Irrigation of the root canal

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In recent years, there have been major advances in improving the properties of root canal instruments. Since the introduction of nickel-titanium alloy (NiTi) into endodontics, nearly every month a new rotary NiTi-system comes onto the dental market. Moreover, there are several new products and root canal filling materials. Unfortunately, all these advances are focused on the technical aspects of the root canal treatment. A more biologically based approach has received less attention. There is a renewed interest in the relationship between mechanical root canal instrumentation and intraradicular disinfection. Infection control during root canal treatment is important for successful outcome in nonsurgical root canal treatment.

Therefore, the aim of this review is to analyse the relevant literature on root canal irrigation. An irrigation protocol for the everyday clinical practice is also proposed, including other relevant recommendations.
atively superficial. In the more apical part of the root canal, most of the pulp tissue will be vital and free of bacteria at this point of the disease.

Due to this microbial aetiology of pulpitis, it is clear that the treatment of irreversible pulpitis, in other words the treatment of vital cases, should focus on prevention of infection rather than an elimination of intraradicular infection. As the apical part of the root canal system is bacteria free, the aim is asepsis. Under clinical conditions, the easiest and by far the most efficient methods to guarantee asepsis during endodontic treatment are coronal disinfection of the tooth and mandatory use of rubber dam. Moreover, sterilised burs and root canal instruments must be used, gloves must be worn, and the root canal obturation should if possible be performed at the first visit. Thus the pulpectomy procedure is basically aimed at preventing the development of a pulpal infection and thereby preventing the development of an apical periodontitis.

In contrast, in cases with radiographic and/or clinical signs of an apical periodontitis there can no longer be any doubts that the root canal system harbours microorganisms. There is overwhelming evidence that periradicular diseases are caused by microorganisms in the necrotic root canal. Hence, antisepsis must be the aim of therapy in order to eliminate all microbes within the root canal system. In cases of infected root canals, the key factor for clinical success – and thus for promoting healing of the apical periodontitis – is efficient, and theoretically the complete, removal of intraradicular microorganisms. Whether or not there is a direct correlation between the number of viable microbes remaining in the root canal at the time of obturation and the outcome of the therapy remains controversial. Some studies have shown a significantly poorer prognosis for cases of apical periodontitis if viable bacteria remain in the canal at the time of filling. This finding has been supported by a clinical study indicating that the treatment outcome was significantly better in cases with apical periodontitis after intraradicular dressing versus one-visit treatment. On the other hand, a recent study failed to show any difference in residual microorganisms between one- and two-visit treatment, an observation that is in agreement with another report in as far as no difference in healing between teeth filled after positive or negative root canal cultures was observed.

### Endodontic infection: localisation of microorganisms

In general, endodontic infection can be divided into primary (primary apical periodontitis) or secondary root canal infection (previously root-filled teeth with apical periodontitis). In general, primary root canal infection is characterised by strictly anaerobic bacteria and is typically polymicrobial. Predominant species are Bacteroides, Porphyromonas, Prevotella, Fusobacterium, Treponema, Peptostreptococcus, Eubacterium and Campylobacter. Facultative streptococci are also commonly found in primary infection. In general, about 110 different microbial species have been detected in the root canal system in cases of apical periodontitis; less than 10–15 different species can be expected in a single tooth. In chronic periradicular lesions, the range of microbial species encountered is wider compared with a more restricted number of species in cases of acute apical periodontitis or acute periapical abscess.

Secondary intraradicular infection is usually caused by microbes that are not normally present in primary infection and have entered the root canal system during or after endodontic treatment. If these microorganisms are successful in colonising the root canal system and survive the prevailing local conditions, a secondary infection becomes established. The composition of intraradicular flora in secondary infection is quite different from that found in primary infection. Usually, in the former, the flora is dominated by a single species, or by fewer species when compared with primary infection. In fact, monoinfection is not common in primary infection. Gram-positive bacteria predominate in secondary infection and fungi can also often be found. Sometimes Enterococcus faecalis is found in pure culture in previously root-filled teeth with persistent apical periodontitis.

In approximately 85–95% of all cases, the infection is restricted to the root canal system containing necrotic pulp tissue; they are protected from the
action of the cellular (phagocytes) and molecular (antibodies, complement system) host defences\textsuperscript{12,29}. The microbes survive on pulp tissue remnants and exudate from the periodontium\textsuperscript{20}, the reason why in most cases intraradicular microbes are mostly located in the apical part of the root canal system\textsuperscript{23}. These microorganisms at the apical part of the root canal are usually delineated from the inflamed periapical tissues either by a dense accumulation of polymorphonuclear neutrophils or by an epithelial plug near the apical foramen\textsuperscript{12,23}. As a result, these microbes are protected from the host defences. Within the root canal these microorganisms are organised on the surface of the canal walls as an aggregation in an extracellular polysaccharide matrix, the so-called ‘biofilm’\textsuperscript{30,31}. Unfortunately, microbes organised in a biofilm are about 1000-fold more resistant to antimicrobial agents than their planktonic counterparts that exist in the root canal\textsuperscript{32}.

When intraradicular infection is not adequately treated, microorganisms will penetrate from the main root canal into dentinal tubules, lateral canals, and other canal irregularities. The invasion of dentine is estimated to occur in approximately 50–80\% of all teeth with apical periodontitis, and the microbes involved are dominantly Gram-positive cocci and rods\textsuperscript{30,33–36} (Fig 1). Bacterial invasion into the dentinal tubules can be observed up to a depth of 400 µm\textsuperscript{29,33,37}, while bacterial components such as lipopolysaccharides can penetrate even up to 1.2 mm into these tubules\textsuperscript{35}. It is also worth remembering that even in the absence of viable bacteria, their products can cause a severe inflammatory response\textsuperscript{12}. These indirect mechanisms of tissue damage are caused by bacteria enzymes (e.g. collagenase, hyaluronidase), exotoxins and metabolites (e.g. butyrate, ammonium, sulphur compounds), and other bacterial components, such as peptidoglycans, teichoic acid and lipopolysaccharides\textsuperscript{12,38}. The lipopolysaccharide, in particular, seems to be capable of causing severe tissue destruction by stimulating the development of host immune reaction\textsuperscript{12}. The endotoxin is located in the outer membrane of Gram-negative bacteria and is able, as mentioned above, to cause tissue damage even in the absence of viable bacteria\textsuperscript{6}. It has been shown in an animal model that placing lipopolysaccharide in empty sterile root canals led to the development of periapical lesions\textsuperscript{39}. In pulp tissue of teeth associated with apical periodontitis, high levels of lipopolysaccharides were found\textsuperscript{40}, and there was a strong association between lipopolysaccharide levels and the prevalence of Gram-negative bacteria\textsuperscript{41}. These observations that bacterial metabolites and breakdown products play a significant role in the pathogenesis of apical periodontitis\textsuperscript{6} confirmed the need for a root canal irrigant capable of neutralising lipopolysaccharides.

Microbes can also reside in the root canal system within the smear layer. This is a thin surface film formed on the root canal wall after instrumentation. A smear layer is produced on areas touched by root canal instruments. The smear layer consists of dentine particles, bacterial components, and remnants of vital or necrotic pulp tissue\textsuperscript{42,43}. Due to these organic remnants, the microbes have easy access to nutrients inside the smear layer. Therefore, it is clear that in infected cases the smear layer should be re-

![Fig 1](image-url) Gram-positive bacteria that have penetrated into the dentinal tubules (Gram; original magnification 400x).
moved to achieve the greatest possible reduction of intraradicular microorganisms.

Extraradicular infections may occur, but they are rare6,12. Usually, they may occur in the form of an acute periradicular abscess or in cases of actinomycosis12. The problem with extraradicular infection is that microbes are established in the periradicular tissues, inaccessible to nonsurgical endodontic treatment procedures. As a result, extraradicular infection may cause endodontic failure. There is overwhelming evidence in the literature that microorganisms left in the root canal system after root canal preparation or re-infection of the root filled tooth (secondary infection), are the main causes of endodontic failure4,7,14,25.

## Root canal instrumentation and bacterial reduction

Due to its complex anatomy, with the multiple fins, isthmuses, ramifications and accessory canals64 (Fig 2), it is virtually impossible for mechanical root canal instrumentation to shape and clean the entire root canal system45. Intraradicular microbes may be lodged in these areas, which are inaccessible to instrumentation6. In addition, this complex environment prevents irrigants from exerting their full antimicrobial potential4. Approximately 79% of all permanent molars have accessory canals in the furcation area46 (Fig 3). These canals may have diameters of up to 200 µm, so microbes can easily penetrate from the root canal system into the periodontal tissues or vice versa. Therefore, nowadays an adhesive seal of the pulp chamber floor, after the obturation of the root canal space, is recommended to avoid re-infection of the root canal system via the periodontium46.

Classic Scandinavian studies by Byström and Sundqvist clearly indicated that mechanical instrumentation is able to reduce significantly the number of microbes in the root canal system47–49. These studies showed that hand instrumentation using stainless steel files and sterile saline resulted in a 100–1000-fold reduction of intraradicular microorganisms, but it was not possible to predictably obtain bacteria-free root canals47. These results were supported by further investigations, which showed that the sole use of manual stainless steel instruments does not render root canals bacteria-free50–52. These investigations convincingly demonstrated the limited antibacterial effect of mechanical preparation.

The introduction of more flexible alloys like NiTi led to the development of rotary systems. It was assumed that these improvements would result in more effective removal of bacteria from infected root canals compared with conventional hand instruments6. Recently, several studies have been conducted to compare the level of intraradicular bacterial reduction of hand instruments (stainless steel or NiTi) compared with rotary NiTi instruments with greater tapers than ISO .02 taper. Nearly all of these studies failed to show significant differences between hand and rotary instrumentation50,51–58. Only about one- third of the instrumented canals were found to be free of bacteria.
and in general both preparation techniques were not able to predictably render canals bacteria-free\textsuperscript{50,51,58}. In summary, predictable and complete bacterial elimination does not appear to be possible, either with traditional hand instrumentation or with newer rotary NiTi-systems\textsuperscript{6,50}. Irrigation of the root canal is essential for effective elimination of bacteria.

- **Objectives and requirements of irrigants**

  The aims of root canal irrigation are:
  
  - Reduction of intraradicular microorganisms and neutralisation of endotoxins.
  - Dissolution of vital or necrotic pulp tissue.
  - Lubrication of canal walls and instruments.
  - Removal of dentine particles.

  The following are requirements of a root canal irrigant:
  
  - A broad antimicrobial spectrum.
  - Biocompatibility.
  - Tissue-dissolution capability.

- **Antimicrobial irrigation solutions**

  Clinical studies performed in Scandinavia showed that copious irrigation with an antimicrobial solution during mechanical root canal preparation has an essential effect on the reduction of intraradicular microorganisms\textsuperscript{47,48,55}. Mechanical root canal preparation using saline as an irrigant leaves only about 20\% of canals bacteria-free. The percentage of bacteria-free canals was increased to up to 50\% when NaOCl was used for irrigation. Ultrasonic activation of sodium hypochlorite achieved a further reduction of intraradicular microbes, with approximately 70\% of all canals bacteria-free. These studies confirmed the paramount importance of antibacterial irrigation solutions. The employment of one or more antimicrobial irrigation solutions during root canal treatment is good clinical practice.

- **Sodium hypochlorite**

  Sodium hypochlorite (NaOCl) is the most widely used irrigation solution in endodontics. Currently available evidence strongly indicates that NaOCl is the irrigant of choice\textsuperscript{7}. In water, NaOCl dissociates into Na\textsuperscript{+} and OCl\textsuperscript{-}, the hypochlorite ion. Between pH 4 and pH 7, chlorine from NaOCl exists predominantly as HClO (hypochlorous acid), whereas above pH 9, OCl\textsuperscript{-} predominates\textsuperscript{59}. Although the antimicrobial effectiveness of hypochlorous acid is greater than that of hypochlorite\textsuperscript{59}, in the clinically used NaOCl solutions, the entire available chlorine is in the form of OCl\textsuperscript{-}, as the pH of the solution is normally about 12\textsuperscript{6,60}. Unfortunately, due to several technical problems (e.g. stability of the solution), NaOCl solutions with a lower pH, which would increase the amount of available hypochlorous acid, are not commercially available at present\textsuperscript{7}.

  In endodontic therapy, NaOCl solutions are used in concentrations varying from 0.5\% to 5.25\%.\textsuperscript{4} Also available are unbuffered solutions at pH 11–12 in concentrations ranging between 0.5\% and 5.25\%, or the so-called Dakin’s solution, which is a buffered 0.5\% solution at pH 9.0\textsuperscript{4,59}. There is no difference between these two solutions with respect to tissue dissolution or antibacterial efficiency\textsuperscript{4,61}. Although allergic reactions to NaOCl are rare\textsuperscript{7}, there have been several case studies on the potential risk of hypersensitivity reactions\textsuperscript{62–64}.

  NaOCl dissolves pulpal remnants (vital and necrotic pulp tissue), organic compounds of dentine, and the organic components of the smear layer\textsuperscript{65–69}. The tissue-dissolving capability of NaOCl is significantly better than all other commonly used irrigants\textsuperscript{65} (Fig 4). Moreover, neutralisation or inactivation of lipopolysaccharides has been reported with NaOCl\textsuperscript{70–72}. However, NaOCl is not able to remove the smear layer\textsuperscript{73,74}.

  Sodium hypochlorite is characterised by having strong antibacterial activity with comparably short contact times\textsuperscript{4,7}. Even the resistant *Candida albicans* was killed in vitro by both 5\% and 0.5\% NaOCl solutions\textsuperscript{75}. Several in vitro investigations\textsuperscript{75–77} and one clinical study\textsuperscript{26} confirmed the susceptibility of *C. albicans* to NaOCl. In addition, several Gram-negative anaerobic bacteria, typically found in primary root canal infection, displayed a high susceptibility to NaOCl in concentrations ranging from 0.5\% to 5\%.\textsuperscript{77} In contrast, based on the results of both laboratory studies\textsuperscript{76,78} and one clinical report evaluating microbiological samples of previ-
ously root-filled teeth with apical periodontitis. E. faecalis is much more resistant to NaOCl than the aforementioned microbes. However, despite the reduced effectiveness of NaOCl against E. faecalis, NaOCl has the unique ability to disrupt or to remove biofilms. In a comparative study on the effect of different irrigants against E. faecalis biofilms, both 6% and 1% NaOCl killed more than 99.7% of bacteria after contact times of 1 or 5 minutes, while 2% chlorhexidine and MTAD killed only 60.5% and 16% of the biofilm bacteria respectively. Therefore current evidence indicate that NaOCl is distinctly more effective in rendering biofilm bacteria nonviable and in physically removing the biofilm compared with other commonly used irrigants.

The tissue-dissolution capability and the antimicrobial efficiency as well as the toxicity of NaOCl is dependent on the concentration of the solution. The higher the concentration of the solution the greater the cytotoxicity. A 5.25% NaOCl solution demonstrated greater cytotoxicity than 0.5% or 1% NaOCl solutions. Most in vivo studies showed no significant difference in antibacterial activity between 0.5%, 1%, 2.5% and 5% solutions against both E. faecalis and a mixed anaerobic flora. Comparative studies failed to show any significant difference in the tissue-dissolving ability between higher and lower concentration NaOCl solutions. In fact, based on laboratory investigations, 1% NaOCl is sufficient for dissolving pulp tissue. The tissue-dissolution capacity is reliant on regularly refreshing the irrigant solution rather than the concentration of the solution. Therefore, the NaOCl solution must be regularly replenished during treatment. In summary, NaOCl at concentrations between 0.5% and 1% is recommended for routine clinical use. These concentrations represent the best balance between tissue-dissolution capability, antimicrobial activity and biocompatibility. There is currently no convincing scientific evidence for using NaOCl at higher concentrations or at 'full strength' (5.25%).

Instead of using a more concentrated solution, the effectiveness of NaOCl could be improved by increasing the temperature of a less concentrated solution. Several studies reported that warmed NaOCl dissolved organic tissues significantly better than unheated solutions. It was found that a 1% solution at 45°C dissolved pulp tissue as effectively as 5.25% NaOCl at 20°C, and a 1% solution at 60°C was significantly more effective than an unheated full-strength solution. Warmed solutions also exhibited significantly greater antimicrobial efficacy compared with unheated solutions. A 100-fold increase in the killing of E. faecalis was observed between corresponding NaOCl solutions (1%, 2.62%, and 5.25%) at 20°C and at 45°C. It seems reasonable to use lower concentration NaOCl solutions in order to ensure a good margin of safety, but its effectiveness may be improved by warming the solution. It must be kept in mind that NaOCl solution once heated but not used must be discarded, as its effectiveness will be exhausted.

Ultrasonic activation of NaOCl is a very promising alternative approach to improve the effectiveness of this irrigant. A detailed review of the use of ultrasound for irrigation can be found in this issue of the journal. The question of the duration required for irrigants to work optimally is still open to debate.
nately, data are limited and most studies are not representative of the clinical situation. In a biofilm model using *C. albicans*, both 1% and 5% NaOCl needed 60 minutes to kill all the microbes. After only 30 minutes, both concentrations failed to remove the biofilm. These observations are in agreement with another study assessing the effect of different irrigation solutions on biofilms of root canal isolates. It was reported that 2.25% NaOCl solution required 60 minutes of contact time to achieve a total elimination of all microorganisms, including *E. faecalis*. Therefore for effective biofilm removal, NaOCl should be allowed a contact time of at least 30 to 60 minutes.

The volume of irrigant is also clinically relevant. The scientific evidence on this matter is sparse. However, an increase in the volume of the irrigant used is correlated with a reduction of intraradicular microorganisms and improved canal cleanliness. Yamada et al. recommended at least 10–20 ml of irrigant for each canal, followed by a final high volume flush after the shaping procedure has been completed (Table 1).

The chemical stability and activity of NaOCl solutions may be adversely affected by many factors. Piskin and Turkun conducted a study on the stability of various NaOCl solutions and pointed out that all solutions showed degradation with time. High-concentration NaOCl (5%) decomposed far more rapidly when stored at 24°C compared with 0.5% NaOCl. Solutions containing 0.5% and 5% chlorine stored at 4°C displayed satisfactory stability at 200 days. Therefore it is recommended to store NaOCl solutions in a refrigerator and in dark bottles to avoid degradation caused by light.

NaOCl is caustic if accidentally extruded into periapical tissues or adjacent anatomical structures such as the maxillary sinus. In the case of accidental injection of NaOCl into periapical tissues, emphysema may develop within 10–20 minutes. Furthermore, oedema and paraesthesia may result due to the tissue-dissolving capability of NaOCl. An even more serious development is ecchymosis, which is associated with severe pain, profuse interstitial bleeding, and haemorrhage under the skin (Fig 5). Fortunately, most of these symptoms will regress within 2 weeks. A more detailed overview of complications occurring during root canal irrigation with NaOCl was published by Hülsmann.

### Chlorhexidine

It is generally accepted that as an irrigant chlorhexidine gluconate (CHX) should be used in a concentration of 2%. CHX has a wide antimicrobial spectrum and is effective against Gram-positive and Gram-negative bacteria as well as yeasts. CHX is able to permeate the cell wall or outer membrane and attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrane.

CHX solutions in concentrations of 0.2–2% are considered toxicologically safe. For instance, a 2% solution has been used as a subgingival irrigant without any adverse effects. In an animal study on rats, the root canals of teeth with experimentally induced apical periodontitis were temporarily filled with 2% CHX gel. Histological evaluations after 7 days

### Table 1  Suggested irrigation protocol.

<table>
<thead>
<tr>
<th>Size of apical preparation: at least size 35</th>
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<tr>
<td>After access cavity: flush the canals with NaOCl</td>
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<tr>
<td>Between instruments: 2–5 ml of NaOCl per canal</td>
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<tr>
<td>After shaping: 5–10 ml of NaOCl per canal</td>
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<tr>
<td>After shaping: irrigation with 5 ml of EDTA per canal for 1 minute (or with citric acid)</td>
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<tr>
<td>Final rinse with 2 ml NaOCl per canal</td>
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<tr>
<td>Optional: final irrigation with chlorhexidine</td>
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<td>Optional: rinse with alcohol before obturation</td>
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**Fig 5** Ecchymosis due to accidentally injection of NaOCl beyond the apex. Clinical situation two weeks after this complication.
revealed that the use of CHX resulted in good periapical regeneration and no indication of inflammation\(^{102}\). These findings were supported by a histopathological study on dogs, evaluating the periapical regeneration using 2% CHX as root canal irrigation\(^{101}\). A more recent study also confirmed the biocompatibility of 2% CHX\(^{104}\). CHX was injected into the peritoneal cavity of mice and the inflammatory response was evaluated. CHX was found to be biocompatible since this solution did not induce a significant inflammatory response\(^{104}\).

Although sensitivity to CHX is rare\(^{65}\), there are reports that CHX has the potential to cause anaphylactic reactions\(^{105}\) and even anaphylactic shock\(^{65}\). Hypersensitivity reactions described in the literature include contact dermatitis and photosensitivity. Both application of CHX to mucous membranes and to intact skin can cause allergic reactions. Therefore it is important to remember this potential risk of CHX.

According to several \textit{in vitro} studies, CHX has a marked antimicrobial effect on \textit{E. faecalis} after a short contact time even in comparatively low concentrations\(^{75,78,97,107}\). Under these \textit{in vitro} conditions, CHX was better at killing \textit{E. faecalis} than NaOCl. CHX was also found to be a very effective antifungal agent. In several studies, CHX was very effective in killing \textit{C. albicans} under \textit{in vitro} conditions\(^{108-111}\). These two microorganisms (\textit{E. faecalis} and \textit{C. albicans}) are reported to be responsible for endodontic failures\(^{4,112,114}\) in approximately 75% of retreatment cases associated with apical periodontitis. Since CHX is highly effective against the Gram-positive bacteria and both \textit{E. faecalis} and \textit{C. albicans}, the use of CHX is recommended for retreatment cases.

Due to its cationic properties, CHX can bind to dentine and enamel\(^{113,114}\) and is gradually released over time. Owing to this phenomenon of substantivity, which has not been observed with any other irrigant, CHX has prolonged antimicrobial activity\(^7,60\). After 10 minutes of irrigation, a prolongation of the antimicrobial effect of about 12 weeks was observed\(^{115}\). Hence, CHX is the only irrigant whose antimicrobial effect outlasts the duration of irrigation.

Although not yet fully understood, the combination of CHX and hydrogen peroxide was reported to be better at killing \textit{E. faecalis} in dentinal tubules compared with NaOCl or CHX alone\(^{116}\). In a further study, the observation of synergistic effect was supported, as the two irrigants in combination killed all \textit{E. faecalis} in concentrations much lower than for each component alone\(^{117}\). Although this synergistic mechanism of CHX and hydrogen peroxide has not been elucidated in detail, it may be speculated that CHX denatures the bacterial cell walls and creates pores in the membrane, resulting in a more permeable cell wall\(^{117}\), which then allows hydrogen peroxide to penetrate the microbes and damage intracellular organelles such as DNA\(^{17}\). A similar synergistic effect has also been shown for CHX and hydrogen peroxide when used as an antiplaque mouth rinse\(^{118}\). However, there are no clinical studies at present that have investigated the potential synergistic effect of these irrigants against intraradicular microbes\(^4\).

Unlike NaOCl, CHX does not possess any tissue-dissolving ability\(^4,120\), and is unable to remove the smear layer or neutralise lipopolysaccharides, which are obvious benefits of NaOCl. It is only because of these differences that CHX cannot be a substitute for NaOCl as the gold standard of root canal irrigants. Also, CHX seems to be less effective against Gram-negative bacteria (which predominate in primary endodontic infection)\(^{121}\). Further weaknesses of CHX include its susceptibility to the presence of organic material; the antimicrobial effect of CHX is strongly reduced by the presence of dentine, inflammatory exudates, serum albumin, dentine matrix, and heat-killed cells of \textit{E. faecalis} and \textit{C. albicans}\(^{58,122-124}\). These findings may explain the poor \textit{in vivo} performance of CHX compared with \textit{in vitro} results\(^4\). As yet, there have been no controlled clinical studies on CHX to show increased antimicrobial activity against \textit{E. faecalis} compared with NaOCl. In a randomised clinical trial, 2.5% NaOCl was significantly more effective than 0.2% CHX\(^{7,60}\). Direct contact between NaOCl and CHX should be avoided, otherwise red CHX crystals will precipitate immediately (Fig 6).

In summary, a 2% CHX solution is an alternative irrigant because of its potent antimicrobial activity\(^4\), but it certainly cannot replace NaOCl. CHX is useful as a final flush (Table 1)\(^7,60\) in retreatment-cases.
Hydrogen peroxide

Hydrogen peroxide (H$_2$O$_2$) is used in dentistry in various concentrations ranging from 1% to 30%\(^4\). For endodontic treatment, a concentration between 3% and 5% is preferred. Solutions of H$_2$O$_2$ are chemically stable and H$_2$O$_2$ is active against bacteria, yeasts, and viruses\(^4\) due to the production of hydroxy free radicals (·OH). These radicals attack several cell components such as proteins and DNA\(^4,59\).

The antimicrobial efficiency and the tissue-dissolving capacity of H$_2$O$_2$ are poor in comparison with NaOCl. It was previously thought that an irrigation protocol employing NaOCl and H$_2$O$_2$ alternately could have beneficial effects on canal cleanliness and reduce intraradicular microorganisms, but this has not been substantiated scientifically. Several studies showed that a combination of NaOCl and H$_2$O$_2$ resulted in a marked reduction in both the tissue dissolution capacity and the antibacterial efficiency of NaOCl\(^116,125\). In fact, a combination of these two solutions resulted in a bubbling effect as a result of the chemical reaction. Oxygen evaporates from the aqueous peroxide and NaOCl reacts with sodium chloride\(^126\), rendering both irrigation solutions useless.

\[ \text{H}_2\text{O}_2 + \text{NaOCl} \rightarrow \text{O}_2 + \text{H}_2\text{O} + \text{NaCl} \]

Although there is no obvious synergism between H$_2$O$_2$ and NaOCl, a combination including H$_2$O$_2$ has been reported to be promising. Recent studies on combining CHX and H$_2$O$_2$ at low concentrations found significantly greater antimicrobial activity against *E. faecalis* than the tested medicaments alone. In other words, the combination of the solutions killed *E. faecalis* in concentrations much lower than each irrigant could alone\(^116,117\). Certainly this topic warrants further investigation as the mechanism has yet to be fully understood\(^4\).

In conclusion, there is no scientific evidence indicating that H$_2$O$_2$ may be superior to other irrigants\(^4\).

Iodine compounds

Iodine can penetrate into microorganisms and attacks cell molecules such as proteins, nucleotides, and fatty acids\(^4\), resulting in cell death. Iodine compounds are bactericidal, fungicidal and virucidal\(^4\). Since aqueous iodine solutions are unstable, and molecular iodine (I$_2$) displays the most marked antimicrobial activity, iodine potassium iodide (IPI) is used (2% iodine in 4% potassium iodine) in endodontics\(^4\).

There seems to be some evidence that IPI is effective against *E. faecalis*\(^28,129\) and biofilms of root canal isolates\(^79\). At the same time, the biocompatibility of this irrigant is good since iodine compounds are less cytotoxic and irritating to vital tissues than other commonly used irrigants\(^82,128\). However, there are two main problems associated with the clinical use of iodine compounds as a root canal irrigant. Firstly, iodine is a very potent allergen so there is a high risk of an allergic reaction. Secondly, substances commonly found in the root canal inhibit the antimicrobial efficiency of iodine. For example, dentine powder, organic dentin matrix, heat-killed cells of *E. faecalis* and *C. albicans* displayed an inhibitory effect on both 0.2% and 0.4% IPI\(^122,129\). A minor problem is that iodine has also the potential to stain dentine. For these reasons, IPI cannot be considered an irrigant of first choice.

MTAD

MTAD is a mixture of tetracycline (doxycycline, 3%), citric acid (4.25%), and detergent (Tween 80, 0.5%), with a pH of 2.15; the commercial product is Biopure (Tulsa Dentsply, Tulsa OK, USA). When assessing the properties of MTAD on the basis of the currently available literature, it should be kept in mind that most of the studies conducted on MTAD were performed by its creators, Mahmoud Torabinejad and co-workers.

MTAD was reported to be effective in removing the smear layer due to its low pH, and showed
tissue-dissolving action as long as the canal was rinsed with NaOCl during mechanical preparation\textsuperscript{130,131}. It is interesting that MTAD displayed good tissue-dissolving capability only when NaOCl was used during instrumentation\textsuperscript{131}. Therefore, the clinical recommendation is to use 1.3% NaOCl during instrumentation, followed by MTAD as a final irrigation. MTAD seems to be less cytotoxic than 3% H\textsubscript{2}O\textsubscript{2}, 5.25% NaOCl, CHX mouthwash, and EDTA, but more cytotoxic than 2.63% and 1.31% NaOCl\textsuperscript{132}. Moreover, MTAD seems to adversely influence the physical properties of dentine or the bonding strength of adhesives to dentine\textsuperscript{133,134}.

When introducing a new irrigant, the key requirement is its antimicrobial efficiency. Some studies found that MTAD has a good antibacterial activity against \textit{E. faecalis}\textsuperscript{135–137}, but this was not supported by other investigations. NaOCl solutions (5%) and CHX were both more effective against \textit{E. faecalis} and \textit{C. albicans} than MTAD\textsuperscript{138,139}. Both 1% and 6% NaOCl were found to be more effective in eliminating \textit{E. faecalis} biofilms than MTAD\textsuperscript{80}; this has been supported by another recently published report\textsuperscript{81}. In this study, the intraradicular content from 10 extracted teeth associated with a chronic apical periodontitis was collected and cultured on sections of root apices to generate a polymicrobial biofilm. The sections were immersed in 6%, 3%, and 1% NaOCl, 2% chlorhexidine, sterile phosphate buffered solution, and 1% NaOCl followed by MTAD. While 6% and 3% NaOCl were able to disrupt and remove the biofilm, 1% NaOCl and 1% NaOCl followed by MTAD were capable of disrupting the biofilm but not eliminating the bacteria. Chlorhexidine was not capable of disrupting the biofilm. Viable bacteria were not found in sections immersed in 6% NaOCl, 2% chlorhexidine, and 1% NaOCl followed by MTAD. The authors concluded that 6% NaOCl was the only irrigant capable of both rendering bacteria nonviable and physically removing the biofilm\textsuperscript{81}.

A preliminary report found the alternating use of NaOCl and MTAD might potentially cause iatrogenic tetracycline staining of teeth\textsuperscript{140}. Another concern is the high concentration of tetracycline in MTAD; resistance to tetracycline is not uncommon among bacteria isolated from root canals\textsuperscript{141}. In fact, a higher incidence of tetracycline-resistant bacteria must be expected in future years. The local application of antibiotics must be viewed very critically, as the spectra of some antibiotics are narrower than commonly used antimicrobial irrigating solutions\textsuperscript{7,59}. According to Zehnder, the "use of antibiotics instead of biocides such as hypochlorite or chlorhexidine appears unwarranted"\textsuperscript{7}.

\section*{Phenolic compounds}

These irrigants are relatively ineffective under clinical conditions\textsuperscript{142}, and from reports of numerous studies there is clear scientific evidence that solutions containing camphorated paramonochlorophenol are irritating and display toxic effects on healthy tissues\textsuperscript{82,143,144}. The application of these irrigants in the root canal results in systemic distribution\textsuperscript{145}. In general, phenolic compounds are assessed as "incompatible with a biologic approach to endodontic treatment"\textsuperscript{146}.

Therefore, phenolic compounds must be seen as obsolete\textsuperscript{147} and will not be discussed further in this review.

\section*{Irrigation solutions to remove the smear layer}

\subsection*{EDTA}

Ethylenediaminetetraacetic acid (EDTA) as a 17% solution (pH 7) effectively removes the smear layer by chelating the inorganic components of the dentine\textsuperscript{4,148}. EDTA has almost no antibacterial activity\textsuperscript{4}, is highly biocompatible, can demineralise intertubular dentine and reduces the surface hardness of root canal wall dentine\textsuperscript{7,149}. Some caution should be exercised when using EDTA inside root canals because prolonged exposure to EDTA may weaken root dentine\textsuperscript{150} and thereby increase the risk of creating a perforation during mechanical root canal instrumentation.

According to the results of preliminary studies, irrigation of the root canal using alternately NaOCl and EDTA appears to be very promising\textsuperscript{151}. This combination seems to enhance the tissue-dissolution capability of NaOCl\textsuperscript{151,152} and is more
efficient in reducing intraradicular microbes than NaOCl alone. EDTA retains its calcium-complexing ability when mixed with NaOCl, but EDTA causes NaOCl to lose its tissue-dissolving capacity. Therefore EDTA and NaOCl should be used separately and EDTA should never be mixed with NaOCl. Furthermore, chelating agents like EDTA can disrupt the biofilm adhering to the root canal wall. After irrigation of the canals with EDTA, 2 ml of NaOCl should be finally used to neutralise the acidic effects of EDTA and to allow NaOCl to penetrate into the dentinal tubules, which are opened after the use of EDTA.

**Citric acid**

Concentrations ranging from 1–40% have been used in endodontics to remove the smear layer after root canal preparation (Fig 7). Compared with EDTA, 10% citric acid seems to be more effective in removing the smear layer and in dissolving powdered dentine. Moreover, citric acid solutions display antimicrobial effects, although it is questionable whether or not this property might have any clinical significance. In contrast, other studies have failed to show a significant difference between EDTA and citric acid in removing the smear layer. As citric acid demineralises the intertubular dentine around the opening of the tubules, they are enlarged.

In summary, both EDTA and citric acid can remove the smear layer effectively. The removal of the smear layer is a crucial step to facilitate disinfection of the root canal system. Firstly, microorganisms embedded in the smear layer are eliminated and canal cleanliness is improved. Secondly, it has been shown that the removal of the smear layer improves the antimicrobial effect of intraradicular medications in the deeper layer of dentine. Therefore, either EDTA or citric acid should be included in the irrigation regimen (Table 1).

**Irrigants for drying the root canal**

Rinsing the root canal with alcohol before obturation has been anecdotally practised. The basic premise is that alcohol reduces the surface tension of irrigants and root canal sealers. Lowering the surface tension of a fluid or a sealer will increase the fluid flow into the dentinal tubules. Thus alcohol will spread into the dentinal tubules and dry the root canal as it evaporates. Therefore alcohol might affect sealer penetration and leakage of the root canal filling. In a recently published study it was shown that a final rinse with 95% alcohol before root canal obturation resulted in increased sealer penetration and consequently decreased leakage. This is in agreement with another study demonstrating that a final rinse with alcohol allowed better sealer coverage than drying with paper points.

Therefore a final rinse of approximately 3 ml of 95% ethyl alcohol per canal can be recommended in order to improve the sealing ability of the root canal filling (Table 1).
Clinical and technical aspects of irrigation

The most important technical aspect of root canal irrigation is the correlation between the diameter of the irrigating needle and the apical preparation size. Inside the root canal the effect of irrigation is limited to 3–4 mm apical from the needle tip. In an in vitro study, it was shown that the introduction of an irrigation needle 1 mm short of working length resulted in significantly fewer remaining bacteria in the root canal compared with using a needle 6 mm short of the working length. The aim is to introduce the needle as near as possible to working length to improve the irrigation efficiency. Since the smallest needle recommended for root canal irrigation is a 30-gauge needle (Fig 8) (diameter of 0.3 mm, corresponding to ISO size 30), the apical preparation should be size 35 to 40. Even in severely curved canals, an apical preparation of size 35 to 40 can be achieved with modern rotary nickel-titanium instruments without the risk of canal aberrations or canal straightening. Flexible irrigation needles with a safety tip are recommended, so that the needle can be pre-bent according to the canal curvature to allow proper cleaning of the apical part of curved root canals (Fig 9).

When trying to insert the needle tip as close as possible to the working length, the needle might become jammed in the root canal and the pressure exerted can easily result in extrusion of NaOCl or H2O2 into the periapical tissue. Hence when resistance to the needle is felt, it should be pulled back approximately 2 mm to ensure space between the canal wall and needle to allow the irrigant to flow out of the canal (Fig 10). This will minimise the risk of injecting irrigation solutions beyond the apex and into the periapical tissue.

Based on this review, the following irrigation protocol is suggested (Table 1):  
- Apical root canal preparation should be to at least size 35 and a 30-gauge needle should be used.  
- After access cavity preparation: flush the cavity and the canals with NaOCl. Canals must always be filled with NaOCl because this will increase working time available for the irrigant. At the same time, cutting efficiency of root canal instruments is enhanced due to the lubrication effect.
• Between instruments: 2–5 ml of NaOCl per canal. NaOCl should always be employed throughout mechanical root canal preparation.
• After root canal shaping: 5–10 ml of NaOCl per canal. When the shaping procedure is completed, flush with a high volume of NaOCl.
• After shaping: irrigation with 5 ml of EDTA per canal for 1 minute (or with citric acid). After a final rinse of NaOCl, the canals should be irrigated with either EDTA or citric acid to remove the smear layer.
• Final rinse: irrigation with 2 ml of NaOCl per canal to neutralise the acidic effect of EDTA and to allow NaOCl to penetrate into the opened tubules.
• Optional, final irrigation – especially in retreatment cases: chlorhexidine. Rinse with water to remove NaOCl and then with a 2% chlorhexidine solution.
• Optional, before root canal filling: rinse with 3 ml of alcohol per canal to dry the root canal.

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