

Universidade Estadual de Campinas  
Faculdade de Odontologia de Piracicaba

**Fernanda Miori Pascon**

Estudo da Permeabilidade Radicular de Dentes Decíduos.  
Avaliação de Substâncias Irrigadoras e Métodos de  
Irrigação.

*Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para a obtenção do título de Mestre em Odontologia – Área de Odontopediatria.*

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Dedico esse trabalho a:

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Um dos principais objetivos do tratamento endodôntico é o debridamento do sistema de canais radiculares através da remoção dos restos pulpare, da *smear layer* e *smear plugs*, para eliminar a contaminação bacteriana e subprodutos da mesma, os quais podem se tornar reservatórios de irritantes no canal tratado. Além disso, as substâncias irrigadoras e os métodos de irrigação utilizados durante o preparo químico-mecânico, podem também contribuir para a remoção da *smear layer* e conseqüentemente alterar a permeabilidade dentinária. Dessa forma, os objetivos desta Dissertação foram: (1) discutir através da revisão da literatura, a eficácia de substâncias irrigadoras, bem como a influência de dois métodos de irrigação (irrigação manual ou ativação ultrasônica) utilizados durante o preparo químico-mecânico de dentes decíduos e permanentes, considerando a permeabilidade da dentina radicular; (2) correlacionar o índice de permeabilidade da dentina radicular de dentes decíduos sob a influência de substâncias irrigadoras associadas ou não a auxiliares de instrumentação (líquido de Dakin, líquido de Dakin associado ao peróxido de hidrogênio, solução salina associada à clorexidina gel e solução salina) e diferentes métodos de irrigação (irrigação manual ou ativação ultrasônica) aos seus aspectos morfológicos (presença ou ausência de *smear layer*). Baseando-se na revisão de literatura pôde-se concluir que as substâncias irrigadoras e associações utilizadas durante o tratamento endodôntico promoveram aumento da permeabilidade dentinária radicular de dentes permanentes e que não houve diferenças quanto à permeabilidade dentinária, quando comparada a irrigação realizada manualmente ou através de vibração ultrasônica. Não há estudos relacionados à permeabilidade radicular de dentes decíduos, quando estes são submetidos a substâncias irrigadoras e diferentes métodos de irrigação. A técnica de irrigação manual foi considerada mais efetiva do que a técnica de irrigação através de sistema de vibração ultrasônica, quanto à permeabilidade dentinária radicular de dentes decíduos. A presença da *smear layer* nas paredes radiculares foi um fator limitante à penetração do corante (índice de permeabilidade) para o terço médio quando dentes decíduos foram irrigados manualmente. O líquido de Dakin apresentou os maiores valores de índice de permeabilidade, nos três

terços radiculares, sugerindo que essa solução irrigadora seja indicada para o tratamento endodôntico de dentes decíduos.

**Palavras-chave:** dentes decíduos, tratamento endodôntico, permeabilidade dentinária radicular, substâncias irrigadoras, métodos de irrigação, *smear layer*.

## ***ABSTRACT***

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One of the main goals of root canal treatment is the cleaning of the entire root canal system, through removal of pulpal debris, smear layer and smear plugs in order to avoid harbor bacteria or bacterial by-products, providing a reservoir of potential irritants in treated root canal. In addition, the cleansers and irrigation methods used in the endodontic treatment, can also contribute with the smear layer removal and with permeability alterations. Thus, the aims of this Thesis was: (1) to discuss the efficacy of some cleansers, as well as the influence of two irrigation systems used during endodontic preparation of primary and permanent teeth (manual or ultrasonic vibration) with regard to root dentin permeability, based on a literature reviewing; (2) to correlate the dye permeability to morphological aspect (presence or absence of smear layer) of the primary root dentin wall, using scanning electron microscopy (SEM), regarding the endodontic preparation and irrigation methods. Based on literature review (1) it can be concluded that among the solutions used for permanent teeth, all solutions and associations studied increased the dentin permeability. There was no difference in the dentin permeability when comparing manual and ultrasonic irrigation. There are not enough papers focusing primary teeth on this subject to definitely establish the patterns of increased dentin permeability in tooth root canal treatment regarding the irrigating solutions and irrigation systems. The results of the second study showed that manual irrigation technique was better than ultrasonic one and that Dakin's liquid, Dakin's liquid associated with hydrogen peroxide, and saline solution showed the highest permeability index averages values. Regarding the correlation study, it could be concluded that the smear layer presence on the root dentin walls was a limiting factor to dye penetration (Permeability Index), in middle third for manual irrigation.

**Key-words:** primary teeth, endodontic treatment, dentin permeability, cleansers, irrigation methods, smear layer.

## INTRODUÇÃO GERAL

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O alto índice de cárie decorrente dos maus hábitos alimentares, associado à higiene bucal deficiente tem grande influência na perda precoce dos dentes decíduos. A manutenção desses dentes no arco e o restabelecimento da condição de saúde dos tecidos afetados por grandes lesões de cárie e conseqüente infecção pulpar são os objetivos principais do tratamento de dentes decíduos. Além disso, este deve ser realizado visando o não comprometimento do desenvolvimento dos dentes sucessores permanentes adjacentes (Camp, 1994).

Estudos *in vitro* e *in situ* têm demonstrado que a formação e a progressão de cárie são mais rápidas em dentes decíduos do que em permanentes (Peterson & Derand, 1981). Evidências epidemiológicas afirmam que a cárie dentária progride do esmalte para a dentina em um período curto de tempo, de 3,4 semanas em média (Ismail & Sohn, 1999). Somado a isso, o *Third National Health and Nutrition Examination Survey* (1988-1994) mostrou que uma em cada cinco crianças entre 2 e 5 anos possuía dentes cariados e que 71% destes dentes não haviam sido restaurados (Ismail *et al.*, 2003).

As lesões de cárie presentes na dentina de dentes decíduos costumam atingir rapidamente a câmara pulpar, levando à necessidade da realização de tratamento endodôntico. A contaminação do tecido pulpar por bactérias e toxinas derivadas do metabolismo destas, exige que o tratamento endodôntico promova, não apenas a remoção de restos pulpares necrosados, como também a desinfecção dos canais radiculares e dos túbulos dentinários. Neste contexto, as substâncias irrigadoras e o método de irrigação são de fundamental importância para o sucesso do tratamento endodôntico. Além de promover a limpeza dos canais radiculares e possuir propriedade antimicrobiana, as substâncias irrigadoras têm por função facilitar a instrumentação e manter os resíduos em suspensão (Marshall *et al.*, 1960; Stewart *et al.*, 1961; Cohen *et al.*, 1970; Rome *et al.*, 1985; Pécora *et al.*, 1987; Vahdaty *et al.*, 1993; Primo 2000; Hata *et al.*, 2001; Ari *et al.*, 2004).

Com base nessa premissa, a permeabilidade dentinária mantém estreitas relações com o preparo químico-mecânico, podendo tornar-se mais evidente, dependendo da melhor limpeza do sistema de canais radiculares. Além disso, a permeabilidade poderia estar

relacionada com a descontaminação dos túbulos dentinários e subsequente penetração das pastas obturadoras. Estas pastas, penetrando nos túbulos dentinários limitariam a contaminação bacteriana e impediriam a reinfecção dos sistemas de canais radiculares. Nesse sentido, um dos principais aspectos que devem ser pesquisados no tratamento endodôntico de dentes decíduos é a permeabilidade dentinária radicular.

As substâncias irrigadoras e métodos de irrigação, utilizados durante o preparo químico-mecânico, podem alterar química (composição) e fisicamente (espessura) a estrutura dentinária (Fogel & Pashley, 1990). Outro aspecto importante da terapia endodôntica refere-se à formação da *smear layer* durante a instrumentação. O uso de instrumentos rotatórios e limas endodônticas levam à formação de uma camada microscópica de resíduos provenientes da dentina instrumentada. A *smear layer* observada em Microscopia Eletrônica de Varredura em dentes permanentes apresenta-se como uma camada uniforme, densa, de estrutura amorfa que oblitera completamente a entrada dos túbulos dentinários podendo reduzir drasticamente a permeabilidade dentinária (Pashley *et al.*, 1981). Portanto, a eliminação completa da *smear layer* permitiria a remoção mais efetiva de irritantes dos canais radiculares, além de promover aumento da permeabilidade dentinária e da superfície de contato entre a dentina e a pasta obturadora, o que contribuiria sobremaneira para o sucesso da terapia endodôntica.

As técnicas de condutância hidráulica e penetração de corante na dentina radicular são utilizadas para avaliação das alterações na permeabilidade dentinária (Marshall *et al.*, 1960; Stewart *et al.*, 1969; Cohen *et al.*, 1970; Fraser & Laws, 1976; Moura & Paiva, 1989; Fogel & Pashley, 1990; Tao *et al.*, 1991; Guignes *et al.*, 1996). Diante da controvérsia se a presença da *smear layer* sobre as paredes dentinárias radiculares afetaria a permeabilidade, Fogel & Pashley (1990) verificaram que a delgada camada de *smear layer* presente na parede dentinária radicular permitiu a penetração dos fluídos para o interior dos canalículos dentinários em baixas proporções. E ainda, Tao *et al.*, (1991) verificaram que a preparação endodôntica convencional reduziu a espessura de dentina e criou *smear layer*, a qual não modificou a permeabilidade dentinária em toda extensão radicular. Entretanto, Scelza *et al.* (2003), afirmaram que o preparo endodôntico formou uma *smear layer*, que afetou diretamente a permeabilidade dentinária radicular.



Estes estudos foram realizados em dentes permanentes, entretanto, é notório que dentes decíduos e permanentes possuem características próprias, sendo que a diferença básica entre eles é o ciclo de vitalidade. Enquanto nos dentes permanentes os odontoblastos apresentam uma fase ativa de 700 dias, nos dentes decíduos esta fase é de apenas 350 dias, possivelmente gerando uma dentina, na primeira dentição, menos densa, com túbulos mais irregulares e apresentando metade da espessura observada nos dentes permanentes (Schour, 1960).

Além disso, a topografia dos sistemas de canais dos dentes decíduos é complexa como demonstrado por Benfatti (1966), que observou uma proporção significativamente maior de molares decíduos com ramificações colaterais, intercomunicantes, bifurcações ou ramificações apicais. Dessa forma, observa-se a estreita relação entre a morfologia dos canais radiculares e o sucesso do tratamento endodôntico. Outro aspecto a ser considerado é a estrutura dentinária, a qual tem papel preponderante no sucesso da terapia endodôntica, em relação ao efeito do preparo químico-mecânico no saneamento das paredes dentinárias.

Devido às diferenças existentes entre dentes decíduos e permanentes, as substâncias irrigadoras e os métodos de irrigação atuam na dentina dos dentes decíduos de maneira diferente em relação à remoção da *smear layer* e a alterações na permeabilidade dentinária radicular durante o preparo químico-mecânico. Além disso, tendo em vista a escassa literatura concernente às técnicas de tratamento endodôntico de dentes decíduos, e ainda, baseados na verificação de Hobson (1970) de que  $\frac{3}{4}$  da dentina radicular de dentes decíduos necrosados encontram-se infectadas, observa-se a necessidade da determinação da técnica de instrumentação e irrigação adequadas aos canais radiculares, visando a eliminação da infecção, o aumento da permeabilidade dentinária e a manutenção da assepsia dos canais realizada através das propriedades antimicrobianas e ainda pela obturação com material compatível que acompanhe o processo de reabsorção fisiológica dos dentes decíduos.

## ***PROPOSIÇÃO GERAL***

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Os objetivos do presente estudo, foram:

1. Discutir, através da revisão da literatura, a eficácia de substâncias irrigadoras, bem como a influência de dois métodos de irrigação (irrigação manual ou ativação ultrasônica) utilizados durante o preparo químico-mecânico de dentes decíduos e permanentes, considerando a permeabilidade da dentina radicular;
2. Correlacionar o índice de permeabilidade da dentina radicular de dentes decíduos sob a influência de substâncias irrigadoras associadas ou não a auxiliares de instrumentação (líquido de Dakin, líquido de Dakin associado ao peróxido de hidrogênio, solução salina associada a clorexidina gel e solução salina) e diferentes métodos de irrigação (irrigação manual ou ativação ultrasônica) aos seus aspectos morfológicos (presença ou ausência de *smear layer*).

Para alcançar esses objetivos, esta Dissertação\* foi dividida em dois capítulos, correspondentes aos objetivos descritos.

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**INFLUENCE OF CLEANSERS AND IRRIGATION METHODS ON PRIMARY AND PERMANENT ROOT DENTIN PERMEABILITY: A LITERATURE REVIEW.\***

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## **Abstract**

This paper review is key issue to known that the adequate endodontic techniques accomplished on primary and permanent teeth regarding the root dentin permeability. Therefore, it discussed the efficacy of some cleansers and the influence of two irrigation systems used during endodontic preparation of primary and permanent teeth (manual or ultrasonic activation). The literature was searched for original papers relating cleansers characteristics, cleansers effect of root dentin permeability and the influence of manual irrigation or ultrasonic activation systems at primary and permanent endodontic therapy. The articles were selected using Bireme and Medline. Manual tracing of references cited in key papers otherwise not elicited. It can be concluded that regarding cleansers and irrigation systems, there are not enough papers focusing primary teeth to establish patterns of change in the root dentin permeability. However, among the solutions used to treat permanent teeth, all solutions and associations studied increased the root dentin permeability. For permanent teeth, there was no difference in the root dentin permeability when comparing manual and ultrasonic preparation.

**Key-Words:** Dentin Permeability, Irrigating Solutions, Irrigation Systems, Primary Teeth, Permanent Teeth.

## **Introduction**

The high rate of tooth-decay derives from bad eating habits. This concept in association with deficient oral hygiene has had great influence on the premature loss of primary teeth. The aim of treatment of primary teeth with large decay lesions enveloping pulp is not only made in order to maintain the teeth in the arch, but also reestablish the healthy condition of the tissues affected by the pulp infection. Therefore, it does not to compromise the development of the permanent successor teeth<sup>1</sup>.

Considering the high level of caries prevalence in primary teeth, their fast development, and consequently the pulp damage caused by the pulpar tissue contamination by bacteria and their derived toxins, it demands an endodontic treatment. This therapy promotes the removal of necrotic pulpal tissues remains, and the disinfection of the root canals and dentin tubules. In this context, the cleansers are very important to the success of the endodontic treatment. Besides promoting the cleansing of the root canals and having antimicrobial properties, the cleansers allow instrumentation and keep the residues in suspension, minimizing the extrusion of pulpal and dentine remains through the apical foramen<sup>2</sup>.

The choice of a cleanser in the pulpal therapy of primary teeth should take into account the differences among the dentin substrata, and the requirement of not being irritating to the periapical tissues. It is important to avoid harming the germ of the permanent successor tooth due to the physiologic root resorption leads the apical extrusion of the cleanser<sup>2</sup>.

Primary and permanent teeth presents its own characteristics. The basic differences between them are concerning their function in life. Primary teeth has less mineralized tissue, short dimensions, and behave different from their successor permanent when they receive similar endodontic therapy<sup>3</sup>. In addition, the cleansers would act in the dentin of the primary teeth in a different way in relation to the dentin permeability, bactericidal action and irritating potential during the endodontic instrumentation.

The use of rotating instruments and endodontic files leads to the formation of a microscopic layer of residues coming from the scoured dentin during the endodontic treatment. The smear layer observed under a Scanning Electron Microscopy (SEM) in permanent teeth comes as a uniform, dense layer of an amorphous structure that completely obliterates the entrance to the dentin tubules and drastically reduces the dentin permeability<sup>4</sup>.

Under clinical conditions, especially during the treatment of infected teeth, viable bacteria and their products can be incorporated into the smear layer, forming a deposit of irritants<sup>5</sup>. Therefore, its complete elimination would allow the most effective removal of irritants from root canals, besides promoting an increase in the dentin permeability and the contact surface between the dentin and the filling paste. This contributes greatly to the success of the endodontic therapy.

Hobson<sup>6</sup> verified that  $\frac{3}{4}$  of the root dentin of necrotic primary teeth are infected. This fact confirms the importance of instrumentation and irrigation endodontic to eliminate the root canal infection, to increase the root dentin permeability and to maintain the asepsis of the canals.

The aims of this review article was to discuss the efficacy of some cleansers, as well as the influence of two irrigation systems used during endodontic preparation of primary and permanent teeth (manual or ultrasonic vibration) with regard to root dentin permeability.

### **Reviewing Methodology**

The authors searched for papers using Bireme and Medline from 1960 to 2005. The search was supplemented by manual searching of reference lists from each relevant paper identified.

The main search terms were “root dentin permeability”, “cleansers”, “irrigation solutions”, “endodontic treatment”, and “irrigation methods”. A total of 203 records were originally identified filters, and then used to allow only for subject papers to be connected, which resulting in 65 articles. These were printed as abstracts or full-text articles if the abstract was missing. Only original papers were considered. Interim reports, abstracts, letters, reviews, and chapters in textbooks were discarded. Articles in Swedish, Danish, Norwegian, Japanese, German, and French were not accepted. Although

65 papers had been selected, only 46 studies were included from the appraisal. The main reasons for exclusion of 19 articles were: papers of reviews and papers that evaluated root dentin permeability associated with laser applications. Out of the 46 papers that were critically assessed, 14 studies were identified from the search and were pointed out due to they related dentin permeability, cleansers and irrigation methods (Table 1).

### **Efficacy of Cleansers**

In root canal treatment, cleaning is the removal all contents of root canal system before and during shaping. Irrigation is presently the best method for lubrication, destruction of microbes, the removal of tissue remnants, and dentin debris during instrumentation. The simple act of irrigation allows the flushes away loose, necrotic, contaminated materials before that they are inadvertently pushed deeper into the canal and apical tissues, compromising the periapical tissue and permanent bud. In this context, the use of cleansers in the irrigation process is very important<sup>7</sup>.

Many researchers have studied the effect of several cleansers on the permeability of the dentine using methods that involve bacteria or radioisotopes, with different methodologies. Those cleansers have been used with the objective of eliminating pulpal remains and residues. In addition, they increase the dentin permeability (removing the smear layer), facilitate the instrumentation and promote the cleaning and disinfection of the root canals<sup>8-16</sup>. In addition, they should be soluble in water and biocompatible to the periapical tissues<sup>17</sup>.

In view of the fact that there is not a single drug that unites all those properties mentioned, a variety of cleansers and their associations have been used such as Sodium Hypochlorite, Urea Peroxide, Hydrogen Peroxide, Ethylenediamine Tetra Acetic Acid, Organic Acids, and Chlorhexidine Gluconate (Figure 1).

#### *Sodium Hypochlorite*

Sodium Hypochlorite (NaOCl) have been used separately or associated with other medicines. NaOCl is a weak alkaline/base that acts on the albumin (remains of pulpal tissue, foods and microorganisms), denaturing them and turning them soluble in water. Like soap, it facilitates the removal of debris from the root canals and, in spite of being a necrosis agent (to act on organic matter) it is little poisonous or irritating to the live tissues<sup>18</sup>. Dakin's liquid (0.5% NaOCl neutralized with boric acid) is the most commonly used solution to irrigate primary teeth, because it is less irritating to the periapical tissue. The NaOCl alkali contacting with organic products in decomposition liberates chlorine and nascent oxygen that promote bactericidal action<sup>18</sup>.

Marshall et al.<sup>9</sup> observed a small increase of the dentin permeability to radioisotopes when the root canals were irrigated with 5.25% NaOCl. However, the association of this solution with 3% hydrogen peroxide solution significantly increased the dentin permeability. Fogel & Pashley<sup>19</sup> found that the use of endodontic files created smear layers, which produced modest reductions in the permeability of inner and outer root dentin. Sodium hypochlorite or saline application did not affect the hydraulic conductance of such smear layers.

#### Urea Peroxide

Another widely used solution to aid instrumentation is Urea Peroxide (Endo-PTC or Gly-Oxide). The peroxides are oxidizing agents that react chemically, liberating great amounts of nascent oxygen that explains their bactericidal action. The effervescence, due to the liberation of oxygen, contributes to the removal of pulp tissue remains and dentin particles during the chemical-mechanic preparation. In Brazil, the trade name of Urea Peroxide is Endo-PTC (10% Urea Peroxide, 15% Tween 80 and 75% Carbowax). International literature shows Urea Peroxide as Gly-Oxide. It is a base of anhydrous glycerol, without any added detergent. Moura & Paiva<sup>20</sup> used Endo-PTC as an auxiliary chemical substance and also considered time and type instruments. They found less dye penetration when an increase of instrumentation was placed, mainly observed in the apical area.



The Urea Peroxide has several desirable characteristics for the irrigation of root canals in primary teeth. It presents detergent and haemostatic properties, besides not being irritating to the periapical tissues and non allergenic. Stewart et al.<sup>21</sup> and Rome et al.<sup>12</sup> observed that the bactericidal activity of the Urea Peroxide (Gly-Oxide) was superior to 3% Hydrogen Peroxide in the preparation of infected root canals. The association of Urea Peroxide/NaOCl maintains the previously described properties<sup>10</sup>. According to Rome et al.<sup>12</sup>, the use of Urea Peroxide is the first choice cleanser in small curved canals. Its properties of lubrication without demineralization the dentin walls<sup>10</sup> avoid the risks of root perforation, common in primary teeth.

The association of Urea Peroxide with NaOCl promotes significant more increase in the dentin permeability index to dye and drugs<sup>21</sup> than when used separately<sup>9</sup>. In spite of promoting increase in the dentin permeability, the association of Urea Peroxide/NaOCl showed less effectiveness in removing the smear layer<sup>12</sup>. In contrast, it is known that the smear layer reduces dentin permeability, and prevents the penetration of root canal disinfectants into the deep area of the root canal wall<sup>22,23</sup>.

#### Hydrogen Peroxide

Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) is an oxidizing agent that acts similarly to Urea Peroxide releasing nascent oxygen and producing effervescence. The H<sub>2</sub>O<sub>2</sub>/NaOCl association produces increased dentin permeability in smaller degree than the association Urea Peroxide/NaOCl<sup>11</sup>. In addition, Urea Peroxide (Gly-Oxide) presented smaller bactericidal activity than H<sub>2</sub>O<sub>2</sub> when used in the irrigation of root canals infected in permanent teeth<sup>10</sup>.

#### Ethylenediamine Tetra Acetic Acid

Ethylenediamine Tetra Acetic Acid (EDTA) is a chelating substance that has been also used. It is capable of removing calcium ions of the dentin, giving rise to demineralization and as a consequence, increasing the dentin permeability of the root canals instrumented and irrigated with it. EDTA is used in concentrations from 10 to 17% and in association with other drugs<sup>21</sup>. The efficiency of chelating agents generally depends on many factors, such as the root canal length, penetration depth

of the material, hardness of the dentin, application time, the pH, and the concentration. Thus, the purpose of Serper & Calt's study<sup>24</sup> was to compare the effects of EDTA (pH and concentration) on root dentin demineralization. The author's results suggest that during prolonged cleaning and shaping of root canals lower concentrations of EDTA (10%) should be preferred at neutral pH. It reduces erosive effects of EDTA solutions. In addition, Nakashima & Terata<sup>25</sup> observed that the permeability of root canal disinfectants increased to similar degrees in the 3% and the 15% EDTA groups. In comparing dentin properties they propose that 3% EDTA is more useful for clinical applications. Zuolo et al.<sup>26</sup> found that the most effective combination to increase root dentin permeability was EDTA associated with Cetavlon (EDTAC).

However, Tao et al.<sup>27</sup> found that EDTA did not modify the root dentin permeability. They suggest that the absence of changes in the root dentin permeability with a conventional endodontic preparation was due to the fact that, even though endodontic preparation reduces dentin thickness, it also created a smear layer that compensated to the extent that there was no overall change in permeability.

A new chelating agent (Glyde File Prep) containing EDTA has been proposed for permanent teeth. Therefore, Grandini et al.<sup>28</sup> evaluated the smear layer, debris, and tubule orifices of root canal walls after being instrumented and irrigated by Glyde File Prep, using a SEM. The results of this study confirm that irrigation with NaOCl alone is not able to totally remove the smear layer, because its action is mainly directed to the organic debris. To obtain the total removal of the smear layer, that is, both organic and inorganic components, the combined use of NaOCl and EDTA is recommended. The chelating agent prepares the canal wall surfaces so that cleansers and medications are really effective with their antibacterial action.

One of the most effective drugs for removing the smear layer is RC-Prep (EDTA/Urea Peroxide). It is a potent bactericidal agent and increases the dentin permeability significantly<sup>11,21</sup>. In contrary, in this literature review only one paper found that Decal (Glover Laboratories, Melbourne,

Australia), Largal Ultra (Septodont, Paris, France), and RC-Prep (Medical Products Laboratories, Philadelphia, USA) significantly reduced the dye penetration into dentin, but that there was no difference among the agents in the degree of reduction of dye penetration<sup>29</sup>. Others substances (organic acids) have been used to remove the smear layer, such as 6 - 10% citric acid, 20% polyacrylic acid, and tannic acid. Salama & Abdelmegid<sup>30</sup> found that irrigation with 6% citric acid for 15 or 30 seconds was effective in removing all smear layer components of the primary root canals. However, further research is needed to investigate the biocompatibility of acids and to test combinations of solutions. Moreover, these acids could have a harmful effect on the periapical tissues of both permanent and primary teeth<sup>28</sup>.

#### Chlorhexidine Gluconate

A solution researched in Endodontics is Chlorhexidine Gluconate that seems to act by adsorbing onto the cell wall of the microorganisms and causing leakage of the intracellular components. At low concentrations, small molecular weight substances will leak out, especially potassium and phosphorus, resulting in a bacteriostatic effect. At high concentrations, chlorhexidine gluconate has a bactericidal effect due to the precipitation and/or coagulation of the cellular cytoplasm, probably caused by cross-linking proteins<sup>31</sup>. Vahdaty et al.<sup>14</sup> evaluated in vitro the antibacterial efficiency of 2% and 0.2% chlorhexidine, comparing them with NaOCl in the same concentrations. These cleansers were used in the infected dentin tubules. The results indicated that both the chlorhexidine and the NaOCl reduced the number of bacteria in the superficial layers of the dentin tubules. Heling & Chandler<sup>32</sup> and White et al.<sup>33</sup> suggested that chlorhexidine can be an excellent antimicrobial endodontic irrigating agent if used alone, or as an aid to NaOCl during the instrumentation. Chlorhexidine Gluconate showed quick residual antimicrobial activity in these in vitro studies.

Gomes et al.<sup>34</sup> evaluated the antimicrobial activity of the two formulations of Chlorhexidine Gluconate (liquid and gel) in three concentrations (0.2%, 1.0% and 2%), and of NaOCl (0.5%, 1.0%,

2.5%, 4.0%). The results showed that chlorhexidine in liquid form eliminated bacterial cells more quickly than the chlorhexidine gel. Even though all tested cleansers possessed antimicrobial activity, the time required to eliminate the studied microorganisms depended on the concentration and of the type of cleansers used. Ferraz et al.<sup>17</sup> evaluated 2% chlorhexidine gluconate gel as an endodontic irrigating agent according to its capacity to disinfect root canals contaminated with *Enterococcus faecalis*. Furthermore, they tested chlorhexidine gel cleaning capacity when compared with solutions commonly used in Endodontics (5.25% NaOCl and 2% chlorhexidine gluconate solution). The results demonstrated that the chlorhexidine gel produced cleaning of the surface of the root canal and presented antimicrobial capacity comparable with the other appraised solutions. It could be concluded that the chlorhexidine in the gel form has satisfactory potential to be used as an endodontic irrigating agent. Most of the studies have been undertaken on permanent teeth in vitro, demonstrating the properties of the cleanser used for the instrumentation of those canals.

This literature review found only three studies regarding primary teeth and root dentin permeability (Table 1). Bengtson et al.<sup>35, 36</sup> concluded that Endo-PTC + Dakin's liquid showed the highest dye penetration index of primary root dentin permeability. Primo<sup>15</sup> verified that 1% NaOCl associated with 10% citric acid was the most effective association to remove the smear layer of anterior primary teeth, followed by the associations Endo-PTC + Dakin's liquid and 4% NaOCl + 3% H<sub>2</sub>O<sub>2</sub>. All of these solutions produced an increase in the primary dentin permeability. Therefore, other researches should be undertaken to indicate an effective substance for the chemical-mechanic preparation of primary teeth, as well as to verify the physiochemical properties of treatment solutions, providing greater sanitation and appropriate preparation of the root canals.

### **Influence of Irrigation Methods**

A variety of instrumentation and irrigation methods have been used in endodontic treatment. According to research literature the instrumentation and the irrigation of the root canals can be accomplished via manual conventional means (endodontic files and Luer syringes) or via endodontic

ultrasonic-vibration-generator systems. The endodontic preparations might induce changes in the root dentin permeability<sup>37</sup>. When a file is ultrasonically activated and placed passively in a canal, a phenomenon called acoustic streaming is produced<sup>38</sup>. Acoustic streaming is one of the purported mechanisms for superior debridement<sup>39</sup>. Biological material that enters the streaming fields would be subjected to large shear stresses and may be disrupted<sup>40</sup>. Ultrasonically prepared teeth showed cleaner canals than the teeth prepared by hand instrumentation<sup>41,42</sup>.

Regarding primary teeth, Seow<sup>43</sup> concluded that a combination of mechanical filling followed by ultrasonication produced the best results, with 95% bacteria removed. The results showed that the ultrasonication might be useful for primary teeth endodontic treatment. However, this article review also found studies that were undertaken on teeth whose instrumentation were accomplished with endodontic files and were irrigated with Luer syringes (NaOCl). It presented little effect in removing the smear layer<sup>12</sup>.

Hata et al.<sup>16</sup> affirmed that the manual irrigation technique was more effective to remove the smear layer when used 15% EDTA syringe irrigation with the instrumentation with 5% NaOCl. However, the most effective irrigation technique to remove debris was the ultrasonic system, regardless of the cleanser used. Cunningham & Martin<sup>41</sup> observed a good rate of smear layer removal from root canals irrigated with 2.5% NaOCl, using the Endosonic system. However Pécora et al.<sup>44</sup>, Vansan et al.<sup>45</sup>, Abbott et al.<sup>46</sup>, Cheung & Stock<sup>47</sup>, and Karadag et al.<sup>48</sup> did not find significant differences among the instrumentation techniques manual and ultrasonic in permanent teeth in reduce the smear layer effectively, and consequently permeability alterations.

Other study<sup>37</sup> that analyzed the variation of hydraulic conductance measured in situ after three endodontic preparations (manual, ultrasonic, and manual with NaOCl and EDTA) verified there was an inverse relationship between variations in dentin permeability and the presence of smear layer. Dentin thickness was a significant factor influencing radicular permeability as well as the smear layer.

The use of EDTA induced a considerable increase in radicular permeability and the use of ultrasonics produced a similar but weaker effect.

Cameron<sup>42</sup> affirmed that the most effective method to increase the root dentin permeability, considering removal of smear layer, was a method in which manual instrumentation (endodontic files), irrigation with EDTAC (EDTA associated to Cetavlon), followed by the use of ultrasound with EDTAC for 1 minute and ultrasound with 4% NaOCl for 2 minutes.

This literature review found no study that demonstrates the close relation among primary teeth, root dentin permeability, and irrigation methods. Further researches should be undertaken to evaluate the best irrigation method of primary teeth, which provide increase of root dentin permeability.

### **Conclusion**

Based on literature reviewed it can be concluded that among the solutions used for permanent teeth, all solutions and associations studied increased the dentin permeability. There was no difference in the dentin permeability when comparing manual and ultrasonic irrigation. There are not enough papers focusing primary teeth on this subject to definitely establish the patterns of increased dentin permeability in tooth root canal treatment regarding the irrigating solutions and irrigation systems.

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**Fig. 1** - Root canals cleansers which have been studied for use in endodontic treatment.

<b>Category</b>	<b>Agents</b>		<b>Ingredients</b>	<b>Major Advantages(s)</b>
Antiseptic and/or Disinfectant	Sodium Hypochlorite		0.5-5.25% available chlorine	Tissue dissolution and antimicrobial
	Chlorhexidine Gluconate		0.1-2.0% Chlorhexidine Gluconate	Antimicrobial
Oxidizing agents	Hydrogen Peroxide		3% Hydrogen Peroxide	Effervescence with NaOCl (beneficial effect in the canal questionable)
	Urea Peroxide	Endo-PTC	10% Urea Peroxide, 15% Tween 80 and 75% Carbowax	Good wetting ability, excellent lubricant
		Gly-Oxide	10% Carbamide peroxide in glycerol	
Chelating agent	EDTA		10–17% recommended	Softens dentine and removes (partially) smear layer
	EDTAC		EDTA with Cetrimide/Cetavlon	Good wetting ability for EDTAC preparations
	RC-Prep		EDTA and Urea Peroxide in a base of carbowax	Excellent lubricant
Organic Acid	Citric acid		10-50% recommended	Removes smear layer
	Polyacrylic acid		5-20% recommended	
	Tannic acid		25% solution	

**Table 1 - Cleansers, Instrumentation/Irrigation Method, Type of teeth, and Evaluations regarding endodontic treatment**

<b>Author</b>	<b>Year</b>	<b>Cleansers</b>	<b>Method</b>	<b>Teeth</b>	<b>Evaluation</b>
Marshall et al. <sup>9</sup>	1960	NaOCl / EDTA / HP	Manual	PT	DP / R
Stewart et al. <sup>21</sup>	1969	EDTA+ Gly-Oxide / Gly-Oxide / Aqueous Peroxide / DW	Manual	PT	DP
Cohen et al. <sup>11</sup>	1970	NaOCl / HP / Gly-Oxide / RC-Prep / EDTAC Zephiran Chloride / Hydrochloric acid / Sulfuric acid	Manual	PT	DP
Fraser & Laws <sup>29</sup>	1976	RC-Prep / Decal / Largal Ultra *	Manual	PT	DP
Bengtson et al. <sup>35</sup>	1983	NaOCl / Endo-PTC / Tergentol / DW	Manual	DT	DP
Bengtson et al. <sup>36</sup>	1985	NaOCl / Endo-PTC / Tergentol / DW	Manual	DT	DP
Zuolo et al. <sup>26</sup>	1987	EDTA / EDTAC / EDTAT** / EDTACP*** / SS	Manual	PT	HM
Pécora et al. <sup>13</sup>	1987	NaOCl / NaOCl + HP / EDTA	Manual	PT	HM
Moura & Paiva <sup>20</sup>	1989	NaOCl / Endo-PTC	Manual	PT	DP
Pécora et al. <sup>42</sup>	1990	NaOCl / DW	Manual/Ultrassom	PT	HM
Vansan et al. <sup>43</sup>	1990	NaOCl / DW / Tergentol	Manual/Ultrassom	PT	HM
Fogel & Pashley <sup>19</sup>	1990	NaOCl / Citric acid / Monopotassium-Monohydrogen oxalate	Manual	PT	HC
Tao et al. <sup>27</sup>	1991	NaOCl / EDTA	Manual	PT	HC
Guignes et al. <sup>37</sup>	1996	NaOCl / EDTA	Manual/Ultrassom	PT	HC / SEM

Hydrogen Peroxide = HP; Distilled Water = DW; Saline Solution = SS.

Permanent Teeth = PT; Primary (Deciduous) Teeth = DT.

Dye penetration = DP; Radioisotope = R; Scanning Electron Microscopy = SEM; Histochemical = H; Morphometric = M; Hydraulic Conductance = HC

\*Decal and Largal Ultra are chelating agents used in Australia and France, respectively.

\*\*EDTAT=EDTA + Tergentol (Lauryl-diethylene-glycol-ether sodium sulphate)

\*\*\* EDTACP= EDTA + Pyridine-phenyl-chlorine

**IS THE PRESENCE OF THE SMEAR LAYER A LIMITING FACTOR FOR ROOT DENTIN PERMEABILITY IN PRIMARY TEETH?\***

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## **ABSTRACT**

**Objectives.** To correlate dye permeability to presence/absence of smear layer in primary root dentin, with regard to endodontic preparation and irrigation methods. **Study design.** 112 extracted roots were distributed into the following groups: Dakin, Dakin+Hydrogen Peroxide, 2% Chlorhexidine, and Saline Solution. Manual (MI) or Ultrasonic (UI) irrigation was performed. The roots were sealed, filled with dye and longitudinally sectioned. The halves were marked in cervical, middle, and apical thirds for dye penetration measurement. The samples were observed under SEM. The data were submitted to linear regression analysis with the dummy variable ( $p < 0.05$ ). **Results.** The data revealed a relationship between decreasing permeability and smear layer presence on root dentin walls for MI in the middle third ( $p = 0.0147$ ). As regards UI, no statistically significant relation was observed ( $p > 0.05$ ). **Conclusion.** Smear layer presence on root dentin walls was a limiting factor for dye penetration in middle third for manual irrigation.

## INTRODUCTION

The smear layer is an important matter in Dentistry. It is linked to bond properties of different materials to tooth structure, their retention, and marginal leakage.<sup>1</sup> In addition, it has been associated with unclean root dentin walls in Endodontics. The smear layer is a debris layer produced during canal instrumentation, which obstructs the underlying dentinal tubule orifices.<sup>2</sup> In the case of infected teeth, this layer contains a high number of organic and inorganic calcified tissue particles and organic elements such as, pulp tissue debris, odontoblastic processes, microorganisms and blood cells from dentinal tubules.<sup>3</sup> The smear layer makes up the underlying dentin matrix and may decrease permeability by blocking tubule openings.<sup>4</sup> In coronal dentin, the smear layer produced by bur can decrease permeability from 70 to 90%, while the radicular dentin allows a smaller decrease (25 to 49%)<sup>2</sup>, due to its lower hydraulic conductance.<sup>5</sup>

However, there is controversy concerning the role of the smear layer in root dentin permeability. Scelza et al<sup>6</sup> stated that endodontic preparations may induce changes in root dentin permeability and smear layer formation after canal instrumentation directly affects root dentin permeability. Fogel and Pashley<sup>2</sup> found that the smear layer that covered the root dentin wall was thick and its presence did not prevent fluid penetration in dentin, although it happened in lower proportions. In addition, Tao et al<sup>7</sup> found that the absence of changes in root dentin permeability with a conventional endodontic preparation was due to the fact that although endodontic preparation reduced dentin thickness, it also created a smear layer that compensated to the extent that there was no overall change in permeability.

One of the main goals of root canal treatment is to clean the entire root canal system, by removal of pulpal debris, smear layer and smear plugs in order to prevent bacteria or bacterial by-products from being harbored and providing a reservoir of potential irritants in the treated root canal.<sup>8-</sup>

<sup>10</sup> The majority of studies have been conducted in permanent teeth.<sup>2,7,11</sup> However, there are differences between primary and permanent teeth as regards root canal morphology. Dentin permeability has a



direct relationship with dentinal tubule diameter and density. Therefore, smear layer presence and permeability alterations produced by endodontic treatment in root dentin should be studied in primary teeth.

This subject is of great significance to primary teeth. The root canal system must be cleaned, decontaminated, shaped, and enlarged, since the filling has to be made with non-set pastes. These pastes have to penetrate the dentinal tubules in order to limit bacterial contamination and not allow re-infection of the root canal system. From this aspect, dentin permeability is one of the main subjects to be researched in primary teeth with regard to endodontic treatment. There are no articles focused on the relationship between smear layer presence and primary root dentin permeability, while only one dealt with permanent teeth.<sup>11</sup>

The cleansing and irrigation method can contribute to efficient smear layer removal and increased permeability. In 1982, Cunningham and Martin<sup>12</sup> 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.<sup>13</sup> In addition, ultrasonic irrigation uses energy as a catalyst to activate the irrigant, both physically and chemically.<sup>12</sup>

Only one study was conducted in primary teeth.<sup>14</sup> However, the authors studied the role of irrigating solutions in removing the smear layer from root dentin, using scanning electron microscopy (SEM). They observed a heavy smear layer at all levels in teeth irrigated with saline solution. Goldman et al<sup>1</sup>, Aktener and Bilkay<sup>15</sup>, and Bitter<sup>16</sup> also found that this solution produced a sludge layer made up of residual debris that occluded the dentinal tubules in both primary and permanent teeth. In addition, Salama and Abdelmegid<sup>14</sup> also verified that irrigation with 5.25% sodium hypochlorite for 30 seconds was effective for removing the smear layer components from primary root canals. But they did not correlate these results with root dentin permeability.

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

## **MATERIAL AND METHODS**

A hundred-twelve (112) infected human maxillary and mandibular posterior deciduous roots were extracted for clinical reasons and selected for this study. The Ethical Committee in Human Research of Piracicaba Dental School/University of Campinas 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 Sorensen buffered solution under refrigeration.

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

The working length was determined visually by using the thinnest #15 K-file (Dentsply/Maillefer, Ballaigues, Switzerland) 1.0 mm shorter than that observed to just perforate apex. All root canals were sequentially manually instrumented using K-files from #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 hydrogen peroxide cream (**DHP**) (8.85% hydrogen peroxide, 14.34% Tween 80, 76.80 % Carbowax), or 1mL of saline solution (**S**) (control group) as irrigants between each instrument, in a total of 5 mL. The solutions and gel were inserted

within the root canals using a 1 mL insulin syringe with 12.7 x 0.33 mm round edge needles (Becton Dickinson and Company, New Jersey, EUA), which were placed at the working length in each canal. For the DHP group, hydrogen peroxide cream was placed into the sectioned pulpal camera and Dakin's liquid was dropped into it. After instrumentation, a final irrigation with 1 mL Dakin's liquid was always performed in order to wash out the hydrogen peroxide cream. For the chlorhexidine group (CL), the root canal was totally filled with 2% chlorhexidine digluconate gel before performing a final irrigation with 1 mL saline solution in order to wash out the chlorhexidine. For UI group the cleansers were inserted at the same time as ultra-sonic activation was performed to increase the efficiency of irrigation by the ultra-sonic system. For this, a Mult-Sonic-s ultra-sonic system was utilized (Gnatus, Ribeirão Preto, Brazil) at 50/60 Hz, Power 40 vA, consumption 20W and frequency of 29KHz.

The root canals were dried with tips of absorbent paper (Tanari FDA, Manaus, Brazil, Batch # 005001P) and the roots were then left to dry for 30 minutes. Roots were externally sealed with two coats of nail varnish (Colorama, Brazil) and, additionally, apically impermeabilized with wax. For the evaluation of permeability index (PI), 2% methylene blue solution (pH 7.0) was placed into root canals using an insulin syringe (Becton Dickinson and Company), and left in 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 two hemisections. Only one of the hemisections was used to verify the dye penetration into the root dentin.

#### Permeability index analyses

All hemisections were observed under a stereomicroscope Leica MZ6 (Leica Microsystems AG, Wetzlar, Germany) at 0.63-3.2x magnification, depending on the hemisection root size. After assessment under the stereomicroscope, forty specimens out of the initial sample were discarded, either because it was impossible to observe the apical third clearly or during the SEM preparations. Thus, the final sample comprised seventy-two hemisections (n=9), of which the images were captured with a

digital camera (Viewse digital VC-813D, São Paulo, Brazil), and sent to Pinnacle Studio DC 10 AV/DV – Version 9 software (São Paulo, Brazil).

The dye penetration areas were measured with the Image Tool 3.0 software (Periodontology Department, University of Texas, and Health Science Center at San Antonio, TX, USA). Every hemisection was divided into thirds (cervical, middle, and apical); for each third, the total and dye penetration areas (mm<sup>2</sup>) were measured, with the exception of the light root area. Thus, the root dentin permeability index (PI) was determined by multiplying the value of dye penetration area (DPA) by 100

and this value was divided for total root dentin area (TA) as the equation:  $PI = \frac{DPA \times 100}{TA}$ .

#### Scanning electron microscopy evaluations (SEM)

After the dye penetration assessment, the specimens were prepared for SEM evaluation. They were dehydrated in ascending grades of ethanol (25% for 20 min, 50% for 20 min, 75% 20 min, 95% 30 min, 100% 60 min). 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.<sup>17</sup> 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 min (SCD050 sputter coater, Balzers, Liechtenstein). They were observed under a Scanning Electron Microscopy (JSM 5600LV, JEOL, Tokyo – Japan) at an accelerating voltage of 10 kV, a working distance of 20 mm, and magnification 2000x.

For each third (cervical, middle, and apical), one image was obtained. Each photomicrograph was evaluated by one calibrated examiner, twice, with a one-week interval in between. In order to calibrate the examiner, 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 intra-examiner coincidence level was found to be 90%.

The photomicrographs were classified according to a score based on the smear layer presence (SL) and the characteristics of the collagen fibril network: 1- no smear layer presence, and dentinal tubules open; 2- Partial smear layer and dentinal tubules open; 3-Total smear layer and/or no open dentinal tubules. (**Figure 1A, B**)

Original data from permeability index means (PI) were transformed (sine arch of the root of  $X/100$ ) before applying the ANOVA and Tukey tests, because variance was not homogeneous. A factorial (a x b) ANOVA was applied to analyze the interactions between the factors (method of irrigation and type of irrigant). In order to assess significant differences within these factors, the Tukey test was applied ( $p < 0.05$ ). The SEM data were submitted to the Kruskal-Wallis test ( $p < 0.05$ ). These statistical tests were performed by SANEST (Statistical Analysis System). The PI and SEM data were submitted to the regression analysis with Dummy variable. The software SAS system (version 8.02, SAS Institute Inc., Cary: NC, 1999) was used and the significance limit was set at 5%.

## **RESULTS**

*Permeability Index:* The null hypotheses were rejected. There was a significant difference between irrigation method and also among the different cleansers used in this study. In addition, there was a significant association between the studied factors (irrigation methods and different cleansers). The mean permeability index values in the different thirds are shown in **Table II**. Results demonstrate that the irrigation method, in association with the cleansers, had a significant influence on PI means. The manual irrigation method produced a higher PI than that observed when the ultrasonic irrigation method was used in the cervical and middle thirds ( $p < 0.05$ ).

*Morphologic aspect of dentinal wall surface:* There was no difference between irrigation methods ( $p = 0.3445$ ), and among cleansers ( $p = 0.4237$ ) used in this study, and the interaction between methods and cleansers ( $p = 0.1941$ ). According to scores used to evaluate the smear layer (SEM), there was no statistically significant difference among the thirds as regards different cleansers and irrigation

methods. Most of the specimens, irrespective of groups, presented a thick smear layer on the root dentin surface (Score 4) (**Figure 2, 3**).

*Regression analyses between PI and Morphologic aspect of dentinal wall surface:* Linear Regression with the Dummy variable test revealed a statistically significant relationship between the decreasing of permeability and the smear layer presence on root dentin walls for manual irrigation in the middle third ( $p=0.0147$ ) (**Table III; Figure 4**). For the cervical third, in both conditions (IM and UI) was observed a statistically significant regression model ( $p= 0.001$ ), however the effect of smear layer presence on root dentin wall for permeability index was not statistically significant ( $p>0.05$ ). For manual irrigation in the apical third, no statistically significant linear regression model was observed between permeability index and the morphologic aspects of the root dentin ( $p>0.05$ ). Similar results were found for regards ultrasonic irrigation, in the middle e 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.<sup>18</sup> Dye penetration into root dentin is frequently used technique for evaluating the increase in dentinal permeability.<sup>19</sup> A critical variable that would affect permeability is the nature of dentin surface and whether or not it is coated with a smear layer.<sup>18</sup>

With regard to permeability index, manual irrigation achieved the highest PI averages when associated with Dakin, Dakin's liquid + hydrogen peroxide, and saline solution, for cervical third. This could be explained by the deproteinizing characteristics of NaOCl-based cleansers. Organic tissue dissolution by sodium hypochlorite solutions is based on chloride action on the proteins, forming chloramines, which are soluble in water. 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.<sup>20</sup> It is probably

the reason for high PI means for all thirds. As regards NaOCl associated with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), it liberates great amounts of nascent oxygen and contributes to pulp tissue remains and dentinal particle removal during the chemical-mechanical preparation.<sup>21</sup> However, the H<sub>2</sub>O<sub>2</sub> cream the end of the canal was not reached, and did not act in an efficient way on apical dentin permeability. Another point to be considered is that saline solution movement maybe has acted mechanically on the root walls, perhaps 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 + hydrogen peroxide 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.<sup>22</sup> Pashely et al<sup>23</sup> 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.<sup>24</sup> This action maybe did not allow high dye permeability.

The decrease in mean PI values (Table II) observed from cervical to apical thirds could be related to the complex root canal morphology in primary teeth.<sup>25</sup> Apical dentin contains more sclerotic dentin, which is less tubular.<sup>26</sup> This may be why apical dentin is much less permeable than either middle or cervical root dentin, even though its dentin thickness is much less than that of the other two zones.

With reference to morphological analyses, the hypothesis that there was difference between irrigation methods, cleansers, and their interaction was rejected. As regards the smear layer presence or absence, the majority of samples showed a thick smear layer covering the root dentinal canal walls. This could be related to non-use of chelating agents. Present study data are in agreement with studies

that have found no significant difference in ability of saline solution, hydrogen peroxide, and NaOCl to remove the smear layer from the surface of instrumented root canals.<sup>1, 14-16,27-34</sup>

The tested hypothesis that there was correlation between the dye permeability and the morphologic aspect of the root dentin in primary teeth was accepted. A clear relationship was observed between decreased permeability and smear layer presence on root dentin walls for manual irrigation in the middle third. This study corroborates the results observed by Fogel and Pashley<sup>2</sup>. They observed that even in the presence of a smear layer, and dentinal tubules occluded by smear plugs, there was low fluid filtration. This present study is also in agreement with Guignes et al,<sup>11</sup> who analyzed the variation of hydraulic conductance measured in situ after three endodontic preparations (manual, ultrasonic, and manual with NaOCl and EDTA) and verified that there was an inverse relationship between variations in dentin permeability and the presence of smear layer. Smear layer was as a significant factor in influencing radicular permeability as dentin thickness.<sup>11</sup>

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 cleanser activation, producing circulation near the tip and not in the entire root canal and consequently this technique did not allow better debridement. This is in agreement with the studies that failed to demonstrate the superiority of ultrasonics as a primary instrumentation technique.<sup>35-37</sup> In addition, Pécora et al<sup>38</sup>, Vansan et al<sup>39</sup>, and Karadag et al<sup>40</sup> 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,<sup>41</sup> but it is a direct function of canal size. Moreover, ultrasonically prepared permanent teeth showed cleaner canals than the teeth prepared by hand instrumentation.<sup>12,42</sup> However, as primary teeth canal diameters are smaller, ultrasonic irrigation



failed. In addition, Seow<sup>43</sup> 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 smear layer presence. This could be explained because the NaOCl-based cleansers and saline solution did not remove the smear layer. In spite of Moorer and Wesselink<sup>20</sup> found that sodium hypochlorite solution removes collagen fibrils, it is not a decisive fact that influence the PI 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.<sup>6</sup> Instead, if NaOCl was associated with EDTA the smear layer would be completely removed.<sup>44</sup> In addition, for the cervical third, the data only suggests that smear layer presence could be connected with decreased dye permeability. The apical third showed no relation between data evaluated, which 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.<sup>45</sup>

Root canal preparation produces a decrease of dentin thickness while induces an increase in the surface area available for permeation. Simultaneously, tubule diameters could be decreased as the root canal was enlarged<sup>45</sup>. This study is in an agreement with current literature with regard to smear layer removal of the apical third showing the worst results with different cleansers, although the majority of these studies were conducted in permanent teeth.<sup>44-47</sup>

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

## **CONCLUSION**

Within the limits of the present study, it can be concluded that the smear layer presence on the root dentin walls was a limiting factor to dye penetration (Permeability Index), in middle third, for manual irrigation.

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**Table I.** Distribution of the groups depending upon the method of irrigation and type of irrigants.

<b>Method Irrigation</b>	<b>Irrigants used</b>	<b>Manufactures*</b>
	Dakin's liquid ( <b>D</b> )	Proderma
Manual	Dakin's liquid + Hydrogen Peroxide ( <b>DHP</b> )	Proderma/ Polidental
<b>(MI)</b>	2% chlorhexidine digluconate gel ( <b>CL</b> )	Endosupport
	Saline solution ( <b>S</b> )	Tayuyna
Manual	Dakin's liquid ( <b>D</b> )	Proderma
+	Dakin's liquid + Hydrogen Peroxide ( <b>DHP</b> )	Proderma/ Polidental
Ultra-sonic activation	2% chlorhexidine digluconate gel ( <b>CL</b> )	Endosupport
<b>(UI)</b>	Saline solution ( <b>S</b> )	Tayuyna

\* Proderma (Laboratory of Manipulation, Piracicaba, Brazil); Polidental Industry and Commercial (São Paulo, Brazil, Batch # 6220); Endosupport (São Paulo, Brazil, Batch # 1802.8295); Tayuyna Laboratory (São Paulo, Brazil, Batch # 035171)



**Table II.** Permeability index (PI) averages percentage for cervical, middle, and apical thirds

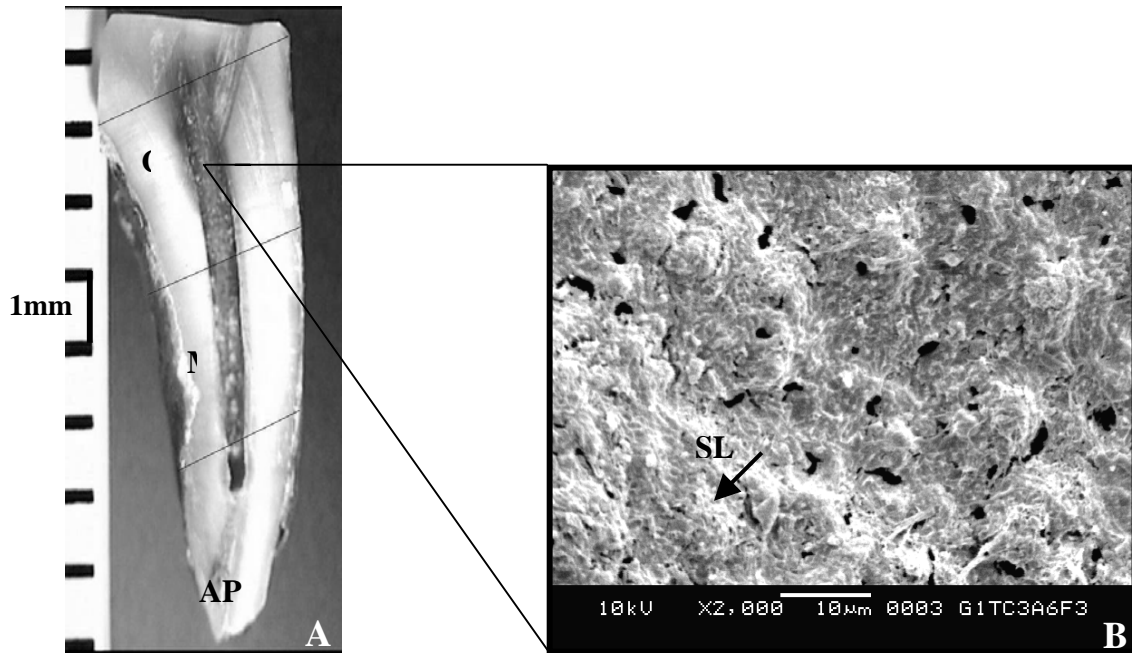
	Cervical Third		Middle Third		Apical Third	
	MI	UI	MI	UI	MI	UI
<b>D</b>	72.1±21.8 <sup>a A</sup>	39.3±18.4 <sup>ab B</sup>	56.5±37.1 <sup>abA</sup>	24.0±17.3 <sup>a B</sup>	42.7±37.4 <sup>a A</sup>	31.6±35.0 <sup>a A</sup>
<b>DHP</b>	78.1±26.1 <sup>a A</sup>	16.6±21.0 <sup>b B</sup>	67.5±22.2 <sup>a A</sup>	35.9±35.8 <sup>a B</sup>	31.4±37.7 <sup>a A</sup>	25.4±36.2 <sup>a A</sup>
<b>CL</b>	10.1±11.4 <sup>b A</sup>	58.8±22.9 <sup>a B</sup>	24.5±34.7 <sup>b A</sup>	31.2±36.2 <sup>a A</sup>	10.4±16.0 <sup>a A</sup>	12.0±12.0 <sup>a A</sup>
<b>S</b>	74.5±32.1 <sup>a A</sup>	66.0±22.1 <sup>a A</sup>	53.4±38.7 <sup>abA</sup>	42.3±25.6 <sup>a A</sup>	22.0±37.3 <sup>a A</sup>	25.0±37.7 <sup>a A</sup>

Similar small letters in column mean no significant statistical difference by factorial (a x b) ANOVA test (p<0.05), regarding each third.

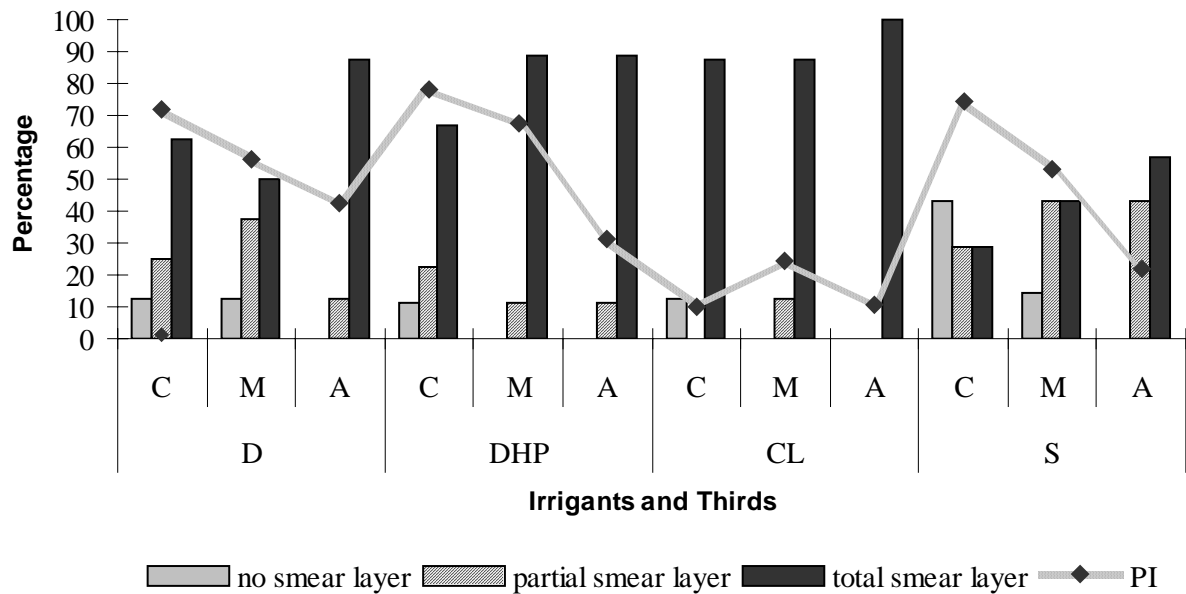
Similar capital letters in line mean no significant statistical difference factorial (a x b) ANOVA test (p<0.05), regarding each third.

**Table III.** Parameter estimated and t test considering the hypothesis that each parameter did not statistically differs from zero for manual irrigation in the middle third.

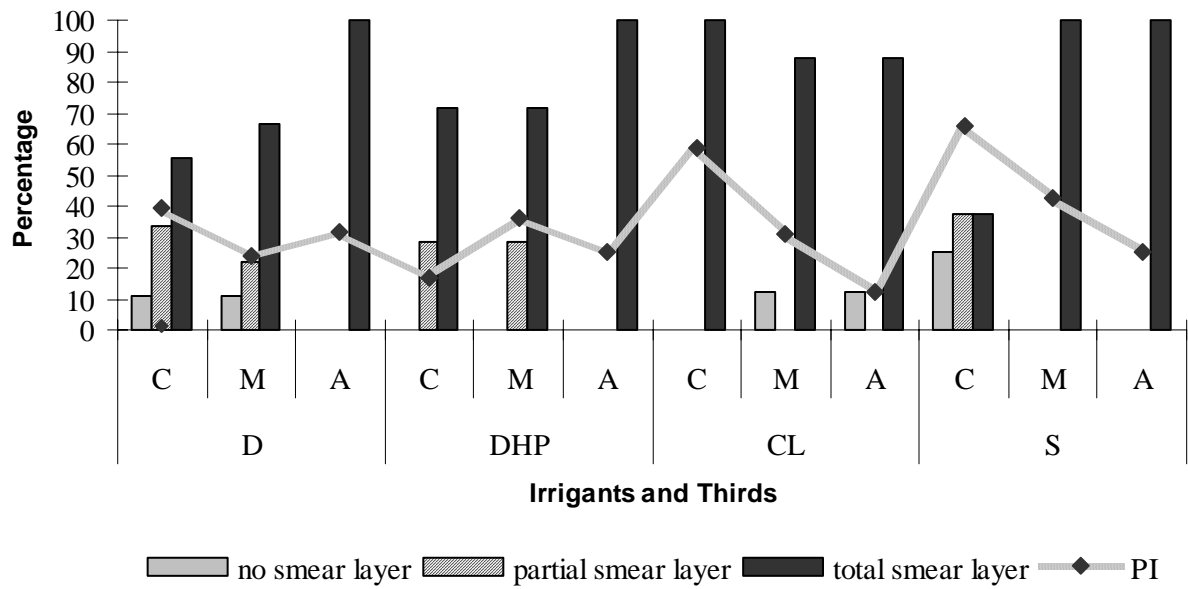
Variable	Label	GL	Parameter	Standard	t Value	Pr >  t
			Estimate	Error		
Intercept	Intercept	1	119.25263	26.35925	4.52	0.0001
DHP	Dummy DHP	1	28.15882	15.70605	1.79	0.0842
CL	Dummy Chlorhexidine 2%	1	-18.87504	16.06714	-1.17	0.2503
S	Dummy Saline Solution	1	-5.45138	15.80937	-0.34	0.7329
Score	Score	1	-26.39742	10.12765	-2.61	0.0147



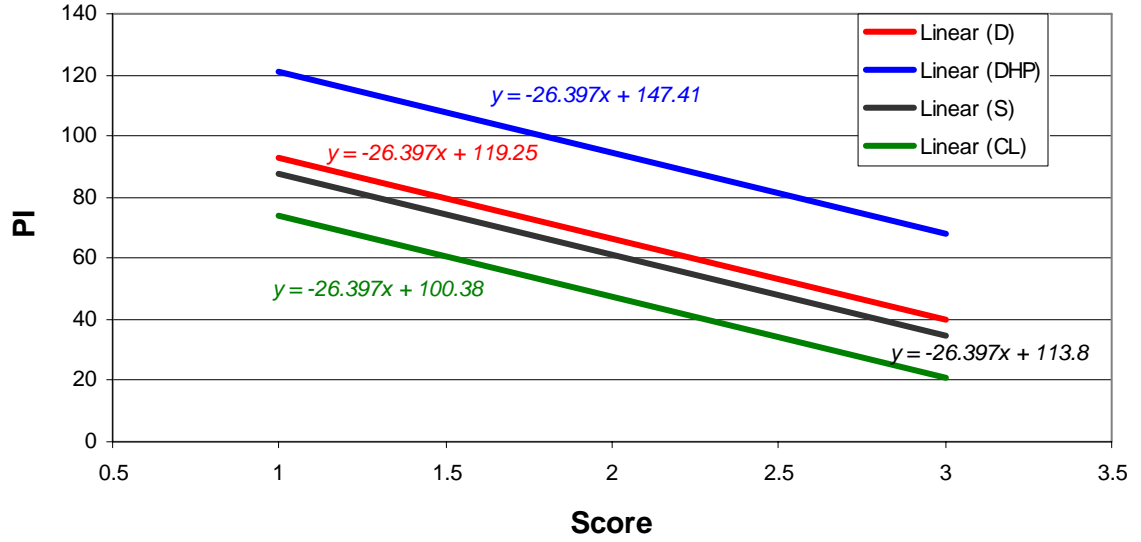
**Fig 1.** Correlation between permeability index and morphological aspects of dentinal wall surface. **A** - Hemisection divided into thirds: (C) cervical third; (M) middle third; (AP) apical third. **B**- Photomicrography of root dentin irrigated with chlorhexidine (SEM). Black arrow means smear layer (SL) presence.



**Fig 2.** Score percentage distribution of smear layer presence and permeability index regarding manual irrigation (MI) associated with irrigants (Dakin-D, Dakin + hydrogen peroxide-DHP, Chlorhexidine-CL, Saline solution-S) and cervical (C), middle (M), and apical (A) thirds.



**Fig 3.** Score percentage distribution of smear layer presence and permeability index regarding ultrasonic irrigation (UI) associated with Irrigants (Dakin-D, Dakin + hydrogen peroxide-DHP, Chlorhexidine-CL, Saline solution-S) and cervical (C), middle (M), and apical (A) thirds.



**Fig 4.** Linear regression model with the Dummy variable represents permeability index and morphological aspects of dentinal wall surface for manual irrigation in the middle third.

Considerando que grande porcentagem da população infantil necessita de tratamento endodôntico para a manutenção dos dentes decíduos no arco dentário, espera-se que este tratamento elimine restos pulpares, facilite a instrumentação, promova a limpeza e desinfecção dos canais radiculares, remova a *smear layer* (Ferraz *et al.*, 2001) e, dessa maneira, altere a permeabilidade dentinária.

Através da revisão de literatura (capítulo 1) pôde-se discutir a eficácia de algumas substâncias irrigadoras, bem como a influência dos sistemas de irrigação manual e de vibração ultrasônica usados durante o tratamento endodôntico de dentes decíduos e permanentes, considerando a permeabilidade dentinária radicular. Embora existam trabalhos relacionando os efeitos das substâncias irrigadoras e métodos de irrigação sobre a permeabilidade radicular em dentes permanentes (Marshall *et al.*, 1960; Stewart *et al.*, 1969; Cohen *et al.*, 1970; Fraser & Laws 1976; Zuolo *et al.*, 1987; Pécora *et al.*, 1987; Moura & Paiva, 1989; Pécora *et al.*, 1990; Vansan *et al.*, 1990; Fogel & Pashley, 1990; Tao *et al.*, 1991; Guignes *et al.*, 1996) poucos foram realizados em dentes decíduos (Bengtson *et al.*, 1983; Bengtson *et al.*, 1985). Os resultados desse presente estudo demonstraram que o hipoclorito de sódio, peróxido de uréia, peróxido de hidrogênio, EDTA e digluconato de clorexidina, sozinhos ou associados, aumentaram a permeabilidade dentinária radicular quando utilizados em dentes permanentes. Observou-se na maioria dos estudos ausência de diferenças na permeabilidade dentinária radicular, quando comparados os métodos de irrigação em dentes permanentes. Entretanto, tornou-se evidente a escassez de trabalhos que focassem o tratamento endodôntico de dentes decíduos.

Neste contexto, poucas são as informações sobre o efeito das substâncias irrigadoras e métodos de irrigação na permeabilidade da dentina radicular de dentes decíduos. Logo, o presente trabalho (capítulo 2) avaliou o impacto de substâncias irrigadoras e métodos de irrigação em dentes decíduos através do índice de permeabilidade radicular. Os resultados demonstraram que tanto as soluções de hipoclorito de sódio a 0,5%, quanto este associado ao peróxido de hidrogênio e ainda a solução salina, foram indicados para a utilização durante o preparo químico-mecânico de canais radiculares de dentes decíduos,

quando irrigados manualmente. Entretanto, a escolha da substância irrigadora também deve considerar as propriedades físicas (tensão superficial, capacidade de molhamento do substrato), químicas (modificação do substrato radicular), microbiológicas (ação antimicrobiana) e biológicas (biocompatibilidade).

Considerando-se as propriedades físicas, a tensão superficial é um fator importante para promover ação efetiva na superfície dentinária radicular. Quanto menor a tensão superficial, melhor a capacidade de molhamento dessa solução (Tasman *et al.*, 2000). A solução de hipoclorito de sódio possui essa característica, bem como a capacidade de dissolução dos tecidos orgânicos baseados na ação do cloro sobre as proteínas, o que altera a configuração dos componentes dentinários e conseqüentemente remove as fibrilas colágenas (Moorer & Wesselink, 1982). Já a clorexidina gel apresenta alta tensão superficial o que poderia dificultar a remoção do gel das paredes dentinárias radiculares. Os resíduos do gel poderiam evitar a penetração do corante utilizado nesse estudo (capítulo 2) para determinação do índice de permeabilidade. Outra característica dessa substância é a potente inibição das metaloproteinases, enzimas que desestabilizam a rede de fibrilas colágenas frente a processos de degradação (Pahsley *et al.*, 2004). Essa é uma provável explicação para os menores valores de índices de permeabilidade quando a clorexidina foi utilizada como agente de irrigação. Por outro lado, embora a solução salina tenha demonstrado altos índices de permeabilidade, é notória a ausência de propriedades químicas e antimicrobianas para a sua indicação clínica em dentes decíduos infectados.

Com relação aos métodos de irrigação, a busca pela eficiência no saneamento dos canais radiculares levou a considerar o uso do ultra-som para a instrumentação/irrigação dos canais radiculares, como meio coadjuvante à terapia endodôntica. Alguns estudos têm demonstrado (Richman, 1957; Martin, 1976) que a instrumentação com o auxílio do ultra-som promove melhor remoção de resíduos e limpeza mais efetiva do sistema endodôntico, quando comparado à instrumentação manual. Porém, dentro dos parâmetros utilizados no Capítulo 2, para dentes decíduos a irrigação manual apresentou os melhores resultados quando comparados à irrigação realizada através de um sistema gerador de vibração ultrasônica. Sabe-se que a efetividade do ultra-som está diretamente relacionada ao diâmetro dos canais radiculares. Uma vez que dentes decíduos apresentam canais de menor diâmetro,



o fenômeno chamado *acoustic streaming* não ocorreu efetivamente, possivelmente mantendo os restos pulpares e/ou substâncias químicas utilizadas durante o tratamento endodôntico, nas paredes das superfícies radiculares.

Uma vez que existiam controvérsias se a presença da *smear layer* afetaria a permeabilidade dentinária radicular de dentes decíduos, foi proposto o estudo de correlação entre os aspectos morfológicos do substrato dentinário radicular (presença ou ausência da *smear layer*) observados através de microscopia eletrônica de varredura, e o índice de permeabilidade dentinária radicular, considerando a utilização de diferentes substâncias irrigadoras e métodos de irrigação (manual ou ativação ultrasônica). Os resultados demonstraram que a presença da *smear layer* nas paredes dentinárias radiculares foi um fator limitante para a penetração do corante azul de metileno, para o terço médio somente quando da irrigação manual.

A solução de hipoclorito de sódio, pela característica desproteinizante, provavelmente atuou sobre os componentes orgânicos presentes na *smear layer*, apesar de não removê-la da entrada dos túbulos dentinários radiculares. Já a clorexidina, além de não possuir ação desproteinizante e ainda, talvez pela alta tensão superficial, contribuiu para que a *smear layer* fosse um fator limitante à permeabilidade dentinária ao corante.

A solução que reuniu características que a tornam indicada para o procedimento de irrigação durante o tratamento endodôntico de dentes decíduos, foi o hipoclorito de sódio (Líquido de Dakin), utilizado através da irrigação manual. Esta permitiu maior permeabilidade ao corante, ainda que na presença da *smear layer*. Ainda, essa solução apresenta atividade antimicrobiana, garantindo a descontaminação inicial da *smear layer*, que poderá ser mantida pela presença e ação da pasta obturadora.

## CONCLUSÕES GERAIS

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Diante dos objetivos e da metodologia empregada no presente estudo, concluiu-se que:

1. A análise da literatura evidenciou que todas as substâncias irrigadoras e associações promoveram aumento da permeabilidade dentinária radicular de dentes permanentes durante o tratamento endodôntico. Não houve diferenças quanto à permeabilidade dentinária, quando comparada irrigação realizada manualmente ou através de vibração ultrasônica.
2. Não há estudos relacionados à permeabilidade radicular de dentes decíduos, quando estes são submetidos a substâncias irrigadoras e diferentes métodos de irrigação.
3. A técnica de irrigação manual foi considerada mais efetiva do que a técnica de irrigação através de vibração ultrasônica, quanto à permeabilidade dentinária radicular de dentes decíduos.
4. O líquido de Dakin apresentou os maiores valores de índice de permeabilidade, nos três terços radiculares, sugerindo que essa solução irrigadora seja indicada para o tratamento endodôntico de dentes decíduos.
5. A presença da *smear layer* nas paredes radiculares foi um fator limitante à penetração do corante (índice de permeabilidade) para o terço médio quando dentes decíduos foram irrigados manualmente.

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\* De acordo com a norma da UNICAMP/FOP, baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

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## DELIBERAÇÃO CCPG – 001/98

Dispõe a respeito do formato das teses de Mestrado e de Doutorado aprovadas pela UNICAMP

Tendo em vista a possibilidade, segundo parecer PG N° 1985/96, das teses de Mestrado e Doutorado terem um formato alternativo àquele já bem estabelecido, a CCPG resolve:

Artigo 1º - Todas as teses de mestrado e de doutorado da UNICAMP terão o seguinte formato padrão:

- I) Capa com formato único, dando visibilidade ao nível (mestrado e doutorado), e à Universidade.
- II) Primeira folha interna dando visibilidade ao nível (mestrado ou doutorado), à Universidade, à Unidade em foi defendida e à banca examinadora, ressaltando o nome do orientador e co-orientadores. No seu verso deve constar a ficha catalográfica.
- III) Segunda folha interna onde conste o resumo em português e o Abstract em inglês.
- IV) Introdução Geral.
- V) Capítulo.
- VI) Conclusão geral.
- VII) Referências Bibliográficas.
- VIII) Apêndices (se necessários).

Artigo 2º - A critério do orientador, os Capítulos e os Apêndices poderão conter cópias de artigos de autoria ou de co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, escritos no idioma exigido pelo veículo de divulgação.

Parágrafo único – Os veículos de divulgação deverão ser expressamente indicados.

Artigo 3º - A PRPG providenciará o projeto gráfico das capas bem como a impressão de um número de exemplares, da versão final da tese a ser homologada.

Artigo 4º - Fica revogada a resolução CCPG 17/97.



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## COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE ESTADUAL DE CAMPINAS  
FACULDADE DE ODONTOLOGIA DE PIRACICABA

## CERTIFICADO



Certificamos que o Projeto de pesquisa intitulado "Influência de Cleansers sobre a Dentina Radicular de Dentes Decíduos", sob o protocolo nº **065/2002**, da Pesquisadora **Fernanda Miori Pascon**, sob a responsabilidade da Profa. Dra. **Regina Maria Puppim Rontani**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP.

Piracicaba, 21 de novembro de 2002

We certify that the research project with title "Study of the Effect of Cleansers on the Root Dentin in Primary Teeth", protocol nº **065/2002**, by Researcher **Fernanda Miori Pascon**, responsibility by Prof. Dr. **Regina Maria Puppim Rontani**, is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Research at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, November 21 2002

  
Prof. Dr. **Pedro Luiz Rosalen**

Secretário  
CEP/FOP/UNICAMP

  
Prof. Dr. **Antonio Bento Alves de Moraes**

Coordenador  
CEP/FOP/UNICAMP



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## BRAZILIAN JOURNAL OF ORAL SCIENCES

Dear Dr. **Fernanda Miori Pascon**

Thank you for submitting your manuscript entitled “**Influence of  
Cleansers and Irrigation Methods on Primary and Permanent Root Dentin  
Permeability: A Literature Review.**” to the *Brazilian Journal of Oral Sciences*.

Please refer to manuscript number **192** on all further correspondence.

We will contact you again as soon as we have the necessary information for an editorial decision.

Sincerely yours,

Jose Francisco Hofling  
Editor

**Figura 1 - Ilustrações da metodologia empregada nos capítulos 2 e 3.**

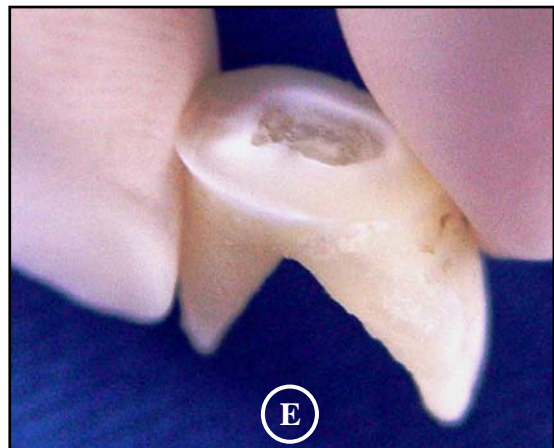
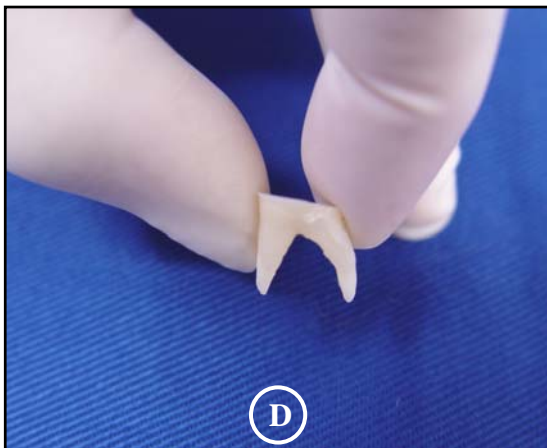
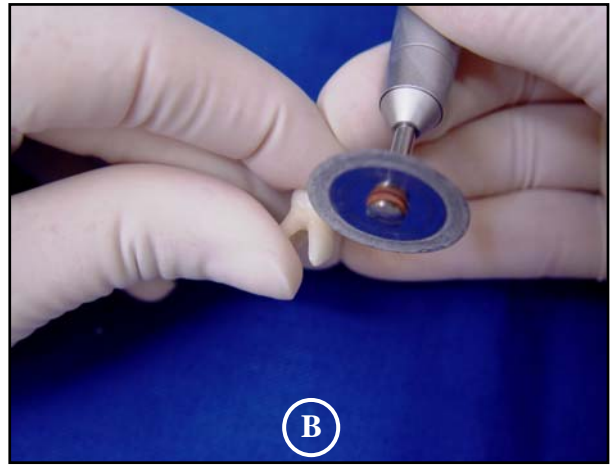
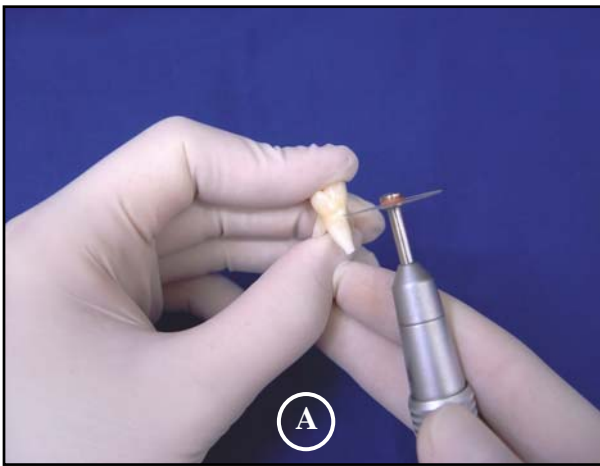
**A** - Secção dos dentes decíduos utilizando disco diamantado (KG Sorensen, São Paulo, Brasil).

**B** - Secção dos dentes decíduos utilizando disco diamantado (KG Sorensen, São Paulo, Brasil)(vista aproximada).

**C** - Dentes decíduos extraídos com e sem as coroas.

**D** - Raiz seccionada.

**E** - Visualização dos canais radiculares após a secção.



**Figura 2 – Ilustrações da metodologia empregada nos capítulos 2 e 3.**

**A** - Lamparina, cera e espátula utilizada para impermeabilização dos ápices radiculares com cera utilidade.

**B / C** - Processo de impermeabilização dos ápices radiculares.

**D** - Ápices radiculares impermeabilizados.

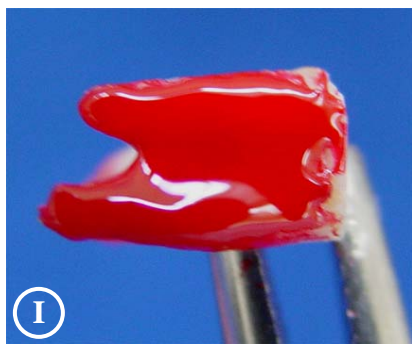
**E** – Cola à base de cianoacrilato (Super Bonder®, Brasil).

**F** - Processo de impermeabilização externa das raízes com o cianocrilato.

**G** - Verniz para unhas (Colorama®, Brasil).

**H** - Processo de impermeabilização externa das raízes com verniz para unhas (Colorama®, Brasil).

**I** - Raízes impermeabilizadas.



**Figura 3 - Sistema gerador de vibração ultrasônica utilizado, método de irrigação e preparo químico-mecânico.**

**A** - Aparelho de ultrassom (Gnatus®, Ribeirão Preto, Brazil).

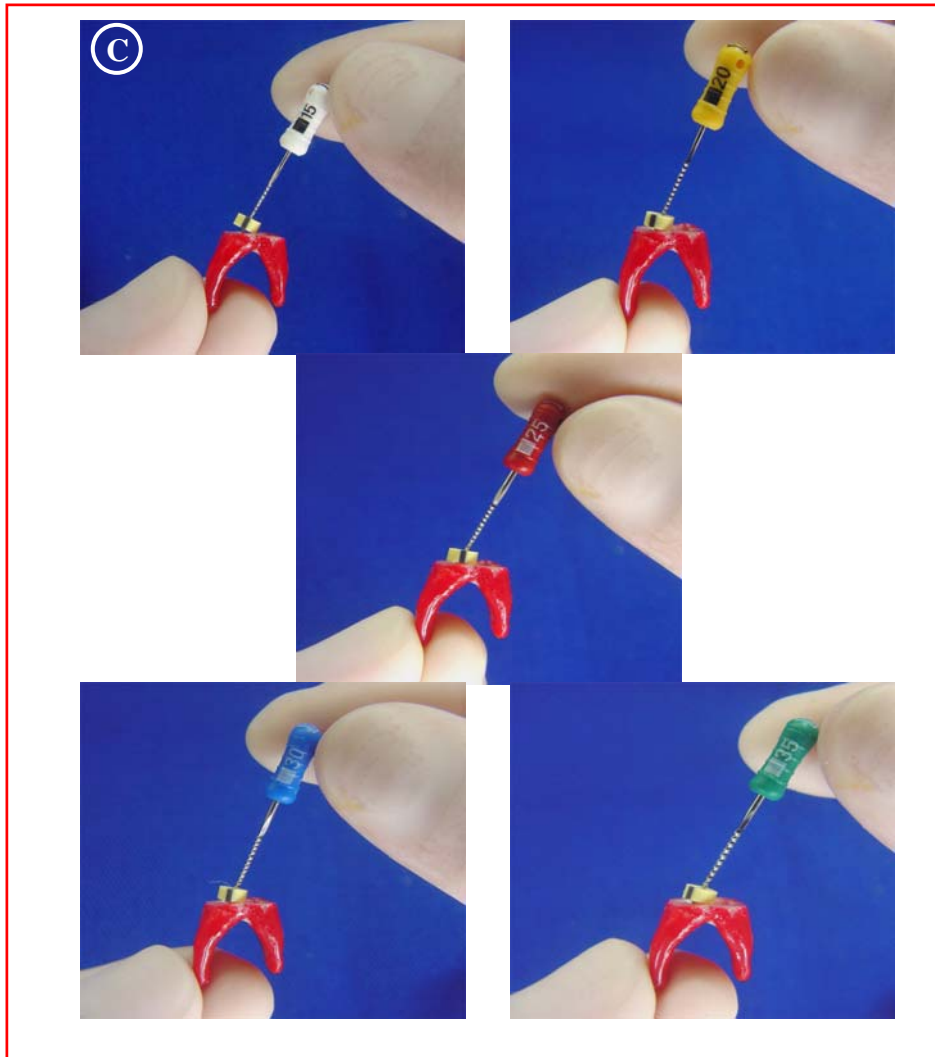
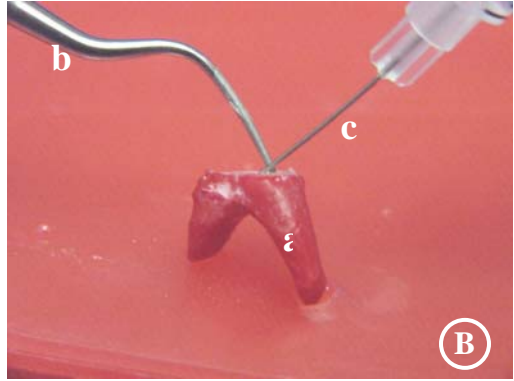
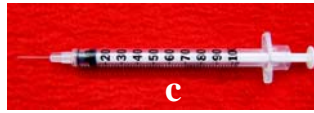
**B** - Realização simultânea da irrigação e vibração ultrasônica.

a) Amostra;

b) Ponteira do sistema de vibração ultrasônica;

c) Agulha utilizada para irrigação (12,7 mm x 0,33 calibre - Becton Dickinson and Company, EUA, Franklin Lakes, New Jersey);

**C** - Seqüência ilustrativa do preparo biomecânico (lima # 15 – 35 - Dentsply/Maillefer, Ballaigues, Switzerland).



#### **Figura 4 - Determinação do Índice de Permeabilidade.**

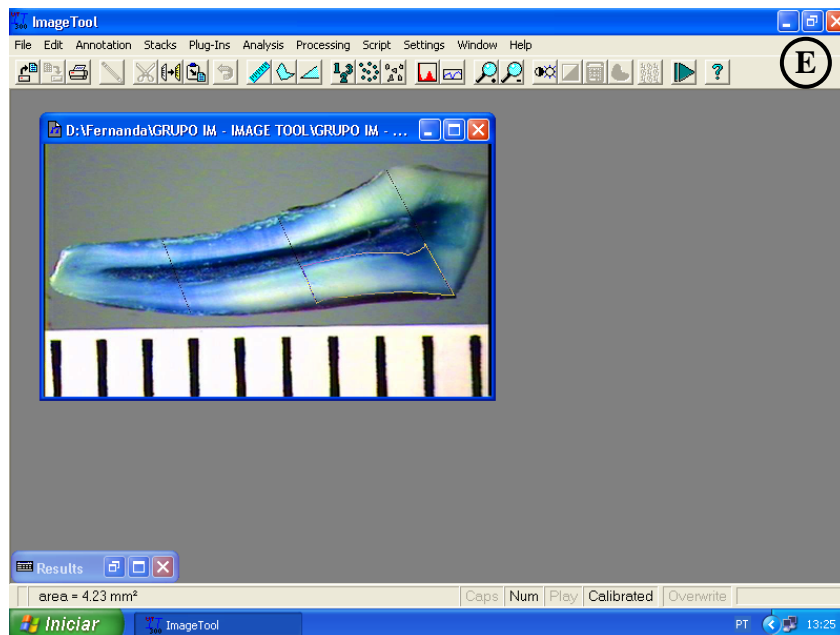
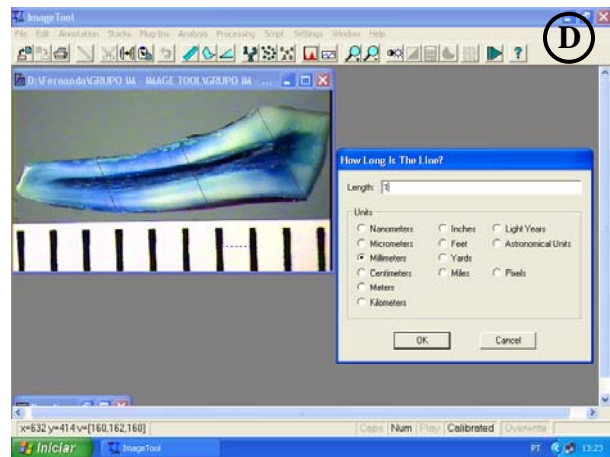
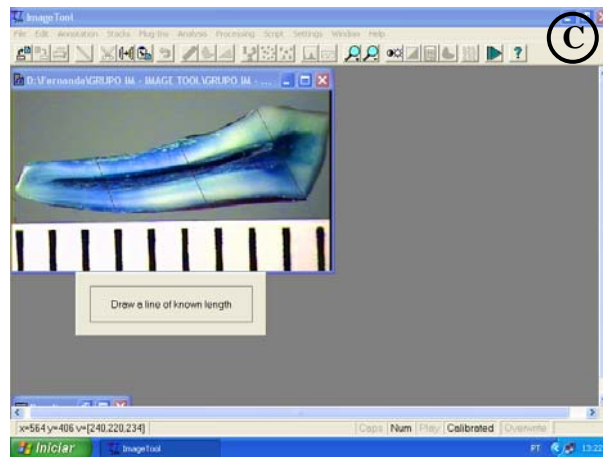
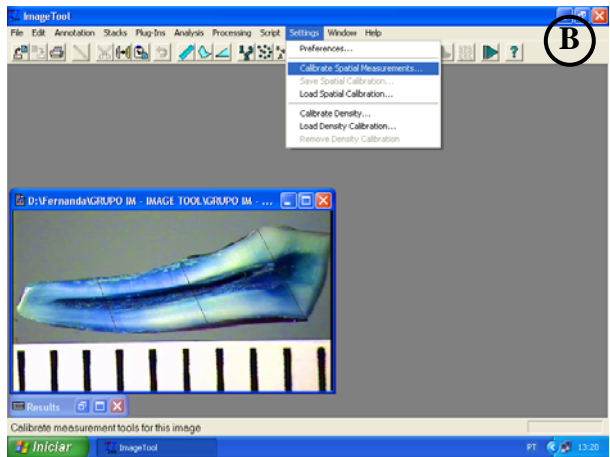
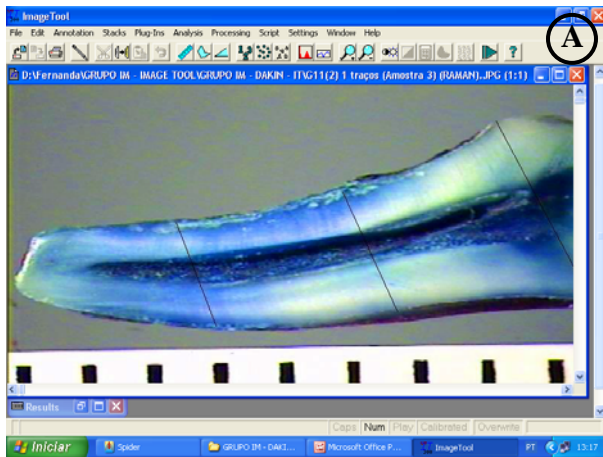
**A** - Software Image Tool 3.0 (Periodontology Department, University of Texas, Health Science Center at San Antonio, TX, USA), utilizado para determinar o índice de permeabilidade.

**B** - As medidas foram transformadas em milímetros (mm) acionando-se o comando de calibração de medidas do Programa.

**C** - Uma linha foi desenhada unindo as barras verticais presentes e utilizadas como referências nas imagens. O número 1 foi digitado na caixa específica que solicita o valor exato da medida utilizada como referência. A partir da calibração, qualquer medida realizada, foi automaticamente convertida em mm. Esse procedimento foi realizado para cada foto.

**D / E** - O comando “área” foi acionado e determinando-se as áreas não coradas e as coradas para o cálculo do índice de permeabilidade.

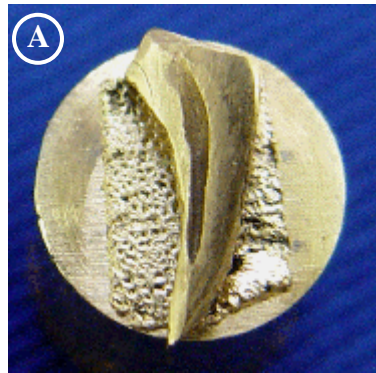




**Figura 5 - Microscopia Eletrônica de Varredura.**

**A** - Amostra metalizada.

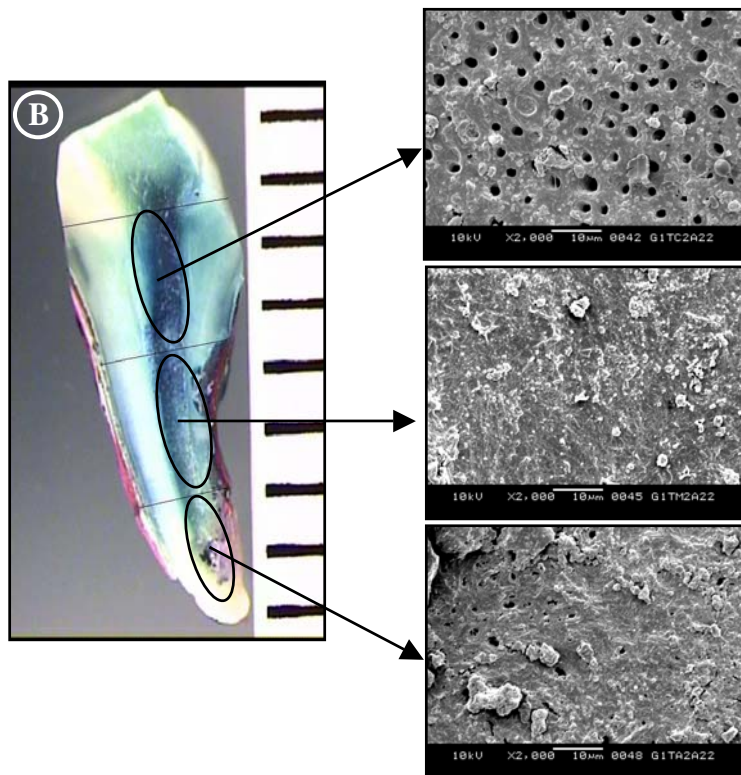
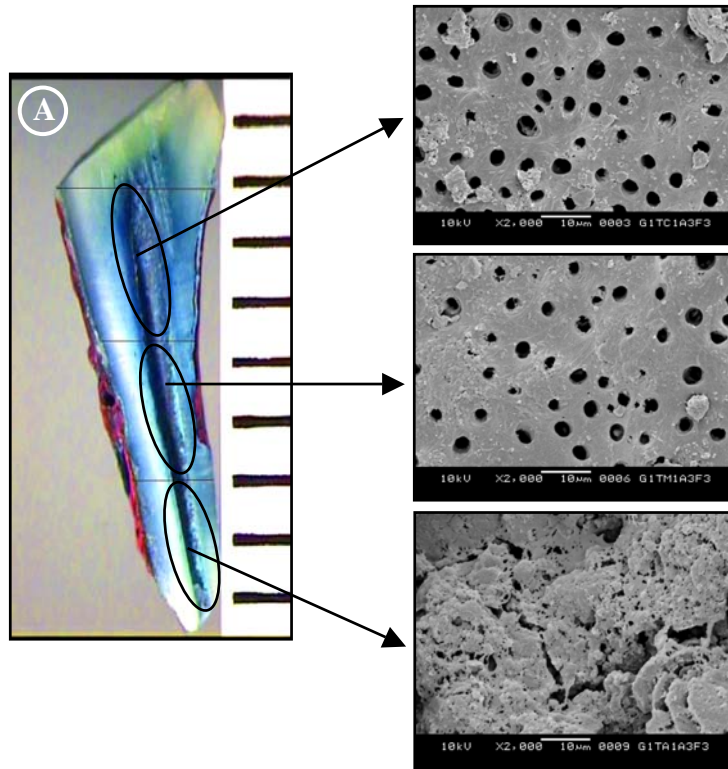
**B** - Microscópio eletrônico de varredura (JSM 5600LV, JEOL, Tokyo – Japan).



**Figura 6 - Fotografias digitalizadas dos espécimes preparados para avaliação do índice de permeabilidade e respectivas fotomicrografias (MEV) em 2000x de aumento, de acordo com a substância irrigadora e método de irrigação.**

**A** - Líquido de Dakin/ Irrigação Manual.

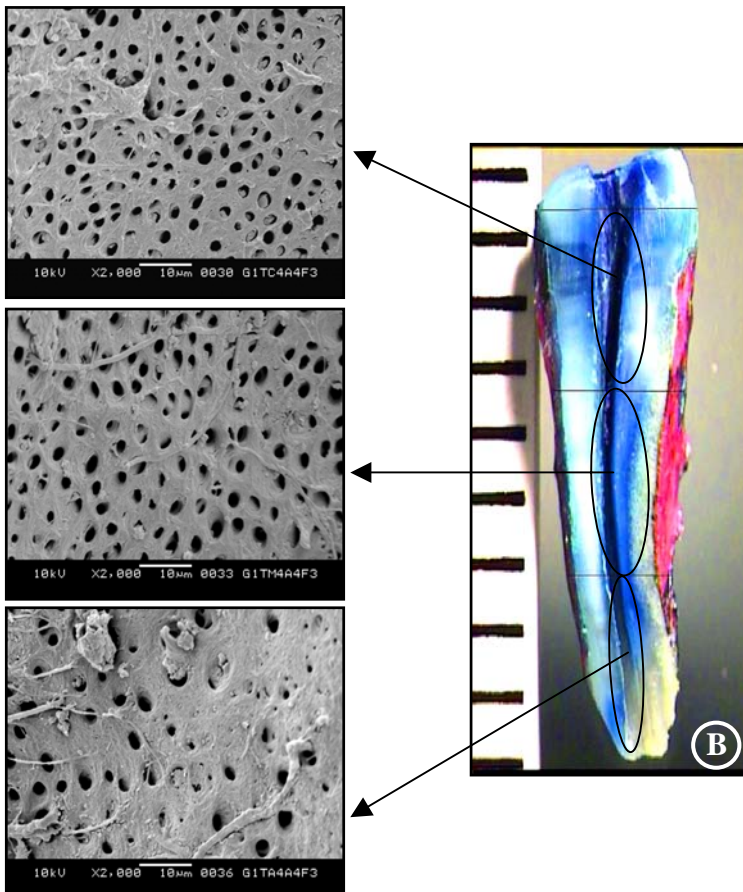
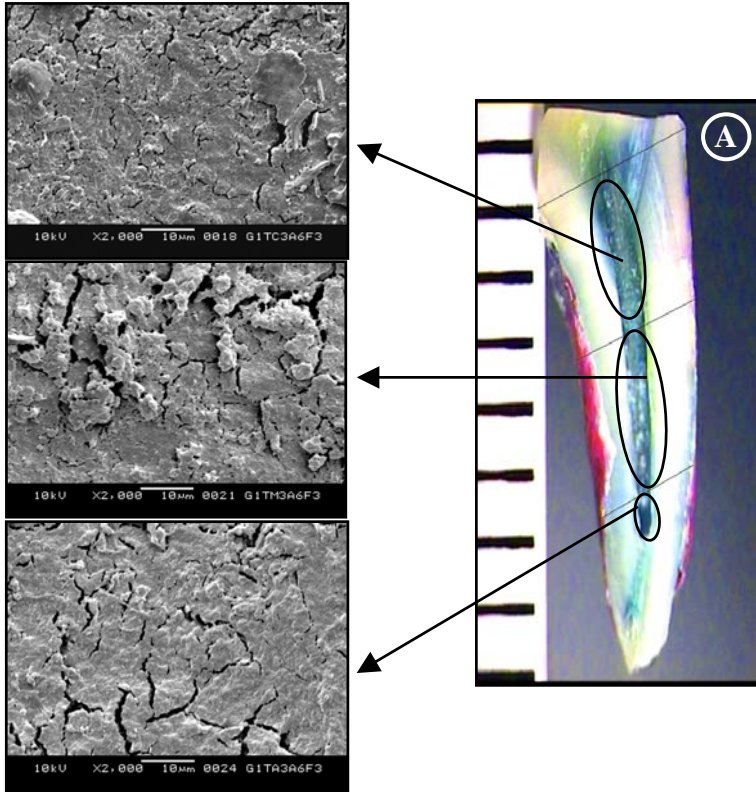
**B** - Líquido de Dakin associado ao peróxido de hidrogênio/ Irrigação Manual.



**Figura 7 - Fotografias digitalizadas dos espécimes preparados para avaliação do índice de permeabilidade e respectivas fotomicrografias (MEV) em 2000x de aumento, de acordo com a substância irrigadora e método de irrigação.**

**A** - Clorexidina gel/ Irrigação Manual.

**B** - Solução salina/ Irrigação Manual.

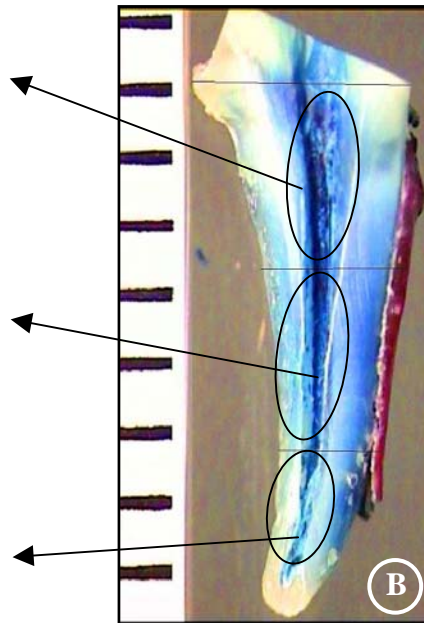
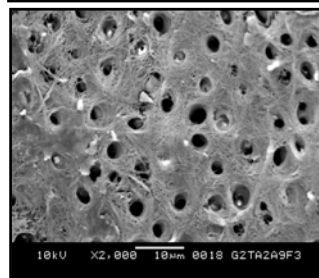
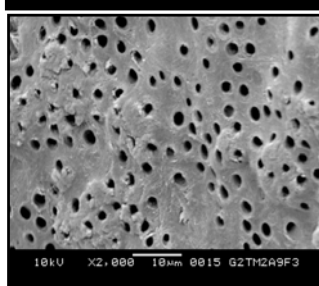
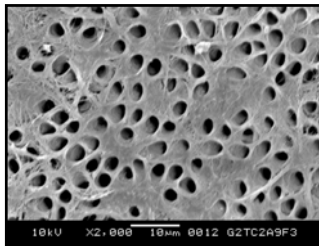
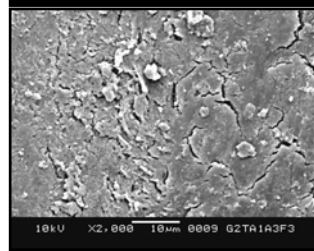
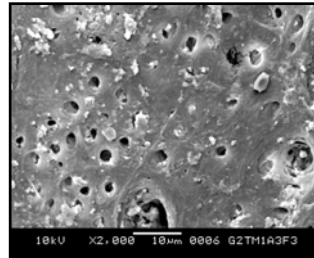
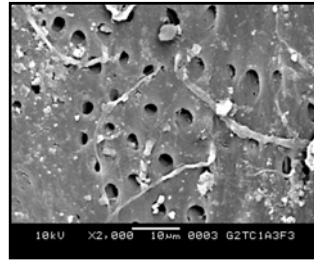
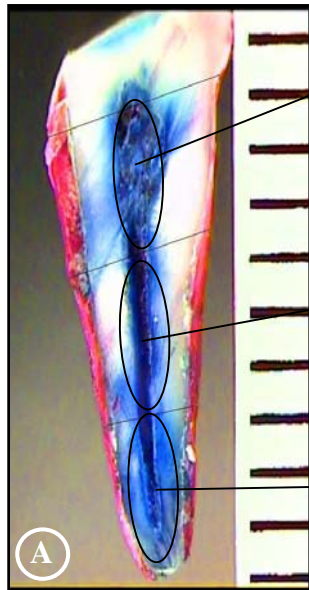


**Figura 8 - Fotografias digitalizadas dos espécimes preparados para avaliação do índice de permeabilidade e respectivas fotomicrografias (MEV) em 2000x de aumento, de acordo com a substância irrigadora e método de irrigação.**

**A** - Líquido de Dakin/ Irrigação Ultrasônica.

**B** - Líquido de Dakin associado ao peróxido de hidrogênio/ Irrigação Ultrasônica.

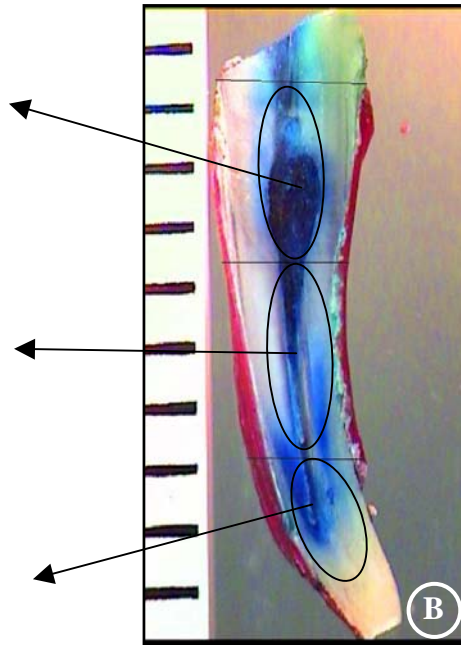
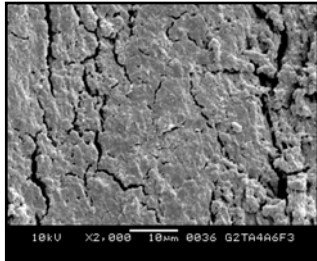
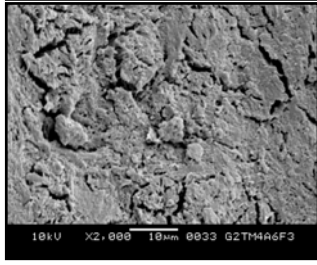
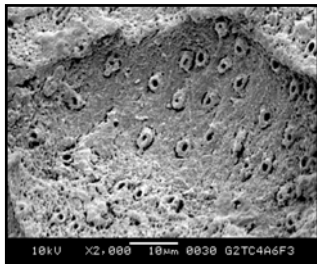
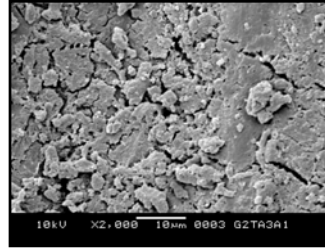
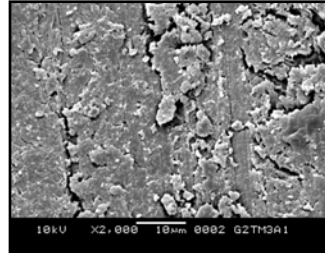
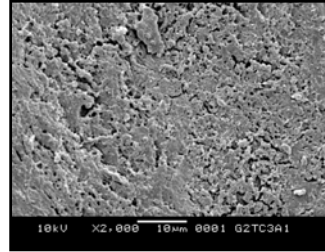
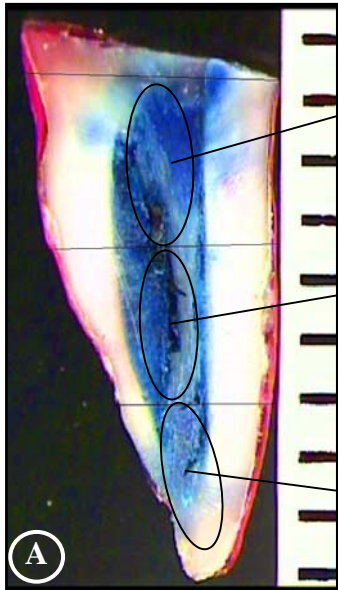




**Figura 9 - Fotografias digitalizadas dos espécimes preparados para avaliação do índice de permeabilidade e respectivas fotomicrografias (MEV) em 2000x de aumento, de acordo com a substância irrigadora e método de irrigação.**

**A - Clorexidina gel/ Irrigação Ultrasônica.**

**B - Solução salina/ Irrigação Ultrasônica.**



**Terço Cervical** – Variável % transformada segundo arco seno da raiz de x/100

**Quadro de Análise de Variância**

Causas da Variação	G.L.	S.Q	Q.M.	Valor F	Prob >F
Irrigação	1	2043.7784279	20437784279	6.5139	0.01290
Tratamento	3	6684.2014449	2228.0671483	7.1012	0.00063
Irr*Trat	3	14914.6826250	4971.5608750	15.8452	0.00001
Resíduo	56	17570.4449407	313.7579454		
Total	63	41007.4859149			

**Teste de Tukey para médias de Irrigação\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias Originais	5%	1%
1	1	Manual	32	53.075147	63.907908	a	A
2	2	Ultra-som	31	41.739803	44.322168	b	A

**Teste de Tukey para médias de Irrigação dentro do fator Dakin (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Manual	8	62.039280	78.016467	a	A
2	2	Ultra-som	9	38.495544	38.744880	b	B

**Teste de Tukey para médias de Irrigação dentro do fator DHP (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Manual	9	67.479416	85.329938	a	A
2	2	Ultra-som	7	19.580631	11.231422	b	B

**Teste de Tukey para médias de Irrigação dentro do fator Clorexidina (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Ultra-som	8	50.507130	59.552677	a	A
2	2	Manual	8	14.381052	6.168745	b	B

**Teste de Tukey para médias de Irrigação dentro do fator Soro Fisiológico (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Manual	7	66.297188	83.840198	a	A
2	2	Ultra-som	8	56.523186	69.573813	a	A

**Teste de Tukey para médias de Tratamento dentro do fator Manual (Irrigação)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	2	DHP	9	67.479416	85.329938	a	A
2	4	Soro	7	66.297188	83.840198	a	A
3	1	Dakin	8	62.039280	78.016467	a	A
4	3	Clorexidina	8	14.381052	6.168745	b	B

**Teste de Tukey para médias de Tratamento dentro do fator Ultra-som (Irrigação)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	4	Soro	8	56.523186	69.573813	a	A
2	3	Clorexidina	8	50.507130	59.552677	a	A
3	1	Dakin	9	38.495544	38.744880	ab	AB
4	2	DHP	7	19.580631	11.231422	b	B

\*Médias seguidas por letras distintas diferem entre si ao nível de significância indicado

**Terço Médio** – Variável % transformada segundo arco seno da raiz de x/100

**Quadro de Análise de Variância**

Causas da Variação	G.L.	S.Q	Q.M.	Valor F	Prob >F
Irrigação	1	3236.6067988	3236.6067988	4.6601	0.03308
Tratamento	3	3729.6899779	1243.2299926	1.7900	0.15825
Irr*Trat	3	3291.1115033	1097.0371678	1.5795	0.20327
Resíduo	56	38894.1694027	694.5387393		
Total	63	49374.3655600			

**Teste de Tukey para médias de Irrigação\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias Originais	5%	1%
1	1	Manual	32	47.063062	53.597623	a	A
2	2	Ultra-som	32	32.798359	29.342178	b	A

**Teste de Tukey para médias de Irrigação dentro do fator Dakin (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Manual	8	52.722260	63.315270	a	A
2	2	Ultra-som	9	25.960127	19.162122	b	A

**Teste de Tukey para médias de Irrigação dentro do fator DHP (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Manual	9	61.181274	76.763752	a	A
2	2	Ultra-som	7	34.411503	31.937498	b	A

**Teste de Tukey para médias de Irrigação dentro do fator Clorexidina (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Ultra-som	8	32.000851	28.082784	a	A
2	2	Manual	8	24.101551	16.675399	a	A

**Teste de Tukey para médias de Irrigação dentro do fator Soro Fisiológico (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Manual	7	49.321987	57.514720	a	A
2	2	Ultra-som	8	38.676834	39.053392	a	A

**Teste de Tukey para médias de Tratamento dentro do fator Manual (Irrigação)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	2	DHP	9	61.181274	76.763752	a	A
2	1	Dakin	8	52.722260	63.315270	ab	A
3	4	Soro	7	49.321987	57.514720	ab	A
4	3	Clorexidina	8	24.101551	16.675399	b	A

**Teste de Tukey para médias de Tratamento dentro do fator Ultra-som (Irrigação)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	4	Soro	8	38.676834	39.053392	a	A
2	2	DHP	7	34.411503	31.937498	a	A
3	3	Clorexidina	8	32.000851	28.082784	a	A
4	1	Dakin	9	25.960127	19.162122	a	B

\*Médias seguidas por letras distintas diferem entre si ao nível de significância indicado

**Terço Apical** – Variável % transformada segundo arco seno da raiz de x/100

**Quadro de Análise de Variância**

Causas da Variação	G.L.	S.Q	Q.M.	Valor F	Prob >F
Irrigação	1	10.3580165	10.3580165	0.0127	0.90688
Tratamento	3	2997.5098595	999.1699532	1.2228	0.30959
Irr*Trat	3	192.4498392	64.1499464	0.0785	0.97060
Resíduo	56	45759.7225489	817.1379027		
Total	63	48959.7151038			

**Teste de Tukey para médias de Irrigação\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias Originais	5%	1%
1	1	Manual	32	24.7722773	17.557172	a	A
2	2	Ultra-som	31	23.965305	16.498499	a	A

**Teste de Tukey para médias de Irrigação dentro do fator Dakin (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Manual	8	35.291359	33.377727	a	A
2	2	Ultra-som	9	30.417860	25.634239	a	B

**Teste de Tukey para médias de Irrigação dentro do fator DHP (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Manual	9	29.069099	23.606396	a	A
2	2	Ultra-som	7	25.719381	18.832469	a	B



**Teste de Tukey para médias de Irrigação dentro do fator Clorexidina (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Ultra-som	8	15.863751	7.472063	a	A
2	2	Manual	8	12.794914	4.904540	a	B

**Teste de Tukey para médias de Irrigação dentro do fator Soro Fisiológico (tratamento)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	2	Ultra-som	8	23.920656	16.440691	a	A
2	1	Manual	7	21.585175	13.533864	a	A

**Teste de Tukey para médias de Tratamento dentro do fator Manual (Irrigação)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Dakin	8	35.291359	33.377727	a	A
2	2	DHP	9	29.069099	23.606396	a	A
3	4	Soro	7	21.585175	13.533864	a	A
4	3	Clorexidina	8	12.794914	4.904540	a	A

**Teste de Tukey para médias de Tratamento dentro do fator Ultra-som (Irrigação)\***

Ordem	Tratamento	Nome	Repetição	Médias Aj.	Médias originais	5%	1%
1	1	Dakin	8	30.417860	25.634239	a	A
2	2	DHP	9	25.719381	18.832469	a	A
3	4	Soro	7	23.920656	16.440691	a	A
4	3	Clorexidina	8	15.863751	7.472063	a	A

\*Médias seguidas por letras distintas diferem entre si ao nível de significância indicado

## Irrigação Manual - Terço Cervical

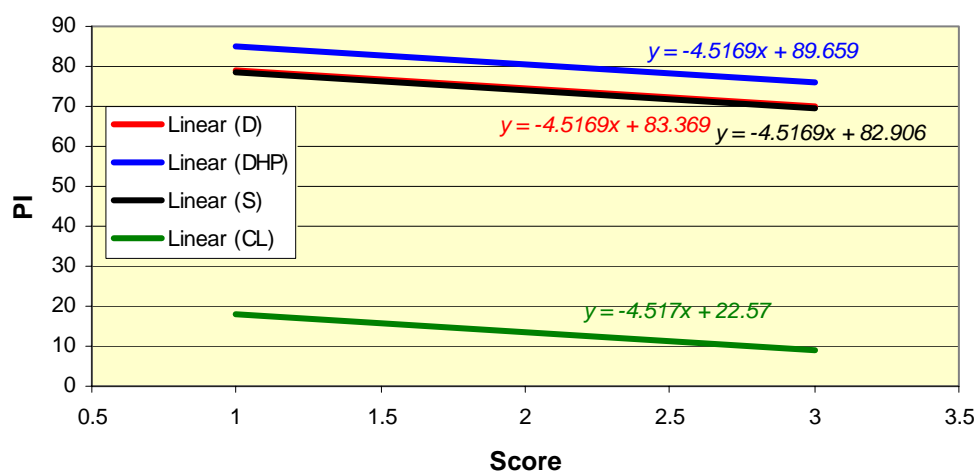
### Análise de variância do modelo de regressão linear com variável Dummy

**Tabela 1.** Quadro de análise de variância do modelo de regressão proposto.

	Causa de variação	GL	Soma de quadrados	Quadrados Médios	Valor F	Pr > F
	Modelo	4	25771	6442.72062	11.50	<.0001
	Resíduo	27	15124	560.13384		
	Total corrigido	31	40894			
Root MSE		23.66715	R-Square	0.6302		
Dependent Mean		58.82688	Adj R-Sp	0.5754		
Coeff Var		40.23186				

**Tabela 2.** Parâmetros estimados e teste t para hipótese de que cada parâmetro não difere significativamente de zero.

Variável	Rótulo	GL	Parâmetro estimado	Erro Padrão	Valor t	Pr >  t
Intercept	Intercept	1	83.36862	16.76821	4.97	<.0001
DHP	Dummy DHP	1	6.29025	11.50470	0.55	0.5890
CL	Dummy Clorexidina 2%	1	-60.79826	11.92246	-5.10	<.0001
S	Dummy Soro Fisiológico	1	-0.46286	12.80617	-0.04	0.9714
escore	Escore	1	-4.51695	5.81249	-0.78	0.4438



**Figura 1.** Modelo de regressão para o estudo de índice de permeabilidade em função do Escore com variáveis Dummy representando o efeito dos 4 tratamentos.

## Irrigação Manual - Terço Médio

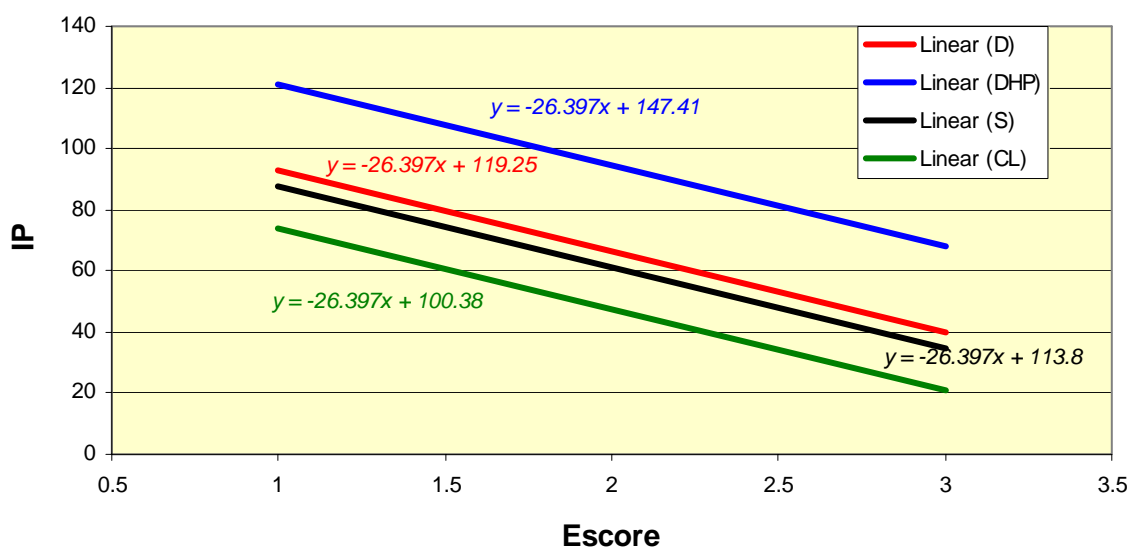
### Análise de variância do modelo de regressão linear com variável Dummy

**Tabela 3.** Quadro de análise de variância do modelo de regressão proposto.

	Causa de variação	GL	Soma de quadrados	Quadrados Médios	Valor F	Pr > F
	Modelo	4	15858	3964.38197	4.26	0.0084
	Resíduo	27	25111	930.04251		
	Total corrigido	31	40969			
Root MSE	30.49660	R-Square	0.3871			
Dependent Mean	51.96781	Adj R-Sp	0.2963			
Coeff Var	58.68363					

**Tabela 4.** Parâmetros estimados e teste t para hipótese de que cada parâmetro não difere significativamente de zero.

Variável	Rótulo	GL	Parâmetro estimado	Erro Padrão	Valor t	Pr >  t
Intercept	Intercept	1	119.25263	26.35925	4.52	0.0001
DHP	Dummy DHP	1	28.15882	15.70605	1.79	0.0842
CL	Dummy Clorexidina 2%	1	-18.87504	16.06714	-1.17	0.2503
S	Dummy Soro Fisiológico	1	-5.45138	15.80937	-0.34	0.7329
escore	Escore	1	-26.39742	10.12765	-2.61	0.0147



**Figura 2.** Modelo de regressão para o estudo de índice de permeabilidade em função do Escore com variáveis Dummy representando o efeito dos 4 tratamentos.

## Irrigação Manual - Terço Apical

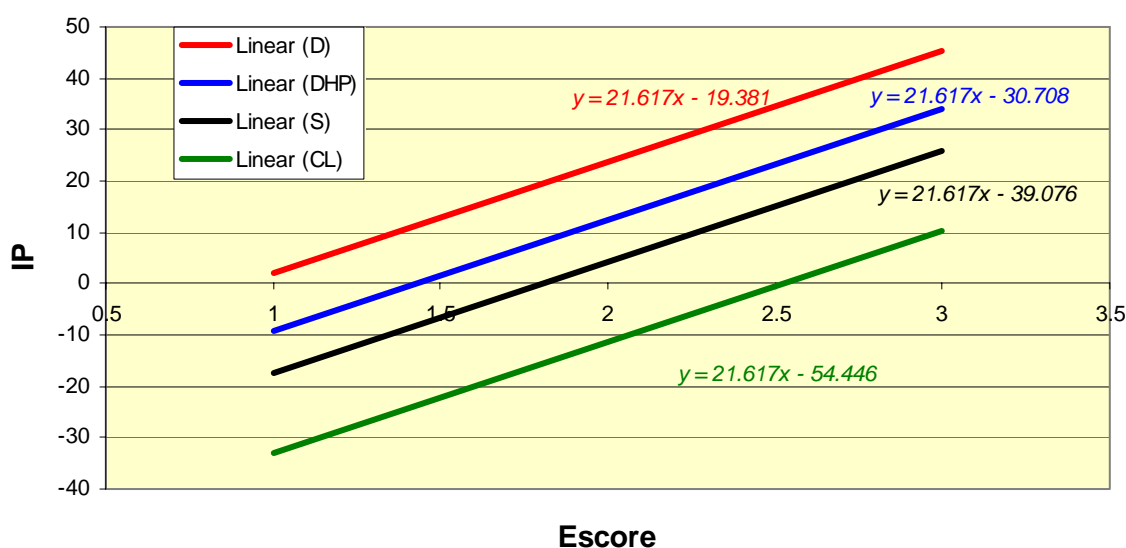
### Análise de variância do modelo de regressão linear com variável Dummy

**Tabela 5.** Quadro de análise de variância do modelo de regressão proposto.

	Causa de variação	GL	Soma de quadrados	Quadrados Médios	Valor F	Pr > F
	Modelo	4	6746.10551	1686.52638	1.61	0.2003
	Resíduo	27	28270	1047.02812		
	Total corrigido	31	35016			
Root MSE	32.35781	R-Square	0.1927			
Dependent Mean	25.83094	Adj R-Sp	0.0731			
Coeff Var	125.26767					

**Tabela 6.** Parâmetros estimados e teste t para hipótese de que cada parâmetro não difere significativamente de zero.

Variável	Rótulo	GL	Parâmetro estimado	Erro Padrão	Valor t	Pr >  t
Intercept	Intercept	1	-19.38143	51.17679	-0.38	0.7079
DHP	Dummy DHP	1	-11.32648	15.72492	-0.72	0.4775
CL	Dummy Clorexidina 2%	1	-35.06457	16.32362	-2.15	0.0408
S	Dummy Soro Fisiológico	1	-19.69407	17.55549	-1.12	0.2718
escore	Escore	1	21.61658	17.35016	1.25	0.2235



**Figura 3.** Modelo de regressão para o estudo de índice de permeabilidade em função do Escore com variáveis Dummy representando o efeito dos 4 tratamentos.

## Irrigação Ultrasônica - Terço Cervical

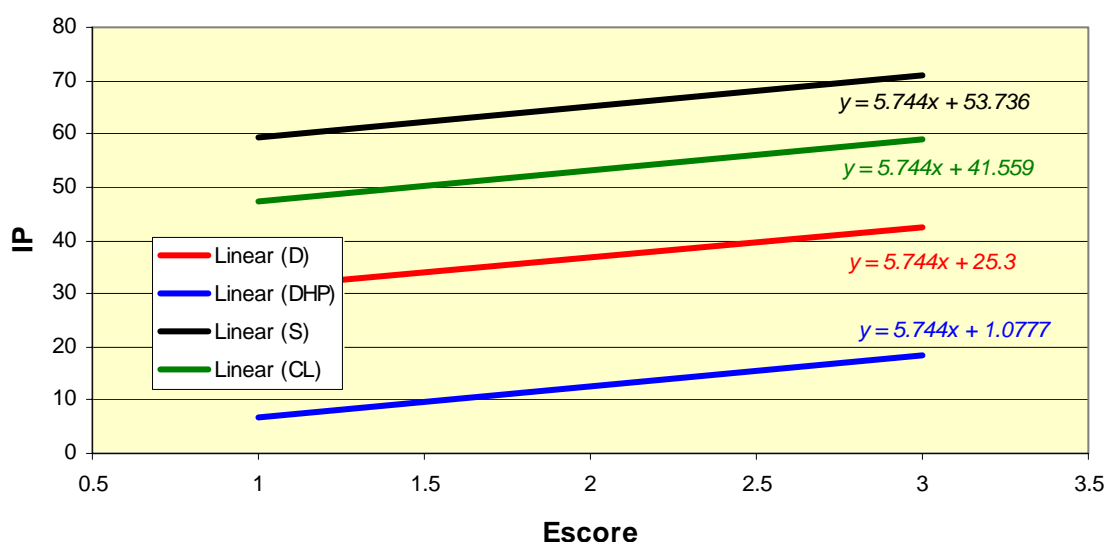
### Análise de variância do modelo de regressão linear com variável Dummy

**Tabela 7.** Quadro de análise de variância do modelo de regressão proposto.

	Causa de variação	GL	Soma de quadrados	Quadrados Médios	Valor F	Pr > F
	Modelo	4	11259	2814.71945	6.27	0.0011
	Resíduo	27	12112	448.59839		
	Total corrigido	31	23371			
Root MSE	21.18014	R-Square	0.4817			
Dependent Mean	45.89438	Adj R-Sp	0.4050			
Coeff Var	46.14975					

**Tabela 8.** Parâmetros estimados e teste t para hipótese de que cada parâmetro não difere significativamente de zero.

Variável	Rótulo	GL	Parâmetro estimado	Erro Padrão	Valor t	Pr >  t
Intercept	Intercept	1	25.30021	17.45009	1.45	0.1586
DHP	Dummy DHP	1	-24.22251	10.81818	-2.24	0.0336
CL	Dummy Clorexidina 2%	1	16.25903	10.91206	1.49	0.1478
S	Dummy Soro Fisiológico	1	28.43628	10.50086	2.71	0.0116
escore	Escore	1	5.74400	6.52832	0.88	0.3867



**Figura 4.** Modelo de regressão para o estudo de índice de permeabilidade em função do Escore com variáveis Dummy representando o efeito dos 4 tratamentos.

## Irrigação Ultrasônica - Terço Médio

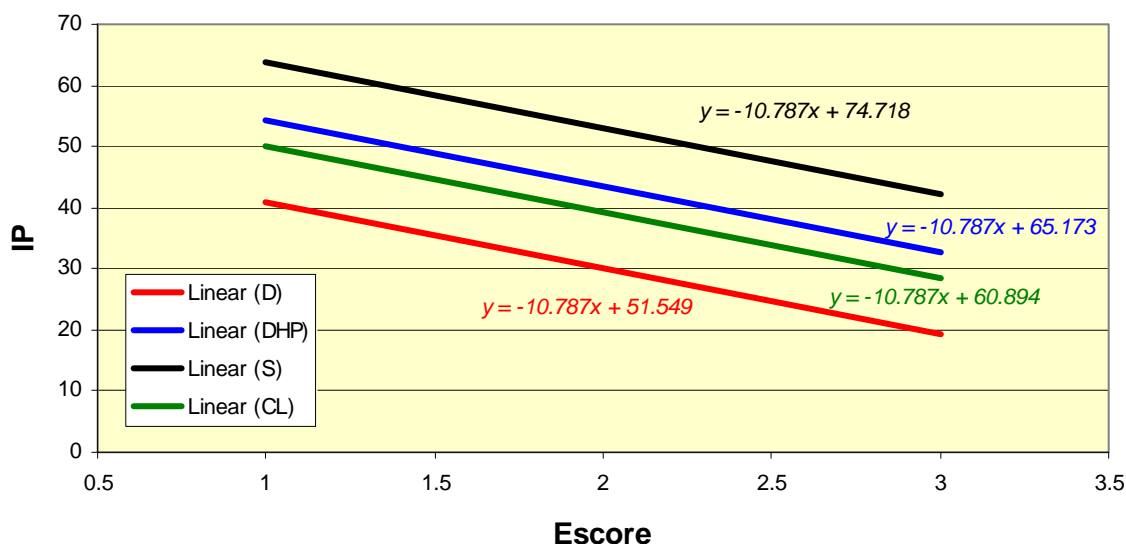
### Análise de variância do modelo de regressão linear com variável Dummy

**Tabela 9.** Quadro de análise de variância do modelo de regressão proposto.

	Causa de variação	GL	Soma de quadrados	Quadrados Médios	Valor F	Pr > F
	Modelo	4	2580.89517	645.22379	0.76	0.5576
	Resíduo	27	22788	843.98908		
	Total corrigido	31	25369			
Root MSE	29.05149	R-Square	0.1017			
Dependent Mean	32.99250	Adj R-Sp	-0.0313			
Coeff Var	88.05483					

**Tabela 10.** Parâmetros estimados e teste t para hipótese de que cada parâmetro não difere significativamente de zero.

Variável	Rótulo	GL	Parâmetro estimado	Erro Padrão	Valor t	Pr >  t
Intercept	Intercept	1	51.54865	26.38421	1.95	0.0612
DHP	Dummy DHP	1	13.62402	14.71972	0.93	0.3629
CL	Dummy Clorexidina 2%	1	9.34517	14.23946	0.66	0.5172
S	Dummy Soro Fisiológico	1	23.16949	14.74767	1.57	0.1278
escore	Escore	1	-10.78730	9.60371	-1.12	0.2712



**Figura 5.** Modelo de regressão para o estudo de índice de permeabilidade em função do Escore com variáveis Dummy representando o efeito dos 4 tratamentos.

## Irrigação Ultrasônica - Terço Apical

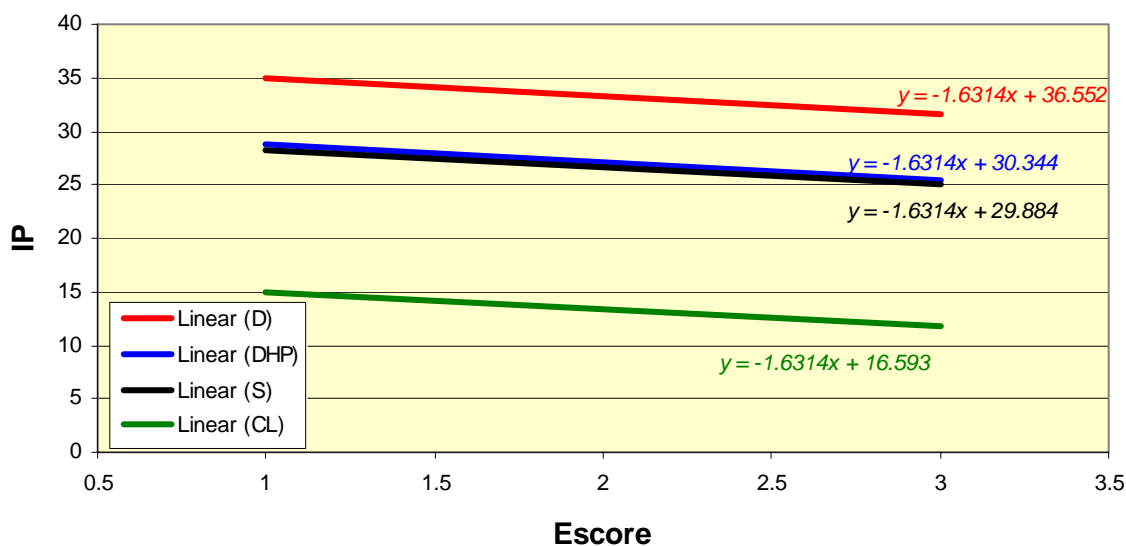
### Análise de variância do modelo de regressão linear com variável Dummy

**Tabela 9.** Quadro de análise de variância do modelo de regressão proposto.

	Causa de variação	GL	Soma de quadrados	Quadrados Médios	Valor F	Pr > F
	Modelo	4	1720.46213	430.11553	0.40	0.8034
	Resíduo	27	28676	1062.06920		
	Total corrigido	31	30396			
Root MSE	32.58940	R-Square	0.0566			
Dependent Mean	23.69406	Adj R-Sp	-0.0832			
Coeff Var	137.54249					

**Tabela 10.** Parâmetros estimados e teste t para hipótese de que cada parâmetro não difere significativamente de zero.

Variável	Rótulo	GL	Parâmetro estimado	Erro Padrão	Valor t	Pr >  t
Intercept	Intercept	1	36.55206	105.08163	0.35	0.7307
DHP	Dummy DHP	1	-6.20778	16.42352	-0.38	0.7084
CL	Dummy Clorexidina 2%	1	-19.95921	16.42352	-1.22	0.2348
S	Dummy Soro Fisiológico	1	-6.66778	15.83560	-0.42	0.6770
escore	Escore	1	1-63143	34.83954	-0.05	0.9630



**Figura 6.** Modelo de regressão para o estudo de índice de permeabilidade em função do Escore com variáveis Dummy representando o efeito dos 4 tratamentos.