Is the Presence of the Smear Layer a Limiting Factor for Root Dentin Permeability in Primary Teeth?

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ABSTRACT

Purpose: The purpose of this study was to correlate the dye permeability to the morphological aspect (presence or absence of a smear layer) of the primary root dentin wall, using scanning electron microscopy, regarding the endodontic preparation and irrigation methods. The hypothesis evaluated was that there was a correlation between the dye permeability and the morphological aspect of the primary root dentin.

Methods: A total of 112 extracted primary roots were distributed into the following groups: Dakin's liquid, Dakin+hydrogen peroxide; 2% chlorhexidine gel; and saline solution. Manual (MI) or Ultrasonic irrigation (UI) was performed. The roots were made impermeable, filled with dye (2% methylene blue), and longitudinally sectioned. The halves were divided in cervical, middle, and apical thirds for dye penetration measurement. The samples were observed under a scanning electron microscope. The data were submitted to linear regression analysis with a dummy variable ($P<.05$).

Results: The data revealed a relationship between decreasing permeability and the presence of a smear layer on root canal dentin walls for MI in the middle third ($P=.0147$). Regarding UI, no statistically significant relation was observed ($P>.05$).

Conclusions: The presence of a smear layer on root canal dentin walls was not a limiting factor to dye penetration in all groups except the middle third for manual irrigation.

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KEYWORDS: DENTIN PERMEABILITY, IRRIGATION, TOOTH PRIMARY, ROOT CANAL, IRRIGANTS, SMEAR LAYER

The smear layer is a debris layer produced during canal instrumentation, which obstructs the underlying dentinal tubule orifices.[2,4] The smear layer is linked to bonding properties of different materials, tooth structure, retention, and marginal leakage.[1] In the case of infected teeth, this layer contains a high number of inorganic calcified tissue particles and organic elements, such as: pulp tissue debris; odontoblastic processes; micro-organisms; and blood cells from dentinal tubules.[3]

There is controversy concerning the role of the smear layer in root dentin permeability. Scelza et al[5] stated that endodontic preparations may induce changes in root dentin permeability and that smear layer formation after canal instrumentation directly affects root dentin permeability. Fogel and Pashley[2] found that the smear layer that covered the root dentin wall was thick and its presence did not prevent fluid penetration in root canal dentin, although it happened in lower proportions. In addition, Tao et al[6] found that the absence of changes in root dentin permeability with a conventional endodontic preparation was due to the

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fact that, although endodontic preparation reduced dentin thickness, it also created a smear layer that compensated the process to the extent that there was no overall change in permeability.

The cleansing and irrigation method can contribute to efficient smear layer removal and increased permeability. In 1982, Cunningham and Martin[7] demonstrated the effect of ultrasonic instrumentation on canal cleansing, which resulted in cleaner canals than those obtained with the conventional technique. Due to agitation, the ultrasonic method enhances the effectiveness of solutions, increasing the wetting ability.[8] In addition, ultrasonic irrigation uses energy as a catalyst to activate the irrigant, both physically and chemically.[7]

The majority of those studies have been conducted in permanent teeth.[2,6-9] There are differences, however, between primary and permanent teeth regarding root canal morphology. Dentin permeability has a direct relationship to dentinal tubule diameter and density. Therefore, the presence of a smear layer and permeability alterations produced by endodontic treatment in root dentin should be studied in primary teeth.

The primary root canal system must be cleaned, decontaminated, shaped, and enlarged, since the filling has to be made with nonset pastes. These pastes have to penetrate the dentinal tubules in order to limit bacterial contamination and do not allow re-infection of the root canal system.

The purpose of this study was to correlate the dye permeability to the morphological aspect (presence or absence of a smear layer) of the primary root dentin wall, using scanning electron microscopy (SEM), and evaluated the correlation between the dye permeability and the morphological aspect of the primary root dentin.

METHODS

A total of 112 infected human maxillary and mandibular posterior primary teeth were extracted for clinical reasons. The Ethical Committee in Human Research of the Piracicaba Dental School at the 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. They were then stored until use in Sorensen buffered solution under refrigeration.

Only teeth with roots that had at least two thirds of 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. The roots were separated from each other using a double-face diamond disk (KG Sorensen, São Paulo, Brazil). The roots were randomly separated into:

a. 2 groups (N=56), depending upon the method of irrigation: (1) manual (MI); or (2) manual+ultrasonic activation (UI); and
b. 4 subgroups (N=14), depending upon the irrigant used (Table 1).

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

Table 1. Distribution of the Groups Depending Upon the Irrigation Method and Irrigants

Irrigation method	Irrigants used	Manufacturers*
Manual (MI)	Dakin's liquid (D; N=14)	Proderma
	Dakin's liquid+ H_2O_2 (DHP; N=14)	Proderma/ Polidental
	2% chlorhexidine digluconate gel (CL; N=14)	Endosupport Tayuyna
	Saline solution (S; N=14)	
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 (CL; N=14)	Endosupport Tayuyna
	Saline solution (S; N=14)	

** 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).*

The root canals were irrigated using:

a. 1 mL of Dakin's liquid (D; 0.5% NaOCl neutralized with boric acid); or
b. 1 mL of Dakin's liquid associated with hydrogen peroxide (H_2O_2) cream (DHP; 8.85% H_2O_2, 14.34% Tween 80, 76.80% Carbowax); or
c. 1 mL of saline solution (S; control group) used 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, NJ), which were placed at the working length in each canal. For the DHP group, H_2O_2 cream was placed into the pulpal chamber 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 the chlorhexidine group (CL), the root canal was totally filled with 2% chlorhexidine digluconate gel before performing a final irrigation with a 1-mL saline solution in order 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-Sonics ultrasonic system was utilized (Gnatus, Ribeirão Preto, Brazil) at 50/60 Hz, 40 vA power, 20 W consumption, and a frequency of 29 kHz.

The root canals were dried with absorbent paper tips (Tanari FDA, batch no. 005001P, Manaus, Brazil). The

roots were then left to dry for 30 minutes. Roots were externally coated with two coats of nail varnish (Colorama, São Paulo, Brazil) and, apically coated with wax. For the evaluation of permeability index (PI), a 2% methylene blue solution (pH=7.0) was placed into the root canals using an insulin syringe (Becton Dickinson and Company), for 4 hours in a closed chamber at 37°C and 100% humidity. Following the storage time, the roots were washed for the removal of excess dye and sectioned longitudinally using a double-face diamond disk (KG Sorensen, São Paulo, Brazil) into 2 root halves. Only one of the halves was used to verify the dye penetration into the root dentin. The halves were randomly selected.

PERMEABILITY INDEX ANALYSES

All halves were observed under a stereomicroscope Leica MZ6 (Leica Microsystems AG, Wetzlar, Germany) at X0.63 to X3.2 magnification, depending on the hemisection root size. After assessment under the stereomicroscope, 40 specimens out of the initial sample were discarded because it was impossible to observe the apical third clearly or during the SEM preparations. Thus, the final sample comprised 72 halves (N=9), the images of which were captured with a digital camera (Viewse digital VC-813D, Shenzhen Viewse Electronics Co Ltd, Shenzhen Guangdong, China) and edited with Pinnacle Studio DC 10 AV/DV software (v. 9, Pinnacle, Mountain View, Calif).

The dye penetration areas were measured using Image Tool 3.0 software (Periodontology Department, University of Texas, and Health Science Center at San Antonio, TX). Every half root was divided into thirds (cervical, middle, and apical); for each third, the total and dye penetration areas (mm^2) 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 for total root dentin area (TA) using the formula:

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

SCANNING ELECTRON MICROSCOPY (SEM) EVALUATIONS

After the dye penetration assessment, the specimens were prepared for SEM evaluation. They were dehydrated in ascending grades of ethanol (25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, 95% for 30 minutes, and 100% for 60 minutes). After the final ethanol step, the specimens were dried by immersion in hexamethyldisilazane (HMDS) for 10 minutes, placed on filter paper inside a covered glass vial, and air-dried at room temperature.[10]

The hemisections were mounted on aluminum stubs with double-sided carbon tape (SEM, NISSHIN EM Co. Ltd, Tokyo, Japan), and sputter coated at 10 mA for 2 minutes (SCD050 sputter coater, Balzers, Liechtenstein). They were observed under SEM (JSM 5600LV, JEOL, Tokyo, Japan) at an accelerating voltage of 10 kV, a working distance of 20 mm, and X2,000 magnification.

For each cervical, middle, and apical third, one image was obtained. The photomicrographs were evaluated twice by one calibrated examiner, with a 1-week interval in between. To calibrate the examiner, 20% of the randomly chosen sample was examined and evaluated twice at a weekly interval. The data were submitted to Pearson's correlation test, and the intraexaminer coincidence level was found to be 90%.

The photomicrographs were classified according to a score based on the presence of a smear layer (SL) and the characteristics of the collagen fibril network:

a. 1=no the presence of a smear layer and dentinal tubules open;
b. 2=partial smear layer and dentinal tubules open; and
c. 3=total smear layer and/or no open dentinal tubules (Figure 1a, b).

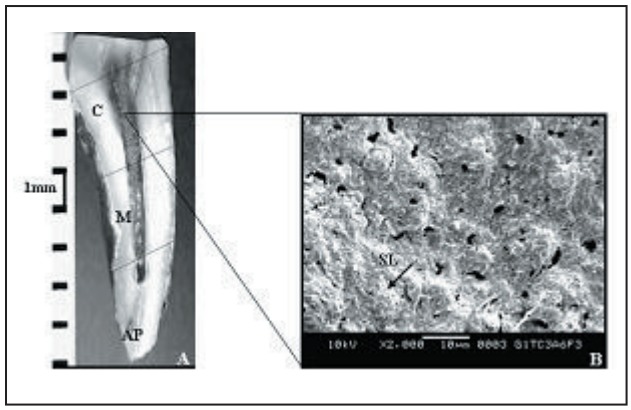

FIGURE 1

Original data from permeability index means (PI) were transformed (sine arch of the root of X/100) before applying the analysis of variance (ANOVA) and Tukey tests, because variance was not homogeneous. A factorial (axb) ANOVA was applied to analyze the interactions between the factors (method of irrigation and type of irrigant). To assess significant differences within these factors, the Tukey test was applied ($P<.05$). The SEM data were submitted to

*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	72.1±21.8aA	39.3±18.4abB	56.5±37.1abA	24.0±17.3aB	42.7±37.4aA	31.6±35.0aA
DHP	78.1±26.1aA	16.6±21.0bB	67.5±22.2aA	35.9±35.8aB	31.4±37.7aA	25.4±36.2aA
CL	10.1±11.4bA	58.8±22.9aB	24.5±34.7bA	31.2±36.2aA	10.4±16.0aA	12.0±12.0aA
S	74.5±32.1aA	66.0±22.1aA	53.4±38.7abA	42.3±25.6aA	22.0±37.3aA	25.0±37.7aA

** MI=manual irrigation; UI=ultrasonic irrigation; PI=permeability index.*
† Similar small letters in column=no significant statistical difference by factorial (axb) 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.

the Kruskal-Wallis test (P<.05). The statistical tests were performed by SAS (Statistical Analysis System, v.8.02, SAS Institute INC, Cary, NC). The PI and SEM data were submitted to the regression analysis with a dummy variable. The SAS software (v. 8.02, SAS Institute Inc, Cary, NC) was used, and the significance limit was set at 5%.

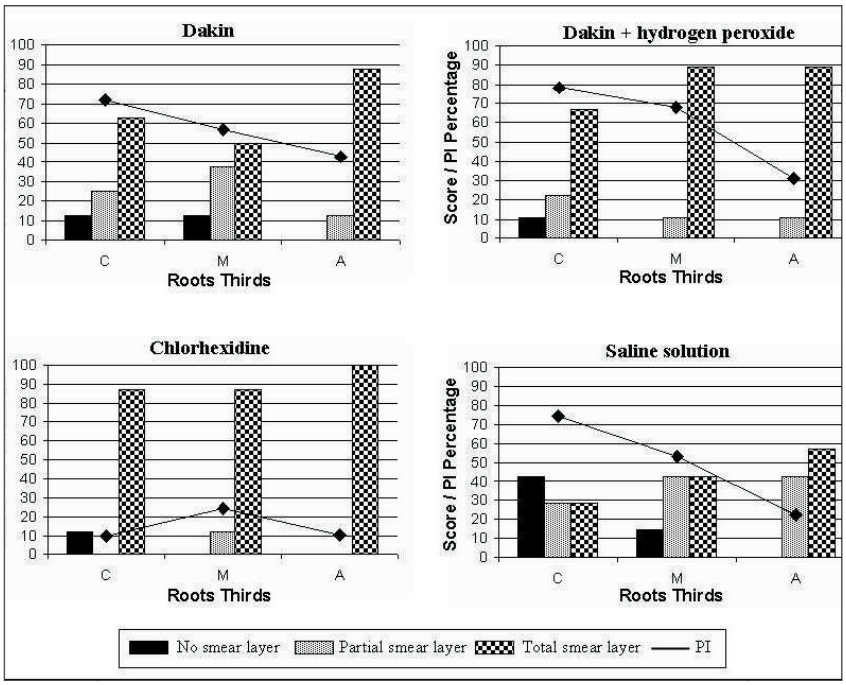

FIGURE 2

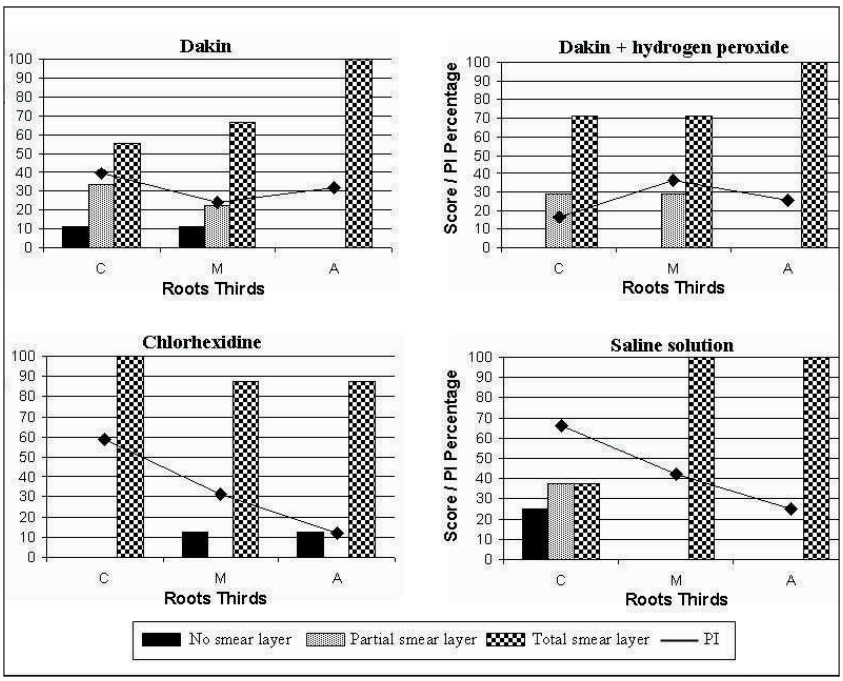

FIGURE 3

RESULTS

PERMEABILITY INDEX

There was a significant association between irrigation methods and different cleansers. The manual irrigation method produced a higher Permeability Index (**PI**) than that observed when the ultrasonic irrigation method was used in the cervical and middle thirds when D, DHP, and S were employed as irrigant solutions (P<.05; Table 2).

MORPHOLOGIC ASPECT OF DENTINAL WALL SURFACE

There was no difference between irrigation methods (P=.3445) and among cleansers (P=.4237) used in this study, or the interaction between methods and cleansers (P=.1941). According to scores (SEM) used to evaluate the smear layer, there was no statistically significant difference among the thirds regarding different cleansers and irrigation methods. Most of the specimens, irrespective of groups, presented a thick smear layer on the root dentin surface (score=4; Figures 2 and 3).

REGRESSION ANALYSES BETWEEN PI AND MORPHOLOGIC ASPECT OF THE DENTINAL WALL SURFACE

Linear regression with the dummy variable test revealed a statistically significant relationship between the decreasing of permeability and the presence of a smear layer on root dentin walls only for manual irrigation in the middle third (P=.0147; Table 3, Figure 4). For the cervical third, a statistically significant regression model (P=.001) was observed in both conditions (IM and UI). The effect of the presence of a smear layer on the root dentin wall regarding the permeability index, however, was not statistically significant (P>.05). For manual irrigation in the apical third, no statistically significant linear regression model was observed between the permeability index and the morphologic aspects of the root dentin (P>.05). Similar results were found concerning ultrasonic irrigation in the middle apical thirds.

DISCUSSION

Root dentin permeability is an important biologic variable that can be measured and used to compare the barrier properties of dentin within teeth or between teeth.[11] Dye penetration into root dentin is a frequently used technique to evaluate the increase in dentinal permeability.[12] A critical variable that would affect permeability is the nature of a dentin surface and whether or not it is coated with a smear layer.[11]

Regarding the permeability index, manual irrigation achieved the highest PI averages when associated with Dakin, Dakin's liquid+ H_2O_2, and saline solution for the cervical

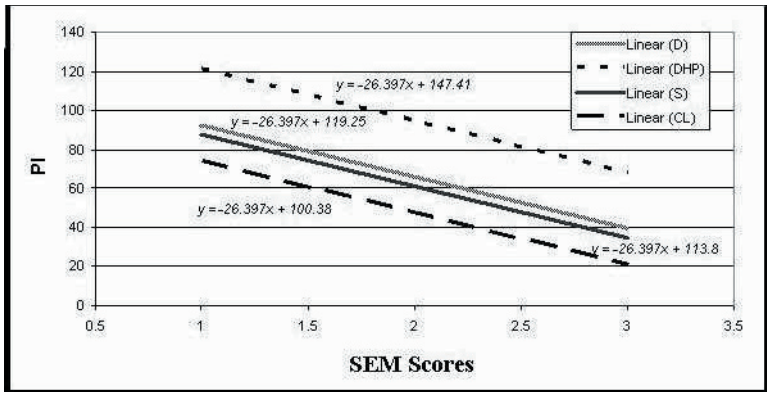

FIGURE 4

Table 3. Parameter Estimated and t Test Considering the Hypothesis That Each Parameter Did Not Statistically Differ From Zero for Manual Irrigation in the Middle Third

Variable	Label	GL	Parameter estimate±(SD)	*t* value	*p*-value
Intercept	Intercept	1	119.25263±26.35925	4.52	.0001
DHP	Dummy DHP	1	28.15882±15.70605	1.79	.0842
CL	Dummy Chlorhexidine 2%	1	-18.87504±16.06714	-1.17	.2503
S	Dummy Saline Solution	1	-5.45138±15.80937	-0.34	.7329
Score	Score	1	-26.39742±10.12765	-2.61	.0147

third. This could be explained by the deproteinizing characteristics of sodium hypochlorite (NaOCl)-based cleansers. Organic tissue dissolution by NaOCl solutions is based on the chloride action on the proteins, forming chloramines, which are water soluble. 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.[13] It is probably the reason for high PI means for all thirds. Concerning NaOCl associated with H_2O_2, it liberates great amounts of nascent oxygen and contributes to pulp tissue remains and dentinal particle removal during the chemical-mechanical preparation.[14] However, the H_2O_2 cream did not reach the end of the canal, and did not act in an efficient way on apical dentin permeability. Another point to consider is that saline solution movement perhaps acted mechanically on the root walls, possibly removing the weakly linked debris bonded to the root structure and allowing the dye to penetrate. This data was similar to that of Dakin and Dakin's liquid+ H_2O_2 action in the cervical and middle area.

In addition, chlorhexidine gel showed the lowest PI averages. This could be explained because the main organic extracellular-dentin matrix molecules are collagen and proteoglycans. Type I collagen forms the fibrillar framework on which other organic molecules and apatite crystals are deposited. Collagen matrix stability might be broken down by host-derived matrix metalloproteinases.[15] Pashely et al[16] found that the use of chlorhexidine inhibited endogenous collagenolytic activity by protease inhibitors, which preserved the structural integrity of the collagen fibrils. Thus, apart from being a commonly known disinfectant, chlorhexidine also acts as a potent matrix metalloproteinases inhibitor.[17] This action may not have allowed high dye permeability.

The decrease in mean PI values (Table 2) observed from cervical to apical thirds could be related to the complex root canal morphology.[18] Apical dentin contains more sclerotic dentin, which is less tubular.[19]

Concerning morphological analyses, the hypothesis that there was a difference between irrigation methods, cleansers, and their interaction was rejected. Regarding the presence or absence of a smear layer, most samples showed a thick smear layer covering the root dentinal canal walls. This could be related to absence of chelating agents. Present study data agree with studies that have found no significant difference in the ability of saline solution, H_2O_2, and NaOCl to remove the smear layer from the surface of prepared root canals.[1,20-25] A clear relationship was observed between decreased permeability and the presence of a smear layer on root dentin walls for manual irrigation in the middle third. This study corroborates the results observed by Fogel and Pashley.[2] The investigators observed that, even with a smear layer and dentinal tubules occluded by smear plugs, there was low fluid filtration. This present study also agrees with Guignes et al,[9] who analyzed the variation of hydraulic conductance measured in situ after 3 endodontic preparations (manual, ultrasonic, and manual with NaOCl and EDTA). They verified that there was an inverse relationship between variations in dentin permeability and the presence of smear layer. Moreover, the smear layer was as significant a factor in influencing radicular permeability as dentin thickness.[9]

This study did not show ultrasonic treatment to be effective for smear layer removal and increasing the dentin permeability index. This could be explained because no file was passively placed in the whole length of the canal. Furthermore, a tip was used in the cervical third, which allowed the cleanser to be activated; producing circulation near the tip and not in the entire root canal. Consequently, this technique did not allow better debridement. This agrees with the studies that failed to demonstrate the superiority of ultrasonics as a primary instrumentation technique.[26-28] In addition, Pécora *et al*,[29] Vansan *et al*,[30] and Karadag *et al*[31] did not find significant differences among manual and ultrasonic techniques in permanent teeth for effectively reducing the smear layer.

Another factor that could have contributed to low PI values is the acoustic streaming phenomenon. This phenomenon is produced when a file is ultrasonically activated. It is one of the mechanisms recommended for superior canal debridement,[32] but it is a directly dependent of canal size.

Moreover, ultrasonically prepared permanent teeth showed cleaner canals than the teeth prepared by hand instrumentation.[7,33] However, as primary teeth canal diameters are smaller, ultrasonic irrigation failed. Also, Seow[34] concluded that the ultrasound treatment was auxiliary to endodontic cleaning during primary tooth therapy, since these teeth have accessory canals which are inaccessible to manual mechanical cleaning.

For the middle third, the data showed an inverse relationship between the variations in dentin permeability and in the presence of a smear layer. This could be explained because the NaOCl-based cleansers and saline solution did not remove the smear layer. In spite of Moorer and Wesselink's[13] findings that NaOCl solution removes collagen fibrils, it is not a decisive fact that the PI was influenced, since the present study showed that the smear layer still remained on the dentinal tubules even when NaOCl was used. Chelating agents have been recommended for chemical and mechanical debridement during root canal therapy for smear layer removal.[5] Instead, if NaOCl was associated with EDTA, the smear layer would be completely removed.[35] In addition, for the cervical third, the data only suggests that the presence of a smear layer could be connected with decreased dye permeability. The apical third showed no correlation between data evaluated. This could be related to primary tooth root canal morphology that has many root canal ramifications, so that it cannot be reached during canal preparation. Another possible explanation for these results is the irregularity of the dentinal wall preparation.[36]

Root canal preparation produces a decrease of dentin thickness while it (root canal preparation) induces an increase in the surface area available for permeation. Simultaneously, tubule diameters could be decreased as the root canal was enlarged.[36] This study agrees with current literature concerning removal of the smear layer of the apical third showing the worst results with different cleansers compared with cervical and middle thirds, although the majority of these studies were conducted in permanent teeth.[35-38]

As mechanical and chemical root canal system cleaning is a fundamental principle of root canal therapy, further in vitro and in vivo studies should be conducted to correlate root dentin permeability and the presence or absence of a smear layer in primary teeth.

CONCLUSION

Within the limits of the present study, it can be concluded that the presence of a smear layer on the root dentin walls was not a limiting factor to dye penetration (permeability index) in all groups, except the middle third of roots that were manually irrigated.

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