Influence of the dentinal wall on the pH of NaOCl during root canal irrigation*

Abstract

**Aim** To evaluate the influence of dentin on pH levels of different concentrations of sodium hypochlorite (NaOCl) solutions over time and to evaluate if pre-conditioning of dentin with 17% EDTA or agitation of the NaOCl solution influences these pH levels. **Methods** A novel clinical representative model that respects the ratio volume of irrigant and dentin surface of a human root canal has been used. Three standardized bovine dentin bars (2mm × 2mm × 10mm) were placed in a plastic test tube. One hundred and fifty tubes were distributed in 29 groups. In the first experiment, the pH of various NaOCl solutions, with different concentrations (3%, 6% and 9%) and pH (5 and 12), was monitored during exposure to dentin from 10 to 300 seconds. In a second experiment, the effect of agitation (45Hz) and pre-treatment of dentin with 17% EDTA on the pH of various NaOCl solutions was studied after 30 seconds of exposure to dentin. Short-term chemical stability of the tested solutions was assessed for both concentration and pH. **Results/Conclusions** Exposure time (P <0.001) and concentration of the NaOCl solution (P <0.01) significantly influence its pH after exposure to dentin. However, the change in pH is too small to induce a change in the irrigant antimicrobial/tissue dissolution capacity. Agitation of irrigant and preconditioning of dentin did not alter the pH (P>0.05). Both the pH5 and pH12 solutions were chemically stable for 1 hour.

Introduction

Sodium hypochlorite (NaOCl) is widely used as primary root canal irrigant of choice (Dutner et al. 2012) due to its unpaired action against microorganisms (McDonnell & Russell 1999) and biofilm (Arias-Moliz et al. 2009) and unique capacity to dissolve pulp tissue (Sirtes et al. 2005) and organic components of the smear layer (Baumgartner & Mader 1987). The reaction of NaOCl with these components in the root canal system (including the root canal wall), will reduce its available chlorine (Macedo et al. 2010). The reaction rate (chemical efficacy) and/or tissue dissolution capacity of NaOCl are significantly influenced by the concentration, exposure time, laser/ultrasonic energy applied (Macedo et al. 2010), contact area (Rosenfeld et al. 1978; Moorer & Wesselink 1982), temperature (Sirtes et al. 2005), interaction with other chemicals (Shiozawa 2000; Zehnder et al. 2002; Zehnder et al. 2005) and pH (Jungbluth et al. 2011). The pH of the solution determines the equilibrium of the free available chlorine, the hypochlorite ion (OCl⁻) and the hypochlorous acid (HOCl) (Baker 1947) (Fig.1). The biological effect of NaOCl, which can be defined as its tissue-dissolving capacity and antimicrobial effect, will be influenced by this equilibrium. In alkaline solutions (pH > 7.5), 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 < pH < 7.5). It has a

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

However, contradicting findings have been reported in the endodontic literature. A reduction of the pH down to 5 neither showed a decrease in the tissue dissolution of a NaOCl solution (Christensen et al. 2008), nor a change in its reaction rate when exposed to dentin (Macedo et al. 2010). The buffer capacity of dentin (Portenier et al. 2001) or any other component of the root canal system like e.g. pulp tissue (Jungbluth et al. 2011) could be an explanation for these results. Therefore, it is important to know the buffer capacity of the different components of the root canal system.

Because the influence of the buffer capacity of dentin on the pH of a NaOCl solution has never been evaluated, the first aim of this study was to evaluate the influence of dentin on pH levels of different concentrations of NaOCl solutions over time. The second aim was to evaluate if pre-conditioning of dentin with 17% EDTA or agitation of the NaOCl solution would influence the pH levels of NaOCl solutions. A clinical representative model that respects the ratio of the volume of the irrigant to the surface of a human root canal has been used. The null hypothesis is that there is no influence of the concentration or agitation of NaOCl solutions or pre-conditioning of dentin with EDTA on the pH levels of NaOCl solutions.

Materials & Methods

Dentin bars were prepared from the roots of bovine incisors immediately after extraction, using a saw microtome (SP1600, Leica Microsystems, Glattbrugg, Switzerland) to a profile of 2mm × 2mm; the length of the bars was controlled using a stereomicroscope (Stemi SV6; Carl Zeiss, Göttingen, Germany) and grinded to 10 mm. For all the dentin bars, the orientation of the tubuli was perpendicular to its long axis. All biological material used was stored overnight in sterile Milli-Q water (Millipore Corporation, Billerica, MA, USA) at 4ºC to prevent bacterial growth.

In total four hundred and fifty dentin bars (2×2×10mm) were prepared, weighed and distributed to 150 plastic sample tubes (volume 1.5 mL; diameter 2.5 mm, taper 4%, length 25 mm) (Thermo Fisher Scientific, Waltham, USA) which were randomly distributed within 29 groups. Three dentin bars were included in each sample tube with an average sum of dentin surface of 264mm² and 527.7±9.9mg of weight. Table 1 represents the distribution of samples per groups. Three different concentrations of NaOCl solutions and two pH values were tested: 12% and 6% at pH 12 and 3% at pH 12 and 5. The pH 12 solutions were obtained by dilution of 10-15% NaOCl - reagent grade, (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) with milliQ water (Millipore Corporation, Billerica, MA, USA) and the pH 5 solutions by titration of perchloric acid (HClO₄ 70-72%) (Merck KGaA, Darmstadt, Germany). The pH and the concentration were measured just before starting and after each test, using a Sentron pH meter (SENTRON Europe B.V., Roden, The Netherlands) with a Sentron microelectrode (Topac Inc, Cohasset, MA, USA) and a standard iodine/thiosulfate titration assay (Vogel 1962), respectively. Using a micropipette (Finnpipettes; MTX Lab Systems, Vienna, VA, USA), the dentin bars, already placed in sample tubes, were irrigated with 0.6mL of the NaOCl solution to be tested for a certain time ranged from 10 to 300 seconds (Table 1).

In a second experiment, the influence of agitation of the NaOCl solutions or pre-conditioning of dentin with 17% EDTA on the pH of the tested solutions was determined (Table 1b). During these
tests, the dentin bars were exposed for 30 seconds to a 3% and 6% NaOCl solution with pH 12 and 3% NaOCl solution with pH 5. Negative control groups without agitation were included for all solutions tested. To determine the influence of agitation a vortex was used at 45Hz (Vortex Genie 2, The Lab Warehouse, London, UK). To pre-condition dentin with EDTA, the bars in the sample tube were immersed for 1 min in 0.6mL EDTA 17% (Lottanti et al. 2009). The dentin bars were immediately rinsed with Milli-Q water to stop the chelation, dried using absorbent papers (Kleenex®, Milsons Point, NSW, Australia) and placed in new sample tubes before the experiments.

Short-term chemical stability of the above mentioned solutions, in 1.5mL plastic tubes (Thermo Fisher Scientific), was assessed by measuring the amount of available chlorine using the aforementioned iodometric titration assay and the pH of the solution was measured over time (10, 30, 45 and 60 min) using a pH meter and electrode. Three measurements were done for each solution at room temperature (21ºC).

The relation of the pH and free chlorine levels of the different NaOCl solutions was monitored with Spearman’s rank correlation coefficient. Kruskal-Wallis one-way analysis of variance was performed to assess differences in final pH levels between groups with different concentration (3%, 6% and 12%). Mann-Whitney U tests were used to compare the influence of agitation and pre-treatment of dentin with EDTA on the pH levels of different NaOCl solutions. For all tests, P-values <0.05 were considered statistically significant.

**Results**

The relation of the pH levels of the tested NaOCl solutions after contact with dentin is represented in Fig.2. Spearman’s rank correlation coefficient was for all solutions a correlation coefficient of 1.00 and P-values <0.001 showing a direct proportion between the contact time of NaOCl-dentin and the buffering of the solutions. Average standard deviation per group was 0.35 and range [0.11-0.63].

A high concentration of NaOCl compensates the buffer effect of dentin. A NaOCl solution of 12% at pH12 shows a significantly lower reduction of the pH than the solutions at 3% and 6% (P-values <0.01) that show no differences in final pH for all tested exposure times (P-values >0.05).

Agitation of the solution or pre-treatment of dentin with EDTA did not influence the pH of the irrigant solutions after 30 s of exposure with dentin (P-values >0.05).

The chemical stability essay showed that lowering the pH of the NaOCl solution to 5 using HClO₄ reduces approximately 50% of the initial concentration of 6%. After preparing the solutions, the concentration (3%) and pH (5) were kept constant during one-hour at 21ºC, therefore the turnover of the freshly made NaOCl solutions, used in this study was, 1 hour.

**Discussion**

This study contributed to the characterization of the influence of the buffer capacity of dentin on the pH of a NaOCl solution during root canal irrigation. It is known that contact of NaOCl with dentin causes depletion of the free available chlorine (Macedo et al. 2010; Macedo et al. 2013), resulting in protein degradation, rise of temperature (Baker 1947) and changes in pH (Jungbluth et al. 2011).

At room temperature, the pKa of hypochlorous acid is 7.5. At this pH, equal amounts of HOCl and OCl⁻ are present in the solution (Fig.1). In this study, NaOCl solutions with different
concentrations and pH levels were exposed to dentin for up to 300 seconds. The exposure time had a significant effect on the pH of the solution (P<0.001), in contrast to agitation and pre-treatment of dentin with 17%EDTA (P=0.05). Nonetheless, even with extended exposure times, not usual in endodontic practice, the pH levels were kept bellow 6.5 for the 3% NaOCl solution at pH 5 and above 9.5 for initial NaOCl solutions at pH 12. At these pH levels the free available chlorine is expected to be predominantly (>90%) HOCl for the acidic solutions and OCl\(^{-}\) for the basic counterparts (Fig.1). So we can conclude that although dentin had a significant buffer effect on the pH levels of different NaOCl solutions, this will most likely not influence the biological effect.

For a typical root canal with a working length of 15mm, prepared until a master apical file size 0.3/taper 0.06, the expected volume is 7.42mm\(^3\) and the expected dentin surface 35.4mm\(^2\). This results in a volume/surface ratio of 0.21mm. The model used in this study is composed of 3 dentin bars with the dimension of 2×2×10mm inside an inert plastic sample tube. The volume used was 0.6mL, which was chosen to guarantee an optimum inclusion of the bars within the liquid and to assure a clinically realistic volume to surface ratio of 0.227mm. Previous studies neglected the typical clinical conditions exposing large amounts of NaOCl with relatively small amounts of substrate to be dissolved (Moorer & Wesselink 1982; Sirtes et al. 2005). Consequently, the expected reduction of the concentration of the irrigant solutions (Macedo et al. 2010) and the eventual buffer effect did not occur.

NaOCl solutions with a lower pH have a higher antimicrobial effect when planktonic microorganisms are concerned (Bremer et al. 2002). However, in the root canal, microorganisms are present in a biofilm (Ricucci & Siqueira Jr 2010). Characteristic of the biofilm is that the microorganisms are protected by a highly hydrated extracellular matrix (Flemming & Wingender 2010). Therefore, the dynamics of the antimicrobial effect is completely different for microorganisms in a biofilm. It has been suggested that penetration of chlorine in a biofilm follows a diffusion-reaction pattern where chlorine will be reduced by the matrix material (DeBeer et al. 1994). A large variation of penetration of chlorine into the biofilm was detected mainly depending on the structure of the biofilm. The study of Bremer (Bremer et al. 2002) showed that a reduction of pH of NaOCl to 6.5 resulted in a better bactericidal effect, than using the same concentration at pH12, in a two-species biofilm for one of the two species of microorganisms present in the biofilm. The effect on biofilm in the root canal is not known. In contrast, OCl\(^{-}\) has proven to be more efficient in tissue dissolution than HOCl (Zehnder 2006). The same authors suggested to alkalize NaOCl solutions using NaOH to compensate for the buffer effect that might occur whenever it is in contact with organic tissue inside the root canal. The contribution of dentin to such buffer effect does not justify this extra step. Nonetheless, the influence of other substances present in the root canal in early stages of the treatment (i.e. pulp tissue and biofilm) or retreatment (i.e. sealer and filling material) were not evaluated here, but should be subject of further studies. For the later stages of the root canal treatment, when the bulk of pulp tissue has been removed, this alkalinization does not seem to be necessary.

The initial concentration of the NaOCl solution was also a factor that influenced the final pH. The lower the concentration, the higher the reduction of pH after exposure. This effect is significant (P<0.01) for the highest concentration (12%), whereas there was no difference between the 3% and 6% NaOCl solutions. Clinically, concentrations between 0.5% and 6% have been recommended, however the optimal clinical concentration is still subject of controversy. In this
work, extending the concentration range up to 12%, does not suggest its use in endodontics but contributes to a full characterization of the influence of the concentration. Agitation of a solution inside the root canal stimulates the refreshment of the free chlorine available at the interface with organic tissues, while pre-treatment of dentin with 17% EDTA removes the inorganic component of dentin and smear layer (Baumgartner & Mader 1987) exposing more collagen fibers. However, the effect of both variables does not influence the final pH of a NaOCl solution after being exposed for 30 s to dentin (P>0.05). The exposure time was selected according to the best current evidence available, which should govern the clinical procedures (Liang et al. 2013). The intermittent flush method with three cycles of refreshment with 2mL of NaOCl solution followed by 20 seconds of ultrasonic activation was found to be the best irrigation protocol to remove dentin debris from an oval shaped canal (van der Sluis et al. 2010). If the flow rate of the refreshment is 0.22mLs⁻¹ as reported by Boutsioukis et al. (2007) then 30 seconds is the approximate clinical time of each cycle. It can be concluded that the exposure time with dentin and concentration of a NaOCl solution significantly influence its pH. However, this change in pH is too limited to induce a biological effect. Agitation of irrigant and pre-conditioning of dentin did not alter the pH. Both the solutions with a pH of 5 and pH12 were chemically stable for 1 hour.

### Tables

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**Table 1:** Distribution of the samples per group. In experiment A the buffer effect of dentin on various NaOCl solutions was monitored over time; in B the influence of agitation and dentin pre-conditioning with 17%EDTA on the buffer effect of dentin on NaOCl solutions was tested.
Figures

**Figure 1:** Free available chlorine in solution with regard to pH. Temperature 25°C. (After Baker RJ 1959)

**Figure 2:** Effect of the buffer capacity of dentin on the pH of NaOCl. Average of various NaOCl solutions’ pH levels as function of the contact time with dentin (n=3). Dashed line represents the pKa of HOCl (pH 7.5) where both forms of active chlorine OCl⁻ and HOCl exist in equilibrium. Spearman's rank correlation coefficient shows for all solutions a correlation coefficient of 1.00 and P-values <0.001. Average standard deviation was 0.35 and range [0.11-0.63].
References


