

DYE DIFFUSION IN INSTRUMENTED ROOT CANALS IRRIGATED WITH DIFFERENT SOLUTIONS

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ABSTRACT

Success in endodontics depends largely on the activity of the irrigation solutions used during canal cleaning and shaping. Sometimes the irrigation solutions should act deep within the dentin, particularly in cases of chronic infectious lesions, in which it has been found that germs can lodge in the depths of the dental tubules. The aim of this study was to compare the penetration of methylene blue in root dentin in instrumented teeth irrigated with different solutions, considering the apical, middle and cervical thirds. Single-rooted teeth were instrumented, irrigated, flooded with 2% methylene blue, washed and cut into thirds. Dye penetration in the dentin was measured by means of two procedures. Linear measurement: maximum dye penetration was recorded towards the four surfaces of each third. Area measurement: the stained surface of each third was measured on dig-

italized images. On analyzing the data with Friedman and Kruskal Wallis tests, it was found that there was greater penetration of methylene blue in the coronary third in all experimental groups, followed by middle and apical thirds. The mean values for the different groups using the linear method were EDTA 17%: 71.69% - 60.10% - 34.55%. NaOCl 2.5%: 54.04% - 41.79% - 27.53%. CHX 0.2%: 44.28%, 37.58%, 17.80%; and using the area method the mean values were: 17% EDTA: 61.52%, 45.44%, 27.08%. 2.5% NaOCl: 38.15%, 30.77%, 14.60%. 0.2% CHX: 40.95%, 35.46%, 12.27%, for the cervical, middle and coronary thirds respectively. Dye penetration was greatest with 17% EDTA, followed by 2.5% NaOCl and 0.2% CHX, whereas none was observed when distilled water was used.

Key words: endodontics, dentine, root canal irrigation.

DIFUSIÓN DE COLORANTE EN CONDUCTOS RADICULARES INSTRUMENTADOS E IRRIGADOS CON DIFERENTES SOLUCIONES

RESUMEN

El éxito endodóntico depende en gran medida de la acción que ejerzan las soluciones de irrigación durante la limpieza y conformación de los conductos. En algunos casos se hace necesario que las soluciones de irrigación actúen en la profundidad de la dentina, especialmente en casos de lesiones infecciosas crónicas en las que se ha comprobado que los gérmenes pueden alojarse en la profundidad de los túbulos dentinarios. El objetivo del presente trabajo fue comparar la penetración de azul de metileno en la dentina radicular en piezas instrumentadas e irrigadas con diferentes soluciones en los tercios apical, medio y cervical. Dientes unirradiculares fueron instrumentados, irrigados, llenados con azul de metileno 2%, lavados y cortados en tercios. La penetración del colorante en dentina se midió empleando dos procedimientos. Medición lineal: se registró la máxima penetración del colorante en las cuatro caras de cada

tercio. Medición de áreas: se midió la superficie coloreada en cada tercio, en imágenes digitalizadas. Al analizar los datos con el test de Friedman y Kruskal Wallis, se observó que el azul de metileno difundió más en el tercio cervical, en todos grupos experimentales, luego medio y apical. La media en los diferentes grupos emplear el método lineal fueron EDTA 17%: 71,69% - 60,10% - 34,55%. NaOCl 2,5%: 54,04% - 41,79% - 27,53%. CHX 0,2%: 44,28%, 37,58%, 17,80% y por el método de áreas: EDTA 17%: 61,52%, 45,44%, 27,08%. NaOCl 2,5%: 38,15%, 30,77%, 14,60%. CHX 0,2%: 40,95%, 35,46%, 12,27%, en los tercios cervical, medio y apical respectivamente. Hubo mayor penetración del colorante con EDTA 17%, luego NaOCl 2,5% y CHX 0,2% mientras que no se observó al emplear agua destilada.

Palabras clave: endodoncia, dentina, irrigación de los conductos.

INTRODUCTION

How clean a prepared root canal is depends of the instrumentation technique, the solution and irrigation procedures used¹, the volume of irrigant and the use of a technique enabling contact between the irrigation solution and the walls of the root canal². Root canal anatomy is so complex that endodontic instrumentation does not reach all its walls, a situation

which is even more complicated in young patients³. According to Evans et al.⁴ the purpose of chemical debridement is to remove residual tissue and bacterial biofilm, mainly from non-instrumented areas. Mjör⁵ describes dentin permeability as the property of dentin that enables the passage of substances or fluids from the outside into the pulp cavity and in the opposite direction. This property is directly linked to

the histological structure of the tissue. Dentinal tubules make dentin a permeable tissue, and their number and diameter are related to the zone of the canal at the depth of the dentin being considered⁶.

Sometimes it is necessary for the irrigation solutions to act on the dentin mass, especially in cases of chronic infectious lesions, where it has been found that germs can lodge deep in the dentinal tubules^{7,8}. This deep action would favor the antiseptic action of the irrigant itself⁹ and of the topical medication¹¹. The aim of this study was to compare the penetration of methylene blue in root dentin in irrigated and instrumented teeth.

Penetration was measured by means of two methods: linear measurement of maximum dye penetration towards each surface, and measurement of stained areas, considering the apical, middle and cervical thirds.

MATERIALS AND METHODS

55 recently extracted upper canines and central incisors were used, they were stored in saline solution at 9°C. Teeth with cervical caries or extracted due to periodontal disease were discarded because of possible alterations in their dentin structure. The pre-selected teeth were examined under stereomicroscope with a 10x micrometer eyepiece (Olympus SZ40 Tokyo-Japan), and any with signs of resorption, lateral canals or fissures were discarded.

The teeth were divided at random into four groups: three groups of 15 for the experimental solutions, and a group of 10 for the control solution.

Group 1 – 2.5% sodium hypochlorite

Group 2 – 17% EDTA

Group 3 – 0.2% chlorhexidine gluconate

Group 4 – Distilled water (control)

All teeth were prepared according to the following procedure. The mesial surface was marked in order to enable subsequent identification during measuring. The crowns were removed at the proximal cemento-enamel junction using a high-speed, water cooled cylindrical diamond bur N° 837-014 (3098 Sorensen Brazil). Pulp tissue was removed by pulpotomy (Micro Mega Switzerland) and the working length determined by direct viewing with a N°15 K file (Beutelrock United Dental Manufactures TULSA USA) at 1 mm from the apical foramen. The teeth were instrumented using step-back technique with K files, to number 45 or 50 and working back

to N° 80, using 3 mL of the solution corresponding between each instrument to give a total of 30 mL with a disposable 5 ml Luer syringe and a needle 0.5 mm in diameter (Terumo 25G x1 Medical Corporation USA), then the canals were dried with paper points and, to prevent the dye from leaking, the apices were sealed with pink wax (Egeo, Argentina).

A solution of 2% methylene blue was placed in each canal thus prepared, using a 1 mL disposable Luer syringe with a needle 0.5 mm in diameter, preventing the dye from flowing out of the canal during the procedure. The dye was kept “in situ” for 2 minutes, after which the canals were vacuumed, rinsed with 10 ml distilled water and dried with absorbent paper points (Meta Dental Co. Ltd Korea).

The teeth were embedded in autopolymerizing acrylic (Subiton Prothoplast, Buenos Aires, Argentina), with the vestibular surface facing upwards, in an ad-hoc silicone tray. When the acrylic had polymerized, indelible ink was used to mark three lines at which the tooth would be cut: one 3 mm from the apex, another 3 mm from the tooth's anatomical neck and a third line equidistant between the other two.

The teeth were cut into sections with a hand saw, and the sections spray washed with water, dried with force air and classified for subsequent observation.

Dye penetration was measured in the apical, middle and cervical thirds by means of two different procedures.

In the first method, maximum linear penetration towards each surface (vestibular, palatine, mesial and distal) was measured using an Olympus SZ40 (Tokyo, Japan) stereomicroscope with a micrometer eyepiece. Two measurements were recorded on the surface of the section towards each of the tooth surfaces – buccal, lingual, mesial and distal: total dentin thickness and maximum linear dye penetration. Figure 1 provides a diagram showing how the measurements were taken.

These two measurements were used to determine the percentage of dye penetration depth towards each surface for each section (apical, middle and cervical).

The values obtained for the four surfaces on each section, corresponding to apical, middle and cervical, were then averaged.

The statistical analysis was performed using Variance Analysis and Kruskal Wallis test. First the overall results for the different solutions were compared

without considering the different thirds; and then the results for each solution at each of the three levels – apical, middle and coronary, and then the permeability of the three thirds were compared to each other. For the second method, the stereomicroscope was connected to an Olympus video-camera (Tokyo, Japan), to obtain an image of each section. An image analysis system was used (Macintosh, Image 1.45) to measure three areas on each image (Fig. 2) – the surface of the canal (area 1), the surface of the canal plus the surface of stained dentin (area 2) and the surface of the canal plus total dentin surface (including stained and unstained dentin) (area 3). The following procedure was applied to determine the percentage of colored surface for each section:

$$\text{Colored surface} = \text{area 2} - \text{area 1}$$

$$\text{Total surface} = \text{area 3} - \text{area 1}$$

$$\frac{\text{Colored surface} \cdot 100}{\text{Total surface}} = \text{Percentage of colored surface}$$

This percentage was determined for the apical, middle and cervical third of each tooth.

These data were analyzed, comparing the behavior of the solutions, without considering the thirds. In addition, the three thirds were compared to each other with each of the solutions used, by Friedman test. Dye penetration percentages obtained with each solution in each third was compared using Kruskal-Wallis test.

Finally, to determine the correlation between the two methods, the results were compared using the Pearson linear correlation test.

RESULTS

Table 1 shows dye penetration percentages using the linear measurement method.

Table 2 shows dye penetration percentages using the area measurement method.

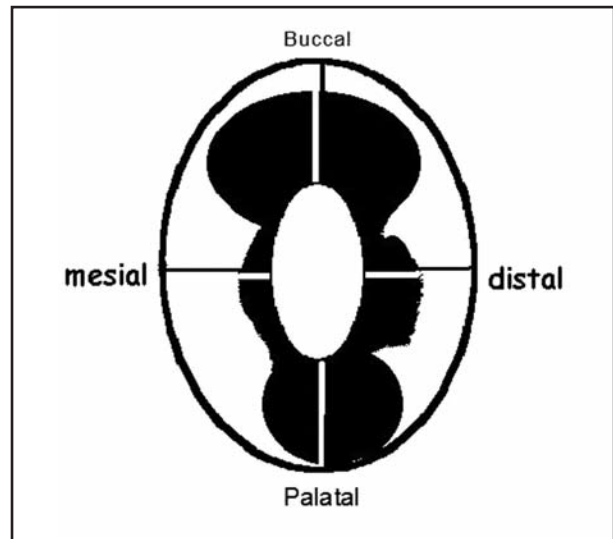


Fig. 1: Graphic measurement lines made in each third. White line (A): maximum dye penetration Black line (B) total dentin thickness.

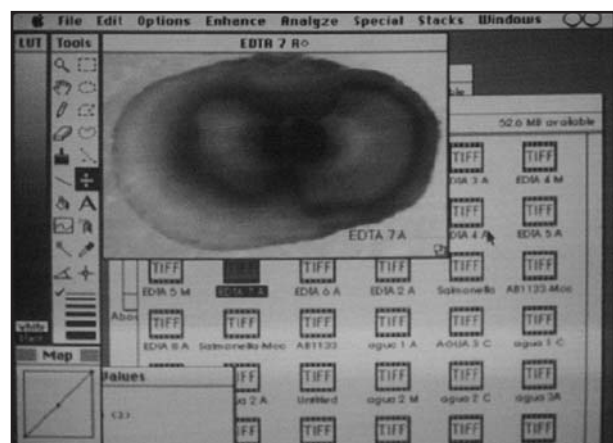


Fig. 2: Digitalized image of each third taken from the PC monitor.

Figure 3 compares all irrigation solutions, showing that mean permeability values change significantly according to the solution when the linear measuring method is used.

Table 1: Dye penetration using the linear measurement method.

	Apical	Middle	Cervical
17% EDTA	34.55 ± 16.51	60.10 ± 17.27	71.69 ± 17.81
2.5% NaOCl	27.53 ± 5.69	41.79 ± 6.46	54.04 ± 12.84
0.2% CHX	17.80 ± 7.68	37.58 ± 8.51	44.28 ± 12.63
Distilled water	5.98 ± 3.83	9.84 ± 3.91	9.92 ± 2.76

Values are expressed in means of percentages ± standard deviation.

Table 2: Dye penetration using the area measurement method.

	<i>Apical</i>	<i>Middle</i>	<i>Cervical</i>
17% EDTA	34.55 ± 16.51	60.10 ± 17.27	71.69 ± 17.81
2.5% NaOCl	27.53 ± 5.69	41.79 ± 6.46	54.04 ± 12.84
0.2% CHX	17.80 ± 7.68	37.58 ± 8.51	44.28 ± 12.63
Distilled water	5.98 ± 3.83	9.84 ± 3.91	9.92 ± 2.76

Values are expressed in means of percentages ± standard deviation.

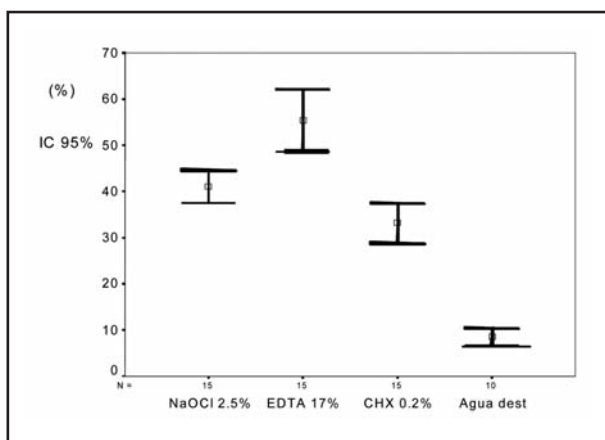


Fig. 3: Linear method. Overall comparison among all irrigation solutions.

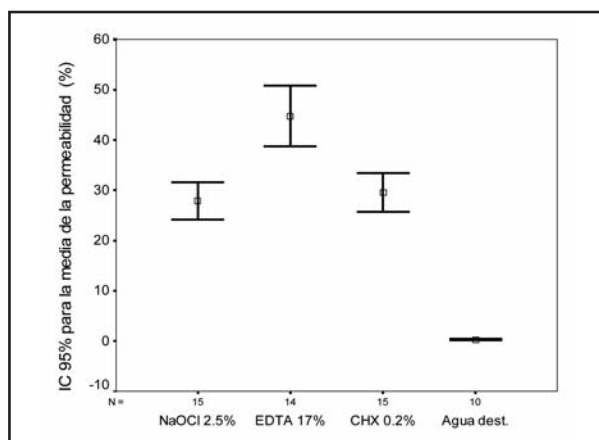


Fig. 4: Area method. Overall comparison among all irrigation solutions.

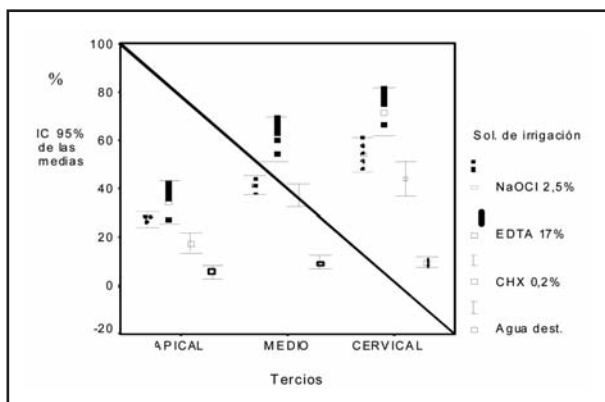


Fig. 5: 95% confidence intervals for mean values of dentin permeability in the different thirds and with the different solutions using the linear method.

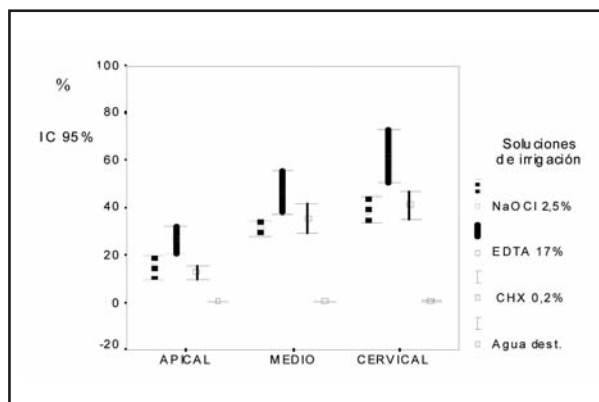


Fig. 6: 95% confidence intervals for mean values of dentin permeability in the different thirds and with the different solutions using the area method.

Figure 4 compares all irrigation solutions, showing that mean permeability values change significantly according to the solution when the area measurement method is used.

Figure 5 shows the mean values and the respective confidence intervals for theoretical mean values for all irrigation solutions in all three thirds, using the linear measurement method.

Figure 6 shows the mean values and the respective confidence intervals for theoretical mean values for all irrigation solutions in all three thirds using the area measurement method.

DISCUSSION

Dentin permeability has been studied from exposed tissue towards the pulp cavity¹², and also in the

opposite direction, i.e. from the canal towards the root surface¹³. The latter is clinically important when deep action of the intra-canal medication is sought, in cases of root resorption or periapical infections.

Several factors can affect dentin permeability. Those related to the tissue itself, such as thickness, diffusion area or extension of exposed dentin, as well as size and number of dentinal tubules, must be related to the characteristics of the substance that will diffuse^{14,15}. Studies of the permeability of dentin should also consider which third of the canal is being analyzed, since each third has certain characteristics regarding number and diameter of dentinal tubules^{16,17}.

This study found differences in the diffusion of the dye in the different thirds. The cervical third was the most permeable, followed by the middle third and finally the apical third, in agreement with the results of Zuolo et al.¹⁸ using a histochemical method, and those of Paqué et al.¹⁹ who used dye diffusion. This could be explained by the statements of Hampson and Atkinson²⁰, Carrigan et al.¹⁷ and Baumgartner et al.²¹, that the histological structure of dentin is less tubular as it approaches the apical zone. Also regarding the zone of the canal being analyzed, Robazza et al.²² used methylene blue to analyze the variations in root dentin permeability in human teeth extracted after instrumentation and irrigation, showing that the apical zone of the canal was the least permeable. Moura et al.²³ arrived at the same conclusion using methylene blue in a study performed *in vitro* and *in vivo*. Other studies^{23,27} also say that the apical third is the least permeable, probably due to tubular sclerosis which occurs in the apical region in adults and progresses with age²⁸. Although apical sclerosis would be an impediment for diffusion of antibacterial substances placed in the canal, fortunately it is also a barrier that hinders the penetration of germs²⁹.

It is important to note that if a medication placed in the root canal does not diffuse through the dentin, it may be due, as have we have pointed out, to dentinal sclerosis as well as to the low diffusion capacity of the substance being tested³⁰. The methylene blue solution used in this study has been successful for this type of research performed by several other authors^{12,25,26,32,33}.

This study proposes two methods for evaluating dye penetration: linear measurement and area measure-

ment. A comparison shows that they both have similar ratios among the tested solutions, both in the global analysis with 0.89 Pearson linear correlation coefficient and at different section levels. The advantage of the linear method is that it evaluates the depth of dye penetration, which is worthwhile knowing in cases where the medication should approach the root surface. Regarding the method used for measuring stained areas, we found differences compared to the procedure used by Pécora³³ and Zuolo et al.¹⁸. In their studies, as in ours, teeth were cut horizontally into thirds. Pécora³³ and Zuolo et al.¹⁸ quantified their results by measuring the areas, taking the distance from the edge of the canal to the point of greatest dye penetration as the radius, and then using a formula to calculate a circular area of this radius, which was established as stained. As the authors themselves point out, the problem with this procedure is that it produces a surface area based on a linear measurement of maximum dye penetration, which could lead to errors, since the stained areas determined are always greater than the real areas. In our study, independent measurements were taken to determine the amount of stained dentin: on the one hand, the linear penetration towards the four root surfaces, which were averaged, and on the other, the real stained area using digitalized images.

In our study, we noted a particular characteristic regarding dye penetration, which had already been noted by Pécora³³ and Paqué et al.¹⁹ as a “butterfly”-shaped image. We noted a dye polar distribution, with the dentin more stained towards the vestibular and lingual surfaces, concluding that permeability is greater in those directions, a feature that was even more noticeable in teeth with oval or ribbon-shaped roots.

The area measuring method showed that the greatest dye penetration occurred using the 17% EDTA solution, followed by 0.2%, chlorhexidine gluconate, and then very closely by 2.5% sodium hypochlorite and finally distilled water (control).

The permeabilizing activity of the EDTA solution found in our study does not match the finding of Marshall et al.²⁴ that 17% EDTA reduced dentin permeability. This discrepancy is probably due to differences in methodology, as Marshall et al.²⁴ used the solution for five minutes after preparing the canals. Similarly, Fraser and Laws³⁴ found that different commercial EDTA preparations reduced dye

penetration, which might be attributed to the procedure employed, since they worked with non-instrumented canals. Our results also differ from those of Galvan et al.³⁰, who found that there was lower penetration of tritiated water in root dentin after applying EDTA. This may be due to the fact that the solution was applied for only one minute and did not exert enough chelating action to dissolve the calcium crystals, which might block the dentinal tubules.

However, Raldi and Lage-Marques³⁵ used an area measurement method similar to ours, and obtained similar values for dye penetration on using 17% EDTA. The result of our study regarding the 17% EDTA solution may be explained by the findings of Mc Comb and Smith³⁶, who by means of scanning electron microscope observed that the walls of the canal remained free of smear layer and superficial debris after placing a commercial EDTA-based preparation (REDTA) inside the canal for 24 hours. Mac Comb et al.³⁷ also obtained the best results using EDTA as an irrigant during canal preparation, as they found little debris and no smear layer.

Baumgartner and Mader³⁸ found that a 17% EDTA solution was more effective in eliminating the smear layer than 2.5% sodium hypochlorite solutions, and noted that when EDTA was used as an irrigant, there was a layer of fibrous texture and exposed dentinal tubules, which probably enabled the penetration of the dye used in this study. Our observations match the findings of Pécora³³ who noted in histochemical studies that EDTA solutions and halogen solutions increased dentin permeability.

In the group irrigated with 2.5% sodium hypochlorite, average dye penetration obtained using the area measurement method was 14.60% in the apical third, 30.77% in the middle third and 8.15% in the cervical third. These low permeability percentages could be explained by the probable formation of sodium chloride crystals reported by Gutierrez et al.⁴⁰. On the other hand, Barbosa et al.⁴⁰ found it was effective in increasing hydraulic conduction. These differences are probably due to the fact that the latter used discs of cervical dentin, which is the most permeable, and soaked them in a concentrated sodium hypochlorite solution (5%) for 1 hour.

Studies by Baumgartner et al.²¹ using a 5.25% sodium hypochlorite solution, and by Garberoglio and Becce⁴¹ using a 5% sodium hypochlorite solution, both found, through observations with a scanning

electron microscope, that the smear layer was not removed. In our study, dye penetration is probably explained by the fact that the solution alters the inter-tubular dentin, making it permeable to dyes⁴². We found that 0.2% chlorhexidine gluconate solution has less permeabilizing activity than 17% EDTA, but that it is similar to 2.5% sodium hypochlorite, except in the middle third, in which it did not differ significantly from 17% EDTA using the area method, although the values were lower. This differs from the results of Hampson and Atkinson⁴³, who used radioactive sulfur and found that chlorhexidine increased dentin permeability. In contrast, Schaller et al.⁴⁴ noted that 0.1% CHX did not change dentin permeability.

We have not found many papers with which to compare our results regarding the permeabilizing activity of chlorhexidine solution, due to the fact that the many of them focus only on its substantivity and activity as an antibacterial agent. In recent years, the cleansing action of chlorhexidine has been analyzed using scanning electron microscope^{45,46}, and it was found to be inefficient. It could be inferred from these results that as it does not eliminate debris from the canal walls and lacks solvent action, as noted by Ferraz et al.⁴⁶, it does not appear to have good permeabilizing activity.

Further research is therefore needed regarding the cleansing and permeabilizing activity of chlorhexidine gluconate solutions in order to clarify these properties.

The natural permeability of dentin may also be altered by the presence of a smear layer^{36,37,47,48}. From the fact that the dye diffused after using our experimental solutions, it may be inferred that either they altered the debris layer sufficiently or that the smear layer does not completely prevent dye diffusion.

Dentin permeability is a property that enables substances to diffuse through its mass, allowing the solutions used in endodontic treatment to act in depth, beyond the surface of the canal.

From the analysis of these results it may be inferred that the dissolving action of 17% EDTA on inorganic matter breaks down the matrix of the smear layer without removing it, thus facilitating the penetration of solutions, whereas sodium hypochlorite would alter it by dissolving its organic components. The action of chlorhexidine gluconate requires further study to clarify its behavior. From the analysis of the behavior of irrigant solutions, it may be

deduced that it is not necessary to remove the smear layer entirely in order to enable the passage of a dye into the depth of the dentine, but only to alter it.

The linear method that we used for weighting dye penetration may be considered valid for the study of the diffusion of substances in dentin, considering that the correlation index was acceptable when

compared to the area measurement method, and in addition, it provides information on penetration depth of the dye towards the surfaces studied. It is important to note that only a stereomicroscope is needed for these measurements, avoiding the need for more complex equipment and procedures such as those used in this study for measuring areas.

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