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**INFLUENCE OF SOME CHEMICAL CHARACTERISTICS ON THE USE OF
CHLORINE-CONTAINING SOLUTIONS AS ROOT CANAL IRRIGANTS**

Thesis submitted as part of the requirements for the PhD in Dentistry – Area of Endodontology

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ABSTRACT

Aim: the goal of the present investigation was to look into chemical interactions and characteristics, other than chlorine concentration, and their effect on the overall performance of chlorine-containing root canal irrigants.

Methodology: Three different searches were performed in the Medline electronic database, with the purpose of identifying publications that: (a) reviewed the influence of pH changes on the efficacy of chlorine-containing endodontic irrigating solutions; (b) studied unwanted chemical interactions between sodium hypochlorite (NaOCl), chlorhexidine (CHX), EDTA and Citric acid; (c) compared NaOCl alone and NaOCl modified with the addition of a surface-active agent in endodontics. A hand search of articles published online, and appearing in the reference list of the articles included (for search c only), was further performed, using the same search criteria as the electronic search.

Four investigations were carried out: (a) to evaluate the antimicrobial effect of Sterilox and sodium hypochlorite against *Enterococcus faecalis* in a bovine root canal model; (b) to evaluate the bovine pulp tissue dissolution ability of Sterilox, HealOzone, and 0.5% sodium hypochlorite, used alone or in combination; (c) to understand the effect of storage conditions on Sterilox's stability. Eight bottles (four completely full, four half-full) of freshly prepared solution were divided into four groups and subsequently stored by being either exposed to or protected from sunlight; (d) to evaluate whether the immersion of CHX impregnated gutta-percha points in chlorine-containing endodontic irrigants causes colour changes and precipitate formation, eighty-one CHX medicated points were immersed in microtubes, containing the following solutions: 0.5 and 5.25% NaOCl or Aquatine (Sterilox). The samples were visually assessed by two independent observers at regular intervals over three weeks to detect colour changes and precipitate formation.

Results: The searches identified: for topic a: 1304 publications, 20 fulfilled the inclusion/exclusion criteria; for topic b: 1285 publications, 19 fulfilled the inclusion/exclusion criteria; for topic c: 302 publications 11 fulfilled the inclusion/exclusion criteria.

For investigation a: statistically significant differences between the groups exposed to sunlight and non-exposed groups ($p < 0.001$) were found, whilst the presence of air did not

affect the chlorine decomposition in the bottles. For investigation b: the groups containing 5.25% NaOCl started to produce a visible precipitate after fourteen days (16.48 ± 0.98) for the impregnated points; no other test group presented with changes.

Conclusions:

Regarding investigation (a), on antimicrobial efficacy, NaOCl appears to be the best preparation amongst chlorine-containing irrigants; this can be enhanced by reducing its pH. Interactions with chelating agents can reduce this action, and there is no clear evidence regarding the effect of surface-tension modification. Investigation (b) on tissue dissolution ability showed that NaOCl is the only chlorine-containing solution with a clinically relevant action. Sodium hypochlorite dissolution ability may be speeded up with the adjunct use of ozone. The literature reviews also pointed out that interaction with chelators reduce this ability, whilst the surface tension of the preparations has no influence. By mixing NaOCl and CHX in liquid or gel forms, a precipitate is formed; this is likely to contain a cancerogenic substance. Investigation (c) showed that Sterilox chlorine concentration is stable for a two-week period if protected from direct sunlight; if exposed to sunlight the degradation process started after 4 days. Investigation (d) allowed stating that the placement of impregnated gutta-percha points in 5.25% NaOCl for a two-week period caused the formation of a precipitate in the experimental conditions of this study.

RESUMO

Objetivo: este estudo buscou avaliar as interações e características químicas, além da concentração de cloro, que afetam o desempenho das soluções irrigadoras contendo cloro na desinfecção dos canais radiculares.

Metodologia: Sete estudos foram realizados, sendo três de revisão de literatura e quatro trabalhos experimentais. (1) revisou a influência das modificações de pH na eficácia das soluções irrigadoras contendo cloro. (2) estudou as interações químicas indesejáveis entre hipoclorito de sódio e clorexidina, EDTA e ácido cítrico. (3) comparou o hipoclorito de sódio usado isoladamente e adicionado com agente surfactante. Uma busca manual foi realizada dos artigos disponíveis online, quando necessário, utilizando o mesmo critério da busca eletrônica. (4) avaliou a atividade antimicrobiana do Sterilox e do hipoclorito de sódio em dentes bovinos infectados por *Enterococcus faecalis*. (5) avaliou a capacidade de dissolução de tecido pulpar bovino do Sterilox, do HealOzone e do hipoclorito de sódio a 0,5%, tanto em uso isolado como em combinação. (6) Buscou compreender o efeito da luz solar na estabilidade do Sterilox comparado ao hipoclorito de sódio. (7) avaliou o efeito da imersão, em hipoclorito de sódio a 0,5% e a 5,25%, de cones de guta-percha impregnados com clorexidina na modificação de cor e na formação de precipitado em tubos de Eppendorf transparentes. As metodologias específicas de cada estudo encontram-se nas publicações.

Resultados: Os estudos de revisão mostraram que o pH pode interferir positivamente na ação antimicrobiana e negativamente na capacidade de dissolução tecidual das soluções contendo cloro (1). Além disso, interações com as substâncias contendo cloro podem afetar positiva ou negativamente nas propriedades dessas substâncias (2). O uso de surfactante ainda necessita maiores estudos para avaliar o potencial no favorecimento da ação dessas substâncias no sistema de canais radiculares (3). Em relação aos estudos experimentais, foram encontradas os seguintes achados: o Sterilox tem atividade antimicrobiana, mas estatisticamente inferior ao hipoclorito de sódio (4); o Sterilox não tem capacidade de dissolver tecido pulpar, mas a velocidade de dissolução do hipoclorito de sódio pode ser aumentada com o uso do ozônio (5); a luz solar interfere no tempo de estabilidade do Sterilox (6); precipitados são formados depois da imersão em hipoclorito de sódio por 14 dias, tanto

em cones sem como com clorexidina, embora a clorexidina propiciasse precipitados bastante expressivos (7).

Conclusões:

Do ponto de vista de ação antimicrobiana, o hipoclorito de sódio parece ser a melhor solução irrigadora. Sua ação pode ser aumentada com a diminuição do seu pH. As interações com outras substâncias podem reduzir a ação do hipoclorito de sódio, e o efeito de um surfactante necessita maiores estudos para avaliar seu efeito. O hipoclorito de sódio é a única substância contendo cloro que tem ação relevante na dissolução de tecido pulpar, mas sua atividade pode ser potencializada por ozônio. A mistura de hipoclorito de sódio com clorexidina forma precipitados e a liberação de substância potencialmente cancerogênica. Este precipitado pode ocorrer inclusive em cones de guta-percha impregnados com clorexidina quando em contato com o hipoclorito de sódio. A concentração de cloro no Sterilox permanece estável por 14 dias se estiver protegida da luz solar.

INTRODUCTION

The aim of endodontic treatment is to preserve functional teeth without prejudice to the patient's health; it encompasses all those procedures that aim to maintain the health of the dental pulp and to prevent and treat apical periodontitis (European Society of Endodontology 2006).

When looking into tooth survival (to preserve functional teeth) following non-surgical root canal treatment, a systematic review followed by a meta-analysis found a success rate in the range of 83 to 93% (Ng *et al.* 2010); similarly an investigation looking into the insurance records of almost one and a half million teeth endodontically treated found that 97% of teeth were retained, with no further treatment after eight years (Salehrabi & Rostein 2004). When looking into the outcome of non-surgical root canal retreatment *per se* (treat apical periodontitis) a systematic review followed by meta-analysis found a complete healing rate of 76.7% in studies that assessed treatment success with traditional criteria (Ng *et al.* 2008). Given that outcome studies included in this study used periapical radiography, which has been shown to be less accurate in detecting apical periodontitis than cone beam computed tomography (Estrela *et al.* 2008) or biopsy, the real success rate of root canal retreatment should be re-evaluated (Wu *et al.* 2009). In consequence, the search for improvement in the different treatment procedures carried out in endodontics, including root canal irrigation, should be justified.

The ideal irrigant solution should have disinfectant and debris dissolving properties, whilst not irritating the periradicular tissues (European Society of Endodontology 2006). Sodium hypochlorite, a chlorine-containing solution, has been recommended as the main root canal irrigant since it covers more of the requirements than any other agent; it has a broad antimicrobial spectrum, as well as the capacity to dissolve tissue remnants and the organic component of the smear layer (Zehnder 2006). Given that no single irrigant is capable of removing both the inorganic and organic components of the smear layer, combinations have been suggested (Baumgartner *et al.* 1984), with the consequent risk of interaction. Furthermore, seminal literature has suggested that sodium hypochlorite has a chlorine concentration-dependent cytotoxic effect (Pashley *et al.* 1985).

Myriad investigations have assessed alternative root canal irrigants such as iodine compounds, chlorhexidine and ozone, amongst others. Conversely, the endodontic literature that reports the chemistry of sodium hypochlorite is sparse, with the exception of studies that examine the effects of chlorine concentration in terms of its antimicrobial and tissue dissolution abilities.

The goal of the present investigation is to look into chemical interactions and characteristics other than chlorine concentration, and their effect on the overall performance of chlorine-containing root canal irrigants. It further seeks to assess whether the clinical capabilities of sodium hypochlorite may be enhanced, and whether alternative chlorine-containing solutions may replace sodium hypochlorite as the main irrigant solution.

Study 1 – Published in International Endodontic Journal

Review

Influence of pH changes on chlorine-containing endodontic irrigating solutions

International Endodontic Journal, 44, 792–799, 2011.

Review

Influence of pH changes on chlorine-containing endodontic irrigating solutions

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Abstract

Rossi-Fedele G, Guastalli AR, Doğramacı EJ, Steier L, De Figueiredo JAP. Influence of pH changes on chlorine-containing endodontic irrigating solutions. *International Endodontic Journal*, **44**, 792–799, 2011.

Chlorine-containing solutions are used for broad disinfection purposes. Water disinfection literature suggests that their disinfectant action depends on pH values as this will influence the available free chlorine forms. Hypochlorous acid (HOCl) has been suggested to have an antimicrobial effect around 80–100 times stronger than the hypochlorite ion. The aim of this paper was to review the influence of pH changes on the efficacy of chlorine-containing endodontic irrigating solutions. An electronic and hand search (articles published through to 2010, including 'in press' articles; English language; search terms 'root canal irrigants AND sodium hypochlorite or hypochlorous acid or superoxidized water or electrochemically activated solution'; 'antimicrobial action AND sodium hypochlorite or hypochlorous acid or superoxidized water or electrochemically activated solution'; 'tissue dissolution AND sodium hypochlorite or hypochlorous acid or superoxidized water or electrochemically activated solution'; 'smear layer AND

sodium hypochlorite or hypochlorous acid or superoxidized water or electrochemically activated solution') was performed to identify publications that compared chlorine water solutions with different pH. Of 1304 publications identified, 20 were considered for inclusion in the review. The search resulted in the retrieval of articles studying sodium hypochlorite (NaOCl), superoxidized waters (SOW) and sodium dichloroisocyanurate (NaDCC). Regarding antimicrobial efficacy, the literature suggested that reducing the pH value of NaOCl to between 6 and 7.5 would lead to improved action; SOW was described as having a lower antimicrobial effect. The tissue dissolution activity NaOCl decreased when the pH reached values between 6 and 7.5; NaDCC and SOW had no clinically relevant tissue dissolution capability. Chlorine solutions of different characteristics appeared to have some cleaning efficacy although they should to be used in conjunction with chelating and/or detergent agents.

Keywords: electrochemically activated solution, hypochlorous acid, pH, sodium hypochlorite, superoxidized water, root canal irrigants.

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Introduction

Chlorine solutions are widely used for disinfection purposes including potable and sewage water, swim-

ming pools, flowers, environmental surfaces, medical equipment and laundry (Rutala & Weber 1997). In dentistry, they are also currently suggested for the disinfection of dental water lines (Martin & Gallagher 2005) and impression materials (Martin *et al.* 2007) and, in the form of sodium hypochlorite (NaOCl), are widely suggested as the main root canal irrigant because of its broad antimicrobial activity, its function

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to prevent formation and to dissolve the smear layer and its ability to dissolve tissue remnants (Zehnder 2006).

Chlorine has a strong tendency to acquire electrons in order to achieve greater stability, and this translates into chlorine's oxidizing activity (Fair *et al.* 1948); its oxidizing capacity is retained by hypochlorous acid (HOCl), its hydrolysis product, which, according to classic water treatment literature (Butterfield *et al.* 1943, Fair *et al.* 1948, Brazis *et al.* 1958), is responsible for the disinfectant action of chlorine solutions. The relative amount of hypochlorite ion and HOCl present in chlorine solutions at a given pH and temperature is constant (Fair *et al.* 1948), as HOCl in water undergoes an instantaneous and reversible ionization into hypochlorite (OCl^-) and hydrogen (H^+) ions, having an ionization constant that depends only on temperature (Fair *et al.* 1948). Subsequently, pH changes will reflect the relative amounts of hypochlorite ion and HOCl present in the solution (Fair *et al.* 1948); if HOCl is consumed, then the balance will shift and new HOCl will form at the expense of OCl^- . Therefore, the OCl^- in the aqueous solution can work as reservoir for the formation of new HOCl and vice versa. By lowering the pH to values below 4 and 5, the relative amount of HOCl diminishes and chlorine gas (Cl_2) dissolved in water increases at the same rate (Fair *et al.* 1948). Chlorine in gas form is unstable because of its volatility (Lee *et al.* 2000); chlorine gas has been suggested to have a noxious odour and to be irritant to the respiratory tract, eyes and mucous membrane and, at higher concentrations, can have fatal effects (Baumgartner & Ibay 1978).

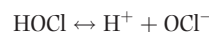
It would be reasonable to have different antimicrobial actions at different pH values, with a decrease in disinfection efficacy with a pH increase starting from neutral to alkaline values (Weber & Levine 1944, Fair *et al.* 1948, Brazis *et al.* 1958, Bloomfield & Miles 1979, Death & Coates 1979). The relative antimicrobial actions of HOCl and OCl^- against waterborne pathogens have been estimated to be around 80–100 : 1 in laboratory conditions (Fair *et al.* 1948, Brazis *et al.* 1958). A pH value around 6 has been proposed as ideal as the HOCl concentration is optimal for disinfection (Rutala & Weber 1997); at pH 7, 78% of the chlorine in a solution is available as HOCl, whilst at pH 8, this drops to 26% (Claesen & Edmonson 2006).

It has been suggested that the reaction of chlorine solutions with inorganic matter is rapid and stoichiometric, whilst that against organic matter is normally slower and depends on excess concentration (Fair *et al.*

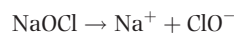
1948). Organic matter reacts strongly with NaOCl, and the reaction depends on the relative amount of NaOCl and organic matter, with initially a relatively fast reaction followed by a slower second phase; the presence of organic matter in excess depletes the solution (Moorer & Wesselink 1982). The reactivity of chlorine is limited to particular organic sites (Deborde & von Gunten 2008) and appears to depend on pH: in an alkaline environment, many biological polymers are susceptible to hydrolysis (Baumgartner & Ibay 1978) and protein removal from apatite surfaces has been suggested to be more efficient (Haikel *et al.* 1994), whilst the hypochlorite ion is more reactive against amines at a pH around 8.5 (Hawkins *et al.* 2003). On the other hand, high HOCl concentrations can induce protein fragmentation (Hawkins *et al.* 2003).

Hypochlorous acid-releasing agents described for root canal treatment include NaOCl (Zehnder 2006), sodium dichloroisocyanurate (NaDCC) (Naenni *et al.* 2004) and 'electrochemically activated solutions/waters' (ECA solutions), denominated also 'superoxidized waters' (SOW), 'oxidative potential waters' (OPW) or 'functional waters' (Marais 2000, Solovyeva & Dummer 2000, Hata *et al.* 2001, Serper *et al.* 2001, Gulabivala *et al.* 2004, Garcia *et al.* 2010).

Hypochlorous acid dissociates in water as follows:

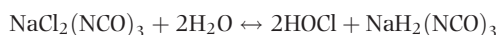


Commercially available NaOCl products are 1–15% aqueous solutions with an alkaline pH (circa 11) and often contain 0.01–0.75% sodium hydroxide salts and other basic salts or buffers to increase its stability (Rutala & Weber 1997). This group includes the so-called Dakin's solution, which has pH 10 and a 0.5% concentration and is obtained by the addition of carbonate (Moorer & Wesselink 1982). Sodium hypochlorite hydrolyses in water as follows:



Sodium dichloroisocyanurate is commonly used for the disinfection of baby-feeding equipment and treatment of drinking water (Claesen & Edmonson 2006). It is an organic compound available in tablet or powder form that releases chlorine when in solution at pH 5.9 (Dychdala 1991); its acidic pH is attributable to the effervescent base of the tablets (Claesen & Edmonson 2006). A NaDCC solution has only 50% of its chlorine available in solution, with the remnant part bound in the organic compound in equilibrium, which serves as the reservoir for free chlorine. NaDCC has been previously shown to have a significantly higher

antimicrobial activity compared with NaOCl, and this is suggested to be dependent not entirely on their pH but also on the differences between their properties and mode of action (Bloomfield & Miles 1979). Sodium dichloroisocyanurate dissolves in water as follows:



Electrochemically activated solutions are a group of disinfectants regularly used in endoscope disinfection containing a mixture of oxidizing substances that have different oxidation–reduction potentials (ORP), chlorine concentrations and/or pH (Fraise 1999). These factors depend on the concentration of the saline precursor, the generation rate and applied potential, as ECA solutions are obtained via electrolysis, a process similar to the commercial production of NaOCl. Production of anolyte and catholyte solutions at the different electrolytic chambers occurs during this procedure (Gulabivala *et al.* 2004). It is necessary to highlight that the chlorine concentrations of ECA solutions are normally smaller than those of NaOCl. For example, a 0.5% NaOCl solution will have a solvent concentration approximately 18 times larger than a 200-ppm ‘high-concentration’ SOW (Rossi-Fedele *et al.* 2010b). Disinfectants vary in their susceptibility to organic matter (Fraise 1999), and the presence of large amounts of organic matter influences the concentration of NaOCl required for disinfection (Bloomfield & Miles 1979). In root canal irrigation, this might be relevant particularly for solutions with low concentrations as there might be the risk that there is insufficient ‘available chlorine’ in order to obtain the desired actions. Dentine by weight consists of seventy per cent of inorganic matter, twenty per cent of organic matter and the remnant is water (Berkovitz *et al.* 2002). Therefore, some of the available chlorine potentially can be consumed by contact with tooth tissues. The use of a solution with an excessive chlorine concentration should be considered more dangerous because of the increased toxicity of higher NaOCl concentrations (Pashley *et al.* 1985) as well as its dentine-weakening action (Grigoratos *et al.* 2001, Sim *et al.* 2001), amongst other reasons. The use of a solution combining a reasonably high antimicrobial effect and low toxicity has been suggested in the absence of an ideal irrigating solution (Spanberg *et al.* 1973). Several studies, mainly *ex vivo* investigations, have been conducted in order to study the use of chlorine solutions as root canal irrigants for its antimicrobial, tissue dissolution, smear layer and debris removal actions. The goal of this paper is to review studies regarding the use of chlorine irrigants that

compare solutions with different pH in order to achieve those desired effects. A literature search in the electronic database MEDLINE was conducted using the following search terms and combinations: ‘root canal irrigants AND sodium hypochlorite or hypochlorous acid or superoxidized water or electrochemically activated solution’; ‘antimicrobial action AND sodium hypochlorite or hypochlorous acid or superoxidized water or electrochemically activated solution’; ‘tissue dissolution AND sodium hypochlorite or hypochlorous acid or superoxidized water or electrochemically activated solution’; and ‘smear layer AND sodium hypochlorite or hypochlorous acid or superoxidized water or electrochemically activated solution’. Furthermore, in order to include the most recent publications, a hand search of articles published online, ‘in-press’ and ‘early view’ in the International Endodontic Journal, Journal of Endodontics, Australian Endodontic Journal and Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology was performed on 31 December 2010 using the same search criteria as the electronic search. Publications were included if they compared chlorine water solutions with different pH and were published in English. Titles and abstracts of the publications identified by electronic database using the search terms mentioned above and those identified from the hand search were screened initially by two independent reviewers (GRF and EJD). Publications were included for full-text evaluation by one reviewer (GRF) if the content of the abstracts met the inclusion criteria. Full-text assessment and data extraction were performed by one reviewer (GRF). Publications were excluded if they did not meet the inclusion criteria (i.e. if they did not study antimicrobial action, tissue dissolution, smear layer removal and root canal irrigation) and also if they did not compare different pH chlorine-containing solutions. Of 1304 publications identified, 20 were included in the review.

Review

Antimicrobial effect

Different acidic and neutral chlorine-containing solutions have been suggested for root canal disinfection. Two kinds of ‘functional waters’ have been tested: strong acid–electrolysed water ‘SAEW’ [pH 2.8, residual chlorine concentrations of 10 ppm (Aoi Engineering Inc, Mishima, Japan)] and hypochlorous water ‘HAW’ [pH 6, residual chlorine concentration of 50 ppm (Tecnomax Corporation, Yoshikawa, Japan)]

with a 3% NaOCl as the control. *Enterococcus faecalis* and *Candida albicans* were used as test organisms in a culture medium, and the effect was measured following changes in colony-forming units (CFUs); different irrigating volumes and the presence or absence of organic substance in the medium were tested. The results showed good microbicidal activity of NaOCl against both microorganisms; HAW's activity was slightly inferior to NaOCl's, whilst SAEW was overall weaker than HAW. The presence of organic matter did not significantly change the antimicrobial capacity of any of the solutions (Gomi *et al.* 2010). Further ECA waters containing chlorine of different pH (pH 3 and 6.5 – chlorine concentrations, ORP and source not described in the article) and 3% NaOCl have been tested in an *ex vivo*-infected root canal model. Following irrigation and serial dilution, CFUs were analysed as ratio against the negative controls. Although NaOCl gave by far the highest bacterial kills, the pH 3 and 6.5 'anolyte solutions' were shown to have antibacterial action against *E. faecalis*, with ultrasonic activation of the solutions enhancing their antibacterial effect (Gulabivala *et al.* 2004). A neutral SOW called Dermacyn (Oculus Innovative Sciences, Petaluma, CA, USA; pH value, chlorine concentration and ORP not described in the manuscript) has been tested on *E. faecalis* following inoculation in agar Petri dishes. The disinfectants were delivered by saturating paper discs and placing them in direct contact with the growth medium. Amongst other medicaments tested, 5.25% NaOCl had larger zones of inhibition than Dermacyn, which showed no microbial inhibition (Davis *et al.* 2007). Another neutral SOW [pH between 5.0 and 6.5 chlorine concentration of 144 mg L⁻¹ (Aquatine Sterilox, Ilkley, UK)] has been tested by comparing it against a 4% NaOCl in a bovine root canal model inoculated with *E. faecalis*. By looking into CFUs following serial dilutions, the SOW was shown to have antimicrobial action; however, only NaOCl was capable of consistently eradicating the infection in the assays (Rossi-Fedele *et al.* 2010a). Other SOWs with different pH (7 and 9), chlorine concentration not described, [STEDS, Radical Waters (Pty) Ltd, Vorna Valley, South Africa], were tested against a 3.5% NaOCl in an *ex vivo* human tooth model inoculated with *E. faecalis*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* and *Porphyromonas gingivalis*. When measuring for CFUs and spectrophotometric values immediately after irrigation and 7 days later, both SOWs were not as effective as NaOCl in eliminating bacteria. NaOCl showed no CFUs, whilst SOWs showed some reduction

only in bacterial number (Marais & Williams 2001). The antimicrobial efficacy of NaOCl 4.2% solutions at pH 12, 7.5 and 6.5 (by adding acetic acid) was tested in infected *ex vivo* root canals by assessing bacterial growth (presence or absence) following irrigation. A significant increase in the disinfecting capacity of the pH 6.5 solution against the pH 12 solution group was shown; however, the intermediate value (pH 7.5) showed no difference with the other group. (Mercade *et al.* 2009). Similarly, different NaOCl solutions [2.5% pH 12 'unbuffered', 0.5% pH 12 'unbuffered', 0.5% pH 9 'buffered' by adding sodium bicarbonate (Dakin's solution) and 0.5% pH 12 'buffered' by adding sodium carbonate] were tested on *E. faecalis*. Studies using filter papers assessed CFU reduction (with different disinfectant concentration and incubation times) and dentinal blocks looking into degree of growth (with different concentrations), which showed no differences between the solutions regarding antibacterial effect (Zehnder *et al.* 2002). Furthermore, Dakin's solution (0.5% pH 10), NaOCl 'unmodified' 2.5% pH 12.5 and 'neutralized' 2.5% pH 7.5 [by the addition of hydrochloric acid (HCl)] were tested in human teeth following infection with *E. faecalis*, with the irrigants left *in situ* for 5 or 20 min. By the analysis of the number of sterile roots per group, it was concluded that the 'neutralized' solution was the most efficient for elimination of intracanal bacteria and that Dakin's solution was less effective than the 2.5% unmodified solution (Camps *et al.* 2009). In terms of the antimicrobial ability of chlorine-containing solutions, there is no alternative to NaOCl with similar proven efficacy. Attempts to enhance the pH and/or concentration of SOW may be considered, provided that they have low toxicity and limited dentine-weakening properties.

Tissue dissolution

Only two studies that included chlorine-containing solutions different than NaOCl were found on the subject of tissue dissolution ability, with both irrigants having a neutral pH value. The first compared 1% (wt/vol) NaOCl against 5% NaDCC (pH and source not described) and found that after 120 min, NaDCC caused a loss of 13% of the original weight of necrotic porcine palatal tissue against 97% when NaOCl solution was used, with significant differences shown consistently over increasing times of incubation in the solutions until 120 min (Naenni *et al.* 2004). The second investigation compared Aquatine [200 ppm pH

5.0–6.5 (Sterilox, Optident, Ilkley, UK)] and 0.5% NaOCl using bovine pulp fragments placed in Eppendorf tubes containing the test solution, with half of the samples 'activated' using an ozone delivery system. The fragments were assessed for total dissolution, and it was found that the pulp tissue was completely dissolved only by NaOCl, whilst application of ozone enhanced the speed of dissolution (Rossi-Fedele et al. 2010b). An investigation analysed alkaline NaOCl solutions [2.5% pH 12 'unbuffered', 0.5% pH 12 'unbuffered', 0.5% pH 9 'buffered by adding sodium bicarbonate' (Dakin's solution) and 0.5% pH 12 'buffered' by adding sodium carbonate] for tissue-dissolving capacity on decayed and fresh porcine palate tissue and assessed the percentage of remaining weight. When comparing solutions of similar concentration (0.5%), Dakin's solution was equally effective on both types of tissues, whilst the other dissolved solutions decayed tissue more rapidly. No differences were found on fresh tissues, but significant differences in necrotic tissue were found between Dakin's solution and the unbuffered solution at 60, 90 and 120 min. Overall, it was concluded that pH changes and buffering NaOCl solutions had modest effects on their tissue-dissolving capacity, as no other significant difference between these three 0.5% NaOCl solutions was found (Zehnder et al. 2002). Sodium hypochlorite at various concentrations has been tested for tissue dissolution capacity following the addition of acids to the original solution in order to lower their pH values. By the addition of boric acid (0.5% at pH 11.6 and pH 9; 0.36% at pH 11.6 and pH 7), the speed for complete dissolution of the pulp fragments was reduced to half for the 0.5% and to a third for the 0.36% groups with lower pH (Spanó et al. 2001). By adding hydrochloric acid, four different investigations have been carried out; first, a study compared percentage of weight loss in porcine muscle three solutions at different pH values (12, 9 and 6) and concentrations (5.25% and 2.6%); no significant differences were found between the pH 12 and 9 groups; however, there was significant difference between the pH 6 group and the other groups with the higher value showing greater weight loss (Christensen et al. 2008). Also, 'neutralized' (pH 7.5) and pH 12 2.5% solutions were tested on porcine palatal mucosa by measuring weight variation every 10 min until 120 min whilst soaking in NaOCl. It was found that the dissolution action of high-pH solution was around five times higher than that of the neutralized one (Aubut et al. 2010). Subsequently, NaOCl 'unmodified' (2.5% pH 12.5) and 'neutralized' (2.5% pH 7.5)

and Dakin's solutions (0.5% pH 10) were tested on bovine dental pulp, with the changes in weight recorded after the immersion of the specimens for 5, 10, 15 or 20 min in a rotating agitator. Overall, the 'unmodified' NaOCl was more effective than the 'neutralized' solution, which was more effective than Dakin's solution; however, no statistically significant difference was present within the 5-min group and, considering that regular replenishment of the irrigants is suggested in routine practice, the authors suggested the differences were not clinically relevant (Camps et al. 2009). Finally, the tissue-dissolving capability of NaOCl at alkaline pH values (12 and 10) was tested on necrotic rabbit liver at different concentrations (3.0%, 1.2% and 0.6%). When measuring percentage of tissue dissolved, it was concluded that concentration of available chlorine rather than pH is the factor that influences the results in the experimental conditions (Moorer & Wesselink 1982). Superoxidized water and NaDCC have no proven tissue dissolution ability. This is a key factor for the option of NaOCl as the main current irrigating solution. Experiments using associations with tissue-dissolving agents may provide light into the use of other chlorine-containing substances in place of NaOCl.

Cleaning effectiveness

Electrochemically activated solutions of different characteristics, including acidic and neutral compounds, have been tested for their smear layer and debris removal ability in *ex vivo* human teeth. Various neutral solutions have been tested for this purpose: Aquatine endodontic cleanser [pH 6, 180–200 ppm available free chlorine (Sterilox Puricore, Malvern, PA, USA)] was compared with a 6% NaOCl solution. When used in association with 17% EDTA, they were similarly effective at removing debris and the smear layer from the entire root canal when assessed using scanning electron microscopy (SEM) and semi-quantitative visual criteria. If no chelating agents were used, then a large amount of smear layer was present for both irrigants (Garcia et al. 2010). A different investigation compared a solution denominated 'Anolyte neutral cathodic (ANC)' [pH 7.7 ± 0.5, active chlorine 300 mg L⁻¹ (STEL-10H-120-01)] alone and together with a catholyte solution (not containing chlorine) against 3% NaOCl used to irrigate during root canal preparation. Smear layer and debris removal was evaluated via SEM on selected sites in the coronal, middle and apical segments of the canals. ANC and

NaOCl had similar debris removal capacity, and both were ineffective in the removal of the smear layer, although ANC affected its thickness and surface. When the ANC was alternated to the catholyte solution, both smear layer and debris removal improved (Solovyeva & Dummer 2000). The cleaning efficacy of an ECA water anolyte solution during instrumentation [pH 7.4, ORP and chlorine concentration not described, STEDS, Radical Waters, Johannesburg, South Africa] followed by a final flush with its catholyte solution (non-chlorine containing) was compared with 2.5% NaOCl alone in canals of *ex vivo* single-rooted teeth when delivered using an ultrasonic unit. ECA solutions produced surfaces cleaned of debris and bacteria and removed the smear layer in larger areas (Marais 2000). The use of an acidic 'OPW' [pH 2.5, chlorine concentration and ORP not reported in literature (NDX-250 KH; Nihon Aqua Co. Ltd, Kyoto, Japan)] in the removal of the smear layer was tested on human single-rooted teeth using a SEM on the root canal's middle and apical thirds. The OPW was tested alone or in association with 17% EDTA or 5% NaOCl, using either a syringe or an ultrasonic unit as irrigation methods. The authors concluded that OPW delivered by syringe after instrumentation effectively removed the superficial smear layer as specimens irrigated using OPW during and after instrumentation showed no smear layer or packing in the tubules. Similarly, OPW irrigation following EDTA showed clean surfaces, no smear layer and some visible tubule openings. Overall, this OPW was found to be as effective as 5% NaOCl or 17% EDTA used separately for the removal and prevention of smear layer formation (Hata *et al.* 1996). In a different investigation, the same OPW was compared when used alone against a combination consisting of 5% NaOCl during instrumentation followed by OPW as final irrigant. This investigation suggested that OPW alone removed the smear layer in the middle third more effectively than the combined irrigants and that neither of the irrigation regimes was capable of removing the smear layer from the apical third and left debris at the dentinal tubule openings (Serper *et al.* 2001). Finally, the effectiveness of the same OPW (pH described as lower than 2.7) in debris and smear layer removal was compared with those NaOCl and 15% EDTA. The different irrigants were delivered using either syringe or an ultrasonic unit using *ex vivo* human maxillary incisor root canals to understand the effects in the apical and middle thirds. The presence of the smear layer and debris was assessed via SEM observation using a three-point scale. NaOCl or OPW was used

during instrumentation, whilst EDTA or OPW was used as a final rinse. This study suggested that the most effective technique for smear layer removal was NaOCl during instrumentation followed by EDTA using a syringe. OPW as a final rinse following NaOCl showed similar effects; however, when used alone, OPW did not remove the smear layer or debris effectively (Hata *et al.* 2001). Considering the large variability in the characteristics of the chlorine solutions tested and the need for a chelating agent in order to remove the smear layer, the association of detergents with chlorine-containing solutions should be tested further to better understand whether deeper penetration within the dentinal tubule system and more effective cleaning can be achieved. The information gathered about the antimicrobial effects, tissue dissolution and cleaning effectiveness of various chlorine-containing endodontic solutions, in a way, explains why NaOCl is still the main choice worldwide. Based on the literature search, it can be proposed that the use of conventional NaOCl for vital pulp treatments, when removing organic contents, is of primary importance. Using an acidified NaOCl for nonvital treatment is recommended when a strong antibacterial effect is required. The undesired properties of this irrigant, however, drive researchers to look at various factors affecting other chlorine solutions and their potential use as substitutes for NaOCl. If tissue dissolution and cleaning effectiveness remain a problem, at least the time of exposure to high pH solutions might be reduced.

Conclusions

Further investigations on chlorine-containing solutions should include chlorine concentration and pH analysis as part of the experimental methodology in order to understand the type of chlorine species present as well as their concentration. By modifying the pH of NaOCl solutions to values around 6 and 7.5 using specific acids, the antimicrobial effect seems to be increased. Low-concentration acidic and neutral chlorine-containing solutions appear to have antimicrobial effect; however, this is lower than currently used NaOCl concentrations. By modifying pH of NaOCl solutions to values below 7.5, the tissue dissolution capability appears to decrease. Sodium dichloroisocyanurate and SOW appear not to have clinically relevant pulp tissue dissolution effects. Neutral and acidic chlorine solutions appear to have potential cleaning effectiveness; however, the use of a chelating agent or detergent in combination might be necessary.

References

- Aubut V, Pommel L, Verhille B *et al.* (2010) Biological properties of a neutralized 2.5% sodium hypochlorite solution. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* **109**, e120–5.
- Baumgartner JC, Ibay AC (1978) The chemical reactions of irrigants used for root canal debridement. *Journal of Endodontics* **13**, 47–51.
- Berkovitz BKB, Holland GR, Moxham BJ (2002) *Oral anatomy, embryology and histology*, 3rd edn. Edinburgh, UK: Mosby Elsevier.
- Bloomfield SF, Miles GA (1979) The antibacterial properties of sodium dichloroisocyanurate and sodium hypochlorite formulations. *Journal of Applied Bacteriology* **46**, 65–73.
- Brazis AR, Leslie JE, Kabler PW, Woodward RL (1958) The inactivation of spores of *Bacillus globigii* and *Bacillus anthracis* by free available chlorine. *Applied Microbiology* **6**, 338–42.
- Butterfield CT, Wattie E, Megregian S, Chambers CW (1943) Influence of pH and the temperature on the survival of coliforms and enteric pathogens when exposed to free chlorine. *Public Health Reports* **58**, 1837–67.
- Camps J, Pommel L, Aubut V *et al.* (2009) Shelf life, dissolving action, and antibacterial activity of a neutralized 2.5% sodium hypochlorite solution. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* **108**, e66–73.
- Christensen C, McNeal ST, Eleazer P (2008) Effect of lowering the pH of sodium hypochlorite on dissolving tissue in vitro. *Journal of Endodontics* **34**, 449–52.
- Claesen T, Edmonson P (2006) Sodium dichloroisocyanurate (NaDCC) tablets as an alternative to sodium hypochlorite for the routine treatment of drinking water at the household level. *International Journal of Hygiene and Environmental Health* **209**, 173–81.
- Davis JM, Maki J, Bahcall JK (2007) An in vitro comparison of the antimicrobial effects of various endodontic medicaments on *Enterococcus faecalis*. *Journal of Endodontics* **33**, 567–9.
- Death J, Coates D (1979) Effect of pH on sporicidal and microbicidal activity of buffered mixtures of alcohol and sodium hypochlorite. *Journal of Clinical Pathology* **32**, 148–52.
- Deborde M, von Gunten U (2008) Reactions of chlorine with inorganic and organic compounds during water treatment – kinetics and mechanisms: a critical review. *Water Research* **42**, 13–51.
- Dychdala RG (1991) Chlorine and chlorine compounds. In: Block SS, ed. *Disinfection, sterilization and preservation*, 4th edn. Philadelphia, PA: Lea & Febinger, pp. 131–51.
- Fair GM, Morris JC, Chang SL, Weil I, Burden RP (1948) The behaviour of chlorine as water disinfectant. *Journal of the American Water Works Association* **1**, 1051–61.
- Fraiese AP (1999) Choosing disinfectants. *Journal of Hospital Infection* **43**, 255–64.
- Garcia F, Murray PE, Garcia-Godoy F, Namerow K (2010) Effect of aquatine endodontic cleanser on smear layer removal in the root canals of ex vivo human teeth. *Journal of Applied Oral Science* **18**, 403–8.
- Gomi K, Makino T, Suzuki S, Hagesawa M, Maeda N, Arai T (2010) Microbicidal and cytotoxic effects of functional water in vitro. *Quintessence International* **41**, e166–72.
- Grigoratos D, Knowles J, Ng Y-L, Gulabivala K (2001) Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *International Endodontic Journal* **34**, 113–9.
- Gulabivala K, Stock CJR, Lewsey JD, Ghori S, Ng Y-L, Spratt D (2004) Effectiveness of electrochemically activated water as an irrigant in an infected tooth model. *International Endodontic Journal* **37**, 624–31.
- Haikel Y, Gorce F, Allemann C, Voegel JC (1994) In vitro efficiency of endodontic irrigation solutions on protein desorption. *International Endodontic Journal* **27**, 16–20.
- Hata G, Uemura M, Weine FS, Toda T (1996) Removal of smear layer in the root canal using oxidative potential water. *Journal of Endodontics* **22**, 643–5.
- Hata G, Hayami S, Weine FS, Toda T (2001) Effectiveness of oxidative potential water as a root canal irrigant. *International Endodontic Journal* **34**, 308–17.
- Hawkins CL, Pattison DI, Davies MJ (2003) Hypochlorite-induced oxidation of amino acids, peptides and proteins. *Amino Acids* **25**, 259–74.
- Lee S-V, Hung Y-C, Chung D, Anderson JL, Erickson MC, Morita K (2000) Effects of storage conditions and pH on chlorine loss in Electrolyzed Oxidizing (EO) Water. *Journal of Agricultural and Food Chemistry* **50**, 209–12.
- Marais JT (2000) Cleaning efficacy of a new root canal irrigation solution: a preliminary investigation. *International Endodontic Journal* **33**, 320–5.
- Marais JT, Williams WP (2001) Antimicrobial effectiveness of electro-chemically activated water as an endodontic irrigation solution. *International Endodontic Journal* **32**, 237–43.
- Martin MV, Gallagher MA (2005) An investigation of the efficacy of super-oxidised (Optident/Sterilox) water for the disinfection of dental unit water lines. *British Dental Journal* **198**, 353–4.
- Martin N, Martin MV, Jedyakiewicz NM (2007) The dimensional stability of dental impression materials following immersion in disinfecting solutions. *Dental Materials* **23**, 760–8.
- Mercade M, Duran-Sindreu F, Kuttler S, Roig M, Durany N (2009) Antimicrobial efficacy of 4.2% sodium hypochlorite adjusted to pH 12, 7.5, and 6.5 in infected root canals. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* **107**, 295–8.
- Moorer WR, Wesselink PR (1982) Factors promoting the tissue dissolving capability of sodium hypochlorite. *International Endodontic Journal* **15**, 187–96.

- Naenni N, Thoma K, Zehnder M (2004) Soft tissue dissolution capacity of current and potential endodontic irrigants. *Journal of Endodontics* **30**, 784–7.
- Pashley EL, Birdsong NL, Bowman K, Pashley DH (1985) Cytotoxic effect of NaOCl on vital tissue. *Journal of Endodontics* **11**, 525–8.
- Rossi-Fedele G, de Figueiredo JAP, Steier L, Canullo L, Steier G, Roberts AP (2010a) Evaluation of the antimicrobial effect of super-oxidized water (Sterilox®) and sodium hypochlorite against *Enterococcus faecalis* in a bovine root canal model. *Journal of Applied Oral Science* **18**, 498–502.
- Rossi-Fedele G, Steier L, Dogramaci EJ, Canullo L, Steier G, de Figueiredo JAP (2010b) Bovine pulp tissue dissolution ability of Healozone®, Aquatine Alpha Electrolyte® and sodium hypochlorite. *Australian Endodontic Journal*, published on line 5 November 2010. DOI: 10.1111/J.1747-4477.2010.00287.x
- Rutala WA, Weber DJ (1997) Use of Inorganic hypochlorite (bleach) in health-care facilities. *Clinical Microbiology Reviews* **10**, 597–610.
- Serper A, Çalt S, Doğan L, Güç D, Özçelik B, Kuraner T (2001) Comparison of the cytotoxic effects and smear layer removing capacity of oxidative potential water, NaOCl and EDTA. *Journal of Oral Science* **43**, 233–8.
- Sim TP, Knowles JC, Ng Y-L, Shelton J, Gulabivala K (2001) Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *International Endodontic Journal* **34**, 120–32.
- Solovyeva AM, Dummer PMH (2000) Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions: a pilot study. *International Endodontic Journal* **33**, 494–504.
- Spanberg L, Engström B, Langeland K (1973) Biologic effects of dental materials. 3 Toxicity and microbial effect of endodontic antiseptics in vitro. *Oral Surgery* **36**, 856–71.
- Spanó JCE, Barbin EL, Santos TC, Guimaraes LF, Pecora JD (2001) Solvent action of sodium hypochlorite on bovine pulp and physico-chemical properties of resulting liquid. *Brazilian Dental Journal* **12**, 154–7.
- Weber GR, Levine M (1944) Factors affecting germicidal efficiency of chlorine and chloramines. *American Journal of Public Health* **34**, 719–27.
- Zehnder M (2006) Root canal irrigants. *Journal of Endodontics* **32**, 389–98.
- Zehnder M, Kosicki D, Luder H, Sener B, Waltimo T (2002) Tissue-dissolving capacity and antibacterial effect of buffered and unbuffered hypochlorite solutions. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* **94**, 756–62.

Study 2 – Published in Journal of Endodontics

Review

Antagonistic Interactions between Sodium Hypochlorite, Chlorhexidine, EDTA, and Citric Acid

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Antagonistic Interactions between Sodium Hypochlorite, Chlorhexidine, EDTA, and Citric Acid

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Abstract

Introduction: Root canal irrigants play a significant role in the elimination of microorganisms, tissue dissolution, and the removal of debris and smear layer. No single solution is able to fulfill these actions completely; therefore, their association is required. The aim of this investigation was to review the antagonistic interactions occurring when sodium hypochlorite (NaOCl), chlorhexidine (CHX), EDTA, and citric acid (CA) are used together during endodontic treatment. **Methods:** A search was performed in the electronic database Medline (articles published through 2011; English language; and the following search terms or combinations: "interaction AND root canal irrigant or endodontic irrigant or sodium hypochlorite or chlorhexidine," "sodium hypochlorite AND EDTA or ethylenediaminetetraacetic acid or citric acid or chelating agent or chlorhexidine," and "chlorhexidine AND EDTA or ethylenediaminetetraacetic acid or citric acid or chelating agent") to identify publications that studied unwanted chemical interactions between NaOCl, CHX, and EDTA and CA. **Results:** The search identified 1,285 publications; 19 fulfilled the inclusion/exclusion criteria of the review. Their research methodology was classified as either *in vitro* or *ex vivo*. **Conclusions:** Antagonistic interactions included the loss of free available chlorine for NaOCl when in contact with chelators, which consequently reduced the tissue dissolution capability and to a lesser extent antimicrobial activities. When CHX and NaOCl are mixed, a precipitate forms that can present detrimental consequences for endodontic treatment, including a risk of discoloration and potential leaching of unidentified chemicals into the periradicular tissues. CHX and EDTA mixtures cause a precipitate, whereas CHX and CA do not exhibit interaction. (*J Endod* 2012;38:426–431)

Key Words

Chlorhexidine, citric acid, EDTA, endodontic irrigant, interaction, root canal irrigants, sodium hypochlorite

Root canal cleaning and disinfection during chemomechanical preparation relies heavily on irrigants because of the anatomic complexities of the pulp canal system. Irrigants should ideally have antimicrobial and tissue-dissolution actions as well as other advantageous properties, such as lubrication, demineralization, and the ability to remove debris and the smear layer (1).

Sodium hypochlorite (NaOCl) is recommended as the main endodontic irrigant because of its ability to dissolve organic matter together with its broad antimicrobial action (2). NaOCl is commercially available as aqueous solutions with concentrations ranging from 1% to 15% and having an alkaline pH with values around 11 (3). Among other salts, they also contain sodium hydroxide salts in order to increase their stability (3), and they might contain surfactants as well as other components that are not always disclosed by the manufacturer (4).

No irrigation solution has been found capable of demineralizing the smear layer and dissolving organic tissue simultaneously (5). Therefore, the adjunctive use of chelating agents such as EDTA or citric acid (CA) is suggested in order to remove and prevent the formation of the smear layer associated with root canal instrumentation (2).

EDTA is a polyprotic acid whose sodium salts are noncolloidal organic agents that can form nonionic chelates with metallic ions (2, 6). Its solutions are normally used at concentrations between 10% and 17%, and its pH is modified from its original value of 4 (7) to values between 7 and 8 to increase its chelating capacity (2, 6). Like many well-known chelating agents, EDTA exists in aqueous solutions as an equilibrium mixture of both protonated and unprotonated forms. CA is an organic acid normally used in endodontics at concentrations between 10% and 50% (2) with a pH value between 1 and 2 (8).

Although the role of smear layer removal has been widely debated, endodontic literature concerning the antimicrobial action of irrigants suggests that the combined use of EDTA and NaOCl is more efficient than NaOCl alone when measuring bacterial survival after multiple appointments (9); bacterial survival analysis is a surrogate measure of treatment outcome. A recently published outcome investigation indicated that 2.5% to 5% NaOCl followed by 17% EDTA had a profoundly beneficial effect on secondary nonsurgical root canal treatment success while having a marginal effect on the original treatment (10).

It has been suggested that variations of NaOCl pH will modify the antimicrobial and tissue-dissolution activities (11). A reduction of the pH to values around 6.0 to 7.5 has been found to improve the antimicrobial efficacy (11–13) but hinders tissue-dissolution action (11, 13–15). If the pH is lowered to values below 4, then the amount of chlorine gas in the solution will increase (16). Chlorine in gas form is volatile and therefore unstable (17). If NaOCl is mixed with other irrigants possessing low pH values, there is a possibility of altering its properties.

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Chlorhexidine (CHX), a bisguanide, is stable as a salt although it dissociates in water at a physiologic pH, releasing the CHX component (18). It is frequently used at concentrations between 0.2% and 2% (2) and exhibits an optimal antimicrobial activity at a pH of 5.5 to 7.0 depending on the buffering agent used and the organism studied (19). The most common preparation is CHX gluconate (20). It has been recommended that CHX be used as either an alternative or an adjunct root canal irrigant because of its antimicrobial qualities. Studies comparing its antimicrobial action versus NaOCl solutions present conflicting results (10, 21–29).

Some investigations suggest that NaOCl is more effective as an antimicrobial agent compared with CHX. One *in vivo* study showed 2.5% NaOCl was a more effective antimicrobial agent compared with 0.2% CHX (21). However, an *in vitro* study (22) using a bovine root model showed that CHX had a similar antimicrobial effect as NaOCl, whereas another investigation into bovine dentinal tubule disinfection comparing NaOCl and CHX 0.2% to 2% found no difference in antimicrobial efficacy between either solution at these concentrations (23). Similarly, an *ex vivo* investigation found no statistically significant difference when comparing 5.25% NaOCl and 2% CHX (24). Contemporary *in vivo* studies comparing 2.5% NaOCl and 0.12% CHX and their ability to reduce the numbers of cultivable bacteria (25) and the presence of bacteria, archaea, and fungi on teeth with apical periodontitis using molecular microbiology procedures (26) suggest no difference in effectiveness between the solutions. On the contrary, an *in vivo* investigation into the percentage of growing bacterial species after irrigation with 5.25% NaOCl or 2% CHX in teeth with pulpal necrosis, apical pathosis, or both found the latter to be significantly more effective at reducing growth (27). Some characteristics of the irrigants investigated are summarized in Table 1.

A seminal investigation comparing the use of 0.2% CHX and 2.5% NaOCl individually and in combination in human teeth presenting with periapical radiolucencies suggested that their combined use produced the greatest percentage reduction in cultures (28). Equally, the addition of 2% CHX to 1% NaOCl in teeth with infected necrotic pulps was found to enhance the disinfection of the root canal system because of the reduction of cultivable bacteria in those cases (29). Ng et al (10) in their outcome investigation suggested that the use of 0.2% CHX in addition to NaOCl significantly reduces the success rate in nonsurgical treatment (10). CHX lacks tissue dissolution capacity (30), an important quality desired from root canal irrigants.

It has been purported that the application of CHX before the application of adhesives prevents resin-dentin bond degradation because of its ability to inhibit collagenolytic enzymes (31). Concerns about the longevity of bonding to root canal dentin have been raised for bonded root filling techniques and resin cemented posts (32) so this should be taken into consideration when resin-based sealers are used even when gutta-percha is used as the core material.

Commercially available NaOCl solutions have an alkaline pH value, with the hypochlorite ion being the main chlorine species present (16). The chemical interactions of NaOCl with EDTA or CHX are redox reactions, with molecular groups being oxidized by NaOCl (20, 33); an acid-base reaction occurs when CHX and NaOCl are mixed because CHX has

the ability to donate protons as a positive component, whereas NaOCl can accept them (20, 28, 34). In regard to EDTA associated with CHX, it may potentially degrade CHX, forming a salt (35). CA and CHX apparently pose no antagonistic reactions (36). Therefore, the purpose of this article was to review the undesired effects after interactions between NaOCl, CHX, and the commonly used chelating agents EDTA and CA.

Materials and Methods

A literature search using electronic database Medline was conducted on June 15, 2011, for articles published through to the date using the following search terms and combinations: “interaction AND root canal irrigant or endodontic irrigant or sodium hypochlorite or chlorhexidine,” “sodium hypochlorite AND EDTA or ethylenediaminetetraacetic acid or citric acid or chelating agent or chlorhexidine,” and “chlorhexidine AND EDTA or ethylenediaminetetraacetic acid or citric acid or chelating agent.” Publications were included if they studied antagonistic interactions between NaOCl, CHX, EDTA, and CA by comparing 1 of the solutions against a mixture of them and were published in English. Titles and abstracts of the publications identified were initially screened by 2 independent reviewers (G.R.F. and E.J.D.). Publications were included for full-text evaluation by 1 reviewer (G.R.F.) if the content of the abstracts met the inclusion criteria. Full-text assessment and data extraction were performed by 1 reviewer (G.R.F.). Publications were excluded if they did not meet the inclusion criteria (ie, if they did not study antagonistic interactions between NaOCl, CHX and CA or EDTA by comparing 1 of these alone and when combined with a substance mentioned previously) or if they were not published in English. Of 1,285 publications identified, 19 were included in the review.

Interactions between NaOCl and Chelating Agents

The addition of chelators to NaOCl reduces its pH in a ratio and time-dependent manner (37–39). This affects the forms of free chlorine in the solution and causes an increase in hypochlorous acid and chlorine gas, which subsequently reduces the amount of the hypochlorite ion (3, 11). When 1% NaOCl was mixed with 17% EDTA (pH = 8) in ratios of 1:1, 1:5, and 5:1, the pH of the solutions ranged between 8.0 and 8.4 (37). The addition of 10% CA to 1% NaOCl in the same ratios resulted in pH values between 1.8 and 4.3 (37). Another study mixed 1% to 2% NaOCl with 17% EDTA in equal proportions, resulting in a final pH value of 8.0 from an initial value of 10 after an elapse of 48 hours. However, when mixed in a 1:3 ratio, although with a larger volume of EDTA, the pH value was stable during the 48-hour experimental time, probably because of an immediate interaction between the solutions (38). The reduction of pH values in the NaOCl solution causes the release of chlorine gas, which has potentially hazardous effects on humans (39). When EDTA is added to NaOCl, chlorine gas can be detected at relatively low levels. When CA is used, significantly more chlorine is detectable and present at a further distance. This is according to a laboratory-based investigation that studied the reactions between NaOCl (5.25%, pH = 12.12) and CA (50%,

TABLE 1. Characteristics of Some Root Canal Irrigants

Compound	Chemical structure	Type	Concentration of solution (%)	Typical pH of solutions	Commonly used oral preparation
Sodium hypochlorite	NaOCl	Chlorine-releasing agent	0.5 to 15	9 to 12	
EDTA	C ₁₀ H ₁₆ N ₂ O ₈	Polyprotic acid	10 to 17	7 to 8	EDTA disodium salt
CHX	C ₂₂ H ₃₀ Cl ₂ N ₁₀	Bisguanide	0.2 to 2	5.5 to 7	CHX (di)gluconate
CA	C ₆ H ₈ O ₇	Organic acid	10 to 50	1 to 2	

pH = 1.28) or EDTA (15%, pH = 7.51). Portions of the chelator were added to the NaOCl at regular time intervals for a total time period of 2 hours; the release of chlorine gas was measured at 6 inches and 6 feet from the container (39).

The consequences of chemical interactions between chelating agents and NaOCl result in a loss in the free available chlorine (FAC) of the mixtures (6, 37, 40). The effects on FAC contents in a 1% NaOCl solution were assayed by mixing it with either 17% EDTA or water (1:1) and measured via an iodine/thiosulfate titration method. NaOCl's FAC was substantially modified by the presence of EDTA with a reduction to 0.06% when compared with 0.5% of the water dilution control (6). This research group subsequently looked into the impact on available chlorine in 1% NaOCl from the interaction with 17% EDTA (pH = 8) and 10% CA using the same methodology and taking into account the time factor. Their results indicated that when mixed with CA, FAC decreased to 0 in less than a minute, whereas EDTA required between 1 and 60 minutes to reduce the FAC to the same level (37). These results were confirmed by a different research group that looked into time-related effects (between 5 and 18 minutes) on active chlorine content because of dilution with a EDTA solution (17%, pH = 7.5, and containing a surfactant). Different NaOCl preparations (1%, 1.5%, 4%, and 4.5% with some containing surfactant) and NaOCl:EDTA ratios (9:1, 3:1, and 1:1) were tested via iodometric titration. Apart from modifications because of dilution, the available chlorine loss was extreme (ie, up to 80% even when adding small amounts of EDTA at the early stages of the process and then becoming more gradual, indicating that a chemical reaction occurs between the solutions). The presence of a surfactant made the reduction of the available chlorine less marked in time and was a far better predictor than the original concentration for chlorine loss (4). When gel-type preparations of chelators containing 15% EDTA and 10% Urea peroxide (RC-Prep; Premier Dental, Philadelphia, PA, and Glyde; DeTrey Dentsply, Konstanz, Germany) were tested for interaction with 1% NaOCl using spectroscopy, it was shown that both compounds depleted the solution from its chlorine content after 5 minutes (40).

The dramatic reduction of FAC in NaOCl mixtures caused by chemical interactions appears to explain the inability of NaOCl and EDTA mixtures to dissolve soft tissues. An investigation looking into bovine tissue dissolution of NaOCl (1%-2.5%) alone and combined with 17% EDTA in different ratios (2:2 and 1:3) showed that after 48 hours only unmixed NaOCl was able to completely dissolve the tissue (38). Similarly, the tissue dissolution effects of the interactions between NaOCl and EDTA were tested on porcine palatal mucosa by assessing the percentage of original tissue weight after different exposure periods up to 120 minutes; 8.5% EDTA, 0.5% NaOCl, and a 1:1 mixture of 17% EDTA together with 1% NaOCl were the test solutions. This investigation suggested that NaOCl alone was substantially more efficient than the other groups, with no statistically significant differences among them (6).

The degradation and consequent deactivation of EDTA after its interaction with NaOCl is extremely slow, and, therefore, it does not compromise its clinical performance with respect to its chelating, smear layer removal, and dentin softening effects (6, 7, 37). This phenomenon has been analyzed via nuclear magnetic resonance with no reactions detected in the first 7 minutes, and the process was not complete after 120 minutes (33).

NaOCl does not reduce the calcium chelating or smear layer ability of EDTA and CA (6, 7, 37). An investigation, using standardized dentin portions immersed into solutions of either 17% EDTA and distilled water or 17% EDTA and 0.5% NaOCl, found greater calcium chelation occurring in the solution containing NaOCl (7). Similarly, the chelating ability of 17% EDTA alone and in a mixture with 5% NaOCl (9:1 mixture)

was compared using a calcium titration method in order to assess the amount of chelated calcium per mole of EDTA. The results indicated that NaOCl had little effect on EDTA's calcium chelating ability (6). Another investigation from the same research group studied calcium chelation and smear layer removal from root canals after irrigation *ex vivo* (37). Human single-rooted teeth were instrumented and subsequently irrigated with mixtures of the chelating agents and 1% NaOCl or water at a 1:1 ratio. After irrigation, the solutions were analyzed for their calcium content using atomic absorption spectrophotometry. No statistically significant differences for EDTA or CA were found between the combinations containing water or NaOCl. The teeth were subsequently split and observed using a scanning electron microscope for the presence or absence of smear layer in a semiquantitative manner; no differences were found among the irrigant combinations described earlier (37). The addition of NaOCl to EDTA does not alter EDTA's ability to decalcify human dentin, and this has been shown through studies assessing Vickers microhardness after adding either NaOCl or distilled water to EDTA in a 1:1 ratio and observing for 7 minutes (7).

Chelators can eliminate NaOCl's antimicrobial efficacy if the original FAC values are modest, whereas EDTA and CA performance does not seem to be jeopardized because of interactions with NaOCl (6, 37). The effects on antimicrobial ability, related to the interactions between EDTA and NaOCl, have been analyzed using an agar diffusion test against *Enterococcus faecalis* and *Candida albicans* using 0.5% NaOCl, 8.5% EDTA, and a mixture with 1% NaOCl and 17% EDTA (1:1 mixture) (6). Pure NaOCl produced smaller zones of inhibition when compared with pure EDTA or the mixture of EDTA/NaOCl, and there were no statistically significant differences among the EDTA-containing groups (6). An *in vitro* investigation testing the impact of CA and EDTA on NaOCl's antimicrobial action against was performed by the same group. *E. faecalis* was suspended in phosphate buffered saline and then added (1:1) to tubes containing chelating agent mixtures with 1% NaOCl and their 1:10 and 1:100 dilutions; after incubation, it was found that 10% CA and 17% EDTA eliminated NaOCl's antimicrobial action at the 1:100 dilutions because growth was present (37).

Interactions between NaOCl and CHX

From the review of the literature, it transpires that mixing NaOCl with liquid CHX results in the instant formation of a flocculate or precipitate (41–49). Basrani et al (41) looked into the minimum NaOCl concentration required to form a precipitate when mixed with 2% CHX (41). Concentrations ranging from 0.023% to 6% were tested, and an instant color change occurred in all samples from dark brown to light orange. A precipitate was induced with 0.19% NaOCl with varying amounts of material formed in the different mixtures (41).

Several investigations have been undertaken to elucidate the chemical composition of the flocculate produced by the association of NaOCl with CHX (41–45, 47–49). Different proportions and concentrations of NaOCl (0.5%, 2.5%, and 5%) and CHX (0.2%–2%) have been mixed, which results in the formation of a brownish flocculate evident when the solutions make contact with each other; atomic absorption spectrophotometry showed the presence of Ca, Fe, and Mg (42). Although most investigations report the presence of parachloroaniline (PCA) in the precipitate, 1 failed to detect its presence. The precipitate was analyzed using X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectrometry, which detected that PCA was present at concentrations directly related to the NaOCl concentration (41). The same researchers used gas chromatography-mass spectrometry in order to further identify the precipitate composition after the mixture of 6% NaOCl with 2% CHX; PCA was detected again although no further

aniline derivatives or chlorobenzene were found (43). Krishnamurthy and Sudhakaran (44) mixed 2.5% NaOCl with 2% CHX and were able to detect PCA in the precipitate by using Beilstein and HCl solubility tests followed by nuclear magnetic resonance. Despite Thomas and Sen (45) using nuclear magnetic resonance spectroscopy, they failed to detect PCA in the precipitate after combining 5.25% NaOCl with 2% CHX acetate. PCA has been suggested to be a toxic and carcinogenic substance, hence the significance of this subject (46).

Three studies have evaluated the cleaning efficacy after irrigation with CHX containing solutions (44, 47, 48). Bui et al (47) investigated the influence of irrigation on debris removal and patent dentinal tubules using 5.25% NaOCl and 2% CHX *ex vivo* and analyzed it with an environmental scanning electron microscope (47). The test group involved irrigation initially with NaOCl, which was either left inside the root canal or aspirated and dried with paper points; after which CHX irrigation was performed. The positive control group consisted of irrigation solely with NaOCl and then aspiration and drying with paper points. There was no difference in remaining debris and a reduction in number of patent dentinal tubules in the coronal and middle third between the 2 test groups. A scanning electron microscopic investigation into the percentage of open and closed tubules after root canal instrumentation on human teeth using 2.5% NaOCl and 2% CHX in liquid or gel forms, intercalated by physiologic saline, with half of the experimental groups receiving a final flush with 17% EDTA was performed by Valera et al (48). Their results indicated that 2% CHX gel produced the largest amount of open dentinal tubules, whereas 2% CHX liquid presented the worst result. The addition of EDTA and physiologic saline as a final flush improved cleaning and debris removal. The presence and thickness of the precipitate formed after irrigation with 17% EDTA followed by 2.5% NaOCl and a final flush with 2% CHX (test group) was compared against the same sequence, but they were intercalated between these other solutions to assess their ability to reduce formation of the precipitate (44). This was performed on *ex vivo* root canals and examined with a stereomicroscope (44). Isopropyl alcohol resulted in completely clean canals, whereas the use of saline or distilled water produced a sparse precipitate. The test group presented deposits all along the canal wall with a mean thickness 2 to 3 times greater than that of the saline and distilled water groups. The precipitate was present mainly in the coronal and middle thirds of the canals.

This precipitate has an effect on dentinal permeability (34) and dye penetration after root canal obturation (49). An *ex vivo* investigation compared the effects of combining 1% NaOCl and 2% CHX on dentinal permeability as measured by Rhodamine leakage in percentage (34). When compared against a "no irrigation" control, the mixture of NaOCl and CHX caused a reduction of permeability only in the apical third. This was explained by the formation of a brown mass suspended in the liquid that becomes a flocculate precipitate, which acts as a "chemical smear layer." Another *ex vivo* investigation assessed dye penetration in clear teeth after preparation using different irrigants and obturation (49). The results suggested that a precipitate formed when combining 1% NaOCl and 2% CHX gel, which stained the dentin and adhered to the canal walls. Therefore, this group presented the largest values of linear dye penetration. Statistically significant differences were found with the other groups, which included NaOCl alone, NaOCl and EDTA, CHX gel alone, and distilled water.

Interactions between CHX and Chelating Agents

CHX is easily mixed with CA, and no modification of its demineralizing ability or precipitation occurs (34, 36). An *in vitro* study on bovine dentin slices using atomic absorption spectrophotometry looked into the effect of adding 1% CHX and 10% to 20% CA on the

TABLE 2. Reported Interactions between Root Canal Irrigants

Mixture	Reaction product or byproduct	Undesirable result	Chemical reaction	First author	Journal	Year	Issue and page numbers
NaOCl + EDTA	Cl ₂ (gas)	Loss on active chlorine content	Breakdown of NaOCl at low pH	Grawehr Zehnder	J Endod J Endod	2003 2005	36:411-5 31:817-20
NaOCl + CHX	Glioxilic acid; EDTA	Degradation of EDTA molecule	Oxidation of EDTA (after 7 minutes of reaction)	Zehnder Baumgartner Clarkson Grande	J Endod J Endod J Endod J Endod	2006 1987 2011 2006	32:389-98 13:47-51 37:538-43 32:460-4
NaOCl + CHX	Orange-brown precipitate	Potentially Toxic compound; color change	Redox reaction	Krishnamurthy Basrani Vivaqua-Gomes Zehnder Bui Marchesan	J Endod J Endod Int End J J Endod J Endod Oral Surg Oral Med Oral Pathol	2010 2007 2002 2006 2008 2007	36:1154-7 33:966-9 35:791-5 32:389-98 34:181-5 103:e103-5
NaOCl + CA CHX + EDTA	Cl ₂ (gas) Salt; white precipitate	Loss on active chlorine content Chemical degradation of CHX	Breakdown of NaOCl at low pH Salt formed by neutralization between CHX and EDTA	Baumgartner Rasimick	J Endod J Endod	1987 2008	13: 47-51 34:1521-3
CHX + CA		No reaction known	No reaction known	González-Lopez	J Endod	2006	32:781-4

demineralizing capacity of the chelator (36). The results after 3, 10, and 15 minutes of immersion showed no alteration of the decalcifying effect (36). Another study looking into modification of dentinal permeability after the irrigation of human teeth found no statistically significant differences when compared against the “no irrigation” group although statistically significant differences were found when compared against the NaOCl and CHX group in the apical third of the root canal (34). It has been shown that 15% CA followed by 2% CHX causes the formation of a “milky” solution, which can be easily removed by using further CHX; no precipitation occurs (34).

When mixing CHX with EDTA, it is difficult to obtain a homogeneous solution; a precipitate composed chiefly of the original components forms (35, 36). It has been shown that it is not possible to obtain a homogeneous solution by mixing 17% EDTA and 1% CHX because a highly insoluble pink powdery precipitate forms (36). An investigation using reverse-phase high-performance chromatography analyzed the precipitate that forms after the combination of 17% EDTA with 2% or 20% CHX in equal volumes and 3 different mixing conditions (35). Over 90% of the precipitate mass was either EDTA or CHX although PCA was not detected. It was suggested that the precipitate was most likely a salt formed by neutralization of the cationic CHX by anionic EDTA (36).

Discussion

This literature review highlights the importance of clinicians having a comprehensive understanding of possible antagonistic interactions among endodontic irrigants used in their routine clinical practice. Because these solutions are used in succession, they come into contact with each other inside the endodontic space. This might impact treatment due to the modifications to tissue dissolution, antimicrobial and cleaning efficacy, seal, the risk of discoloration, and most importantly the potential adverse effects to a patient’s general health as a consequence of leaching chemicals in the periradicular tissues. Table 2 summarizes the deleterious effects of the associations described earlier.

Preventive Strategies

Apart from avoiding use of the aforementioned chemicals together to prevent or reduce the occurrence of the detrimental reactions described, the following strategies have been suggested:

1. NaOCl and EDTA: rinse out with copious amounts of NaOCl (37), making sure that fluid exchange occurs at all levels in the canal to prevent stratification of the solutions through the canal, which will lead to different mixtures of the irrigants at different levels (4). Alternatively, evacuation or drying before the placement of the next irrigant (4) can also help.
2. NaOCl and CHX: to prevent the formation of a precipitate associated with CHX and NaOCl interactions, a rinse with intermediate solutions after NaOCl has been suggested. They include saline (48); water (47, 48); alcohol (42, 47); isopropyl alcohol (44); or a demineralizing solution, which can be CA (34) or EDTA (42). Finally, if the flocculate is formed, then acetic acid can be used to dissolve the precipitate (41).
3. CHX and chelating agents: CA can be used in association with CHX because no interactions occur (34, 36). Alternatively, maleic acid can be used because it has been shown that this combination does not cause the formation of a precipitate, and only a marginal reduction of CHX availability occurs (50).

In summary, chelating agents have a dramatic effect on the free available chlorine contents of NaOCl and subsequently on its tissue dissolution capability, whereas its antimicrobial effect is reduced only when the

initial NaOCl concentrations are modest. EDTA and CA do not suffer from a reduction of their chelating ability in mixtures containing NaOCl. CHX and NaOCl-containing solutions develop a precipitate that might contain toxic substances that have an influence on root canal cleaning; however, further research is required to better understand its nature. When mixing CHX and EDTA, it is difficult to obtain a homogeneous solution, and a precipitate composed mainly of those substances is formed. CA is not influenced by CHX, and no precipitate is formed when mixed with it.

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References

1. Gutmann JL, Dumsha TC, Lovdahl PE. *Problem Solving in Endodontics*. 4th ed. St Louis, MO: Elsevier; 2006.
2. Zehnder M. Root canal irrigants. *J Endod* 2006;32:389–98.
3. Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health-care facilities. *Clin Microbiol Rev* 1997;10:597–610.
4. Clarkson RM, Podlich HM, Moule AJ. Influence of ethylenediaminetetraacetic acid on the active chlorine content of sodium hypochlorite solutions when mixed in various proportions. *J Endod* 2011;37:538–43.
5. Baumgartner JC, Brown CM, Mader CL, et al. A scanning electron microscopic evaluation of root canal debridement using saline, sodium hypochlorite, and citric acid. *J Endod* 1984;10:525–30.
6. Grawehr M, Sener B, Waltimo T, et al. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. *Int Endod J* 2003;36:411–5.
7. Saquy PC, Maia Campos G, Sousa Neto MD, et al. Evaluation of chelating action of EDTA in association with Dakin’s solution. *Braz Dent J* 1994;5:65–70.
8. Haznedaroğlu F. Efficacy of various concentrations of citric acid at different pH values for smear layer removal. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003;96:340–4.
9. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 1985;18:35–40.
10. Ng Y-L, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of non surgical root canal treatment: part 1: periapical health. *Int Endod J* 2011;44:583–609.
11. Rossi-Fedele G, Guastalli AR, Doğramacı EJ, et al. Influence of pH changes on chlorine-containing endodontic irrigating solutions. *Int Endod J* 2011;44:792–9.
12. Mercade M, Duran-Sindreu F, Kuttler S, et al. Antimicrobial efficacy of 4.2% sodium hypochlorite adjusted to pH 12, 7.5 and 6.5 in infected root canals. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;107:295–8.
13. Camps J, Pommel L, Aubut V, et al. Shelf life, dissolving action, and antibacterial activity of a neutralized 2.5% sodium hypochlorite solution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;108:e66–73.
14. Christensen C, McNeal ST, Eleazer P. Effect of lowering the pH of sodium hypochlorite on dissolving tissue *in vitro*. *J Endod* 2008;34:449–52.
15. Aubut V, Pommel L, Verhille B, et al. Biological properties of a neutralized 2.5% sodium hypochlorite solution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;109:e120–5.
16. Fair GM, Morris JC, Chang SL, et al. The behaviour of chlorine as water disinfectant. *J Am Water Works Assoc* 1948;1:1051–61.
17. Lee SV, Hung YC, Chung D, et al. Effects of storage and pH on chlorine loss in Electrolyzed Oxidizing (EO) water. *J Agric Food Chem* 2000;50:209–12.
18. Greenstein G, Berman C, Jaffin R. Chlorhexidine: and adjunct to periodontal therapy. *J Periodontol* 1986;57:370–6.
19. Block S, Seymour B. Disinfectant and antiseptic. *Kirk-Othmer Encyclopedia of Chemical Technology*. New York: Wiley & Sons; 1998.
20. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. *Int Endod J* 2009;42:288–302.
21. Ringel AM, Patterson SS, Newton CW, et al. *In vivo* evaluation of chlorhexidine gluconate solution and sodium hypochlorite solution as root canal irrigants. *J Endod* 1982;8:200–4.
22. Helling I, Chandler NP. Antimicrobial effect of irrigant combinations within dentinal tubules. *Int Endod J* 1998;31:8–14.
23. Vahdaty A, Pitt Ford TR, Wilson RF. Efficacy of chlorhexidine in disinfecting dentinal tubules *in vitro*. *Endod Dent Traumatol* 1993;9:243–8.
24. Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. *J Endod* 1994;20:276–8.
25. Siqueira JF, Roças IN, Paiva SSM, et al. Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;104:122–30.

26. Roças IN, Siqueira JF. Comparison of the *in vivo* antimicrobial effectiveness of sodium hypochlorite and chlorhexidine used as root canal irrigants: a molecular microbiology study. *J Endod* 2011;37:143–50.
27. Ercan E, Özenkici T, Atakul F, et al. Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: *in vivo* study. *J Endod* 2004;30:84–7.
28. Kuruvilla JR, Kamath P. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. *J Endod* 1998;24:472–6.
29. Zamany AZ, Safavi K, Spånberg LS. The effect of chlorhexidine as an endodontic disinfectant. *Oral Surg Oral Med Oral Pathol* 2003;96:578–81.
30. Okino LA, Siqueira EL, Santos M, et al. Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel. *Int Endod J* 2004;37:38–41.
31. Liu Y, Tjäderhane L, Breschi L, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. *J Dent Res* 2011;90:953–68.
32. Santos J, Carrilho M, Tervahartala T, et al. Determination of matrix metalloproteinases in human radicular dentin. *J Endod* 2009;35:686–9.
33. Grande NM, Plotino G, Falanga A, et al. Interaction between EDTA and sodium hypochlorite: a nuclear magnetic resonance analysis. *J Endod* 2006;32:460–4.
34. Akisue E, Tomita V, Gavini G, et al. Effect of the combination of sodium hypochlorite and chlorhexidine on dentinal permeability and scanning electron microscopy precipitate observation. *J Endod* 2010;36:847–50.
35. Rasimick BJ, Nekich M, Hladek M, et al. Interaction between chlorhexidine digluconate and EDTA. *J Endod* 2008;34:1521–3.
36. Gonzalez-Lopez S, Cornejo-Aguilar D, Sanchez-Sanchez P, et al. Effect of CHX on the decalcifying effect of 10% citric acid, 20% citric acid, or 17% EDTA. *J Endod* 2006;32:781–4.
37. Zehnder M, Schmidlin P, Sener B, et al. Chelation in root canal reconsidered. *J Endod* 2005;31:817–20.
38. Irala LED, Grazziotin-Soares R, Azevedo Salles A, et al. Dissolution of bovine pulp tissue in solutions consisting of varying NaOCl concentrations and combined with EDTA. *Braz Oral Res* 2010;24:271–6.
39. Baumgartner JC, Ibay AC. The chemical reactions of irrigants used for root canal debridement. *J Endod* 1987;13:47–51.
40. Girard S, Paque F, Badertscher M, et al. Assessment of a gel-type chelating preparation containing 1-hydroxyethylidene-1, 1 biphosphonate. *Int Endod J* 2005;38:810–6.
41. Basrani BR, Manek S, Sodhi RN, et al. Interaction between sodium hypochlorite and chlorhexidine gluconate. *J Endod* 2007;33:966–9.
42. Marchesan MA, Pasternak Junior B, Afonso MM, et al. Chemical analysis of the flocculate formed by the association of sodium hypochlorite and chlorhexidine. *Oral Surg Oral Med Oral Pathol* 2007;103:e103–5.
43. Basrani BR, Manek S, Mathers D, et al. Determination of 4-chloroaniline and its derivatives formed in the interaction of sodium hypochlorite and chlorhexidine by gas chromatography. *J Endod* 2010;36:312–4.
44. Krishnamurthy S, Sudhakaran S. Evaluation and prevention of the precipitate formed on interaction between sodium hypochlorite and chlorhexidine. *J Endod* 2010;36:1154–7.
45. Thomas JE, Sem DS. An *in vitro* spectroscopic analysis to determine whether para-chloroaniline is produced from mixing sodium hypochlorite and chlorhexidine. *J Endod* 2010;36:315–7.
46. Chhabra RS, Huff JE, Haseman JK, et al. Carcinogenicity of p-chloroaniline in rats and mice. *Food Chem Toxicol* 1991;29:119–24.
47. Bui TB, Baumgartner JC, Mitchell JC. Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. *J Endod* 2008;34:181–5.
48. Valera MC, Chung A, Menezes MM, et al. Scanning electron microscope evaluation of chlorhexidine gel and liquid associated with sodium hypochlorite cleaning on the root canal walls. *Oral Surg Oral Med Oral Pathol* 2010;110:e82–7.
49. Vivacqua-Gomes N, Ferraz CCR, Gomes BPFA, et al. Influence of irrigants on the coronal microleakage of laterally condensed gutta-percha root filling. *Int Endod J* 2002;35:791–5.
50. Ballal NV, Moorkhot S, Mala K, et al. Evaluation of chemical interactions of maleic acid with sodium hypochlorite and chlorhexidine gluconate. *J Endod* 2001;37:1402–5.

Study 3 – Published in International Endodontic Journal

Review

The effect of surface tension reduction on the clinical performance of sodium hypochlorite in endodontics

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Review

The effect of surface tension reduction on the clinical performance of sodium hypochlorite in endodontics

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Abstract

Rossi-Fedele G, Prichard JW, Steier L, de Figueiredo JAP. The effect of surface tension reduction on the clinical performance of sodium hypochlorite in endodontics. *International Endodontic Journal*.

Sodium hypochlorite (NaOCl) is recommended as an endodontic irrigant in view of its broad antimicrobial and tissue dissolution capacities. To enhance its penetration into inaccessible areas of root canals and to improve its overall effect, the addition of surface-active agents has been suggested. The aim of this investigation was to review the effect of the reduction of the surface tension on the performance of NaOCl in endodontics. A search was performed in the Medline electronic database (articles published up to 28 July 2012, in English) with the search terms and combinations as follows: 'sodium hypochlorite AND surface tension or interfacial force or interfacial tension or surface-active agent or amphiphilic agent or surface active agent or surfactant or tenside or detergent'.

The purpose of this search was to identify publications that compared NaOCl alone and NaOCl modified with the addition of a surface-active agent in endodontics. A hand search of articles published online ('in-press' and 'early view'), and appearing in the reference list of the articles included, was further performed, using the same search criteria as the electronic search. The search identified 302 publications, of which 11 fulfilled the inclusion/exclusion criteria of the review. The evidence available suggests that surface-active agents improve the penetration of NaOCl in the main canal and have no effect on its pulp tissue dissolution ability. There are, however, insufficient data to enable a sound conclusion to be drawn regarding the effect of modifying NaOCl's surface tension on lubrication, antimicrobial and smear layer or debris removal abilities.

Keywords: sodium hypochlorite, surface tension, surface-active agent, surfactant.

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Introduction

The key role of endodontic irrigants is to clean the root canal system during the enlarging and shaping process (Gutmann *et al.* 2006). It has been suggested that root canal preparation has the role of gaining 'radicular access' to the uninstrumented

root canal system, so as to disperse irrigants (Gulabivala *et al.* 2005). Because of the complexity of root canal morphology, some intra-radicular areas remain inaccessible to chemo-mechanical preparation. Following one-visit root canal treatment in teeth with apical periodontitis, the presence of bacteria has been detected in the recesses, diverticula and isthmuses of the main canals, as well as accessory canals in the mesial roots of human mandibular molars (Nair *et al.* 2005). In root canal treatment involving the removal of vital pulp tissues, the presence of incompletely treated or filled

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canals is suggested as a potential cause of persistent pain (Nixdorf *et al.* 2010).

A possible way of increasing the debridement of root canals that are inaccessible to instrumentation is to improve the delivery and agitation of irrigants. The use of various devices for this purpose has been shown to improve root canal cleanliness *in vitro* when compared with the use of conventional syringe and needle irrigation (Gu *et al.* 2009).

It has been further suggested that modification of the surface tension of irrigating solutions may improve irrigation efficacy by allowing irrigants to flow into remote areas, as surface tension inhibits the spread of a liquid over a surface and its ability to permeate capillary tubes (Cameron 1986). The spread or concentration of a liquid in contact with a surface depends on forces of attraction between the liquid molecules and those forces that the surface molecules exert on contact with those molecules of the liquid (Pècora *et al.* 1991). Moreover, the addition of surface-active agents promotes an increase in surface area, thereby making surfaces more easily wetted than in their absence. Those surface-active agents used in the cleaning processes are referred to as detergents (Williams & Elliot 1978). Amongst the purported benefits of reducing the surface tension of root canal irrigants are the increase of debris-free root canal dentine, adaptation to the dentinal walls, better tubule penetration (Cameron 1986, Taşman *et al.* 2000, Giardino *et al.* 2006) and more rapid exchange with fresh solution (Palazzi *et al.* 2012).

Sodium hypochlorite (NaOCl) solutions are recommended as the primary irrigant, because of their broad antimicrobial efficacy and their ability to dissolve tissue remnants (Zehnder 2006), which have been assumed to be enhanced by reducing its surface tension (Palazzi *et al.* 2012). NaOCl is commercially available as an aqueous solution with concentrations ranging from 0.5% to 15% (Rutala & Weber 1997), and may contain surfactants, as well as other components that are not always readily available (Clarkson *et al.* 2011). The surface tension values of NaOCl preparations reported in the literature are highly variable, owing to differences in the solutions tested, the temperature and the methodologies used to measure its values (Abou-Rass & Patonai 1982, Pècora *et al.* 1991, Spanó *et al.* 2001, Giardino *et al.* 2006). The purpose of this article is to review the effects of lowering the surface tension of NaOCl solutions and the clinical significance of this in endodontics.

Materials and methods

A literature search using the Medline electronic database was conducted for articles published up to the 28 July 2012, using the following search terms and combinations: 'sodium hypochlorite AND surface tension or interfacial force or interfacial tension or surface-active agent or amphiphilic agent or surface active agent or surfactant or tenside or detergent'.

Publications were included if the effects of surface-active agents on the clinical performance of NaOCl in root canal treatment were stated, if solutions of NaOCl containing surface-active agents were compared with plain solutions and if they were published in English. The titles and abstracts of the publications identified were initially screened by two independent reviewers (GRF and JP). Publications were included for full text evaluation by one reviewer (GRF). Full text assessment and data extraction were performed by one reviewer (GRF) if the content of the abstracts met the inclusion criteria. Furthermore, to include the most recent publications, a hand search of articles published online, 'in-press' and 'early view' was performed using the same search criteria as the electronic search for the International Endodontic Journal, Journal of Endodontics, Dental Traumatology, Australian Endodontic Journal and Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology. The date of the last search was 28 July 2012. The reference lists of those articles included were checked for additional articles of relevance. 302 publications were identified using the database, and 11 were included in the review.

Review

Table 1 summarizes the reported effect of the addition of surfactants to NaOCl solutions.

Irrigant penetration

Modification of NaOCl through the addition of surface-active agents increases the ability of this irrigant to penetrate the main root canal *in vitro* (Abou-Rass & Patonai 1982, Cunningham *et al.* 1982). When ethanol was added to NaOCl, it was found that this combination moved further in the capillary tubes when compared with NaOCl alone, with mean distances of combinations ranging from 30.15 to 45.93 mm, depending on the amount of ethanol contained in the mixtures (Cunningham *et al.* 1982). Similar results were obtained by adding the chemical

Table 1 Reported effects of surface tension reduction of sodium hypochlorite

Preparations tested	Result	Authors	Journal	Year	Volume, pages
Penetration and spread NaOCl+Ethanol NaOCl+polysorbate 80	Increased penetration in glass micropipettes Increased flow into root canals after 5 min. No differences after 7 days	Cunningham et al. Abou-Rass & Patonai	<i>Oral Surgery</i> <i>Oral Surgery</i>	1982 1982	54, 333-5 54, 524-6
Antibacterial activity Chlor-Xtra	No difference in bacterial activity on biofilms of <i>E. faecalis</i>	Williamson et al.	<i>Journal of Endodontics</i>	2009	35, 95-7
Chlor-Xtra, NaOCl+cetrimide	Antibacterial activity on dentine specimens infected with <i>E. faecalis</i> improved for 2% NaOCl only; no differences for 6% NaOCl	Wang et al.	<i>Journal of Endodontics</i>	2012	38, 948-53
NaOCl+Proteolytic enzyme+ surfactant Tissue dissolution NaOCl+Fluorad 99	Reduced antimicrobial efficacy against some bacterial strains in an agar diffusion test No difference in total human pulp dissolution time	Poggio et al. Cameron	<i>The International Journal of Artificial Organs</i> <i>Australian Dental Journal</i>	2010 1986	33, 654-9 31, 364-8
Domestic and proprietary solutions	Reduced total porcine pulp dissolution times for 1% concentration. No difference for 4% preparations	Clarkson et al.	<i>Australian Dental Journal</i>	2006	51, 245-51
Chlor-Xtra Chlor-Xtra and a household bleach HypoChlor	Increased bovine muscle tissue weight loss No difference in bovine pulp tissue weight reduction No difference in total porcine pulp dissolution times	Stojicic et al. Jungbluth et al. Clarkson et al.	<i>Journal of Endodontics</i> <i>Journal of Endodontics</i> <i>Journal of Endodontics</i>	2010 2012 2012	36, 1558-62 38, 372-5 38, 1257-60
Free available chlorine (FAC) stability NaOCl+Ethanol	Marked loss in 15-30 min, depending on ethanol%	Cunningham et al.	<i>Oral Surgery</i>	1982	54, 333-5
NaOCl+Fluorad 99	Less stable than unmixed solution over a 2-month period. Colour changes, formation of a precipitate and gas release at completion of the tests	Cameron	<i>Australian Dental Journal</i>	1986	31, 364-8
FAC loss prevention because of interactions with EDTA Proprietary solutions and household bleach	Varies depending on proportions and preparations	Clarkson et al.	<i>Journal of Endodontics</i>	2011	37, 538-43

polysorbate 80 to reduce the surface tension of NaOCl. The solutions were placed in the pulp chamber of the teeth and allowed to penetrate passively into the root canals; the average distance from the apex was reduced for the modified solutions. Most of the significant findings were obtained after 5 min of experimental time, with the distance from apex to solution level of 6.4 mm against 2.6 mm for the 2.6% NaOCl groups, whilst for the 5.25% NaOCl the values were 7.7 and 3.6 mm, respectively. A further measurement taken a week later revealed no significant increase of flow into the canals (Abou-Rass & Patonai 1982).

Antibacterial activity

Overall, the evidence remains inconclusive regarding the effects of reducing the surface tension of NaOCl on its antimicrobial effect. Indeed, an investigation into an *Enterococcus faecalis* biofilm model on glass slides found no significant differences in bactericidal activity when comparing various 6% NaOCl solutions. The solutions tested were Chlor-Xtra (Vista Dental Products, Racine, Wisconsin, USA), containing a wetting agent and proprietary surface modifiers, and a regular preparation (Williamson *et al.* 2009). Similar results were obtained using a dentine infection model, with the same microorganism. This group compared 2% and 6% NaOCl solutions, with or without cetrimide (a cationic surfactant with antibacterial effect commonly used for skin disinfection) as well as Chlor-Xtra. No differences were found between the three 6% solutions; however, differences were apparent amongst the 2% NaOCl solutions, with an increased antibacterial effect for the cetrimide-containing solution. The authors suggest two explanations for the differences in the lower concentration preparation: the antibacterial action of the cetrimide *per se* and improved penetration into the dentinal tubules of the irrigant in the model (Wang *et al.* 2012). A different investigation looking into the antimicrobial activity of a conventional 5.25% NaOCl solution and another modified with the addition of a proteolytic enzyme and a surfactant found that the conventional preparation gave larger average inhibition zones in an agar disc diffusion tests, when tested against different bacterial strains (Poggio *et al.* 2010). Apart from the 6% solution, where a modification of the surface tension appeared to have no effect on its antimicrobial action, it is not possible to reach a definitive conclusion for other concentrations; this is because of inconsistent

results and the presence of confounding factors, such as the addition of a proteolytic enzyme or a detergent with antibacterial action (cetrimide) in the test solutions with lowered surface tension.

Tissue dissolution

Several investigations (Cameron 1986, Clarkson *et al.* 2006, 2012, Stojicic *et al.* 2010, Jungbluth *et al.* 2012) have considered the effects of adding surfactants to NaOCl and its consequences on tissue dissolution ability, with contrasting results. Overall, based on the most current literature, it seems clear that reducing the surface tension of NaOCl preparations has no influence on tissue dissolution capability.

An investigation looking into the human pulp tissue dissolution ability of a modified household bleach with 4% free available chlorine (FAC) by adding a surface-active agent (Fluorad 99), was compared with the original preparation. When looking at complete tissue dissolution, no difference was found between the solutions (Cameron 1986). The porcine pulp dissolution capacity of some NaOCl solutions commercially available in Australia as 'domestic chlorine bleach', infant sanitizer and proprietary solutions locally approved for endodontic treatment (Hypochlor, Dentalife, Croydon, Victoria, Australia) was tested. The products included concentrations of 1% and 4%, and most included a surface tension agent. When investigating total dissolution, greater concentrations provided shorter dissolution times, whilst solutions with 1% concentration and containing surface-active agents were more effective than that which contained none (Clarkson *et al.* 2006). Similar results were obtained when a NaOCl product containing a surface-active agent (Chlor-Xtra) was compared with conventional products for bovine muscle tissue weight loss. The solutions were tested at different temperatures and concentrations (1–5.8%), and with agitation by sonic, ultrasonic or pipetting. The preparation containing the surface-active agent was shown to dissolve significantly more tissue than other solutions in every temperature and concentration, as well as under agitation at the maximum concentration tested (Stojicic *et al.* 2010). The recently published literature suggests that reduced surface tension does not affect bovine pulp dissolution (Clarkson *et al.* 2012, Jungbluth *et al.* 2012). One group investigated NaOCl solutions from dental suppliers (Chlor-Xtra), a technical-grade solution and household bleaches. They considered reduction in tissue weight following

immersion of the tissues in the solutions, in identical experimental conditions. Chemical assessment of different batches of bottles of Chlor-Xtra showed a marked difference in chlorine content. Subsequently, the authors considered questionable the results in publications where the FAC of NaOCl solutions was not evaluated (Jungbluth *et al.* 2012). A second investigation tested the effect of surface-active agents on porcine pulpal dissolution of 1% and 4% NaOCl solutions, with and without surfactants. Those with surfactants are specifically produced for endodontic use in Australia (Hypochlor) and contain a surfactant approved for pharmaceutical use. Their results found no variation of dissolution times because of the presence of surface-active agents. This group highlighted the methodological errors in the literature suggesting that modification of the surface tension would improve the tissue dissolution of NaOCl solutions, such as failure to control the FAC and osmolality of the solutions tested; furthermore, it was mentioned that differences in the quantity and type of the surfactant used can give different results (Clarkson *et al.* 2012).

Stability

The addition of surface-active agents modifies the stability of sodium hypochlorite (Cunningham *et al.* 1982, Cameron 1986). Information is only available regarding experimental preparations, and appears not to have been looked into in terms of commercially available preparations within the endodontic literature. Ethanol reduces the FAC with a greater and faster effect with higher alcohol concentration (Cunningham *et al.* 1982), and when 50% ethanol is added to 2% NaOCl, the solutions are almost depleted from their FAC in 15 min, whilst 30% ethanol mixtures had a 70% loss after 30 min (Cunningham *et al.* 1982). Mixtures containing a different agent (Fluorad 99) showed superior stability over a 2-month period when compared to those containing ethanol. In this investigation, over a one- and 2-month period, respectively, the test solution lost 5% and 26% of the original FAC (Cameron 1986). On completion of these tests, the sample containing Fluorad 99 changed colour from clear, light yellow to a dull brown, with a brown deposit formed; furthermore, gas was present in the solution and was liberated in the container (Cameron 1986).

The influence of surfactants on the chlorine loss of NaOCl solutions due to interactions with EDTA has been investigated. The time-related effect (between 5

and 18 min) of an EDTA solution (17%, containing a surfactant) was tested when in contact with NaOCl preparations, including 'domestic chlorine bleaches', solutions designed for endodontic use (Hypochlor) and an infant sanitizer (concentrations 1, 1.5, 4 and 4.5% with some containing surfactant). This investigation found that products containing surface-active agents exhibited a markedly lower FAC reduction at some dilutions, and *vice versa*. Differences between solutions with surfactants were found: the 'domestic bleach' often performed better when compared to preparations for endodontic use (Clarkson *et al.* 2011). Thus, the results appear to be inconclusive.

Discussion

The present review included only NaOCl with modified surface tension, compared with standard solutions. Several investigations excluded from the review studied the use of a surface tension agent prior to the use of NaOCl, suggesting that this leads to enhanced debridement (Gambarini 1999) and bacterial elimination from dentinal tubules (Berutti *et al.* 1997). These publications were not included, because the tensioactive agent was used to lower the surface tension and prepare the canal walls for the subsequent NaOCl, thus comparing different sequences rather than different NaOCl solutions (Berutti *et al.* 1997, Gambarini 1999). None of the investigations included considered other desired irrigant actions such as the effect of surface tension on flushing action and the prevention of the formation and removal of the smear layer by NaOCl (Zehnder 2006).

NaOCl solutions may vary according to chemical characteristics such as FAC, pH, presence of surface-active agents and osmolality, and this has subsequent implications for research and practice. The role of FAC on NaOCl's tissue dissolution effect is well known (Hand *et al.* 1978, Spanó *et al.* 2001, Clarkson *et al.* 2006); likewise, pH has been shown to have an effect on NaOCl's antimicrobial and tissue dissolution actions (Rossi-Fedele *et al.* 2011). Because NaOCl solutions are inherently unstable (Rutala *et al.* 1998), investigations of chlorine-containing solutions should include a chemical assessment, including chlorine concentration, as part of the methodology, so as to obtain valid results. This analysis was not carried out in most of the literature included in the review. Nonetheless, clinicians should know the characteristics of the NaOCl solutions used in their daily routine and understand its influence on clinical performance.

The root canal system is not a dry surface due to its intrinsic moisture content, with a value of approximately 12% suggested for vital and endodontically treated teeth (Papa *et al.* 1994), and due to potential seepage of interstitial fluids. As surface tension reduction is able to improve rewetting of dry surfaces only, the diffusion of chlorine to the targeted areas should be the deciding factor for the action of NaOCl in areas not accessible to mechanical preparation (Jungbluth *et al.* 2012). In the experimental models that have looked into the effects of surface-active agents on NaOCl's clinical performance in endodontics, this factor has not so far been taken into consideration and needs further investigation. For example, the effects of decreasing surface tension on NaOCl penetration have been studied using glass tubes (Cunningham *et al.* 1982) or extracted teeth that might have desiccated in the laboratory (Abou-Rass & Patonai 1982), and thus might not correspond to the naturally wet tooth.

Conclusion

The evidence available suggests that surface-active agents improve the penetration of NaOCl in the main canal and have no effect on its pulp tissue dissolution ability. There are, however, insufficient data to enable a sound conclusion to be drawn regarding the effect of modifying NaOCl's surface tension on lubrication, antimicrobial and debris or smear layer removal capabilities.

References

- Abou-Rass M, Patonai FJ (1982) The effects of decreasing surface tension on the flow of irrigating solutions in narrow root canals. *Oral Surgery* **54**, 524–6.
- Berutti E, Marini R, Angeretti A (1997) Penetration ability of different irrigants into dentinal tubules. *Journal of Endodontics* **23**, 725–7.
- Cameron JA (1986) The effect of a fluorocarbon surfactant on the surface tension of the endodontic irrigant, sodium hypochlorite. *Australian Dental Journal* **31**, 364–8.
- Clarkson RM, Moule AJ, Podlich HM *et al.* (2006) Dissolution of porcine incisor pulps in sodium hypochlorite solutions of varying compositions and concentrations. *Australian Dental Journal* **51**, 245–51.
- Clarkson RM, Podlich HM, Moule AJ *et al.* (2011) Influence of ethylenediaminetetraacetic acid on the active chlorine content of sodium hypochlorite solutions when mixed in various proportions. *Journal of Endodontics* **37**, 538–43.
- Clarkson RM, Kidd B, Evans GE, Moule AJ (2012) The effect of surfactant on the dissolution of porcine pulpal tissue by sodium hypochlorite solutions. *Journal of Endodontics* **38**, 1257–60.
- Cunningham WT, Cole JS, Balekjian AY (1982) Effect of alcohol on the spreading ability of sodium hypochlorite endodontic irrigant. *Oral Surgery* **54**, 333–5.
- Gambarini G (1999) Shaping and cleaning the root canal system: a scanning electron microscopic evaluation of a new instrumentation and irrigation technique. *Journal of Endodontics* **25**, 800–3.
- Giardino L, Ambu E, Becce C, Rimondini L, Morra M (2006) Surface tension of four common root canal irrigants and two new irrigants containing antibiotics. *Journal of Endodontics* **32**, 1091–3.
- Gu LS, Kim JR, Ling J, Choi KK, Pashley DH, Tay FR (2009) Review of contemporary irrigant agitation techniques and devices. *Journal of Endodontics* **35**, 791–804.
- Gulabivala K, Patel B, Evans G, Ng Y-L (2005) Effects of mechanical and chemical procedures on root canal surfaces. *Endodontic Topics* **10**, 103–22.
- Gutmann JL, Dumsha TC, Lovdahl PE (2006) *Problem Solving in Endodontics*, 4th edn. St Louis, MO, USA: Elsevier.
- Hand RE, Smith NL, Harrison JW (1978) Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. *Journal of Endodontics* **4**, 60–4.
- Jungbluth H, Peters C, Peters O, Sener B, Zehnder M (2012) Physicochemical and pulp tissue dissolution properties of some household bleach brands compared with a dental sodium hypochlorite solution. *Journal of Endodontics* **38**, 372–5.
- Nair PNR, Henry S, Cano V, Vera J (2005) Microbial status of root canal system of human mandibular first molars with primary apical periodontitis after “one visit” endodontic treatment. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology* **99**, 231–52.
- Nixdorf DR, Moana-Filho EJ, Law AS, McGuire LA, Hodge JS, John MT (2010) Frequency of persistent tooth pain after root canal therapy: a systematic review and meta-analysis. *Journal of Endodontics* **36**, 224–30.
- Palazzi F, Morra M, Mohammadi Z, Grandini S, Giardino L (2012) Comparison of the surface tension of 5.25% sodium hypochlorite solution with three new sodium hypochlorite-based endodontic irrigants. *International Endodontic Journal* **45**, 129–35.
- Papa J, Cain C, Messer HH (1994) Moisture content of vital vs endodontically treated teeth. *Endodontics and Dental Traumatology* **10**, 91–3.
- Pêcora JD, Guimarães LF, Savioli RN (1991) Surface tension of several drugs used in endodontics. *Brazilian Dental Journal* **2**, 123–7.
- Poggio C, Arciola CR, Dagna A, Chiesa M, Sforza D, Visai L (2010) Antimicrobial activity of sodium hypochlorite-based solution. *The International Journal of Artificial Organs* **33**, 654–9.

- Rossi-Fedele G, Guastalli AR, Dođramacı EJ, Steier L, De Figueiredo JAP (2011) Influence of pH changes on chlorine-containing endodontic irrigating solutions. *International Endodontic Journal* **44**, 792–9.
- Rutala WA, Weber DJ (1997) Uses of inorganic hypochlorite (bleach) in health-care facilities. *Clinical Microbiology Reviews* **10**, 597–610.
- Rutala WA, Cole EC, Thomann CA, Weber DJ (1998) Stability and bactericidal activity of chlorine solutions. *Infection Control and Hospital Epidemiology* **19**, 323–7.
- Spanó JC, Barbin EL, Santos TC, Guimarães LF, Pêcora JD (2001) Solvent action of sodium hypochlorite on bovine pulp and physico-chemical properties of resulting liquid. *Brazilian Dental Journal* **12**, 154–7.
- Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M (2010) Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. *Journal of Endodontics* **36**, 1558–62.
- Taşman F, Çehreli Z, Oğan C, Etikan I (2000) Surface tension of root irrigants. *Journal of Endodontics* **26**, 586–7.
- Wang Z, Shen Y, Ma J, Haapasalo M (2012) The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. *Journal of Endodontics* **38**, 948–53.
- Williams RAD, Elliot JC (1978) *Basic and Applied Dental Biochemistry*, 1st edn. Edinburgh, UK: Churchill Livingstone.
- Williamson AE, Cardon JW, Drake DR (2009) Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of *Enterococcus faecalis*. *Journal of Endodontics* **35**, 95–7.
- Zehnder M (2006) Root canal irrigants. *Journal of Endodontics* **32**, 389–98.

Study 4 – Published in Journal of Applied Oral Science

Review

Antagonistic Interactions between Sodium Hypochlorite, Chlorhexidine, EDTA, and Citric Acid

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Evaluation of the antimicrobial effect of super-oxidized water (Sterilox[®]) and sodium hypochlorite against *Enterococcus faecalis* in a bovine root canal model

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ABSTRACT

Ideally root canal irrigants should have, amongst other properties, antimicrobial action associated with a lack of toxicity against periapical tissues. Sodium hypochlorite (NaOCl) is a widely used root canal irrigant, however it has been shown to have a cytotoxic effect on vital tissue and therefore it is prudent to investigate alternative irrigants. Sterilox's Aquatine Alpha Electrolyte[®] belongs to the group of the super-oxidized waters; it consists of a mixture of oxidizing substances, and has been suggested to be used as root canal irrigant. Super-oxidized waters have been shown to provide efficient cleaning of root canal walls, and have been proposed to be used for the disinfection of medical equipment. Objective: To compare the antimicrobial action against *Enterococcus faecalis* of NaOCl, Optident Sterilox Electrolyte Solution[®] and Sterilox's Aquatine Alpha Electrolyte[®] when used as irrigating solutions in a bovine root canal model. Methodology: Root sections were prepared and inoculated with *E. faecalis* JH2-2. After 10 days of incubation the root canals were irrigated using one of three solutions (NaOCl, Optident Sterilox Electrolyte Solution[®] and Sterilox's Aquatine Alpha Electrolyte[®]) and subsequently sampled by grinding dentin using drills. The debris was placed in BHI broth and dilutions were plated onto fresh agar plates to quantify growth. Results: Sodium hypochlorite was the only irrigant to eliminate all bacteria. When the dilutions were made, although NaOCl was still statistically superior, Sterilox's Aquatine Alpha Electrolyte[®] solution was superior to Optident Sterilox Electrolyte Solution[®]. Conclusions: Under the conditions of this study Sterilox's Aquatine Alpha Electrolyte[®] appeared to have significantly more antimicrobial action compared to the Optident Sterilox Electrolyte Solution[®] alone, however NaOCl was the only solution able to consistently eradicate *E. faecalis* in the model.

Key words: Sodium hypochlorite. Super-oxidized water. Electrochemically activated solutions. Root canal irrigation. *Enterococcus faecalis*.

INTRODUCTION

Endodontic irrigants should have, amongst other properties, a broad antimicrobial spectrum of activity against anaerobic and facultative microorganisms growing in biofilms and a relative absence of toxicity against periapical tissues and

oral mucosa²⁵.

Sodium hypochlorite (NaOCl) is recommended as the main root canal irrigant because of its broad antimicrobial activity, the capacity to prevent formation of and dissolve the smear layer, in association with chelating agents, and its ability to dissolve tissue remnants²⁵. However, NaOCl has

been shown to have a cytotoxic effect on vital tissue and can therefore elicit inflammatory reactions if it reaches the periapex¹³. Furthermore, NaOCl has been shown to cause a change in the force required to fracture dentin²⁴, and a reduction of the elastic modulus and flexural strength of dentin^{5,21}. Furthermore NaOCl corrodes Protaper NiTi Rotary¹ (Dentsply/Maillefer, Baillagues, Switzerland) and carbon steel instruments³, cause early fractures of ProTaper (Dentsply/Maillefer) instruments¹ and, when heated, reduce resistance to cyclic fatigue of nickel-titanium files¹⁶.

Sterilox's Aquatine Alpha Electrolyte® (Optident Dental, Ilkley, West Yorkshire, UK) is a super-oxidized water that consists of a mixture of oxidizing substances including hypochlorous acid (HOCl) at a concentration of 144 mg/L, with a pH of 5.0-6.5 and a redox potential of >950 mV²⁰. The manufacturer suggests that the production of HOCl in the Sterilox Dental System (Optident Dental) does not produce free radical Cl and that the available free chlorine in the solution is 200 PPM, that is larger than the concentration reported in literature.

Super-oxidized water has been suggested as an alternative to NaOCl, as it provides efficient cleaning of root canal walls²², and has been recommended for the disinfection of endoscopes¹⁹, dental unit water lines¹⁰ and dental impression materials¹¹.

The aim of this study was to compare the antimicrobial action against *Enterococcus faecalis* of NaOCl, Optident Sterilox Electrolyte Solution® (Optident Dental) and Sterilox's Aquatine Alpha Electrolyte® when used as irrigating solutions in an *E. faecalis* infected bovine root canal model.

MATERIAL AND METHODS

Bovine incisors were used throughout this study. The study exerted no influence on the animal's fate at any stage as they were previously slaughtered in an Italian slaughterhouse for commercial purposes. The apical 5 mm and the crown of each incisor were dissected and the remaining root was cut into 1 cm slices with a diamond disc (Abrasive Technology Inc, Westerville, OH, USA). Subsequently the canal lumen was widened to a minimal diameter of 1.4 mm using the ParaPost® XP™ Endodontic post system drills (Coltene/Whaledent, Konstanz, Germany).

Finally the smear layer was removed via copious irrigation in an EDTA solution (Smear Clear, SybronEndo, Scafati, Italy) (4 min) and NaOCl (Teepol Bleach, Teepol, Orpington, UK) (4 min) in an ultrasonic bath.

Fifteen roots were placed individually in 10 mL of Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, UK) and autoclaved. These were left to cool to room temperature and then incubated overnight at 37°C to verify the sterility of the samples. The BHI broths containing the roots were

inoculated with 100 µL of an overnight culture of *E. faecalis* JH2-2⁷ and incubated for 10 days at 37°C to allow for bacterial growth, infiltration of the dentin tubules and *E. faecalis* JH2-2 biofilm formation.

The roots were divided into 3 groups, according to the irrigant used: group 1 was irrigated with the Optident Sterilox Electrolyte Solution® (this is essentially saline and was used as our negative control), group 2 was irrigated with 4% NaOCl (Teepol Bleach) and group 3 was irrigated with freshly prepared Sterilox's Aquatine Alpha Electrolyte® solution. The concentration of the NaOCl solution was tested by iodometric titration. The obtained value amounted to 3.9.

After sealing the apical portion with autoclaved physiowax (RA Lamb Ltd, Eastbourne, UK), 5 cc of the selected irrigant was dispensed using a 27 gauge Monoject syringe (Kendall, Tyco, Mansfield, MA, USA) in an up-and-down motion, and left *in situ* for 3 min.

Following the removal of the apical seal to allow for the irrigation solution to drain, the coronal 5 mm portion of the specimen was sampled by grinding dentin and canal contents using ParaPost® XP™ Endodontic post system drill (Coltene/Whaledent) with a diameter of 1.5 mm.

Debris collected in the flutes of each drill was placed in a 1.5 mL microcentrifuge tube containing 1 ml BHI broth. After vortexing for 10 sec, a serial dilution of the debris containing BHI broth was made and 100 µL of neat, 10⁻², 10⁻⁴ and 10⁻⁶ dilutions were plated in duplicate onto fresh BHI agar plates and incubated overnight at 37°C. To confirm the morphology and Gram group of the bacterial cells, Gram staining was performed.

In order to determine if the carry-over of NaOCl could prevent the growth of cells in the broths an additional experiment was carried out on sterile bovine teeth. These had been treated the same as the teeth used above except that they had not been inoculated with *E. faecalis*. After irrigation with NaOCl, the debris from the drill flutes were put into 900 µL of BHI and 100 µL of stationary phase *E. faecalis* culture was added. This was serially diluted as above and dilutions plated out as before. In addition, one group of teeth was irrigated with sterile water to provide a negative control.

All data were compared stratified by dilutions. Colony-forming units (cfu) with too many to count (TMTc) (defined here as >800 cfu per agar plate) were attributed the highest rank in a non-parametric approach with ANOVA on ranks with a Duncan *post-hoc*. Differences were considered significant at p<0.05. To double check the results an additional non parametric approach was conducted. All data was compared stratified by dilutions. The Kruskal-Wallis test was used to compare the three groups involved followed by Mann-Whitney's test as a *post-hoc* procedure adjusted with Bonferroni correction for multiple comparisons. Significance

Table 1- Colony-forming units (cfu) in serial dilution plates. Each group contained 5 roots and serial dilutions and plating were carried out in duplicate. Whilst NaOCl (Group 2) was the only irrigant to eradicate the *E. faecalis* a significant difference was seen between the Optident Sterilox Electrolyte Solution® (Group 1) and the Sterilox’s Aquatine Alpha Electrolyte® irrigation (Group 3)

Group 1 - Optident Sterilox Electrolyte Solution® irrigation (negative control)										
Dilution	Root 1		Root 2		Root 3		Root 4		Root 5	
Neat	TMTC ^a	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	Root Shattered sample lost
10 ⁻²	178	188	285	49	532	578	28	38		
10 ⁻⁴	9	0	0	0	7	3	2	0		
10 ⁻⁶	0	0	0	0	0	0	0	0		
Group 2 - Sodium hypochlorite irrigation										
Dilution	Root 1		Root 2		Root 3		Root 4		Root 5	
Neat	0	0	0	0	0	0	0	0	0	0
10 ⁻²	0	0	0	0	0	0	0	0	0	0
10 ⁻⁴	0	0	0	0	0	0	0	0	0	0
10 ⁻⁶	0	0	0	0	0	0	0	0	0	0
Group 3 - Sterilox’s Aquatine Alpha Electrolyte® irrigation										
Dilution	Root 1		Root 2		Root 3		Root 4		Root 5	
Neat	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	TMTC	146	230
10 ⁻²	4	4	6	14	42	50	17	16	1	2
10 ⁻⁴	0	0	0	0	0	0	0	0	0	0
10 ⁻⁶	0	0	0	0	0	0	0	0	0	0

^a TMTC = too many to count (>800 cfu per plate).

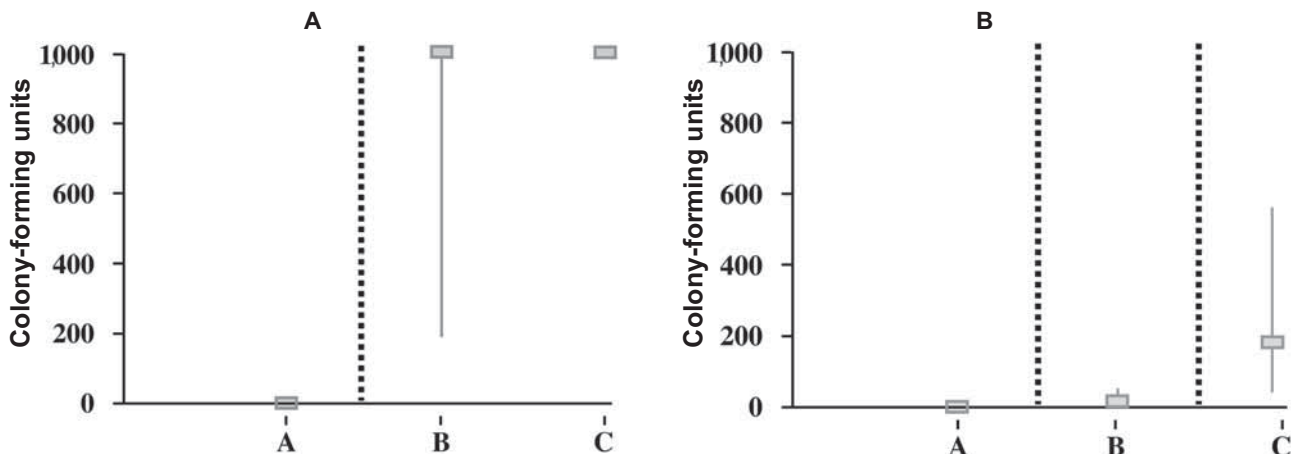


Figure 1- Mean number of colony-forming units recovered from debris after dentine grinding and root canal irrigation with NaOCl (A) - Sterilox’s Aquatine Alpha Electrolyte® (B) - or Optident Sterilox Electrolyte Solution® (C) - 1A; Neat broth (no dilution). 1B; 10⁻² dilution. The vertical dashed line shows significant difference between the groups on either side (P<0.05).

level was set at $\alpha=0.05$. Data were analyzed using SPSS software v. 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

The overnight incubation of the sectioned bovine root canals resulted in no growth in any of the samples. This indicates that all the root sections were sterile at the start of the experiment.

Gram staining of a number of the resulting colonies showed the presence of Gram-positive cocci, consistent with the *E. faecalis* inoculum. The

bacteria were morphologically identical to the *E. faecalis* used in the inoculum.

Results of the dilution series are presented in Table 1, while statistical analysis of the raw data for neat and 10⁻² dilution is presented in Figure 1. Results for statistical analysis coincided for both approaches.

NaOCl was the only irrigant to eliminate all bacteria and was significantly better at killing *E. faecalis* than both the Optident Sterilox Electrolyte Solution® (saline) and Sterilox’s Aquatine Alpha Electrolyte®. Additionally Sterilox’s Aquatine Alpha Electrolyte® solution was superior to Optident

Sterilox Electrolyte Solution®.

The experiment to determine the effect of carry-over of NaOCl resulted in similar colony counts for the samples from teeth irrigated with NaOCl and water (results not shown) indicating that carry over of NaOCl had no noticeable effect in our experiment.

DISCUSSION

This study evaluated the antimicrobial action of Sterilox's Aquatine Alpha Electrolyte®, a commercially available super-oxidized water in the United Kingdom, in bovine root canals.

E. faecalis was selected as the test species, because it is commonly detected in asymptomatic, persistent root canal infections^{12,15}. The bovine root model was chosen as it is clinically relevant, although the large root canal preparation size allows for more favorable dynamics of irrigation for the solution tested than is likely to occur *in vivo*. Additionally the number of bacteria present is likely to be artificially high compared to the *in vivo* situation. Despite these limitations, the *ex vivo* model has been successfully used previously to test the ability of *E. faecalis* to survive diverse root canal irrigations¹⁷.

Our study suggested that the protocol followed was either able to prevent carry-over of the antimicrobial effect of NaOCl onto the BHI plates, possibly due to drainage of the solutions after irrigation or any carry over had no effect on the viability of the organisms, possibly due to the immediate dilution of the samples in the BHI broth. Furthermore, based on pilot studies, it was decided to collect samples at a single depth as no difference was found between different depths of sampling when NaOCl was used as irrigant; this is consistent with the results from other investigators on a similar bovine tooth model⁸.

Sterilox's Aquatine Alpha Electrolyte® is obtained by passing a sodium chloride solution (Optident Sterilox Electrolyte Solution®) over coated titanium electrodes at 9 amps in a specifically made device (Optident Sterilox Dental Generator®; Optident Dental). Optident Sterilox Electrolyte Solution® (non-activated) was used as the negative control as we did not expect any antimicrobial action from this irrigant. NaOCl was tested, because it is largely recommended as the main root canal irrigant²⁵.

Endodontic literature suggests that infection of the root canal at the time of obturation has a negative influence on the prognosis of endodontic treatment²³; NaOCl was the only irrigant tested which was consistently associated with negative cultures in our study. However, Sterilox's Aquatine Alpha Electrolyte® might be able to reduce the bacterial load to levels that could influence treatment outcome.

These results are not consistent with those of a previous study where different irrigants, including

NaOCl, were tested against *E. faecalis* in a bovine tooth model, in fact Krause, et al.⁸ (2007) suggest that 5.25% NaOCl was not able to render the dentinal shavings obtained sterile, it was however, significantly more effective than the other solutions tested⁸. The major difference between the models is the difference in volumes of irrigation used; 60 µL twice against 5 cc in our study, therefore suggesting a role for the amount of irrigant used on the ability to eliminate root canal infection in the bovine root model; in the same way a previous investigation indicates that the volume of irrigation has a significant influence in removing a bio-molecular film from root canal walls⁶.

The disinfecting actions of super-oxidized water are heavily reduced in the presence of organic contamination^{11,20}. The model used in our study allows for a greater bacterial growth than one might expect in an *in vivo* situation. It further excludes the mechanical aspect of root canal preparation, so that the bacterial biomass present in the root canal is likely to be greater than in normal clinical conditions. Consequently, its elimination will depend exclusively on the flushing and chemical effects of the irrigation solution tested. The importance of instrumentation in obtaining a significant reduction in bacterial content has been shown¹⁴. Therefore we hypothesize that, in the presence of a reduced bacterial load, as a result of a chemo-mechanical preparation super-oxidized water irrigation might have the ability to eradicate a more clinically relevant root canal infection.

The result of a previous study² showed that super-oxidized water had no ability to prevent the growth of *E. faecalis* using paper disks as the delivery method on Petri dishes, a protocol more favorable to the irrigant when compared to the bovine root model due to long time of contact with the micro-organisms, absence of interaction with dentin and cells in a metabolically active phase, therefore more susceptible to antimicrobials⁹. Nonetheless, a different irrigation source was tested (Dermacyn, Oculus Innovative Sciences, Petaluma, CA, USA). Different super-oxidized waters are produced by a similar electrolysis process but, due to a difference in the active concentration and the pH of the final solution, the product can have a different antimicrobial activity¹⁸.

One of the suggested advantages of super-oxidized water, when compared to NaOCl, is its level of toxicity²⁰. It is worth noting, that the mechanism of action of super-oxidized water involves oxidative damage which might cause ageing and irreversible dysfunctions that eventually produce cellular death⁴. A pH-neutral super-oxidized solution (Microcyn; Dermacyn, Oculus Innovative Sciences, Petaluma, CA, USA) has been tested. It was found to be significantly less cytotoxic than antiseptic hydrogen peroxide concentrations (used as a positive control for oxidative damage) because it does not induce genotoxicity or accelerated ageing

*in vitro*⁴. However, Microcyn has a different pH than Sterilox's Aquatine Alpha Electrolyte® and this needs to be taken into account when comparing the two irrigants.

CONCLUSIONS

Under the conditions of this study Sterilox's Aquatine Alpha Electrolyte® appeared to have significantly more antimicrobial action when used as an irrigant in the root canal system compared to the non-activated Optident Sterilox Electrolyte Solution®, but NaOCl was the only irrigant able to eliminate all bacteria in our experiments. Sterilox's Aquatine Alpha Electrolyte® caused a bacterial load decrease although being less effective than NaOCl.

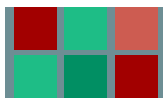
REFERENCES

- 1- Berutti E, Angelini E, Rigolone M, Migliaretti G, Pasqualini D. Influence of sodium hypochlorite on fracture properties and corrosion of ProTaper Rotary instruments. *Int Endod J.* 2006;39(9):693-9.
- 2- Davis JM, Maki J, Bahcall JK. An *in vitro* comparison of the antimicrobial effects of various endodontic medicaments on *Enterococcus faecalis*. *J Endod.* 2007;33(5):567-9.
- 3- Gallegos AG, Bertolotti RL. Effect of sodium hypochlorite on the strength of carbon steel endodontic instruments. *J Endod.* 1981;7(9):828-30.
- 4- González-Espinosa D, Pérez-Romano L, Guzmán-Soriano B, Arias E, Bongiovanni CM, Gutiérrez AA. Effects of pH-neutral, superoxidised solution on human dermal fibroblasts *in vitro*. *Int Wound J.* 2007;4(3):241-50.
- 5- Grigoratos D, Knowles J, Ng YL, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *Int Endod J.* 2001;34(2):113-9.
- 6- Huang TY, Gulabivala K, Ng YL. A bio-molecular film ex-vivo model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation. *Int Endod J.* 2008;41(1):60-71.
- 7- Jacob AE, Hobbs SJ. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zimogenes*. *J Bacteriol.* 1974;117(2):360-72.
- 8- Krause TA, Liewehr FR, Hahn CL. The antimicrobial effect of MTAD, sodium hypochlorite, doxycycline, and citric acid on *Enterococcus faecalis*. *J Endod.* 2007;33(1):28-30.
- 9- Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol.* 2001;9(1):34-9.
- 10- Martin MV, Gallagher MA. An investigation of the efficacy of super-oxidised (Optident/Sterilox) water for the disinfection of dental unit water lines. *Br Dent J.* 2005;198(6):353-4.
- 11- Martin N, Martin MV, Jedynakiewicz NM. The dimensional stability of dental impression materials following immersion in disinfecting solutions. *Dent Mater.* 2007;23(6):760-8.
- 12- Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J.* 1988;31(1):1-7.
- 13- Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effects of NaOCl on vital tissue. *J Endod.* 1985;11(12):525-8.
- 14- Pataky L, Iványi I, Grigár A, Fazekas A. Antimicrobial efficacy of various root canal preparation techniques: an *in vitro* comparative study. *J Endod.* 2002;28(8):603-5.
- 15- Peculiene V, Balciulienė I, Eriksen HM, Haapasalo M. Isolation of *Enterococcus faecalis* in previously root-filled canals in a Lithuanian population. *J Endod.* 2000;26(10):593-5.
- 16- Peters OA, Roehlike JO, Baumann MA. Effect of immersion in sodium hypochlorite on torque and fatigue resistance of nickel-titanium instruments. *J Endod.* 2007;33(5):589-93.
- 17- Rossi-Fedele G, Roberts AP. A preliminary study investigating the survival of tetracycline resistant *Enterococcus faecalis* after root canal irrigation with high concentrations of tetracycline. *Int Endod J.* 2007;40(10):772-7.
- 18- Sampson MN, Muir AV. Not all super-oxidized waters are the same. *J Hosp Infect.* 2002;52(3):228.
- 19- Selkon JB, Babb JR, Morris R. Evaluation of the antimicrobial activity of a new super-oxidized water, Sterilox®, for the disinfection of endoscopes. *J Hosp Infect.* 1999;41(1):59-70.
- 20- Shetty N, Srinivasan S, Holton J, Ridgway GL. Evaluation of microbicidal activity of a new disinfectant: Sterilox® 2500 against *Clostridium difficile* spores, *Helicobacter pylori*, vancomycin resistant *Enterococcus* species, *Candida albicans* and several *Mycobacterium* species. *J Hosp Infect.* 1999;41(2):101-5.
- 21- Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *Int Endod J.* 2001;34(2):120-32.
- 22- Solovyeva AM, Dummer PM. Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions; a pilot study *Int Endod J.* 2000;33(6):494-504.
- 23- Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1988;85(1):86-93.
- 24- White JD, Lacefield WR, Chavers LS, Eleazer PD. The effect of three commonly used endodontic materials on the strength and hardness of root dentin. *J Endod.* 2002;28(12):828-30.
- 25- Zehnder M. Root canal irrigants. *J Endod.* 2006;32(15):389-98.

Study 5 – Published in Australian Endodontic Journal

Bovine pulp tissue dissolution ability of HealOzone®, Aquatine Alpha Electrolyte® and sodium hypochlorite

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ORIGINAL RESEARCH

Bovine pulp tissue dissolution ability of HealOzone[®], Aquatine Alpha Electrolyte[®] and sodium hypochlorite

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Keywords

dental pulp, ozone, sodium hypochlorite, superoxidised water, tissue dissolution.

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Abstract

The aim of this study was to evaluate the bovine pulp tissue dissolution ability of HealOzone, Aquatine Alpha Electrolyte[®] and 0.5% sodium hypochlorite, used alone or in combination. Thirty bovine pulp fragments were weighed, divided into six groups and placed individually in Eppendorf tubes containing the tested solution until total dissolution occurred. The groups were: G1: saline (negative control), G2: Aquatine Alpha Electrolyte[®], G3: 0.5% NaOCl (positive control), G4: Saline + HealOzone, G5: 0.5% NaOCl + HealOzone, G6: Aquatine Alpha Electrolyte[®] + HealOzone. HealOzone was activated for 2 min with a #6 cup covering the test tube opening on a fixed platform. Two blinded observers using 2× loupes magnification assessed the samples continuously for the first 2 h, and then every hour for the next 8 h. Dissolution speed was calculated by dividing pulp weight by dissolution time (mg min⁻¹). G3 (NaOCl) and G5 (NaOCl + HealOzone) dissolved the pulp tissue completely. The mean dissolution speed for G3 was 0.396 mg min⁻¹ (SD 0.032) and for G5 was 0.775 mg min⁻¹ (SD 0.2). Student's *t*-test showed that G5 dissolved bovine pulp tissue faster than G3 (*P* = 0.01). Only groups containing sodium hypochlorite dissolved pulp tissue, whilst HealOzone enhanced speed of dissolution.

Introduction

Root canal irrigants should have the ability to dissolve pulp remnants (1), as removal of pulpal tissue is inadequate with mechanical preparation alone because of the morphological complexities of root canals (2). It is suggested that post-operative pain is more prevalent in vital than in non-vital cases (3) and pulp remnants can cause post-operative pain (4).

Sodium hypochlorite (NaOCl) is recommended as the main irrigant because of its broad antimicrobial activity, its capacity to prevent formation of and dissolve the organic part of the smear layer, and its ability to dissolve tissue remnants (1). However, it has been shown to have a cytotoxic effect on vital tissues, eliciting severe inflam-

matory reactions if it reaches the periapex (5), with 5.25% producing greater toxic and caustic effects than 0.5% and 1% solutions (5). Low NaOCl concentrations have reduced tissue dissolution capability (6) although this can be improved through increasing its temperature (3,4). It has been postulated that such solutions have reduced systemic toxicity when compared to higher concentration solutions (7).

Aqueous and gaseous ozone have been tested as antimicrobial agents for endodontic treatment although different studies reveal varying results. An investigation using the HealOzone delivery system in extracted teeth after carriage in the oral cavity suggests that ozone has good potential as an antimicrobial in endodontics (8) and it has been shown to be dose-, strain- and

Table 1 Test solutions' characteristics and manufacturers

Name of chemical	Description	Manufacturer
Aquatine Alpha Electrolyte	Chlorine concentration 200 p.p.m., pH 5	Optident Dental, Ilkley, West Yorkshire, UK
Optident Sterilox electrolyte solution	10% sodium chloride	Optident Dental, Ilkley, West Yorkshire, UK
Sodium hypochlorite	0.5% by dilution, confirmed via iodometric titration	Teepol Bleach, Teepol, Orpington, Kent, UK
HealOzone	$4.2 \times 10^6 \mu\text{g m}^{-3}$ ozone concentration	KaVo, Biberach, Germany

time-dependent against biofilm cells (9). Conversely a different investigation using a positive/negative culture analysis with longer time exposure to gaseous ozone and ozonated water found that these substances were not able to inactivate *Enterococcus faecalis* (10).

Ozonated water had no negative effect on periodontal cells (11) and when compared against other established antimicrobials ozonated water was shown to have the highest level of biocompatibility (12).

Superoxidised water is a form of disinfectant which is generated at the point of use by passing a saline solution over titanium-coated electrodes. According to the manufacturer Aquatine Alpha Electrolyte® (Optident Dental, Ilkley, West Yorkshire, UK) is a solution which is described as 'brine' consisting of a mixture of oxidising substances including hypochlorous acid, with 200 p.p.m. available free chlorine (AFC) at a pH of 5.0–6.5.

Electrochemically activated solutions have been suggested as irrigants as they provide efficient cleaning of root canal walls (13); Aquatine has been shown to have an antimicrobial effect against *E. faecalis* biofilms in a bovine root model (14).

The aim of this study was to evaluate the bovine pulp tissue dissolution ability of HealOzone, Aquatine and 0.5% NaOCl, used solely or in associations.

Materials and methods

Thirty bovine pulp fragments were used in this study. The animals were slaughtered for commercial purpose and therefore this study exerted no influence on the animals' fate. The teeth were stored frozen and left to thaw overnight at room temperature (20°C circa) before being split. The pulps were removed and divided into fragments using a #12 scalpel blade (Swann-Morton, Sheffield, UK) and weighed using precision scales (Sartorius BP61S, Göttingen, Germany). The pulp fragments were then divided into six groups of five and placed individually in 1.5 mL Eppendorf tubes filled with the test substance.

The groups were as follows: G1: saline (negative control) which is Optident Sterilox electrolyte solution® (Optident Dental, Ilkley, West Yorkshire, UK), not activated, G2: Aquatine (Optident Dental, Ilkley, West Yorkshire, UK), G3: 0.5% NaOCl (positive control)

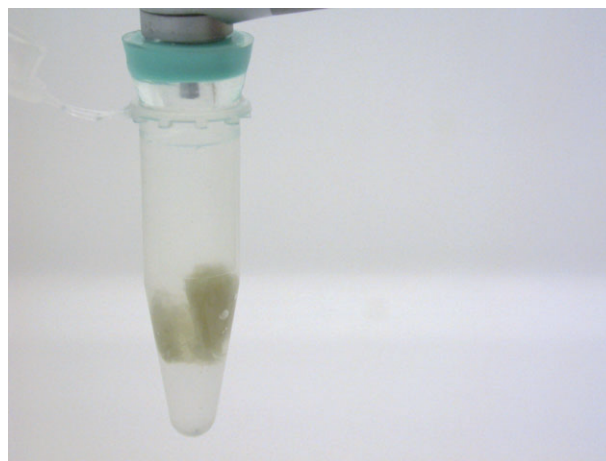


Figure 1 Pulp fragment immersed in solution with a HealOzone delivery cup achieving an airtight seal against the Eppendorf tube opening.

(Teepol Bleach, Teepol, Orpington, Kent, UK), G4: HealOzone + Saline, G5: HealOzone + 0.5% NaOCl, G6: HealOzone + Aquatine. Details of the above solutions are presented in Table 1.

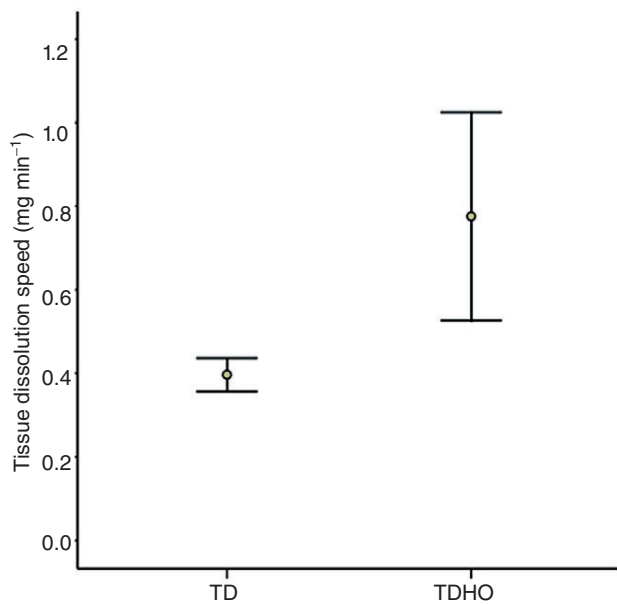
Following complete immersion of pulp fragments in the solutions, HealOzone gaseous ozone delivery system (KaVo, Biberach, Germany) with a $4.2 \times 10^6 \mu\text{g m}^{-3}$ ozone concentration was activated for 120 s with a #6 cup covering providing an airtight seal against the Eppendorf tube opening (Fig. 1) while resting in a fixed platform. All other groups remained in a resting state in the same fixed platform.

Two observers blinded to the experimental groups visually assessed the samples using 2× loupes for magnification. The samples were continuously monitored for the first 2 h, and then every hour for the next 8 h, or until complete dissolution occurred. Time taken for dissolution was recorded in minutes (min) and dissolution speed was calculated by dividing pulp weight (mg) by dissolution time (mg min^{-1}).

The NaOCl concentration was tested by iodometric titration and was 0.5%. Statistical analysis involved use of Student's *t*-test ($\alpha = 0.05$) to compare the means between the NaOCl groups.

Table 2 Mean weight (mg) and dissolution time (min) amongst test groups

Solution	G1 Saline	G2 Sterilox	G3 0.5% NaOCl	G4 HealOzone + Saline	G5 HealOzone + NaOCl	G6 HealOzone + Sterilox
Weight (mg)	12.2	13.2	13.4	13.4	12.4	13.2
Time to dissolution	no dissolution	no dissolution	34 min	no dissolution	16.4 min	no dissolution

**Figure 2** Mean speed (mg min^{-1}) of pulp dissolution within the NaOCl groups. TD: Group 3 (NaOCl), TDHO: Group 5 (NaOCl + HealOzone).

Results

Results are summarised in Table 2 and Figure 2.

Only G3 (NaOCl) and G5 (NaOCl + HealOzone) were able to dissolve pulpal tissue; no dissolution activity was evident in the remaining groups. The mean dissolution speed for G3 was $0.396 \text{ mg min}^{-1}$ (SD 0.032) and for G5 was $0.775 \text{ mg min}^{-1}$ (SD 0.2), and thus NaOCl + HealOzone dissolved bovine pulp tissue faster than NaOCl alone and this was statistically significant ($P = 0.01$).

Discussion

The aim of this study was to evaluate the bovine pulp tissue dissolution ability of HealOzone, Aquatine and 0.5% NaOCl, used solely or in associations.

Bovine pulp tissues are regarded as comparable to human pulp tissues despite some minor differences (15), and have been previously used to test the dissolution ability of different endodontic irrigants (16,17).

As greater NaOCl volume and contact surface area leads to greater tissue dissolution ability (2,18), it could be speculated that the Eppendorf tubes used in these experiments could have reduced the dissolution time compared with normal clinical (*in vivo*) conditions, as is most likely the case in previously reported *in vitro* investigations (2–4,6,16–18).

The results of this study appear to be in line with previous investigations where only NaOCl has shown tissue dissolution ability (16,19); one study has shown the mean dissolution speed for 0.5% sodium hypochlorite solutions as being 0.31 mg min^{-1} (16), which is very close to the dissolution speeds obtained in our assays. This might be explained by the differences in the volume of irrigant in contact with the pulp fragments (20 mL vs. 1.5 mL in our research) and perhaps a difference in the temperature of the solutions. An investigation into the tissue dissolution capacity of 5% dichloroisocyanurate, which in water releases hypochlorous acid that contains 'free chlorine', and therefore a potential for tissue dissolution, did not have any significant action against necrotised porcine palates (19); this is similar to our findings using Aquatine.

It has been suggested that 0.5% NaOCl solutions are ineffective as necrotic tissue solvents after contact for 7 min (6). Our investigations showed that when 0.5% NaOCl was evaluated beyond 7 min, its dissolution capacity was efficient as no pulp fragments remained; they were completely dissolved.

A previous investigation suggests that a similar time to the one used in our research to activate the irrigants (2.5 min vs. 2 min in our research) was able to achieve total elimination of microorganisms in an *in vitro* model with a low gas concentration (4 g m^{-3}) (9). HealOzone application for 40 s had similar antimicrobial ability when compared to NaOCl and MTAD (8); 2 min is a clinically relevant time period and, in our model, halved the time required to totally dissolve the pulp fragments.

The results in this study were reported as dissolution speed to compensate for fragment weight variability even though there was no statistical difference between total fragment weights amongst groups.

It has been suggested that ozone is approximately 10 times more soluble in water than oxygen and its half life in pyrogen-free water is 9–10 h (at pH 7 and 20°C) (20).

Conversely a different investigation suggests that when ozonated water is maintained at 22°C for 180 min its concentration decreases significantly with time and after 10 min is almost halved (21). Therefore, it is unclear whether using the HealOzone generating device, 2 min activation is likely to produce ozone present in solution for a clinically relevant period of time.

The mechanism for ozone to activate sodium hypochlorite is not fully understood. Volume of irrigation, agitation, temperature and solution concentration, mechanisms previously suggested to activate NaOCl, were unchanged between the groups and ozone activation did not cause an increase in temperature during the assays.

It has been suggested that the amount of available chlorine is responsible for the tissue-dissolving properties of hypochlorite solutions (22). We can hypothesise that ozone, being a strong oxidising agent, might be able to activate NaOCl directly, leading to the formation of oxygen molecules as well as extremely reactive atomic oxygen that possibly enhances the tissue dissolution capacity of the solution.

When comparing the tissue dissolution capacity of 0.5% NaOCl and Aquatine solutions it is necessary to take into account the differences in solvent concentration, as well as the differences in behaviour of the compounds. A 0.5% NaOCl solution will have a solvent concentration (mainly dissociated as hypochlorite ion, because of its high dissolution constant) 18 times larger than a 200 p.p.m. AFC Aquatine solution (AFC consists of hypochlorite ion and a larger proportion of HOCl because of a very low dissolution constant). Subsequently tissue dissolution might depend on the amount of hypochlorite ion rather than the AFC.

The ozone gas concentration provided by the HealOzone system has been shown to be slightly less cytotoxic than 2.5% NaOCl (23). An aqueous form of ozone at a concentration of 1.25–20 µg mL⁻¹ showed less cytotoxicity on human oral epithelial and gingival fibroblast cells when compared with 0.2% and 2% chlorhexidine digluconate, 2.25% and 5.25% sodium hypochlorite, 3% hydrogen peroxide (12). Ozonised water has no negative effect on periodontal cells remaining on tooth surfaces after irrigation for 2 min at a concentration of 2.5–3.5 ng mL⁻¹ (11).

Exposure to ozone causes acute changes in pulmonary function and development of symptoms (24) and therefore the HealOzone system has a safety mechanism that will stop the delivery of the gas if the seal between the delivery cup and the tooth is broken.

Reduced toxicity is one of the proposed advantages of superoxidised waters when compared to NaOCl (25). Another electrochemically activated water (Microcyn,

Oculus Innovative Sciences, Petaluma, CA, USA) was tested and found to be significantly less cytotoxic than antiseptic hydrogen peroxide concentrations (26). Microcyn is different to Aquatine and therefore results may not be directly transferable.

It is worth noting that it is not completely clear if 0.5% NaOCl solutions activated with HealOzone would minimise known side-effects such as tissue irritation and loss of resistance to mechanical stress of dentine when compared to more concentrated solutions that have a similar tissue dissolution capability.

In conclusion, only NaOCl was able to dissolve pulp tissue and HealOzone contributed to reducing the time for this. It could be speculated that dissolution properties of NaOCl could be enhanced by HealOzone allowing clinical use of more diluted concentrations with potentially reduced side-effects.

References

1. Zehnder M. Root canal irrigants. *J Endod* 2006; 32: 389–98.
2. Thé SD. The solvent action of sodium hypochlorite on fixed and unfixed necrotic tissue. *J Endod* 1979; 47: 558–61.
3. Abou-Rass M, Oglesby SW. The effects of temperature, concentration, and tissue type on the solvent ability of sodium hypochlorite. *J Endod* 1981; 7: 376–7.
4. Cunningham WT, Balekjian AY. Effect of temperature on collagen-dissolving ability of sodium hypochlorite irrigant. *Oral Surg Oral Med Oral Pathol* 1980; 49: 175–7.
5. Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effect of NaOCl on vital tissue. *J Endod* 1985; 11: 525–8.
6. Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. *J Endod* 1978; 4: 60–4.
7. Sirtes G, Waltimo T, Schaetzle M, Zehnder M. The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. *J Endod* 2005; 31: 669–71.
8. Virtej A, MacKenzie CR, Raab WHM, Pfeffer K, Barthel CR. Determination of the performance of various root canal disinfection methods after in-situ carriage. *J Endod* 2007; 33: 926–9.
9. Huth KC, Quirling M, Maier S *et al.* Effectiveness of ozone against endopathogenic microorganisms in a root canal biofilm model. *Int Endod J* 2009; 49: 3–13.
10. Estrela C, Estrela CRA, Decurcio DA, Hollanda AC, Silva JA. Antimicrobial efficacy of ozonated water, gaseous ozone, sodium hypochlorite and chlorhexidine in infected human root canals. *Int Endod J* 2007; 40: 85–93.

11. Ebensberger U, Pohl Y, Filippi A. PCNA-expression of cementoblasts and fibroblasts on the root surface after extraoral rinsing for decontamination. *Dent Traumatol* 2002; 18: 262–6.
12. Huth KC, Jakob FM, Saugel B *et al.* Effect of ozone on oral cells compared with established antimicrobials. *Eur J Oral Sci* 2006; 114: 435–40.
13. Solovyeva AM, Dummer PMH. Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions; a pilot study. *Int Endod J* 2000; 33: 494–504.
14. Rossi-Fedele G, Figueiredo JAP, Steier L, Canullo L, Steier G, Roberts AP. Evaluation of the antimicrobial effect of super-oxidised water (Sterilox®) and sodium hypochlorite against *Enterococcus faecalis* in a bovine root canal model. *J Appl Oral Sci* 2010; 18: 498–502.
15. Koskinen KP, Stenvall H, Uitto VJ. Dissolution of bovine pulp tissue by endodontic irrigants. *Scand J Dent Res* 1980; 88: 406–11.
16. Okino LA, Siqueira EL, Santos M, Bombana AC, Figueiredo JAP. Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel. *Int Endod J* 2004; 37: 38–41.
17. Rossi-Fedele G, Figueiredo JAP. Use of a bottle warmer to increase 4% sodium hypochlorite dissolution on bovine pulp. *Aust Endod J* 2008; 34: 39–42.
18. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. *Int Endod J* 1982; 15: 187–96.
19. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential irrigants. *J Endod* 2004; 30: 785–7.
20. Baysan A, Whiley RA, Lynch E. Antimicrobial effect of a novel ozone-generating device on micro-organisms associated with primary carious lesions in vitro. *Caries Res* 2000; 34: 498–501.
21. Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T. Efficacy of ozone on survival and permeability of oral microorganisms. *Oral Microbiol Immunol* 2004; 19: 240–6.
22. Zehnder M, Kosicki D, Luder H, Sener B, Waltimo T. Tissue-dissolving capacity and antibacterial effect of buffered and unbuffered hypochlorite solutions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; 94: 765–62.
23. Filippi A. The effect of ozonized water on epithelial wound healing. *Dtsch Zahnarzt Z* 2006; 54: 104–8.
24. Bates DV, Bell GM, Burnham CD *et al.* Short term effects of ozone on the lung. *J Appl Physiol* 1972; 32: 176–81.
25. Selkon JB, Babb JR, Morris R. Evaluation of the antimicrobial activity of a new super-oxidised water, Sterilox®, for the disinfection of microscopes. *J Hosp Infect* 1999; 41: 59–70.
26. González Espinosa D, Pérez Romano L, Guzmán Soriano B, Arias E, Bongiovanni CM, Gutiérrez AA. Effects of pH-neutral, superoxidised solution on human dermal fibroblasts in vitro. *Int Wound J* 2007; 4: 241–50.

Study 6 – Published in British Dental Journal

Some factors influencing the stability of Sterilox®, a super-oxidised water

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Some factors influencing the stability of Sterilox[®], a super-oxidised water

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IN BRIEF

- This article studies the stability of Sterilox solutions and some factors that might cause changes in chlorine concentration and pH
- Sterilox was found to be stable for a 14-day period when stored in ideal conditions
- Sunlight exposure accelerates the decomposition rate of chlorine
- The presence of air in the bottle does not influence chlorine decomposition

Super-oxidised waters, particularly Sterilox[®], have been suggested for the disinfection of dental unit water lines and dental impression materials owing to their antimicrobial efficacy. One of the previously suggested characteristics is their short shelf life. The purpose of this investigation was to understand the effect of storage conditions on Sterilox[®]'s stability. Eight bottles (four completely full, four half-full) of freshly prepared solution were divided into four groups and subsequently stored by being either exposed to or protected from sunlight. The chlorine concentration was monitored using chlorine test strips until the concentration reached zero, or until the thirteenth week. Statistically significant differences between the groups exposed to sunlight and the non-exposed groups ($p < 0.001$) were found. The mean loss of chlorine per day for the non-exposed samples was 1.01 mg/L, whilst the mean for the exposed samples was 2.42 mg/L. The presence of air did not affect the chlorine decomposition in the bottles. The results of this investigation indicate that when the solution is exposed to sunlight, the decrease of chlorine starts at day 4, whilst for the groups sheltered from sunlight, the process started after day 14. Therefore, Sterilox[®] solutions appear to be more stable than previously surmised.

INTRODUCTION

Super-oxidised waters (also known as electrochemically activated solutions) are produced from saline solutions following electrolysis by passing over titanium electrodes.¹ In dentistry they have been proposed for the removal of bacteria from dental unit water supplies¹ and disinfection of common impression materials,² and their antimicrobial and cleaning effectiveness in root canals have been studied.^{3,4}

Sterilox[®] is a super-oxidised water currently used to decontaminate endoscopes.

Its main component is hypochlorous acid (HOCl),^{5,6} the recommended concentration of which has been suggested to be between 144 mg/L^{5,6} and 240 mg/L,⁷ whilst the manufacturer suggests 200 mg/L chlorine concentration in the solution (personal communication). Its pH has been described as 5–6.5,^{5,6} 6.3,⁷ and between 2 and 14, but this can be controlled between 2.7 and 6.8.⁸ Meanwhile, the manufacturer suggests a pH of between 5 and 7.⁹

The decomposition and evaporation of chlorine solutions leads to a loss of chlorine, and hence to a loss of antimicrobial activity.¹⁰ It has been suggested that Sterilox[®] solutions are inherently unstable and are highly antimicrobial when freshly generated.^{5,6,11} However, the effects of time and storage conditions on Sterilox[®]'s chlorine concentration have not been reported in the literature. A different super-oxidised water, Microcyn (Oculus Innovative Sciences, California, USA), according to its manufacturer, has a neutral pH (between 6.2 and 7.8), lower active chlorine (51–85 ppm) and a one-year shelf life.¹²

A direct association between pH and stability of hypochlorous acid solutions has previously been shown; the stability

decreases dramatically with the change from alkaline to acidic.¹³ A further factor which has been suggested to influence the stability of chlorine solutions is their concentration: higher concentrations are more stable.¹⁰

The purpose of this study was to investigate the effects of time, sunlight and head air on the chemical stability (chlorine concentration and pH) of a freshly prepared Sterilox[®] solution.

MATERIALS AND METHODS

A freshly prepared and electrochemically activated solution (Aquatine Alpha Electrolyte, Sterilox Dental, Ilkley, West Yorkshire) was tested for chlorine concentration using 25–500 mg/L chlorine test strips (Merckoquant, Merck, Darmstadt, Germany) and for pH using non-bleeding pH indicator strips pH 0–14 (Merckoquant, Merck, Darmstadt, Germany). The solution was then dispensed into eight 250 ml plastic transparent bottles, in duplicates, as follows: two bottles containing 250 ml solution protected completely from sunlight by storage in a light-proof cupboard (group 1), two bottles containing 125 ml solution, also protected completely from sunlight by storage in a light-proof

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cupboard (group 2), two bottles containing 250 ml solution exposed to direct sunlight (group 3) and two bottles containing 125 ml solution, also exposed to direct sunlight (group 4). The bottles were closed with a screw cap-type lid. The groups were randomly allocated to the storage conditions, but kept in the same room at a constant temperature of $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Chlorine concentration and pH were measured daily during the first week, on days 10 and 14, and then at weekly intervals, either up to the 13th week or until the chlorine concentration value reached zero, whichever was the sooner.

Two observers blinded to the experimental groups visually assessed the chlorine and pH indicator strips. Where the observers were unable to agree on the findings, discussions were held until they agreed on a result.

The statistical approach for the findings, considering the chlorine concentration in mg/L and the variables exposure to light, volume of solution and time (days), was a multiple linear regression model at $\alpha = 0.05$.

RESULTS

Results are presented in Figure 1. These show the decrease of chlorine starting at day 4 for the groups exposed to sunlight and at day 14 for the groups protected from sunlight. The volume of air left above the Sterilox[®] solution in the storage containers (ie 0 ml *versus* 125 ml) did not affect the rate of chlorine decomposition. However, there were statistically significant differences between the groups exposed to sunlight and the non-exposed groups ($p < 0.001$). The mean loss of chlorine/day for the non-exposed samples was 1.01 mg/L, whilst the mean for the exposed samples was 2.42 mg/L. Therefore, the loss of chlorine was statistically greater when Sterilox[®] was exposed to sunlight. As for pH, this remained unchanged throughout the duration of the assays with a value of 5 for all samples.

DISCUSSION

Paper strips were used to measure chlorine concentration. This is a more clinically representative process than the titration technique as it is a simple and repeatable procedure which does not require specific

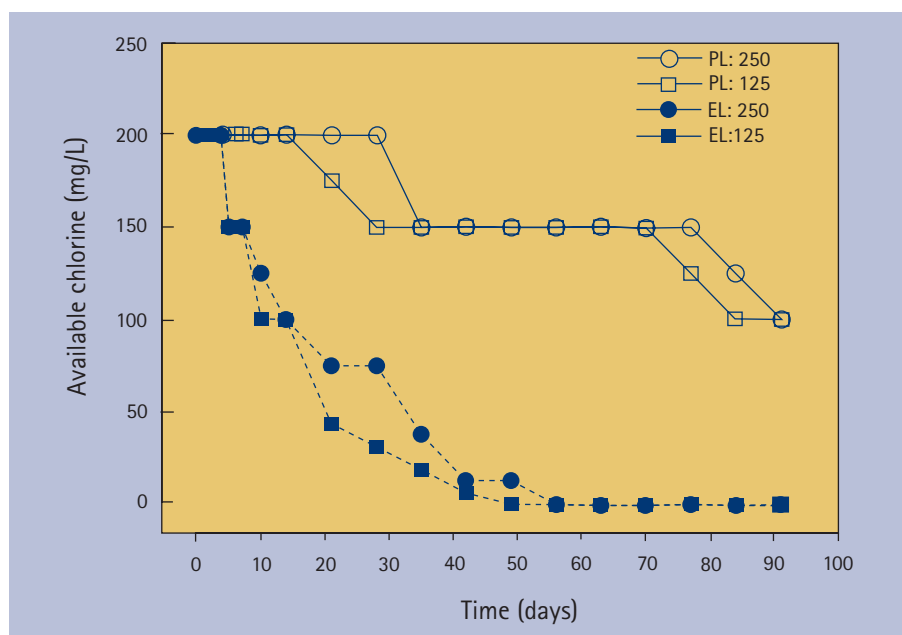


Fig. 1 Graph displaying the chlorine concentration in mg/L amongst groups considering the time of exposure in days. PL 250: Protected from sunlight, 250 ml. PL 125: Protected from sunlight, 125 ml. EL 250: Exposed to sunlight, 250 ml. EL 125: Exposed to sunlight, 125 ml

laboratory equipment and skills. In the present study, the counts were consistent and the duplicates were almost identical.

The different amounts of solution in the bottle were tested to replicate clinical usage and storage conditions.

The chlorine decomposition rate depends on the solution's pH, concentration, temperature, presence of impurities in the solution and exposure to sunlight.^{10,13}

Chlorine decomposition rates and sunlight

In our investigation, the bottles were exposed to direct sunlight on a windowsill around the northern hemisphere equinox in southern England. It is difficult to quantify the amount of sunlight as this would vary in intensity on a temporal and geographical basis, therefore sunlight exposure in different conditions might cause a different rate of decrease in chlorine concentration. A previous investigation using diffused fluorescent light found this to be an important factor in causing chlorine loss in electrolysed oxidised water in similar experimental conditions.¹³ That investigation tested a pH 2.5–2.6 53–56 mg/L chlorine concentration super-oxidised water in a sealed jar and showed that approximately 60% of the chlorine was lost after 1400 hours in diffused light. About 40% was lost in the solutions that were protected from light.¹³ Our study revealed a statistically

significant difference in the loss of chlorine when there was exposure to sunlight. The solutions were stable only until day 4 under sunlight exposure, whilst solutions in non-exposed bottles were stable until day 14. Sterilox[®]'s prolonged stability may be clinically relevant and therefore allow for greater use, even if the renewal of the solution is easy.

Another study tested the action of direct sunlight *versus* diffused sunlight on the decomposition of chlorine solutions and found that direct sunlight greatly accelerated the decomposition of the solution at a rate that was three to four times faster.¹⁴ It is worth noting that the solutions were kept at a much higher temperature ($42\text{--}45^{\circ}\text{C}$) than in our assays. Also, *in vitro* models involving specimens exposed to sunlight might subsequently experience an increase in temperature, resulting in acceleration in the rate of chlorine decomposition.

Chlorine decomposition rates and head space

Chlorine loss occurs as a result of the evaporation of chlorine gas dissolved in the solution in addition to HClO decomposition.¹³ In 'closed' conditions, as in our experiments, it has been suggested that the primary mechanism of chlorine loss could be the self-decomposition of chlorine species in the solution because chlorine evaporation is normally limited.^{13,15}

The presence of 'head space' in the 'half-full' bottles and the fact that the containers were opened during sampling might have speeded up the process as repeated opening of the bottles might have increased the evaporation and loss of chlorine gas into the environment, especially in the 'half-full' bottles. One must bear in mind that it will only be possible to use the solution if the bottle is opened, therefore this procedure has clinical relevance.

Chlorine decomposition rates and pH

The influence of pH on chlorine solution stability has been explained by the fact that with an increase in pH the equilibrium in the solution will shift towards the formation of HClO and therefore a consequent decrease in volatile chlorine gas, resulting in a reduction of chlorine evaporation.¹³ However, this seems to be important in the presence of a solution exposed to air, while in sealed solutions and higher pH, the chlorine loss will depend on the self-decomposition of the chlorine species.¹³ pH increase also influences the dissociation of HClO to hypochlorite ions (OCl⁻), a less antimicrobial form, therefore reducing its disinfecting efficacy.¹⁰

Regarding pH changes in super-oxidised waters, a previous investigation showed similar results to our assays with the pH almost unchanged during storage for a two-month period.¹³ This trend was also observed in our study.

Clinical and experimental applications

Previous publications suggest different cut-off points for the use of Sterilox[®] after production: 5 hours,⁵ 24 hours.¹⁶ It has also been suggested that 'the solution needs to

be generated on site, its pH and redox potential confirmed, and all old disinfectant replaced every 24 hours.'¹⁶ Sterilox[®] was considered to be more suitable to a centralised service¹⁷ and one of the suggested advantages is that it can be generated on site.¹³ However, this might require the availability and maintenance of multiple Sterilox[®] activator machines instead of the delivery of the activated solution from a centralised source.

Regular confirmation of the microbial activity of Sterilox[®] by biological tests or by determination of chlorine levels has been suggested.¹⁶ The authors agree with this suggestion, given that preliminary investigations showed that different Sterilox[®] generators produced inconsistent chlorine concentration solutions (data not shown).

Furthermore, it would be ideal if any further investigations on chlorine-containing solutions could include chlorine concentration analysis as part of the experimental methodology. More importantly, this needs to be carried out regularly in clinical conditions in order to confirm that the solution fulfils the required criteria. However, the 'ideal' pH, chlorine concentration and redox potential for Sterilox[®] and other super-oxidised waters have yet to be established, if indeed these exist, considering that they might also influence super-oxidised waters' toxicity and corrosive action against metals.

The study suggests that, if stored protected from sunlight, Sterilox[®] solutions are stable for at least a two-week period. The solution thus needs to be replaced less frequently than previously thought.

1. Martin M V, Gallagher M A. An investigation of the efficacy of super-oxidised (Optident/Sterilox) water for the disinfection of dental unit water lines. *Br Dent J* 2005; **198**: 353–354.

2. Martin N, Martin M V, Jedynekiewicz N M. The dimensional stability of dental impression materials following immersion in disinfecting solutions. *Dent Mater* 2007; **23**: 760–768.
3. Marais J T, Williams W P. Antimicrobial effectiveness of electro-chemically activated water as an endodontic irrigation solution. *Int Endod J* 2001; **34**: 237–243.
4. Solovyeva A M, Dummer P M. Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions: a pilot study. *Int Endod J* 2000; **33**: 494–504.
5. Shetty N, Srinivasan S, Holton J, Ridgway G L. Evaluation of microbicidal activity of a new disinfectant: Sterilox 2500 against *Clostridium difficile* spores, *Helicobacter pylori*, vancomycin resistant *Enterococcus* species, *Candida albicans* and several *Mycobacterium* species. *J Hosp Infect* 1999; **41**: 101–105.
6. Selkon J B, Babb J R, Morris R. Evaluation of the antimicrobial activity of a new super-oxidised water, Sterilox, for the disinfection of microscopes. *J Hosp Infect* 1999; **41**: 59–70.
7. Loshon C A, Melly E, Setlow B, Setlow P. Analysis of the killing of spores of *Bacillus subtilis* by a new disinfectant, Sterilox. *J Appl Microbiol* 2001; **91**: 1051–1058.
8. Zinkevich V, Beech I B, Tapper R, Bogdarina I. The effect of super-oxidised water on *Escherichia coli*. *J Hosp Infect* 2000; **46**: 153–156.
9. PuriCore plc. How PuriCore's technology works. Available at http://www.puricore.com/technology_works.aspx.
10. Rutala W A, Cole E C, Thomann C A, Weber D J. Stability and bactericidal activity of chlorine solutions. *Infect Control Hosp Epidemiol* 1998; **19**: 323–327.
11. Ayliffe G, Minimal Access Therapy Decontamination Working Group. Decontamination of minimally invasive surgical endoscopes and accessories. *J Hosp Infect* 2000; **45**: 263–277.
12. Landa-Solis C, González-Espinosa D, Guzmán-Soriano B et al. Microcyn: a novel super-oxidised water with neutral pH and disinfectant activity. *J Hosp Infect* 2005; **61**: 291–299.
13. Lee S-V, Hung Y-C, Chung D, Anderson J L, Erickson M C, Morita K. Effects of storage conditions and pH on chlorine loss in Electrolyzed Oxidizing (EO) Water. *J Agric Food Chem* 2000; **50**: 209–212.
14. El Din A M S, Arain R A, Hammoud A A. On the chlorination of seawater. *Desalination* 2000; **129**: 53–62.
15. Shimada K, Ito K, Murai S. A comparison of the bacterial effects and cytotoxic activity of three types of oxidizing water, prepared by electrolysis as chemical dental plaque control agents. *Int J Antimicrob Agents* 2000; **15**: 49–53.
16. Clark J, Barrett S P, Rogers M, Stapleton R. Efficacy of super-oxidized water fogging in environmental decontamination. *J Hosp Infect* 2006; **64**: 386–390.
17. Cooke R P D, Goddard S V, Whymant-Morris A, Sherwood J, Chatterly R. An evaluation of Cidex OPA (0.55% ortho-phthaldehyde) as an alternative to 2% glutaraldehyde for high-level disinfection of endoscopes. *J Hosp Infect* 2003; **54**: 226–231.

Study 7 – Published in International Endodontic Journal

Interaction between chlorhexidine-impregnated gutta-percha points and several chlorine-containing endodontic irrigating solutions

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Interaction between chlorhexidine-impregnated gutta-percha points and several chlorine-containing endodontic irrigating solutions

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Abstract

Rossi-Fedele G, Doğramacı EJ, Steier L, de Figueiredo JAP. Interaction between chlorhexidine-impregnated gutta-percha points and several chlorine-containing endodontic irrigating solutions. *International Endodontic Journal*.

Aim To evaluate if the immersion of chlorhexidine-impregnated gutta-percha points in chlorine-containing endodontic irrigants causes colour changes and precipitate formation.

Methodology Eighty-one size 25 chlorhexidine medicated (Activ Points, Roeko, Langenau, Germany) and 27 size 25 standard gutta-percha points (Roeko) were immersed in microtubes, containing the following solutions: 0.5 and 5.25% NaOCl or Aquatone (Optident, Ilkley, UK). The samples were visually assessed, by two independent observers, at regular intervals over 3 weeks, to detect colour changes and precipitate formation. A score system was used to grade the precipitate intensity. To compare the number of days required for a precipitate to form mean and standard differences were calculated; the means between the groups were compared using a one-way

analysis of variance test. Mean intensity scores were calculated and compared between groups using Kruskal–Wallis procedure followed by the Mann–Whitney test.

Results The groups containing 5.25% NaOCl started to produce a visible precipitate after fourteen days (16.48 ± 0.98) for the Activ points; this process was first noticed with the standard gutta-percha group on day 17 (18.33 ± 1.22 days), with a statistically significant difference ($P = 0.002$). When looking at the intensity scores from day 17 onwards, these two groups differed statistically, with a greater amount for the AP group ($P < 0.001$). No other test group presented with changes.

Conclusion The placement of impregnated gutta-percha points, in 5.25% NaOCl for a 2-week period, caused the formation of a precipitate in the experimental conditions of this study.

Keywords: chlorhexidine, chlorhexidine medicated gutta-percha point, electrochemically activated solution, interaction, sodium hypochlorite, super-oxidized water.

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Introduction

The role of bacteria in initiating and maintaining periapical inflammation has been established using an animal model (Kakehashi *et al.* 1965) and confirmed

in humans (Sundqvist 1976). Consequently, one main goal of root canal treatment is bacteria elimination in the root canal system, and this is pursued by root canal instrumentation, as well as using antimicrobial agents, including root canal irrigants and inter-appointment medicaments. Chlorhexidine (CHX) is one of the chemicals available in endodontics. It has been compared against established antimicrobial agents *in vivo*, to show that it is an effective antiseptic in solution (Roças & Siqueira 2011), and gel-type

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preparations (Manzur *et al.* 2007, Wang *et al.* 2007). However, CHX is less effective on Gram-negative bacteria, which predominate in primary endodontic infections (Zehnder 2006).

Sodium hypochlorite (NaOCl) and CHX in liquid (Basrani *et al.* 2007, Mortenson *et al.* 2012, Rossi-Fedele *et al.* 2012) or gel-type preparations (Valera *et al.* 2010), when in contact, develop a precipitate, which has an influence on root canal cleaning, and might contain toxic substances (Mortenson *et al.* 2012, Rossi-Fedele *et al.* 2012).

CHX embedded gutta-percha points (Activ Points, Roeko, Langenau, Germany) (AP) are suggested as an interim dressing as they are easy to insert and retrieve from the root canals, and also they have the ability, according to the manufacturer, to release large quantities of CHX diacetate from their surface in a time-dependent fashion (http://roeko.com/download.php?file_id=3851) [accessed on 1 November 2012]. According to the manufacturer NaOCl or alcohol might have a synergistic action and present no risk for the patient; subsequently, if these have been used during preparation, the canals can be partially dried prior to AP placement (http://roeko.com/download.php?file_id=3851) [accessed on 1 November 2012].

AP antimicrobial activity has been compared *in vitro* against other medicated gutta-percha points and well-established antiseptics, with contrasting results. It has been suggested that it performs better than calcium hydroxide (CH) containing points (Barthel *et al.* 2002, Lin *et al.* 2006, Ebert *et al.* 2008) and paste (Lucena JMVM *et al.* 2012) or a 0.25% CHX solution (Lin *et al.* 2003). An investigation found overall no differences when compared to CH-containing points (Öztan *et al.* 2005). Finally, several medicaments have been suggested to perform better than AP, including: points containing a mixture of CH and CHX (Öztan *et al.* 2005), CH paste (Barthel *et al.* 2002, Lui *et al.* 2004) and a 5% CHX gel (Barthel *et al.* 2002) or Octenidine gel (Lucena JMVM *et al.* 2012).

Aquatine Endodontic Cleanser (Aquatine) belongs to the group of the super-oxidized waters or electrochemically activated waters/solutions (Rossi-Fedele *et al.* 2011b); it has been approved by the US Food and Drug administration for use as root canal irrigant (Ring *et al.* 2008). In the literature, it is also referred to as 'Sterilox' (Martin & Gallagher 2005, Martin *et al.* 2007, Rossi-Fedele *et al.* 2011a) or 'Aquatine Alpha Electrolyte' (Rossi-Fedele *et al.* 2010a). Aquatine has been suggested as suitable for regenerative endodontic treatment because it is highly biocompatible, and thus,

it allows the pulp stem cells to survive and attach (Ring *et al.* 2008). Aquatine is a chlorine-containing solution with a pH value between 5 and 6 and a concentration of around 200 ppm (Rossi-Fedele *et al.* 2011a). Thus, the main chlorine form present in the solution is that of hypochlorous acid (HClO) (Fair *et al.* 1948). According to the literature on water treatment, the germicidal activity of HClO is 100 times more effective than its ionization product, the hypochlorite ion (OCl^-), which is the main component of NaOCl solutions (Fair *et al.* 1948, Dychdala 1991). This concept cannot necessarily be transferred to an endodontic milieu: in a preliminary investigation, using bovine teeth infected with *Enterococcus faecalis*, it was found that although Aquatine has an antimicrobial effect and it is inferior to NaOCl (Rossi-Fedele *et al.* 2010a). When looking into other requirements for irrigating solutions, it has been suggested that it is similar to 6% NaOCl in removing debris and the smear layer, when used together with EDTA (Garcia *et al.* 2010). Aquatine is unable to dissolve bovine pulp tissue (Rossi-Fedele *et al.* 2010b), possibly due to its low chlorine concentration and the prevalence of HClO, which has been suggested as being less able to dissolve tissues when compared to OCl^- (Rossi-Fedele *et al.* 2011b).

The aim of the investigation was to determine whether the CHX released by AP causes colour change and precipitate formation when immersed in some chlorine-containing solutions.

Materials and methods

Three flat-top 1.5-mL microtubes were used per group; the groups were as follows:

Negative controls: containing 0.5 and 5.25% NaOCl (Chlorhexid 5.25%, Cercamed, Nisko, Poland) alone, 2% CHX (GlucocheX 2.0%, P.P.H Cercamed) alone, and freshly prepared Aquatine (Optident, Ilkley, UK) alone, respectively. Aquatine was prepared *in situ* from its precursor (Optident Sterilox Electrolyte Solution (Optident) via electrophoresis using the Optident Sterilox Dental Generator (Optident) following manufacturer's instructions. Positive controls: containing 1 mL of 0.5 and 5.25% NaOCl (Cercamed, Nisko, Poland) or Aquatine (Optident), with the addition of 0.5 mL of 2% CHX (GlucocheX 2.0%, P.P.H Cercamed).

Six different test groups were established: Aquatine+AP ($n = 9$), Aquatine+gutta-percha points (GP) ($n = 3$), 0.5% NaOCl+AP ($n = 9$), 0.5% NaOCl+GP ($n = 3$), 5.25% NaOCl+AP ($n = 9$) and 5.25% NaOCl+GP ($n = 3$). In the 'AP' groups, one size 25

1 Activ point (Roeko, Langenau, Germany) from dif-
 2 ferent lots (numbers 296757, C34901 and C40196)
 3 was immersed in a solution, which was completely
 4 filling the microtubes. In the 'GP' groups, a size 25,
 5 0.04 greater taper gutta-percha point (Roeko,
 6 Langenau, Germany) was used. The points were
 7 obtained from freshly opened boxes and were ran-
 8 domly allocated to the groups.

9 The 0.5% NaOCl was obtained via dilution, using
 10 distilled water from the original NaOCl 5.25%, with
 11 their final concentrations confirmed using iodometric
 12 titration. Aquatine's chlorine contents and pH were
 13 tested using test strips (Merckoquant, Darmstadt,
 14 Germany), as suggested by the manufacturer, giving
 15 values of 200 ppm and a pH 5, respectively. The
 16 specimens were completely protected from sunlight
 17 and kept in the same room at a constant temperature
 18 of $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.

19 The microtubes were visually assessed by two inde-
 20 pendent observers every 15 min for the first 2 h, then
 21 hourly for the following 6 h, and finally daily for
 22 21 days, during which period the time required for
 23 colour changes and/or deposit precipitate formation
 24 was recorded. Disagreements were resolved, where
 25 required, by discussion or by the involvement of a
 26 third observer. The observers were blind to the test
 27 solution but not to the type of point, because of their
 28 obvious differences in colour, shape and size. The test
 29 was repeated 3 times.

30 To compare the number of days required for a pre-
 31 cipitate to form mean and standard differences were
 32 calculated; subsequently, the means between the
 33 groups were compared using a one-way analysis of
 34 variance test (ANOVA). The alpha level was set at
 35 $P < 0.05$ for all statistical procedures.

36 To evaluate precipitate intensity, a score system
 37 was devised, ranging from 0 (zero) to 2, in an ascen-
 38 ding scale relating to none, mild and intense precipi-
 39 tates. It was decided to use the mean instead of the
 40 median as the data contained a considerable number
 41 of ties. In such situations, the mean can be considered
 42 more informative (Campbell & Maclin 1993). Mean
 43 scores were calculated and compared between groups
 44 using Kruskal–Wallis procedure followed by the Mann–
 45 Whitney test. The level of significance was set at
 46 $\alpha = 0.05$.

48 Results

49 The NaOCl-positive controls were associated with an
 50 immediate formation of a precipitate, with the higher
 51

concentration having a larger amount and a more
 intense ochre-red colour. The Aquatine-positive con-
 trol had an instant, cloudy appearance of yellow
 colour. A precipitate was formed after 5 days.

No changes occurred in the negative controls or in
 the test groups containing either 0.5% NaOCl or Aqu-
 atine. The points, immersed in 5.25% NaOCl, became
 progressively discoloured with time. After 14 days,
 the 5.25% NaOCl specimens of the AP groups started
 to become increasingly cloudy, and a precipitate was
 formed (16.48 ± 0.98 days, with no significant differ-
 ences between the lots). A similar process started on
 the GP group on day 17 (18.33 ± 1.22 days), with a
 significant difference between the GP group and the
 AP groups ($P = 0.002$). After 21 days, all microtubes
 from the test groups containing 5.25% NaOCl had a
 white opaque precipitate, with a greater amount for
 the AP group.

A summary of the results for the precipitate inten-
 sity scores can be seen in Table 1 and Fig. 1. Table 1
 shows the mean values and standard deviation for
 the scores of precipitate formation, whilst Fig. 1 is a
 graph illustrating the behaviour of the two groups
 that displayed precipitation (5.25% NaOCl + AP and
 5.25% NaOCl + GP) compared with all other groups
 with no precipitation. From day 17 onwards, these
 two groups differed statistically ($P < 0.001$).

Discussion

These experiments aimed to assess the interactions
 between chlorine-containing endodontic irrigants and
 a CHX embedded gutta-percha point, proposed as
 intracanal medicaments.

Table 1 Mean and standard deviation for scores of precipi-
 tate formation

Day	5.25% NaOCl+AP <i>n</i> = 27	5.25% NaOCl+GP <i>n</i> = 9	Other groups* <i>n</i> = 72
up to 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
14	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
15	0.3 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
16	0.7 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
17	1.9 ± 0.4	0.3 ± 0.5	0.0 ± 0.0
18	2.0 ± 0.0	0.6 ± 0.5	0.0 ± 0.0
19	2.0 ± 0.0	0.8 ± 0.4	0.0 ± 0.0
20	2.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0
21	2.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0

*Aquatine+AP; Aquatine+GP; 0.5% NaOCl+AP; 0.5% NaOCl+GP
 AP: active points, GP: Gutta-Percha points.

COLOR

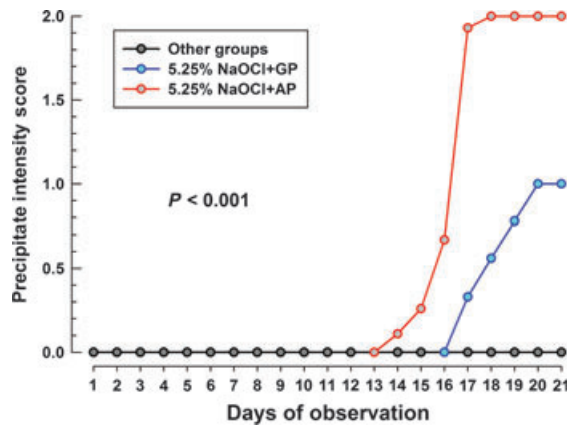


Figure 1 precipitate intensity scores of the different groups in relation to the number of days of observation.

The use of traditional root canal dressings may not give advantages in terms of outcome, as treatment in single or multiple visits has been suggested to have similar effectiveness when looking into radiological success and immediate postoperative pain (Figini *et al.* 2007). Considering that calcium hydroxide is currently the most popular intracanal medication (Figini *et al.* 2007), there is justification for the search for alternative dressings, including AP.

The methodology of this investigation sought to replicate as closely as possible that of Basrani *et al.* (2007). It was decided to completely fill the microtube for the experimental groups, to make sure that the points were fully submerged. It is worth noting that a larger volume of solution when compared with the clinical reality of a prepared root canal was used in the assays; as a limited volume fluid may hinder CHX release because of drug accumulation and saturation (Huang *et al.* 2000). Previous investigations have tested AP in small volumes of various solutions or media as follows: saline ('a drop') (Barthel *et al.* 2002) or (0.01 mL) (Lin *et al.* 2003), distilled water (5 μ L) (Lui *et al.* 2004), diluted serum (100 μ L) (Öztan *et al.* 2005) and agar plates (Lin *et al.* 2003), or was 'placed in the canal following manufacturer's instructions' (Lucena JMVM *et al.* 2012). No investigation has looked into whether AP has a synergistic relationship with NaOCl or alcohol. AP can also come in contact with NaOCl if the pulp chamber is flooded with irrigant prior to their removal or because of inadequate evacuation or drying prior to its placement; this can occur because of root canal anatomy complexities such as isthmuses and fins (Mortenson *et al.* 2012).

The Aquatine+CHX containing group may not be considered as a positive control, as their interactions have not been previously described in the literature. However, based on basic scientific knowledge of chlorine-containing solutions, this was the expected behaviour. Aquatine's chlorine concentration is similar to the lowest NaOCl concentration tested by Basrani *et al.* (2007), which caused colour to change but did not form any precipitate after 1 week. The formation of a precipitate in the Aquatine control group can be explained by differences in behaviour between HClO and OCl⁻.

The literature suggests a content of 4–5% of CHX diacetate for AP (Szep *et al.* 2002, Öztan *et al.* 2005), with the remaining composition in weight consisting of: gutta-percha 30%, zinc oxide 65%, pigments 1% and CHX acetate 4%, with an undeclared amount of barium sulphate (Szep *et al.* 2002). The gutta-percha point used as a control has the same components, apart from CHX. A possible explanation for the lack of colour changes and the formation of a precipitate, because of interactions, can be that the amount of CHX released by the points is insufficient to form a reaction product to a detectable level via observation. This methodology was used to determine the minimum concentration of NaOCl to form a precipitate with 2% CHX (Basrani *et al.* 2007).

The clinical significance of the formation of a white opaque precipitate in the 5.25% NaOCl test groups needs to be better understood and can be explained by the degradation of gutta-percha cones. This process is time and concentration dependent, with 5.25% causing increasing deterioration and changes in physical properties (Valois *et al.* 2005a,b, Pang *et al.* 2007). In line with the present results, in a previous investigation, a 0.5% NaOCl caused no points degradation in a 5 min experimental time (Valois *et al.* 2005b). The degradation depends on the lysis and loss of some components of the point (Valois *et al.* 2005a, Pang *et al.* 2007) together with oxidation of the gutta-percha polymer (Valois *et al.* 2005b). Therefore, it is likely that the precipitate is made of those components of the points that are lost, because of the deterioration process. Including CHX acetate in AP appears to speed up the process in our experiments.

A three weeks dressing period with AP is the maximum suggested by the manufacturer. The solutions tested are stable in the experimental conditions of this study (Rutala *et al.* 1998, Rossi-Fedele *et al.* 2011a); therefore, gutta-percha degradation is likely to have

occurred during the total time length of the experiments. However, *in vivo*, this process might be hindered because of potential seepage of interstitial fluid or blood (Öztan *et al.* 2005) and chlorine loss, due to the interaction with organic and inorganic matter (Rossi-Fedele *et al.* 2011b).

To prevent the formation of a precipitate if 5.25% NaOCl is used as the final irrigant during canal preparation, the dressing of teeth with AP for more than 2 weeks should be avoided; alternatively, the canal should be rinsed out with 0.5% NaOCl, saline or distilled water prior to the placement of AP.

Conclusions

4 Activ Points and standard gutta-percha points immersed in 5.25% NaOCl caused a precipitate to form after a period of between 2 and 3 weeks. No colour changes or precipitate formation arose from immersion in the other chlorine-containing solutions tested.

Acknowledgements

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References

- Barthel CR, Zimmer S, Zilliges S, Schiller R, Göbel UF, Roulet JF (2002) In situ antimicrobial effectiveness of chlorhexidine and calcium hydroxide: gel and paste versus gutta-percha points. *Journal of Endodontics* **28**, 427–30.
- Basrani BR, Manek S, Sodhi RN, Fillery E, Manzur A (2007) Interaction between sodium hypochlorite and chlorhexidine gluconate. *Journal of Endodontics* **33**, 966–9.
- Campbell MJ, Maclin D (1993) *Medical Statistics - A common-sense approach*. 2nd edn Chichester, UK: John Wiley & Sons.
- Dychdala RG (1991) Chlorine and chlorine compounds. In: Block SS, ed. *Disinfection, sterilization, and preservation*. 4th edn. Philadelphia, PA, USA: Lea & Febinger, pp. 131–51.
- Ebert J, Roggendorf MJ, Frank K, Petschelt A (2008) Antimicrobial activity of various “active” gutta-percha points against *Enterococcus faecalis* in simulated root canals. *International Endodontic Journal* **41**, 249–57.
- Fair GM, Morris JC, Chang SL, Chang SL, Weil I, Burden RP (1948) The behaviour of chlorine as water disinfectant. *Journal of the American Water Works Association* **1**, 1051–61.
- Figini L, Lodi G, Gorni F, Gagliani M (2007) Single versus multiple visits for endodontic treatment of permanent teeth. *Cochrane Database of Systematic Reviews* 2007, Issue 4. Art. No.: CD005296. DOI: 10.1002/14651858.CD005296.pub2
- Garcia F, Murray PE, Garcia-Godoy F, Namerow K (2010) Effect of aquatine endodontic cleanser on smear layer removal in the root canals of ex vivo human teeth. *Journal of Applied Oral Science* **18**, 403–8.
- Huang J, Wong HI, Zhou Y, Grad H, Komorowsky R, Friedman S (2000) In vitro studies and modelling of a controlled-release device for root canal therapy. *Journal of Controlled Release* **67**, 293–307.
- Takehashi S, Stanley H, Fitzgerald R (1965) The effect of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surgery Oral Medicine Oral Pathology* **20**, 340–9.
- Lin S, Zuckermann O, Weiss EI, Mazor Y, Fuss Z (2003) Antibacterial efficacy of a new chlorhexidine slow release device to disinfect dentinal tubules. *Journal of Endodontics* **29**, 416–18.
- Lin S, Levin L, Weiss EI, Peled M, Fuss Z (2006) In vitro antibacterial efficacy of a new chlorhexidine slow-release device. *Quintessence International* **35**, 391–4.
- Lucena JMVM de, Decker EM, Walter C, Boeira LS, Löst C, Weiger R (2012) Antimicrobial effectiveness of intracanal medicaments on *E. faecalis*: chlorhexidine versus octenidine. *International Endodontic Journal* ‘accepted article’ DOI: 10.1111/j.1365-2591.2012.02093.x
- Lui JN, Sae-Lim KP, Song P, Chen NN (2004) In vitro antimicrobial effect of chlorhexidine-impregnated gutta-percha points on *Enterococcus faecalis*. *International Endodontic Journal* **37**, 105–13.
- Manzur A, Gonzalez AM, Pozos A, Silva-Herzog D, Friedman S (2007) Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. *Journal of Endodontics* **33**, 114–18.
- Martin MV, Gallagher MA (2005) An investigation of the efficacy of super-oxidised (Optident/Sterilox) water for the disinfection of dental unit water lines. *British Dental Journal* **198**, 353–4.
- Martin N, Martin MV, Jedynekiewicz NM (2007) The dimensional stability of dental impression materials following immersion in disinfecting solutions. *Dental Materials* **23**, 760–8.
- Mortenson D, Sadilek M, Flake NM *et al.* (2012) The effect of using an alternative irrigant between sodium hypochlorite and chlorhexidine to prevent the formation para-chloroaniline within the root canal system. *International Endodontic Journal* **45**, 878–92. **6**
- Öztan MD, Kiyani M, Gerçeker D (2005) Antimicrobial effect, in vitro, of gutta-percha points containing root canal medications against yeast and *Enterococcus faecalis*. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology* **102**, 410–16.
- Pang NS, Jung IY, Bae KS, Baek SH, Lee WC, Kum KY (2007) Effects of short term chemical disinfection of gutta-percha cones: identification of affected microbes and

- alterations in surface texture and physical properties. *Journal of Endodontics* **33**, 594–8.
- Ring KC, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F (2008) The comparison of the effect of endodontic irrigation on cell adherence to root canal dentin. *Journal of Endodontics* **34**, 1474–9.
- Roças IN, Siqueira JF (2011) Comparison of the *in vivo* antimicrobial effectiveness of sodium hypochlorite and chlorhexidine used as root canal irrigants: a molecular microbiology study. *Journal of Endodontics* **37**, 143–50.
- Rossi-Fedele G, de Figueiredo JAP, Steier L, Canullo L, Steier G, Roberts AP (2010a) Evaluation of the antimicrobial effect of super-oxidized water (Sterilox®) and sodium hypochlorite against *Enterococcus faecalis* in a bovine root canal model. *Journal of Applied Oral Science* **19**, 498–502.
- Rossi-Fedele G, Steier L, Doğramacı E, Canullo L, Steier G, de Figueiredo JAP (2010b) Bovine tissue dissolution ability of Healozone®, Aquatine Alpha Electrolyte® and sodium hypochlorite. *Australian Endodontic Journal* Article first published online: 5 NOV 2010 | DOI: 10.1111/j.1747-4477.2010.00287.x
- Rossi-Fedele G, Doğramacı EJ, Steier L, de Figueiredo JAP (2011a) Some factors influencing the stability of Sterilox®, a super-oxidized water. *British Dental Journal* **4**, e27.
- Rossi-Fedele G, Guastalli AR, Doğramacı EJ, Steier L, de Figueiredo JAP (2011b) Influence of pH changes on chlorine-containing endodontic irrigating solutions. *International Endodontic Journal* **44**, 792–9.
- Rossi-Fedele G, Doğramacı EJ, Guastalli AR, Steier L, de Figueiredo JAP (2012) Antagonistic interactions between sodium hypochlorite, chlorhexidine, EDTA and citric acid. *Journal of Endodontics* **38**, 426–31.
- Rutala WA, Cole EC, Thomann CA, Weber DJ (1998) Stability and bactericidal activity of chlorine solutions. *Infection Control and Hospital Epidemiology* **19**, 323–7.
- Sundqvist G (1976) Bacteriological studies of necrotic dental pulps (PhD Thesis-University Odontological dissertation) Umeå, Sweden.
- Szep S, Grumann L, Ronge K, Schriever A, Schultze M, Heidemann D (2002) In vitro cytotoxicity of medicated and non medicated gutta-percha points in cultures of gingival fibroblasts. *Journal of Endodontics* **29**, 36–40.
- Valera MC, Chung A, Menezes MM *et al.* (2010) Scanning electron microscope evaluation of chlorhexidine gel and liquid associated with sodium hypochlorite cleaning on the root canal walls. *Oral Surgery Oral Medicine Oral Pathology and Endodontology* **110**, e82–7.
- Valois CRA, Silva LP, Azevedo RB (2005a) Effects of 2% chlorhexidine and 5.25% sodium hypochlorite on gutta-percha cones studied by atomic force microscopy. *International Endodontic Journal* **38**, 425–9.
- Valois CRA, Silva LP, Azevedo RB (2005b) Structural effects of sodium hypochlorite solutions on gutta-percha cones: atomic force microscopy study. *Journal of Endodontics* **31**, 749–51.
- Wang CS, Arnold RR, Trope M, Teixeira FB (2007) Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria. *Journal of Endodontics* **33**, 1283–9.
- Zehnder M (2006) Root canal irrigants. *Journal of Endodontics* **32**, 389–98.

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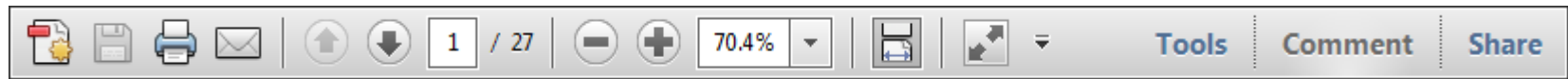
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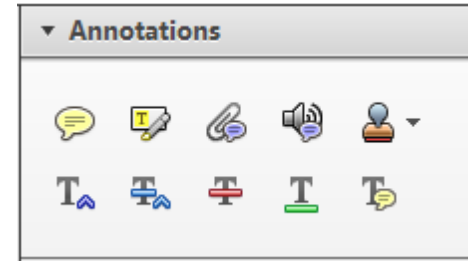
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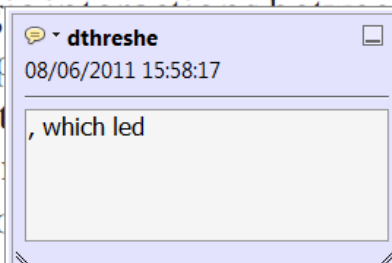


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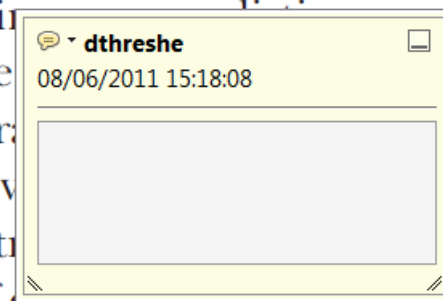


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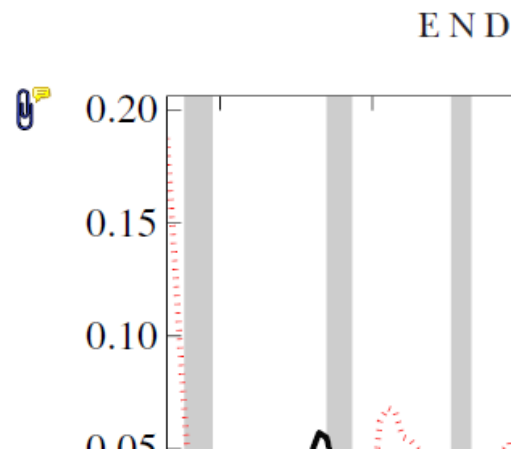
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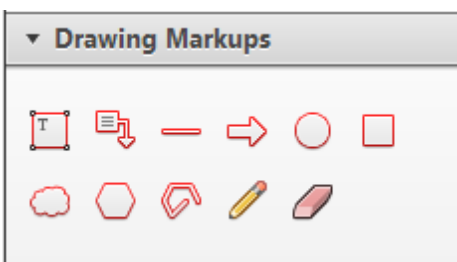
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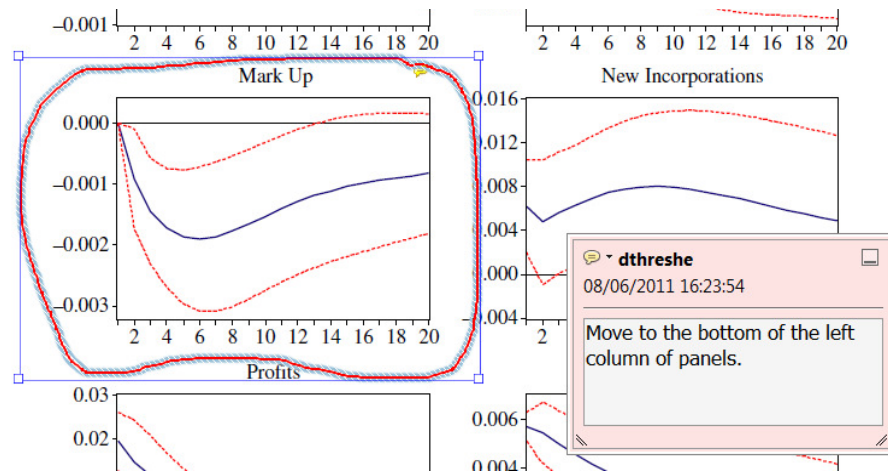


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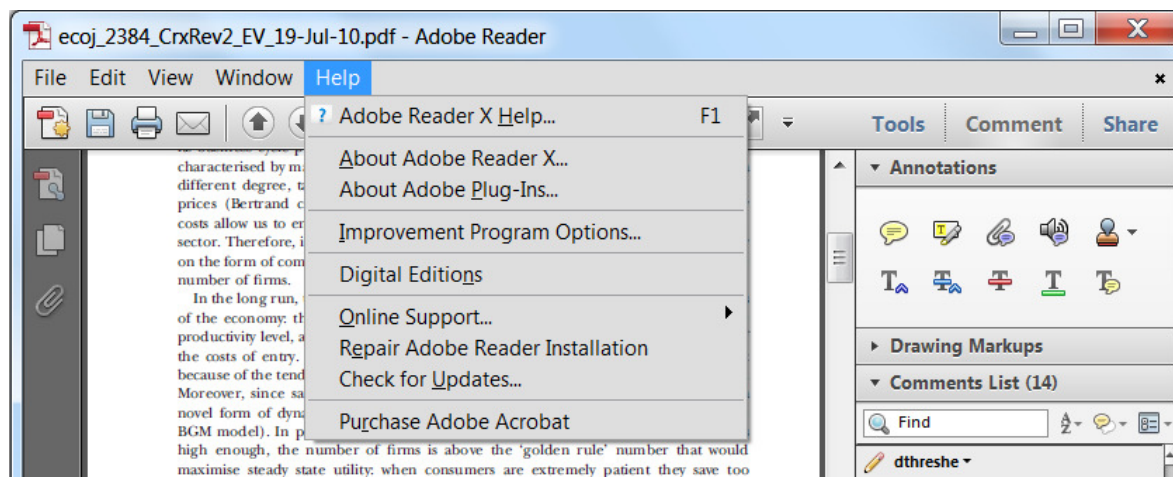
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FINAL CONSIDERATIONS

This thesis consists of three review and four research papers. A fourth review paper was planned that considered the role of osmolarity in the clinical performance of chlorine-containing solutions. However, no records were generated when different search terms and combinations related to the topic were entered into the Medline electronic database. A more recent search, carried out on 10th December 2012, found only the manuscript from Jungbluth *et al.* (2012), which has been included in the surface-tension review paper. Review papers are popular amongst editors and readers, in that they summarise other papers and allow for the rapid assimilation of large amounts of information by readers, although no new knowledge is generated. The two research papers were designed to fill some of the gaps present in the literature, as revealed by the review stage.

Main conclusions

Antimicrobial effect

Sodium hypochlorite seems to be the best chlorine-containing solution in terms of antimicrobial ability; indeed, its antimicrobial effect appears to be increased by modifying its pH to values below 6 and 7.5.

Reducing the surface tension of sodium hypochlorite does not improve the antimicrobial effect of high concentration solutions; it is not possible to reach a final conclusion for low concentrations because of inconsistent results and confounding factors.

The antimicrobial efficacy of low concentration sodium hypochlorite preparations may be eliminated through interactions with EDTA or Citric acid.

Tissue dissolution

Sodium hypochlorite is the only chlorine-containing solution with tissue dissolution ability; yet its efficacy appears to decrease by modifying its pH to values to below 6 and 7.5.

Reducing the surface tension of sodium hypochlorite, meanwhile, has no influence on tissue dissolution capability.

The tissue dissolution ability of sodium hypochlorite is reduced dramatically in view of the interactions with EDTA or Citric acid.

Cleaning effectiveness

Overall, chlorine-containing solutions appear to have good cleaning effectiveness; however association with a chelating agent is required to achieve the removal of the smear layer.

Irrigant penetration

The reduction of the surface tension of sodium hypochlorite preparations improves the main canal penetration *in vitro*.

Stability

Experimental preparations suffer from free available chlorine loss because of the addition of surface-active agents, which varies depending on the agent. There are no published studies into the effect on commercially available solutions. Interactions between EDTA or Citric acid and NaOCl cause a sudden chlorine-contains loss. The presence of surface active agents in commercially available preparations may reduce the chlorine loss because of interactions with EDTA.

Aquatine (Sterilox) chlorine concentration is stable for a two-week period if protected from direct sunlight; if exposed to direct sunlight the degradation process starts after 4 days.

Precipitate formation

Mixing sodium hypochlorite and chlorhexidine in liquid or gel forms leads to the formation of a flocculate or precipitate, which has been suggested to contain carcinogenic substances.

When chlorhexidine medicated gutta-percha points are immersed in 5.25 % sodium hypochlorite, the solution becomes cloudy, and a precipitate forms after 14 days in our experimental conditions. No other chlorine-containing solution tested caused similar changes. The interactions between Aquatine and chlorhexidine lead to colour changes, and the formation of a precipitate at a later stage.

These precipitates have a negative influence on the cleaning efficacy of mixtures of sodium hypochlorite and chlorhexidine.

Italicized conclusions result from primary research; non-italicised sections refer to those conclusions drawn from review papers.

Further investigations on chlorine-containing solutions should include chlorine concentration and pH analysis as part of the experimental methodology in order to understand the type of chlorine species present as well as their concentration. By modifying the pH of NaOCl solutions to values around 6 and 7.5 using specific acids, the antimicrobial effect seems to be increased. Low-concentration acidic and neutral chlorine containing solutions appear to have antimicrobial effect; however, this is lower than currently used NaOCl concentrations. By modifying pH of NaOCl solutions to values below 7.5, the tissue dissolution capability appears to decrease. Sodium dichloroisocyanurate and SOW appear not to have clinically relevant pulp tissue dissolution effects. Neutral and acidic chlorine solutions appear to have potential cleaning effectiveness; however, the use of a chelating agent or detergent in combination might be necessary.

Chelating agents have a dramatic effect on the free available chlorine contents of NaOCl and subsequently on its tissue dissolution capability, whereas its antimicrobial effect is reduced only when the initial NaOCl concentrations are modest. EDTA and CA do not suffer from a reduction of their chelating ability in mixtures containing NaOCl. CHX and NaOCl-containing solutions develop a precipitate that might contain toxic substances that have an influence on root canal cleaning; however, further research is required to better understand its nature. When mixing CHX and EDTA, it is difficult to obtain a homogenous solution, and a precipitate composed mainly of those substances is formed. CA is not influenced by CHX, and no precipitate is formed when mixed with it.

Surface-active agents improve the penetration of NaOCl in the main canal and have no effect on its pulp tissue dissolution ability. There are, however, insufficient data to enable a sound conclusion to be drawn regarding the effect of modifying NaOCl's surface tension on lubrication, antimicrobial and debris or smear layer removal capabilities.

Sterilox's Aquatine Alpha Electrolyte® appeared to have as an irrigant in the root canal system compared to the non-activated Optident Sterilox Electrolyte Solution®, but NaOCl was the only irrigant able to eliminate all bacteria in our experiments. Sterilox's Aquatine Alpha Electrolyte® caused a bacterial load decrease although being less effective than NaOCl.

Only NaOCl was able to dissolve pulp tissue and HealOzone contributed to reducing the time for this. It could be speculated that dissolution properties of NaOCl could be enhanced by HealOzone allowing clinical use of more diluted concentrations with potentially reduced side-effects.

It would be ideal if any further investigations on chlorine-containing solutions could include chlorine concentration analysis as part of the experimental methodology. More importantly, this needs to be carried out regularly in clinical conditions in order to confirm that the solution fulfils the required criteria. However, the 'ideal' pH, chlorine concentration and redox potential for Sterilox® and other super-oxidised waters have yet to be established, if indeed these exist, considering that they might also influence super-oxidised waters' toxicity and corrosive action against metals. If stored protected from sunlight, Sterilox® solutions are stable for at least a two-week period. The solution thus needs to be replaced less frequently than previously thought.

Activ Points and standard gutta-percha points immersed in 5.25% NaOCl caused a precipitate to form after a period of between 2 and 3 weeks. No colour changes or precipitate formation arose from immersion in the other chlorine-containing solutions tested. To prevent the formation of a precipitate if 5.25% NaOCl is used as the final irrigant during canal preparation, the dressing of teeth with AP for more than 2 weeks should be avoided; alternatively, the canal should be rinsed out with 0.5% NaOCl, saline or distilled water prior to the placement of AP.

The search for a robust substitute for sodium hypochlorite needs to continue. Despite the disadvantages of this solution, it is still the number 1 choice of endodontists all over the world. The attempts are leading to further research which may be on the enhancement of the chlorine-containing solutions' concentrations, and the possible effects on antimicrobial effect, biocompatibility, resistance to fracture and other physical properties, and the associations in which these solutions can be of further help as an adjunct of the root canal treatment. A myr-

iad of new investigations must come, maybe addressing the problem with fewer difficulties than the ones contained in this thesis.

Additional Bibliography

Baumgartner JC, Brown CM, Mader CL, Peters DD, Shulman JD (1984) A scanning electron microscopic evaluation of root canal debridement using saline, sodium hypochlorite, and citric acid. *Journal of Endodontics* **10**, 525-30.

Estrela C, Bueno MR, Leles CR, Azevedo B, Azevedo JR (2008) Accuracy of cone beam computed tomography and panoramic and periapical radiography for detection of apical periodontitis. *Journal of Endodontics* **34**, 273-9.

European Society of Endodontology (2006) Quality guidelines. *International Endodontic Journal* **39**, 921-30.

Ng YL, Mann V, Gulabivala K (2008) Outcome of secondary root canal treatment: a systematic review of the literature. *International Endodontic Journal* **41**, 1026-46.

Ng YL, Mann V, Gulabivala K (2010) Tooth survival following non-surgical root canal treatment: a systematic review of the literature. *International Endodontic Journal* **43**, 171-89.

Pashley EL, Birdsong NL, Bowman K, Pashley DH (1985) Cytotoxic effects of NaOCl on vital tissue. *Journal of Endodontics* **11**, 525-8.

Salehrabi R, Rotstein I (2004) Endodontic treatment outcome in a large patient population in the USA: an epidemiological study. *Journal of Endodontics* **30**, 846-50.

Wu MK, Shemesh H, Wesselink PR (2009) Limitations of previously published systematic reviews evaluation the outcome of endodontic treatment. *International Endodontic Journal* **42**, 656-66.

Zehnder M (2006) Root canal irrigants. *Journal of Endodontics* **32**, 389-98.