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The influence of irrigant activation, concentration and contact time on sodium hypochlorite penetration into root dentine: an *ex vivo* experiment

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Abstract

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Aim To establish whether irrigant activation techniques, namely manual dynamic activation (MDA), passive ultrasonic irrigation (PUI) and sonic irrigation (SI), improve the tubular penetration of sodium hypochlorite (NaOCl) into root dentine when compared with conventional needle irrigation (CNI). Secondly, investigate if increasing NaOCl concentration and/or contact time improves the performance of these techniques.

Methodology A total of 83 extracted human maxillary permanent canines were decoronated to 15 mm, and root canals prepared to a size 40, .10 taper. Root dentine was stained with crystal violet for 72 h and embedded in silicone. Eighty specimens were randomly distributed into 16 groups ($n = 5$) according to the irrigant activation technique, NaOCl concentration (2%; 5.25%) and irrigant contact time (10 min; 20 min). All activation techniques were used for 60 s in the last minute of irrigation. Additionally, three teeth were not exposed to NaOCl to confirm adequate dentine staining had occurred (i.e. negative control). All specimens were subsequently dissected, observed under a light microscope and NaOCl penetration depth (μm) determined by measuring the average width of bleached dentine using ImageJ software. Statistical comparisons were made with paired and unpaired *t*-tests, ANOVAs followed by *post hoc* Tukey's and Dunnett's tests, and a general linear model ($\alpha < 0.05$).

Results Overall, NaOCl penetration ranged from 38.8 to 411.0 μm with MDA, PUI and SI consistently resulting in significantly greater tubular infiltration than CNI ($P < 0.05$). The deepest measurements in the coronal, middle and apical segments were all recorded in the MDA; 5.25%; 20 min group and the least in the CNI; 2%; 10 min group. Increasing either irrigant concentration or contact time resulted in significantly greater NaOCl penetration depths for all techniques and segments of the canal ($P < 0.05$). However, when irrigant concentration and contact time were increased together, a significant interaction effect between these two independent variables was observed on overall NaOCl penetration ($P < 0.05$).

Conclusions Agitating irrigants with MDA, PUI or SI, as well as using greater irrigant concentrations or contact times, potentiated NaOCl penetration into root

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dentine. However, longer durations of NaOCl exposure at lower concentrations resulted in similar depths of tubular penetration as those achieved at higher concentrations.

Keywords: irrigant penetration, manual dynamic activation, passive ultrasonic irrigation, root dentine, sonic irrigation.

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Introduction

Microbial infection of the pulp is a prerequisite to the development of apical periodontitis (Kakehashi *et al.* 1965, Möller *et al.* 1981). Current treatment strategies therefore focus largely on reducing the bacterial load within the root canal system to levels that induce periradicular healing (Siqueira & Rôças 2008). Generally, this is achieved through the use of instruments to enlarge the canal and antibacterial solutions (Schilder 1974, Haapasalo *et al.* 2005). Greater emphasis is placed on the latter as up to 35% of the canal wall can remain uninstrumented (Peters *et al.* 2001). Sodium hypochlorite (NaOCl) is currently the irrigant of choice, due to its efficacy against a broad spectrum of microbes and tissue dissolution capabilities (Haapasalo *et al.* 2014). It is routinely deposited into root canals at concentrations of 0.5%–5.25% (Baumgartner & Cuenin 1992), by way of conventional needle irrigation (CNI).

Although NaOCl substantially reduces the number of microorganisms within superficial layers of root dentine via CNI, bacteria more deeply embedded within tubules often remain unaffected (Wong & Cheung 2013, Azim *et al.* 2016, Vatkar *et al.* 2016). This could negatively impact the prognosis of root canal treatment as enduring pathogens, which have been found residing at depths of up to 420 μm in human dentine (Love 1996, Kakoli *et al.* 2009), may later contribute to persistent periradicular disease (Siqueira & Rôças 2008). Deeper irrigant penetration is therefore desirable as some *ex vivo* experiments have demonstrated CNI only allows NaOCl infiltration up to 250 μm into root dentine (Ghorbanzadeh *et al.* 2016, Faria *et al.* 2019).

The aforementioned limitations of CNI could, however, be overcome through the use of irrigant activation techniques. Manual dynamic activation (MDA), passive ultrasonic irrigation (PUI) and sonic irrigation (SI) are currently some of the most widely used and studied methods (Gu *et al.* 2009, Virdee *et al.* 2018). MDA involves repeatedly inserting a well-fitting gutta-percha (GP) cone to the working length of an instrumented canal to produce hydrodynamic displacing forces within irrigants (McGill *et al.* 2008). Passive ultrasonic irrigation uses freely oscillating files at ultrasonic frequencies (25–30 kHz) to generate acoustic cavitation and streaming forces (van der sluis *et al.* 2007). Sonic irrigation devices create hydrodynamic phenomenon within irrigants by oscillating a smooth flexible polymer file at frequencies of 1–10 kHz (Gu *et al.* 2009).

Currently, only a few studies directly compare the NaOCl tubular penetration achieved by MDA, PUI or SI to that of CNI (Ghorbanzadeh *et al.* 2016, Faria *et al.* 2019). Furthermore, even fewer studies report how irrigant concentration or contact time influences the performance of these techniques. In studies which have investigated NaOCl penetration into root dentine, floating dentine segments (Zou *et al.* 2010, Palazzi *et al.* 2016) or irrigant regimes spanning durations of only 1–2 min (Ghorbanzadeh *et al.* 2016, Generali *et al.* 2018, Faria *et al.* 2019) have often been used. These do not mimick *in vivo* conditions or irrigant protocols well (Darcey *et al.* 2016). Therefore, investigating more commonly used irrigant regimes in a more clinically representative model system would better inform clinicians on effective endodontic disinfection strategies.

The primary aim of this *ex vivo* study was to establish whether the use of MDA, PUI and SI significantly improve the tubular penetration of NaOCl into root dentine when compared with CNI. Secondly, this study investigates if increasing NaOCl concentration and/or contact time improves the performance of the aforementioned irrigant activation techniques. The null hypotheses tested were as follows: (i) MDA, PUI or SI do not increase NaOCl tubular penetration when compared with CNI and (ii) raising irrigant concentration and/or contact time does not significantly increase NaOCl tubular penetration.

Material and methods

This study was performed under the ethical approval (14/SW/1148) for the University of Birmingham's Dentistry Research Tissue Bank (DRTB), and the workflow is summarized in Fig. 1.

Specimen selection

A total of 83 human maxillary canines were selected from a pool of teeth that were extracted, for reasons not related to this study and stored under controlled

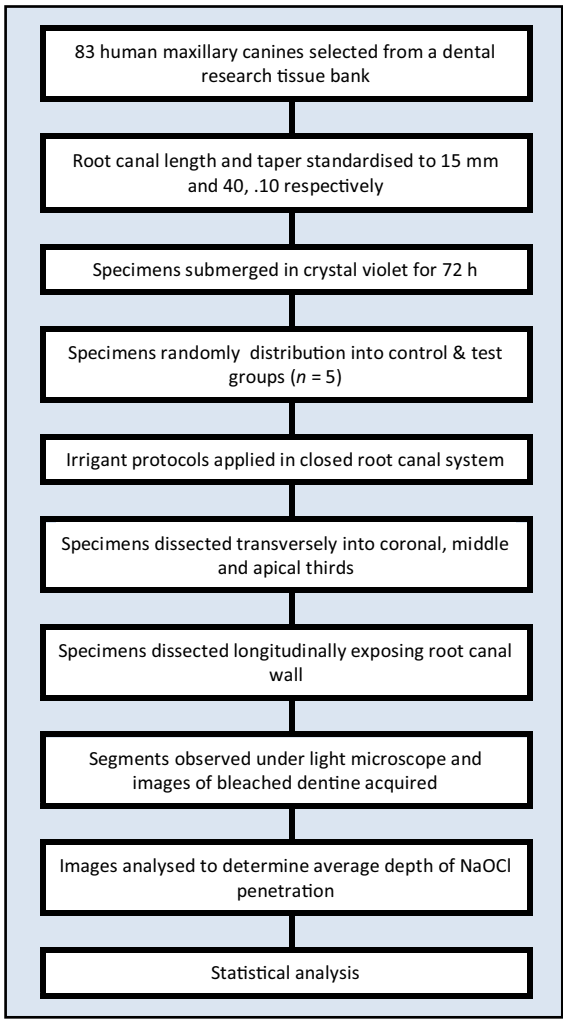

Figure 1 A flow chart depicting the key stages of the experimental protocol.

conditions at −20 °C in the DRTB. Mesio-distal and bucco-lingual digital radiographs were taken to ensure only permanent teeth with single canals and root lengths of ≥16 mm were included. Teeth with caries, root fractures, open apices, root curvatures >10°, calcified canals, resorptive defects, posts and previous root fillings were excluded (Fig. 2a).

Specimen preparation

Tubular penetration of NaOCl was evaluated based on the stained dentine model proposed by Zou *et al.* (2010), but with modifications to better reflect *in vivo* conditions. Briefly, teeth were decoronated with a slow-speed diamond disc (ContacEZ, Vancouver, Washington, USA) and the remaining root adjusted to 15 mm using cooled silicon carbide grinding paper (Struers, Pederstrupvej, Denmark). Residual periodontal tissues were removed using an ultrasonic scaler and the working length (WL) subsequently determined by inserting a size 10 K-File (Dentsply Sirona, Ballaigues, Switzerland) into the root canal until the tip was visible beyond the apex under magnification. One millimetre was then subtracted from this distance, and all root canals were prepared to this WL up to a ProTaper Gold F4 rotary file (size 40, .10 taper) at speeds and torques recommended by the manufacturer (Dentsply Sirona). During instrumentation, 1 mL of 5.25% NaOCl (Cerkamed, Stalowa-Wola, Poland) was administered between files.

A slow-speed diamond disc (ContacEZ) was used to prospectively scribe grooves, without penetrating into the canal space, along the planes at which specimens would later be dissected. First, transverse grooves were made circumferentially at locations of 5 and 10 mm from the anatomical apex so that the root could be divided into coronal, middle and apical thirds. Vertical grooves were then made mesio-distally along the entire length of the tooth so that the longitudinal axis of the root canal could be exposed. Bucco-lingual vertical grooves were also scribed into the coronal and middle segments so that NaOCl penetration could be analysed in two planes (Fig. 2b).

Staining protocol

To remove both organic and inorganic components of the intracanal smear layer, each specimen was immersed in 10 mL of 5.25% NaOCl for 5 min followed by 10 mL in 17% ethylenediaminetetraacetic acid (EDTA; Cerkamed) for a further 5 min. Thereafter, dentine blocks were washed in distilled water for 5 min to remove residual irrigant solutions, dried using paper towels and then immersed in 10 mL of crystal violet (ThermoFisher Scientific, Waltham, Massachusetts, USA) for 72 h at room temperature. To maximize dye penetration, every 24 h the crystal violet was renewed and irrigated into canals via CNI (Fig. 2c). After this period, teeth were rinsed under tap water for 30 min and sealed in Aquasil soft putty silicone (Dentsply Sirona) to simulate a closed root canal system (Tay *et al.* 2010, Fig. 2d).

Control and test groups

To evaluate the efficacy of MDA, PUI and SI in relation to CNI, 80 prepared teeth were randomly distributed into 16 groups ($n = 5$) depending on the

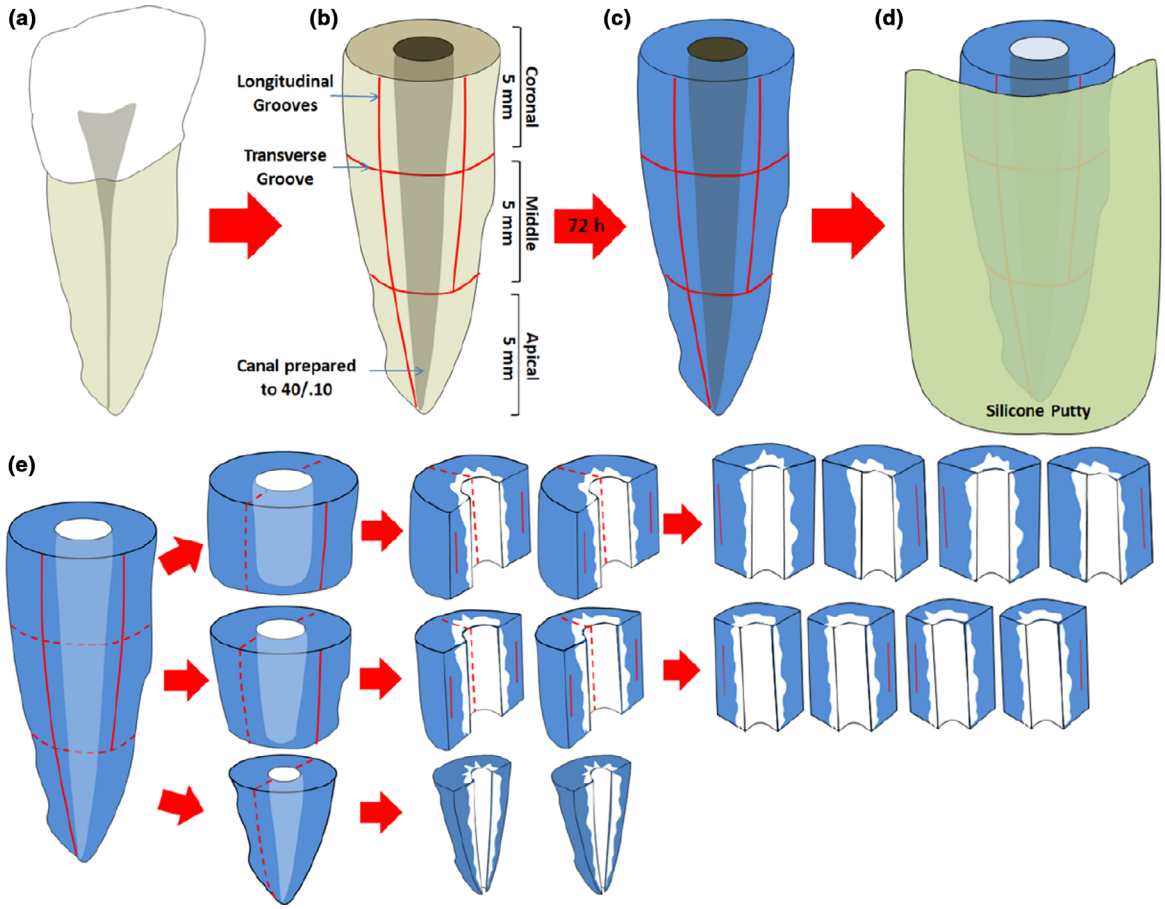

Figure 2 A schematic diagram displaying the stages of preparing root canal blocks used in this study. (a) Maxillary canines selected. (b) Teeth decoronated and splitting grooves included (indicated by solid red lines) using slow speed diamond disc (c) Specimens dyed in crystal violet for 72 h. (d) Specimens sealed in silicone putty and irrigant protocols applied. (e) Transverse and longitudinal dissections made (indicated by broken red lines) as per splitting grooves. Orientation grooves were also scribed on root canal walls in mesio-distal plane.

method of irrigant agitation, NaOCl concentration (%) and irrigant contact time (min). Briefly, each irrigant activation technique was used alongside 2% or 5.25% NaOCl at room temperature for a period of 10 or 20 min (Faria *et al.* 2019). The CNI groups acted as controls and a further three specimens were dyed without being exposed to NaOCl to confirm adequate dentine staining had occurred (i.e. negative control). The individual groups are displayed in Table 1.

Irrigant activation protocols

For all groups, NaOCl was deposited into the canal via CNI with a 27 gauge side vented needle and 3 mL monoject syringe (Covidien, Dublin, Ireland). The needle tip was placed 2 mm short of WL, and the irrigant solution replenished with 1 mL of irrigant every 2 min to prevent depletion of NaOCl activity (Moorer & Wesselink 1982, Boutsioukis *et al.* 2010). For test groups, the irrigant was agitated in the final minute of NaOCl exposure by a single trained operator in accordance with protocols outlined by McGill *et al.* (2008) and Mancini *et al.* (2013). Briefly, for MDA a single Protaper F4 GP cone (Dentsply Sirona) was repeatedly inserted to WL, via short 2–3 mm longitudinal push–pull strokes, for 60 s at a rate of 100 strokes per minute. Passive ultrasonic irrigation was completed using a size 15 K-file (Dentsply Sirona) which was activated via a MiniEndo II device (SybronEndo, Orange County, California, USA) at half power for 1 min at 1 mm from WL. Finally, for SI an Endoactivator device (Dentsply

Sirona) was used for 1 min with a size 15/ .02 point 1 mm from the WL. Following irrigation, canals were flushed with 5 mL distilled water and then dried with sterile paper points.

Specimen dissection

Dentine blocks were removed from the silicone putty and a chisel and hammer used to transversely separate roots into coronal, middle and apical sections using the aforementioned splitting grooves as guides. Sections were further divided mesio-distally, and the coronal and middle regions once again bucco-lingually, to expose the longitudinal axis of the root canal which created four coronal, four middle and two apical segments per tooth. Thereafter, the internal surfaces were grooved mesio-distally for orientation purposes and then polished to remove topographical irregularities using a medium-grit abrasive polishing disc (3M, St. Paul, Minnesota, USA; Fig. 2e). With five teeth per group and three negative controls, a total of 830 sections were generated for experimental study.

Evaluating NaOCl penetration

Using a custom silicone jig, specimens were positioned under a light microscope (Zeiss Axiophot, Carl Zeiss, Oberkochen, Germany) so that the longitudinal plane of the root canal wall could be observed. Two images at random locations either side of the canal were captured, alongside a calibrated scale bar, for coronal and middle segments using a digital camera (Apple, Cupertino, California, USA). Only a single image was acquired for apical segments, due to reduced dye penetration in this region (Paqué *et al.* 2006, Russell *et al.* 2013). For the purposes of standardization, all images were captured using the same objective at a fixed resolution and optimal focus, saved in.tiff format and then uploaded onto ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

Quantitative morphometric image analysis was conducted to calculate the average NaOCl penetration depth for each image (Fig. 3a). Briefly, the 'polygon' and 'clear outside' tools were used to outline and isolate the internal canal wall. The image was then separated via 'split channel' function and a manual 'threshold' applied to the green image, which provided the greatest contrasts between bleached and dyed dentine (Fig. 3b). A calibrated 'straight' line, spanning perpendicularly from the inner canal wall towards the periphery, was then used to obtain 10 measurements from each image at 150 μm vertical increments (Fig. 3c). The average NaOCl penetration depth for the coronal, middle and apical segments of each group was then calculated in micrometres (μm), and the results presented as averages (μm) ± standard deviations alongside 95% confidence intervals for the mean.

Statistical analyses

All statistical analyses were performed using SPSS (V.25) software (IBM, New York, USA). Initially, a preliminary screen for data normality was conducted using histograms and normal plots. A majority

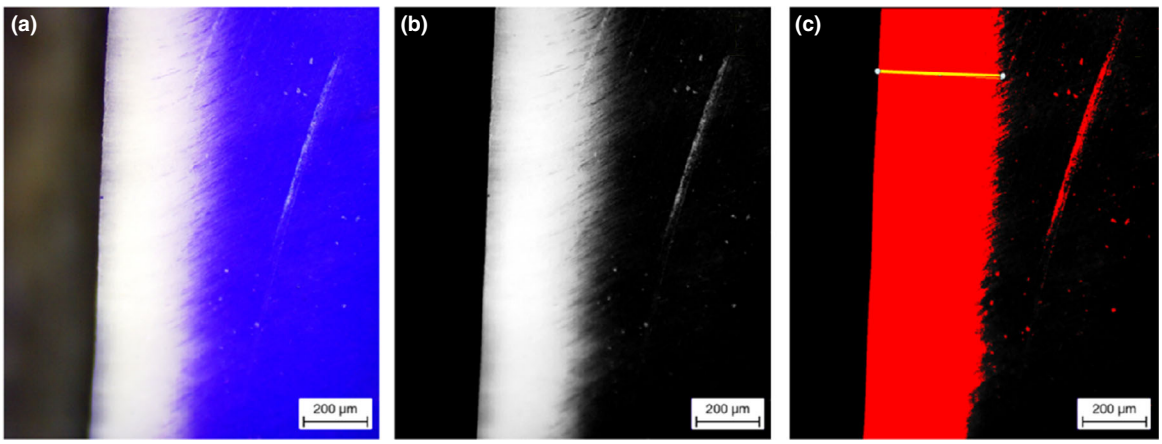

Figure 3 Morphometric analysis calculating average depth of NaOCl penetration (a) Original image. (b) Green image following 'split channel' function. (c) Manual 'threshold' applied and calibrated 'straight line' tool used 10 times at 150 μm vertical increments to calculate average NaOCl penetration per images.

normal distribution was revealed, and subsequent comparisons between groups were made using unpaired *t*-tests and ANOVAs followed by *post hoc* Tukey's and Dunnett's tests. Comparisons within groups (intra-tooth) were made using paired *t*-tests.

The effects of irrigant concentration and contact time were explored using a general linear model. The 'main effects' included in this model were (i) irrigant concentration and (ii) irrigant contact time, and the 'interaction effect' was considered the simultaneous effect of increasing both of these independent variables (i.e. irrigant concentration and contact time) on NaOCl penetration. These main and interaction effects were explored for the overall NaOCl penetration (averaged across all techniques) as well as for the individual methods of irrigant agitation (CNI, MDA, PUI and SI) across all thirds of the canal. In this model, comparisons were made only between groups (inter-tooth) and not within (intra-tooth).

The intra-rater reliability for image analysis was conducted in each group using the intra-class correlation coefficient, and the alpha value for all tests was set at a 5% level of significance ($\alpha = 0.05$).

Results

The intra-class correlation coefficient demonstrated 'excellent' intra-rater agreement with coefficients

Table 1 Average NaOCl penetration into root dentine following various irrigation protocols

	NaOCl irrigation protocol			Average depth of tubular penetration (μm)		
Group	IAT	Conc. (%)	Time (m)	Coronal	Middle	Apical
1	CNI	2	10	179.4 ± 67.2[a,b,c] [172.8–186.0]	158.7 ± 70.3[a,b,c,d] [151.8–165.5]	38.8 ± 38.1[a,b,c,d] [31.3–46.2]
2	CNI	2	20	265.2 ± 71.1[a,b,c] [258.2–272.1]	246.5 ± 82.1[a,b,c,d] [238.4–254.5]	124.5 ± 47.2[a,b,c,d] [115.2–133.7]
3	CNI	5.25	10	255.4 ± 72.0[a,b,c] [248.4–262.5]	226.8 ± 62.9[a,b,c,d] [220.5–233.0]	113.1 ± 49.6[a,b,c,d] [103.4–122.9]
4	CNI	5.25	20	272.6 ± 76.1[a,b,c] [265.1–280.0]	259.8 ± 71.2[a,b,c,d] [252.8–266.8]	171.2 ± 54.9[a,b,d] [160.5–182.0]
5	MDA	2	10	240.6 ± 71.9 [b,c] [233.5–247.7]	237.8 ± 87.1[c] [229.2–246.3]	159.9 ± 63.5[c,d] [147.5–172.4]
6	MDA	2	20	300.4 ± 100.8[c] [290.6–310.1]	294.7 ± 96.7[b] [285.7–304.3]	162.2 ± 75.7[d] [147.4–177.0]
7	MDA	5.25	10	279.9 ± 65.4[b,c] [273.4–286.3]	325.6 ± 108.8[b,c,d] [314.7–336.4]	212.9 ± 76.0[b,c,d] [198.1–227.8]
8	MDA	5.25	20	379.0 ± 110.8[b,c] [368.0–390.0]	411.0 ± 132.3[b,c,d] [398.2–423.8]	232.7 ± 78.2 [c,d] [217.3–248.0]
9	PUI	2	10	258.5 ± 78.7[a,c] [250.9–265.6]	241.9 ± 69.9 [c,d] [235.0–248.9]	172.3 ± 51.3 [c,d] [162.3–182.9]
10	PUI	2	20	298.3 ± 64.9[c] [294.6–308.2]	322.1 ± 87.1[a,d] [313.5–330.7]	169.2 ± 55.8[d] [158.3–180.1]
11	PUI	5.25	10	306.5 ± 85.2[a] [298.1–314.9]	303.5 ± 83.4[a] [295.4–311.7]	154.9 ± 40.4[a,d] [147.0–162.9]
12	PUI	5.25	20	300.0 ± 69.5[a,c] [293.2–306.8]	296.5 ± 77.3[a] [289.1–303.9]	216.3 ± 92.3 [c,d] [198.3–234.4]
13	SI	2	10	280.3 ± 72.8[a,b] [273.1–287.4]	275.3 ± 85.6[a,b] [267.1–284.0]	121.0 ± 51.6[a,b,d] [110.9–131.2]
14	SI	2	20	326.8 ± 83.2[a,b] [318.5–335.1]	310.1 ± 80.1[d] [302.3–317.9]	149.5 ± 37.4[d] [142.2–156.9]
15	SI	5.25	10	302.4 ± 93.3[a] [293.2–311.6]	296.1 ± 86.5[a] [287.8–304.5]	165.7 ± 66.9[a,d] [152.6–178.8]
16	SI	5.25	20	329.2 ± 81.2[a,b] [321.5–337.0]	306.3 ± 83.0[a,d] [298.2–314.5]	161.9 ± 82.5[a,b,d] [145.7–178.1]

Results are presented as means (μm) ± standard deviations and [95% confidence intervals] for the mean.
[a]Versus corresponding MDA segment ($P < 0.05$).
[b]Versus corresponding PUI segment ($P < 0.05$).
[c]Versus corresponding SI segment ($P < 0.05$).
[d]Versus preceding coronal segment ($P < 0.05$).

spanning upwards of 0.87. Results in NaOCl penetration for the different irrigant protocols are summarized in Table 1 and Figure 4, with representative light microscopy images for each group displayed in Fig. 5.

The overall NaOCl dentine penetration ranged from 38.8 to 411.0 μm. With exception to the apical third in group 16 ($P > 0.05$), CNI displayed significantly less NaOCl penetration than MDA, PUI and SI in all locations for any given irrigant protocol ($P < 0.05$). The deepest measurements across the entire canal were all recorded in group 8 (MDA; 5.25%; 20 min) and the least in group 1 (CNI; 2%; 10 min). Individual comparisons between MDA, PUI and SI revealed no single superior method of irrigant agitation across all protocols and locations, however; SI consistently performed the poorest apically after CNI.

For all irrigant protocols, the apical region exhibited significantly lower NaOCl penetration compared with the respective coronal and middle sections ($P < 0.05$). By contrast, differences between coronal and middle regions were varied with groups exhibiting positive, negative or no significant differences. Furthermore, there were no significant differences in NaOCl penetration between mesio-distal and buccolingual planes for the coronal or middle segments of dentine ($P > 0.05$).

The general linear model indicated significantly deeper NaOCl penetration for each technique across all regions of the canal when the main effect of either concentration or contact time was increased ($P < 0.05$). A more pronounced effect was demonstrated for CNI and MDA compared with PUI or SI. Additionally, when increasing both irrigant concentration and contact time, a highly significant interaction effect was observed in the coronal and middle segments ($P < 0.05$), but not apically ($P > 0.05$).

Discussion

This study demonstrates that MDA, PUI and SI substantially improve NaOCl penetration across all regions of the canal for any given irrigant regime compared with CNI. Additionally, when NaOCl concentration or contact time is increased, the performance of these techniques is further improved. Finally, longer periods of exposure to lower concentrations of NaOCl result in similar depths of tubular penetration as those achieved by higher concentrations. Both null hypotheses have therefore been rejected.

A broad range of methodologies have been used previously to determine NaOCl penetration into root dentine. Several studies have added fluorescent dyes to irrigants for subsequent confocal laser scanning microscopy analysis (Llena *et al.* 2015, Vadhana *et al.* 2015, Gu *et al.* 2017). Others have substituted NaOCl completely for fluorescent solutions, which have then been viewed under a light microscope (Galler *et al.* 2019). These approaches were not employed here as the oxidizing nature of NaOCl could affect the fluorescent capacity of the dye and surrogate solutions may not have the same penetrative properties as the test irrigant. Alternatively, the stained dentine model proposed by Zou *et al.* (2010) was used in this study as it overcame the aforementioned limitations. Whilst this experimental model has been used in several previous investigations, significant efforts were made in this study to improve its biofidelity so that it better mimicked *in vivo* conditions. For instance, human teeth were used instead of bovine (Faria *et al.* 2019), irrigants were deposited into intact root canals in a clinically representative manner rather than dentine blocks being submerged in pools of NaOCl (Zou *et al.* 2010, Palazzi *et al.* 2016) and the durations of disinfection were more akin to those delivered throughout root canal treatment (Ghorbanzadeh *et al.* 2016, Generali *et al.* 2018). Thus, this model allowed for more robust analysis of the various irrigant protocols, including the complex interaction effects between NaOCl concentration and contact time which at the time of this study have not been previously reported. Furthermore, the number of segments (10), images (18) and data points (180) per tooth, the multiple planes analysed and the highly reproducible image threshold techniques applied in the present study also allowed for more accurate and sensitive evaluation of NaOCl penetration than any preceding investigation. Nevertheless, several limitations of this methodology are still present and acknowledged. For example, the method for selecting teeth did not allow the baseline conditions of root dentine (i.e. age, tubular density or size) to be standardized and further investigations would be required to establish whether the reported findings are applicable to curved canals where irrigant flow dynamics are different (Nguy & Sedgley 2006). Additionally, the limited sample size in this study, which was based on previous experiments by Zou *et al.* (2010) and Palazzi *et al.* (2016), may also increase the risk of type 2 errors (false negatives). It is important to note, however, highly significant differences were consistently identified between the various irrigant regimes indicating

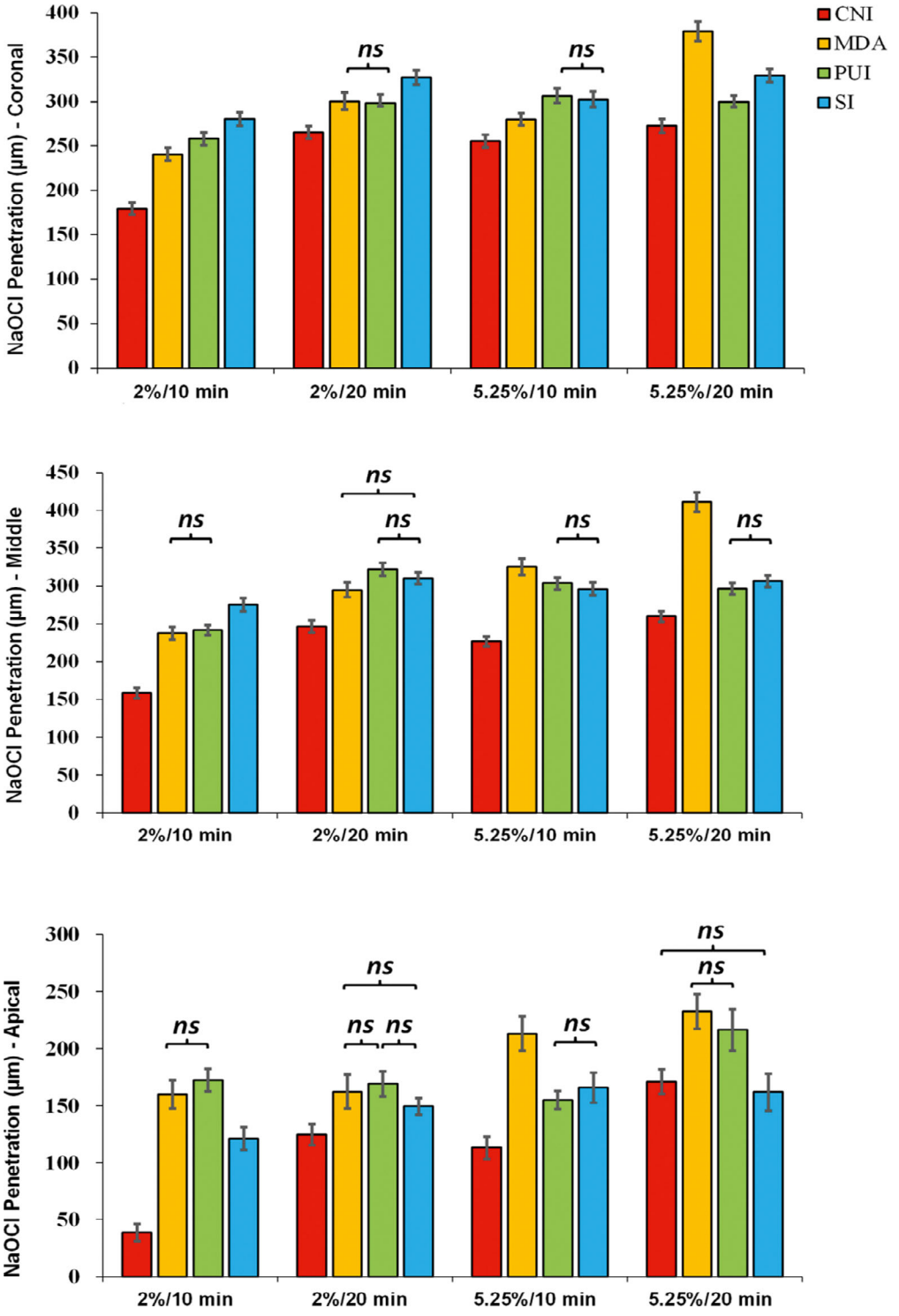

Figure 4 Average NaOCl penetration (µm) in the coronal, middle and apical regions of the canal for each agitation technique compared with of CNI. The error bars indicate the 95% confidence intervals for the mean. Those comparisons with respect to technique (CNI versus MDA versus PUI versus SI) in each group that are not significant are indicated by '*ns.*' All other comparisons within each group are significant ($P < 0.05$).

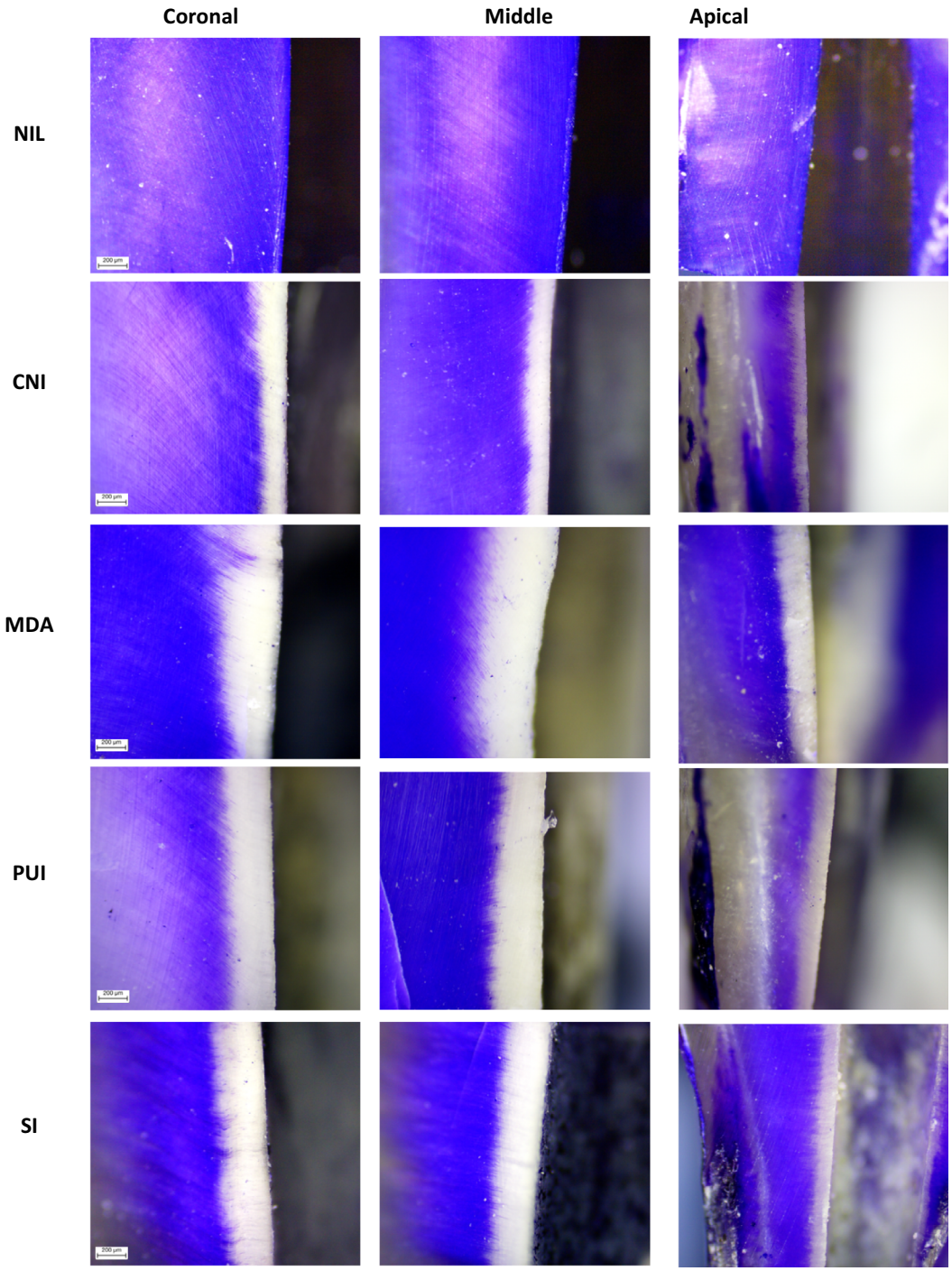

Figure 5 Representative light microscopy images of dentinal tubular penetration in the coronal, middle and apical segments of the root canal following use of various agitation techniques with 5.25% NaOCl for 20 min. The white/bleached region of dentine, which was stained with crystal violet, demonstrates that which has been penetrated by NaOCl. CNI, conventional needle irrigation; MDA, manual dynamic activation; Nil, negative control; PUI, passive ultrasonic irrigation and SI, sonic irrigation. Scale bar represents 200 μm.

high statistical power. Nevertheless for the reasons described above, caution must still be taken when extrapolating outcomes of this laboratory experiment into the clinical setting.

The results of this study demonstrate that the tubular infiltration of NaOCl was considerably improved in all segments of the canal when irrigants were agitated with MDA, PUI or SI. Similar trends are present in other investigations that have made comparisons in irrigant penetration between CNI and any of the aforementioned agitation techniques (Ghorbanzadeh *et al.* 2016, Faria *et al.* 2019). This discrepancy could be attributed to the superior irrigant flow dynamics of the latter which help promote more intimate interactions between solutions and the internal surfaces of the root canal wall. For instance, Chen *et al.* (2014) reported the shear stresses and hydrodynamic pressures generated within irrigants during PUI were significantly greater and more evenly distributed across a larger area of the canal wall than those produced by CNI. Conversely, Munoz & Camacho-Cuadra (2012) demonstrated that irrigants delivered via conventional syringes passively backflow towards the pulp chamber soon after they have been expressed into canals, thus limiting their penetrative potential. The use of these agitation techniques is therefore likely to assist clinicians achieve optimum disinfection throughout the entirety of the canal during treatment.

In this study, NaOCl infiltration was consistently lower apically when compared to the corresponding coronal and middle segments, where there was relatively even penetration in both bucco-lingual and mesio-distal planes. Despite the use of different methodologies, similar observations have also been reported by Paqué *et al.* (2006), Giardino *et al.* (2017) and Galler *et al.* (2019). This regional variation can be attributed to characteristic features of apical dentine, which include increased peritubular sclerosis that advances in a coronal direction from 30 years of age, as well as reduced tubular density (Mjör & Nordahl 1996, Mjör *et al.* 2001, Paqué *et al.* 2006). Additionally, the dentinal sclerosis that physiologically develops along the mesio-distal direction of teeth has been shown to be more pronounced apically than in any other segment of the root canal (Russell *et al.* 2013, Giardino *et al.* 2017, Generali *et al.* 2018). This 'butterfly effect' is also likely to have contributed significantly to the reduced penetration observed in this region.

No single technique consistently achieved the greatest effect across the full length of the root canal. However, SI generally exhibited the highest NaOCl penetration coronally, all techniques performed relatively equally in the middle third and MDA and PUI outperformed SI apically. These regional differences in penetrative efficacy can likely be attributed to the agitating mechanisms of these techniques. For example, with MDA the simultaneous poor coronal and tight apical adaption of a single GP cone when placed within an instrumented canal would lead to greater differences in irrigant penetration apically, with respect to CNI, compared with any other region. This would suggest that each technique is suited for a specific region of the canal and a combination of agitation methods may be required to achieve the maximum irrigant penetration across the entire length of the root canal. Data reported by Spoorthy *et al.* (2013) and Ismail *et al.* (2016) support this hypothesis as they found deeper infiltration of irrigants and sealers, respectively, when several agitation techniques were used in a single canal. Further investigations are, however, required to identify the most efficacious combination of agitation techniques.

Highly significant increases in tubular penetration were measured across all irrigant activation techniques and canal locations when either NaOCl concentration or contact time was exclusively increased from 2% to 5.25% and from 10 min to 20 min, respectively. Despite methodological variations, these trends are consistent with findings from other studies that have investigated similar variables (Zou *et al.* 2010, Palazzi *et al.* 2016, Faria *et al.* 2019). These outcomes would also be expected as the amount of freely available chlorine increases when the NaOCl concentration is raised or when the solution is repeatedly replenished over a longer period of time (Siqueira *et al.* 2000). Interestingly, these effects were found to be much more pronounced for CNI and MDA than PUI or SI. This could be attributed to the fact that these are manually operated techniques and so greater time may be required for maximum effect on tubular penetration to be achieved, which in turn could be compensated for by using greater concentrations of NaOCl. There was also a highly significant interaction between irrigant concentration and contact time when both were increased in the coronal and middle thirds of canals. Therefore, the additional effect of raising either irrigant concentration or contact time will be of less magnitude than the initial effect of raising one of these parameters alone. For example, in this experiment, raising the irrigant contact time from 10 to 20 min with 2% NaOCl produced an estimated marginal mean increases in penetration of 58.2 μm coronally and 64.7 μm in the middle third. However,

when raising the irrigant contact time with 5.25% NaOCl, estimated marginal mean increases in penetration of only 34.1 and 30.4 μm were observed in the coronal and middle regions, respectively. This could be explained by the fact that NaOCl penetration occurs more quickly at higher concentrations and consequentially the results saturate at a given depth earlier. Therefore, whilst clinicians can use higher concentrations of NaOCl during root canal treatment to improve tubular penetration, the findings suggest similar outcomes can be achieved when lower concentrations are administered over longer periods of time. Given the harmful cytotoxic and caustic properties of more concentrated NaOCl solutions (Kleier *et al.* 2008, Martin *et al.* 2014), it may be more desirable to adopt this strategy for the purposes of patient safety.

Conclusions

Within the limitation of this study, three main conclusions can be drawn:

1. Manual dynamic activation, passive ultrasonic irrigation and sonic irrigation significantly improve tubular penetration of sodium hypochlorite throughout the root canal, including apically, when compared to conventional needle irrigation.
2. Increasing sodium hypochlorite concentration or contact time further improves tubular penetration for each of the aforementioned techniques.
3. Longer durations of sodium hypochlorite exposure at lower concentrations result in similar depths of tubular penetration as those achieved at higher concentrations.

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Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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