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The influence of the irrigating solution on the percentage of dentinal tubule sealer penetration: Evaluation with Rhodamine B

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Abstract:

Introduction: The main objectives of endodontic therapy are the complete removal of residual pulpal tissue, the elimination of bacteria from the root canal system and the prevention of recontamination after the treatment that could lead to unsuccessful outcomes. Chemical irrigants such as Sodium Hypochlorite or Chlorhexidine are needed to aid in the debridement of the root canals. Furthermore, the use of chelating agents has been advocated. Sealer penetration into the dentinal tubules could influence the sealing ability of the root filling considering that an increase of the contact surface between dentin and filling material is accompanied by an improvement of sealability. Additionally, sealer penetration can contribute to an antimicrobial effect in the tubules. In search for endodontic sealers that combined the ideal properties, new materials have been proposed such as MTA Fillapex®.

Aim: The purpose of this study was to investigate and compare the percentage of sealer penetration into dentinal tubules using different endodontic irrigating solutions under *in vitro* conditions.

Methods: twenty-nine extracted human single-rooted teeth were divided into 3 groups according to the main irrigating solution used: (1) the Sodium Hypochlorite group: 3,0% Sodium Hypochlorite + 17% Ethylenediamine tetraacetic acid, (2) the Chlorhexidine group: 2,0% Chlorhexidine + 17% Ethylenediamine tetraacetic acid and (3) the Control group: Saline Solution + 17% Ethylenediamine tetraacetic acid. All teeth were obturated using cold lateral condensation technique with gutta-percha and MTA Fillapex® sealer labeled with rhodamine B. The teeth were sectioned at the apical and middle thirds. Total percentage of sealer penetration was measured using confocal laser scanning microscopy.

Results: The Kruskal-Wallis analysis results showed that there was no significant difference in the percentage of sealer penetration among all groups in all sections ($p < 0{,}05$). The Group G1 and the Control Group obtained a higher mean percentage of sealer penetration in the apical section (Graphic 4). The Group G2 obtained a higher mean percentage of sealer penetration in the middle section.

Conclusions: The results of this study suggest that there appears to be no significant difference in the percentage of sealer penetration between the irrigants tested, when smear layer was removed with 17% EDTA ($p = 0.05$).

Keywords:

Irrigating solutions, Sodium Hypochlorite, Chlorhexidine, rhodamine B, sealer penetration, confocal, MTA Fillapex

1.1 Introduction

The main objectives of endodontic therapy are the complete removal of residual pulpal tissue, the elimination of bacteria from the root canal system and the prevention of recontamination after the treatment that could lead to unsuccessful outcomes.(1-12)

The complex anatomy of the root canal systems may limit the mechanical action of the endodontic instruments,(2, 7, 12-23) thus, chemical irrigants are needed to aid in the debridement of the canals.(7, 12, 17, 20, 22, 24)

Consequently, the objectives of irrigation should be mechanical, thus involving flushing out debris, lubricating the canal and dissolving organic and inorganic tissue, as well as biological due to their antimicrobial effect.(18)

The list of the ideal properties of an endodontic irrigating solution is extensive and was outlined by several authors.(8, 12, 18, 22, 23, 25-28) Currently, no solution meets all these characteristics.(8, 29, 30)

Sodium hypochlorite (NaOCl) is the most commonly used irrigant.(5, 7-9, 12, 19, 23, 26, 29-50) It has numerous advantages: excellent antibacterial agent, mechanical flushing of debris from the canal, ability to dissolve vital and necrotic tissues, antimicrobial action, lubricating action, inexpensive, long-shelf life and easily available.(5, 7, 8, 12, 25, 28, 33, 43, 49, 51-56) This solution is, however, highly irritating to periapical tissues, especially at high concentration(7, 9, 29, 34, 44, 47, 49-51, 56-58) and doesn't remove the inorganic component of the smear layer.(12, 19, 23, 28, 30, 32, 46, 48, 59-65)

Chlorhexidine gluconate (CHx) is an effective oral antimicrobial agent active against gram-positive and gram-negative bacteria as well as yeasts.(18, 23, 33-35, 37, 47, 52, 66-70) This agent holds substantivity(7, 9, 17-19, 28, 41, 47, 50, 51, 58, 71) and has low grade of toxicity.(9, 18, 19, 47, 71, 72) Still, it is not a tissue solvent (7, 8, 17, 25, 28, 29, 47, 53, 71) and cannot remove the smear layer.(25, 53, 73)

The smear layer is an amorphous irregular layer containing inorganic debris and organic material that is formed as a result of the biomechanical preparation.(3, 5, 8, 11, 13, 22, 27, 33, 47, 61-65, 74-88) Although controversial, it is generally advocated that the smear layer should be removed prior to the insertion of the root canal filling material.(15, 33, 47, 60, 74, 76-78, 89-95) This is assumed to facilitate adaptation of the filling material to the canal walls, improve adhesion and enhance resistance to bacterial penetration.(5, 11, 15, 59-62, 65, 77-79, 84-86, 89, 90, 92, 93, 95-104)

To completely remove the smear layer, the use of irrigating solutions that can dissolve both its organic and inorganic components is required. However, no single solution is known to provide both effects alone, thus the use of chelating agents has been advocated.(33, 85, 92, 103, 105)

Ethylenediamine tetraacetic acid (EDTA) is a polyprotic acid whose sodium salts are noncolloidal organic agents that can form nonionic chelates with metallic ions. Its concentrations vary between 10% and 17%, and its pH is modified from its original value of 4 to values between 7 and 8 to increase its chelating capacity.(75, 105, 106)

Since it is not possible to sterilize the root canal or remove all debris, the goal of obturation is to eliminate leakage pathways from the coronal and apical directions and entomb remaining bacteria in the canal.(84, 107-109) Ingle and Bakland (2002)(110, 111) addressed the fact that inadequate filling of the root canal is one of the most important causes of endodontic failure. Numerous materials have been used for root filling, gutta-percha being the most commonly used.(110) Because gutta-percha does not bond spontaneously to the root canal walls, a sealer applied concurrently is generally used to achieve an impervious sealing.(10, 110, 112-114)

Sealer penetration into the tubules could influence the sealing ability of the root filling considering that an increase of the contact surface between dentin and filling material is accompanied by an improvement of sealability.(115, 116) Furthermore, sealer penetration can contribute to an antimicrobial effect in the tubules, which increases when in closer contact with the microbes.(116)

In search for endodontic sealers that combined the ideal properties, new materials have been proposed. MTA Fillapex® (Angelus, Londrina, Brazil) is composed of silicate resin, resin diluent, natural resin, bismuth oxide, Epiphany/Resilon system, pigments and MTA (mineral trioxide aggregate).(117-121)

The aim of this study was to investigate and compare the percentage of dentinal tubule sealer penetration using different endodontic irrigating solutions under *in vitro* conditions.

1.2 Materials and Methods

Collection of teeth

Twenty-nine extracted human single-rooted teeth, with apex completely formed were used in this study. These teeth were stored in a 0,9 % sodium chloride solution containing 0,02% sodium azide at 4ºC until use, to prevent bacterial growth. Radiographs were exposed from facial and proximal views to ensure the presence of a single canal.

Root canal preparation and fillings

Subsequently, the crowns were sectioned with a high-speed burr and water spray, in order for all the roots to be approximately 15 mm long.

Apical patency was established with a K file, ISO size #10 (Dentsply Maillefer, CH-1338 Ballaigues, Switzerland) into the canal until tip was visible at the apical foramen. The working length was established at 1 mm short of the apex, using a K file size #15 (Dentsply Maillefer, CH-1338 Ballaigues, Switzerland).

The roots were randomly divided into two experimental groups of 10 teeth each and one control group of 9 teeth designated Control Group (Table I).

Group	Main Irrigating Solution
G1 (n=10)	3,0 % Sodium Hypoclorite
G2 (n=10)	2,0% Chlorhexidine Gluconate
Control (n=9)	Saline Solution

Table I: Samples division, groups and type of irrigant utilized

Root canal preparation was performed using ProTaper® nickel-titanium rotary instruments (Dentsply Maillefer, CH-1338 Ballaigues, Switzerland), with a crown-down technique. The handpiece was used with an electric engine (X-Smart, Dentisply Maillefer, CH-1338 Ballaigues, Switzerland) at 250 rpm. Apical patency was verified with a K file, ISO size #10 (Dentsply Maillefer, CH-1338 Ballaigues, Switzerland) throughout the instrumentation. All irrigation throughout the study was accomplished by using 3 mL endodontic irrigation syringes with 27 gauge endodontic needles (Kendall Monoject™, Tyco/Healthcare) at 3mm from total length. Instrumentation was completed with a F3 ProTaper® file up to the working length.

After the use of each instrument, the samples from the NaOCl group were irrigated using 10 mL of 3,0% NaOCl (CanalPro™, Coltène/Whaledent Inc., Cuyahoga Falls, OH 44223) as the main irrigating solution. Additionally, to remove the smear layer from all the samples it was used 5 mL of 17% EDTA (CanalPro™, Coltène/Whaledent Inc., Langenau/Germany) for 1 minute. Finally, the main irrigant was used again - 10 ml of 3,0% NaOCl.

The samples from the Group G2 and the Control Group were irrigated using the same protocol, only the main irrigating solution differed. In the Group G2, the samples were irrigated using 10 mL of 2,0% CHx (CanalPro™, Coltène/Whaledent Inc., Langenau/Germany) and in the Control Group the samples were irrigated with Saline Solution (SS). Before the final rinse with the main solution from each group, all the samples from every group were irrigated using 5 mL of 17% EDTA (Coltène/Whaledent Inc., Langenau/Germany, D-89122) for 1 minute.

Before and after the chelating agent was used, the samples were irrigated with 10 mL of saline solution to neutralize and prevent a possible reaction between the EDTA and the main irrigant utilized.

Before obturation, the root canals were dried with sterile paper points.

To facilitate fluorenscence under confocal laser microscopy, MTA Fillapex® sealer (Angelus, Londrina, PR, Brazil) was mixed with fluorescent Rhodamine B dye (Panreac®) to an approximate concentration of 0,1%.

The root canals were filled with cold lateral condensation technique, using a #30 spreader (Dentsply, Maillefer, Ballaigues, Switzerland) inserted 2 mm short of the working lengths and a master cone gutta-percha point, ISO size #30, (Dentsply, Maillefer, Ballaigues, Switzerland) with tug-back at the working length.

The apical section of the master gutta-percha cone was coated with a thin film of sealer and placed into the canal at full working length. Auxiliary size ISO #10, #15 and #20 points (Dentsply, Maillefer, Ballaigues, Switzerland) coated with sealer were placed in the voids created by the spreader. This was repeated until the spreader could not penetrate more than 1–2 mm into the canal orifice. The gutta-percha was excised with a hot instrument at the canal orifice and final compaction was completed with vertical pressure with a #60 hand plugger (Dentsply, Maillefer, Ballaigues, Switzerland).

The root canal orifices were sealed using Cavit® (3M, ESPE AG, Seefeld, Germany).

Sectioning

The filled root segments were stored for 1 week at 37ºC and 100% relative humidity to allow the sealer to set completely.

One-millimetre transverse sections were cut from the middle and apical thirds of each root resulting in 6 distinct groups as explained by Graphic 1 (G1: middle and apical sections, G2: middle and apical sections, and Control Group: middle and apical sections).

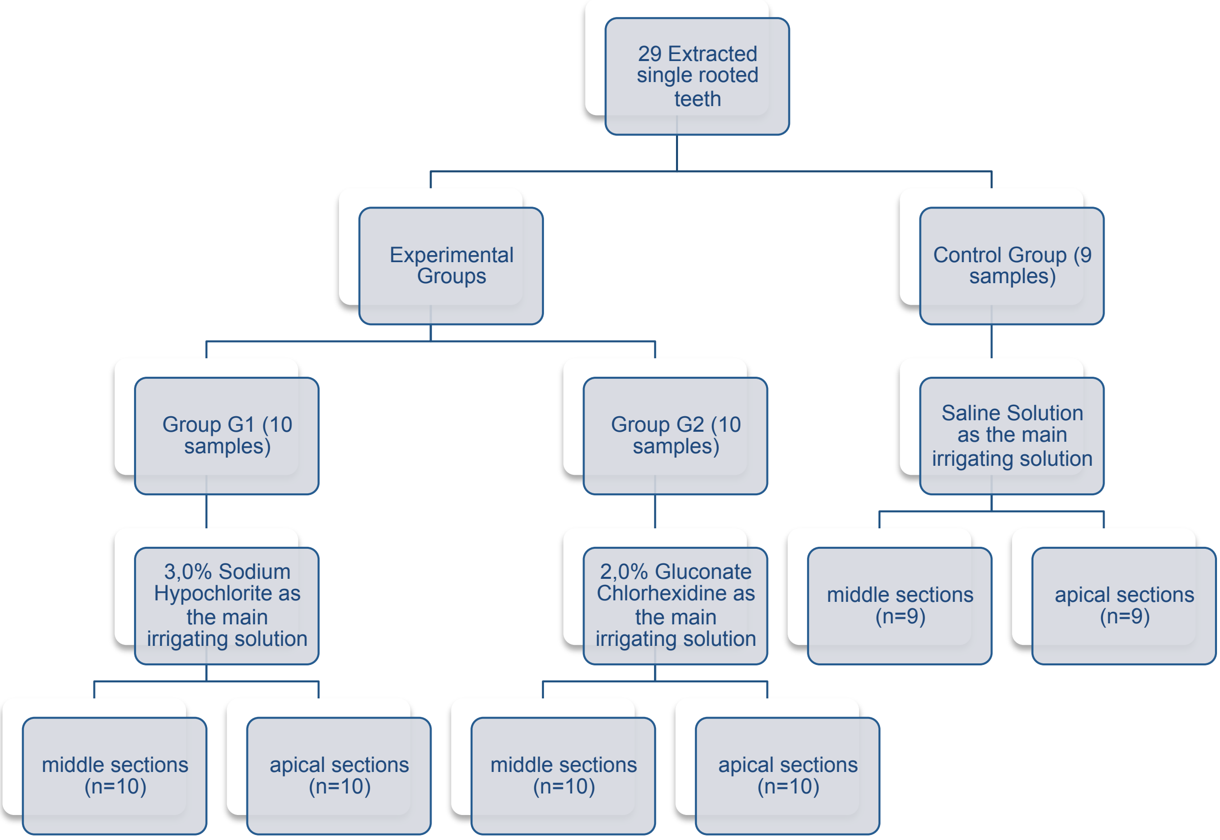
All sections were sequentially polished and the specimens were mounted onto glass slides.

Confocal Laser Scanning Microscopy (CLSM) Analysis

Samples were examined with a Zeiss 710 Laser Scanning Microscope (Carl Zeiss, Gottingen, Germany), using the excitation laser line at 561 nm. Images were recorded in the fluorescent mode with a EC-Plan-Neofluor 10X/0.3 M27 objective. The size of the 10 X images recorded was 1414.22 X 1414.22 mm^2, and the resolution was 512 X 512 pixels. Each sample was evaluated for a consistent fluorescent ring around the canal wall indicating MTA Fillapex®-sealer distribution. The multiple images obtained from each sample were imported to Adobe Photoshop® 7.0 (Adobe Systems, San Jose, CA) and an overlay of all the different images obtained from each sample was executed, thus resulting in a single image to be analysed.
The sealer penetration was then measured using Image J® (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA). For this, the area of the circumference of each canal was outlined and measured (Figure 1). Then, the areas along the canal walls in which sealer penetrated into dentinal tubules any distance were outline and measured using the same method (Figure 2). The outlined areas were divided by the area of the canal circumference to calculate the percentage of canal wall sealer penetration in that section.

The percentages for each group were statistically analysed with Kruskal-Wallis non-parametric test to determine the differences among sealer penetration percentages, with statistical significance at $p = 0.05$. This analysis was obtained in the apical and middle sections, independently.

The software employed was SPSS® version 20.



Graphic 1: Samples division (groups, irrigant utilized and sections)

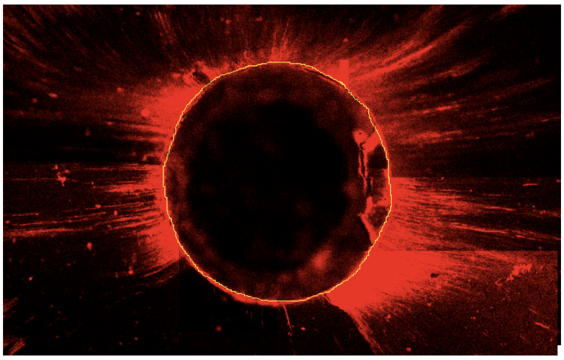


Figure 1: measurement of the circumference area of the root canal

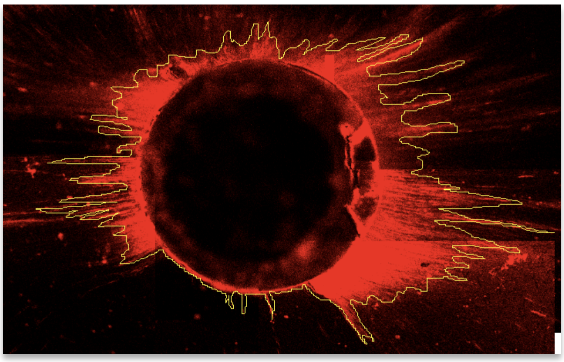


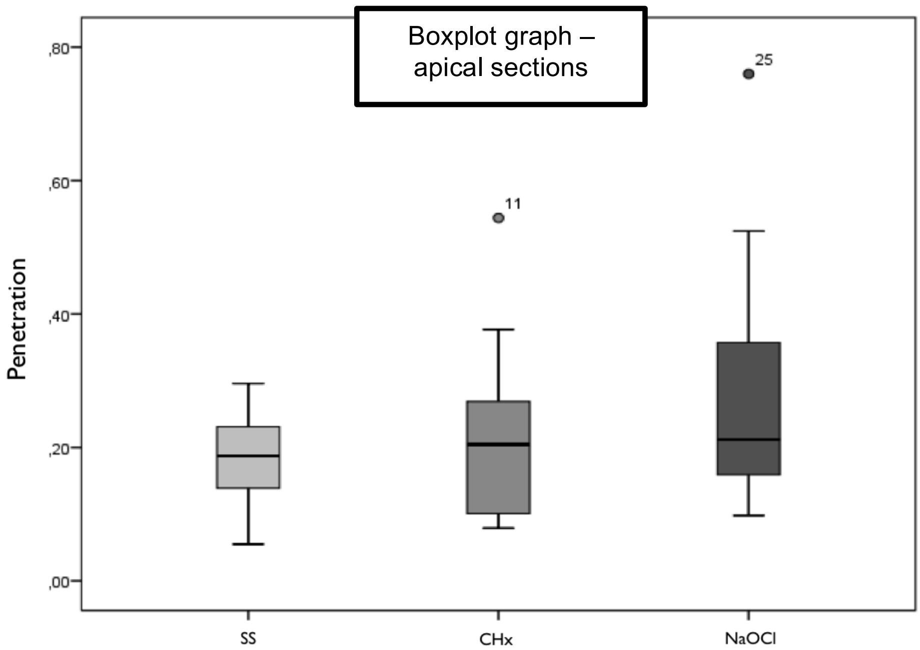
Figure 2: measurement of the area in which sealer penetrated into dentinal tubules using ImageJ®

1.3 Results

The sealer penetration percentages for apical and middle sections are presented in Table II.

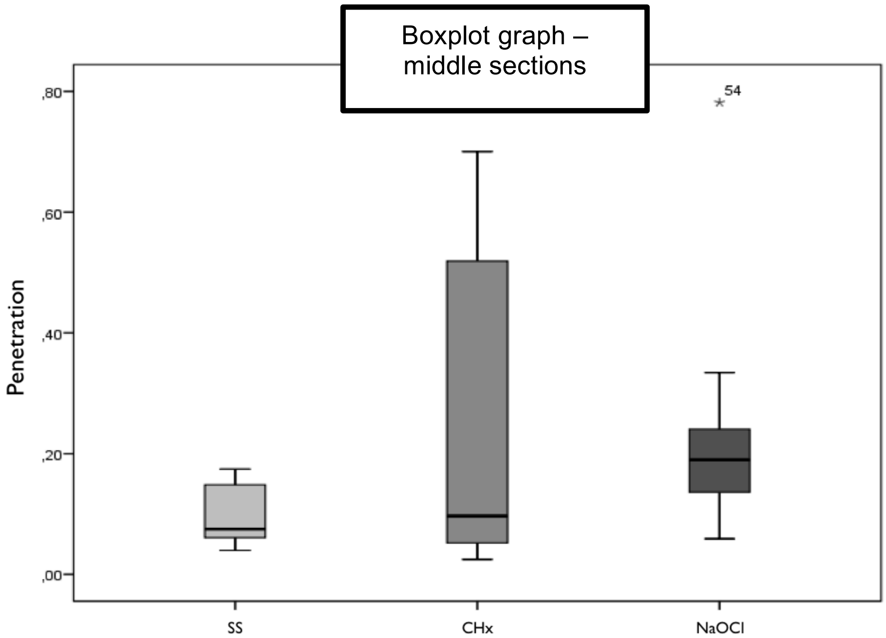
section	irrigating solution	mean	median	standard deviation	minimum	maximum
Apical	SS	0.1846	0.1871	0.0788	0.0549	0.2957
	CHx	0.2258	0.2045	0.1469	0.0788	0.5440
	NaOCl	0.2868	0.2117	0.2081	0.0978	0.7598
Middle	SS	0.0999	0.0750	0.0531	0.0396	0.1743
	CHx	0.2402	0.0965	0.2726	0.0247	0.7005
	NaOCl	0.2459	0.1897	0.2164	0.0589	0.7816

Table II: sealer penetration percentages for apical and middle section



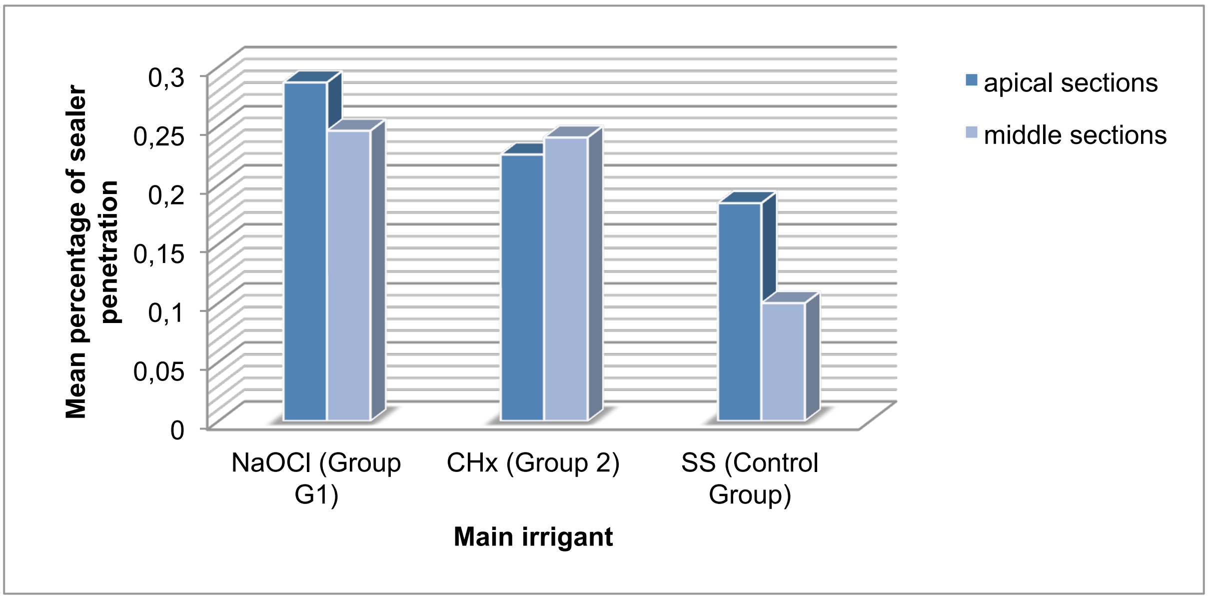
Graphic 2: sealer penetration in the apical sections

In the apical sections, there was no significant difference between samples from groups G1, G2 or Control, with respect to sealer penetration. In these observations, Group G1 (NaOCl) obtained the highest percentage of sealer penetration, followed by Group G2 (CHx), although these values do not show a statistically significant difference (Graphic 2).



Graphic 3: sealer penetration in the middle sections

In the middle sections, there was no significant difference between samples from groups G1, G2 or Control, with respect to sealer penetration. In these observations, Group G1 (NaOCl) obtained the highest percentage of sealer penetration, followed by Group G2 (CHx), although these values do not show a statistically significant difference (Graphic 3).



Graphic 4: mean percentage of sealer penetration among the three main irrigating solutions in the middle and apical sections

In terms of regional variance, all the main solutions used in this study showed different results between the mean percentage of sealer penetration in the apical section and in the middle section. The group that used 3,0% NaOCl as the main irrigant (Group G1) and the group that used saline solution as the main irrigant (Control Group) obtained a higher mean percentage of sealer penetration in the apical section (Graphic 4).

The group that used 2,0% CHx as the main irrigant (Group G2) obtained a higher mean percentage of sealer penetration in the middle section (Graphic 4).

1.4 Discussion

Various factors have been shown to influence the efficacy of root canal irrigation, including apical preparation size,(45, 92) taper,(38, 42, 75, 92) chemical nature, age, surface tension, contact time and temperature of the solution,(65) distance of the irrigation needle to the apex,(27, 42, 45, 65) irrigation volume,(13, 36, 42, 45, 78) and dimension of the irrigation needle. (13, 38, 42, 65, 122)

The irrigating solutions tested in this study were 3,0% NaOCl and 2,0% CHx. Although they present antibacterial activity, both substances have distinct characteristics. Numerous research results have shown disagreement when comparing the antimicrobial effect of these solutions.(6, 25, 29, 35, 37, 44, 52, 66, 69, 72) Different experimental methods, concentrations, biological indicators or the period of analysis may have caused these differences.(66)

No single irrigant is capable of removing both inorganic and organic materials.(33, 85, 92, 103, 105)

NaOCl shows antiseptic properties due to the formation of hypochlorous acid and the subsequent release of chlorine, which is a very active bactericide. Free chlorine in NaOCl dissolves necrotic tissue by breaking down proteins into amino acids.(12) Additionally, Zou *et al.* (2010)(24) concluded that temperature, time and concentration play a role in determining the depth of hypochlorite penetration into dentinal tubules. In addition, deepest irrigant penetration was obtained when these factors were present simultaneously, suggesting an additive effect.(24)

CHx lacks the tissue dissolution capabilities of NaOCl.(103) Hence, it has been suggested that CHx should not be used as a replacement irrigant of NaOCl, but as a supplemental final irrigation step after NaOCl and EDTA irrigation(17, 23, 35, 40, 41, 51, 53, 123, 124) because of its antimicrobial action, substantivity, and easy removal from the root canal system.(40) Furthermore, the use of CHx as a final irrigant has been shown to be favorable because it increases the levels of adhesion to dentin,(40) which can be explained by the presence of surface surfactant in CHx composition, increasing the dentin surface energy and, consequently, its wettability, a property required for adhesion.(41)

EDTA retains its calcium-complexing ability when mixed with NaOCl, but EDTA causes NaOCl to lose its tissue-dissolving capacity. Therefore, EDTA and NaOCl should be used separately and EDTA should never be mixed with NaOCl.(23) Additionally, when CHx and EDTA interact, a precipitate is formed, however its clinical significance is largely unknown.(18) Consequently, in the present study, we neutralized the root canals by irrigating with 10 mL of saline solution between the main irrigant and the 17% EDTA solution.

In general, *in vitro* studies suggest that CHx and NaOCl have equivalent antimicrobial effectiveness when used in similar concentrations.(25, 29, 33, 35, 37, 52, 66) Additionally, Scelza *et al*. (2000)(13, 125) reported that the volume is more important than the type of the solution, due to the mechanical action created by the flux and reflux of the solution inside the canal, removing debris left in suspension after biomechanical procedures.

Although the results were not statistically significant, our study showed that the group using NaOCl as the main irrigant (Group G1) demonstrated the highest percentage of sealer penetration. This could be explained by the fact that EDTA acts upon the inorganic components of the smear layer, causes the decalcification of peri- and intertubular dentine, and leaves the collagen exposed. Subsequently, the use of NaOCl dissolves the collagen leaving the entrances to the dentinal tubules more open and exposed.(85) Furthermore, Hu *et al. (*2010)(41, 126) observed that the use of EDTA improved the wettability of the sealer by removing the smear layer and exposing the dentinal tubules, which increased the roughness of the dentin surface. Hence, authors like Baumgartner and Mader (1987),(127) found alternating NaOCL with EDTA to be effective in eliminating the smear layer and producing clean root canal walls.(11, 51, 55, 56, 61, 63, 64, 83, 86, 94, 100, 109, 128, 129)

In our study, we opted to remove the smear layer. This decision is supported by investigators such as Brannstrom (1984)(130) and Perez-Heredia *et al*. (2008)(131) who believed that the smear layer feeds microorganisms and helps them colonize. Some researchers also believed that the smear layer prevents or delays action of canal irrigating solutions for disinfection of the root canal.(11, 61) Others showed endodontic sealers to have a superior penetration into dentinal tubules and a better adhesion to the root canal wall after smear layer removal.(5, 11, 15, 59-62, 65, 77-79, 85, 86, 89, 90, 92, 93, 95-104) Additionally, the smear layer is non-homogenous and weakly adherent to the tooth structure; therefore, it might slowly disintegrate in the presence of fluids from a leaking filling material.(61, 89)

The results of this study suggest that there appears to be no significant difference between the irrigants used, when smear layer was removed with 17% EDTA. This observation is in agreement with Menezes *et al.*(47) who concluded that the use of 17 % EDTA improved significantly the removal of the smear layer regardless of the solution evaluated. Additionally, Yamashita et al. (2003)(33) found that using a regimen of irrigation with CHx associated with EDTA allows similar leakage results as the regimen of irrigation with NaOCl associated with EDTA.

According to Shahravan *et al*. (2007),(56, 97) smear-free obturated canals leaked significantly less than groups with intact smear layer. Cobankara *et al. (2004)*(132) showed that the presence of the smear layer could negatively influence both coronal and apical leakage of root canal treated teeth obturated with different methods and materials. Moreover, Oksan *et al.* (1993)(102) observed that the smear layer obstructed the penetration of the tubules by the sealers. Kokkas *et al*. (2004)(55, 133) demonstrated that this could be explained by the fact that complete removal of the smear layer allows sealers to penetrate into dentinal tubules. Conversely, excessive demineralization of dentin could create more difficulties in the adaptation of the filling materials to the canal walls.(55)

Additionally, De Deus *et al.* (2002)(10, 101) showed that the removal of smear layer allowed significant sealer penetration into dentinal tubules. Also, Moon *et al.* (2010)(2, 134) reported that sealer penetration may serve as an indicator of the extent to which the smear layer was removed. Furthermore, it is suggested that the decreased microleakage associated with smear layer removal might be attributable to the penetration of sealer into dentinal tubules.(10)

However, Galvan *et al.* (1994)(114, 135) reported that the presence of smear layer resulted in reduced leakage as compared to those without smear layer. Evans and Simon *et al.* (1986)(114, 136) showed that the presence or absence of smear layer has no significant effect on the apical seal. In addition, Paqué *et al.* (2006)(90, 137) reported that the smear layer does not appear to be a diffusion barrier. They observed microscopically that irrigant penetration was not influenced by the presence of the smear layer, but was rather a function of tubular sclerosis.(90, 137) Bertacci *et al.* (2007)(82, 138) reported that the thickness of the endodontic smear layer is probably 1-5μm and can be easily be pushed with enough pressure by warm gutta-percha inserted inside the root canal. Moreover, Saleh *et al.* (2003)(61, 139) suggested that the penetration of the endodontic sealer into dentinal tubules, whose smear layer was removed, was not related to higher bond strengths.

There is no agreement in the literature concerning the volume of chelating agent or the contact time required in the final rinse protocols (75, 85, 92). Additionally, Crumpton *et al.* (2005)(55, 140) reported that the volume of irrigation does not influence the quality of smear layer removal, since this is a function of contact time.

Nevertheless, to minimize destructive effects on dentin reported by some investigators, in the present study we used 5 mL of 17% EDTA for 1 minute, since several studies have reported that the application of EDTA for more than 1 minute causes erosion of dentinal tubules, thus reducing the dentin microhardness and consequently causing root fragility.(11, 30, 75, 76, 78, 92, 106, 141)

Numerous investigators showed that coronal and middle areas of the canals irrigated with EDTA were cleaner than the apical part.(11, 63, 74, 85, 103) Such findings could be related to the comparatively smaller apical canal dimensions, which hinders the penetration of irrigants, resulting in limited contact between the root canal walls and the irrigating solutions.(3, 103) Also, dentin in the apical third is much more sclerosed and the number of dentinal tubules present is less.(74, 75) To overcome the potential limited irrigation in the apical area, enlargement of this area has been advocated for better cleansing.(38, 56, 75, 92)

A crown-down instrumentation technique was executed in the present study as it may tend to allow removal of the majority of radicular pulp tissue early in the root canal preparation, increasing the volume of irrigant in the canal.(86) However, it has been reported that neither instruments nor instrumentation techniques in canal preparation achieve complete cleanliness of root canal walls.(98, 142)

The choice of using ProTaper® instrumentation is supported by various studies suggesting that root canal preparation with nickel-titanium rotary files produces a more consistent, uniform, centered and round canal form than with hand instrumentation.(143-145) Also, inferior leakage results have been found with canal preparation using rotary nickel-titanium instruments.(93, 146) Nevertheless, studies have also reported superior results when using hand instrumentations for creating a well-shaped root canal.(143, 147, 148) Additionally, the use of rotary instruments may pack debris further into dentinal tubules, thus making their remotion by irrigant more difficult.(61, 78, 86)

Although in the present study we did not use alcohol, rinsing the root canal with this solution before obturation has been anecdotally practiced.(23, 149) The basic premise is that alcohol reduces the surface tension of irrigants and root canal sealers.(23, 150) Lowering the surface tension of a fluid or a sealer will increase the fluid flow into the dentinal tubules. Thus alcohol will spread into the dentinal tubules and dry the root canal as it evaporates. Therefore, alcohol might affect sealer penetration and leakage of the root canal filling.(23) In a study published by Stevens *et al.* (2006)(149) it was shown that a final rinse with 95% alcohol before root canal obturation resulted in increased sealer penetration and consequently decreased leakage.

Sealer cements create a union between the core material and the canal wall by filling any residual spaces. Additionally, they often have the ability to penetrate areas such as lateral canals and dentinal tubules.(120) The endodontic sealer used in this experiment was MTA FillApex®. According to the manufacturer, this sealer has high radiopacity, low solubility in contact with tissue fluids, low expansion during setting and excelled viscosity for insertion. This sealer does not stain the tooth and promotes deposition of hard tissue at the root apex and perforation sites.(120) Silva *et al.* (2013)(151) reported that MTA FillApex® showed significantly superior flow values compared with AH Plus® ($p < 0,5$) and, because of this property, MTA Fillapex® will probably penetrate easier into the ramifications and irregularities of the root canal walls than AH Plus®. However, its high flow may cause overfilling beyond apical foramen, which might come as a disadvantage.(152)

The penetration of sealer into dentinal tubules is considered to be a desirable outcome for various reasons as it will increase the interface between material and dentine, thus improving the sealing ability, and retention of the material may be improved by mechanical locking, which potentially reduces leakage (10, 116) Since chemical adhesion between different kind of sealers, pastes, plastics or cements and dentin cannot be achieved, it has been suggested that a mechanical block might be the solution.(128) Sealers within dentinal tubules might also entomb any residual bacteria within the tubules and the chemical components of sealer cements may exert an antibacterial effect that will be enhanced by closer approximation to the bacteria.(10, 116) Furthermore, sealer penetration in *in vitro* models is comparable to *in vivo*.(10, 116)

Longitudinal sections of the tooth samples were taken for evaluation. The disadvantage of this orientation is that it does not allow for complete observation of all of the dentine surrounding the canal and there is potential to miss areas of deep penetration.(10)

Regional variation in the depth of tubular penetration was shown by a number of authors (Balguerie *et al.* 2011, Weis *et al.* 2004).(93, 116) Deepest penetration of sealer cement has been demonstrated in the middle third of the root canal, since apical dentine displays less tubule density, the tubules have smaller diameter or they are more often closed. Furthermore, the apical portion shows a pronounced variation in structure, with some areas completely devoid of tubules.(10, 116)

Our results are in incongruity with these previous studies, since they revealed an increased sealer penetration in the apical third in comparison with the middle third, aside from the group that used CHx as the main irrigating solution (Group G2). These results might be due to the introduction of artifacts probably caused by rhodamine B dye leakage in the cutting section process that led to higher mean percentages of sealer penetration in the apical sections. Moreover, the number of samples used in the present study is relatively low, thus, further studies addressing the subject should be performed.

A maximum volume of gutta-percha and a thin layer of sealer are preferred because sealer might shrink during setting and dissolve, thus causing leakage.(143)

In the present study, the obturation was performed using a cold lateral condensation technique, which is in understanding with Wu *et al.* (2000)(2, 153) who evaluated the effect of obturation techniques on sealer distribution and found that the area of sealer-coated root canal wall in the coronal area was significantly higher when lateral condensation was used than when vertical condensation was used. Additionally, Facer and Walton *et al.* (2003)(110, 154) have shown that lateral condensation of gutta-percha may provide close adaptation to the canal walls resulting in the gutta-percha coming into direct contact with the canal wall.

However, Gharib *et al.* (2007)(107, 108) studied the percentage of the sealer penetration in root canals filled with a vertical compaction technique using CLSM. Their results showed a higher percentage of sealer penetration and greater depth of sealer penetration in the coronal (88%) and middle (74%) thirds compared with the apical third of the root (46%).(107, 108) As formerly mentioned, this might be explained by the fact that in the root's coronal third the density of dentinal tubules is higher and their diameters are greater compared with the middle and apical thirds.(2, 104, 107, 112) Similar experiments found that the percentage of sealer penetration using a lateral condensation technique ranged from 46 % to 63%.(107, 155)

Furthermore, Gilbert *et al.* (2001)(77) found that vertical compaction leacked significantly less than lateral condensation during bacterial challenge and De Deus *et al.* (2004)(2, 156) reported that the core-carrier-based Thermafill technique with warm condensation technique produced significantly deeper sealer penetration than the cold lateral condensation technique.

These studies might show that the filling technique has an influence in the percentage of sealer penetration to the root canal walls (108). However, in a study by Weis *et al.* (2004)(93) it was concluded that the frequency and depth of sealer penetration were unrelated to the obturation technique employed. Additionally, Keçeci *et al.* (2005)(143) concluded that there were no statistical differences found between any of the preparation/filling combinations in terms of distribution of sealer, gutta-percha or voids and that the distribution of filling material was not influenced by preparation technique. In a similar study, Shahravan *et al.* (2007)(97, 113) found that smear layer removal improves the fluid-tight seal of the root canal system, whereas other factors, such as the obturation technique or the sealer used did not produce significant effects.

Ultimately, regarding several studies analysis, the sealer penetration depth might depend on numerous factors such as smear layer removal, dentinal permeability,(61, 116) root canal dimension,(61, 116) the physical and chemical properties of the sealer,(10, 61, 101, 102, 116) the surface tension of filling materials,(61, 112, 128) the obturation technique, the angle between the dentinal wall and the dentinal tubule and the diameter of the tubule.(101)

CLSM used in the present study offered multiple advantages over scanning electron microscopy (SEM) as it did not require special specimen processing, thus it was non-destructive and had less potential to produce artifacts.(107, 108) It also allowed visualization of the sealer-dentin interface using fluorescence and the interpretation of CLSM data was directly related to the penetration of the fluorescently labelled MTA FillApex® sealer.(107) Earlier studies using CLSM have indicated that the incorporation of rhodamine B into the sealer is essential to observe the extent of sealer adaptation and penetration.(108, 157)

Despite the advantages of confocal microscopy, there is a possibility that the dye leached from the sealer or altered the properties of MTA FillApex®.(107, 108) Further studies should address the influence of the rhodamine dye on the physicochemical properties of different sealers.(107, 108)

1.5 Conclusions

Although the results were not statistically significant (*$p = 0.05$*), our study showed that the group that used NaOCl (Group G1) demonstrated the highest percentage of sealer penetration, followed by the group that used CHx (Group G2).

Recognizing the inherent limitations of an *in vitro* experiment, the results of this study suggest that there appears to be no significant differences in the percentage of sealer penetration between the irrigants tested, when smear layer was removed with 17% EDTA (*$p = 0.05$*).

The number of samples used in the present study is relatively low; hence, further studies addressing the subject should be performed to determine whether the irrigating solution used has an effect on the percentage of sealer penetration into dentinal tubules.

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