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Penetration depth of a dye marker into dentine using a novel hydrodynamic system (RinsEndo®)

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Abstract

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Aim To investigate the efficiency of a hydrodynamic irrigation system compared with conventional cleansing techniques in root canals.

Methodology Forty-five freshly extracted single-rooted teeth were de-coronated and their root canals were enlarged to size 30 at the apex. The teeth were randomly divided into three groups ($n = 15$) for the final rinsing sequence using 2% NaOCl plus acid fuchsin: group I: static application of irrigant, 3 min; group II: flushing with a syringe; 5-mL NaOCl, 1 min; group III: RinsEndo®-system; 5-mL NaOCl, 50 s. Apical extrusion was documented photographically. The roots were sectioned at 2, 4, 6 and 8 mm from their apices and the penetration depths of dye into dentine measured, using a stereomicroscope. Wilcoxon's test and Pearson's chi-squared test were employed to prove statistic relevance.

Results Greater dye penetration depth into the dentinal tubules was achieved when employing hydrodynamic rinsing procedures. Using this technique, 23% of the specimens were penetrated for more than 50% of their dentine thickness, whereas the results for flushing with a syringe were 12% (static application, 7%). No penetration of dentine occurred in 63% of specimens with static application, 39% flushing with a syringe and 15% using the hydrodynamic system ($P < 0.05$ Pearson's chi-squared test). Apical extrusion occurred more frequently after hydrodynamic rinsing (extruded specimens: RinsEndo® = 80%; static application/flushing with a syringe = 13%; $P < 0.05$ Pearson's chi-squared test).

Conclusions Hydrodynamic rinsing demonstrated an improvement over conventional methods in terms of dentine penetration of a dye marker. A higher risk of apical extrusion with the RinsEndo®-system was evident.

Keywords: apical extrusion, cleansing efficacy, cleansing techniques, dentinal tubules, dye penetration, hydrodynamic irrigation.

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Introduction

Since sodium hypochlorite (NaOCl) was introduced into dentistry in 1936, it has become the recommended irrigating solution because of its ability to dissolve organic material and to disinfect the root canal (Baumgartner & Ibay 1987, Zehnder 2006). The irrigation of root canals with NaOCl is thus a critical component of root canal treatment to remove pulp debris as well as to decontaminate the root canal system when infected (Byström & Sundqvist 1981). When testing three different irrigation solutions for efficacy in removing debris, it was suggested that the flushing component might be more important than the tissue dissolving capability of the irrigant (Baker *et al.* 1975). Therefore, the efficacy of NaOCl might also be influenced by the method by which it is introduced. For example, Cheung & Stock (1993) demonstrated an increase in the cleaning potential of NaOCl when combined with ultrasonic activation. When conven-

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tional methods are used, the irrigating solution passes only 1 mm deeper than the tip of the needle (Ram 1977).

The penetration depth of the irrigating solution and therefore its ability to disinfect dentinal tubules are limited. Hence, bacteria are likely to remain in dentinal tubules following intra-canal shaping, irrigation and medication (Wu *et al.* 2006). In a recent study by Nair *et al.* (2005), bacteria, mostly incorporated into biofilms, were found in inaccessible inter-canal isthmi and accessory canals in 14 out of 16 teeth. It would thus be an advantage to develop new application systems which increase dentine tubular penetration depths, thereby ensuring a thorough rinsing of the prepared root canal system whilst minimizing apical extrusion, to eliminate cytotoxic effects on the periapical tissues (Bradford *et al.* 2002, Serper *et al.* 2004). Ultrasonic systems have the potential to achieve these standards (Alaçam 1987, Cameron 1995). Their acoustic streaming effects have been shown to produce sufficient shear forces to dislodge debris in instrumented canals and to produce significantly cleaner canals than those produced by hand filing alone (Ahmad *et al.* 1987). Adding 1 min of ultrasonically activated irrigation significantly improved overall mean canal cleanliness values at all apical levels (Gutarts *et al.* 2005). These results were equal or superior to these using ultrasonic step-back techniques (Lev *et al.* 1987, Metzler & Montgomery 1989, Archer *et al.* 1992). Sabins *et al.* (2003) evaluated the amount of debris remaining after passive sonic and ultrasonic irrigation and were able to demonstrate that significantly less debris was evident in comparison with the control group which had been simply syringed. Jensen *et al.* (1999) and Lee *et al.* (2004a,b) describe similar results. However, the efficacy of this technology may be limited in small and curved canals because of their resonance behaviour (Krell *et al.* 1988, Stock 1991). Indeed, ultrasonic irrigation tends to be more effective in removing artificially placed dentine debris from extensions in wide canals than in small canals (Van der Sluis *et al.* 2005).

Recently, a novel device has been introduced for root canal irrigation. Employing a hydrodynamic working principle, this system (RinsEndo®, Co. Duerr-Dental, Bittigheim-Bissingen, Germany) has the potential to be more effective than conventional irrigating techniques. Unfortunately, no data or systematic clinical studies are currently available to support its use. Therefore, the aim of the present *ex vivo* study was to compare this newly developed hydrodynamic irrigation system to conventional techniques. The evaluation was focused on the penetration depth of irrigating solution into the circumpulpal dentine of root canal sections. The apical extrusion of the irrigating solution was also assessed.

Materials and methods

The crowns of 45 freshly extracted teeth (maxillary anteriors, single canal pre-molars and palatal roots of maxillary molars, extracted by dentists not included into the study) were removed at the cervical level. Because of anonymity, the reason for extraction of the teeth was not known. Teeth with obliterated root canal systems, root fillings and an apical curve >10° were excluded. After extraction, the teeth were immediately stored in 0.9% saline solution with an addition of 0.0001% sodium azide (NaN_3, Co. Merck-Schuchardt, Hohenbrunn, Germany) at 4 °C. The working lengths of the root canals were measured by using size 10 K-files and by deducting 1 mm from the lengths of the files when extending just beyond the apical foramina; all apical foramina were patent. The root canals were then prepared to a size 30 .02 taper, flushing the root canal with 5-mL NaOCl after every file had been used (needle diameter 0.50 × 25 mm). The volumes of the canals were determined by weighing the tooth before and after being filled with distilled water ensuring that no air remained in the canal. The teeth were then distributed into three groups, balanced in respect of tooth type and the volumes of the root canals. Each group consisted of three maxillary central incisors, two maxillary lateral incisors, two maxillary canines, four maxillary or mandibular single rooted pre-molars and four palatal roots of maxillary molars (Table 1). Additionally, the volumes of the canals in each group were randomized as follows: each group contained three roots with volumes <0.0049 mL, six roots with volumes of 0.0050–0.0099 mL, four roots with volumes of 0.0100–0.0149 mL and two roots with volumes >0.0150 mL (Table 2).

Table 1 Types of teeth used in the present study and distribution into the experimental groups

Type of tooth	Total number of teeth	Number of teeth in each group
Maxillary central incisor	9	3
Maxillary lateral incisor	6	2
Maxillary canine	6	2
Pre-molar	12	4
Palatal root of maxillary molar	12	4

Table 2 Canal volumes of the samples

Canal volumes	Total number of teeth	Number of teeth in each group
0.0000–0.0049 mL	9	3
0.0050–0.0099 mL	18	6
0.0100–0.0149 mL	12	4
>0.0150 mL	6	2

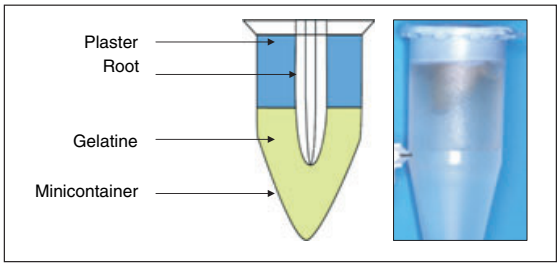

Figure 1 Experimental root socket: schematic (left) and original (right).

The specimens were embedded in an experimental root socket, which was filled with gelatine (15-g gelatine solved in 100-mL distilled water) up to 5 mm above the particular tooth length (Fig. 1). The space remaining between the edge of the container and the root was filled with plaster. The junction between tooth-plaster-container was sealed with lacquer to prevent the irrigating solution from entering from the outside. Between all stages of specimen preparation, the teeth were stored (4 °C) in 0.9% saline solution and additional sodium azide (NaN_3, 0.0001%).

The irrigating solution used as a final rinse during testing was NaOCl (2%) and a dye marker (5-g acid fuchsin dissolved in 100 mL of 2% NaOCl). Pilot experiments demonstrated that an addition of acid fuchsin to the NaOCl solution was sufficient to achieve an intense colouration. All experiments were carried out by one operator under standardized conditions, following the same time schedules. The rinsing syringe was inserted as far into the prepared root as was possible without binding. Excess solution was aspirated with a surgical suction apparatus. Groups I and II were used for comparison. Group I was treated with a static application of rinsing solution. In other words, the root canal was filled with the stained experimental solution with a syringe (needle Ø: 0.50 × 25 mm) up to the coronal orifice of the root canal. Bubbles were eliminated with a size 10 K-file. A silicone stop adapted 1 mm shorter than the working lengths prevented apical over-instrumentation. The solution was left in the root canals for 3 min and was then removed with a surgical suction system. Group II was treated by flushing with a syringe and needle (Ø: 0.50 × 25 mm) with a defined rate (5 mL within 1 min) into the root canal. Group III was subjected to cleansing with the RinsEndo®-system for hydrodynamic root canal irrigation. This system works on the basis of a pressure–suction technology: 65 μL of a rinsing solution oscillating at a frequency of 1.6 Hz (generated by a pneumatic element) is drawn from an attached syringe and transported to the root canal via an adapted cannula. During the suction phase, the used solution and air are extracted from the root canal and automatically merged with fresh rinsing solution. The pressure–suction cycles change approximately 100 times per minute. The irrigating volume was 6.2 mL min^{-1}. The RinsEndo®-system was charged with a 5-mL syringe and cannula (Ø: 0.45 × 12 mm), containing the experimental rinsing solution.

Immediately after each experiment, all specimens were dried with five size 30 paper points and pictures of the apical region were taken to document any dye penetration into the surrounding gelatine. Apical extrusion was assessed by a yes/no-decision on dye colouration in the apical gelatine. The assessment was performed by two persons. They were given a short introduction on their role and were instructed to note any visible colouration of the gelatine as a 'yes', whilst no visible colouration was to be noted as a 'no'. The data were blinded. The same two persons double checked the results by examining the apices for signs of dye using a light microscope. No disagreements were found.

Directly afterwards, the specimens were processed with a separating-cutting-system (Co. Exact, Norderstedt, Germany) into canal cross-sections (at 2, 4, 6 and 8 mm from the apex) and were assessed under a stereomicroscope (Co. Wild, Heerburg, Switzerland) with an incident illumination modus. Micrographs of the specimens were used for standardized documentation and metric assessment. The stereomicroscope was calibrated by using a measuring scale (Co. Wild, Heerburg, Switzerland, serial number 310345). To evaluate the results of the dye penetration depth on the four cross-sections (at 2, 4, 6 and 8 mm from the apex), the maximal dye penetration was measured in two directions: mesial-distal and buccal-lingual resulting in four measurements per cross-section (Fig. 2).

Statistical analysis was performed with SPSS for Windows 10.0 (SPSS Inc. Chicago, IL, USA). All results were tested for normal distribution with the Shapiro–

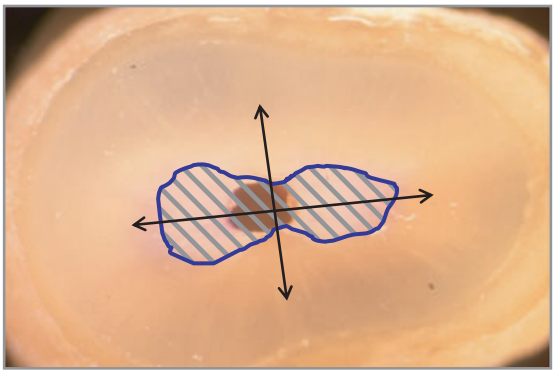

Figure 2 Microscopic specimen showing the penetration depth of the irrigating solution into the circumpulpal dentine (specimen of group III).

Wilk test. As not all values were normally distributed, the nonparametric Wilcoxon's test was used to analyse the results of dentine penetration. The Mann–Whitney *U*-test was used to assess the combination capability of measuring directions mesial-distal and buccal-lingual. Cross-tables concerning the circumpulpal and apical dye penetration results were generated and analysed by means of the Pearson's chi-squared test to test the statistical relevance of the yes/no-decision. Values were considered as statistically different at $P < 0.05$.

Results

Apical extrusion

Apical extrusion of coloured rinsing solution into the apical gelatine was documented as a prominent character of root canal irrigation. In group I (static application of irrigation solution) and group II (rinsing with a syringe), only two specimens had apical extrusion (13%; $n = 15$). Group III (hydrodynamic irrigation) had apical extrusion in 12 cases (80%; $n = 15$). The differences were statistically significant ($P < 0.05$; Pearson's chi-squared test).

Dentine penetration depth

Following statistical analysis, it was shown that the depths of penetration in a mesial-distal and buccal-lingual direction were not significantly different ($P > 0.05$; Mann–Whitney *U*-test). Therefore, the two mesial-distal and two buccal-lingual measurements were combined.

Within groups I, II and III, dye penetration in the cross-sections (2, 4, 6 and 8 mm) differed significantly from each other (Figs 3 and 4, Table 3; $P < 0.05$; Wilcoxon's test) with no statistical difference between the penetration in the mesial-distal and buccal-lingual directions ($P > 0.05$, Mann–Whitney *U*-test). At the 2- and 4-mm levels, no significant differences were observed within group I ($P > 0.05$; Wilcoxon's test). All specimens between groups I, II and III were statistically different (Table 3).

Categorizing the penetration depth into 'no penetration', '<50%' and '>50%' of dentine thickness gave the following results: Group I (static application of irrigating solution) – no movement of dye solution into dentine in 63.3%; group II (flushing with a syringe) – 38.7%; and group III (hydrodynamic irrigation) – 15.0%. Overall 30.0% of the specimens in group I, 50.0% in group II and 61.6% in group III had penetration of dye up to 50% of the circumferential dentine. Penetration depths more than 50% of the circumferential dentine were: group I: 6.7%; group II: 11.7%; group III: 23.3% (Fig. 5). Groups I, II and III differed significantly from each other comparing the particular cross-sections (2, 4, 6 and 8 mm from the apex; $P < 0.05$; Pearson's chi-squared test).

Discussion

The novel irrigation system tested in this study was compared with the more conventional cleansing technique of static application of irrigating solution and syringe irrigation. A favourable penetration depth within dentine was observed with the new device. Many authors have focused on irrigating efficacy when determined by the amount of debris remaining on various root canal sections (Alaçam 1987, Lussi *et al.* 1993, Scelza *et al.* 2000, Hata *et al.* 2001, Attin *et al.* 2002, Grandini *et al.* 2002, Mayer *et al.* 2002, Torabinejad *et al.* 2003). In the present study, attention focused on the microscopic evaluation of dye penetration into pulpal wall dentine at levels 2, 4, 6 and 8 mm from the apex. By creating similar experimental groups in terms of tooth type and canal volume, comparison between the groups was possible.

Pilot experiments demonstrated that the addition of acid fuchsin to NaOCl solution was sufficient to achieve an intense colouration of the NaOCl. In this way, the effective concentration of NaOCl was only changed to a minor degree. Additionally, the pilot experiments revealed that the dye marker was not bleached by NaOCl, with the conclusion that there was no interaction between dye and the NaOCl vehicle.

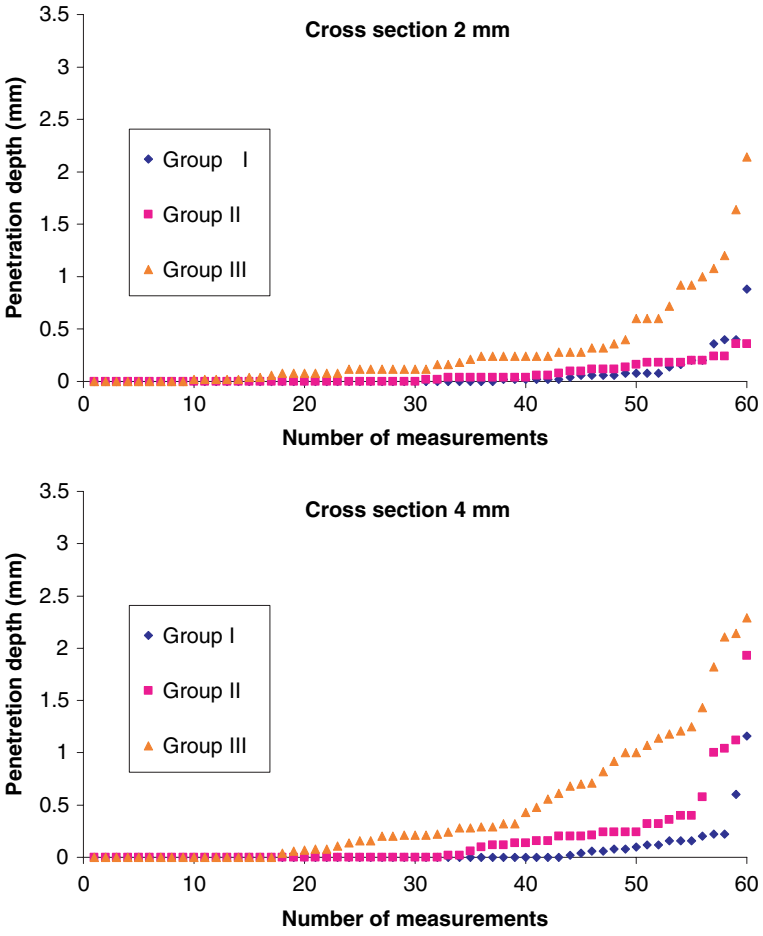

Figure 3 Group comparison of the penetration depth into root wall dentine between the experimental groups on the cross-sections 2 (top) and 4 mm (bottom).

Age of the teeth and thus variation in terms of dentine sclerosis could not be taken into account because of the anonymity of the teeth. Sclerotic dentine structure might lead only to minor dye penetration because of obliterated dentinal tubules, and it was hoped that randomly distributing the teeth to the three experimental groups overcome this potential throwback.

Canal preparation included only taper 0.02 K-files. In canals with greater tapers, ultrasonic irrigation has a tendency to remove artificially placed dentine debris from simulated canal extensions more effectively (Van der Sluis *et al.* 2005). Therefore, adding experimental groups with tapers of 0.04 and 0.06 might have improved the results.

Dye penetration was used as a marker for penetration of NaOCl, but it has not been established beyond doubt that the penetration depth of the dye matched the penetration depth of the NaOCl. It has been reported that the surface tension of NaOCl limits its ability to spread within the canal (Cunningham & Balekjian 1982). Adding acid fuchsin to NaOCl might have had an effect on its surface tension, which should be investigated in future studies. Because of the small molecular size of NaOCl (molecular weight of NaOCl dissolved in distilled water: 74.45 g mol^{-1}), it might be possible that the penetration depth of NaOCl was deeper than that of the dye itself (molecular weight of acid fuchsin: 585.54 g mol^{-1}). Thus, the advanced front of the irrigant remained only detectable indirectly. Therefore, no absolute results were considered, instead a standardized intraexperimental comparison between the experimental group (hydrodynamic irrigation) and the comparative groups (static application of irrigant and flushing with a syringe) was established.

Peters *et al.* (2001) reported that bacteria were present *in vivo* in 62% of cases up to the root cementum. In 24% of cases, more than 50 000 CFU g^{-1} were demonstrated at the root

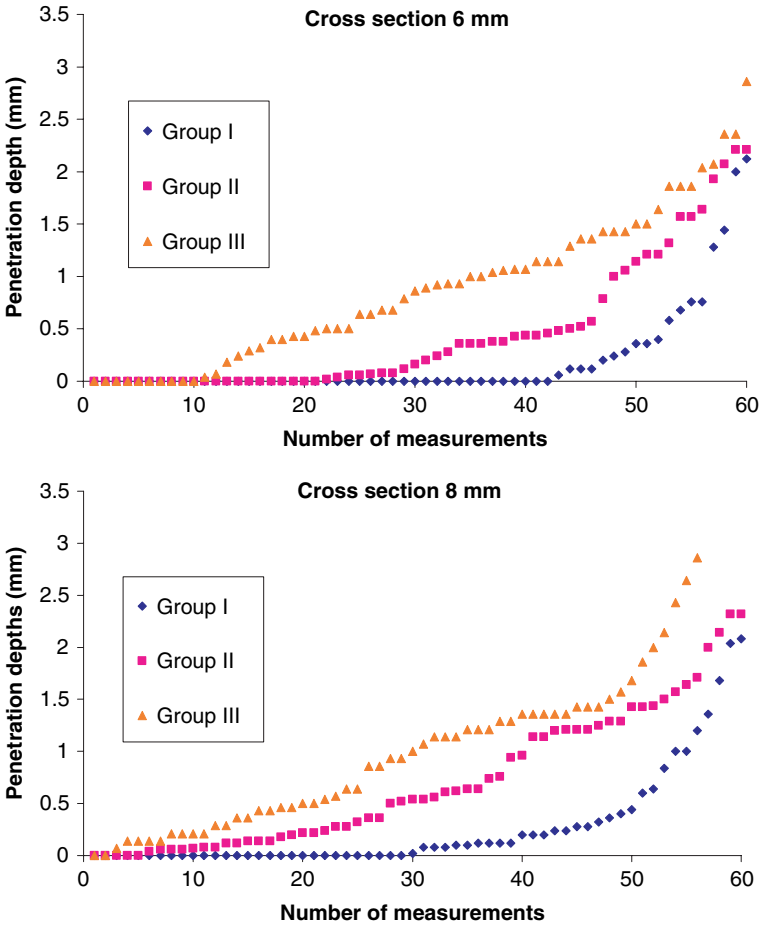

Figure 4 Comparison of the circumferential penetration depth amongst groups I, II and III on the cross-sections 6 (top) and 8 mm (bottom).

Table 3 Minimum and maximum values and the SD of group I, II and III at the cross-sections at 2, 4, 6 and 8 mm from the apex

	Group I			Group II			Group III		
	Min	Max	SD	Min	Max	SD	Min	Max	SD
2 mm	0	0.88	0.14	0	0.36	0.09	0	2.14	0.42
4 mm	0	1.16	0.17	0	1.93	0.34	0	2.29	0.59
6 mm	0	2.12	0.46	0	2.21	0.64	0	2.86	0.71
8 mm	0	2.08	0.50	0	2.32	0.66	0	2.86	0.69

cementum level. In other studies, the maximal penetration depth of bacteria into pulp wall dentine was 0.25 mm (Sirén *et al.* 1997, Basrani *et al.* 2003, Schäfer & Bössmann 2005). Those findings lead to the conclusion that it is necessary to achieve penetration of at least 50% of the dentine and that in many cases even a penetration depth of more than 50% would not achieve complete disinfection of root dentine. Therefore, penetration depths of '<50%' and '>50%' were used to visualize the potential cleaning effects of the different experimental techniques.

The static application of irrigant led to a poor dentine penetration. Flushing with a syringe (group II) revealed no dye penetration in 38.7% with the results for hydrodynamic irrigation being 15%.

A size 30 apical preparation was chosen to provide a realistic challenge for the irrigating method, as it is known for example that ultrasonics are less effective in small canals (Krell *et al.* 1988, Stock 1991, Van der Sluis *et al.* 2005). In oval root canals, 42% of dentine may remain unprepared (Wu *et al.* 2003). No technique presently available is able to remove the entire inner layer of infected dentine from root canals; in other words, bacteria are likely to remain in dentinal tubules after instrumentation (Leonardo *et al.* 1994, Ricucci & Langeland 1998). Root canal preparation up to size 30 is a clinically accepted protocol, considering the anatomic root configurations of the teeth under study. To compare the irrigating effects of the experi-

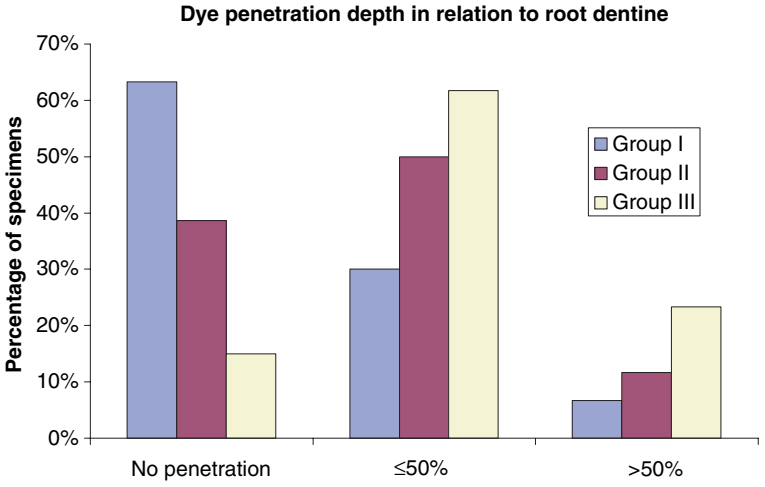

Figure 5 Distribution of the penetration depths in percent in groups I, II and III.

mental device with conventional techniques, similar diameters of needles were used in all groups. Knowing that it was not possible to place the tip of the cannula to the apical stop, this protocol allowed an intraexperimental comparison between the groups. The calculated difference of the apical position of the experimental needle (Ø 0.45 mm) to the standard needle (Ø 0.50 mm) using 0.02 taper is 0.72 mm, which has no clinical relevance.

Tissue necrosis can develop as a consequence of apical extrusion of irrigants (Hülsmann & Hahn 2000) and the tissue solving properties of NaOCl. Additionally, patients described severe pain immediately afterwards (when no local anaesthesia had been used) and swelling (Herrmann & Heicht 1979, Reeh & Messer 1989, Sabala & Powell 1989, Becking 1991, Gatot *et al.* 1991, Joffe 1991, Ehrich *et al.* 1993, Kavanagh & Taylor 1998). In this context, group III (hydrodynamic irrigation) had the worst results with 80% of the specimens having apical dye extrusion. In group I (static application of irrigant) and in group II (flushing with a syringe), only 13% had apical extrusion. The use of constant gentle pressure and preventing the needle from binding have been proposed to limit apical extrusion of irrigants (Hülsmann & Hahn 2000). Providing exclusive side-openings to the cannula and closing the opening of the tip could improve the hydrodynamic system and reduce the chance of apical extrusion.

Conclusion

In a laboratory setting, hydrodynamic rinsing enhanced the penetration depth of a dye marked rinsing solution into root canal wall dentine. However, apical extrusion of NaOCl was a common occurrence.

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