

# Sodium hypochlorite in endodontics: an update review

Zahed Mohammadi  
Yazd, Iran

The major objective in root canal treatment is to disinfect the entire root canal system. This requires that the pulpal contents be eliminated as sources of infection. This goal may be accomplished using mechanical instrumentation and chemical irrigation, in conjunction with medication of the root canal between treatment sessions. Microorganisms and their by-products are considered to be the major cause of pulpal and periradicular pathosis. In order to reduce or eliminate bacteria and pulpal tissue remnants, various irrigation solutions have been suggested to be used during treatment. Sodium hypochlorite, an excellent non-specific proteolytic and antimicrobial agent, is the most common irrigation solution used during root canal therapy. The purpose of this paper was to review different aspects of sodium hypochlorite use in endodontics.

*Key words:* Antibacterial, antifungal, proteolytic, sodium hypochlorite, toxicity, vital pulp therapy

The essential role of microorganisms in development and perpetuation of pulpal and periapical diseases has been demonstrated clearly in animal models and human studies<sup>1-3</sup>. Elimination of microorganisms from infected root canals is a difficult task. Numerous measures have been described to reduce the numbers of root canal microorganisms, including the use of various instrumentation techniques, irrigation regimens and intra-canal medicaments. There is no evidence in the literature to show that mechanical instrumentation alone results in a bacteria-free root canal system<sup>4</sup>. Considering the complex anatomy of the root canal pulp space<sup>5,6</sup>, this is not surprising. It is assumed, but not demonstrated, that any pulp tissue left in the root canals can serve as bacterial nutrient. Furthermore, tissue remnants also impede the antimicrobial effects of root canal irrigants and medicaments. Therefore some sort of irrigation / disinfection is necessary to remove tissue from the root canals and to kill microorganisms. Simply, chemical treatment of the root canal can be arbitrarily divided into irrigants, rinses, and inter-visit medicaments.

## History of chlorine-releasing agents

Chlorine exists in combination with sodium, potassium, calcium, and magnesium<sup>7</sup>. In the human body, chlorine compounds are part of the nonspecific immune system.

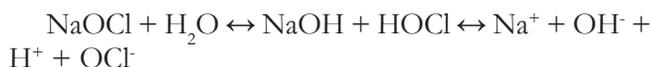
They are generated by neutrophils via the myeloperoxidase-mediated chlorination of a nitrogenous compound or set of compounds<sup>8</sup>.

Potassium hypochlorite was first chemically produced in France by Claude Louis Berthollet as an aqueous chlorine solution. This solution was produced industrially by Percy in Javel near Paris, hence the name 'Eau de Javel'<sup>7</sup>. Hypochlorite solutions were first used as bleaching agents. Subsequently, sodium hypochlorite was recommended by Labarraque to prevent childbed fever and other infectious diseases<sup>7</sup>. Based on the controlled laboratory studies by Koch and Pasteur, hypochlorite then gained wide acceptance as a disinfectant by the end of the 19<sup>th</sup> century<sup>7</sup>. In World War I, chemist Henry Drysdale Dakin and the surgeon Alexis Carrel extended the use of a buffered 0.5% sodium hypochlorite solution to the irrigation of infected wounds, based on Dakin's meticulous studies on the efficacy of different solutions on infected necrotic tissue<sup>9</sup>. Beside their wide-spectrum, non-specific killing effects on all microbes, hypochlorite preparations are sporicidal, viricidal<sup>10</sup> and show far greater tissue dissolving effects on necrotic than on vital tissues<sup>11</sup>. These features prompted the use of aqueous sodium hypochlorite in endodontics as the main irrigant as early as 1919 as recommended by Coolidge<sup>12</sup>. Furthermore, sodium hypochlorite solutions are cheap, easily available, and demonstrate good shelf

life<sup>13</sup>. Other chlorine-releasing compounds have been advocated in endodontics, such as chloramines-T and dichloroisocyanurate (NaDCC)<sup>14,15</sup>. These, however, have never gained wide acceptance, and appear to be less effective than sodium hypochlorite at comparable concentrations<sup>7</sup>.

### Mechanism of action

Sodium hypochlorite exhibits a dynamic balance as shown by the following reaction<sup>16</sup>:



Interpreting these chemical reactions, sodium hypochlorite acts as a solvent for organic and fat degrading fatty acids, transforming them into fatty acid salts (soap) and glycerol (alcohol) that reduces the surface tension of the remaining solution<sup>16</sup>.

Sodium hypochlorite neutralises amino acids forming water and salt (neutralisation reaction). With the exit of hydroxyl ions, there is a reduction in pH. Hypochlorous acid, a substance present in sodium hypochlorite solution, when in contact with organic tissue acts as a solvent and releases chlorine that, combined with the protein amino group, forms chloramines (chloramination reaction) that interfere in cell metabolism. Hypochlorous acid (HOCl) and hypochlorite ions (OCl<sup>-</sup>) lead to amino acid degradation and hydrolysis<sup>16</sup>.

Chlorine (a strong oxidant) presents antimicrobial action inhibiting bacterial enzymes leading to an irreversible oxidation of SH groups (sulphydryl group) of essential bacterial enzymes<sup>16</sup>.

Considering the physico-chemical properties of sodium hypochlorite when in contact with organic tissue, these reactions can be verified. Sodium hypochlorite is a strong base (pH>11). At 1% concentration, sodium hypochlorite presents a surface tension equal to 75 dynes/cm, stickiness equal to 0.986cP, conductivity of 65.5mS, density of 1.04g/cm<sup>3</sup> and moistening capacity equal to 1h and 27min. Its antimicrobial mechanism of action can be observed verifying its physico-chemical characteristics and its reaction with organic tissue<sup>16</sup>.

The antimicrobial effectiveness of sodium hypochlorite, based in its high pH (hydroxyl ions action), is similar to the mechanism of action of calcium hydroxide<sup>17</sup>. The high pH of sodium hypochlorite interferes in the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism and phospholipid degradation observed in lipidic peroxidation<sup>16</sup>. The amino acid chloramination reaction forming chloramines interfere with cellular metabolism. Oxidation promotes irreversible bacterial enzymatic inhibition replacing hydrogen with chlorine. This enzyme inactivation can be observed in the reaction of chlorine with amino groups (NH<sub>2</sub>) and an

irreversible oxidation of sulphydryl groups (SH) of bacterial enzymes (cystein)<sup>16</sup>.

Thus, sodium hypochlorite presents antimicrobial activity with action on bacterial essential enzymatic sites promoting irreversible inactivation originated by hydroxyl ions and chloramination action. Dissolution of organic tissue can be verified in the saponification reaction when sodium hypochlorite degrades fatty acids and lipids resulting in soap and glycerol<sup>16</sup>.

### Antibacterial activity

#### *In vitro* studies

Several *in vitro* studies have been performed on the antibacterial activity of NaOCl. Walker<sup>18</sup>, in 1936, introduced the use of double-strength chlorinated soda (5% NaOCl) solution as a root canal irrigant in endodontic practice, which has continued worldwide ever since with no study definitively showing any other irrigant to be more effective. Siqueira *et al.*<sup>19</sup> evaluated the effectiveness of 4% NaOCl against *Enterococcus faecalis in vitro* reporting that it was significantly more effective than saline solution (control group) in disinfecting the root canal. In another study, Siqueira *et al.*<sup>20</sup> compared the antibacterial activity of several irrigants against four black-pigmented anaerobic bacteria and four facultative bacteria through an agar diffusion test. Their findings showed that the antibacterial effectiveness of 4% NaOCl and 2.5% NaOCl was significantly greater than other tested agents. In another study, they showed that there was no difference in the antibacterial activity of 1%, 2.5%, and 5% NaOCl<sup>21</sup>.

Gomes *et al.*<sup>22</sup> evaluated the effectiveness of five concentrations of NaOCl (0.5%, 1%, 2.5%, 4% and 5.25%) and two forms of chlorhexidine gluconate (CHX) (gel and liquid) in three concentrations (0.2%, 1% and 2%) in the elimination of *E. faecalis*. They found that all irrigants were effective in killing *E. faecalis*, but at different times. CHX in the liquid form at all concentrations tested (0.2%, 1% and 2%) and NaOCl (5.25%) were the most effective irrigants. However, the time required by 0.2% chlorhexidine liquid and 2% chlorhexidine gel to promote negative cultures was only 30s and 1min, respectively. Vianna *et al.*<sup>23</sup> investigated the antimicrobial activity of five concentrations of NaOCl (0.5%, 1%, 2.5%, 4%, and 5.25%) and compared the results with those achieved by 0.2%, 1%, and 2% CHX. All tested irrigants eliminated *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, and *Prevotella intermedia* in 15s. The timing required for 1.0% and 2.0% CHX liquid to eliminate all microorganisms was the same required for 5.25% NaOCl. Berber *et al.*<sup>24</sup> assessed the efficacy of 0.5%, 2.5% and 5.25% NaOCl as intracanal irrigants associated with hand and rotary instrumentation techniques against *E. faecalis* within root canals and dentinal tubules. They found that 5.25% concentration was the

most effective solution followed by 2.5% concentration. Oliveira and associates<sup>25</sup> compared the efficacy of two different concentrations of NaOCl (5.25% and 1.5%) with 2% CHX gel against *E. faecalis*. Results showed that 5.25% NaOCl and 2% CHX gel had good potential to keep a low *E. faecalis* CFU count immediately and 7 days after instrumentation, whereas 1.5% NaOCl reduced the *E. faecalis* CFU only after instrumentation.

### In vivo studies

Bystrom and Sundqvist<sup>26</sup> evaluated the antibacterial effect of 0.5% NaOCl on fifteen single-rooted teeth. Each tooth was treated at five appointments, and the presence of bacteria in the root canal was studied on each occasion. No antibacterial intracanal dressings were used between the appointments. When 0.5% hypochlorite was used no bacteria could be recovered from twelve of fifteen root canals at the fifth appointment. This should be compared with eight of fifteen root canals when saline solution was used as an irrigant. Ercan *et al.*<sup>27</sup> compared the antibacterial efficacy of 2% CHX and 5.25% NaOCl as root canal irrigants. Their findings demonstrated that both solutions were significantly effective in reducing the microorganisms in teeth with necrotic pulp, periapical lesions, or both. Vianna *et al.*<sup>28</sup> investigated the degree of microbial reduction after chemo-mechanical preparation of human root canals containing necrotic pulp tissue when using NaOCl solution or CHX gel with real-time quantitative-polymerase chain reaction (RTQ-PCR) and culture techniques. They found that using both identification techniques, the bacterial reduction in the NaOCl group was significantly greater than in the CHX group. Siqueira *et al.*<sup>29</sup> compared the effectiveness of 2.5% NaOCl and 0.12% CHX as irrigants in reducing the cultivable bacterial populations in infected root canals of teeth with apical periodontitis. Their findings revealed that chemo-mechanical preparation using either solution substantially reduced the number of cultivable bacteria in the canals. No significant difference was observed between the NaOCl and CHX groups with regard to the number of cases yielding negative cultures or quantitative bacterial reduction. In another study, Siqueira *et al.*<sup>30</sup> investigated the bacterial reduction after instrumentation using 2.5% NaOCl as an irrigant and further interappointment dressing with a calcium hydroxide (Ca(OH)<sub>2</sub>)/camphorated paramonochlorophenol (CPMC) paste. Results showed that chemo-mechanical preparation with 2.5% NaOCl significantly reduced the number of bacteria in the canal but failed to render the canal free of cultivable bacteria in more than one-half of the cases. A 7-day intracanal dressing with Ca(OH)<sub>2</sub>/CPMC paste further significantly increased the number of culture-negative cases. These findings were confirmed in another investigation<sup>31</sup>. On the whole, it can be concluded that NaOCl, in both *in vitro* and *In vivo* conditions, exhibits excellent antibacterial activity.

### Antifungal activity

Fungi constitute a small part of the oral microbiota. The largest proportion of the fungal microbiota is made up of *Candida* species. *Candida albicans* is the fungal species most commonly detected in the oral cavity of both healthy (30-45%) and medically compromised (95%) individuals. Fungi have occasionally been found in primary root canal infections, but they seem to be more common in the root canals of obturated teeth in which the treatment has failed<sup>32</sup>. Overall, the occurrence of yeasts reported in infected root canals varies between 1% and 17%<sup>33</sup>.

Because fungi may be involved in cases of persistent and secondary infections associated with recalcitrant periradicular lesions, the spectrum of antimicrobial activity of endodontic medicaments and irrigants should include these microorganisms. Thus, strategies with medicaments that have antifungal effectiveness may assist in the successful management of persistent or secondary endodontic infections caused by fungi<sup>32,33</sup>. Sen *et al.*<sup>34</sup> evaluated the antifungal properties of 1% NaOCl, and 5% NaOCl and 0.12% CHX against *Candida albicans* using cylindrical dentine tubes. They found that found *C. albicans* to be more resistant in the presence of smear layer than in the absence of smear layer. When smear layer was absent, NaOCl started to display antifungal activity after 30 minutes. Waltimo *et al.*<sup>35</sup> evaluated the susceptibility of seven strains of *C. albicans* to four disinfectants: NaOCl, IKI, CHX acetate and calcium hydroxide. In addition, all possible pairs of the disinfectants were tested to compare the effect of the combination and its components. *C. albicans* cells were highly resistant to calcium hydroxide. NaOCl (5% and 0.5%) and iodine (2%) potassium iodide (4%) killed all yeast cells within 30s, whilst CHX acetate (0.5%) showed complete killing after 5min. Combinations of disinfectants were equally or less effective than the more effective component. All *C. albicans* strains tested showed similar susceptibility to the medicaments tested. Ferguson *et al.*<sup>36</sup> sought to determine the *in vitro* susceptibility of *C. albicans* to various irrigants and medicaments. The minimum inhibitory concentrations of NaOCl, hydrogen peroxide, CHX digluconate, and aqueous calcium hydroxide were determined. Their results revealed that NaOCl, hydrogen peroxide, and CHX digluconate were effective against *C. albicans* even when significantly diluted. Aqueous calcium hydroxide had no activity. Some other studies have shown that NaOCl demonstrated complete antifungal activity in a range from 15s to 5min<sup>37,38</sup>. Ruff *et al.*<sup>39</sup> found that 6% NaOCl was equally effective and statistically significantly superior to BioPure MTAD and 17% EDTA in antifungal activity. Radcliffe *et al.*<sup>40</sup> demonstrated that four concentrations of NaOCl lowered CFU below the limit of detection after 10s in the case of *C. albicans*. This finding was confirmed by Ayhan *et al.*<sup>41</sup> and taken together, it could be argued that the antifungal activity of NaOCl is superior to or at least equal to other common irrigation solutions.

## NaOCl and biofilms

The term biofilm was introduced to designate the thin-layered condensations of microbes that may occur on various surface structures in nature. Free-floating bacteria existing in an aqueous environment, so-called planktonic microorganisms are a prerequisite for biofilm formation<sup>42</sup>. Such films may thus become established on any organic or inorganic surface substrate where planktonic microorganisms prevail in a water-based solution. In the dental context bacteria free in saliva (planktonic organisms) serve as the primary source for the organisation of this specific biofilm<sup>42</sup>. However, in endodontics the biofilm concept has so far gained limited attention. It has been discussed mainly within the framework of bacterial appearances on root tips of teeth with non-vital pulps. Such bacterial aggregations have been thought to be the cause of therapy-resistant apical periodontitis<sup>42</sup>. However, microbial communities grown in biofilms are remarkably difficult to eradicate with anti-microbial agents and microorganisms in mature biofilms can be notoriously resistant for reasons that have yet to be adequately explained<sup>42</sup>. There are reports showing that microorganisms grown in biofilms could be 2-1,000-fold more resistant than the corresponding planktonic form<sup>43</sup>.

Spratt *et al.*<sup>44</sup> evaluated the effectiveness of NaOCl (2.25%), 0.2% CHX, 10% povidone iodine, 5ppm colloidal silver and phosphate buffered solution (PBS) (as a control) against monoculture biofilms of five root canal isolates including *P. intermedia*, *Peptostreptococcus micros*, *Streptococcus intermedius*, *F. nucleatum*, *E. faecalis*. Results showed that NaOCl was the most effective anti-microbial followed by the iodine solution. Clegg *et al.*<sup>45</sup> evaluated the effectiveness of three concentrations of NaOCl (6%, 3%, and 1%), 2% CHX and BioPure MTAD on apical dentine biofilms *in vitro*. Their findings indicated that 6% NaOCl was the only irrigant capable of both rendering bacteria nonviable and physically removing the biofilm.

Ozok *et al.*<sup>46</sup> compared growth and susceptibility to different concentrations of NaOCl of mono- and dual-species biofilms of *Fusobacterium nucleatum* or *Peptostreptococcus micros* *in vitro* at 24 hours or 96 hours. Results revealed that although at 24 hours dual-species biofilms had similar viable counts to those of monospecies, they were more resistant to NaOCl. At 96 hours, both microorganisms had higher viable counts and were more resistant to NaOCl in dual-species biofilms than in monospecies biofilms. Mixed-species biofilms of *F. nucleatum* and *P. micros* showed a time-dependent synergy in growth and resistance to NaOCl. Dunavant *et al.*<sup>47</sup> evaluated the efficacy of 6% NaOCl, 1% NaOCl, Smear Clear™, 2% CHX, REDTA, and BioPure™ MTAD™ against *E. faecalis* biofilms using a novel *in vitro* testing system. Biofilms grown in a flow cell system were submerged in test irrigants for either 1 or 5 minutes. There was a significant relationship between test agent and

percentage kill of the biofilm bacteria. No significant relationship between time and percentage kill was found. The percentage kill of the biofilms bacteria was: 6% NaOCl (>99.99%), 1% NaOCl (99.78%), Smear Clear™ (78.06%), 2% CHX (60.49%), REDTA (26.99%), and BioPure™ MTAD™ (16.08%). There was a significant difference between 1% and 6% NaOCl, and all other agents including Smear Clear™, 2% CHX, REDTA, and BioPure™ MTAD™. Therefore, both 1% NaOCl and 6% NaOCl were more efficient in eliminating *E. faecalis* biofilm than the other solutions tested.

Giardino *et al.*<sup>48</sup> evaluated the efficacy of 5.25% NaOCl and MTAD against against *E. faecalis* biofilm and found that only 5.25% NaOCl can disgregate and remove the biofilm at every time. On the whole, it seems that NaOCl be the only endodontic irrigant that can disrupt and remove microbial biofilm from the infected root canals.

## NaOCl for decontamination of the microbial sampling field

Studies of root canal infection may be compromised at various stages, such as decontamination of the field, access cavity preparation, during sampling, transportation of the sample to the laboratory, and finally during laboratory processing of the cultivation. The protocols used at each of these stages should ideally be optimal and standardised both within and between studies, enabling valid comparison of the results<sup>49</sup>. Decontamination of the sampling field is mandatory to avoid false-positive results. Traditionally, decontamination of the field is performed with 30% hydrogen peroxide followed by swabbing with 5% or 10% iodine tincture before root canal sampling<sup>49</sup>. Considering its excellent antimicrobial and tissue dissolving properties, NaOCl can be a potential alternative for the above mentioned disinfectant regime.

Ng *et al.*<sup>50</sup> compared the effectiveness of 2.5% NaOCl and 10% iodine for decontamination of the operation field using cultivation and polymerase chain reaction (PCR) techniques. They found that bacterial DNA could be detected significantly more frequently from the tooth surfaces after iodine (45%) compared with NaOCl (13%) decontamination and concluded that root canal sampling for PCR might be better preceded by NaOCl decontamination than by iodine. It should be noted for avoidance of false-negative results, NaOCl should be inactivated with sodium thiosulfate<sup>51</sup>. On the other hand, the thioisulfate-NaOCl reaction produces sodium salts<sup>52</sup> that could inhibit the PCR reaction, raising the question of possible false-negative PCR results<sup>51</sup>.

## Buffering effect of dentine on NaOCl

Bone apatite has long been known to be a major carbonate reservoir, providing buffering for all acid-base

reactions and maintaining the body's acid-base balance<sup>53</sup>. With a quite similar chemical composition, dentine can be expected to have a corresponding buffering effect on acids and bases. Wang and Hume<sup>54</sup> showed that dentine was a strong buffer against acids. Buffering against alkali (NaOH) was weaker but nevertheless considerable. Dentine chips weighing 250mg were able to keep the pH unchanged after the addition of the 3mmol of HCl or 2mmol of NaOH. Inorganic apatites are supposed to be mainly responsible for the buffering effect of dentine. However, the fact that whole dentine is a more effective buffer than hydroxyapatite suggests that other inorganic and even organic components also contribute to the buffering. Camps and Pashley<sup>55</sup> found that organic components of dentine alone accounted for 1.5% of the total buffering capacity.

The root canal milieu is a complex mixture of a variety of organic and inorganic compounds. Hydroxyapatite, the main component of dentine, is the major representative of inorganic components present. In addition, inflammatory exudate, entering the apical root canal in purulent infections, is rich in proteins such as albumin. The relative importance of the various organic and inorganic compounds in the inactivation of root canal disinfectants have been studied restrictively<sup>56</sup>. Difficulty in designing experiments that will give reliable and comparable data was one of the great challenges for researchers for many years. Ultimately, Haapasalo *et al.*<sup>56</sup> introduced a new dentine powder model for studying the inhibitory effect of dentine on various root canal irrigants and medicaments.

NaOCl is a strong base and nonspecific oxidiser. It reacts with amino acid by neutralisation and chloramination reactions, leading to degradation of amino acids<sup>16</sup>. The deproteinisation effect has been used in endodontic treatment. An immunohistochemical study demonstrated that type I collagen and glycosaminoglycan lost their immunoreactivity after NaOCl treatment when a demineralised dentine model was used<sup>57</sup>. However, in intact dentine this effect was minimal, suggesting that hydroxyapatite has a protective role by embedding collagen and other proteins against the oxidative activity of NaOCl. As mentioned earlier the antibacterial effect of sodium hypochlorite is well established. However, a number of *in vivo* studies have clearly shown that instrumentation and irrigation with sodium hypochlorite fail to predictably produce sterile root canals<sup>58,60</sup>. Studies with dentine powder have shown that dentine has an inhibitory effect on the antibacterial effectiveness of 1% sodium hypochlorite<sup>56</sup>. Dentine powder (18% v/w) greatly delayed the killing process of *E. faecalis*, which was used as a test organism<sup>56</sup>. When hypochlorite was pre-incubated with dentine in a closed test tube for 24 hours before adding the bacteria, killing all of the bacteria required 24 hours incubation with hypochlorite, whereas after 1 hour's incubation all of the bacterial cells were still viable<sup>56</sup>. There are no data available about

the effect of hydroxyapatite on the antibacterial effect of sodium hypochlorite. On the whole, it seems that dentine, reduces or inhibits the antibacterial activity of NaOCl.

### Tissue solubility of NaOCl

Several studies have been conducted to find an irrigant that possesses four major properties: antimicrobial activity, non-toxicity to periapical tissues, water solubility and capacity to dissolve organic matter. Therefore, an ideal irrigant should dissolve the organic matter inside the root canal system. Grossman and Meiman<sup>61</sup> reported that 5% sodium hypochlorite dissolves this tissue in 20min to 2h. Moorer and Wesselink<sup>62</sup> showed that tissue dissolution was dependent on three factors: frequency of agitation, amount of organic matter in relation to amount of irrigant in the system and surface area of tissue that was available. Okino *et al.*<sup>63</sup> evaluated the tissue dissolving ability of 0.5, 1.0 and 2.5% sodium hypochlorite; 2% aqueous solution of CHX; 2% CHX gel (Natrosol™); and distilled water as control. Bovine pulp fragments were weighed and placed in contact with 20mL of each tested substance in a centrifuge at 150rpm until total dissolution. Dissolution speed was calculated by dividing pulp weight by dissolution time. Distilled water and both solutions of CHX did not dissolve the pulp tissue within 6h. Mean dissolution speeds for 0.5, 1.0 and 2.5% sodium hypochlorite solutions were 0.31, 0.43 and 0.55mg/min, respectively.

In another study, Naenni *et al.*<sup>64</sup> assessed the necrotic tissue dissolution capacity of 1% (wt/vol) sodium hypochlorite (NaOCl), 10% chlorhexidine, 3% and 30% hydrogen peroxide, 10% peracetic acid, 5% dichloroisocyanurate (NaDCC), and 10% citric acid. Standardised necrotic tissue samples obtained from pig palates were incubated in these solutions, and their weight loss was measured over time. None of the test solutions except sodium hypochlorite had any substantial tissue dissolution capacity. It was concluded that this might be important when considering the use of irrigants other than NaOCl. Clarkson *et al.*<sup>65</sup> evaluated the tissue-dissolving ability of two concentrations of NaOCl on porcine incisor pulps. They found that greater concentrations provided more rapid dissolution of tissue. Taken together, NaOCl is a strong proteolytic agent, which exhibits the best tissue dissolving ability as an endodontic irrigant.

### Effect of NaOCl on endodontic instruments

Canal preparation requires a continuous and progressively tapered shape, so as to allow NaOCl to be delivered to the apical section of canal and perform its bactericidal action and to dissolve organic debris. Nickel-Titanium instruments come into contact with

NaOCl when the solution is present in the pulp chamber and root canal during instrumentation. NaOCl is corrosive to metals involving selective removal of nickel from the surface creating micropitting<sup>66</sup>. It is supposed that these microstructural defects can lead to areas of stress concentration and crack formation, weakening the structure of the instrument<sup>66</sup>. O'Hoy *et al.*<sup>67</sup> evaluated the effect of cleaning procedures using NaOCl, and detected significant corrosive phenomena of NiTi instruments exposed to 1% NaOCl for up to 10 cleaning cycles. However, no significant reduction of torque at fracture or number of revolutions to flexural fatigue was found. Busslinger *et al.*<sup>68</sup> used 5% NaOCl for 30 or 60min and Lightspeed rotary instruments, and found corrosion patterns, even if the authors were not sure of the clinical implications. Berutti and Marini<sup>69</sup> evaluated the influence of immersion in NaOCl on resistance to cyclic fatigue fracture and corrosion of ProTaper NiTi rotary instruments. They concluded that if NiTi rotary instruments operate immersed in a NaOCl solution contained in the pulp chambers of teeth restored with metals or alloys having different electrochemical nobility values, galvanic corrosion may occur. These coupling phenomena may cause pitting and cracks that alter the integrity of the instrument surface, decreasing its resistance to fracture because of cyclic fatigue. Haikel *et al.*<sup>70,71</sup> reported that the mechanical properties of Ni-Ti instruments were not affected by NaOCl, nor was the cutting efficiency. This is probably the result of very slow corrosion of NiTi alloy alone, when the galvanic effect does not potentiate it, as occurs where other metals are present.

### Effect of heating on the antimicrobial and tissue solubility of NaOCl

An alternative approach to improve the effectiveness of sodium hypochlorite in the root canal system could be to increase the temperature of low-concentration NaOCl solutions thereby improving their immediate tissue-dissolution capacity<sup>7</sup>. Furthermore, heated hypochlorite solutions remove organic debris from dentine shavings more efficiently than unheated counterparts<sup>72</sup>. The antimicrobial properties of heated NaOCl solutions have also been discussed. As early as 1936, the effect of NaOCl temperature on *Mycobacterium tuberculosis* survival was demonstrated<sup>73</sup>. With the taxa tested so far, bactericidal rates of sodium hypochlorite solutions are more than doubled for each 5°C rise in temperature in the range of 5-60°C<sup>7</sup>. This was corroborated in a recent study using steady-state planktonic *E. faecalis* cells; a temperature raise of 25°C increased NaOCl efficacy by a factor 100<sup>74</sup>. The capacity of a 1% NaOCl at 45°C to dissolve human dental pulps was found to be equal to that of a 5.25% solution at 20°C<sup>74</sup>. On the other hand, with similar short-term efficacy in the immediate environment, i.e. the root canal system, the systemic toxicity

of pre-heated NaOCl irrigants should be lower than the one of the more concentrated non-heated counterparts as temperature equilibrium is reached relatively quickly<sup>75</sup>. However, there are no clinical studies available to support the use of heated NaOCl.

### Effect of NaOCl on the composition and structure of dentine

One NaOCl side effect has received relatively little attention in the endodontic literature: the impact on the dentine matrix. Dentine is composed of approximately 22% organic material by weight. Most of this consists of type I collagen, which contributes considerably to the mechanical properties of dentine<sup>76</sup>. Sodium hypochlorite is known to fragment long peptide chains and to chlorinate protein terminal groups; the resulting N-chloramines are broken down into other species<sup>77</sup>. Consequently, hypochlorite solutions may affect the mechanical properties of dentine by the degradation of organic dentine components<sup>78</sup>. A study on bovine dentine suggested that, within the time frame of a root canal treatment, concentrated hypochlorite solutions cause untoward effects on dentine biomechanics<sup>79</sup>. Slutzky-Goldberg *et al.*<sup>79</sup> evaluated the effect on root dentine microhardness of 2.5% and 6% sodium hypochlorite solutions for various irrigation periods (5, 10, or 20 min). They found that there was a significant difference in groups irrigated for 10 or 20 min. Furthermore, the decrease in microhardness was more marked after irrigation with 6% NaOCl than 2.5% NaOCl. A 2h exposure of dentine to NaOCl solutions of more than 3% (w/v) significantly decreases the elastic modulus and flexural strength of human dentine compared to physiological saline<sup>80,81</sup>.

Mountouris *et al.*<sup>82</sup> evaluated the deproteination potential of 5% aqueous NaOCl solution applied with a rubbing action on the molecular composition and morphology of smear-layer covered and acid-etched human coronal dentine surfaces. They found that in both groups, NaOCl treatment reduced the organic matrix (amide I, II, III peaks), but did not affect carbonates and phosphates. Di Renzo *et al.*<sup>83</sup> evaluated chemical alterations on the dentine surface after treatment with NaOCl using a photoacoustic FTIRS (PA-FTIRS) technique. Results showed that NaOCl-treated dentine samples demonstrated a slow and heterogeneous removal of its organic phase, leaving calcium hydroxyapatite and carbonate apatite unchanged. A combined sequential 2min treatment of dentine with both maleic acid and NaOCl indicated that this treatment could produce a surface region which was neither significantly demineralised nor deproteinated. In another study, the effects of NaOCl on dentine collagen and glycosaminoglycans were evaluated immunohistochemically<sup>57</sup>. The results showed that 5% NaOCl induced alterations in dentine collagen and glycosaminoglycans and hydroxyapatite demonstrated

a protective role of on organic matrix stability<sup>57</sup>. Recently, Marending *et al.*<sup>78</sup> evaluated the effects of NaOCl on the structural, chemical and mechanical properties of human root dentine. They found that NaOCl caused a concentration-dependent reduction of elastic modulus and flexural strength in human root dentine. Furthermore, both the carbon and nitrogen content of the specimens were significantly reduced. In addition, intertubular dentine permeability to basic fuchsin dye altered, although no effect on inorganic dentine components could be detected in backscattered SEMs and the peripheral dentine matrix was severely altered.

### Effect of NaOCl on bonding to dentine

Dentine is degenerated by NaOCl treatment because of the dissolution of dentinal collagen<sup>84</sup>. Moreover, residual NaOCl may interfere with polymerisation of bonding resin due to oxygen generation<sup>84</sup>. The bond strength of resin following NaOCl treatment before etching decreased when a MMA-TBB resin system was employed<sup>85</sup>. The decreased bond strength is improved when an ascorbic acid or a sodium thiosulfate solution is applied after NaOCl treatment. These solutions remove NaOCl by the oxidation-reduction reaction<sup>85</sup>. Nikaido *et al.*<sup>86</sup> evaluated the bonding strength at the buccal dentine surface after NaOCl treatment on the root canal wall dentine; the bonding strength of single bond (SB) was significantly decreased after NaOCl treatment, while that of self-etching primer system (Clearfil Mega Bond) did not change. Ishizuka *et al.*<sup>84</sup> found that the bonding strength of self-etching primer system decreased following NaOCl treatment whereas that of SB did not change. Perdigao *et al.*<sup>87</sup> assessed the effect of 10% commercial NaOCl gel on the dentine shear bond strengths and HL ultra-morphology of two total-etch adhesive systems (Prime&Bond NT and Single Bond). Results demonstrated that the increase in the NaOCl application time resulted in a progressive decrease in shear bond strengths for both dentine adhesives.

Frankenberger *et al.*<sup>88</sup> compared the dentine bond strength and marginal adaptation of direct composite resins with and without additional NaOCl treatment after the etching process. They found that after hypochlorite treatment, dentine bond strength and marginal adaptation decreased significantly. Saboia *et al.*<sup>89</sup> investigated the effect of 10% NaOCl for one minute after acid conditioning on the shear bond strength of two acetone-based single-bottle adhesive systems and found that collagen removal improves the bond strength for these systems. Pioch *et al.*<sup>90</sup> evaluated the effect of NaOCl treatment of acid-etched dentine on the tensile bond strength of adhesive resins. They found that the removal of the collagen layer with NaOCl could enhance or decrease bond strengths, depending on the bonding agent used. Osorio *et al.*<sup>91</sup> evaluated the effect of NaOCl treatment on the shear bond strength

and microleakage of a polyalkenoic acid-containing adhesive system. Results showed that adverse chemical interactions could have occurred between the remnant collagen matrix and/or mineralised dentine after NaOCl treatment. There was no additional advantage in using NaOCl treatment with this adhesive. Ari *et al.*<sup>92</sup> evaluated the effect of NaOCl on the regional bond strengths of four adhesive systems to root canal dentine. They found that, depending on the adhesive system, NaOCl enhanced the bond strength. Erdemir *et al.*<sup>93</sup> indicated that NaOCl, decreased bond strength of C&B Metabond to root canal dentine significantly.

Shinohara *et al.*<sup>94</sup> found that depending on the adhesive system used, the application of NaOCl increased microleakage along dentine margins. Correr *et al.*<sup>95</sup> demonstrated that dentine surface treatment with NaOCl did not affect the resin-dentine bonding strength in primary teeth. Vongphan *et al.*<sup>96</sup> indicated that NaOCl significantly reduced the bond strengths of the adhesive when a total etching was applied. The application of sodium ascorbate on NaOCl treated dentine significantly improved the bond strengths. Wachlarowicz *et al.*<sup>97</sup> examined the effects of commonly employed endodontic irrigants on Epiphany (root canal sealer)-dentine bond strengths. They found that only NaOCl improved the bond strengths.

### Haemostatic property of NaOCl

In addition to its excellent antimicrobial and tissue dissolving properties, NaOCl is also successful in controlling tissue amputation haemorrhage. Since the late 1950s, histological studies have reported that NaOCl is biologically compatible with exposed pulp tissues and highly successful when used as a haemostatic agent in direct pulp capping<sup>98-100</sup>. Clinical studies have reported that proper haemorrhage control appears to be perhaps the single most important factor to influence the clinical success of direct pulp capping, regardless of the pre-existing clinical symptoms<sup>101,102</sup>. Senia *et al.*<sup>103</sup> demonstrated that 5.25% NaOCl is more effective for removal of normal non-infected tissue than saline and that the solvent effect of NaOCl on normal subjacent tissues seems to be limited by the effervescence of the solution; this limits its solvent effect to only those superficial cells and renders it ineffective in deeper pulpal tissues. Hafez *et al.*<sup>104</sup> showed that 3% NaOCl was biocompatible as a haemorrhage control agent, because pulps treated with this concentration demonstrated no evidence of pulpal necrosis after 7- and 27-days. It has been shown that with increased amounts and concentration of NaOCl, the protein component of the supernatant breaks down into lower molecular weight fragments on SDS-PAGE analysis and protein bands are difficult to detect at 5% concentrations of NaOCl<sup>105</sup>. It is known that certain components of haemoglobin change into bilirubin pigments within the cell microsomes *In vivo*<sup>105</sup>. It can be

concluded that NaOCl can be used for the disinfection and chemical amputation of operative debris and clot and coagulum debris as well as for the establishment of a dentine-pulp interface free of organic biofilm before adhesive capping.

### Toxicity of NaOCl

Sodium hypochlorite has a pH 11–12 approx and when hypochlorite contacts tissue proteins, nitrogen, formaldehyde and acetaldehyde are formed within a short time and peptide links are broken resulting in dissolution of the proteins<sup>106</sup>. During the process, hydrogen in the amino groups (-HN-) is replaced by chlorine (-NCl-) thereby forming chloramine, which plays an important role in antimicrobial effectiveness. Necrotic tissue and pus are thus dissolved and the antimicrobial agent can reach and clean the infected areas better. An increase in temperature of the solution significantly improves the antimicrobial and tissue-dissolving effects of sodium hypochlorite. As a consequence to these properties, NaOCl is highly toxic at high concentrations and tends to induce tissue irritation on contact<sup>106</sup>.

The minimum amount of a household bleach (3.12–5.25% NaOCl) to cause a caustic burn in dog oesophagus was found to be 10mL over a 5-min period. Results from this study suggest that provided the time of contact with sodium hypochlorite is minimal, it does not have the same untoward effect on the mucosal surface as is evidenced when it is introduced interstitially<sup>107</sup>. In a similar study, Weeks and Ravitch<sup>108</sup> observed severe oedema with areas of haemorrhage, ulceration, and necrosis and stricture formation in the cat oesophagus when undiluted household bleach was placed on the mucosal surface. Pashley *et al.*<sup>109</sup> demonstrated the cytotoxicity of NaOCl using three independent biological models. They found that a concentration as low as 1:1000 (v/v) NaOCl in saline caused complete haemolysis of red blood cells *in vitro*. As the solution used in this study was isotonic and thus excluded an osmotic pressure gradient, the observed haemolysis and loss of cellular protein was due to the oxidising effects of NaOCl on the cell membrane. Undiluted and 1:10 (v/v) dilutions produced moderate to severe irritation of rabbit eyes whilst intradermal injections of undiluted, 1:2, 1:4 and 1:10 (v/v) dilutions of NaOCl caused skin ulcers. Kozol *et al.*<sup>110</sup> proved Dakin's solution to be detrimental to neutrophil chemotaxis and toxic to fibroblasts and endothelial cells.

Heggors *et al.*<sup>111</sup> examined wound healing relative to irrigation and bactericidal properties of NaOCl in *in vitro* and *In vivo* models. They concluded that 0.025% NaOCl was the safest concentration to use because it was bactericidal but not tissue-toxic.

Zhang *et al.*<sup>112</sup> evaluated the cytotoxicity of four concentrations of NaOCl (5.25%, 2.63%, 1.31%, and 0.66%), eugenol, 3% H<sub>2</sub>O<sub>2</sub>, Ca(OH)<sub>2</sub> paste and MTAD.

Results showed that toxicity of NaOCl was dose-dependent. Barnhart *et al.*<sup>113</sup> measured the cytotoxicity of several endodontic agents on cultured gingival fibroblasts using the CyQuant assay. The results showed that IKI and Ca(OH)<sub>2</sub> were significantly less cytotoxic than NaOCl.

Most complications of the use of sodium hypochlorite appear to be the result of its accidental injection beyond the root apex which can cause violent tissue reactions characterised by pain, swelling, haemorrhage, and in some cases the development of secondary infection and paresthesia<sup>106</sup>. A great deal of care should therefore be exercised when using sodium hypochlorite during endodontic irrigation. Ehrlich *et al.*<sup>114</sup> suggested that a clinician should check, both clinically and radiographically for immature apices, root resorption, apical perforations or any other conditions that may result in larger than normal volumes of irrigant being extruded from the root-canal system into the surrounding tissue. Irrigation should be performed slowly with gentle movement of the needle to ensure that it is not binding in the canal<sup>106</sup>. In an *in vitro* study by Brown *et al.*<sup>115</sup>, the use of a reservoir of irrigation fluid in the coronal access cavity and carried into the root canal during filing resulted in significantly less apical extrusion of irrigation solution than with deep delivery with an irrigation needle.

### Complications during irrigation

As discussed above sodium hypochlorite has toxic effects on vital tissues resulting in haemolysis, skin ulceration and necrosis. Some of the most important complications related to irrigation with NaOCl are listed below.

#### Damage to clothing

As sodium hypochlorite is a common household-bleaching agent, even small amounts may cause severe damage. When using an ultrasonic device for root canal irrigation the aerosol may also cause damage. These mishaps should be prevented by proper protection of the patient's clothing. When using hand irrigation, one should ensure that the irrigation needle and syringe are securely attached and will not separate during transfer or irrigation in order to prevent leakage over clothing<sup>116</sup>.

#### Damage to the eye

Irrigant in contact with the patient's or operator's eyes results in immediate pain, profuse watering, intense burning, and erythema. Loss of epithelial cells in the outer layer of the cornea may occur. Immediate ocular irrigation with large amounts of tap water or sterile saline should be performed by the dentist and the patient referred to an ophthalmologist for further examination and treatment<sup>117</sup>.

### **Injection of sodium hypochlorite beyond the apical foramen**

Inadvertent injection of sodium hypochlorite beyond the apical foramen may occur in teeth with wide apical foramina or when the apical constriction has been destroyed during root canal preparation or by resorption. Additionally, extreme pressure during irrigation or binding of the irrigation needle tip in the root canal with no release for the irrigant to leave the root canal coronally may result in contact of large volumes of the irrigant to the apical tissues. If this occurs, the excellent tissue-dissolving capability of sodium hypochlorite will lead to tissue necrosis<sup>116</sup>.

In a case report, after wedging the irrigation needle in the root canal, 5.25% sodium hypochlorite was forced beyond the apex of a maxillary right cuspid which led to immediate strong reactions with extreme pain<sup>118</sup>. Within a few seconds the patient's cheek and upper lip showed signs of haematoma and ecchymosis inferior to the right zygoma and profuse haemorrhage from the root canal. Wet compresses continuously applied to the face relieved the pain and the burning sensation felt by the patient. The patient was given antibiotics and analgesics, and the root canal was left open for drainage. Although the swelling increased during the next few hours, the pain had diminished. The patient was advised to replace the cold compresses by hot compresses to stimulate local systemic circulation. One month after the incident the patient's face had returned to normal and root canal therapy could be completed<sup>118</sup>.

After iatrogenic perforation of the root canal of a lateral maxillary incisor, a 3% NaOCl solution was injected inadvertently beyond the apex<sup>119</sup>. The patient experienced 'heavy' spontaneous pain followed by a rapid swelling of the left cheek. Eight days later an abscess had developed, probably due to the spread of infected material from the root canal into the periapical tissue; this had to be treated surgically. Large amounts of pus and necrotic tissue were found. Four years later the patient still reported hyperaesthesia and extreme sensitivity to cold temperatures.

Reeh and Messer<sup>120</sup> reported on a case of injection of sodium hypochlorite (1%) through a midroot perforation of a maxillary central incisor. The patient experienced the typical symptoms of immediate severe pain and swelling, followed by fistulation and erythema extending to the infraorbital area. Paraesthesia of the floor and ala of the patient's nose persisted for more than 15 months.

In a case report presented by Sabala and Powell<sup>121</sup> 5.25% sodium hypochlorite was injected into the periapical tissues of a left maxillary second premolar. The patient experienced symptoms of sudden, severe pain and a swelling rapidly developed, followed by ecchymosis of the skin. Root canal treatment was completed at the same appointment. To prevent secondary infection, antibiotics were prescribed and a surgical drainage performed. Nine days later the symptoms had resolved.

Following injection of 5.25% NaOCl during endodontic treatment of a right maxillary central incisor, Gatot *et al.*<sup>122</sup> reported that the patient immediately experienced severe pain and marked oedema developed extending from the lip to the right eye. The patient received hydrocortisone intravenously and penicillin. Thirty-six hours later there was large ecchymosis under the right orbit and diffuse ecchymosis over the upper lip, as well as epithelial necrosis. Surgical debridement with excision of a large amount of necrotic tissue had to be performed under general anaesthesia. Healing took more than two weeks, leaving a scar on the right cheek and right infraorbital nerve anaesthesia.

Becking<sup>123</sup> presented three cases of sodium hypochlorite injection into the periapical soft tissues. In the first case NaOCl of unknown concentration was extruded through the apical foramen of a mandibular left second molar with a perforation at the cemento-enamel junction, resulting in a progressive swelling of the left side of the mandible extending to the patient's neck. After one day, necrosis of the mucosa and anaesthesia of the mental nerve was apparent. After antibiotic and analgesic therapy, pain and swelling diminished after 5 days, paraesthesia of the nerve resolved after 10 days and healing of the mucosa took 2 months. In the second case NaOCl of unknown concentration was injected into the periapical tissues of a left maxillary second molar, causing irritation behind and below the patient's left eye and severe pain in the left cheek, eye, and temporal region. Additionally, the patient reported a chlorine taste and irritation of the throat. It was presumed that the irrigant had been pressed into the maxillary sinus. In this case no antibiotics were given, only analgesics; the symptoms resolved completely within two weeks. In the third case apical overextrusion of NaOCl occurred during root canal preparation of a mandibular left second premolar, resulting in severe pain, swelling, and anaesthesia of the mental nerve. Again, no antibiotics were prescribed initially. Four days later necrosis and infection became evident and antibiotic therapy was initiated. Resolution of pain and swelling took one month, anaesthesia changed into hyperaesthesia, which slowly resolved.

Following inadvertent injection of 5.25% NaOCl via the palatal root canal of a maxillary first molar into the maxillary sinus, Ehrich *et al.*<sup>114</sup> reported that the patient only complained of a taste of sodium hypochlorite but no further symptoms developed. The root canal and the sinus were irrigated with sterile water through the palatal root canal. The water passed through the maxillary sinus into the nasal cavity and then into the pharynx. The patient was prescribed antibiotics and was asymptomatic four days later.

A similar case was presented by Kavanagh and Taylor<sup>124</sup> during endodontic treatment of a right second maxillary premolar; sodium hypochlorite of unknown concentration was injected beyond the apex. The patient

experienced acute severe facial pain and swelling. Occipito-mental radiographs demonstrated an air fluid level in the right maxillary sinus. The antrum was drained surgically under general anaesthesia. Three weeks later most of the symptoms had resolved and only the premolar presented with localised discomfort; this led to the decision to extract the tooth. Further healing was uneventful and complete.

### Allergic reactions to NaOCl

Hypersensitivity reactions to sodium hypochlorite have also been reported. Kaufman and Keila<sup>125</sup> reported a case which was detected before initiation of endodontic therapy so that the patient was referred to an allergist. Following a skin patch test, the allergist diagnosed a hypersensitivity to household materials containing NaOCl and recommended not using NaOCl during root canal treatment. The canals were irrigated with Solvidont and the procedure was uneventful. In a second case, sodium hypochlorite (1%) was used for irrigation of a maxillary central incisor with midroot horizontal fracture. The patient immediately reported severe pain and a burning sensation, within a few seconds the upper lip and cheek up to the infraorbital area became swollen, accompanied by ecchymosis and profuse haemorrhage from the root canal. Pain diminished after a few minutes but the patient complained about problems in breathing and was referred to an emergency care unit. Systemic corticosteroid and antihistamine were administered intravenously and antibiotics were prescribed. Swelling resolved after three days, but a paraesthesia on the left side of the face remained for 10 days. Further endodontic therapy was performed with hydrogen peroxide and sterile saline and was uneventful. A skin scratch test was performed some days after the incident and gave a very rapid positive allergic reaction<sup>126</sup>.

### Conclusions

- Sodium hypochlorite has a wide range activity against both Gram positive and Gram negative bacteria
- Sodium hypochlorite is the strongest antifungal agent among root canal irrigations and medications
- Sodium hypochlorite is the only root canal irrigant that can destroy the microbial biofilm effectively
- Utilising sodium hypochlorite for decontamination of the microbial sampling field is controversial
- It seems that dentine reduces or inhibits the antibacterial activity of sodium hypochlorite
- Sodium hypochlorite is a non-specific proteolytic agent with an excellent tissue dissolving ability
- Effects of sodium hypochlorite on the mechanical properties and the cutting efficiency of Nickel-Titanium instruments are still controversial
- It seems that heating sodium hypochlorite increases its antimicrobial and tissue dissolving abilities
- Sodium hypochlorite exerts deteriorative effects on mechanical properties and chemical composition of dentine
- Effects of sodium hypochlorite on the bond strength of bonding systems are still controversial
- Excellent haemostatic activity of sodium hypochlorite may enhance the prognosis of vital pulp therapy
- Cytotoxicity and tissue toxicity of sodium hypochlorite is well documented
- Most complications of the use of sodium hypochlorite appear to be the result of its inadvertent injection beyond the root apex which can cause violent tissue reactions (hypochlorite accident)
- In rare cases sodium hypochlorite may cause allergic reactions.

### References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposure of dental pulps in germ-free and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol* 1965 **18**: 340-348.
2. Moller AJ, Fabricius L, Dahlen G *et al.* Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. *Scand J Dent Res* 1981 **89**: 475-484.
3. Sundqvist G. Ecology of the root canal flora. *J Endod* 1992 **18**: 427-430.
4. Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res* 1981 **89**: 321-328.
5. Hess, W. Anatomy of root canals of the teeth of the permanent dentition. Sons and Danielson Ltd, 1925.
6. Peters OA, Laib A, Gohring TN *et al.* Changes in root canal geometry after preparation assessed by high resolution computed tomography. *J Endod* 2001 **27**: 1-6.
7. Zehnder M. Root canal irrigants. *J Endod* 2006 **32**: 389-398.
8. Test ST, Lampert MB, Ossanna PJ *et al.* Generation of nitrogen-chlorine oxidants by human phagocytes. *J Clin Invest* 1984 **74**:1341-1349.
9. Dakin HD. On the use of certain antiseptic substances in treatment of infected wounds. *BMJ* 1915 **2**: 318-320.
10. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action and resistance. *Clin Microbiol Rev* 1999 **12**: 147-179.
11. Austin JH, Taylor HD. Behavior of hypochlorite and chloramines-T solutions in contact with necrotic and normal tissue *In vivo*. *J Exp Med* 1918 **27**: 627-633.
12. Coolidge ED. The diagnosis and treatment of conditions resulting from diseased dental pulps. *J Nat Dent Assoc* 1919 **6**: 337-349.
13. Frai S, Ng YL, Gulabivala K. Some factors affecting the concentration of available chlorine in commercial sources of sodium hypochlorite. *Int Endod J* 2001 **34**: 206-215.
14. Lambjerg-Hansen H, Fiehn NE, Krogh P. Endodontiske medikamenter. *Tandlaegebladet* 1982 **86**: 467-473.
15. Heling I, Rotstein I, Dinur T *et al.* Bactericidal and cytotoxic effects of sodium hypochlorite and dichloroisocyanurate solutions *in vitro*. *J Endod* 2001 **27**: 278-280.
16. Estrela C, Estrela CR, Barbin EL *et al.* Mechanism of action of sodium hypochlorite. *Braz Dent J* 2002 **13**: 113-117.

17. Estrela C, Sydney GB, Bammann LL *et al.* Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. *Braz Dent J* 1995 **6**: 85-90.
18. Walker A. Definite and dependable therapy for pulpless teeth. *J Am Dent Assoc* 1936 **23**: 1418-1424.
19. Siqueira JF Jr, Machado AG, Silveira RM *et al.* Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal, *in vitro*. *Int Endod J* 1997 **30**: 279-282.
20. Siqueira JF Jr, Batista MM, Fraga RC *et al.* Antibacterial effects of endodontic irrigants on black-pigmented gram-negative anaerobes and facultative bacteria. *J Endod* 1998 **24**: 414-416.
21. Siqueira JF Jr, Rocas IN, Favieri A *et al.* Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. *J Endod* 2000 **26**: 331-334.
22. Gomes BP, Ferraz CC, Vianna ME *et al.* *In vitro* antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *Int Endod J* 2001 **34**: 424-428.
23. Vianna ME, Gomes BP, Berber VB *et al.* *In vitro* evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004 **97**: 79-84.
24. Berber VB, Gomes BP, Sena NT *et al.* Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing *Enterococcus faecalis* within root canals and dentinal tubules. *Int Endod J* 2006 **39**:10-17.
25. Oliveira DP, Barzibam JV, Trope M *et al.* *In vitro* antibacterial efficacy of endodontic irrigants against *Enterococcus faecalis*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007 **103**: 702-706.
26. Bystrom A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 sodium hypochlorite in endodontic therapy. *Oral Surg Oral Med Oral Pathol* 1983 **55**: 307-312.
27. Ercan E, Ozekinci 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**: 84-87.
28. Vianna ME, Horz HP, Gomes BP *et al.* *In vivo* evaluation of microbial reduction after chemo-mechanical preparation of human root canals containing necrotic pulp tissue. *Int Endod J* 2006 **39**:484-492.
29. Siqueira JF Jr, Rocas IN, Paiva SS, *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**: 122-130.
30. Siqueira JF Jr, Magalhaes KM, Rocas IN. Bacterial reduction in infected root canals treated with 2.5% NaOCl as an irrigant and calcium hydroxide/camphorated paramonochlorophenol paste as an intracanal dressing. *J Endod* 2007 **33**: 667-672.
31. Siqueira JF Jr, Guimaraes- Pinto T, Rocas IN. Effects of chemomechanical preparation with 2.5% sodium hypochlorite and intracanal medication with calcium hydroxide on cultivable bacteria in infected root canals. *J Endod* 2007 **33**: 800-805.
32. Siqueira JF Jr, Sen BH. Fungi in endodontic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004 **97**: 632-641.
33. Waltimo TMT, Haapasalo M, Zehnder M *et al.* Clinical aspects related to endodontic yeast infections. *Endod Topics* 2004 **9**: 66-78.
34. Sen BH, Safavi KE, Spangberg LS. Antifungal effects of sodium hypochlorite and chlorhexidine in root canals. *J Endod* 1999 **25**: 235-8.
35. Waltimo TM, Orstavik D, Siren EK *et al.* *In vitro* susceptibility of *Candida albicans* to four disinfectants and their combinations. *Int Endod J* 1999 **32**: 421-429.
36. Ferguson JW, Hatton JF, Gillespie MJ. Effectiveness of intracanal irrigants and medications against the yeast *Candida albicans*. *J Endod* 2002 **28**: 68-71.
37. Smith JJ, Wayman BE. An evaluation of the antimicrobial effectiveness of citric acid as a root canal irrigant. *J Endod* 1986 **12**: 54-58.
38. Harrison JW, Wagner GW, Henry CA. Comparison of the antimicrobial effectiveness of regular and fresh scent Clorox. *J Endod* 1990 **16**: 328-330.
39. Ruff ML, McClanahan SB, Babel BS. *In vitro* antifungal efficacy of four irrigants as a final rinse. *J Endod* 2006 **32**: 331-3.
40. Radcliffe CE, Potouridou L, Qureshi R, *et al.* Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms *Actinomyces israelii*, *A. naeslundii*, *Candida albicans* and *Enterococcus faecalis*. *Int Endod J* 2004 **37**: 438-446.
41. Ayhan H, Sultan N, Cirak M *et al.* Antimicrobial effects of various endodontic irrigants on selected microorganisms. *Int Endod J* 1999 **32**: 99-102.
42. Bowden GH, Hamilton IR. Survival of oral bacteria. *Crit Rev Oral Biol Med* 1998 **9**: 54-84.
43. Svensater G, Bergenholtz G. Biofilms in endodontic infections. *Endod Topics* 2004 **9**: 27-36.
44. Spratt DA, Pratten J, Wilson M *et al.* An *in vitro* evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. *Int Endod J* 2001 **34**: 300-307.
45. Clegg MS, Vertucci FJ, Walker C *et al.* The effect of exposure to irrigant solutions on apical dentine biofilms *in vitro*. *J Endod* 2006 **32**: 434-437.
46. Ozok AR, Wu MK, Luppens SB *et al.* Comparison of growth and susceptibility to sodium hypochlorite of mono- and dual-species biofilms of *Fusobacterium nucleatum* and *Peptostreptococcus* (*micromonas*) *micros*. *J Endod* 2007 **33**: 819-822.
47. Dunavant TR, Regan JD, Glickman GN *et al.* Comparative evaluation of endodontic irrigants against *Enterococcus faecalis* biofilms. *J Endod* 2006 **32**: 527-531.
48. Giardino L, Ambu E, Savoldi E *et al.* Comparative evaluation of antimicrobial efficacy of sodium hypochlorite, MTAD, and Tetraclean against *Enterococcus faecalis* biofilm. *J Endod* 2007 **33**: 852-855.
49. Moller AJ. Microbiological examination of root canals and peri-apical tissues of human teeth. Methodological studies. *Odontol Tidskr* 1966 **74**: Suppl: 1-380.
50. Ng YL, Spratt D, Sriskantharajah S *et al.* Evaluation of protocols for field decontamination before bacterial sampling of root canals for contemporary microbiology techniques. *J Endod* 2003 **29**: 317-320.
51. Young G, Turner S, Davies JK *et al.* Bacterial DNA persists for extended periods after cell death. *J Endod* 2007 **33**:1417-1420.
52. Willson VA. Determination of available chlorine in hypochlorite solutions by direct titration with sodium thiosulfate. *Industrial and Engineering Chemistry Analytical Edition* 1935 **7**: 44-45.
53. Haapasalo M, Qian W, Portenier I *et al.* Effects of dentin on the antimicrobial properties of endodontic medicaments. *J Endod* 2007 **33**: 917-925.
54. Wang JD, Hume WR. Diffusion of hydrogen ion and hydroxyl ion from various sources through dentine. *Int Endod J* 1988 **21**: 17-26.
55. Camps J, Pashley DH. Buffering action of human dentine *in vitro*. *J Adhes Dent* 2000 **2**: 39-50.
56. Haapasalo HK, Siren EK, Waltimo TM. Inactivation of local root canal medicaments by dentine: an *in vitro* study. *Int Endod J* 2000 **33**: 126-131.

57. Oyarzun A, Cordero AM, Whittle M. Immunohistochemical evaluation of the effects of sodium hypochlorite on dentine collagen and glycosaminoglycans. *J Endod* 2002 **28**: 152-156.
58. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 1985 **18**: 35-40.
59. Card SJ, Sigurdsson A, Orstavik D *et al.* The effectiveness of increased apical enlargement in reducing intracanal bacteria. *J Endod* 2002 **28**: 779-783.
60. McGurkin-Smith R, Trope M, Caplan DJ *et al.* Reduction of intracanal bacteria using GT rotary instrumentation, 5.25% NaOCl, EDTA, and Ca(OH)<sub>2</sub>. *J Endod* 2005 **31**: 359-363.
61. Grossman LI, Meiman BW. Solution of pulp tissue by chemical agents. *J Am Dent Assoc* 1941 **28**: 223-225.
62. Moorer WR, Wesselink PR. 110th year Nederlands Tijdschrift voor Tandheelkunde. 2. Root canal treatment, intra-canal disinfectants and bacterial culture: past and present. *Ned Tijdschr Tandheelkd.* 2003 **110**: 178-180. Dutch.
63. Okino LA, Siqueira EL, Santos M, Bombana AC *et al.* Dissolution of pulp tissue by aqueous solution of chlorhexidine diglucanate and chlorhexidine diglucanate gel. *Int Endod J* 2004 **37**: 38-41.
64. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. *J Endod* 2004 **30**: 785-787.
65. Clarkson RM, Moule AJ, Podlich H *et al.* Dissolution of porcine incisor pulps in sodium hypochlorite solutions of varying compositions and concentrations. *Aust Dent J* 2006 **51**: 245-251.
66. Sarkar NK, Redmond W, Schwaninger B *et al.* The chloride corrosion of four orthodontic wires. *J Oral Rehabil* 1983 **10**: 121-128.
67. O'Hoy PY, Messer HH, Palamara JE. The effect of cleaning procedures on fracture properties and corrosion of NiTi files. *Int Endod J* 2003 **26**: 724-732.
68. Busslinger A, Sener B, Barbakow F. Effects of sodium hypochlorite on nickel-titanium Lightspeed instruments. *Int Endod J* 1998 **31**: 290-294.
69. Berutti E, Marini R. A scanning electron microscopic evaluation of the debridement capability of sodium hypochlorite at different temperatures. *J Endod* 1996 **22**: 467-470.
70. Haikel Y, Serfaty R, Wilson P *et al.* Mechanical properties of nickel-titanium endodontic instruments and the effect of sodium hypochlorite treatment. *J Endod* 1998 **24**: 731-735.
71. Haikel Y, Serfaty R, Wilson P *et al.* Cutting efficiency of nickel-titanium endodontic instruments and the effect of sodium hypochlorite treatment. *J Endod* 1998 **24**: 736-739.
72. Kamburis JJ, Barker TH, Barfield RD *et al.* Removal of organic debris from bovine dentine shavings. *J Endod* 2003 **29**: 559-561.
73. Costigan SM. Effectiveness of hot hypochlorites of low alkalinity in destroying Mycobacterium tuberculosis. *J Bacteriol* 1936 **32**: 57-63.
74. Sirtes G, Waltimo TM, Schaetzle M *et al.* The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. *J Endod* 2005 **31**: 669-671.
75. Cunningham WT, Balekjian AY. Effect of temperature on collagen-dissolving ability of sodium hypochlorite endodontic irrigant. *Oral Surg Oral Med Oral Pathol* 1980 **49**: 175-177.
76. Currey JD, Brear K, Zioupos P. Dependence of mechanical properties on fibre angle in narwhal tusk, a highly oriented biological composite. *J Biomech* 1994 **27**: 885-897.
77. Stoward PJ. A histochemical study of the apparent deamination of proteins by sodium hypochlorite. *Histochem* 1975 **45**: 213-226.
78. Marending M, Luder UH, Brunner TJ *et al.* Effect of sodium hypochlorite on human root dentine – mechanical, chemical and structural evaluation. *Int Endod J* 2007 **40**: 786-793.
79. Slutzky-Goldberg I, Maree M, Liberman R *et al.* Effect of sodium hypochlorite on dentin microhardness. *J Endod* 2004 **30**: 880-882.
80. Grigoratos D, Knowles J, Ng YL *et al.* Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *Int Endod J* 2001 **34**: 113-119.
81. Sim TP, Knowles JC, Ng YL *et al.* Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *Int Endod J* 2001 **34**: 120-132.
82. Mountouris G, Silikas N, Eliades G. Effect of sodium hypochlorite treatment on the molecular composition and morphology of human coronal dentin. *J Adhes Dent* 2004 **6**: 175-182.
83. Di Renzo M, Ellis TH, Sacher E *et al.* A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. Deproteinization. *Biomaterials* 2001 **22**: 793-797.
84. Ishizuka T, Kataoka H, Yoshioka T *et al.* Effect of NaOCl treatment on bonding to root canal dentin using a new evaluation method. *Dent Mater J* 2001 **20**: 24-33.
85. Kataoka H, Yoshioka T, Suda H *et al.* Effect of sodium hypochlorite on adhesion of 4-META/MMA-TBB resin to dentine. *Jpn J Conser Dent* 1999 **42**: 241-247.
86. Nikaido T, Takano Y, Sasafuchi Y *et al.* Bond strength to endodontically treated teeth. *Am J Dent* 1999 **12**: 177-180.
87. Perdigao J, Lopes M, Geraldini S *et al.* Effect of a sodium hypochlorite gel on dentin bonding. *Dent Mater* 2000 **16**: 311-323.
88. Frankenberg R, Kramer N, Oberschachtsiek H *et al.* Dentin bond strength and marginal adaptation after NaOCl pre-treatment. *Oper Dent* 2000 **25**: 40-45.
89. Saboia V de P, Almeida PC, Ritter AV *et al.* 2-year Clinical evaluation of sodium hypochlorite treatment in the restoration of non-carious cervical lesions: a pilot study. *Oper Dent* 2006 **31**: 530-535.
90. Pioch T, Kobaslija S, Schagen B *et al.* Interfacial micromorphology and tensile bond strength of dentin bonding systems after NaOCl treatment. *J Adhes Dent* 1999 **1**: 135-142.
91. Osorio R, Cebalios L, Tay FR *et al.* Effect of sodium hypochlorite on dentin bonding with a polyalkenoic acid-containing adhesive system. *J Biomed Mater Res* 2002 **60**: 316-324.
92. Ari H, Yasar E, Belli S. Effects of NaOCl on bond strengths of resin cements to root canal dentin. *J Endod* 2003 **29**: 248-251.
93. Erdemir A, Eldeniz AU, Belli S *et al.* Effect of solvents on bonding to root canal dentin. *J Endod* 2004 **30**: 589-592.
94. Shinohara MS, Bedran-de-Castro AK, Amaral CM *et al.* The effect of sodium hypochlorite on microleakage of composite resin restorations using three adhesive systems. *J Adhes Dent* 2004 **6**: 123-127.
95. Correr GM, Puppini-Rontani RM, Correr-Sobrinho L *et al.* Effect of sodium hypochlorite on dentin bonding in primary teeth. *J Adhes Dent* 2004 **6**: 307-312.
96. Vongphan N, Senawongse P, Somsiri W *et al.* Effects of sodium ascorbate on microtensile bond strength of total-etching adhesive system to NaOCl treated dentine. *J Dent* 2005 **33**: 689-695.
97. Wachlarowicz AJ, Joyce AP, Roberts S *et al.* Effect of endodontic irrigants on the shear bond strength of epiphany sealer to dentin. *J Endod* 2007 **33**: 152-155.
98. Tsuneda Y, Hayakawa T, Yamamoto H *et al.* A histopathological study of direct pulp capping with adhesive resins. *Oper Dent* 1995 **20**: 223-229.
99. Cox CF, Hafez AA, Akimoto N *et al.* Biocompatibility of primer, adhesive and resin composite systems on non-exposed and exposed pulps of non-human primate teeth. *Am J Dent* 1998 **11**: S55-63.

100. Hafez AA, Kopel HM, Cox CF. Pulpotomy reconsidered: application of an adhesive system to pulpotomized permanent primate pulps. *Quintessence Int* 2000 **31**: 579-589.
101. Dummer PM, Hicks R, Huws D. Clinical signs and symptoms in pulp disease. *Int Endod J* 1980 **13**: 27-35.
102. Matsuo T, Nakanishi T, Shimizu H *et al.* A clinical study of direct pulp capping applied to caries-exposed pulps. *J Endod* 1996 **22**: 551-556.
103. Senia ES, Marshall FJ, Rosen S. The solvent action of sodium hypochlorite on pulp tissue of extracted teeth. *Oral Surg* 1971 **31**: 96-103.
104. Hafez AA, Cox CF, Tarim B *et al.* An *in vivo* evaluation of hemorrhage control using sodium hypochlorite and direct capping with a one- or two-component adhesive system in exposed nonhuman primate pulps. *Quintessence Int* 2002 **33**: 261-272.
105. Yamaguchi H, Hosoya N, Kobayashi K *et al.* The influence of two concentrations of sodium hypochlorite on human blood: changes in haemolysis, pH and protein. *Int Endod J* 2001 **34**: 231-236.
106. Hauman CH, Love RM. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 1. Intracanal drugs and substances. *Int Endod J* 2003 **36**: 75-85.
107. Yarrington CT. The experimental causticity of sodium hypochlorite in the esophagus. *Annals Otol Rhinol Laryngol* 1970 **79**: 895-899.
108. Weeks RS, Ravitch MM. The pathology of experimental injury to the cat esophagus by liquid chlorine bleach. *Laryngoscope* 1971 **81**: 1532-1541.
109. Pashley EL, Birdsong NL, Bowman K, *et al.* Cytotoxic effects of NaOCl on vital tissue. *J Endod* 1985 **11**: 525-528.
110. Kozol RA, Gillies C, Elgebaly SA. Effects of sodium hypochlorite (Dakin's solution) on cells of the wound module. *Arch Surg* 1988 **123**: 420-423.
111. Heggers JP, Sazy JA, Stenberg BD *et al.* Bactericidal and wound-healing properties of sodium hypochlorite solutions: the 1991 Lindberg Award. *J Burn Care Rehabil* 1991 **12**: 420-424.
112. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method. *J Endod* 2003 **29**: 654-657.
113. Barnhart BD, Chuang A, Lucca JJ *et al.* An *in vitro* evaluation of the cytotoxicity of various endodontic irrigants on human gingival fibroblasts. *J Endod* 2005 **31**: 613-615.
114. Ehrlich DG, Brian JD Jr, Walker WA. Sodium hypochlorite accident: inadvertent injection into the maxillary sinus. *J Endod* 1993 **19**: 180-182.
115. Brown DC, Moore BK, Brown CE Jr. *et al.* An *in vitro* study of apical extrusion of sodium hypochlorite during endodontic canal preparation. *J Endod* 1995 **21**: 587-591.
116. Hulsmann M, Hahn W. Complications during root canal irrigation – literature review and case reports. *Int Endod J* 2000 **33**: 186-193.
117. Ingram TA. Response of the human eye to accidental exposure to sodium hypochlorite. *J Endod* 1990 **16**: 235-237.
118. Becker GL, Cohen S, Borer R. The sequelae of accidentally injecting sodium hypochlorite beyond the root apex. *Oral Surg Oral Med Oral Pathol* 1974 **38**: 633-638.
119. Grob R. Zwischenfall mit Natriumhypochlorit – nur mein Fehler? *Schweizer Monatschrift für Zahnmedizin* 1984 **94**: 661-662.
120. Reeh ES, Messer HH. Long-term paresthesia following inadvertent forcing of sodium hypochlorite through perforation in maxillary incisor. *Endod Dent Traumatol* 1989 **5**: 200-203.
121. Sabala GL, Powell SE. Sodium hypochlorite injection into periapical tissues. *J Endod* 1989 **15**: 490-492.
122. Gatot A, Arbelle J, Leibermann M *et al.* Effects of sodium hypochlorite on soft tissues after its inadvertent injection beyond the root apex. *J Endod* 1991 **17**: 573-574.
123. Becking AG. Complications in the use of sodium hypochlorite during endodontic treatment. Report of three cases. *Oral Surg Oral Med Oral Pathol* 1991 **71**: 346-348.
124. Kavanagh CP, Taylor J. Inadvertent injection of sodium hypochlorite into the maxillary sinus. *Br Dent J* 1998 **185**: 336-337.
125. Kaufman AY, Keila S. Hypersensitivity to sodium hypochlorite. *J Endod* 1989 **5**: 224-226.
126. Çalışkan MK, Türkün M, Alper S. Allergy to sodium hypochlorite during root canal therapy: a case report. *Int Endod J* 1994 **27**: 163-167.

Correspondence to: Dr Zahed Mohammadi, Assistant Professor, Department of Endodontics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Email: mohammadi\_zahed@yahoo.com