Effect of Cleansers and Irrigation Methods on Primary Root Dentin Permeability

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ABSTRACT

Purpose: The purpose of this study was to evaluate the effects of cleansers and irrigation methods on the permeability index (PI) in primary root dentin.
Methods: One hundred twelve teeth were extracted and sectioned transversely, discarding their crowns. Then, the roots were distributed according to the cleansers: (1) Dakin (D); (2) Dakin+hydrogen peroxide (DHP); (3) 2% chlorhexidine gel (C); and (4) saline (S). The canals were manually instrumented. Irrigation was performed manually (MI) or ultrasonically (UI). The roots were: (1) made impermeable; (2) filled with 2% methylene blue solution; and (3) longitudinally sectioned. The halves were marked in thirds (cervical, middle, and apical), and the areas of dye penetration were measured. PI data were submitted to factorial analysis of variance and Tukey tests ($P<.05$).
Results: A significant effect of MI/UI, in association with cleansers, was seen on PI. For all thirds, the PI of MI was superior to UI. In the cervical third, MI achieved a high PI when associated with D, DHP, and S. UI associated with S and C produced the highest PI. In the middle third, MI associated with D and DHP produced the highest PI. In the apical third, MI associated with D demonstrated the highest PI. None of the UI and cleanser associations induced differences in PI for the middle and apical thirds.
Conclusion: MI produced better results than the UI. The D, DHP, and S cleansers demonstrated the highest PIs, suggesting that the combination of MI and these cleansers is recommended for primary teeth to maintain dentin permeability. (J Dent Child 2007;74:30-5)
KEYWORDS: PRIMARY TEETH, ENDODONTIC TREATMENT, CLEANSERS, IRRIGATION METHODS, PERMEABILITY INDEX, ROOT CANAL, IRRIGANTS, DENTIN

The fast development of dental caries in primary teeth produces rapid pulp damage due to the pulpal tissue contamination by bacteria and their derived toxins.[1] The infected root canals are a common problem in the primary dentition, which require endodontic treatment.[1] The most important component of successful therapy is tissue, bacteria, and inflammatory products debridement within the root canal system.[2] In this context, the use of cleansers in the irrigation process is very important. These materials have been used as an aid in the partial or total removal of the smear layer produced during instrumentation.[3] Compatibility with clinical use in terms of physical-chemical properties, antibacterial capacity, tissue dissolution, cleaning effect, chelating action, and tissue tolerance must also be considered when selecting an irrigating solution.[4]

Several cleansers, all of which have been used as irrigants in pulp therapy, may be used to enable debris removal within permanent canal systems for effective endodontic therapy[6]:

1. sodium hypochlorite (NaOCl);
2. hydrogen peroxide (H_2O_2) used alone or the combination of NaOCl+H_2O_2[5]; and
3. NaOCl with acidic or basic substances.

Chlorhexidine has been studied for its various properties:

1. antimicrobial activity;
2. residual antimicrobial activity;

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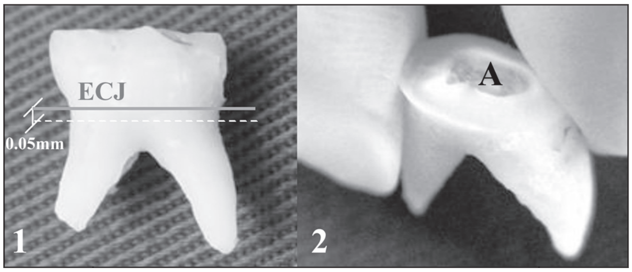


Figure 1a. Primary teeth with two thirds of intact root. The white line is the transversal section.
Figure 1b. Primary teeth transversely sectioned; A=sectioned pulpal chamber.

3. biocompatibility; and
4. action on bacterial lipopolysaccharide as an alternative to sodium hypochlorite in permanent teeth.[7]

In addition, cleansers acting on the smear layer produced by instrumentation probably alter the permeability of canal walls and permit greater penetration of medicaments into dentinal tubules.[8] Also, this denser propriety is important, particularly for primary teeth in which filling material must be reabsorbed during root resorption.[9]

Two factors that directly affect permanent root dentin permeability are the:
1. reduction in dentin thickness after instrumentation of root canal; and
2. formation of smear layer.[10]

The smear layer:
1. reduces dentin permeability; and
2. prevents the penetration of disinfectants into the deep area of the root canal wall.[11]

Alterations in the primary root dentin permeability are very important to both physiopathology and endodontic therapy. Studies concerning the action and effects on root dentin permeability on primary teeth are necessary, since the filling paste must penetrate into dentin tubules to prevent canal recontamination. There is no known study, however, focusing on primary teeth.

This study's purpose was to evaluate the permeability of root canals of primary teeth following the action of cleansers and using different methods of irrigation. The authors tested the first hypothesis that there was no difference between irrigation methods (manual or ultrasonic system). Then, the second hypothesis tested was that different irrigating solutions (0.5% NaOCl, 0.5% NaOCl associated with hydrogen peroxide, and saline solution) and gel (2% chlorhexidine digluconate) affect dentin permeability in the primary tooth root canal.

METHODS

One hundred twelve (112) infected human maxillary and mandibular posterior primary teeth were extracted for clinical reasons, as the periapical lesion involving the crypt of the subjacent tooth and/or the teeth restorations could not possibly be accomplished. The Ethical Committee of the Piracicaba Dental School, University of Campinas, Piracicaba, São Paulo, Brazil approved the study. The teeth were stored in 2.5% glutaraldehyde phosphate buffered (pH 7.4) for 24 hours before washing and storage until use in a Sorensen buffered solution under refrigeration.

Table 1. Group Distribution According to Irrigation Method and Irrigant Type

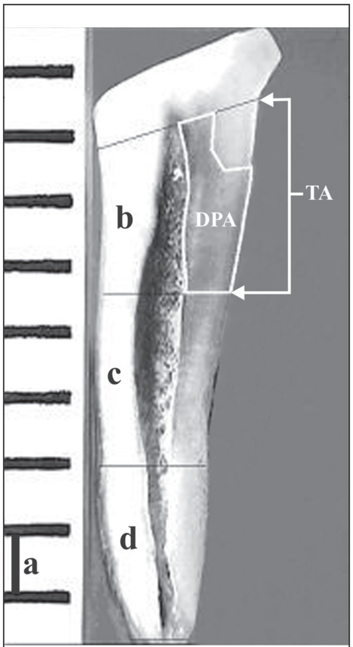
Method irrigation	Irrigants used	Manufacturers*
Manual (MI)	Dakin's liquid (D; n=14)	Proderma
	Dakin's liquid+H2O2 (DHP; n=14)	Proderma/ Polidental
	2% chlorhexidine digluconate gel (C; n=14)	Endosupport
	Saline solution (S; n=14)	Tayuyna
Manual+ultrasonic activation (UI)	Dakin's liquid (D; n=14)	Proderma
	Dakin's liquid+H_2O_2 (DHP; n=14)	Proderma/ Polidental
	2% chlorhexidine digluconate gel (C; n=14)	Endosupport
	Saline solution (S; n=14)	Tayuyna

****Proderma (Laboratory of Manipulation, Piracicaba, Brazil); Polidental Industry and Commercial (São Paulo, Brazil, batch no. 6220); Endosupport (São Paulo, Brazil, batch no. 1802.8295); Tayuyna Laboratory (São Paulo, Brazil, batch no. 035171).***

Only roots with at least two thirds of the intact root and the same length were selected. The teeth were sectioned transversely at the cementoenamel junction (approximately 0.5 mm below the enamel-cementum junction), and the crowns were discarded. (Figure 1) The roots were randomly separated into 2 groups (N=56) depending upon the method of irrigation (manual [MI] or manual+ultrasonic activation [UI]), and into 4 subgroups (N=14) depending upon the irrigant used (Table 1).

The working length was determined visually using the thinnest no. 15 K-file (Dentsply/Maillefer, Ballaigues, Switzerland) 1.0 mm shorter than that observed to just perforate the apex. All root canals were sequentially manually instrumented using K-files from nos. 15 to 35 (Dentsply/Maillefer). Each canal was prepared by the same operator (FMP).

The root canals were irrigated using 1 mL of Dakin's liquid (D) (0.5% NaOCl neutralized with boric acid), or 1 mL of Dakin's liquid associated with H_2O_2 cream (DHP; 8.85% H_2O_2, 14.34% Tween 80, 76.80% Carbowax), or 1 mL of saline solution (S; control group) as irrigants between each instrument, for a total of 5 mL. The solutions and gel were inserted within the root canals using a 1-mL insulin syringe with 12.7x0.33 mm round edge needles (Becton Dickinson and Company, Franklin Lakes, New Jersey), which were placed at the working length in each canal. For the DHP group, H_2O_2 cream was placed into the pulpal chamber (Figure 1) and Dakin's liquid was dropped into it. After instrumentation, a final irrigation with 1 mL Dakin's liquid was always performed to wash out the H_2O_2 cream. For



$$PI = \frac{DPA \times 100}{TA}$$

Figure 2. Hemisection divided into thirds (cervical, middle, and apical) to measure the permeability index (PI); (a) 1 mm scale; (b) cervical third; (c) middle third; (d) apical third; (DPA=dye penetration area; TA=total root dentin area.

the C group, the root canal was totally filled with 2% chlorhexidine digluconate gel before performing a final irrigation with 1 mL saline solution to wash out the chlorhexidine. For the UI group, the cleansers were inserted at the same time, as ultrasonic activation was performed to increase the efficiency of irrigation by the ultrasonic system. For this, a Mult-Sonic-s ultrasonic system was utilized (Gnatus, Ribeirão Preto, Brazil) at 50/60 Hz, 40 vA power, 20 W consumption, and 29 kHz frequency.

The root canals were dried with tips of absorbent paper (Tanari FDA, Manaus, Brazil, batch no. 005001P), and the roots were left to dry for 30 minutes. Roots were then externally coated twice using nail varnish (Colorama, São Paulo, SP, Brazil) and apically coated with wax. To evaluate the permeability index (PI), 2% methylene blue solution (pH 7.0) was placed into root canals using an insulin syringe (Becton Dickinson and Company, Franklin Lakes, New Jersey), and left in for 4 hours in a closed chamber at 37°C and 100% humidity. Following storage, roots were washed to remove dye excess and sectioned longitudinally using a double-face diamond disk (KG Sorensen, São Paulo, Brazil) into hemisections. Only one of the hemisections was used to verify the dye penetration into the root dentin. The hemisections were randomly selected through lottery method.

PERMEABILITY INDEX ANALYSES

The hemisections were observed in a stereomicroscope Leica MZ6 (Leica Microsystems AG, Wetzlar, Germany) at X0.63 to X3.2 magnification, depending on the hemisection root size. From the initial sample, after they were evaluated in a stereomicroscope 16 specimens were discarded since it was impossible to observe the apical third clearly. Thus, the final sample was accomplished by 96 hemisections (N=12). The image was captured with a digital camera (Viewse digital VC-813D, São Paulo, Brazil), which sent the image to DC 10 AV/DV–version 9 software (Pinnacle Studio, São Paulo, Brazil).

The permeability index was defined as the measure in percentage of the dye penetration area in each radicular third.12 The areas of dye penetration were measured using the Image Tool 3.0 software (Periodontology Department, University of Texas Health Science Center at San Antonio, Tex). The hemisection was divided into thirds (cervical, middle, and apical). For each third, the total and dye penetration areas (mm2) were measured, with the exception of the light root area. Thus, the root dentin permeability index (PI) was determined by multiplying the value of the dye penetration area (DPA) by 100. This value was then divided by the total root dentin area (TA) as the equation: $P = \frac{DPA \times 100}{TA}$, and according to Figure 2.

For intraexaminer reliability, 20% of the randomly chosen sample was examined and twice evaluated at a weekly interval. The data were submitted to Pearson's correlation test, and the intraexaminer coincidence level was found to be 90%. The permeability index was determined by one calibrated examiner.

STATISTICAL ANALYSIS

The null hypotheses tested were that there was no difference between irrigation methods used in this study and among the different cleansers. The averages of the permeability index for each irrigation method and cleansers were submitted to the factorial (axb) analysis of variance test ($P<.05$). A pair-wise multiple comparison was performed by independent samples Tukey test ($P<.05$) for different irrigation methods (manually x ultrasonic activation) and different cleansers, regardless of each third (cervical, middle, and apical). All statistical tests were performed by Sanest (Statistical Analysis System, Pelotas Federal University, UFPel, Pelotas, RG, Brazil).

Table 2. Permeability Index (PI) Averages Percentage for Cervical, Middle, and Apical Thirds

	Cervical third		Middle third		Apical third	
	MI (PI %)*	UI (PI %)*	MI (PI %)*	UI (PI %)*	MI (PI %)*	UI (PI %)*
D	68.5±19.8aA	41.2±21.0abB	63.3±34.4aA	26.8±21.8aB	51.9±34.0aA	26.8±21.8aB
DHP	81.5±23.2aA	23.9±23.1bB	72.5±20.2aA	33.4±36.7aB	29.4±32.2bA	24.2±32.7aA
C	20.8±27.8bA	58.1±24.6aB	23.5±30.7bA	31.1±32.2aA	10.0±16.1bA	10.2±12.2aA
S	66.5±31.5aA	66.3±24.6aA	49.5±30.1abA	49.0±26.3aA	21.4±30.2bA	26.0±35.0aA

**** PI=permeability index, obtained by multiplying dye penetration area by 100 and divided to the total root dentin area, in percentage. Similar small letters in column mean no significant statistical difference by factorial (axb) analysis of variance (ANOVA) test (P<.05), regarding each third. Similar capital letters in line mean no significant statistical difference factorial (axb) ANOVA test (P<.05), regarding each third.***

RESULTS

The null hypotheses were rejected. There was a significant difference between irrigation methods and among the different cleansers used in this study. In addition, there was a significant association between the studied factors (irrigation methods and different cleansers) for the cervical, middle, and apical third, respectively (P=.00001; P=.010; P=.049). The mean permeability index values in the different thirds are shown on Table 2. Results demonstrate that the irrigation method, in association with the cleansers, had a significant influence on mean PI (P<.05). The manual irrigation method produced a higher PI than that observed when the ultrasonic irrigation method was used in the cervical and middle thirds (P<.05).

In the cervical third, manual irrigation achieved the highest PI means when associated with Dakin, Dakin's liquid+H_2O_2, and saline solutions and the lowest PI mean when associated with chlorhexidine. Ultrasonic irrigation associated with saline, chlorhexidine, and Dakin produced the highest PI mean (P<.05). In the middle third, MI associated with Dakin or Dakin's liquid+H_2O_2 produced the highest PI means, while all UI and cleansers associations did not induce different PI means (P>.05). In the apical third, MI associated with Dakin allowed the highest PI means (P<.05) and all UI and cleansers associations did not induce different PI means (P>.05).

DISCUSSION

The hypotheses tested in this study were accepted. The PI evaluation showed that there was a significant difference between irrigation methods and among the different cleansers. The study's most important finding, however, was a significant association between irrigation methods and different cleansers. The manual irrigation method produced a higher PI than ultrasonic irrigation in the cervical and middle thirds. The use of ultrasonic energy for irrigant activation did not improve PI compared to the manual groups. These findings are in accordance with other studies that have failed to demonstrate the superiority of ultrasonics as a primary instrumentation technique.[13-15] Yamada[16] stated that the volume of cleansers influenced the cleanliness of the root canal when ultrasound was used. This might explain the results obtained in the present study, in which different volumes of cleansers were not used. Setlock et al[17] and Karadag et al,[18] however, did not find significant differences between manual instrumentation and ultrasonic techniques in permanent teeth in effectively reducing the smear layer and, consequently, alterations in the permeability.

The effects of ultrasound include cavitations and acoustic microstreaming. During cavitation, bubbles are generated in the liquid that implode with great force, creating a pressure-vacuum effect that cleans the root canal walls and has a bactericidal effect. Acoustic microstreaming describes the hydrodynamic shear stress generated in the ultrasonic field, aiding in the removal of debris and smear layers from the root canal walls. Passive activation implies that no attempt is made to instrument, plane, or contact the canal walls with the file to achieve maximum benefits from acoustic streaming.[19]

In this study, a passive activation by ultrasound was used during the irrigation procedure. In addition, since the operator placed a tip in the cervical third, the cleanser activation did not occur in the same manner as in the total length of the root canal. It can be considered as the main limitation of this study. Seow,[20] however, showed that the use of ultrasound also greatly enhances the efficiency of root canal cleaning in primary teeth. He observed that this procedure, using a plain endosonic probe, conventional probe, or a combination of both techniques removed 60%, 40%, and more than 95% of bacteria, respectively. This finding is important, since the primary teeth have accessory canals which are inaccessible to manual mechanical cleaning. There are no other known studies that compare ultrasonic technique and cleansers association used in primary teeth with regard to permeability index.

Another point to be considered is the canal size; the enlargement of the canals determines the amplitude of the oscillating instrument tip. Since the primary teeth have a small diameter in the root canal, the oscillation of the ultrasonic instrument's tip was probably limited by the amplitude of the canal, and did not play a large role in the cleanliness.[21]

In the present study, manual irrigation achieved the highest PI averages when associated with Dakin, Dakin's liquid+H_2O_2, and saline solution and the lowest PI averages when associated with chlorhexidine, in the cervical third. Regarding the use of Dakin, 69% of the root cervical area was permeable to dye penetration, while 82% was permeable to Dakin's liquid+H_2O_2, and 66% to saline solution. Only 21% of the total cervical area, however, became permeable following chlorhexidine use. From the chemical and mechanical point of view, the solutions and methods used showed efficiency in producing permeability in the root walls of primary teeth.

Sodium hypochlorite (0.5% NaOCl neutralized with boric acid—Dakin´s liquid) is the most commonly used irrigant in primary root canal treatment, since it is less irritating to the periapical tissue[22,23] and permanent tooth buds[24] than 1% to 5.25% NaOCl concentrations. Moreover, Dakin´s liquid has proven to be an excellent irrigating solution due to its tissue-dissolving capability and microbicidal activity.[25] The NaOCl-based cleansers, besides their mechanical actions determined by the solution's flow ability, act chemically on the dentinal wall. This solution showed deproteinizing characteristics; the dissolution of organic tissues by sodium hypochlorite solutions is based on the chloride's action on the proteins, which form water-soluble chloramines. This reaction is directly proportional to the active chloride concentration present in the solution. Sodium hypochlorite solution alters the configuration and, consequently, removes the organic components of dentin, especially the collagen fibrils.[26] NaOCl could be used separately or in association with other substances.

Another substance used as auxiliary of instrumentation is the H_2O_2 cream. It was placed into the pulpal chamber to achieve the instrumentation. Then, Dakin's liquid was dropped into it to wash out the H_2O_2 cream. The peroxides are oxidizing agents that react chemically, liberating great amounts of nascent oxygen, explaining their bactericidal action. The effervescence, due to the liberation of oxygen, contributes to the removal of pulp tissue remnants and dentinal particles during the chemical-mechanic preparation. This study's results are in accordance with those of Marshall et al,[12] who observed that H_2O_2 and hypochlorite solutions used alternately (DHP) produced a significant increase in root dentin permeability to isotopes in permanent teeth.

Another result found in the present study was that, in the cervical and middle thirds, H_2O_2 action was more evident than in the apical third. It seems that the H_2O_2 cream did not reach the end of the canal and did not act efficiently to achieve dentinal permeability. This might be considered an advantage, due to the contact of the H_2O_2 with the apical area and its consequent O_2 release and formation of bubbles, which could take debris to the periapex and cause damage to the permanent bud.

Regarding saline solution, the solution movement apparently acted mechanically on the root walls, possibly removing the weakly linked debris bonding to the root structure and, thus, allowing the dye to penetrate 66% of the root cervical area.

Chlorhexidine gluconate has been researched more recently for use in endodontics as an alternative to NaOCl.[24,27] This solution has the possible clinical advantage of being relatively nontoxic to vital tissue.[28] Chlorhexidine gluconate causes the loss of osmotic balance by attaching to bacterial cytoplasmic membranes, resulting in the leakage of intracellular material.[29] It also binds to hydroxypatite and soft tissues, changing their electrical field to compete with bacterial bindings.[30] Furthermore, it has been reported that chlorhexidine also has substantive antimicrobial activity when used as an endodontic irrigant.[31] The major disadvantage of using chlorhexidine gluconate as the primary endodontic irrigant is that it lacks the ability to dissolve necrotic pulp tissue.[32]

In the present study, 2% chlorhexidine gluconate gel demonstrated low PI averages compared to the other groups in all thirds when associated with manual irrigation. It may be presumed that the high surface tension caused by the gel might not have allowed it to make contact with the root canal walls. Another concern is that the chlorhexidine gel, in contrast to H_2O_2 cream, might be difficult to rinse from the canal surfaces. Residual gel and its products in the fluid may contaminate the dentin surface, leading to reduced dye penetration. When the gel was associated with ultrasonic irrigation, however, it presented higher PI averages. This could be explained by the acoustic stream action produced by ultrasonic system that may rinse the chlorhexidine gel easily from root canals.

In the apical third, manual irrigation associated with D, resulted in the highest PI averages. None of the UI and cleanser associations achieved differences in PI averages. For the apical third, no difference was found between manual irrigation and ultrasonic irrigation, possibly due to the complex morphology of root canals in primary teeth.[33] Apical dentin contains more sclerotic dentin, which is less tubular,[34] possibly explaining why apical dentin is much less permeable than either middle or cervical root dentin, even though its dentin thickness is much less than the other 2 zones.

Further studies should be carried out to verify whether the permeability increase is a primary effect of smear layer removal and whether the dye permeability may represent a similar permeability to bacteria and their toxins.

CONCLUSIONS

Within the limits of the present study, it could be concluded that:

1. The association between manual irrigation and Dakin, Dakin's liquid+hydrogen peroxide, and saline solutions produced better permeability index values in all root thirds, although performances for the different thirds were not similar.
2. Manual irrigation associated with Dakin, however, presented a similar performance in all root thirds evaluated.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Pediatric Dentistry and Dental Materials, Piracicaba Dental School, University of Campinas, São Paulo, Brazil, for their cooperation, especially Dr. Mario Fernando de Goes for his assistance with this paper and Dr. Mario Alexandre Coelho Sinhoreti, who collaborated with the statistical analysis. This research was supported by the State of São Paulo Research Foundation, FAPESP, (Grant number 03/09877-0).

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