorganisms by damaging essential cellular molecules (64). Most photosensitisers are activated by red light between a wavelength of 630 and 700 nm. Some of the photosensitisers commonly used in PDT include phenothiazine dyes (methylene blue and toluidine blue), phthalocyanines, chlorines, porphyrins, xanthenes and monoterpene (64). The photosensitiser is commonly used in a low concentration to avoid tooth pigmentation. Diode laser systems have been widely used in PDT because they are easy to handle, portable and cost-effective.

Because the PDT technique does not promote root canal enlargement, it has been recommended as a supplementary disinfecting approach to be used following chemomechanical preparation. *In vitro* (65–73) and *in vivo* (74,75) studies using PDT have demonstrated that this approach has the potential to enhance root canal disinfection. However, while most studies have demonstrated the antibacterial effectiveness of PDT, only a few have evaluated its effects after chemomechanical procedures. An *in vitro* study (76) investigating the antibacterial effects of PDT with either methylene blue or toluidine blue (both at 15 $\mu g\ mL^{-1}$) as a supplement to instrumentation/irrigation revealed that the tested protocols did not significantly enhance disinfection beyond the levels reached by chemomechanical preparation using NaOCl as irrigant. No significant differences were observed between the two photosensitisers. The authors concluded that adjustments in the PDT protocol are required to enhance predictability in bacterial elimination before clinical use is recommended.

In an *ex vivo* study in extracted teeth with apical periodontitis, Ng *et al*. (77) evaluated the antimicrobial effects of chemomechanical preparation with 6% NaOCl followed or not by PDT with methylene blue (50 $\mu g\ mL^{-1}$). A better performance was reported for the protocol using PDT (86.5% of negative cultures as compared with 49% when PDT was not used). The authors also concluded that PDT, if further enhanced by technical improvements, may serve as an adjunct to chemomechanical procedures.

A possible improvement in PDT is the use of nanoparticles encapsulated with photoactive drugs. Pagonis *et al*. (78) studied the *in vitro* effects of poly(lactic-co-glycolic acid) nanoparticles loaded with the photosensitiser methylene blue and activated by light against *E. faecalis* in planktonic phase and in experimentally infected root canals. Methylene blue-loaded nanoparticles concentrated mainly on the bacterial cell walls. The authors reported that PDT using methylene blue-loaded nanoparticles led to approximately 2 and 1 $\log_{10}$ reduction of colony-forming units in planktonic phase and root canals, respectively. Studies are necessary to compare conventional PDT using free photosensitisers with PDT using photosensitiser-loaded nanoparticles.

Concluding remarks

Reduction of the bacterial populations to levels compatible with periradicular tissue healing is the ultimate microbiological goal of the endodontic treatment of infected teeth. New systems and substances have been proposed to improve disinfection either by replacing conventional chemomechanical procedures or by supplementing their effects. This review dealt with the latter and suggests that some OSD approaches may have the potential to improve disinfection in a single visit (Table 1). Nonetheless, most of the results available so far represent low-level evidence, as they come from *in vitro* or *ex vivo* studies. Actually, there is no consistent clinical evidence showing that any of the OSD approaches reviewed herein can predictably reduce the bacterial bioburden in infected root canals to levels below that achieved by chemomechanical procedures and to the point of eliminating the need for an antimicrobial inter-appointment medication. Therefore, while expediting disinfection is a goal that has been pursued by many clinicians and researchers, there is a need for the currently proposed protocols to be consistently evaluated for clinical effectiveness or new ones to be devised and tested before OSD can be considered as a predictable approach.

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Table 1 State-of-the-art efficacy of procedures used to supplement the antimicrobial effects of chemomechanical procedures

Procedure	Antimicrobial efficacy	References
Inter-visits		
Inter-appointment medication	Supported by evidence *in vivo*	(4,5,8,11,13,19)
Intra-visit		
$Ca(OH)_2$ for 10 min	No significant effect *in vivo*	(4)
5.25% NaOCl for 30 min	No significant effect *in vivo*	(5)
Final rinse with iodine potassion iodide	Promising *in vivo* results	(42,43)
Final rinse with chlorhexidine	Promising *in vivo* results	(33)
Final rinse with MTAD	Inconclusive *in vitro* results	(39–41)
	No significant effect *in vivo*	(38)
Sonic activation of NaOCl	No significant effects *in vitro* and *in vivo*	(13,63)
Ultrasonic activation of NaOCl	Inconclusive *in vitro* results	(34,50–52)
	Promising *in vivo* results	(54,55)
Ultrasonic activation of NaOCl followed by final rinse with CHX	Promising *in vitro* results	(34)
Photodynamic therapy	Inconclusive *in vitro* results	(65,67,68,70–72,76,78)
	Promising *ex vivo* and *in vivo* results	(74,77)

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