

# Reaction rate of NaOCl in contact with bovine dentine: effect of activation, exposure time, concentration and pH

R. G. Macedo<sup>1</sup>, P. R. Wesselink<sup>1</sup>, F. Zaccheo<sup>2</sup>, D. Fanali<sup>2</sup> & L. W. M. van der Sluis<sup>1</sup>

<sup>1</sup>Department of Cariology Endodontology Pedodontology, Academic Centre for Dentistry Amsterdam, Amsterdam; and <sup>2</sup>Visiting Researcher, Department of Cariology Endodontology Pedodontology, Academic Centre for Dentistry Amsterdam, Amsterdam, the Netherlands

## Abstract

**Macedo RG, Wesselink PR, Zaccheo F, Fanali D, van der Sluis LWM.** Reaction rate of NaOCl in contact with bovine dentine: effect of activation, exposure time, concentration and pH. *International Endodontic Journal*.

**Aim** To determine the influence of activation method (ultrasound or laser), concentration, pH and exposure time on the reaction rate (RR) of NaOCl when in contact with dentinal walls.

**Methodology** The walls from standardized root canals in bovine incisors were exposed to a standardized volume of sodium hypochlorite (NaOCl) with different concentrations (2% and 10%), pH (5 and 12) and exposure times (1 and 4 min). Two irrigation protocols were tested: passive ultrasonic irrigation or laser activated irrigation with no activation as the

control. The activation interval lasted 1 min followed by a rest interval of 3 min with no activation. The RR was determined by measuring the iodine concentration using an iodine/thiosulfate titration method.

**Results** Exposure time, concentration and activation method influenced the reaction rate of NaOCl whereas pH did not.

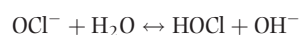
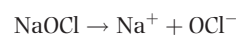
**Conclusions** Activation is a strong modulator of the reaction rate of NaOCl. During the rest interval of 3 min, the consumption of available chlorine increased significantly. This effect seems to be more pronounced after irrigant activation by laser. pH did not affect the reaction rate of 2% NaOCl.

**Keywords:** activation, convection, irrigation, laser, reaction rate, ultrasonic.

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## Introduction

Sodium hypochlorite (NaOCl) solutions are commonly used in endodontics because of at least three important properties: organic tissue-dissolving capacity, bactericidal effect and when properly used, the absence of clinical toxicity (Moorer & Wesselink 1982). In alkaline solutions, when pure NaOCl is dissolved in water, the following reaction takes place:



The free available chlorine consists of hypochlorous acid (HOCl) and the hypochlorite ion ( $\text{OCl}^-$ ), which exist in an equilibrium, depending on the pH of the solution (Baker 1947). The biological activity of NaOCl can be defined as its tissue-dissolving capacity and bactericidal effect and will be influenced by this equilibrium. In alkaline solutions ( $\text{pH} > 7$ ),  $\text{OCl}^-$  prevails, which has a powerful oxidative effect and therefore a higher tissue dissolving capacity than HOCl (Baker 1947). On the other hand, HOCl prevails in acidic solutions ( $3 < \text{pH} < 7$ ) and has a powerful

Correspondence: Ricardo Macedo, DMD, Academic Centre for Dentistry Amsterdam, CEP-Endodontology, Louwesweg 1, 1066 EA Amsterdam, the Netherlands (Tel.: +31 20 5188367; fax: +31 20 6692881; e-mail: r.macedo@acta.nl).

bactericidal effect because it is a smaller uncharged molecule, which can easily penetrate the bacterial membrane. After penetration, it can result in protein degradation (Winter *et al.* 2008).

In the root canal system, NaOCl reacts with organic matter, such as pulp tissue, microorganisms and organic components of the root canal wall, resulting in a loss of its available chlorine ( $\Delta[\text{NaOCl}]$ ). The average velocity of this chlorine consumption is defined as reaction rate (RR) and can be determined by the quotient between the difference in the concentration of NaOCl before and after exposure time ( $\Delta[\text{NaOCl}]$ ) and the total exposure time ( $\Delta t$ ) ( $\text{RR} = \Delta[\text{NaOCl}]/\Delta t$ ).

The chemical efficacy of NaOCl will be influenced significantly by many factors, including: its concentration (Hand *et al.* 1978, Thé 1979, Cunningham & Balekjian 1980, Koskinen *et al.* 1980, Abou-Rass & Oglesby 1981, Sirtes *et al.* 2005, Christensen *et al.* 2008), the surface contact (Rosenfeld *et al.* 1978, Moorer & Wesselink 1982), refreshment (Moorer & Wesselink 1982), exposure time (Thé 1979, Cunningham & Balekjian 1980, Moorer & Wesselink 1982, Christensen *et al.* 2008), type of reactant (Baumgartner & Mader 1987), pH (Baker *et al.* 1947, Christensen *et al.* 2008), energy of the activation (Moorer & Wesselink 1982, van der Sluis *et al.* 2007, Jiang *et al.* 2010a,b), temperature (Cunningham & Balekjian 1980, Abou-Rass & Oglesby 1981, Sirtes *et al.* 2005) and interaction with other chemicals (Shiozawa 2000, and Zehnder *et al.* 2002, 2005). However, the effect of the factors on the RR is currently unknown.

The mechanical aspect of the irrigation procedure (Weller *et al.* 1980, de Groot *et al.* 2009) and the tissue dissolution (Moorer & Wesselink 1982) is improved by passive ultrasonic irrigation (PUI) or laser activated irrigation (LAI), especially when NaOCl solutions are used (van der Sluis *et al.* 2010). No evidence was found in the literature of the influence of ultrasonic or laser activation of NaOCl solutions on the chemical kinetics of the reaction.

The chemical efficacy of NaOCl depends on its free chlorine form and its reactivity. Reaction rate, available chlorine consumption and percentage of chlorine loss ( $\Delta[\text{NaOCl}]/[\text{NaOCl}]_0$ ) are important variables when studying the reactivity of NaOCl in the presence of dentine and to understand its mode of action during root canal treatment.

The aim of this study is to determine the influence of activation method (PUI and LAI), concentration, pH and exposure time on the reactivity of NaOCl in contact with dentinal wall. The null hypothesis is that there is

no difference in reactivity between 2% NaOCl and 10% NaOCl; no activation, PUI and LAI; 1- and 4- min exposure time; and pH 5 and 12 in 2% NaOCl solutions.

## Materials and methods

### Sample selection and standardization

Sixty-six intact, freshly extracted, bovine maxillary central incisors were used. Prior to inclusion, the root canals of the teeth were evaluated radiographically (Advanced 5.4; Emago, Amsterdam, the Netherlands) from two directions (bucco-palatal and mesio-distal) at 1, 8, 16 and 24 mm from the root apex, to ascertain that the canals were smaller than the final root canal preparation. Roots shorter than 26 mm were excluded.

The teeth were decoronated using a diamond cylindrical high-speed bur (Komet, Lemgo, Germany) with air-spray cooling. Pulp tissue was removed entirely using cotton pliers and size 80–100 Hedström files (Dentsply Maillefer, Ballaigues, Switzerland). The contact area between the NaOCl solution and root dentine was standardized by limiting the root and working length (WL) to 24 mm and by a standardized enlargement of the root canal with a round carbide handpiece bur 023 (Komet). All samples were irrigated with Milli-Q water (Millipore Corporation, Billerica, MA, USA) and brushed with a 5.0-mm-diameter interdental brush (Lactona, Hatfield, PA, USA) for blood and debris removal.

The apex of the roots was closed using light-cured composite (Clearfill Photo Core; Kuraray Dental, Frankfurt am Main, Germany) without a bonding system, and the apical 20 mm was embedded in self-curing resin for handling purpose.

### Independent variables

#### *Irrigation methods: activation procedures*

Two irrigation activation protocols were tested: PUI and LAI with no activation of the irrigant as the control. For irrigant placement, a syringe with a 21G needle (Terumo, Leuven, Belgium) was used. The needle was inserted to WL; total irrigant volume was 0.18 mL.

Passive ultrasonic irrigation was performed with a 25-mm, stainless steel, noncutting wire (diameter 0.20 mm, 0 taper) (Irrisafe; Acteon, Merignac, France) powered by a piezoelectronic unit (PMax; Acteon). The oscillation of the wire was directed in bucco-palatal direction, and the power setting was 'Red 10'. Accord-

ing to the manufacturer, the frequency used under these conditions was 30 kHz; the intensity 8 W and the displacement amplitude varied between 20 and 30  $\mu\text{m}$ .

Laser activation was performed by laser radiation (KEY2 laser; KaVo Dental GmbH, Biberach, Germany) from an optical fibre laser tip with outer diameter 280  $\mu\text{m}$  and length 30 mm (type Gr. 30  $\times$  28; Kavo Dental GmbH). Calibration by the manufacturer showed that the optical fibre has a reduction factor of 0.36, which results in a fluence of 146  $\text{mJ mm}^{-2}$  for a laser pulse energy setting of 100 mJ. The Er:YAG laser emits at a wavelength of 2.94  $\mu\text{m}$ , which coincides with the major absorption band of water (Robertson & Williams 1971). A pilot study demonstrated that the optimal settings for chemical activation of NaOCl in bovine teeth are a power setting of 180 mJ per pulse and a pulse repetition frequency of 15 Hz.

In the control group, the irrigant was left in the root canal without any activation procedure.

Wires (PUI) and optical fibres (LAI) were inserted 1 mm coronal from the WL and were moved slowly up and down 4 mm within the apical one-quarter of the root canal (de Groot *et al.* 2009); the activation time was 1 min. The irrigant loss during the activation of the irrigant was tested during a pilot study. The results from this pilot showed that the mean irrigant loss during the activation protocols was 3  $\mu\text{L}$ , which is 1.7% of the irrigant volume. Based on this information, it was concluded that irrigant loss would not interfere with the results.

### Concentration and pH

Two distinct concentrations and pH values were tested: 10% at pH 12 and 2% at pH 12 and 5. The pH 12 solutions were obtained by dilution of  $155 \pm 5$  g/L NaOCl (Boom, Meppel, the Netherlands) with milliQ water and pH 5 solution by titration of HCl 32% (Merck KGaA, Darmstadt, Germany).

The pH and the concentration were measured just before starting each test, using a pH meter – pHM220 MeterLAB (Radiometre analytical, Villeurbanne Cedex, France) and an iodometric titration assay respectively.

### Exposure time

For all variables outlined earlier (activation procedure, concentration and pH), total exposure time to bovine dentine was 1 and 4 min. In the groups with an exposure time of 4 min, the 1 min of activation was followed by a 3- min rest interval (no activation). After

exposure, the solution was collected by negative pressure using a sterile syringe and a 21-gauge needle and put in an Eppendorf tube for quantification of available chlorine.

### Study design

Sixty-six bovine maxillary central incisors were randomly divided into 14 groups: eight experimental groups (laser or ultrasound activation) ( $n = 6$ ) and 6 control groups (no activation) ( $n = 3$ ) (Fig. 1). The RR of different concentrations and pH values of NaOCl was assessed after exposure to bovine dentine after the different irrigant activation protocols. Measurements were taken after 1 and/or 4 min.

### Reaction rate assay

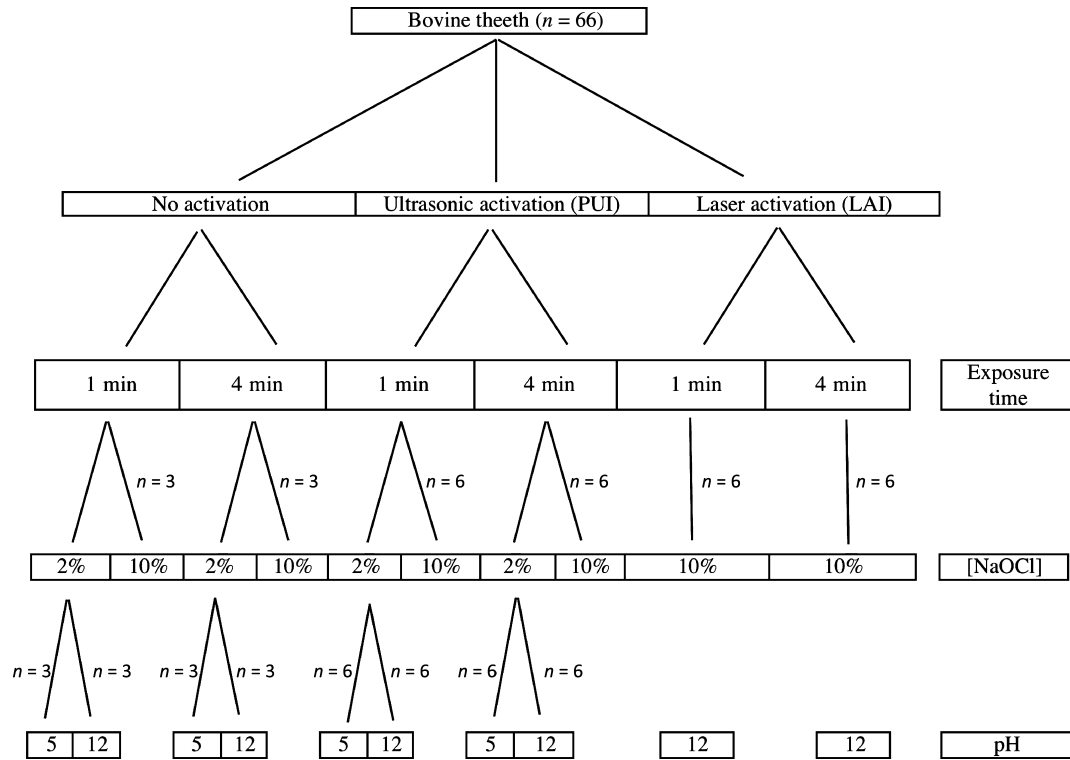
The RR of the NaOCl solutions was assessed by measuring the amount of total available chlorine (%v/v) in solution before and after exposure to bovine dentine, using a standard iodine/thiosulfate titration method (Vogel 1962).

As this method was described and validated only for large volumes (25 mL), smaller volumes (from 25 to 0.01 mL) of a 10%-NaOCl solution were tested for validation. A Pearson product–moment correlation coefficient of 0.996 ( $P < 0.001$ ) between the NaOCl sample volume and sodium thiosulfate titrate volume was obtained validating the iodine/thiosulfate titration method also for sample sizes within the referred range.

Measurements were carried out in triplicate for each sample. Micropipettes (Finnpipettes; MTX Lab Systems, Vienna, VA, USA) were used for titration to increase the accuracy of the method. The reliability was tested using interclass correlation coefficient amongst the three measurements of each sample. A coefficient score of 0.999 for average measurements with  $P < 0.001$  was found, assuring a high reliability.

### Statistics

Student's *t*-tests for independent samples were performed to assess differences in final total available chlorine ( $[\text{NaOCl}]_f$ ) between groups with the same initial concentration. To compare groups with different initial concentration (2% vs. 10%), the percentage of chlorine reduction ( $\Delta[\text{NaOCl}]/[\text{NaOCl}]_0$ ) was used as dependent variable. For all tests, *P*-values  $< 0.05$  were considered statistically significant.



**Figure 1** Distribution of the samples per groups.

**Table 1** Mean  $\pm$  SD of total available chlorine (%v/v) after 1- or 4- min exposure. In bold, comparison between means express the effect of exposure time and activation method

Solution	Activation	Exposure time		1 vs. 4 min (P value)
		1 min	4 min	
NaOCl 2% pH5	No Act	1.5 $\pm$ 0.06	1.33 $\pm$ 0.16	<b>0.157</b>
NaOCl 2% pH5	PUI	1.28 $\pm$ 0.06	0.89 $\pm$ 0.09	<b>&lt;0.001</b>
<b>NaOCl 2% pH5</b>		<i>P = 0.002</i>	<i>P = 0.001</i>	
<b>No Activation versus PUI</b>				
NaOCl 2% pH12	No Act	1.65 $\pm$ 0.04	1.39 $\pm$ 0.05	<b>0.002</b>
NaOCl 2% pH12	PUI	1.38 $\pm$ 0.17	0.89 $\pm$ 0.07	<b>&lt;0.001</b>
<b>NaOCl 2% pH12</b>		<i>P = 0.01</i>	<i>P &lt; 0.001</i>	
<b>No Activation versus PUI</b>				
NaOCl 10% pH12	No Act	8.16 $\pm$ 0.09	7.44 $\pm$ 0.10	<b>0.001</b>
NaOCl 10% pH12	PUI	7.04 $\pm$ 0.45	4.64 $\pm$ 0.36	<b>&lt;0.001</b>
<b>NaOCl 10% pH12</b>		<i>P = 0.004</i>	<i>P &lt; 0.001</i>	
<b>No Activation versus PUI</b>				
NaOCl 10% pH12	LAI	7.43 $\pm$ 0.22	3.82 $\pm$ 0.27	<b>&lt;0.001</b>
<b>NaOCl 10% pH12</b>		<i>P &lt; 0.001</i>	<i>P &lt; 0.001</i>	
<b>No Activation versus LAI</b>				
<b>NaOCl 10% pH12 PUI versus LAI</b>		<i>P = 0.081</i>	<i>P = 0.001</i>	

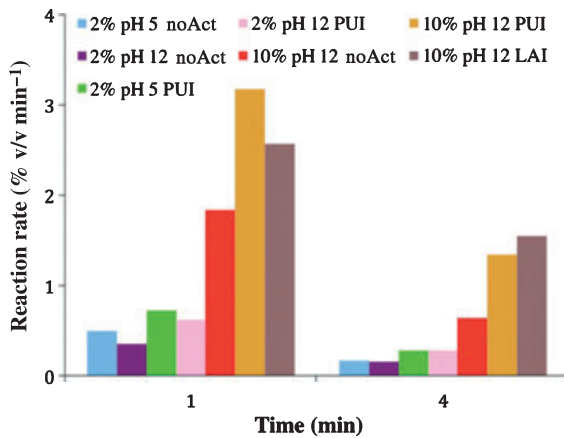
LAI, laser activated irrigation; PUI, passive ultrasonic irrigation.

## Results

The total amount of available chlorine in the NaOCl solutions after 1- or 4- min exposure with bovine

dentine is presented in Table 1 and the reaction rates in Fig. 2.

The difference between the exposure times was statistically significant ( $P < 0.05$ ) for all NaOCl solu-



**Figure 2** Effect of exposure time, activation method, concentration and pH on the reaction rate of sodium hypochlorite solution, measured after 1 and 4 min of contact with dentine.

tions and irrigation activation protocols tested, except the 2% pH 5 without activation ( $P = 0.157$ ).

Activated NaOCl solutions (PUI and LAI) showed a higher RR than nonactivated NaOCl solutions ( $P < 0.05$ ). LAI was more effective than PUI after the 3-min rest interval ( $P = 0.001$ ), but there was no difference between systems after the 1-min activation interval ( $P = 0.081$ ).

The effect of activation on short-term exposure is presented in Table 2. There was no difference in total available chlorine consumption between 1-min exposure of activated solution and 4 min without activation ( $P > 0.05$ ).

High concentration (10% vs. 2%) result in significant higher consumption of available chlorine (Table 1) with no difference in the percentage of chlorine reduction (Table 3).

**Table 2** Mean  $\pm$  SD of total available chlorine (%v/v) after 1- or 4- min exposure. In bold, comparison between means express the effect of activation on short-term exposure

Solutions	Activation	Exposure		1 vs. 4 min ( $P$ value)
		4- min Exposure	1- min Exposure	
2% pH 5	PUI	1.33 $\pm$ 0.16	1.28 $\pm$ 0.06	<b>0.486</b>
2% pH 12	PUI	1.39 $\pm$ 0.05	1.38 $\pm$ 0.17	<b>0.915</b>
10% pH 12	PUI	7.44 $\pm$ 0.10	7.04 $\pm$ 0.45	<b>0.173</b>
10% pH 12	LAI	7.44 $\pm$ 0.10	7.43 $\pm$ 0.22	<b>0.906</b>

LAI, laser activated irrigation; PUI, passive ultrasonic irrigation.

**Table 3** Mean  $\pm$  SD of the percentage of chlorine reduction (%v/v) of 2% and 10% NaOCl solutions after 1- or 4- min exposure and with or without activation of the solutions

Exposure time (min)	Solutions Activation	Solutions		$P$ value
		2% pH 12	10% pH12	
1	No Activation	17.33% $\pm$ 2	18.33% $\pm$ 1	0.507
4	No Activation	30.67% $\pm$ 2	26.33% $\pm$ 1	0.034
1	PUI	31.33% $\pm$ 9	29.83% $\pm$ 4	0.920
4	PUI	55.83% $\pm$ 4	53.5% $\pm$ 4	0.286

PUI, passive ultrasonic irrigation.

## Discussion

Bovine incisor dentine is considered a suitable substitute for human molar dentine (Schilke *et al.* 2000) because of the similar dentinal structure and number of tubuli. However, when used as a root canal model, the root canals are larger than human teeth; therefore, lumen size, capacity and the contact area are significantly increased. After the standardization procedures, the prepared root canal had a cylindrical shape with a diameter of 2.3 mm (radius  $r = 1.15$  mm) and a WL of 24 mm  $h$ . Equation (1) was used to calculate the contact area  $S$  between dentine and NaOCl.

$$S = 2 \cdot \pi \cdot r \cdot (h + r/2) \quad (1)$$

The volume  $V$  of the solution was determined using equation (2)

$$V = \pi \cdot r^2 \cdot h \quad (2)$$

For the bovine teeth,  $S = 177.5 \text{ mm}^2$  and  $V = 99.7 \text{ mm}^3$  (ratio  $V/S = 0.56$ ), whereas a human root canal with, for example, a parallel arranged preparation of size 60 (van der Borden *et al.* 2010) with a length of 15mm has virtually  $S = 57.7 \text{ mm}^2$  and  $V = 4.2 \text{ mm}^3$  (ratio  $V/S = 0.07$ ).

The bovine teeth model used has more free chlorine available to react and relatively less organic matter. Such differences could predict a higher rate of consumption of the reactant – dentine organic matter – in the present model compared to human teeth, whereas the RR of the reagent (NaOCl) would be lower because of the relatively higher volume/surface ratio (Dreybrodt *et al.* 1996). Therefore, a higher effect of activation on the RR is expected in a clinical situation compared to the results of the current study, without the need of high-energy settings and possibly within a shorter activation time.

The increased lumen size (diameter of the canal in a cross-section) increases the distance between the file tip

and dentinal walls. This reduces the streaming (convection) from the oscillating file towards the dentinal walls, thereby possibly reducing the liquid-wall shear stress. Consequently, there is less exposure of active chlorine molecules to dentine. Also, the occurrence of cavitation on the root canal wall will be less likely.

To balance the effect of such dimensional differences, energy of activation was set to power 'Red 10' for ultrasound and energy per pulse of 180 mJ for laser. These settings proved to be optimal in a pilot study.

In general, the RR of a chemical reaction can be defined as the amount of a reactant reacted or the amount of a product formed per unit time. The RR of NaOCl can be expressed by the velocity of the consumption of its available chlorine during a period of time. Its study is vital for understanding the behaviour of a NaOCl solution, when it is in contact with organic components of the root canal system.

Sodium hypochlorite is highly reactive by nature (Dychdala 1977). In the setup used in this study, available chlorine loss may be explained by the following: (i) reaction with organic content of dentine, (ii) reaction with residual pulp, debris and/or blood, (iii) reaction with the material of the activated instrument and (iv) activation, which causes degassing of the solution (Laugier *et al.* 2008).

To minimize the impact of factor (ii), the teeth were thoroughly cleaned trying to limit the amount of smear layer by focussing on the removal of the complete pulp and flushing out of the residual blood. The small standard deviation (Table 1) indicates this procedure was effective.

In a pilot study, all energy settings, activation times and protocols (ultrasonic or laser activation) were tested with NaOCl solutions in 0.5 -mL plastic Eppendorf tubes. Activation of NaOCl solutions, for the volumes studied, resulted in no changes in the RR of the NaOCl solution regardless the initial concentration of available chlorine or pH. These results exclude the influence of factors (iii) and (iv) in the outcome, which is in accordance with the results of Duckhouse *et al.* (2004).

Sodium hypochlorite acts by direct contact between free available chlorine molecules and organic matter (Moorer & Wesselink 1982). In this context, the movement of molecules within fluids plays a major role. Increasing the movement of molecules in the root canal system increases the contact of active chlorine molecules and organic matter and therefore the chemical efficacy of the irrigant. The flux of molecules within a liquid takes place through two mechanisms: diffusion

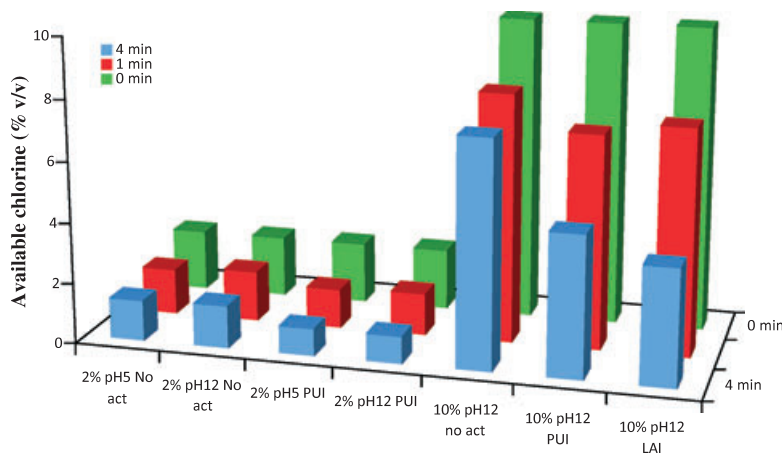
and convection. Diffusion is the random movement of individual particles in the fluid. This process is slow and dependent on temperature and concentration gradients. On the other hand, convection is a faster and more efficient transport mechanism, in which molecules are transported by the motion of fluid (Incropera & de Witt 1990). In the nonactivated solution, diffusion seems to be the main mechanism of molecular transport. To what extent and how fast this occurs will depend on the chemical concentration distribution in the solution. For the activated irrigants, the situation is different. Here, the molecular flow through the root canal is a convection process sustained by acoustic microstreaming.

Activation of NaOCl influences its RR probably by a synergistic effect of temperature and convection in the irrigant/root canal system. The relative importance of these factors, however, is not fully understood, although some aspects have been clarified. Activation increases convection to the dentinal wall by acoustic streaming (Jiang *et al.* 2010a,b). It also increases the temperature of irrigants (Zeltner *et al.* 2009), but this rise of temperature alone cannot explain the tissue dissolution capacity of activated NaOCl in lateral canals (Al-Jadaa *et al.* 2009). The reported temperature rise during ultrasonic activation of NaOCl without refreshment is  $\pm 8$  °C depending on the location of the measurement (Cameron 1988). Looking at the tissue dissolution capacity of NaOCl, an increase of 25 °C was needed to compensate for the effect of the concentration comparing a 1% to a 5.25% NaOCl solution (Sirtes *et al.* 2005). In this study, the temperature rise will be less because of the larger dimension of the root canals, and also the effect in the rest interval cannot be explained by the temperature. Therefore, the temperature will probably not affect the results; however, it is interesting to know what the effect of activation will be on NaOCl solutions with a high temperature.

Mason *et al.* (1996) showed that high-intensity ultrasound doubles the rate of hypochlorite catalytic decomposition in aqueous solutions. These results highlight the capacity of ultrasound to provide unique conditions to drive chemical reactions. These conditions predominantly derive from acoustic cavitation.

Although cavitation has been observed to occur in root canal models during PUI (Lumley *et al.* 1988) or LAI (de Groot *et al.* 2009), its chemical effect on root canal cleaning is poorly studied.

Activation was one of the most significant modulators of the reaction rate. For all exposure times,



**Figure 3** Illustration of the effect of activation method, concentration and pH on the total available chlorine (%v/v) of the irrigation solution, measured after 1 and 4 min of contact with dentine.

concentrations and pH tested, activation during 1 min either by ultrasound or laser increased the reactivity of NaOCl (Tables 1 and 3). Activation compensates 3 min of exposure time (Table 2), which is in line with the findings of Moorer & Wesselink (1982).

An increase in the RR was also found in the rest interval of the activated NaOCl solutions (Fig. 2), which has never been reported before in the literature. Activation, activation type and concentration of the NaOCl solution were the major modulators of this phenomenon. The type of the activation also seems to play a role in the mechanism of action as LAI sustained a higher reactivity over time. The causes of such difference are not yet fully understood thus will be the subject of further investigations.

The efficacy of NaOCl depends on the efficiency of its free chlorine form and its reactivity. In distinctly acid solution, chlorination predominates over oxidation, whereas in alkaline solution oxidation is more pronounced. As the reaction dentine-NaOCl is predominantly oxidative, a high efficacy of an alkaline over an acidic NaOCl (Baker 1947) can be expected even though no difference in reactivity between pH 12 ( $\text{OCl}^-$ ) and pH 5 ( $\text{HOCl}$ ) solutions was found in this study.

For all groups, 10% solutions reacted in a direct proportion with 2% as no differences regarding the rate of consumption between both concentrations (Table 3) were found. This means that in 10% NaOCl solutions there is approximately five times more chlorine consumption than 2% (Fig. 3). Such results are in accordance with the findings of Moorer & Wesselink (1982) and may explain the reported direct proportion between NaOCl concentration and its tissue dissolution

capacity (Hand *et al.* 1978, Thé 1979, Cunningham & Balekjian 1980, Koskinen *et al.* 1980, Abou-Rass & Oglesby 1981, Moorer & Wesselink 1982, Sirtes *et al.* 2005, Christensen *et al.* 2008).

## Conclusions

Activation of NaOCl solutions significantly increased the RR of these solutions. This increase was not limited to the activation interval but extended to the rest interval after activation. Here, an association was found between the RR of NaOCl solutions and the activation, activation type and concentration of NaOCl. In general, pH did not seem to be a contributory factor. Furthermore, the concentration of NaOCl solutions did not affect the percentage of chlorine loss but only the amount of chlorine consumed.

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