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The effects of sodium hypochlorite and calcium hydroxide on tissue dissolution and root canal cleanliness

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Summary

In this *in vitro* study, we investigated the efficacy of sodium hypochlorite (NaOCl) and calcium hydroxide ($Ca(OH)_2$) in dissolving necrotic tissue and cleaning root canals. In the first part of the study, 0.5% NaOCl solution and $Ca(OH)_2$ paste and solution were tested with samples of necrotic bovine muscle in different treatment modes and for different periods. The necrotic tissue was weighed before and after the test and the percentage of weight change calculated. In the second part of the study, 40 extracted single-rooted human teeth were hand instrumented and then subjected to different irrigation regimens. The cleansing efficacy in root canals of 0.5% NaOCl with $Ca(OH)_2$ pretreatments and ultrasonics was examined using scanning electron microscopy. A solution of 5% NaOCl was significantly more effective than 0.5% NaOCl as a solvent of necrotic tissue. Calcium hydroxide was an effective solvent for necrotic tissue as a paste but not as a solution. Pretreatment of necrotic tissue with $Ca(OH)_2$ increased its solubility in 0.5% NaOCl. While 5% NaOCl plus ultrasonic irrigation produced cleaner root-canal walls at the middle and apical thirds, 0.5% NaOCl used with the same technique achieved no root-canal cleaning. However, pretreatment of root canals with $Ca(OH)_2$ paste increased the effectiveness of 0.5% NaOCl plus ultrasonic irrigation, except in the coronal third of the root canal.

Keywords: calcium hydroxide, smear layer, sodium hypochlorite, solvent action, ultrasonic activation.

Introduction

Irrigation of the root-canal system is one of the most important steps in root-canal treatment. Chemical solutions are normally used to enhance the efficacy of mechanical cleansing. Sodium hypochlorite (NaOCl) is the most favoured endodontic irrigant in modern practice because of its tissue-dissolving, antibacterial and lubricant properties (Grossman & Meiman 1941; Shih *et al.* 1970; Hand *et al.* 1978; Grossman 1981; Harrison & Hand 1981; Ingle & Taintor 1985). However, effective concentrations of this solution (2.6–5.25%) are cytotoxic (Spangberg *et al.* 1973; Lamers *et al.* 1980; The & Plasschaert 1980; Pashley *et al.* 1985; Spangberg *et al.* 1988). The cytotoxicity of sodium hypochlorite is reduced at lower concentrations, but dilution impairs its tissue dissolution, canal debridement and antimicrobial properties (McComb & Smith 1975; Hand *et al.* 1978; Harrison & Hand 1981; Harrison 1984; Osetek 1988).

Calcium hydroxide ($Ca(OH)_2$) is also widely used in endodontics because it has antimicrobial activity and stimulates mineralization (Byström *et al.* 1985; Foreman & Barnes 1990; Porkaew *et al.* 1990; Sjöngren *et al.* 1991; Cvek 1992). The tissue-dissolving properties of NaOCl are well documented, but those of $Ca(OH)_2$ were first reported only in 1988 by Hasselgren and colleagues. These authors also reported that the tissue-dissolving effect of NaOCl was enhanced by pretreatment of the tissue with $Ca(OH)_2$.

Martin and colleagues (1980) and Martin & Cunningham (1983, 1985) have claimed that ultrasound and NaOCl act synergistically when used together for root-canal debridement. Moorer & Wesselink (1982) have shown the effectiveness of ultrasonic activation in enhancing the tissue-dissolving and disinfecting action of NaOCl solutions.

In the present study, it was planned to investigate whether the cleansing efficacy of 0.5% NaOCl could be improved by using it in combination with $Ca(OH)_2$ pretreatment and ultrasonics. The first part of the study provided more detailed information about tissue-dissolving capacities of 0.5% NaOCl and $Ca(OH)_2$ when used alone or in combination. In the second part of the study, scanning electron microscopy (SEM) was used to

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determine the presence of smear layer and debris on the wall of the root canal after various irrigation regimens.

Materials and methods

Part 1 of the study

Preparation of the test materials. $Ca(OH)_2$ powder (Merck, Darmstadt, Germany) and distilled water were mixed thoroughly to produce a thick paste of calcium hydroxide (0.9 g mL^{-1}). Calcium hydroxide irrigating solution was prepared according to the technique described by Morgan *et al*. (1991). The powder to liquid ratio was 14.5 g to 3.785 L. A household bleaching agent (Domex, Lever, Istanbul, Turkey) was used as a source of 5% NaOCl as supplied by the manufacturer and 0.5% NaOCl prepared by dilution (1:10) with distilled water. Before the experiments, the solutions were checked for available chlorine by iodometric titration, as described in the document TS 3664 (1980).

Experimental procedure. Bovine muscle that had refrigerated at 100% humidity for 2 weeks was used as a necrotic tissue sample. Frozen muscle was cut into 2 mm sections, from which pieces 6 mm in diameter were cut using a stainless-steel punch. Each sample thus had an equal surface area, and each weighed 80 mg. The ratio of the tissue mass to the volume of the test material was 80 mg/10 mL. All experiments were performed at 37°C. When the test material was a solution, the tissue-solution complex was agitated in a shaker (GD, Milan, Italy) throughout the test period to simulate fluid movement during root-canal instrumentation.

Ten samples of bovine muscle tissue were used in each of the experimental groups (Table 1). Distilled water (Group 1) and 5% NaOCl (Group 2) were used as the negative and positive controls, respectively. The test materials (0.5% NaOCl, $Ca(OH)_2$ paste, $Ca(OH)_2$ solution) were used alone in groups 3–12, and the same test materials were used in combination in Groups 13–15. The necrotic tissues was placed in 0.5% NaOCl after pretreatment with $Ca(OH)_2$ paste for 30 min (in Group 13) and 24 h (in Group 14). In Group 15 the necrotic tissue was pretreated with $Ca(OH)_2$ solution for 30 min before storing in 0.5% NaOCl. The necrotic tissue was weighed before and after the test and the percentage of weight change calculated. The pieces kept in $Ca(OH)_2$ paste were rinsed in distilled water and all the tissue samples were blotted on a dry paper towel for 30 s before weighing.

Statistical analyses applied to the data were analysis of variance and the least significant difference (LSD) test.

Table 1 Experimental groups in Part 1 of the study, investigating the efficacy of sodium hypochlorite and calcium hydroxide in dissolving necrotic muscle

Groups	Experimental procedure
1 (negative control)	Distilled water (30 min)
2 (positive control)	5% NaOCl (30 min)
3	0.5% NaOCl (30 min)
4	$Ca(OH)_2$ paste (30 min)
5	$Ca(OH)_2$ paste (24 h)
6	$Ca(OH)_2$ paste (2 days)
7	$Ca(OH)_2$ paste (3 days)
8	$Ca(OH)_2$ paste (4 days)
9	$Ca(OH)_2$ paste (5 days)
10	$Ca(OH)_2$ paste (6 days)
11	$Ca(OH)_2$ paste (7 days)
12	$Ca(OH)_2$ irrigating solution (30 min)
13	$Ca(OH)_2$ paste (30 min) + 0.5% NaOCl (30 min)
14	$Ca(OH)_2$ paste (24 h) + 0.5% NaOCl (30 min)
15	$Ca(OH)_2$ irrigating solution (30 min) + 0.5% NaOCl (30 min)

Part 2 of the study

Forty recently extracted single-rooted human teeth were used in the second part of the study. After removing the crowns at the cemento–enamel junction, the working length was established at 1 mm short of the apical foramen. The apical foramen of each canal was sealed with casting wax and all canals sequentially enlarged to a standard size of 50 using K-type files. Before changing to a larger file, each canal was flushed with 2 mL of distilled water. To facilitate fracture of the roots, two parallel, longitudinal grooves were made on both buccal and lingual surfaces of the teeth, without penetrating the root canals.

Experimental groups were established according to the parameters obtained in Part 1 of the study. Five instrumented teeth were randomly allocated to each of the eight experimental groups as follows.

1 Negative control: no irrigation.
2 Positive control: 5% NaOCl (ultrasonic irrigation).
3 0.5% NaOCl (ultrasonic irrigation).
4 $Ca(OH)_2$ paste (24 h) + 0.5% NaOCl (conventional irrigation).
5 $Ca(OH)_2$ paste (24 h) + 0.5% NaOCl (ultrasonic irrigation).
6 $Ca(OH)_2$ paste (7 days) + 0.5% NaOCl (ultrasonic irrigation).
7 $Ca(OH)_2$ solution (conventional irrigation) + 0.5% NaOCl (ultrasonic irrigation).
8 $Ca(OH)_2$ paste (7 days) + distilled water (ultrasonic irrigation).

Group 2. Ultrasonic energy was provided by a Cavi-Endo unit (Dentsply International, York, PA, USA) and transmitted through a smooth broach placed in an endosonic insert P105 (Dentsply International). The broach was allowed to oscillate unconstrained at the maximum power setting. The tip of the broach was positioned 1–2 mm short of the apex, taking care that the tip did not touch the canal wall. Each canal was irrigated for 3 min with a continuous flow of 5% NaOCl. The irrigant flow rate was 25 mL min^{-1}.

Group 3. The same method was used as in Group 2, but with the concentration of NaOCl reduced to 0.5%.

Group 4. The root canals were filled $Ca(OH)_2$ paste using a lentulo spiral carrier. The roots were sealed with Cavit (Espe GmbH, Seefeld/Oberbay, Germany) and stored for 24 h at 37°C and 100% humidity. After removal of the Cavit, the root canals were irrigated with a conventional technique using 0.5% NaOCl. The irrigation was carried out with a 27-gauge needle attached to a 10 mL syringe placed at two-thirds of the working length. Each canal received 10 mL of irrigant over 3 min.

Group 5. The same procedures were used as in Group 4, except that canals were irrigated ultrasonically using 0.5% NaOCl after insertion of $Ca(OH)_2$ paste.

Group 6. The $Ca(OH)_2$ paste was left in the root canal for 7 days. Irrigation procedures were the same as those used in Group 5.

Group 7. The root canals were irrigated conventionally with $Ca(OH)_2$ solution for 3 min and then ultrasonically with 0.5% NaOCl.

Group 8. After application of $Ca(OH)_2$ paste for 7 days the root canals were irrigated ultrasonically with distilled water as described above.

After completion of the irrigation regimen, each canal received a final flush of 2 mL of distilled water to halt any further action of the test materials and was dried with sterile absorbent paper points. Each tooth was split with a hammer and chisel. The specimens were dried and coated with 20 nm of gold before viewing with scanning electron microscopes (Jeol/JSM-840 and Jeol/JSM-5200, Tokyo, Japan). The coronal, middle and apical parts of the roots were observed and representative areas of each group photographed. The photographs were evaluated blindly by two investigators. We used the rating system developed by Rome *et al.* (1985): 0, no smear layer, dentinal tubules open and free of debris; 1, moderate smear layer, outlines of dentinal tubules visible or partly filled with debris; 2, heavy smear layer, outlines of dentinal tubules obliterated.

The Kruskal–Wallis test, which is non-parametric, was used to determine whether there were significant differences between the scores obtained from groups at different levels. Pairs of groups were compared using the Mann–Whitney U test.

Results

Part 1 of the study

The percentage weight changes (mean values and standard deviation) of necrotic tissues are shown in Table 2.

Statistical analysis showed that 5% NaOCl dissolved the tissue pieces more effectively than 0.5% NaOCl ($P < 0.05$). However, no statistically significant difference was found between the tissue-dissolving properties of 5% NaOCl and those of 0.5% NaOCl after pretreatments with $Ca(OH)_2$ paste for 24 h and as a solution for 30 min ($P > 0.05$). Pretreatment with $Ca(OH)_2$ paste for 30 min enhanced the tissue-dissolving ability of 0.5% NaOCl but not up to the level achieved by 5% NaOCl. A 0.5% solution of NaOCl dissolved necrotic tissue significantly better than did distilled water ($P < 0.05$).

Tissue pieces kept in the $Ca(OH)_2$ paste increased in weight for 2 days, but after 3 days of the experiment weight loss was observed. On the sixth and seventh days the tissue had become soluble and autolysed in the paste,

Table 2 Mean weight change of necrotic muscle pieces after the treatments listed in Table 1. SD, standard deviation

Group	Weight change (%)	SD
1	–16.42	1.73
2	–98.77	2.14
3	–34.47	9.00
4	+71.09	19.10
5	+52.89	9.83
6	+16.78	8.17
7	–15.63	12.39
8	–23.14	10.97
9	–25.60	9.23
10	Dissolved	
11	Dissolved	
12	+101.74	13.44
13	–72.99	19.49
14	–99.03	2.12
15	–91.66	6.14

and so could not be weighed. This was described as total dissolution of the tissue (Table 2). An irrigating solution of $Ca(OH)_2$ did not dissolve the necrotic tissue during the 30-min test period. On the contrary, it caused the tissue to swell and more than double its original weight. The weight increase was more significant in the specimens stored in $Ca(OH)_2$ solution than it was in those kept in $Ca(OH)_2$ paste ($P < 0.05$).

In the 30-min test period, pretreatment of necrotic tissue with $Ca(OH)_2$ irrigating solution was significantly more effective than pretreatment with $Ca(OH)_2$ paste in enhancing the tissue-dissolving capacity of 0.5% NaOCl ($P < 0.05$).

Part 2 of the study

Group 1 (negative controls). All specimens were heavily smeared at all levels. There was also organic debris on the surface of the smear layer.

Group 2. In most specimens, the coronal third was covered with a smear layer, whereas in the middle third of the samples most of the tubule openings were patent. The apical third of all specimens showed almost complete removal of the smear layer (Fig. 1).

Group 3. Smear material was present in all specimens. In one specimen, uninstrumented areas showed undissolved organic fibrils (Fig .2).

Group 4. An intact smear layer was present at all levels, and covered with $Ca(OH)_2$ crystals of 0.8–12 μm (Fig 3).

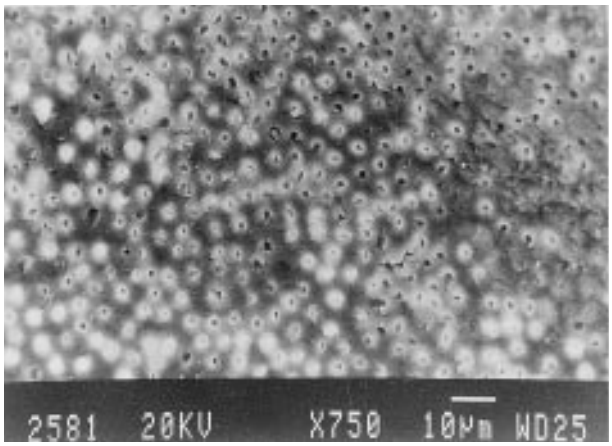

Fig. 1 Group 2 (see text for irrigation procedure): apical third of root canal, smear layer removed and patent dentinal tubule openings (magnification ×750).

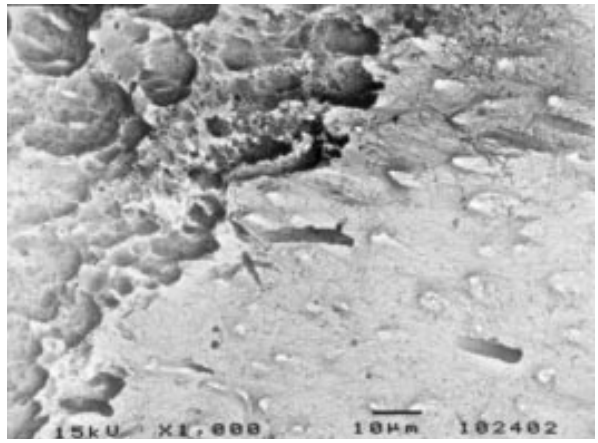

Fig. 2 Group 3 (see text for irrigation procedure): undissolved organic fibrils in an uninstrumented area of root canal (magnification ×1000).

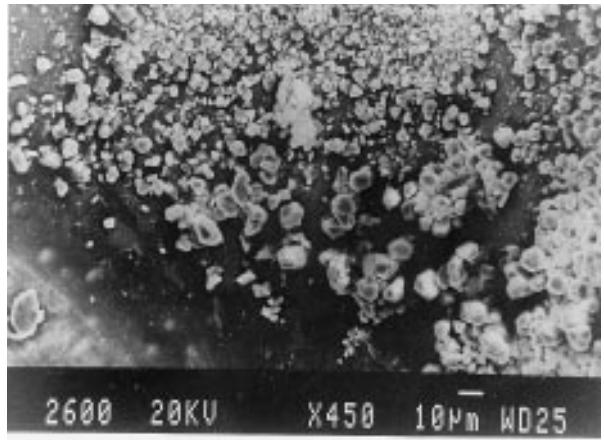

Fig. 3 Group 4 (see text for irrigation procedure): $Ca(OH)_2$ crystals covering the smear layer of a root canal (magnification ×450).

Group 5. The surface of all specimens smeared and partly covered with $Ca(OH)_2$ crystals in the coronal third. The crystals were distributed more sparsely towards the middle third of the canal (Fig 4). When the superficial smear layer was removed from the middle third of the samples, most of the tubule openings were patent. The apical surfaces were consistently cleaner, and dentinal tubules clearly seen without crystal accumulation (Fig. 5).

Group 6. The findings in this group were similar to those in Group 5.

Group 7. The findings in this group were similar to those in Group 3.

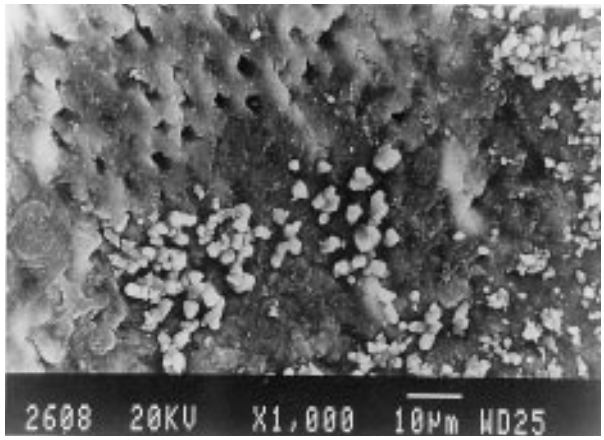

Fig. 4 Group 5 (see text for irrigation procedure): $Ca(OH)_2$ crystals more sparsely distributed towards the middle third of the root canal (magnification ×1000).

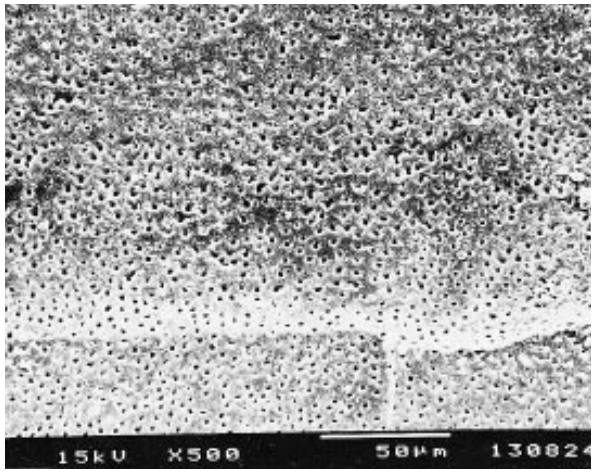

Fig. 5 Group 6 (see text for irrigation procedure): apical third of root canal, smear layer removed and patent dentinal tubule openings (magnification ×500).

Group 8. An intact smear layer was present at all levels in the samples. Crystals were present in decreasing amounts towards the apex. In one specimen, uninstrumented areas showed calcospherites on which $Ca(OH)_2$ crystals were seen (Fig. 6)

Table 3 shows the mean scores for each group at various levels. The experimental groups showed no significant differences at the coronal level ($P > 0.05$). There were significant differences, however, between the middle and apical levels of the specimens ($P > 0.05$) (Tables 4 and 5). Group 2, 5 and 6 showed significantly cleaner surfaces at the middle and apical levels when compared with the other groups (Tables 4 and 5).

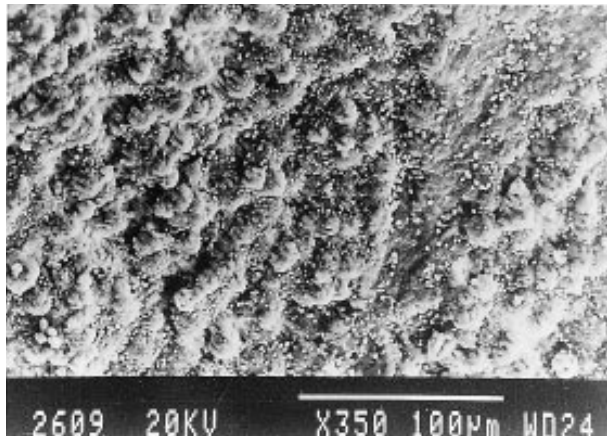

Fig. 6 Group 7 (see text for irrigation procedure): calcospherites with $Ca(OH)_2$ crystals in an uninstrumented area of root canal (magnification ×350).

Discussion

The tissue-dissolving capability of any irrigating solution is important, because it potentially enhances root-canal cleansing. In this study we tested the tissue-dissolving and root-canal cleansing actions of sodium hypochlorite and calcium hydroxide.

The surface area of tissue exposed to the test solution was important, because dissolution is a function of surface contact (Hand *et al.* 1978). In the present study, use of muscle allowed us to standardize the surface area of tissue samples. Had we used pulp tissue, it would have been difficult both to obtain sufficient tissue and standardize the surface area of samples.

The solvent effect on necrotic tissue of an irrigating solution depends on the following factors: concentration, pH, volume, temperature, time, exchange/refreshment, mechanical agitation, amount and surface area of tissue, and tissue type (Senia *et al.* 1971; Hand *et al.* 1978; The 1979; Cunningham & Balekjian 1980; Gordon *et al.* 1981; Abou-Rass & Oglesby 1982; Moorer & Wesselink 1982; Nakamura *et al.* 1985). The variation of these factors between previous studies makes it hard to compare their results with those of the present study. However, the finding that 5% NaOCl was a powerful solvent of necrotic tissue but that a 0.5% solution had little solvent action agrees with previously published reports (Trepagnier *et al.* 1977; Hand *et al.* 1978; Koskinen *et al.* 1980a, Koskinen *et al.* 1980b).

Under certain conditions, pretreatment with $Ca(OH)_2$, paste or solution enhanced the tissue-dissolving activity of 0.5% NaOCl to the level achieved with 5% NaOCl. These findings concur with those of Hasselgren *et al.*

Table 3 Mean scores for root-canal smear layer after different irrigation procedures. Each group contained five single-rooted human teeth; see text for procedures. SD, standard deviation

Group	Coronal third		Middle third		Apical third	
	Mean	SD	Mean	SD	Mean	SD
1	2.00.	0.000	2.00	0.000	2.00	0.000
2	1.80	0.274	0.60	0.418	0.20	0.447
3	1.90	0.224	1.70	0.447	1.60	0.418
4	1.90	0.224	1.80	0.447	1.80	0.447
5	1.80	0.447	0.50	0.500	0.30	0.447
6	1.80	0.224	0.40	0.548	0.20	0.447
7	1.90	0.224	1.50	0.500	1.10	0.224
8	1.90	0.224	1.80	0.274	1.50	0.500

Rating system (Rome *et al.* 1985): 0, no smear layer, dentinal tubules open and free of debris; 1, moderate smear layer, outlines of dentinal tubules visible or partly filled with debris; 2, heavy smear layer, outlines of dentinal tubules obliterated.

Table 4 Significance of the difference between treatment groups (see Table 3) in smear-layer scores for the middle third of the root canals

Group	1	2	3	4	5	6	7	8
1		$P < 0.05$	$P < 0.05$	$P > 0.05$	$P < 0.05$	$P < 0.05$	$P > 0.05$	$P > 0.05$
2			$P < 0.05$	$P < 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.05$	$P < 0.05$
3				$P > 0.05$	$P < 0.05$	$P < 0.05$	$P > 0.05$	$P > 0.05$
4					$P < 0.05$	$P < 0.05$	$P < 0.05$	$P > 0.05$
5						$P > 0.05$	$P < 0.05$	$P < 0.05$
6							$P < 0.05$	$P < 0.05$
7								$P > 0.05$

Table 5 Significance of the difference between treatment groups (see Table 3) in smear-layer scores for the apical third of the root canals

Group	1	2	3	4	5	6	7	8
1		$P < 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P > 0.05$
2			$P < 0.05$	$P < 0.05$	$P > 0.05$	$P > 0.05$	$P < 0.05$	$P < 0.05$
3				$P < 0.05$	$P < 0.05$	$P < 0.05$	$P > 0.05$	$P > 0.05$
4					$P < 0.05$	$P < 0.05$	$P > 0.05$	$P < 0.05$
5						$P > 0.05$	$P < 0.05$	$P < 0.05$
6							$P < 0.05$	$P < 0.05$
7								$P > 0.05$

(1988), but conflict with those of Morgan *et al.* (1991). Pretreament for 30 min with the $Ca(OH)_2$ solution was sufficient to enhance the tissue-dissolving capacity of 0.5% NaOCl; this was not so for the paste. According to Hasselgren *et al.* (1988), $Ca(OH)_2$ causes the tissue to swell and thus become more accessible to the NaOCl. Hence the greater efficacy of $Ca(OH)_2$ solution in enhancing the tissue-dissolving property of 0.5% NaOCl, as the necrotic tissues kept in the solution swelled more than those kept in the paste. The finding that $Ca(OH)_2$ paste was able to dissolve necrotic tissue after 1 week is also consistent with the observations of Hasselgren *et al.* (1988) and Andersen *et al.* (1992).

In the present study that 5% NaOCl with ultrasound removed the smear layer from all instrumented surfaces except the coronal third. Ahmad *et al.* (1987b) suggest that ultrasound achieved excellent cleanliness in the apical third because acoustic streaming is of greater magnitude and faster at the apical region of the endosonic file. Authors who found ultrasound beneficial had used the technique only for final irrigation of the root canal after instrumentation, and were careful not to touch the ultrasonic file on to the canal wall, so as to allow free oscillation (Ahmad *et al.* 1987b; Alaçam 1987; Cameron 1987; Cameron 1988), contrary to the technique recommended by Martin & Cunningham (1985). Ahmad *et al.* (1987a, b) claimed that direct physical contact of the file with canal walls throughout the ultrasonic instrumentation reduced acoustic streaming. This may be the reason why some studies have shown that the use of ultrasonics did not remove the smear layer.

In the present study, ultrasonic irrigation with 0.5% NaOCl did not remove the smear layer. This concurs with a report by Cameron (1988), who investigated the effect of the concentration of NaOCl used with ultrasonic irrigation for removal of the smear layer. However, when we carried out ultrasonic irrigation of root canals with 0.5% NaOCl after pretreatment with $Ca(OH)_2$ paste for 24 h or 7 days, we obtained satisfactory results except in the coronal third.

No significant differences in respect of cleanliness were noted between root canals pretreated with $Ca(OH)_2$ paste for 24 h and those pretreated for 7 days. This finding indicated that 24-h pretreatment with $Ca(OH)_2$ paste before ultrasonic root canal irrigation with 0.5% NaOCl was sufficient to produce clean root canal surfaces.

After pretreatment of root canal walls with $Ca(OH)_2$ paste for 24 h, conventional root canal irrigation performed with 0.5% NaOCl was not efficient in producing a smear-free root canal surface. Pretreatment with $Ca(OH)_2$ paste did not therefore improve the cleanliness of root canals irrigated with 0.5% NaOCl unless an ultrasonic method was used. Metzler & Montgomery (1989) reported that when the root canals were irrigated with 2.6% NaOCl after pretreatment with $Ca(OH)_2$ paste for 7 days, an ultrasonic technique provided no significant improvement in cleanliness compared with the conventional technique. The differences between the results of these studies may be related to the methods used. Histological evaluation as used by Metzler & Montgomery (1989) allows detection only of organic tissue remnants in the root canals, whereas scanning electron microscopy allows detailed evaluation of the cleanliness of root canal.

Conventional irrigation of root canals with $Ca(OH)_2$ solution before ultrasonic irrigation with 0.5% NaOCl did not enhance the cleanliness of the root canals, although pretreatment with $Ca(OH)_2$ solution enhanced the ability of of 0.5% NaOCl to disolve necrotic muscle. A 3-min exposure of the root canals during irrigation allows little time for the $Ca(OH)_2$ irrigating solution to show its effects compared with those of 24 h or 7 days of pretreatment with $Ca(OH)_2$ paste. However, in clinical practice it is not possible to keep an irrigating solution in the root canal for longer than 3 min.

As $Ca(OH)_2$ paste could dissolve tissue after 6–7 days, it might be expected that $Ca(OH)_2$ paste, as a long-term intracanal dressing, could give smear-free root-canal surfaces when used with distilled water applied with ultrasonics. However, unless 0.5% NaOCl was used, this irrigation regimen was not successful in removing smear layer, but dissolved predentin in an uninstrumented surface.

Conclusion

The present study showed that, under certain conditions, pretreatments with $Ca(OH)_2$ enhanced the tissue-dissolving and cleansing efficacies of 0.5% NaOCl to the level achieved with 5% NaOCl. Therefore, it may be concluded that 0.5% NaOCl in combination with $Ca(OH)_2$ pretreatment and ultrasonics can be used effectively in root canal irrigation instead of 5% NaOCl which is proved to be highly cytotoxic.

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