Influence of refreshment/activation cycles and temperature rise on the reaction rate of sodium hypochlorite with bovine dentine during ultrasonic activated irrigation*

Abstract

Aim To evaluate the effect of multiple refreshment/activation cycles and temperature on the reaction rate of sodium hypochlorite (NaOCl) with bovine dentine during ultrasonic activated irrigation (UAI) under laboratory conditions. Methodology The root canal walls of twenty-four standardized root canals in bovine incisors were exposed to a standardized volume of NaOCl at different temperatures (24°C and 38°C) and exposure times (20, 60 and 180 s). The irrigant was refreshed and ultrasonically activated 4 times for 20 s followed by a 40 s rest interval, with no-refreshment and no-activation as the controls. The reaction rate was determined by measuring the amount of active chlorine in the NaOCl solution before and after being exposed to dentine during the specific experimental conditions. Calorimetry was used to measure the electrical-to-sonochemical conversion efficiency during ultrasonic activation. Results Refreshment, activation and exposure time all increased the reaction rate of NaOCl (p<0.05). During activation, the temperature of the irrigant increased up to 10°C. Such temperature rise was insufficient to enhance the reaction rate of NaOCl (p>0.125). Conclusions The reaction rate of NaOCl with dentine is enhanced by refreshment, ultrasonic activation and exposure time. Temperature rise of irrigant during ultrasonic activation was not sufficient to alter the reaction rate.

Introduction

Sodium hypochlorite (NaOCl) is the main irrigation solution used in endodontics because of its antimicrobial activity, tissue dissolving capacity and, when properly used, the absence of clinical toxicity (Zehnder 2006). Sodium hypochlorite is highly reactive by nature (Dychdala 1977). In the root canal system, NaOCl reacts with pulp tissue, microorganisms, biofilm and organic components of the root canal wall, resulting in loss of its available chlorine (Moorer & Wesselink 1982). The reaction rate (chemical efficiency) and/or tissue dissolution capacity (chemical efficacy) of NaOCl are significantly influenced by the concentration, exposure time, laser/ultrasonic energy applied (Macedo et al. 2010), contact area (Rosenfelt 1978, Moorer & Wesselink 1982), temperature (Sirtes et al. 2005) and interaction with other chemicals (Shiozawa 2000, Zehnder et al. 2002, 2005). Various studies have shown that ultrasonic activated irrigation (UAI) improves the tissue dissolution (Moorer & Wesselink 1982) as well as removal of dentine debris (van der Sluis et al. 2010). Ultrasonic activation of NaOCl is believed to enhance its reaction rate by a synergistic effect of a temperature rise, enhanced streaming (resulting in mixing of the irrigant) and cavitation of the irrigant (Zehnder 2006). However, the exact working mechanism has not been clarified.

During UAI, the Intermittent Flush Method (IntFM) can be applied to refresh the irrigant (Cameron 1988, van der Sluis et al. 2010). Using IntFM, the irrigant is delivered into the root canal with a syringe subsequently, the irrigant is activated ultrasonically. These two steps are repeated in a multiple refreshment/activation cycle protocol. Depending on the irrigation time, this method is equally or more effective than refreshment with a continuous flow of irrigant into the pulp chamber (van der Sluis et al. 2009). In previous studies where IntFM was applied, a plateau in debridement efficacy over three refreshment/activation cycles was observed (Al-Jaada et al. 2009, van der Sluis et al. 2010). However, the effect of refreshment/activation cycles on the chemical efficiency of NaOCl is still unknown.

The reaction rate can be determined by the change in NaOCl concentration within a certain time interval due to the reaction with organic tissue. It has been proven that the reaction rate of NaOCl is directly correlated with organic tissue dissolution efficiency (Moorer & Wesselink 1982) mainly due to its proteolytic effect (Baker 1947). In the current study, dentine is used as the substrate to react with NaOCl. It is composed of a complex organic and inorganic structure where type I collagen dominates the organic matrix (20% of weight percentage and 33% of volume) and hydroxylapatite the inorganic part (Nanci 2008). NaOCl has predominantly an impact on dentine's organic matrix (Driscoll et al. 2002). It splits up the long peptide chains by chlorination of the final protein groups (Davies et al. 1993).

Calorimetry is used to measure the ultrasonic power transferred into a solution. It provides a good measure to evaluate the mechanical and electrical efficiency of the sonochemical reactor system (Iida et al. 2005), as the mechanical effects of microstreaming and cavitation are directly correlated with ultrasonic power (Mason & Lorimer 2002).

The aim of this study is to evaluate the effect of a) temperature b) the influence of four ultrasonic refreshment/activation cycles on the chemical reaction of NaOCl with bovine dentine, and c) to measure temperature variations and the ultrasonic power of ultrasonic activated irrigation under laboratory conditions. The null hypothesis were that there was no difference in reactivity between NaOCl at room temperature (24ºC) and preheated to 38ºC with or without ultrasonic activation; and between refreshment (intermittent flush method) and no refreshment of NaOCl in ultrasonic activated irrigation.

**Materials and Methods**

The chemical reaction of NaOCl at different temperatures was assessed after exposure to bovine dentine in different irrigation sequences.

**Sample selection and standardization**

Twenty-four intact, freshly extracted, bovine maxillary central incisors were randomly divided in seven groups: one experimental group (IntFM including 4 refreshment/activation cycles) with 6 teeth and six control groups with 3 teeth each. The groups are described in Figure 1 and Table 1. For the negative control groups, the NaOCl solution was placed in plastic Eppendorf tubes (0.2 mL). Prior to inclusion of the teeth, the root canals were radiographically evaluated (Advanced 5.4; Emago, Amsterdam, The Netherlands) from two directions (bucco-lingual and mesio-distal) at 1, 8, 16 and 24 mm from the root apex, to ascertain the included root canals were smaller than the final root canal preparation dimension. Roots shorter than 26 mm were
excluded. The teeth were prepared and standardized as described elsewhere (Macedo et al. 2010) limiting the root length to exactly 24 mm, parallel walls and the lumen diameter to 2.3 mm.

Concentration, pH and temperature

A 2.5% NaOCl solution, with a pH of 12 and an initial temperature of 24°C (room temperature) or 38°C (pre-heated in a warm bath) was used as root canal irrigant. The solution was obtained by dilution of 155±5 g/L NaOCl (Boom, Meppel, the Netherlands) with milli-Q water (Millipore Corporation, Billerica, MA, USA). The pH, temperature and the concentration were measured just before starting each test, using a pH meter (pHM220 MeterLAB, Radiometre analytical, Villeurbanne, France), thermometer inside a root canal (HI 93552R, HANNA instruments, IJsselstein, The Netherlands) and an iodometric titration assay (Vogel 1962), respectively.

Activation procedures

The irrigant was delivered by syringe with a 21G needle (Terumo, Leuven, Belgium) inserted to working length (WL). The total delivered irrigant volume was 0.18 mL for each refreshment/activation cycle. Thereafter, UAI was performed with a stainless steel, non-cutting wire (diameter 0.20, taper 0.00, length 25 mm) (Irrisafe; Acteon, Merignac, France) powered by a piezoelectric unit (PMax, Acteon, Merignac, France) at 1 mm coronal from the WL. The oscillation of the wire was directed in a bucco-lingual direction, and the power setting was “red 10”. The frequency used under these conditions was 30 kHz; the electronic power was measured to be 4 Watt and the displacement amplitude 65 μm (Jiang et al. 2011). The irrigant loss during its activation was 3 μL, which equals 1.7% of the total volume and thus not considered as significant. This value was determined by measuring the weight (Genius ME235P, Sartorius, Göttingen, Germany) loss of the irrigant-filled tooth during 1 min of activation, taking into account the density of the solution (NaOCl=1.11 g/cm³). Activation time ranged from 20 to 180 s (Figure 1). In the control groups without activation, the irrigant was just left in the root canal without any activation procedure.

Reaction rate essay

The reaction rate of NaOCl was assessed by measuring the amount of total available chlorine in solution before and after exposure to bovine dentine, using a standard iodine/thiosulfate titration method (Vogel 1962). Exposure times ranged from 20 to 180 s (Figure 1). A previous study (Macedo et al. 2010) has shown high reliability and validity of this method for the bovine sample volumes used in this study. Measurements were done three times for each sample.

Calorimetry

A bovine tooth was used for calorimetric characterization of the reaction. An opening was drilled across radicular dentine to the canal with a round carbide bur (diameter 1.0 mm) at 1 mm coronal from the WL, in order to mount T-type thermocouples (Labfacility, Dinnington, UK) flush with the canal walls. The thermocouple was fixed in position with composite (Clearfil photo core, Kuraray dental, New York, USA), and its final position was verified with radiographs. The tooth was fixed by its coronal third. The thermocouple was connected to
a data recorder HI 93552R (HANNA instruments, IJsselstein, The Netherlands) operated by HI92000 software, recording the temperature every 2 s. The resolution of the measurement tool was 0.1°C and the accuracy ±0.5°C. A 2.5% NaOCl solution, pH 12 at 24°C, was activated for 60 s. A control group with 38°C preheated solutions was monitored for the same time period (60 s) with no activation. Measurements were done three times. The average ultrasonic power (W) dissipated was measured during 1 min using the following equation (1):

\[
\text{Power} = C_w M_w \frac{\Delta T}{\Delta t}
\]

where \(C_w\) is the heat capacity of water (4.18 J/g °C), \(M_w\) is the mass of water (g), and \((\Delta T/\Delta t)\) is the temperature rise per second (°C/s). The observed ultrasonic power dissipated into solution (W/mL) was calculated by dividing the ultrasonic power by the volume of liquid inside the bovine tooth (0.18 mL). The electrical-to-sonochemical power conversion efficiency was obtained by the ultrasonically dissipated power divided by the electronic power input (4W).

Statistical analysis

T-tests for independent samples were performed to assess the differences in the final total available chlorine ([NaOCl]_f) between groups. To compare the total available chlorine after each cycle a paired t-test was used. For all tests, P-values<0.05 were considered statistically significant.

Results

The total amount of available chlorine remaining in the NaOCl solution after exposure to bovine dentine is presented in Table 1 and the comparison between the groups in Table 2. The total amount of chlorine consumed during each activation/refreshment cycle (Fig. 3) decreased significantly (P<0.05). A cumulative effect in the amount of chlorine consumed was observed over 3 activation/refreshment cycles, with an increase of 28% over the non-IntFM group (P=0.041). Activation (UAI vs no activation) and longer exposure time (60 vs 20 s) both resulted in a significantly higher consumption of available chlorine (P<0.05).

UAI increased the temperature by 3.9°C and 9.9°C after 20 and 60 s, respectively, of activation of irrigant solution inside the bovine tooth. However, pre-heating NaOCl to a temperature of 38°C did not result in a significant increase of its reaction rate when compared to irrigant at 24°C, for both activated and non-activated solutions (Table 2).

The acoustic power transmitted to the solution was 1.1 W/mL, which corresponds to a power conversion efficiency of 4.95% at 30 kHz, ‘red 10’ and at 4W input condition.

Discussion

In this study, the amount of chlorine available in NaOCl was found to decrease during ultrasonic activation. This may be explained by: a) reaction with organic content of dentine, b) reaction with residual pulp, debris and/or blood, c) reaction with the material of the activation instrument and d) activation itself, which could cause decomposition of the molecule (Mason et al. 1996) and also degassing of the solution (Laugier et al. 2008). To minimize the impact of factor (b) the teeth were thoroughly cleaned, in order to limit the amount of smear layer, pulp
and residual blood. The small standard deviation in the total remaining amount of available chlorine suggests that this procedure was effective. Two negative control groups using plastic Eppendorf tubes were included to test the influence of activation of irrigant on the reaction with the activation instrument itself (c) and on degassing and decomposition of the NaOCl (d). Exposure of the irrigant to the plastic walls of the control-microreactors resulted in no changes in the NaOCl concentration regardless of the activation protocol. These results exclude the influence of factors c) and d) in the outcome, which is in accordance with the results of Duckhouse et al. (2004). Therefore, it can be concluded that the reaction rate of NaOCl was based on the reaction of NaOCl with dentine only. This is confirmed by the observation that a progressive reduction of the total amount of chlorine that has reacted was seen after each activation/refreshment cycle of the intermittent flush protocol (Fig. 3). This indicates that the reaction is limited by the amount of available organic substance to react, in this case in the root canal wall. In this study, bovine incisor dentine was used as it is considered a suitable substitute for human molar dentine (Schilke et al. 2000) due to the similar dentinal structure and number of tubules. Here, the organic substance is bound to inorganic material inhibiting the consumption of the organic content (Baumgartner & Mader 1987). When smear layer, dentine debris, pulp tissue or biofilm are present in the root canal, these structures will dictate the reaction of the NaOCl solution. Increasing the movement of molecules of irrigant in the root canal system improves the contact between 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 result of the random movement of individual particles in the fluid. This process is slow and depends on temperature and concentration gradients (Squires & Quake 2005). Convection is a faster and more efficient transport mechanism with which molecules are transported by the motion of fluid (Incropera & de Witt 1990). During ultrasonic activation the flow of irrigant through the root canal is dominated by convection through acoustic microstreaming (Ahmad et al. 1987, Roy et al. 1994, Jiang et al. 2010, Verhaagen et al. 2013), leading to mixing of the consumed and unused irrigant. This explains the chemical enhancement of NaOCl during UAI observed in this study and in earlier publications (Moorer & Wesselink 1982, Macedo et al. 2010). In the non-activated groups and in the rest period of the activated groups, diffusion will be the main mechanism of molecular transport. To what extent and how fast this occurs depends on the concentration distribution in the solution, but also on the activation period, which can influence the reaction rate of the rest period (Macedo et al. 2010). In this study the intermittent flush protocol increased the reaction rate of NaOCl by 28% (P<0.05). This can be explained by the delivery of fresh NaOCl (active chlorine) into the root canal during each rest phase. Delivery of irrigant solution by a syringe induces convection of irrigant solution in the root canal (Boutsioukis et al. 2010). However, this convection most probably does not contribute to the overall efficacy of the chemical reaction of NaOCl, because of its short duration, limited volume and inefficiency in mechanical debridement of a simulated oval canal (van der Sluis et al. 2010).

Refreshment has been claimed to be an effective method to compensate the loss of chemical efficiency of a lower concentration of NaOCl solutions (Moorer & Wesselink 1982, Zenhder 2006), but this has never been proven. From the results presented here and previously (Macedo et al. 2010) (Fig. 4), it can be concluded that the reaction rate of 2.5% NaOCl during 4 minutes of contact with dentine with refreshment is approximately five times less than 10% NaOCl without refreshment, and also two times less efficient compared to 1 min-
ute of contact of the 10% NaOCl without refreshment. Such loss of efficiency observed at low concentrations cannot be compensated by a combination of ultrasonic activation and multiple refreshments. Figure 4 shows that the reaction rate of 2.5% NaOCl with refreshment and ultrasonic activation during 4 minutes of contact is approximately two times less than 10% NaOCl without refreshment or activation, and also 1.55 times less efficient compared to 1 minute of contact with the same solution, under similar experimental conditions (Macedo et al. 2010). A possible explanation is that penetration of NaOCl deep into the dentine is a diffusion process, dependent on temperature and concentration gradients. It is often thought that ultrasonic activation of NaOCl enhances its reaction rate by a synergistic effect of a temperature rise and mechanical effects (streaming and cavitation) of the irrigant (Zehnder 2006). Here, it was found that UAI increased the temperature of the irrigant by 3.9°C and 9.9°C after 20 or 60 s of activation, respectively. This is in line with previous reports that measured a temperature increase of approximately 8°C (Cameron et al. 1988, Zeltner et al. 2009). Such temperature rises do not influence the reaction rate of NaOCl, as was shown here. This observation is in accordance with the data of Al-Jadaa et al. (2009) who demonstrated that the temperature rise measured during ultrasonic activation of the irrigant did not enhance the dissolution of bovine pulp tissue in lateral canals. Therefore, it can be concluded that the increase in chemical effect by ultrasonic activation of the irrigant is due to mechanical effects such as microstreaming (improved mixing of irrigant resulting in refreshment of active molecules at the contact surface) and cavitation and not due to a temperature rise. The contribution of cavitation to ultrasonic cleaning of root canals is still controversial (Ahmad et al. 1988, Lumley et al. 1988), although recently its occurrence has been demonstrated (Macedo et al. 2013) under the conditions used in this study and in human-sized root canal models (Jiang et al. 2011). The ultrasonic power transmitted into NaOCl in the bovine teeth in this study (1.1W/mL) using a high ultrasonic intensity is comparable to human teeth when, according to manufacturer instructions, low intensity is used (1.74W/mL). Therefore, the outcomes of this study are most probably similar for human teeth were a low intensity ultrasound is used. Nevertheless, inside a human tooth, the irrigant temperature could be influenced by the temperature of the surrounding structures (34-37°C), which may affect the heat transport. This should be the subject for future studies.

**Conclusions**

After three consecutive cycles of refreshment/ultrasonic activation of a NaOCl solution, a cumulative increase of its reaction rate was found. Activation and the total exposure time increased reaction rate of NaOCl. During activation, the temperature of the irrigant increased by up to 10°C. However, such a temperature rise was insufficient to enhance the reaction rate, suggesting that mechanical effects such as acoustic streaming and cavitation are the dominant factors in ultrasonic activated irrigation.
### Tables

#### Table 1: Measurement group characteristics and mean ± standard deviation of total available chlorine [% (m/v)] after dentine exposure and corresponding chlorine consumption during the reaction.

<table>
<thead>
<tr>
<th>N</th>
<th>Activation Time</th>
<th>Exposure Time</th>
<th>Temperature °C</th>
<th>Total Available Chlorine</th>
<th>Consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>IntFM (Cycles 1+2+3)</td>
<td>6</td>
<td>60 sec</td>
<td>180 sec</td>
<td>24</td>
<td>1.37 mol</td>
</tr>
<tr>
<td>Cycle 1</td>
<td>6</td>
<td>20 sec</td>
<td>60 sec</td>
<td>24</td>
<td>1.91% ± 0.08</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>6</td>
<td>20 sec</td>
<td>60 sec</td>
<td>24</td>
<td>2.17% ± 0.06</td>
</tr>
<tr>
<td>Cycle 3</td>
<td>6</td>
<td>20 sec</td>
<td>60 sec</td>
<td>24</td>
<td>2.32% ± 0.05</td>
</tr>
<tr>
<td>Cycle 4</td>
<td>6</td>
<td>20 sec</td>
<td>60 sec</td>
<td>24</td>
<td>2.43% ± 0.03</td>
</tr>
<tr>
<td>No IntFM</td>
<td>3</td>
<td>60 sec</td>
<td>180 sec</td>
<td>24</td>
<td>1.67% ± 0.16</td>
</tr>
<tr>
<td>Control 1</td>
<td>3</td>
<td>60 sec</td>
<td>180 sec</td>
<td>38</td>
<td>1.66% ± 0.13</td>
</tr>
<tr>
<td>Control 2</td>
<td>3</td>
<td>20 sec</td>
<td>20 sec</td>
<td>24</td>
<td>2.20% ± 0.11</td>
</tr>
<tr>
<td>Control 3</td>
<td>3</td>
<td>0 sec</td>
<td>180 sec</td>
<td>24</td>
<td>1.96% ± 0.04</td>
</tr>
<tr>
<td>Control 4</td>
<td>3</td>
<td>0 sec</td>
<td>180 sec</td>
<td>38</td>
<td>1.92% ± 0.08</td>
</tr>
<tr>
<td>Control 5</td>
<td>3</td>
<td>0 sec</td>
<td>60 sec</td>
<td>24</td>
<td>2.11% ± 0.05</td>
</tr>
<tr>
<td>Negative Control 1</td>
<td>3</td>
<td>180sec</td>
<td>180sec</td>
<td>38</td>
<td>2.49% ± 0.03</td>
</tr>
<tr>
<td>Negative Control 2</td>
<td>3</td>
<td>0sec</td>
<td>180sec</td>
<td>38</td>
<td>2.48% ± 0.02</td>
</tr>
</tbody>
</table>

#### Table 2: Influence of independent (clinical) variables in the reaction rate of NaOCl. Bold font indicates statistical significant difference between groups (P < 0.05). Differences between irrigation protocols were determined using the formula [(A/B) - 1] × 100% where A is the total chlorine consumption (mol) in the most chemical efficient group and B in the least.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Groups</th>
<th>Difference %</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation Protocol</td>
<td>IntFM &gt; No IntFM</td>
<td>28</td>
<td><strong>0.041</strong></td>
</tr>
<tr>
<td>Temperature</td>
<td>Control 4 &gt; Control 3</td>
<td>6</td>
<td>0.152</td>
</tr>
<tr>
<td></td>
<td>Control 1 &gt; No IntFM</td>
<td>1</td>
<td>0.958</td>
</tr>
<tr>
<td>Activation</td>
<td>Cycle 1 &gt; Control 5</td>
<td>41</td>
<td><strong>0.015</strong></td>
</tr>
<tr>
<td></td>
<td>No IntFM &gt; Control 3</td>
<td>53</td>
<td><strong>0.039</strong></td>
</tr>
<tr>
<td></td>
<td>Control 1 &gt; Control 4</td>
<td>46</td>
<td><strong>0.028</strong></td>
</tr>
<tr>
<td>Exposure Time</td>
<td>Cycle 1 &gt; Control 2</td>
<td>85</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td></td>
<td>Control 3 &gt; Control 5</td>
<td>37</td>
<td><strong>0.022</strong></td>
</tr>
</tbody>
</table>

**Notes:**
- In Table 1, the time values for Activation Exposure Temperature and Total Available Chlorine are given in seconds (sec) and degrees Celsius (°C), respectively.
- In Table 2, the differences are calculated based on the formula [(A/B) - 1] × 100%.
Figures

Figure 1: Distribution of the samples per group.

Figure 2: Example of the calorimetry data in a bovine tooth under in vitro conditions. The average temperature during the experiments was 28.8°C in the activated group and 34.6°C in the preheated group.
Figure 3: Effect of refreshment in the total available chlorine involved in the reaction of NaOCl with dentine. Mean of the total available chlorine consumed during the reaction, after 1 min of exposure time.

Figure 4: Influence of concentration [% (m/v)], ultrasonic activated irrigation and refreshment in the reaction rate of NaOCl with dentine. Mean of the total available chlorine consumed in the reaction, after 1 and 4 min of exposure time. *Exposure time 3 min; **Data published in Macedo et al. 2010 under similar experimental conditions.
References


