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Abstract

Aim To monitor chlorine consumption from non-agitated aqueous sodium hypochlorite (NaOCl) solutions in human root canals using a recently developed assay, which can determine the order of magnitude of available chlorine in small volumes of liquid.

Methodology The root canals of 80 extracted single-rooted human teeth were instrumented to ProTaper Universal F4 and irrigated using 1% NaOCl. Subsequently, canals were irrigated with copious amounts of deionized water to rinse out the residual chlorine. Subsequently the teeth were sealed externally and placed in a water bath of 37°C. Root canals were filled with NaOCl of 1%, 2.75%, 5.5%, or distilled water for 1 min, 10 min, 100 min, or 1000 min (n = 5 teeth per solution and time). Consumption of chlorine was measured using paper points pre-impregnated with 15% potassium iodide. Colour change of the paper points was determined photo-electronically, assessing their red value after absorbing solutions from root canals. Measurements were compared to a standard series of NaOCl down to 0.001% (n = 5 paper points per concentration).

Results Red values of the paper points inserted into the root canal were affected by initial NaOCl concentration and time (two-way ANOVA, $P < 0.05$). If NaOCl concentrations above 0.1% are considered to be clinically relevant, then 5.5% NaOCl retained its activity in the root canal for more than 100 min, whereas 1% NaOCl lost it's activity between 10 min and 100 min.

Conclusions Non-agitated NaOCl solutions can remain biologically active in human root canals for extended time periods.

Introduction

Sodium hypochlorite (NaOCl or NaClO) irrigation serves different purposes during root canal procedures. NaOCl shows a strong antimicrobial activity against a broad spectrum of microorganisms and has an exceptional capacity to dissolve organic tissue (Zehnder 2006). From a chemical perspective, both these features are related to the strong oxidizing effect of the OCl^-/HOCl system, i.e. to the available chlorine in the system (Zehnder *et al.* 2002). In theory, the effects of NaOCl in the root canal can be measured by the consumption of available chlorine. This, however, has not been possible in human root canals due to their minute volume of merely a few µL (Peters *et al.* 2001). The consumption of available chlorine from a root canal irrigant has been measured in bovine teeth (Macedo *et al.* 2010, Macedo *et al.* 2014). Bovine teeth offer the advantage that they contain a high root canal volume, and available chlorine can thus be measured using standard iodometric titration (Vogel 1962, Macedo *et al.* 2010). However, bovine teeth differ greatly from human counterparts in their surface-area-to-volume ratio. In other words, the relative area of the canal wall to the irrigant that can be contained in a root canal system is much greater in human than in bovine teeth. Consequently, any chemical reaction between an irrigant and the root canal wall will occur much faster in human teeth.

To overcome the problem of measuring NaOCl in human root canals, a new assay has recently been developed to assess available chlorine levels in small volumes of liquid (Rechenberg *et al.* 2014). This assay is based on the reaction between NaOCl and potassium iodide (KI). Both chemicals by themselves are colourless, but their combination results in the generation of elemental iodine (I_2), which is brown. Based on standard iodometric titration, the assay uses paper points impregnated with potassium iodide, and detects the available chlorine photo-electronically via the colour change of the paper point.

In the current first pre-clinical trial using this assay, available chlorine kinetics of non-agitated aqueous NaOCl irrigants in instrumented, single-rooted human teeth were

investigated. The experiment related to the potential clinical concept of placing a NaOCl irrigant in the canal system after instrumentation as an intra-visit (meaning: during the visit) medication. Interestingly, this has been tried successfully with the application of iodine potassium iodide for 10 min (Kvist *et al.* 2004, Molander *et al.* 2007), yet not with NaOCl, which is a stronger antiseptic but more reactive than iodine potassium iodide (McDonnell & Russell 1999).

Materials and methods

Preparation of Teeth

Eighty extracted single-rooted human teeth with fully formed apices were selected for this study from the Department's collection of extracted teeth. Teeth were extracted according to individual treatment plans, which were not influenced by the current investigation. Patients gave informed written consent that their extracted teeth could be used for study purposes. The teeth were stored in 0.1% thymol at 5°C. All teeth contained one single root canal, which was verified radiographically (Digora, Soredex, Helsinki, Finland). Roots were cleaned of soft tissue remnants using dental curettes. If present, coronal carious lesions were excavated and restored with a total-etch dental adhesive (Optibond FL, Kerr, Romulus, MI, USA) and composite resin (Filtek Supreme, 3M ESPE, St. Paul, MN, USA). Crowns were shortened using a diamond bur to create a plateau at the coronal equator. Subsequently, access cavities were prepared using a cylindrical diamond bur under water-cooling. The working length was set at 1 mm short of full length (size-10 K-file flush with the root surface). All root canals were then prepared using ProTaper Universal instruments (Dentsply Maillefer, Ballaigues, Switzerland) to size F4. One mL of a 1% NaOCl solution (Kantonslabor, Zürich, Switzerland) was applied between each instrument using a side-vented 30-gauge irrigation tip (Kerr Hawe, Bioggio, Switzerland). Starting from the F3 instrument, the irrigating tip was applied to full working length. After the F4 instrument, a final irrigation was performed with 5 mL of

deionized water to rinse out residual NaOCl.

Following root canal preparation, external root surfaces were sealed with a total-etch dental adhesive (Optibond FL, Kerr). Composite resin (Filtek Supreme, 3M ESPE) was placed over the apical 3-4 mm to seal off the foramen.

The mean working length ± standard deviation of the 80 teeth was 17.5 mm ± 2.1 mm. The teeth were stratified into 16 similar groups of 5 teeth each, which were filled with different concentrations of NaOCl or deionized water for different time periods (see below). All groups had a mean working length between 17 mm and 18 mm. During the whole preparation and testing period the teeth were stored in tap water between processes to avoid dehydration.

Preparation of Paper Points

For assessment of available chlorine in the liquid contained in root canals the method according to Rechenberg *et al.* (2014) was applied . In brief, paper points of ISO size 40 (ORBIS Dental, Münster, Germany) were dried at 110°C in an incubator (HORO Dr. Hoffmann GmbH, Ostfildern, Germany) and then immersed in 15% KI solution for 1 min. The paper points were subsequently dried again for 4 h in the incubator at 110°C.

Solutions

Three different concentrations of NaOCl were prepared for the main experiment: 5.5%, 2.75%, and 1% (wt/vol). These solutions were made by diluting a 14% stock solution (Kantonslabor, Zürich, Switzerland) with ultrapure water. The NaOCl content of each solution was iodomterically titrated (Vogel 1962). NaOCl solutions were kept in amber glass bottles at 5°C, and warmed to room temperature (25°C) before the experiment.
To assess potential effects of interactions between thymol and NaOCl on the assay, the 0.1% thymol solution that was used to store the teeth was mixed at a 1:1 (wt/wt) ratio with a 5.5%

NaOCl solution. Available chlorine was titrated immediately, and after 1 h and 4 h of storage in an incubator (HORO Dr. Hoffmann GmbH) at 37°C.

Main Experiment

Teeth were placed in a putty mold (Optosil Comfort Putty, Heraeus Kulzer, Hanau, Germany) for ease of handling. The molds containing 5 teeth each were placed in a pre-heated water bath (M3, Lauda, Lauda-Königshofen, Germany) set to 37°C. Preliminary experiments were performed by immersing thermocouples (Z2-T-2M, Labfacility, Hanau, Germany) into the root canals filled with water. Thermocouples were connected to a data logger (Agilent 34970, Agilent, Santa Clara, CA) running proprietary software (BenchLink, Agilent). Accuracy of temperature measurements was verified against calibrated thermometer readings. After 10 min, a steady-state temperature of 34°C to 35°C was reached. This corresponds to the temperature measured in human root canals *in situ* (Cunningham & Balekjian 1980).
The following procedures were performed four-handedly by two investigators (KTR and DKR). Times were meticulously monitored using a stopwatch. The canals were initially dried with size 40 paper points and pressurized air. Root canals were then filled with 1%, 2.75%, 5.5% NaOCl or deionized water using a 30-gauge side-wanted irrigation tip (Kerr Hawe) at working length and kept in the water bath for either 1 min, 10 min, 100 min or 1000 min. Excess irrigation fluid was removed from the access cavity using cotton pellets so that all the liquid was confined to the root canal. After the test time was completed the impregnated paper points were placed to working length and kept in the canal for 10 s. Within 2 min subsequently, in a darkened room, the paper points were then placed on a gray background (www.digigrey.com) and photographed in a standardized manner as described (Rechenberg *et al.* 2014). Using the DigitalColor Metric program (Apple, Cupertino, CA, USA) at the settings 8x magnification and Adobe RGB, the mean red (R) levels were measured in an area 287-299 pixels from the tip of the paper point. The images were transformed to image

processing software and adjusted to the standardized background (Photoshop Element, Adobe Systems, San Jose, CA, USA). Red values from the root canals were compared against a 10-fold dilution series of 10% NaOCl down to .001% obtained with the paper points (n = 5 per concentration) from the same batch, dipped into the solutions and photographed as described above.

Data Presentation and Analysis

Because the current assay cannot provide exact NaOCl concentrations in the percent (%) range, but rather their order of magnitude (Rechenberg *et al.* 2014), statistical analysis was performed on red values, i.e. the original values. For subsequent assessment of the relationship of these values to NaOCl concentrations, mean red values were plotted against the range of red values that corresponded to a specific NaOCl concentration from the 10-fold dilution series obtained with 5 different KI-impregnated paper points (Fig. 1). Red values were subjected to two-way repeated measures ANOVA to consider the impact of time and initial NaOCl concentration. Red values at each time point were compared between the NaOCl solutions and water using one-way ANOVA followed by Tukey's HSD test. The alpha-type error was set at 5% ($P < 0.05$).

Results

In a preliminary study, it was assessed iodometrically whether or not thymol could have an impact on available chlorine levels, as the experimental teeth had been stored in 0.1% thymol prior to the experiment. A 5.5% NaOCl solution mixed at a 1:1 (wt/wt) ratio with 0.1% thymol and stored at 37°C still contained 98% and 95% of the initially available chlorine after 1 h and 4 h, respectively.

Two-way repeated measures ANOVA indicated that both time and NaOCl

concentration had a significant ($P < 0.05$) impact on the red values of the KI-impregnated paper points, which were inserted into the root canals to assess available chlorine. The red values of the paper points did not differ between the different NaOCl concentrations when the irrigants were kept in the root canals for 1 min or 10 min (Table 1). After 100 min, the red values related to the 5.5% NaOCl solution were significantly higher than those of the 1% NaOCl counterpart. After 1000 min, these values were again statistically similar ($P > 0.05$) between the 3 initial NaOCl concentrations under investigation. There was a significant ($P < 0.05$) difference between the NaOCl-related red values and those related to the water control at all times, indicating that there was available chlorine in the system for up to 1000 min (Table 1).

To relate the red values in the current experiment to NaOCl concentrations, the red values obtained in the experiment were compared to those of a dilution series of NaOCl down to .001%. The results showed that, if concentrations above .1% NaOCl are considered clinically relevant, then 5.5% NaOCl remained active for more than 100 min, whereas 1% NaOCl lost its activity between 10 min and 100 min (Fig. 1).

Discussion

The current study showed that NaOCl solutions at concentrations commonly used in clinical endodontics maintain available chlorine levels that can still be considered clinically relevant for extended time periods. This information should be useful to plan future clinical trials.

The current study is limited by two main factors. First, the results were obtained in a laboratory environment using single-rooted teeth. Results may look different in roots with more complex canal systems, as there will be more organic substrate for reaction with NaOCl (Koskinen *et al.* 1980). Also, the fact that teeth were stored in thymol might have affected the results. However, considering the minor effect of thymol on NaOCl levels and the fact that all

teeth were washed, instrumented, and their root canals copiously rinsed before the experiment, an impact of thymol on the outcomes can be excluded. The second limiting factor of this investigation is the assay itself, which is not able to exactly determine the concentration of NaOCl solutions in the % range. The higher the dilution of a NaOCl solution, the better the absolute accuracy of this assay becomes. The measurement error is never greater than 3 times the determined concentration (Rechenberg *et al.* 2014). This can be appreciated in Fig. 1. Consequently, the assay can soundly determine when a NaOCl solution falls below the 0.1% mark. This information appears to be clinically useful.

In the context of the current results and their interpretation, it should be cautioned that 0.1% is thus a somewhat arbitrary value in determining the border between effectiveness and non-effectiveness. The value was chosen because Dakin's solution is in this order of magnitude; it contains 0.5% NaOCl (Dakin 1915). This concentration has been used successfully for decades in clinical root canal procedures (Byström & Sundqvist 1983). However, NaOCl solutions at dilutions down to 0.001% NaOCl kill planktonic bacteria (Zehnder *et al.* 2002). In swimming pool water, the NaOCl concentration is in the order of magnitude of 1 ppm, i.e. 0.0001% (Borgmann-Strahsen 2003). At least in terms of reducing planktonic bacteria or preventing microbial regrowth, low concentrations of NaOCl can thus still be expected to be effective. Furthermore, concentrations below 0.5% appear to exert minimal effects on the collagen in the root dentine, and thus pose little threat to mechanical tooth integrity (Sim *et al.* 2001, Marending *et al.* 2007). Therefore, the information gathered in this communication appears to be of some clinical relevance.

To know the consumption rate for sodium hypochlorite of different concentrations might help to determine the required refreshment rate after root canal shaping for each concentration. The issue of timing disinfection concepts correctly has gained importance in view of the fact that current instrumenting techniques take very little time to shape a root canal system (Paqué *et al.* 2011, Bürklein *et al.* 2013). Theoretically, to save chair-time, a

two-visit disinfection approach could be followed by applying a sodium hypochlorite solution mixed with calcium hydroxide powder for the interim. This should be possible because $Ca(OH)_2$ does not interfere with available chlorine levels (Zehnder *et al.* 2003). Alternatively, an intra-visit dressing could be performed with NaOCl rather than iodine potassium iodide (Kvist *et al.* 2004). As suggested by the current results, both approaches mentioned above appear promising in terms of root canal disinfection, and should be evaluated in clinical trials.

Conclusions

According to the results of the present investigation, and within the limitations of the current study design, non-agitated sodium hypochlorite solutions can remain biologically active in human root canals for extended time periods.

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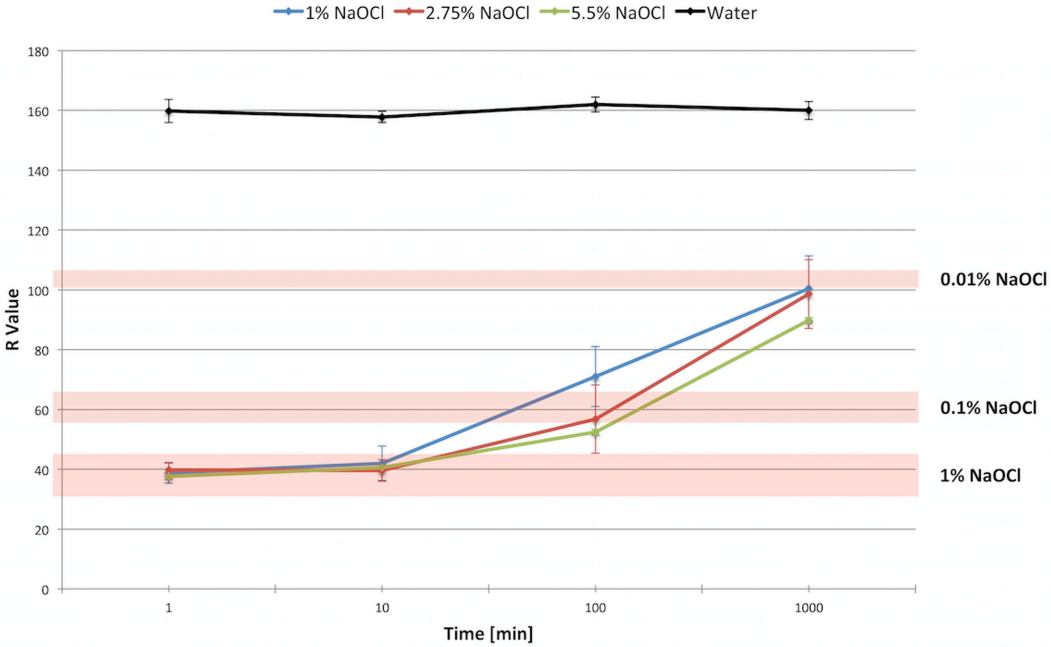
Legends

Figure 1 Line graph depicting the change of red (R) values (means ± SD) of paper points over time. Paper points were inserted into root canals (n = 5 per measuring point) filled with different concentrations of NaOCl or mere distilled water. For comparison, the pink horizontal bars indicate the ranges of values obtained in the 10-fold dilution series of 10% NaOCl (1% NaOCl, 0.1% NaOCl, 0.01% NaOCl, n = 5 paper points per dilution). Note that after 100 min, the mean red values of the 5.5% solution still contained between 0.1% and 1% NaOCl.

Table 1 Red hues (mean values ± SD) of the KI-impregnated paper points dipped into solutions kept in root canals for different time periods

Solution	1 min	10 min	100 min	1000 min
Water	$160 \pm 4^{A*}$	158 ± 2^{A}	162 ± 3^{A}	160 ± 3^{A}
1% NaOCl	39 ± 3^{B}	42 ± 6^{B}	71 ± 10^{B}	100 ± 11^{B}
2.75% NaOCl	40 ± 2^{B}	40 ± 4^{B}	$57 \pm 11^{B,C}$	99 ± 11^{B}
5.5% NaOCl	38 ± 3^{B}	41 ± 8^{B}	52 ± 11^{C}	90 ± 7^{B}

*Values that share a superscript letter did not differ significantly ($P < .05$) at a given point in time (ANOVA, Tukey's HSD).


1% NaOCl
2.75% NaOCl
5.5% NaOCl
Water
180
160
140
120
100
80
60
40
20
0
R Value
1
10
100
1000
Time [min]
0.01% NaOCl
0.1% NaOCl
1% NaOCl