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REVIEW ARTICLE

Present status and future directions – irrigants and irrigation methods

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Abstract

Irrigation is considered the primary means of cleaning and disinfection of the root canal system. The purpose of this review was to set the framework for the obstacles that irrigation needs to overcome, to critically appraise currently used irrigants and irrigation methods, to highlight knowledge gaps and methodological limitations in the available studies and to provide directions for future developments. Organization of bacteria in biofilms located in anatomic intricacies of the root canal system and the difficulty to eliminate them is the main challenge for irrigants. Sodium hypochlorite remains the primary irrigant of choice, but it needs to be supplemented by a chelator. Delivery of the irrigants using a syringe and needle and activation by an ultrasonic file are the most popular irrigation methods. There is no evidence that any adjunct irrigation method, including ultrasonic activation, can improve the long-term outcome of root canal treatment beyond what can be achieved by instrumentation and syringe irrigation. It is necessary to redefine the research priorities in this field and investigate in greater depth the penetration of the irrigants, their effect on the biofilm and the long-term treatment outcome. New studies must also focus on clinically relevant comparisons, avoid methodological flaws and have sufficiently large sample sizes to reach reliable conclusions. Future multidisciplinary efforts combining the knowledge from basic sciences such as Chemistry, Microbiology and Fluid Dynamics may lead to more effective antimicrobials and improved activation methods to bring them closer to the residual biofilm in the root canal system.

KEYWORDS

Activation, delivery, irrigant, sodium hypochlorite, syringe, ultrasonic

INTRODUCTION

Bacteria play a key role in the development of pulpal and periapical disease (Chávez de Paz, 2007; Kakehashi et al., 1965; Möller et al., 1981), so infection control is an essential goal of root canal treatment in order to prevent or cure apical periodontitis (Ørstavik, 2019). For a long time, the debridement and disinfection of the root canal system had been considered primarily a function of the instruments whilst less attention was given to irrigants (Schilder, 1974). However, accumulated evidence gradually revealed that instruments are unable to reach a large portion of the root canal system (Peters, 2004). As a result, the perceived importance of irrigation grew considerably

over the past decades and this eventually led to a paradigm shift. Nowadays, instrumentation is largely considered a means of providing access to the apical anatomy for the irrigants, which are then expected to accomplish most of the cleaning and disinfection (Gulabivala et al., 2005).

The change of paradigm motivated a renewed interest in root canal irrigation, which is manifested by the large number of studies that have been published within the last 20 years and the upward trend (Figure 1). Irrigation appears to be one of the hot topics in Endodontology (Kolahi et al., 2020) with hundreds of new studies being published every year. However, conflicting findings are often reported and the resultant information overload may confuse clinicians, researchers and decision makers. Therefore, the purpose of this review was to set the framework for the challenges that need to be tackled, to critically appraise the most widely used irrigants and irrigation methods, to highlight knowledge gaps and methodological limitations in the available studies and to provide directions for future developments.

CHALLENGES FOR ROOT CANAL IRRIGATION

Root canal infections are caused by multi-species microbial biofilms attached to dentinal surfaces (Svensäter & Bergenholtz, 2004), and this forms the primary challenge for root canal irrigants. A mature biofilm consists of multiple layers of microorganisms embedded in a self-produced extracellular polymeric substance (EPS) and utilizes various mechanisms in order to resist the action of antimicrobial agents (Costerton et al., 1999). The EPS matrix creates a physical barrier that hinders the diffusion of antimicrobials into the biofilm and also neutralizes them (Costerton et al., 1999; del Pozo & Patel, 2007). Organization of the microorganisms in a multilayer structure also leads to concentration gradients of nutrients and oxygen across the biofilm, which force the cells in the inner layers to enter slow-growing or starved metabolic states (dormant cells) that are inherently less susceptible to antimicrobials (Chávez de Paz et al., 2008; Costerton et al., 1999; Hall-Stoodley et al., 2004; Lewis, 2007). In addition, exposure to stress (such as low-level antimicrobials) triggers the differentiation of some cells into a highly persistent phenotypic variant. These *persister* cells are well adapted to the stress conditions; they remain in a stationary phase of growth and exhibit multi-drug resistance. When the conditions become more favourable, persister cells can proliferate and form a new population with normal susceptibility (Costerton et al., 1999; Hall-Stoodley et al., 2004; Lewis, 2007).

An additional problem arises from the complex anatomy of the root canal system. Apart from the main root canal, biofilm may also reside in fins extending laterally from the main canal, isthmuses connecting adjacent root canals in the same root (Vertucci, 2005), accessory canals and apical ramifications (Gulabivala et al., 2005; Ricucci et al., 2013). Dentin debris produced during instrumentation may also accumulate in these areas and is believed to act as protective insulation for the underlying biofilm (Paqué et al., 2009, 2011). Furthermore, bacteria invade patent dentinal tubules, which are located mainly in the middle and coronal third of the root canal system (Vasiliadis et al., 1983a, 1983b), to varying depths (Love & Jenkinson, 2002). Microorganisms evading the action of instruments and irrigants are currently considered the primary cause of failure following both primary treatment and non-surgical

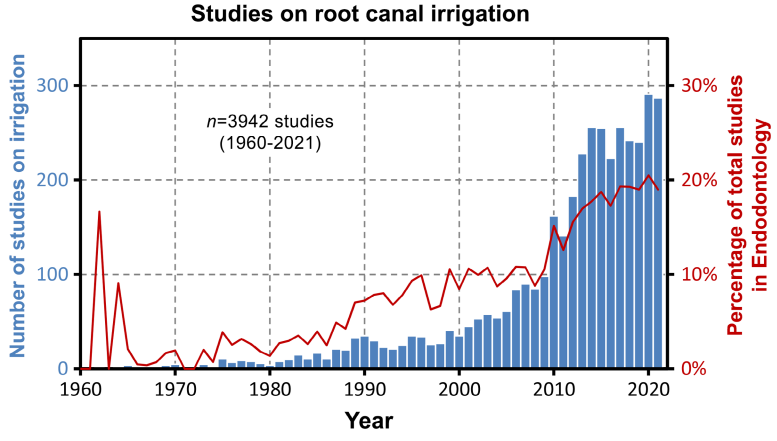

FIGURE 1 Approximate number of studies on root canal irrigation published per year between 1960 and 2021 according to the PubMed database (blue columns). The red line indicates what percentage this number is of the total number of studies in endodontology per year. The studies on root canal irrigation were retrieved using the search terms "root canal" AND (irrigation OR irrigant), the latter two being limited to the title or abstract, whilst those retrieved using the search term "root canal" were used as a proxy for the total number of studies in endodontology. None of these searches is exhaustive, but the findings may give an idea of the research interest over the past six decades.

retreatment (Gorni & Gaglianni, 2004; Zehnder & Paqué, 2011).

The anatomy of the root canal system creates a number of physical obstacles for the irrigants. The main root canal and most of the anatomical intricacies, including dentinal tubules, are closed-ended cavities, so irrigant penetration is inherently difficult. It should be noted here that entrapment of air bubbles near the distal end of such cavities (*vapor lock*) is probably the result and not the cause of the poor irrigant penetration (Boutsioukis et al., 2014a). In addition, bulk irrigant flow, the most efficient transport mechanism and an important means of mechanical disruption and removal of biofilm, is mostly limited to the main root canal and wide adjacent areas due to constraints imposed by the available space and the viscosity of the irrigant (Boutsioukis, 2019). The alternative, diffusion of the active molecules and ions, is an extremely slow and inefficient process (Verhaagen et al., 2014a). Evidently, even the most potent irrigant will not be effective if it cannot reach its targets inside the root canal system in sufficient quantity.

Most currently used irrigants are chemically-active solutions, and their direct reaction with biofilm is considered the foundation of their antimicrobial effect. However, irrigants also react with a variety of other substrates inside the root canal system, for instance dentine or other irrigants. Such reactions are often considered as side-effects of irrigation, not only because the active molecules/ions of the irrigant are consumed in undesired reactions instead of targeting the biofilm (Haapasalo et al., 2000; Portenier et al., 2001; Tejada et al., 2019) but also because, in theory, alterations of the organic and inorganic components of dentin could affect its mechanical properties or the adaptation of filling materials (Augusto et al., 2021; Pascon et al., 2009). Discoloration of the tooth as a result of irrigant interactions is also a concern in some cases (Tay & Mazzoni, 2006).

Undesired chemical effects of irrigants can extend beyond the root canal system. Inadvertent extrusion of the irrigant through the apical foramen may lead to damage of the periapical tissues and pronounced symptomatology (Boutsioukis et al., 2013a; Guivarc'h et al., 2017). Thus, irrigants must reach as much of the root canal system as possible in order to exert their desirable actions but not come in contact with the periapical tissues, at least not in large volume. This is a particularly delicate balance to maintain given the constraints to irrigant penetration.

PROPERTIES OF THE IDEAL IRRIGANT

Taking the abovementioned challenges into account, the main requirements for root canal irrigants are as follows:

- Strong antimicrobial action against a broad spectrum of microorganisms, both planktonic and those organized in biofilms
- Inactivation of bacterial virulence factors, such as endotoxins and lipoteichoic acids
- Disruption or removal of the biofilm
- Dissolution of pulp tissue remnants
- Removal of accumulated hard-tissue debris and the smear layer or prevention of their formation
- Lack of adverse effects, both local (on dentine and the periapical tissues) and systemic (toxicity, allergic reactions)
- Wide availability at low cost

CURRENTLY USED IRRIGANTS

Sodium hypochlorite

Sodium hypochlorite (NaOCl) is by far the most popular root canal irrigant, and it is widely considered the primary irrigant of choice (Dutner et al., 2012) because of its exceptional antimicrobial action particularly against bacteria organized in biofilms (Arias-Moliz et al., 2009, 2014; Ruiz-Linares et al., 2017; Wong & Cheung, 2014; Yang et al., 2016) and its unique ability to dissolve biofilm components and pulp tissue remnants (Busanello et al., 2019; Naenni et al., 2004; Tawakoli et al., 2017; Tejada et al., 2019) (Figure 2). Moreover, it can reduce bacterial virulence factors such as endotoxins and lipoteichoic acids (Hong et al., 2016) and also serve as an effective lubricant for rotary instruments (Boessler et al., 2007). Its low cost and wide availability may have also contributed to its widespread use.

The chemical effects of NaOCl are produced by the contained free available chlorine, which consists of hypochlorite (OCl^-) and hypochlorous acid (HOCl) (Baker, 1947; Davies et al., 1993). Both are strong oxidizers and their relative amounts depend on the pH. Ordinary (unbuffered) NaOCl solutions have a pH close to 11–12 (Jungbluth et al., 2011), so hypochlorite predominates. It has been hypothesized that the antimicrobial activity may be boosted by lowering the pH, which increases the amount of the hypochlorous acid in the solution, but the benefits of such buffering were shown to be insignificant and came at the expense of solution stability (Jungbluth et al., 2011; Zehnder et al., 2002).

There is still no consensus on the optimum concentration of NaOCl solutions, with proposed values ranging from 0.5 to 8.25% (Cullen et al., 2015; Demenech et al., 2021; Gazzaneo et al., 2019; Stojicic et al., 2010). Clinicians' preferences also vary considerably between countries (Clarkson et al., 2003; Dutner et al., 2012; de Gregorio

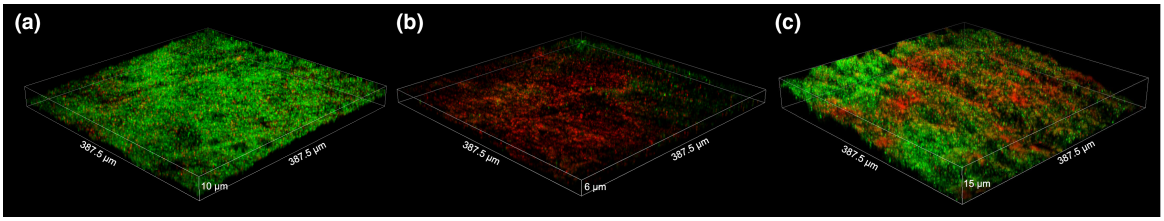

FIGURE 2 Three-dimensional reconstruction of Confocal Laser Scanning Microscopy scans of natural multi-species biofilms grown from an infected root canal sample on dentine for 3 weeks: (a) untreated control, (b) after treatment with 2.5% NaOCl for 1 minute, (c) after treatment with 2% CHX for 1 minute. Green-coloured bacteria are cells with intact membranes and red-coloured bacteria are cells with damaged membranes following Live/Dead staining (BacLight; Invitrogen, Eugene, OR, USA).

et al., 2015; Neukermans et al., 2015; Willershausen et al., 2015). According to laboratory studies, the desirable effects of NaOCl are a function of its concentration (Arias-Moliz et al., 2009; Chau et al., 2015; Macedo et al., 2010; Moorer & Wesselink, 1982; Petridis et al., 2019b; Stojicic et al., 2010). A systematic review also concluded that higher concentrations may provide an advantage, although the evidence was weak (Fedorowicz et al., 2012). Recent clinical studies have not detected a significant difference in the antimicrobial effect or healing of apical periodontitis between different concentrations of NaOCl (Ulin et al., 2020; Verma et al., 2019), but the results may have been biased by the lack of proper randomization and blinding, the flexibility of the instrumentation and irrigation protocols (Ulin et al., 2020), the two-dimensional imaging, the non-verified precision of the radiographic interpretation and the inadequate sample size (Verma et al., 2019). For instance, insufficient enlargement of the root canal can mask even the difference between saline and 2.5% NaOCl concerning their antimicrobial activity *in vivo* (Rodrigues et al., 2017).

Increasing the concentration may also amplify the solution's undesirable effects. NaOCl reacts with the collagen in the dentine matrix, especially after prior exposure to a chelating agent, and this may alter the modulus of elasticity, the tensile and flexural strength and the microhardness of dentine (Pascon et al., 2009). However, such findings should not be interpreted as irrefutable evidence that teeth are rendered more susceptible to fracture. Published *in vitro* experiments investigating the effect of NaOCl on the mechanical properties of dentine often deviated considerably from the *in vivo* conditions; thin partially or totally dehydrated dentine bars were completely immersed in NaOCl for extended periods of time. In addition, dentine is a heterogeneous material and microhardness values measured by indentation methods reflect mostly the condition of the surface, where the effect of the irrigant is expected to be more pronounced, but reveal very little information about the rest of the material. Time dependence of the produced indentations has also been reported (Herkströter et al., 1989). Finally, the true indicator of the ability of dentine to resist fracture is its toughness and not its elastic modulus or strength (Kishen, 2006).

NaOCl is also caustic (Pashley et al., 1985), and its inadvertent extrusion towards the periapical tissues may result in a *NaOCl accident* (Boutsioukis et al., 2013a; Guivarc'h et al., 2017). Even so, contrary to popular belief, there is no clinical evidence correlating the concentration of NaOCl with the risk or severity of such accidents. Case reports have shown that accidents may occur even when a 1% solution is used (Boutsioukis et al., 2013a; Guivarc'h et al., 2017). On the other hand, a higher NaOCl concentration seems to result in slightly more inter-appointment pain according to one study (Mostafa et al., 2020), but another study reported that concentration (2.25–8.25%) was not correlated tooperative pain when the treatment was completed in a single session (Demenech et al., 2021).

Although root canals are usually rinsed with a few millilitres of NaOCl (Boutsioukis et al., 2007), only a very small amount actually remains in place between rinses; the volume of a root canal can be estimated to ≤30 µL for most cases (assuming that a large root canal can be approximated by a conical frustum with an apical size 60, 0.06 taper and a length of 22 mm). Given the rapid consumption of the free available chlorine in chemical reactions with biofilm, dentine, pulp tissue and other irrigants (Macedo et al., 2010; Moorer & Wesselink, 1982; Ragnarsson et al., 2015; Tejada et al., 2019; Zehnder et al., 2005a), frequent exchange with fresh irrigant during chemomechanical preparation is generally advised (Macedo et al., 2010; Moorer & Wesselink, 1982). Nevertheless, this should not be regarded as a complete remedy for using less concentrated solutions. Even if such solutions are refreshed more often, phenomena driven by concentration gradients, such as the diffusion of molecules and ions in the root canal system or through the biofilm, will still be weakened. Prolonging the exposure of the biofilm to NaOCl seems to facilitate its removal *in vitro*, as long as the chlorine is not depleted, and this effect also seems to be intensified by the concentration (Chau et al., 2015; Petridis et al., 2019a). Thus, the volume of the NaOCl that

should be delivered, the required exposure time and frequency of exchange and the concentration of the solution are interrelated parameters strongly dependent on the highly variable conditions in the root canal, and it comes as no surprise that there are still no unanimous guidelines on them.

Preheating NaOCl to 50–60°C prior to irrigation has been suggested as another way to improve the efficacy of low-concentration solutions (Sirtes et al., 2005). Despite the promising *in vitro* and *ex vivo* findings (Dumitriu & Dobre, 2015; Sirtes et al., 2005; Stojicic et al., 2010), the temperature of the solution drops to 37°C very soon after intracanal delivery *in vivo* (de Hemptinne et al., 2015), so only a short-term effect of questionable clinical value may be exerted. Uncontrolled heating of the solution inside the root canal has been proposed as an alternative in order to compensate for the rapid temperature buffering (Bartolo et al., 2016; Leonardi et al., 2019), but the associated risks have not been evaluated in full yet. It should also be noted that the observed temperature-dependent enhancement of the solution's activity is most likely a result of the accelerated diffusion and chemical reactions, both of which are non-specific. Therefore, it is not reasonable to expect a selective boost of the desired actions (antimicrobial effect, tissue dissolution) but not of the undesired ones (effect on dentine collagen, caustic effect on periapical tissues upon contact). A preheated NaOCl solution will likely react faster with all available substrates for as long as its temperature remains elevated.

Chelators

Even though NaOCl is the primary irrigant of choice, it cannot dissolve hard-tissue debris created during instrumentation or the inorganic components of the smear layer, so the supplementary action of a demineralizing agent is considered necessary. Ethylenediamine tetraacetic acid (EDTA) is the most common choice for this role (Dutner et al., 2012; Willershausen et al., 2015). A 15–17% solution of its disodium salt has a neutral or slightly alkaline pH (~7–8), and it is a strong chelator able to dissolve both hard-tissue debris and the smear layer when applied at the end of instrumentation (Calt & Serper, 2002; De-Deus et al., 2008a; Hülsmann & Heckendorff, 2003) (Figure 3). EDTA exerts only a weak antimicrobial effect (Arias-Moliz et al., 2008, 2009; Ordinola-Zapata et al., 2012), but it seems to disrupt the biofilm matrix thereby promoting its detachment (Bryce et al., 2009; Busanello et al., 2019), so it may also supplement the anti-biofilm effect of NaOCl. Despite the fact that a small proportion of clinicians seem to use it as the primary irrigant (Dutner et al., 2012), currently there is no evidence supporting the use of EDTA or any other chelator instead of NaOCl during chemomechanical preparation. Alternate irrigation with NaOCl and disodium EDTA is also contraindicated because these two solutions react and the free available chlorine is lost very rapidly (Grawehr et al., 2003; Zehnder et al., 2005a). EDTA is more biocompatible than NaOCl (Vouzara et al., 2016) and also inexpensive and widely available.

Other less popular strong chelators that could be used instead of EDTA are citric acid (Wayman et al. 1979, Pérez-Heredia et al., 2006, Prado et al., 2011) and maleic acid (Ballal et al., 2009a, 2016). Both are biocompatible (Amaral et al., 2007; Ballal et al., 2009b; Malheiros et al., 2005), but they also react with NaOCl and consume its available chlorine (Ballal et al., 2011; Zehnder et al., 2005a). The antimicrobial activity of citric acid is very limited (Arias-Moliz et al., 2009), but maleic acid is able

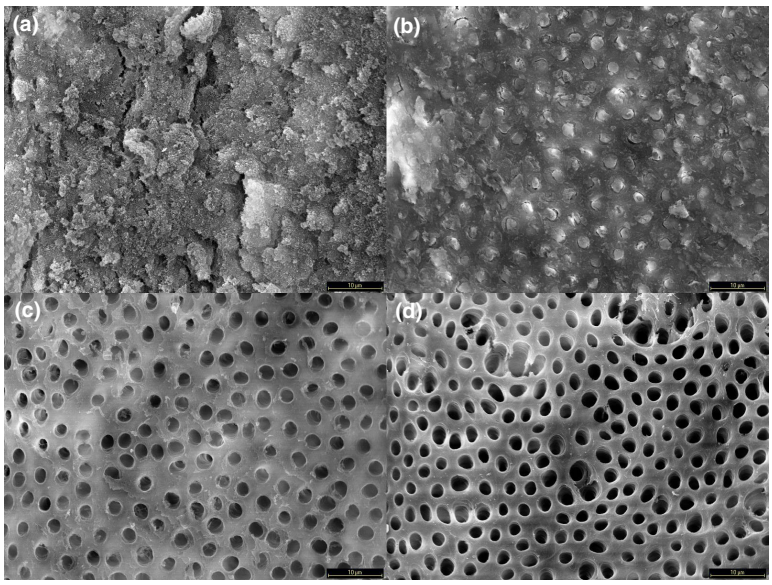

FIGURE 3 Scanning electron microscope photomicrographs of dentine following chemomechanical preparation. A thick contaminated smear layer was evident when distilled water was used as irrigant (a). Irrigation with 2.5% NaOCl during preparation resulted only in partial removal of the smear layer (b) whilst an additional final rinse with 17% disodium EDTA (c) or continuous chelation with a mixture containing 2.5% NaOCl and 9% etidronic acid throughout preparation (d) resulted almost in complete removal.

to kill bacteria organized in biofilms (Ferrer-Luque et al., 2010).

Various weak chelators have recently gained attention as candidates for *continuous chelation* in an effort to simplify the irrigation protocol (Wright et al., 2020a; Zehnder et al., 2005a). These agents can be mixed with NaOCl without consuming its free available chlorine in the short term (Biel et al., 2017; Solana et al., 2017; Tartari et al., 2017; Wright et al., 2020a; Zehnder et al., 2005a), so the mixture maintains the antimicrobial and tissue-dissolving properties of NaOCl and it can also remove hard-tissue debris and the smear layer, albeit after a longer exposure (De-Deus et al., 2008a; Lottanti et al., 2009; Paqué et al., 2012; Wright et al., 2020a, 2020b) (Figure 3). Thus a freshly prepared mixture can be used as the sole irrigant throughout root canal preparation (Arias-Moliz et al., 2014, 2015; Biel et al., 2017; Tartari et al., 2015; Wright et al., 2020b; Zehnder et al., 2005a). HEDP (1-hydroxyethylidene 1,1-disphosphonate), also known as *etidronic acid* or *etidronate*, was one of the first weak chelators to be proposed (Zehnder et al., 2005a), but other solutions, such as tetrasodium EDTA (Solana et al., 2017; Tartari et al., 2017) and clodronate (Wright et al., 2020a, 2020b), are also under investigation.

Chlorhexidine

Chlorhexidine gluconate (CHX) is a cationic bisbiguanide that has been mostly advocated as a final irrigant (Haapasalo et al., 2012; Zehnder, 2006) because the lack of any tissue-dissolving action (Naenni et al., 2004; Okino et al., 2004) precludes its use as the primary irrigant, except for very rare cases (Dandakis et al., 2000). Early studies concluded that it is equally or more effective than NaOCl against bacteria (Gomes et al., 2001; Menezes et al., 2004; Vianna et al., 2004), but these findings were probably a consequence of the overreliance on *Enterococcus faecalis* as a test species (Swimberghe et al., 2019a). *E.faecalis* is particularly susceptible to CHX, but it is not present in many cases of failed root canal treatment, and, when found, it is hardly ever amongst the most prevalent species (Siqueira et al., 2016; Zandi et al., 2018). Thus, its role as the main cause of root canal treatment failure has been much disputed (Zehnder & Paqué 2008, Zehnder & Guggenheim, 2009). More recent work using multi-species biofilm models that resemble the *in vivo* conditions more closely has clearly demonstrated that CHX is a much weaker antimicrobial than NaOCl (Busanello et al., 2019; Ruiz-Linares et al., 2017), and it cannot disrupt the EPS matrix (Busanello et al., 2019; Tawakoli et al., 2017) (Figure 2). The inconsistent findings of clinical studies (Ruksakiet et al., 2020) could be attributed to the well-recognized limitations of paper-point sampling (Sathorn et al., 2007) along with lack of statistical power and poor standardization of the instrumentation and irrigation protocols.

One of the main arguments in favour of CHX is its ability to bind to dentine and exert a prolonged antimicrobial effect (*substantivity*), which may prevent bacterial recolonization after root canal treatment (Komorowski et al., 2000; Rosenthal et al., 2004). However, the substantivity of CHX appears to have been investigated under rather unrealistic conditions, including prolonged total immersion of dentine blocks in CHX, the use of *E.faecalis* as the single test species, and omission of root canal filling (Boutsioukis et al., 2022). Even under these extremely favourable conditions, the antimicrobial activity lasted only up to 12 weeks following exposure to CHX (Baca et al., 2012; Barrios et al., 2013; Komorowski et al., 2000; Parsons et al., 1980; Rosenthal et al., 2004), which seems insignificant compared to the period of time that a treated tooth is expected to survive and function *in vivo*.

Contrary to popular belief, CHX is equally or more cytotoxic than NaOCl at the same concentration (Scott et al., 2018; Vouzara et al., 2016). In addition, it reacts with residual NaOCl in the root canal and forms a potentially toxic orange-brown precipitate that may also cause discoloration (Basrani et al., 2007; Jeong et al., 2021; Prado et al., 2013) (Figure 4). In summary, the currently available evidence does not support the use of CHX as a final irrigant.

Mixtures

Irrigants need to perform a variety of roles, and since the ideal irrigant is yet to be found, mixtures of two or more solutions have been developed in order to combine their desired properties. One example is the mixtures of NaOCl and weak chelators that were already discussed.

Commonly used irrigants such as NaOCl, EDTA and CHX are sometimes combined with surfactants in order to reduce their surface tension. This idea stems from the widespread misconception that a lower surface tension can enhance the penetration of the irrigant in the root canal system (Abou-Rass & Patonai, 1982; Giardino et al., 2006; Palazzi et al., 2012; Taşman et al., 2000). However, surface tension only acts on interfaces formed between immiscible fluids and no such interfaces restrict irrigant penetration in the root canal *in vivo* (Boutsioukis, 2019). The addition of surfactants does not enhance the antimicrobial activity of NaOCl (Baron et al., 2016; Wang et al., 2012) or its tissue dissolution capacity (Clarkson et al., 2012; De-Deus et al., 2013; Jungbluth et al., 2012). On the contrary, it may even accelerate the consumption of its free available chlorine (Guastalli et al., 2015). The combination of CHX with

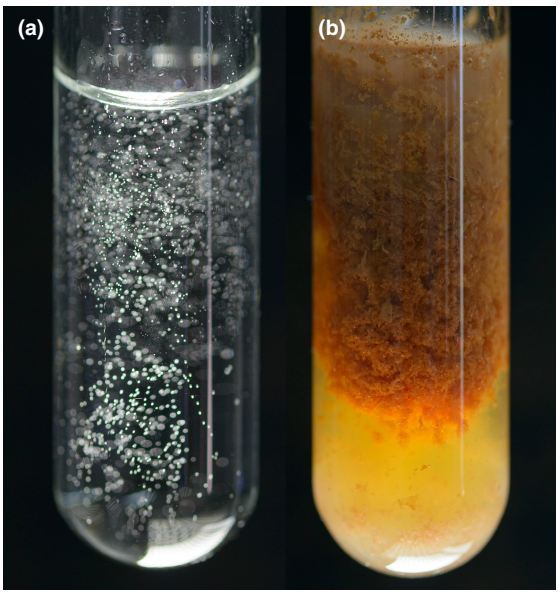

FIGURE 4 (a) Bubble formation a few seconds after mixing equal amounts of 5% NaOCl and 17% disodium EDTA indicating their chemical reaction. (b) Orange-brown mass formed due to the interaction between 5% NaOCl and 2% CHX (mixed in equal amounts).

surfactants appears to have a stronger effect against biofilm than CHX alone (Shen et al., 2011; Wang et al., 2012), but this is likely due to the very weak action of CHX being supplemented by the direct antimicrobial effect of the surfactant rather than the reduction of the surface tension (Wang et al., 2012). Removal of accumulated hard-tissue debris and the smear layer or calcium chelation also seem to be unaffected by the addition of surfactants to various irrigants (De-Deus et al., 2008b; Guerreiro et al., 2020; da Silva et al., 2008; Zehnder et al., 2005b). However, surface tension may erroneously appear to limit the penetration of root canal irrigants in laboratory studies. Dentine can lose a significant amount of free water in a dry environment within a few minutes due to dehydration (Jameson et al., 1994) and become far more hydrophobic than wet dentine (Rosales et al., 1999), thereby exaggerating the effect of irrigant surface tension. Nonetheless, such findings should be regarded as artefacts caused by the unrealistic experimental conditions *ex vivo*.

Some available mixtures, such as BioPure MTAD (Denstply Sirona, Charlotte, NC, USA), Tetraclean (Ogna Laboratori Farmaceutici, Muggiò, Italy) and QMix (Denstply Sirona, Charlotte, NC, USA), contain an antimicrobial, a chelating agent and one or more surfactants. Even though these mixtures have been mostly recommended for a final rinse at the end of the preparation instead of EDTA in order to remove the smear layer and to supplement the antimicrobial effect of NaOCl (Giardino et al., 2007; Newberry et al., 2007; Stojicic et al., 2012; Torabinejad et al., 2003), the currently available evidence suggests that they provide no clear advantage over the concerted use of EDTA and NaOCl after instrumentation (Baumgartner et al., 2007; Dai et al., 2011; Dunavant et al., 2006; Giardino et al., 2007; Kho & Baumgartner, 2006; Malkhassian et al., 2009; Ordinola-Zapata et al., 2012, 2013; Stojicic et al., 2012; Tay et al., 2006; Ye et al., 2018). Bacterial resistance and tooth discoloration because of the contained doxycycline (Tay & Mazzoni, 2006) are further concerns regarding MTAD and Tetraclean.

DESIRED FEATURES OF IRRIGATION METHODS

Similarly to the requirements for root canal irrigants, the challenges discussed at the beginning of this review also inform the requirements for irrigation methods. Consequently, the ideal method should be able to:

- deliver the irrigant to the complete root canal system so that it comes in close contact with its targets;
- refresh the irrigant frequently in order to compensate for its consumption;
- apply shear stress on the targets to detach them from the root canal wall;
- develop a reverse flow to carry detached materials and the depleted irrigant out of the root canal system;
- prevent inadvertent extrusion of the irrigant through the apical foramen.

CURRENTLY USED IRRIGATION METHODS

Syringe irrigation

Syringe irrigation remains the most popular technique for delivering irrigants inside root canals amongst both endodontists and general dentists (Dutner et al., 2012; de Gregorio et al., 2015; Savani et al., 2014; Willershausen et al., 2015). The efficacy of syringe irrigation depends on the proximity of the needles to the apical terminus of the root canal (Boutsioukis et al., 2010a, 2010b; Chen et al., 2014), the space available in the apical third (Boutsioukis et al., 2010c; Boutsioukis & Gutierrez Nova, 2021) and, in certain cases, also on the flow rate of the irrigant (Boutsioukis & Gutierrez Nova, 2021; Boutsioukis et al., 2009; Pereira et al., 2021), parameters that are still ignored in several studies.

The current evidence suggests that irrigation needles are of two types: needles that allow the irrigant to flow straight through their tip irrespective of its particular

shape (*open-ended*) and needles with a closed tip preventing direct outflow, so the irrigant flows through one or more side vents (*closed-ended*) (Boutsioukis et al., 2010a) (Figure 5). Owing to the direction and intensity of the created irrigant jets, open-ended needles seem more effective than closed-ended needles of the same size in terms of irrigant penetration and exchange (Boutsioukis & Gutierrez Nova, 2021; Boutsioukis et al., 2010a, 2010b; Shen et al., 2010; Verhaagen et al., 2012), but they also bear a higher risk of inadvertent irrigant extrusion through the apical foramen (Psimma et al., 2013a, 2013b). The optimum position for the open-ended needles is at 2–3 mm short of working length (WL), whereas closed-ended needles need to be placed within 1 mm from WL (Boutsioukis et al., 2010a, 2010b, 2010c; Chen et al., 2014), always without binding (Psimma et al., 2013b). Thus it is imperative to use flexible fine needles (27-31G) that can reach these positions even in curved root canals. Nowadays, the 30G needle may be considered as the clinical standard but, given the trends in root canal instrumentation (Gluskin et al., 2014), 31G needles may become the standard in the near future. It is noteworthy that the large needles (21-25G) so commonly used in the past (Brown & Doran, 1975; Chow, 1983; Druttman & Stock, 1989; Ram, 1977; Salzgeber & Brilliant, 1977; Teplitsky et al., 1987) only allowed the irrigant to reach up to the middle third of the root canal, which may have had implications for the effectiveness of the irrigants.

When 30-31G needles are used, the root canal needs to be enlarged to a minimum apical size 30–35 in order to prevent their binding. Enlargement up to this size is also important for irrigant penetration apically to the needle due to the viscosity of the irrigant that limits the flow in narrow areas of the root canal system (Boutsioukis, 2019; Boutsioukis et al., 2010c; Boutsioukis & Gutierrez Nova, 2021; Hsieh et al., 2007). The irrigant cannot reach up to the WL in root canals prepared to apical size 25 or smaller irrespective of the type and size of the needle (Boutsioukis & Gutierrez Nova, 2021) (Figure 6). Penetration is greatly improved in larger root canals (Boutsioukis et al., 2010c; Chen et al., 2014) and allows the irrigant to better demonstrate its antimicrobial activity. A clinical study found no significant difference in the reduction of the bacterial counts following instrumentation to an apical size 20–25 and either 2.5% NaOCl or saline irrigation, but the difference became significant after further preparation to size 35–50 (Rodrigues et al., 2017). An increase in the apical size also reduces the risk of inadvertent irrigant extrusion through the apical foramen (Psimma et al., 2013a). Root canal taper, on the other hand, appears to be less important for irrigant penetration in the apical third (Boutsioukis et al., 2010d).

The irrigant flow rate is perhaps one of the most overlooked parameters in root canal irrigation. Firstly, it affects irrigant penetration apically to a closed-ended needle; at flow rates <0.05 mL/s, the irrigant hardly reaches up to the tip of the needle, whereas it can reach up to 1–1.5 mm apically to the tip when the flow rate is increased to 0.15–0.20 mL/s (Boutsioukis & Gutierrez Nova, 2021; Boutsioukis et al., 2009; Verhaagen et al., 2012). When open-ended needles are used, irrigant penetration is less susceptible to changes in the flow rate (Boutsioukis & Gutierrez Nova, 2021; Park et al., 2013). Secondly, the flow rate affects the velocity gradient near the root canal wall irrespective of the type of needle used and, therefore, also the wall shear stress, which is responsible for the mechanical cleaning effect (Boutsioukis & Gutierrez Nova, 2021). A relatively high irrigant flow rate (0.17 mL/s) and the

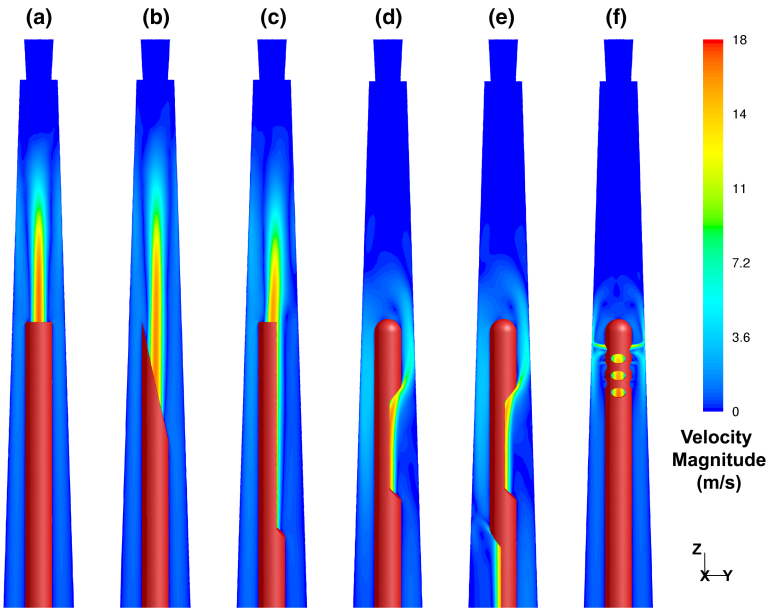

FIGURE 5 Time-averaged contours of irrigant velocity in the apical part of a size 45/.06 taper root canal during syringe irrigation using different types of needles, according to computer simulations [open-ended needles: (a) flat, (b) bevelled, and (c) notched; closed-ended needles: (d) side-vented, (e) double side-vented, and (f) multi-vented]. All needles are positioned at 3 mm short of WL, and they are coloured in red. Reprinted and modified with permission from Elsevier (Boutsioukis et al., 2010a).

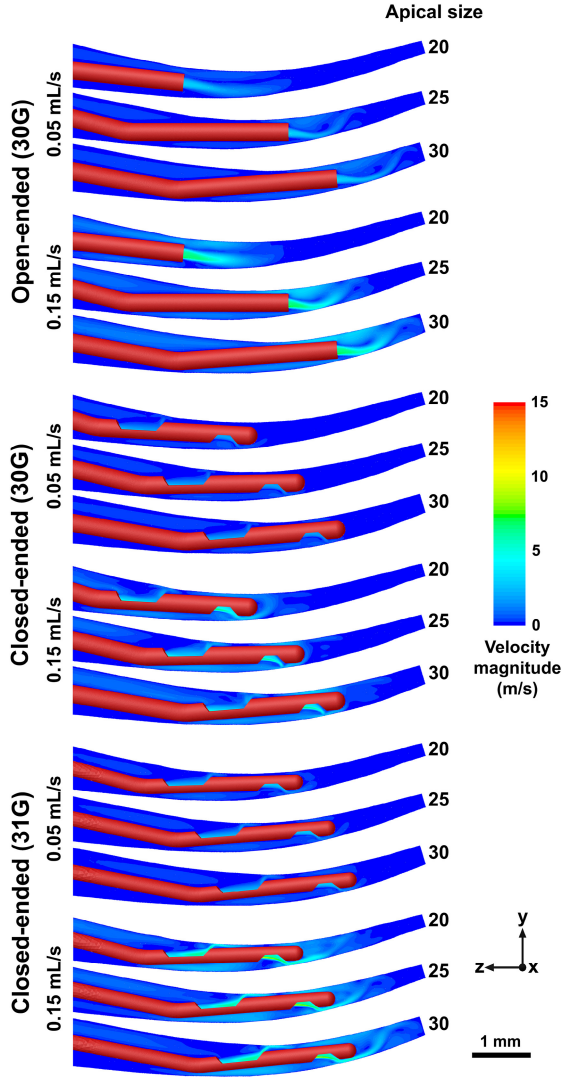

FIGURE 6 Time-averaged contours of irrigant velocity in a mesial root canal of a mandibular molar prepared to apical size 20, 25 and 30/.06 taper during syringe irrigation at 0.05 and 0.15 mL/s using a 30G open-ended, a 30G closed-ended and a 31G closed-ended needle inserted 1 mm short of the binding point, according to computer simulations. The needles are coloured in red. Reprinted and modified with permission from Elsevier (Boutsioukis & Gutierrez Nova, 2021).

resulting mechanical cleaning seem to be more important than NaOCl concentration concerning biofilm removal during syringe irrigation *in vitro* (Pereira et al., 2021).

Syringe irrigation appears to be quite effective in the main root canal when the aforementioned requirements are met. Several *ex vivo* studies and one clinical trial did not find any significant difference between syringe irrigation and a variety of other methods, including negative-pressure irrigation, sonic and ultrasonic activation, regarding the removal of soft-tissue remnants, hard-tissue debris, bacteria or biofilm from the main root canal or the healing of apical periodontitis in teeth with a single root canal and relatively simple anatomy (Adcock et al., 2011; Bhuva et al., 2010; Brito et al., 2009; Howard et al., 2011; Johnson et al., 2012; Klyn et al., 2010; Liang et al., 2013; Sarno et al., 2012; Versiani et al., 2016). In contrast, studies reaching the opposite conclusion usually did not enlarge the canals to an adequate size or placed the needles too far away from WL (Azim et al., 2016; Hockett et al., 2008; Huang et al., 2008; Kishen et al., 2018; McGill et al., 2008; Nielsen & Baumgartner, 2007; Villalta-Briones et al., 2021). Therefore, syringe irrigation appears to be a sufficient irrigation method for teeth with a single root canal and simple anatomy. However, the developed flow cannot penetrate very far inside anatomic irregularities such as fins (Amato et al., 2011; Conde et al., 2017; Jiang et al., 2012; Rödig et al., 2010a; van der Sluis et al., 2010), isthmuses (Adcock et al., 2011; Burleson et al., 2007; Gutarts et al., 2005; Leoni et al., 2017; Paqué et al., 2011; Versiani et al., 2016) and lateral canals (Al-Jadaa et al., 2009a; de Gregorio et al., 2010, 2012), so irrigant activation methods may be helpful in cases with more complex anatomy.

Ultrasonic activation

Ultrasonic activation is presently the most popular irrigant activation method and the second most popular irrigation method (Dutner et al., 2012; de Gregorio et al., 2015; Savani et al., 2014; Willershausen et al., 2015). For many years, this method was erroneously described as "passive activation" or "passive ultrasonic irrigation", despite the self-contradictory meaning of these terms, because it was believed that ultrasonic files could oscillate in the root canal without making any physical contact with the wall (Jensen et al., 1999; van der Sluis et al., 2007). This hypothesis has been refuted repeatedly (Al-Jadaa et al., 2009b; Boutsioukis & Tzimpoulas, 2016; Boutsioukis et al., 2013b; Kanaan et al., 2020; Retsas et al., 2016).

Despite the frequent wall contact (Boutsioukis et al., 2013b), ultrasonic files act primarily by agitating the surrounding irrigant rather than a direct physical effect that would be inevitably limited to the main root canal. Their oscillatory motion at ~30 kHz generates *acoustic streaming* (Jiang et al., 2010a; Verhaagen et al., 2014b), which stirs up the irrigant in the main canal, it transports the irrigant farther into remote areas of the root canal system and it improves the mechanical cleaning by increasing the wall shear stress (Retsas & Boutsioukis, 2019). Under certain conditions, the rapidly changing irrigant pressure may also give rise to *transient acoustic cavitation*, which can be particularly useful because of the emitted shockwaves, the even higher shear stress applied to the wall, and the locally increased pressure and temperature that may produce sonochemical effects (Brennen, 1995; Macedo et al.,

2014a, 2014b; Tiong & Price, 2012). During activation, a part of the kinetic energy is converted to heat (Cameron, 1988; Zeltner et al., 2009), which may also accelerate chemical reactions (Sirtes et al., 2005; Stojicic et al., 2010).

A variety of ultrasonic files, smooth wires and needles have been used for this purpose without any particular type being clearly superior to the others, but K-files and Irrisafe files seem to be the most popular ones (Căpută et al., 2019). The files need to be inserted within 2–3 mm from the WL in order for the streaming to reach the apical end of the root canal (Malki et al., 2012). Enough space should be available at that position for both the file and its unobstructed oscillation, so activation should take place only after chemomechanical preparation is completed and small-size ultrasonic files should be preferred. Given their average oscillation amplitude when driven at this frequency (~50–80 μm), the minimum apical preparation size can be estimated to 30–35 (Retsas & Boutsioukis, 2019).

The phenomena produced during ultrasonic activation depend on the power setting of the ultrasound device. Higher power results in more intense streaming and improved cleaning (Jiang et al., 2011), but the risks of file fracture (Ahmad & Roy, 1994; Craig Rhodes, 2021) and inadvertent dentin removal (Boutsioukis & Tzimpoulas, 2016; Retsas et al., 2016) should also be taken into account. The latter is a universal problem for all types of ultrasonic files and smooth wires. Most manufacturers recommend using approximately 30–50% of the maximum available power for irrigant activation (Acteon-Satelec, 2018; Electro Medical Systems, 2012; NSK, 2017).

Intermittent activation for short periods combined with delivery of fresh irrigant by a syringe and needle in between appears to be more widely used than continuous activation (Căpută et al., 2019). The repeated start-up of the oscillation enhances the cleaning efficacy and possibly also the biofilm removal compared to uninterrupted activation for the same period of time (Jiang et al., 2010b; Retsas et al., 2022; van der Sluis et al., 2006, 2009, 2010), and the frequent irrigant replenishment compensates for its consumption in chemical reactions (Macedo et al., 2014c) and for the irrigant lost because of splashing out of the pulp chamber (Macedo et al., 2014a). A popular protocol for intermittent activation is 3 periods of 20 s, although even shorter protocols are in use (3×10 s) (Căpută et al., 2019). At the moment it remains unclear whether continuous delivery at high flow rate and simultaneous activation of the irrigant at high power by an ultrasonically oscillating needle is more effective than the widely used intermittent activation protocols.

The effectiveness of ultrasonic activation appears to have been overrated in early *in vitro* and *ex vivo* studies (van der Sluis et al., 2007), which probably contributed to its premature adoption by a large portion of endodontists and general dentists. The current evidence indicates that it is clearly more effective than syringe irrigation regarding the debridement of uninstrumented oval extensions, fins, isthmuses and lateral canals, but very limited information is available regarding its antimicrobial effect in those areas and no clinical trial has found yet any improvement in the long-term treatment outcome (Căpută et al., 2019; Retsas & Boutsioukis, 2019).

Sonic agitation

Devices employing plastic tips oscillating at low frequency have long been proposed for irrigant agitation as alternatives to ultrasonic files (Jiang et al., 2010a; Neuhaus et al., 2016). Notwithstanding that sonic agitation is consistently ranked as the third most popular irrigation method (Dutner et al., 2012; de Gregorio et al., 2015), the advantages of such an approach remain unclear. Agitation by these plastic tips creates an oscillatory flow in the main root canal, but the frequency is too low and the oscillation amplitude too large to lead to acoustic streaming or transient acoustic cavitation (Jiang et al., 2010a; Macedo et al., 2014b; Verhaagen et al., 2014b). The oscillation amplitude of the EndoActivator tips (Dentsply Sirona, Charlotte, NC, USA) is approximately 1,200 μm (Jiang et al., 2010a) and that of the more recently introduced EDDY (VDW, Munich, Germany) is approximately 350 μm (Neuhaus et al., 2016; Swimberghe et al., 2019b). Therefore, a minimum of 2,550 μm and 900 μm of free space are needed within 1–2 mm from WL, respectively, for their unobstructed oscillation inside a root canal. This is rarely feasible, so very frequent wall contact is inevitable (Jiang et al., 2010a) and a large portion of the cleaning and disinfection produced in the main root canal *in vitro* and *ex vivo* may be due to this direct physical effect rather than irrigant agitation. Clearly, such an effect cannot reach beyond the main root canal and seems to be redundant when preceded by mechanical preparation and syringe irrigation (Hoedke et al., 2021). Tip-to-wall contact also dampens the oscillation and, contrary to commonly held belief (Haapasalo et al., 2012), these plastic tips are also able to cut dentin and create a smear layer (Kanaan et al., 2020).

Regarding the effectiveness of this approach, a number of *ex vivo* studies found no difference between the EndoActivator (oscillating at 160–190 Hz) and syringe irrigation regarding the cleaning and disinfection of the main root canal, uninstrumented fins or isthmuses (Brito et al., 2009; Duque et al., 2017; Klyn et al., 2010; Rödig et al., 2018; Varela et al., 2019). EndoActivator was also less effective than ultrasonic activation when

applied for the same period of time (Al-Jadaa et al., 2009b; Jiang et al., 2010a; Varela et al., 2019). In contrast, the performance of EDDY, which oscillates at higher frequency (~6 kHz) and smaller amplitude, is reportedly better than syringe irrigation and may approach the effectiveness of ultrasonic activation (Conde et al., 2017; Swimberghe et al., 2019b), even though conflicting results have also been published (Linden et al., 2020). Consequently, an increase in the oscillation frequency and the associated decrease in the amplitude seem to improve the performance of agitation systems that rely on oscillating files or tips. This trend raises further doubt over the rationale of sonic agitation.

Other techniques

A variety of other irrigation techniques are currently in use, but they have not gained much traction except in certain countries (Dutner et al., 2012; de Gregorio et al., 2015; Virdee et al., 2020; Willershausen et al., 2015). Negative-pressure irrigation, for instance, is a method to deliver the irrigant in the root canal but not to agitate it. It employs suction through a fine cannula placed near WL to draw the irrigant from the pulp chamber into the root canal (Adorno et al., 2016; Nielsen & Baumgartner, 2007). Negative-pressure systems can be very complex as they often include several components, tubes and connectors. Irrigant penetration is similar or inferior to that achieved by syringe irrigation (Adorno et al., 2016) and the maximum flow rate is limited, thus the irrigant exchange inside the root canal is slower and the mechanical cleaning effect is reduced (Boutsioukis et al., 2007; Brunson et al., 2010; Chen et al., 2014). Currently there is no clear evidence that negative-pressure irrigation is superior to syringe irrigation apart from very specific cases (Konstantinidi et al., 2017). Its main advantage is that less irrigant is extruded through the apical foramen (Boutsioukis et al., 2013a). The difference may not be clinically relevant in routine cases of root canal treatment, but it could become relevant when a NaOCl accident has already occurred, so the risk of another accident involving the same tooth is increased (Psimma & Boutsioukis, 2019).

Laser-activated irrigation (LAI) relies on rapid heating of the irrigant by Er:YAG or Er, Cr:YSGG lasers, which produces *optic cavitation* (de Groot et al., 2009; Matsumoto et al., 2011; Meire et al., 2014). Laboratory studies have shown that, when the laser tip is placed close to the WL, this technique is more effective than ultrasonic activation regarding the removal of biofilm (De Meyer et al., 2017) or hard-tissue debris (De Moor et al., 2010; de Groot et al., 2009). Variants of LAI, such as Photon-Initiated Photoacoustic Streaming (PIPS) and Shock-Wave Enhanced Emission Photoacoustic Streaming (SWEEPS), which employ slightly different device settings and special laser tips placed in the pulp chamber, have been advocated for the cleaning of minimally shaped root canals (DiVito et al., 2012; Yang et al., 2020), but the evidence is still limited and conflicting findings are not unusual. Some studies found that PIPS was inferior to LAI and, in some cases, equally effective to syringe irrigation when NaOCl was used (De Meyer et al., 2017; Deleu et al., 2015; Pedullà et al., 2012), but others could not detect a difference between PIPS and LAI (Verstraeten et al., 2017). Positioning the laser tip in the pulp chamber as opposed to the apical third of the root canal seems to be a limiting factor, at least for the antimicrobial effect of PIPS (De Meyer et al., 2017). Likewise, contradictory evidence has been published on the comparison between SWEEPS and PIPS (Galler et al., 2019; Yang et al., 2020). Additionally, laser activation seems to extrude more irrigant through the apical foramen than techniques relying on the transverse oscillation of files or tips (Yost et al., 2015).

Multisonic activation (GentleWave; Sonendo, Laguna Hills, CA, USA) has been promoted as a stand-alone irrigation method that does not require any root canal preparation in order for the irrigant to reach, clean and disinfect the complete root canal system (Zhang et al., 2019), although in most published studies the root canals have generally been enlarged to apical size 15–25 (Chan et al., 2019; Molina et al., 2015; Ordinola-Zapata et al., 2022; Sigurdsson et al., 2016, 2018; Zhang et al., 2019). The main innovation of this technique is the production of acoustic waves with a broad range of frequencies during the collapse of hydrodynamic cavitation bubbles. These waves are believed to contribute to the cleaning and disinfection of the root canal (Sigurdsson et al., 2016, 2018). Early studies reported very promising findings and concluded that this technique is clearly superior to syringe irrigation and ultrasonic activation (Molina et al., 2015; Zhang et al., 2019), but more recent studies by independent research groups came to the opposite conclusion (Chan et al., 2019; Ordinola-Zapata et al., 2022).

A simple technique to agitate the irrigant by push-pull movements of well-fitting gutta-percha points (*manual dynamic agitation*) has also been proposed (Machtou, 2015) and seems to improve the cleaning of uninstrumented fins and oval extensions compared to syringe irrigation (Deleu et al., 2015; Jiang et al., 2012; Passalidou et al., 2018). However, it also appears to extrude significant amounts of irrigant through the apical foramen (Boutsioukis et al., 2014b).

SUGGESTED IRRIGATION PROTOCOL

NaOCl remains the primary irrigant of choice, and it should be used throughout chemomechanical preparation in order to kill microorganisms, disrupt the biofilm, dissolve the pulp tissue remnants, remove the organic components of the smear layer and lubricate the instruments (Gulabivala et al., 2005; Zehnder, 2006). Given the anatomical complexity of the root canal system and the time constraints of a typical treatment session, it is strongly advisable to employ irrigant flow as the primary means of transport in order to deliver NaOCl at least to the complete main root canal and only rely on diffusion to reach remote areas where the flow is inherently limited. Syringe irrigation with a fine needle placed close to WL seems to be the most cost-effective irrigant delivery method. Copious amounts of NaOCl should be delivered to compensate for the rapid consumption of the free available chlorine in reactions with organic matter. Even though there is a need to remove hard-tissue debris, the alternate use of NaOCl and strong chelators during instrumentation is contraindicated (Grawehr et al., 2003; Wright et al., 2020a; Zehnder et al., 2005a). Instead, the root canal should be rinsed with a chelator such as EDTA after instrumentation in order to remove accumulated hard-tissue debris and the inorganic components of the smear layer, and, in part, also to disrupt the biofilm matrix.

Nevertheless, this step should not be considered as the final rinse. NaOCl must be reintroduced in the root canal system in order to flush out any remaining chelator, to penetrate farther in uninstrumented areas and dentinal tubules that have now been cleared of accumulated dentine debris and smear layer and to act on the remaining biofilm. The current evidence does not support the use of CHX or any other irrigant instead of NaOCl for the final rinse. The main argument against a final rinse with NaOCl after EDTA is that it attacks the exposed dentine collagen and causes erosion on the root canal wall (Haapasalo et al., 2012) (Figure 7). However, the clinical significance of such erosion remains unclear. Until now there is no evidence that it increases the risk of fracture (not to be confused with changes in the elasticity, strength, or microhardness, as already explained) or that it is anything more than a morphological alteration of the dentine surface. If activation is deemed necessary, intermittent ultrasonic irrigant activation seems to be the most reasonable choice, and it should be applied in this step.

This irrigation protocol could be further simplified by replacing NaOCl and EDTA by a mixture of NaOCl and a weak chelator, such as HEDP, that can be used throughout chemomechanical preparation (Wright et al., 2020a; Zehnder et al., 2005a). However, the clinical evidence on such an approach is still limited.

CURRENT PROBLEMS

A well-recognized problem in root canal irrigation is that randomized clinical trials, especially those focusing on the long-term treatment outcome, are scarce. The use of most solutions and techniques is based entirely on the findings of laboratory studies, which are regarded as the lowest level of evidence (Haapasalo, 2016) and fit within the category of "mechanism-based" reasoning (Howick et al., 2010; OCEBM Levels of Evidence Working Group, 2022). The inferential chain linking an irrigant or irrigation method with a clinical outcome is often incomplete. In addition, the employed laboratory models are rarely validated and, in some cases, they may be noticeably oversimplified and unrealistic (Boutsioukis et al., 2022). Thus extrapolation of the findings of laboratory studies to the clinical setting requires great caution.

The lack of clinical trials is inevitably coupled to the studied outcomes. Prevention or healing of apical periodontitis is the primary outcome of interest in clinical endodontology (Azarpazhooh et al., 2022; Ørstavik, 2019), but easier-to-measure surrogate end-points are usually preferred in experimental studies in order to shorten the post-operative observation period or to conduct the experiments in a laboratory. The reduction of the intracanal microbial load is the most relevant surrogate end-point, and there is evidence that it is correlated to the healing of apical periodontitis, at least to some extent (Sjögren et al., 1997). Other commonly used end-points, such as the removal of pulp tissue remnants, hard-tissue debris or the

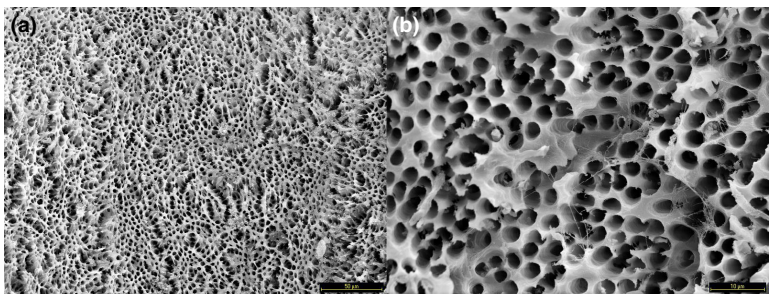

FIGURE 7 SEM photomicrographs of eroded dentine following alternate irrigation with 2.5% NaOCl and 17% disodium EDTA.

smear layer, have not been directly correlated to the primary outcome. Instead, their use is based on a number of hypotheses and assumptions that link them to the reduction of the microbial load. Pulp tissue remnants may serve as nutrients for surviving bacteria (Love, 2012), and they could also interact with the irrigants and limit their action (Haapasalo et al., 2007). Accumulated hard-tissue debris could hinder the access of irrigants to intact biofilm residing in isthmuses and other uninstrumented areas (Gulabivala et al., 2005; Paqué et al., 2009; Siqueira et al., 2018). In instrumented areas, the smear layer may also harbour bacteria or hinder the access of irrigants to them (Gulabivala et al., 2005; Paqué et al., 2009). However, a plausible hypothesis is not enough to validate a surrogate end-point, as recently demonstrated for the apical extrusion of debris, a commonly used end-point in root canal preparation studies (Pappen et al., 2019). The inferential chain must be coherent and it should be based on evidence rather than hypotheses (Howick et al., 2010). Conflicting findings when comparing irrigation methods using different outcome measures are not uncommon (Căpută et al., 2019), and they have raised doubt over the value of the removal of pulp tissue remnants or hard-tissue debris as predictors of the antimicrobial effect of an irrigation method. In addition, SEM studies on the removal of the smear layer have been repeatedly criticized because of their fundamental methodological limitations (De-Deus et al., 2011; Gulabivala et al., 2005; Zehnder, 2012), so their conclusions are not considered reliable (Boutsioukis et al., 2022).

The pooled findings of two recent systematic reviews on different irrigation methods (Căpută et al., 2019; Konstantinidi et al., 2017) can help to estimate the relative use of each outcome/end-point in the literature on this topic. SEM studies on smear layer removal – which had been excluded from the reviews – were added for the purposes of this estimation, leading to a total of 107 studies (both clinical and laboratory). Only 1% of these studies evaluated the healing of apical periodontitis, 22% focused on the antimicrobial effect, 36% investigated the removal of pulp tissue remnants or hard-tissue debris and a remarkable 41% examined the removal of the smear layer under SEM. Thus there seems to be an overreliance on unvalidated or unreliable surrogate end-points that may have led to erroneous conclusions about the effectiveness of certain irrigants or irrigation methods.

Evidently, the most important requirements for effective irrigation are that the irrigant must reach the biofilm and act against it physically and chemically. Therefore, it is imperative that future laboratory studies focus primarily on irrigant penetration and its anti-biofilm effect, particularly in teeth with multiple root canals and complex anatomy. The development of new irrigants and their optimum clinical use will benefit greatly from advances in the bacteria sampling and detection methods and a deeper understanding of biofilm physiology and biofilm–host interactions. The most promising irrigants and irrigation methods should be further tested in clinical trials focusing on the healing of apical periodontitis.

Another common problem stems from the sample size. *A priori* calculation of the necessary sample size is currently a universal requirement for both clinical and laboratory studies (Nagendrababu et al., 2020, 2021), but it is inevitably based on partially subjective decisions about the minimum clinically relevant difference between the compared groups that should be detected with enough power. A recent randomized controlled clinical trial (Verma et al., 2019) compared two irrigants (1% and 5% NaOCl) in terms of the healing of apical periodontitis following root canal treatment and found no significant difference. Nonetheless, a close inspection of the results reveals that there was actually a difference of 9.3% in the success rate in favour of the high-concentration group, but the chi-squared test produced a *P*-value of 0.31, despite the *a priori* sample size estimation. A power analysis shows that the study only had enough power (≥0.80) to detect a difference of at least 21% in the success rate between the two groups, even though a 10% difference would have been considered by many a very clinically relevant difference that was worth detecting. Underpowered studies are unlikely to detect true differences of a clinically relevant magnitude between irrigants or irrigation methods, and even when they succeed, they tend to produce imprecise estimates of the effect. For a binary outcome variable such as success/failure, the minimum required sample size in order to detect a 20% difference between two independent groups is 49 patients per group (χ^2 test, two-tailed, α=0.05, 1-β=0.8, baseline success rate=75%). The number increases to 250 or 1094 patients if a 10% or 5% difference are of interest. Therefore, large clinical trials are needed in order to confirm or refute such hypotheses. Such numbers of patients may be difficult to recruit within a single institution, which underscores the value of collaboration and multicentre trials.

The choice of irrigants or irrigation methods to be compared in a new study, be it laboratory or clinical, is of great importance. Assuming that there are just 15 different main irrigants or irrigation methods and that four of them are compared in each study, it is possible to conduct 1365 original studies per surrogate end-point without ever repeating the comparison of the same four irrigants or methods. The futility of such an approach is obvious. Still, the fact that some particular irrigants or methods have not been compared in the same study yet is a commonly used argument to justify further research. Luckily, not all such comparisons are interesting or relevant. As it has been

demonstrated in other areas of endodontology (Herbst et al., 2019), when there are too many potential combinations, certain comparators serve as clinical standards or benchmarks and are included more often in studies, thereby providing a common point of reference. In root canal irrigation, there are well-established clinical standards. NaOCl, EDTA, syringe irrigation and ultrasonic irrigant activation are the most widely used irrigants and irrigation methods (Dutner et al., 2012; Eleazer et al., 2016; de Gregorio et al., 2015; Savani et al., 2014; Willershausen et al., 2015). Therefore, depending on the particular focus of each study and in order to assist the interpretation of its findings, it is essential to include one or more of these clinical standards as additional controls. A comparison between two irrigants that are rarely used or between these irrigants and no irrigation at all provides very little useful information. It should be also kept in mind that there are no unanimously accepted protocols for most irrigants and irrigation methods, including the clinical standards. The wide variation in the protocols is a potential source of bias. For instance, the performance of a new irrigant or method may be overestimated when compared to a clinical standard that is applied according to a suboptimal protocol (Konstantinidi et al., 2017). Thus it is imperative that optimized protocols are followed when using these clinical standards as comparators. Finally, irrigants and irrigation methods must be presented with a sufficient challenge, for example a mature multi-species *ex vivo* biofilm located in difficult-to-reach areas or a long-standing *in vivo* root canal infection with obvious clinical signs, to demonstrate their full potential. An easy task accomplished equally well by all compared irrigants or methods is not a meaningful challenge for such a comparison and could lead to the incorrect conclusion of equivalence.

FUTURE DIRECTIONS

Irrigants

In spite of its well-known limitations, NaOCl has proven to be a very resilient primary irrigant. Several solutions or mixtures have been introduced to endodontology as "revolutionary" and potential "substitutes" of NaOCl, usually accompanied by very promising early research findings. As more evidence came to light concerning their effectiveness and limitations, their role was downgraded from substitution to supplementation of NaOCl. Further scrutiny of the new irrigants, particularly by research groups unrelated to their introduction, cast doubt even about their use as supplements. It is likely that NaOCl will not be replaced in the foreseeable future due to its outstanding properties, and therefore it will be necessary to fine-tune its use and examine its potential adverse effects on dentine and on the periapical tissues in greater depth. The action of NaOCl will probably continue to be complemented by a chelator applied either as a single rinse at the end of instrumentation (strong chelator) or perhaps in a mixture with NaOCl used throughout instrumentation (weak chelator). Continuous chelation using a variety of solutions that do not interfere with NaOCl in the short term is a topic of interest, so new options may appear in the coming years.

The final irrigation regime is another area that may see changes. NaOCl is currently the most reasonable choice of an antimicrobial solution to be applied after the chelator. However, in the future it could be supplemented or even replaced by new irrigants. The current strategies against root canal biofilms are focused on bacterial killing and biofilm elimination but, taking into account the complexity of endodontic infections, they may be overly simplistic. A multifaceted strategy aiming to degrade or disrupt the protective EPS matrix, kill persister and dormant cells, disrupt cell-to-cell communication and/or modify the dentine surface could prove far more effective (Koo et al., 2017). For instance, antimicrobial peptides could be deployed as part of such a strategy. These biomolecules are naturally produced by the immune cells of a variety of organisms and seem to be effective against a broad spectrum of oral bacteria even at low concentration and without triggering resistance (Mai et al., 2017). The potential use of synthetic antimicrobial peptides in root canal treatment is already being investigated, and early results have shown that some of them are able to suppress the expression of virulence and stress-associated bacterial genes and to inhibit biofilm growth even in the presence of saliva (Li et al., 2020; Wang et al., 2015). Peptides could also be combined with EPS-synthesis inhibitors or EPS-degrading enzymes in order to enhance both their access to the biofilm and their antimicrobial effect (Liu et al., 2016). Moreover, dentine surface modification by a coating containing a biocide, such as benzalkonium chloride, could prevent the recolonization by bacteria after root canal treatment (Busscher et al., 2012; Jaramillo et al., 2012), provided that the effect can be sustained for a prolonged period of time. However, most of these innovative anti-biofilm approaches are still in the basic-research stage and any clinical application will probably require several more years of development.

Irrigation methods

Conventional irrigant delivery by a syringe and needle will probably continue to be widely used in the near future along with affordable activation systems like ultrasonics.

So far, several combinations of an irrigant delivery and an activation/agitation method have been proposed as ways to simplify irrigation protocols and to enhance the cleaning and disinfection of the root canal system (Gutarts et al., 2005; Malentacca et al., 2018; Rödig et al., 2010b; Sigurdsson et al., 2016). This trend may eventually lead to combinations of activation methods. Ultrasonic and laser activation are potential candidates for such an approach since they have already been successfully combined in other fields to control the occurrence and location of cavitation more accurately (Feng et al., 2015).

Another direction that could revolutionize root canal irrigation in the future is the development of customized irrigation protocols. At the moment, it is not uncommon for clinicians to employ slightly different protocols depending on the diagnosis of each case. Future advances in the bacteria sampling and detection methods and a deeper understanding of biofilm physiology and its interaction with the host could allow for optimization of the protocols and even their customization based on the microbiome in each particular case. The idea of varying the irrigation protocol according to the anatomy of the root canal system has also been proposed (Gulabivala et al., 2019; Gulabivala & Ng, 2014). In the short term, systematic research on the effectiveness of irrigation in different types of root canal systems *ex vivo* could provide some guidelines on the most suitable protocol for each type. Evidently, an important obstacle would be the correct identification of the type of root canal system in each clinical case. Initially, this could be based on observation under the dental operating microscope coupled with the knowledge from studies on root canal anatomy. Later on, a high-resolution three-dimensional scan of the root canal system using either a low-radiation CBCT scanner or a method relying on non-ionizing radiation, such as magnetic resonance imaging, could become the standard of care for every case. This detailed scan could be fed into a computer algorithm along with microbiological data and could lead to a customized patient-specific irrigation protocol. Pre-operative computer-based simulations of the outcome of different treatment strategies in individual patients are already being tested as an aid to treatment planning in vascular surgery (Chiastra et al., 2016; Chung & Cebral, 2015).

CONCLUDING REMARKS

Root canal irrigation is a common theme in the endodontic literature, but progress in a field is not a mere function of the number of published studies. Certain topics, such as debris and smear layer removal, have been investigated very extensively, whereas others, such as the penetration of the irrigants in the root canal system and their effect on the biofilm or on the long-term treatment outcome, have gained much less attention. Hence there is a clear need to redefine the research priorities in this field. New studies must also focus on clinically relevant comparisons, avoid methodological flaws and have sufficiently large sample sizes to reach valid conclusions. A systematic search of the literature on almost any topic on root canal irrigation will provide numerous examples of studies that did not adhere to these basic principles but were nevertheless published. This problem is neither new nor specific to root canal irrigation (Altman, 1994). Therefore, instead of striving to produce more studies, the attention should be put on producing better studies.

Based on the current body of knowledge, NaOCl and EDTA delivered by a syringe and needle and possibly activated by an ultrasonic file remain the cornerstone of root canal irrigation protocols. Future multidisciplinary efforts combining the knowledge from basic sciences such as Chemistry, Microbiology and Fluid Dynamics could eventually lead to more effective antimicrobials and improved activation methods to bring them closer to the residual biofilm in the root canal system.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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