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Necrotic pulp tissue dissolution by passive ultrasonic irrigation in simulated accessory canals: impact of canal location and angulation

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Abstract

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Aim To evaluate whether passive ultrasonic irrigation (PUI) of 2.5% NaOCl would dissolve necrotic pulp tissue from simulated accessory root canals (SACs) better than passive placement of the irrigant, when temperature was equilibrated between the two treatments.

Methodology Transparent root canal models ($n = 6$) were made from epoxy resin. SACs of 0.2 mm diameter were placed at defined angles and positions in the mid-canal and apical area. SACs were filled with necrotic bovine pulp tissue. PUI was performed five times for 1 min each with irrigant replenishment after every minute. Main canal temperature was measured after each minute, and a digital photograph was taken. In control experiments, mock treatments were performed with the same set-up without activation of the file using heated NaOCl to mimic the temperature created by PUI. Experiments were repeated five times. Digital photographs were analysed for the distance of dissolved tissue into the SACs in mm. Overall comparison (sum of dissolved tissue from all five accessory canals) between treatments was performed using paired *t*-test. Differences between SAC angulation and position after PUI were investigated using ANOVA/Bonferroni (alpha < 0.05).

Results Passive ultrasonic irrigation caused a rise in irrigant temperature in the main canal to 53.5 ± 2.7 °C after the fifth minute. PUI dissolved a total of 6.4 ± 2.1 mm, mock treatment controlled for heat: 1.4 ± 0.6 mm ($P < 0.05$). No significant influence of SAC position or angulation was found.

Conclusions Passive ultrasonic irrigation promotes positive tissue-dissolving effects beyond a rise in irrigant temperature.

Keywords: sodium hypochlorite, passive ultrasonic irrigation.

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Introduction

Disinfection and debridement of root canals is an important aspect of endodontic treatment. Based on the fact that mechanical preparation alone cannot fully achieve this aim (Byström & Sundqvist 1981), the chemo-mechanical principle using topically applied substances during and after instrumentation was established. In this context, the correct choice of the chemicals to be used and their ideal mode of application are of interest. Sodium hypochlorite is the root canal irrigant of choice for many practitioners, as it dissolves necrotic tissue (Naenni *et al.* 2004) and has a superior antimicrobial effect compared with most other disinfectants that have been used in the root canal system (Vianna *et al.* 2006). It has been shown that the local efficacy of hypochlorite preparations can be improved

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by heating the solution to be applied (Sirtes *et al.* 2005). Alternatively, the irrigant can be activated mechanically. Amongst the mechanical methods for irrigant activation, passive ultrasonic irrigation (PUI) is probably the most established method (van der Sluis *et al.* 2007).

Ultrasound was first introduced to endodontics in 1957 for mechanical root canal and root-end preparation (Richman 1957). Later, it was realized that ultrasonic activation could be beneficial in enhancing the efficacy of irrigants in the root canal (Martin 1976, Martin & Cunningham 1985). The main effects in this context are (transitional) cavitation and streaming (Walmsley 1987). Both phenomena are well known to enhance the effectiveness of antiseptics, especially sodium hypochlorite (Martin & Cunningham 1985, Blume & Neis 2005). Whilst streaming undoubtedly occurs, it is unclear whether cavitation actually occurs in the root canal system (Ahmad *et al.* 1987, Lumley *et al.* 1988). A third, often overlooked, effect of the application of ultrasonic energy in the root canal is the general increase in irrigant temperature (Cunningham *et al.* 1982, Cameron 1988).

Researchers have extensively studied the influence of ultrasonic irrigant activation on the appearance of root canal walls as observed by scanning electron microscopy (Ahmad *et al.* 1987, Abbott *et al.* 1991). Others used a scoring model of the stained organic debris and smear layer (Cheung & Stock 1993). It was found that ultrasonic activation increases the debridement activity of sodium hypochlorite (Cameron 1987). Using artificially prepared grooves filled with dentine debris in the walls of human root canals as well as in artificial canals, it has been shown that PUI has the potential to remove debris from canal extensions and irregularities (van der Sluis *et al.* 2005). It was also shown *in situ* that the soft tissue debridement of sodium hypochlorite is greatly enhanced by ultrasonic activation in the isthmus areas of human mandibular molars (Burleson *et al.* 2007). However, until now the impact of PUI on accessory canals is still unclear because of the lack of studies with such observations. It has been shown that clinically, these areas are especially difficult to clean (Nair *et al.* 2005). The lack of studies on irrigant action in lateral or accessory canals can be related to the difficulty in carrying out such investigations on natural teeth, as the accessory canal position and status before treatment are difficult to determine. Consequently, there appears to be a need for standardized models simulating accessory canals with multiple controlled variables yielding repeatable results. The aim of this study was to establish a model especially tailored for this purpose.

Materials and methods

Fabrication of model

A suitable model that would allow the observation and direct quantitative measurement of pulp tissue before and after irrigation was not available. A transparent model was prepared using a wax mould that was filled with epoxy resin (Stycast, Emerson & Cuming, Westerlo, Belgium). To ensure reproducibility of the model, a sheet of paper with a drawing representing the main canal, position and angulation of accessory canals was used as reference to assemble the parts in the proper position using super glue before transferring them to a box made of pink plate wax with a dimension of 30, 20 and 15 mm length, width and height, respectively (Fig. 1a,b). The main canal was simulated using a D-size finger spreader (Dentsply Maillefer, Ballaigues, Switzerland). This instrument had a length of 25 mm, a tip diameter of 0.35 mm, and a 0.06 taper (Briseño Marroquín *et al.* 2001). Accessory canals were created by 0.2-mm stainless steel wires (Fig. 1b,c). The length of the canal was determined by allowing 5 mm of the wire to extrude from a 22-gauge needle (Ultradent Products, Inc. South Jordan, UT, USA), The needle was used later to carry the necrotic pulp tissue and apply it into the canal by means of injection. A pair of canals were placed at distances of 1 mm and 9 mm from the main canal apex opposing each other, one of these was made perpendicular to the main canal, the other created at a 45° angle with the apical extension of the main canal. In addition, an accessory canal that continued in the direction of the main canal (180°) was created. A millimetric paper scale was placed parallel to the long access of each simulated accessory canal to ensure a precise measurement of the length of tissue dissolution. Eight models were fabricated to be used in the study. Before any of the models were used, continuity of simulated accessory canals with the main canal was ensured by introducing a 0.2-mm wire inside each accessory canal until it appeared in the main canal. Finally, a simulated pulp chamber and reservoir for the passively placed irrigant was created using a rubber tube with a length of 7 mm and 3 mm internal diameter, which was glued over the main canal entrance. This reservoir ensured that the whole canal remained filled with irrigant after the passive ultrasonic activation procedure described below. A model ready to

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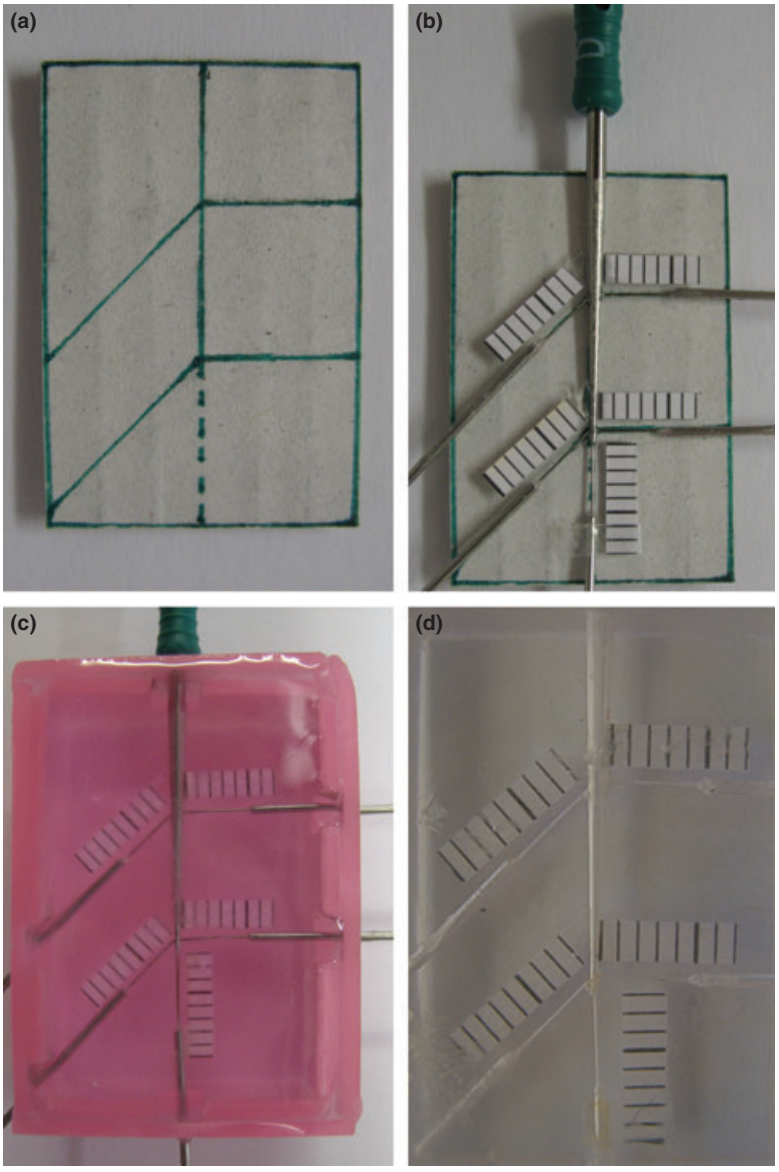

Figure 1 Preparation of an epoxy resin model used in this study: (a) template to ensure similar simulated accessory canal position and angulation between the models; (b) positioning of the finger spreader and the wires; (c) mould made of pink wax filled with epoxy resin; (d) finished model.

be filled with necrotic pulp tissue is depicted in Fig. 1, panel d.

Bovine pulp tissue preparation

The accessory canals of seven models were filled with bovine pulp tissue. The tissue was obtained from bovine anterior teeth of animals that were raised and slaughtered for food production according to the Swiss standards of animal welfare. Consequently, this study was not considered an animal study and the internal review board had no objections to the current protocol. Pulps were extirpated after decoronation of the teeth and then frozen at −20 °C. Frozen tissue was thawed, dried with paper tissues, and then each piece was immersed in liquid nitrogen to achieve a solid dry material. Subsequently, tissue was transformed into fine particles using a scalpel to scratch the hard surface. Sometimes it was required to re-immerse the piece into liquid nitrogen several times to maintain its solid consistency. When a sufficient amount of tissue was prepared, a 22-gauge needle (Ultradent Products) was used to aspirate part of it and then the needle was inserted in its place in the model until it reached the outer end of the simulated accessory canal. The tissue was injected in the accessory canal until part of it

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extruded into the main canal. Excess tissue was placed in the wide entrance of the carrying needle to obtain a passive closure simulating a pathosis rather than a tight seal of the simulated accessory canals. This procedure was repeated in all the five simulated accessory canals in each model.

The models were re-filled for the control experiments with heated NaOCl and NaOCl at room temperature (see below) after removing the old tissue from accessory canals and extensive rinsing with tap water.

Control experiments on temperature

It is well known that ultrasonic irrigant activation is associated with heat generation (Cunningham *et al.* 1982, Cameron 1988). An increase in temperature can enhance the efficacy of NaOCl (Sirtes *et al.* 2005). To discern between pure temperature and other ultrasonic effects on NaOCl, the temperature associated with PUI in the current model was determined. A preliminary study was carried out using one of the models fabricated for the study. The temperature was recorded after each 1 min of activation and also after each flush with 1 mL 2.5% (wt/vol) NaOCl using a thin couple wire connected to a calibrated temperature measuring device (Testo Term 9010, Lenzkirch, Germany). This procedure was carried out over 5 min and repeated thrice. After the intracanal temperature created by PUI in the current set-up was known, the irrigant temperature to be used in the second part of the study was determined by trial. The irrigant was heated by placing the irrigation syringe inside a water bath and the temperature was measured after each irrigation by 1 mL of 2.5% NaOCl and after 1 min of irrigation. After that 1 min the syringe was returned to the water bath to ensure a stable temperature. The irrigant temperature inside the syringe was measured by introducing the couple wire through its opening just before irrigation. The temperature was raised gradually until the suitable temperature inside the canal was achieved. This procedure was repeated thrice.

Main experiment

The model was held on a cone especially designed to direct light through it to have a contrast facilitating the interpretation of results and to prevent artefacts caused by over-exposure of light. Halogen light (Intralux 4000-1, Volpi AG, Schlieren, Switzerland) was introduced from behind the model and through the cone. An initial photograph using a 10-megapixel camera (Nikon D200, Tokyo, Japan) mounted on a stand in front of the model was taken to ensure the complete filling of the simulated accessory canals with pulp tissue and to allow comparison later on. The irrigation protocol was as follows: 1 mL of 2.5% NaOCl at room temperature was introduced to full canal length by a long irrigation needle with 30-gauge diameter (Max-i-Probe, Hawe Neos, Bioggio, Switzerland). Care was exercised that the opening at the needle tip was not directed towards the accessory canals directly. An ultrasonic device (EMS 400, EMS, Nyon, Switzerland) with its power set at the ¼ of the scale, with an ultrasonic stainless steel K-type file size 15 (Endosonore, Dentsply Maillefer) mounted on an ultrasonic adaptor (Piezon, 90° Endo File Holder, EMS) was used to activate the irrigant in the canal with an up and down motion by hand at a ratio of 10 mm s^{-1} to the full length of the canal minus half a millimeter, for 1 min. Subsequently, a photo was taken and the main canal was irrigated with 1 mL of sodium hypochlorite at room temperature. The same procedure was repeated every minute for 5 min. At the end of the fifth minute, the temperature inside the canal was measured to ensure that the ultrasonic file was active. The ultrasonic file was replaced for each model to avoid fracture, whilst the ultrasonic adaptor was replaced after two models. This protocol was carried out on the seven models. In the control experiments, the models were refilled with tissues as described before and the same procedure was carried out except for the NaOCl temperature which was 68–69 °C in the second experiment and at room temperature the third time. The file was introduced in the canal without ultrasonic activation in these two experiments. The experiment for the second and third parts was carried out only on six models because one of the models was lost because of a fractured file in the first part. Results from that model were discarded.

Data generation and analysis

Data from the temperature experiments are presented as means and standard deviations ($n = 3$).

The photos were analyzed using the ImageJ program (nih.gov; National Institute of Health, Bethesda, MD, USA). The outcome variable assessed here was distance of tissue dissolution in simulated accessory canal, measured from the canal entrance to the closest tissue-irrigant interface. Measurements were performed by one operator, who was tested for his accuracy by analysing the same images ten times after different

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intervals. The error of the individual measurement was < 0.05 mm. Consequently, data pertaining to tissue dissolution were rounded to 0.1 mm. To compare overall tissue dissolution at room temperature with the corresponding values obtained by PUI and in the temperature-controlled experiments, the sums of distances of tissue dissolution in all accessory canals per model were averaged for each mode ($n = 6$) and compared by a paired *t*-test. To compare the impact of accessory canal position and angulation on tissue dissolution by PUI, mean values per simulated accessory canal were compared by one-way analysis of variance (ANOVA). Bonferroni's correction was applied for multiple testing. The alpha-type error was set at 0.05.

Results

Temperature

Passive ultrasonic irrigation caused a rise in hypochlorite temperature in the main canal to 53.5 ± 2.7 °C after the fifth min (Fig. 2). For the temperature-control experiment, the suitable irrigant temperature in the syringe was found to be 68–69 °C, which was achieved by placing the 5-mL irrigation syringe in a water bath of 75 °C for 5 min. This resulted in an overall temperature in the canal that was similar to the one observed with PUI (Fig. 2).

One of the observations, which might affect the clinical usability of PUI, was that after multiple usage of the ultrasonic adaptor (usually after 12–14 min of activation), the temperature suddenly dropped, indicating a loss of ultrasonic energy transmitted to the irrigant in the canal. After multiple trials and by exclusion it was found that the rubber ring between the two parts of the ultrasonic adaptor wore out so that there was less activation of the ultrasonic file. This observation necessitated a regular replacement of the adaptor. As an extra precaution the temperature was measured after the fifth and final minute of PUI in each individual model as an indicator of the ultrasonic activity inside the canal.

Tissue dissolution

The mean sums of dissolved tissue from simulated accessory canals after 5 min of PUI or the mock treatments were: PUI: 6.4 ± 2.1 mm, mock treatment at room temperature: 0.8 ± 0.3 mm, and mock treatment controlled for heat: 1.4 ± 0.6 mm. The difference between the heated irrigant and the counterpart administered at room temperature was not significant at the 0.05 level, whilst there was a significant ($P < 0.05$) difference between both these treatments and PUI, indicating a clear PUI effect.

When the influence of simulated accessory canal position and angulation on tissue dissolution by PUI was studied (Table 1), it was noted that regardless of accessory canal position or angulation, a plateau was reached after the third minute of activation. Furthermore, there was no significant difference in tissue dissolution between different simulated accessory canals at any time.

Discussion

The current study showed a positive effect of PUI in conjunction with a sodium hypochlorite irrigant on pulp tissue dissolution from simulated accessory canals in an epoxy resin model. This effect was not explained by a simple rise in overall irrigant temperature.

The current study is limited by the fact that epoxy resin is a completely different material from human dentine, and direct clinical conclusions can therefore not be drawn from the results presented here. Further-

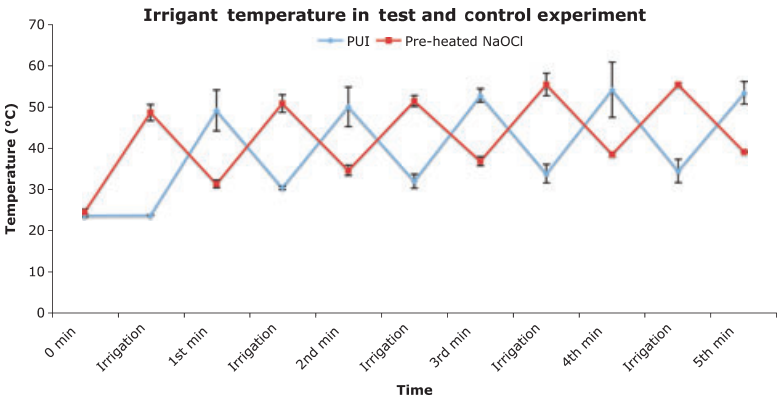

Figure 2 Temperatures (°C) measured in the simulated main canal after passive ultrasonic irrigation (blue) and during the mock treatment with a heated sodium hypochlorite solution (red) over time. Dots indicate means, error bars standard deviations ($n = 3$).

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Table 1 Distance in mm of dissolved tissue as measured from the simulated accessory canal entrance after passive ultrasonic irrigation (means and standard deviations, $n = 6$)

Time	90°, mid-canal	45°, mid-canal	90°, apex	45°, apex	180°, apex
1st min	0.2 ± 0.3	0.1 ± 0.2	0.0 ± 0.0	0.4 ± 0.6	0.1 ± 0.1
2nd min	1.1 ± 0.5	0.8 ± 0.6[a]	0.9 ± 0.3	1.3 ± 0.8	0.5 ± 0.6
3rd min	1.4 ± 0.5	1.0 ± 0.6	1.1 ± 0.3	1.5 ± 0.9	0.7 ± 0.7
4th min	1.4 ± 0.5	1.1 ± 0.6	1.2 ± 0.3	1.6 ± 0.8	0.8 ± 0.8
5th min	1.5 ± 0.6	1.2 ± 0.6	1.3 ± 0.3	1.7 ± 0.8	0.9 ± 0.9

No statistically significant differences were found between canals at any given time ($P > 0.05$, ANOVA, Bonferroni).

more, the simulated main canal in the current model was straight. This type of anatomy is rarely encountered in natural teeth. However, the aim of this study was to discern between mere temperature and other PUI effects in the cleansing of accessory canals. For this purpose, the model appeared adequate. However, despite the standardization of the models that were used, data variation pertaining to the distance of dissolved tissue in simulated accessory canals was still relatively large as indicated by the high standard deviations (Table 1). This can be explained by the difficulty in obtaining completely homogenous and standardized fills of necrotic tissue in these thin canals. On the other hand, the density of necrotic tissue in infected natural accessory canals might also vary. It is a common observation when dealing with natural tissues such as the bovine pulps that were used in the current investigation that outcomes vary. In addition, because the ultrasonic tip was guided by hand, it was impossible to control where it touched the canal wall, which may also have contributed to the variance in outcome. A further limitation of this study is the fact that the average width of accessory canals is not known or published (De Deus 1975). However, based on our own observations on micro-computer tomographies of human teeth, 200 μm appeared to be a fair approximation.

The temperature that was measured in the current study was somewhat higher than that measured in natural teeth, which may be because of the fact that thermal transducing properties of dentine differ from those of epoxy resin (Brown *et al.* 1970) and also because of the potential cooling effect of the blood circulation around natural teeth. Using PUI with intermittent flushes, temperatures of up to 45 °C were measured in root canals of natural teeth after 30 s of ultrasonic irrigant activation (Cameron 1988). Considering the shorter activation times, there appears to be little variance between these published data and the current results. However, other researchers found the temperature rise in the root canal promoted by PUI to be minimal (Ahmad 1990). However, a root canals were widened to an ISO-size 80 in that study, and there was continuous flow of irrigant during ultrasonic activation which might explain the differences.

The exact mechanism by which ultrasonic hypochlorite activation can affect the tissue in accessory canals is still unclear. One hypothetical mechanism is the collapse of bubbles during transient cavitation that produces a pressure-vacuum effect, which sucks the canal content to the inside rather than pushing it further in the canal. This will be followed by diffusion of the irrigant in the main canal to substitute the space created (Martin & Cunningham 1985). Another possibility is that the streaming around the activated file because of the cohesion between fluid particles inside the accessory canal and the irrigant in the main canal sucks the content of the accessory canals into the main canal with fluid flow toward the main canal (Ahmad *et al.* 1992). The third possibility is a local temperature effect because of the collapse of bubbles during transitional cavitation. It has been shown that locally, the temperature can reach up to 5000 °C with heating and cooling rates greater than 10^9 K/s during cavitation (Suslick 1990). Consequently, a great part of the ultrasonic effect may still be thermal, but just not measurable by assessing the overall irrigant temperature. However, it is still unclear at this point whether transient cavitation occurs in the root canal. Based on preliminary observations with dye solutions of different colours in the model described here, it was noted that little streaming occurred in the apical area, especially in the simulated accessory canal at 180° at the apical end of the main canal (not shown). Nevertheless, tissue dissolution was similar regardless of accessory canal position or angulation in the current study. Consequently, it may be so that cavitation was, at least in part, responsible for the observed phenomenon of tissue dissolution by PUI. This again highlights what has been pointed out more than 20 years ago, namely that further studies are required to elucidate the phenomena behind ultrasonic effects that might or might not occur in the root canal.

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One further observation that was made during the current study was that because of the high corrosive potential of hypochlorite and the heat that is generated during ultrasonic activation, material wear out occurred rapidly. Initially, noncutting nickel-titanium tips were used, but these fractured so frequently that it was decided to use the cheaper stainless-steel files. Results between the two types of instruments were similar (data not shown).

Conclusions

- A model allowing the quantitative assessment of necrotic pulp tissue dissolution in simulated accessory canals was presented.
- The temperature generated in the main canal of this model by passive ultrasonic activation of a 2.5% NaOCl solution was over 50 °C.
- This rise in overall temperature could not be responsible for the effectiveness of PUI.
- Tissue dissolution by PUI was irrespective of simulated accessory canal position or angulation.

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