MAÍRA DO PRADO

“AVALIAÇÃO DA INTERAÇÃO ENTRE AS DIFERENTES SUBSTÂNCIAS QUÍMICAS AUXILIARES UTILIZADAS EM ENDODONTIA E SEUS EFEITOS NAS ETAPAS DO TRATAMENTO ENDODÔNTICO”

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UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

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Orientador (a): Profª. Drª. Brenda Paula Figueiredo de Almeida Gomes

TESE DE DOUTORADO APRESENTADA A FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNICAMP PARA OBTENÇÃO DO TÍTULO DE DOUTORA EM CLÍNICA ODONTOLÓGICA NA ÁREA DE ENDODONTIA.

Este exemplar corresponde à versão final
da tese defendida pela aluna e orientada
pela Profª. Drª. Brenda Paula F. A. Gomes

_____
Assinatura da Orientadora

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Renata Antoun Simão
Heloisa Carla Dell\'Santo Gusman
Caio Cezar Randi Ferraz
Alexandre Augusto Zaia

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Profa. Dra. RENATA ANTOUN SIMÃO

Profa. Dra. HELOISA CARLA DELL' SANTO GUSMAN

Prof. Dr. CAIO CEZAR RANDI FERRAZ

Prof. Dr. ALEXANDRE AUGUSTO ZAIA

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RESUMO

Introdução: O objetivo do presente trabalho foi avaliar a interação entre as diferentes substâncias químicas auxiliares, utilizadas em endodontia, e seus efeitos nas etapas do tratamento endodôntico. **Métodos:** Soluções de hipoclorito de sódio (NaOCl) a 0,16%, 1%, 2,5% e 5,25%, clorexidina 2% solução e gel, EDTA 17%, ácido cítrico 10% e ácido fosfórico 37% foram utilizadas isoladamente ou associadas entre si na proporção 1:1. As mesmas foram analisadas quimicamente por espectrometria de massa; e microbiologicamente pelo método de difusão em ágar, contra diferentes patógenos. Adicionalmente, foram avaliados onze protocolos de irrigação em relação à formação de smear layer química por microscopia eletrônica de varredura. Por fim, foram avaliados 9 protocolos de irrigação associados a dois diferentes sistemas de obturação, guta-percha/ AH Plus e Resilon/ Real Seal SE, em relação à microinfiltração coronária e à resistência de união da dentina aos materiais obturadores. **Resultados:** O NaOCl, em todas as concentrações, associado à clorexidina em ambas as formulações, levou à formação de precipitado, assim como a associação entre a clorexidina e o EDTA e entre clorexidina e solução salina. Todas as associações avaliadas apresentaram algum grau de inibição contra os patógenos testados. Irrigação intermediária, com 10 mL de água destilada, entre as soluções de NaOCl e clorexidina, não foram capazes de inibir a formação de smear layer química, assim como irrigações com EDTA e ácido cítrico. Dos diferentes protocolos de irrigação avaliados, aqueles que apresentaram uma irrigação final com solução de clorexidina 2% tiveram níveis reduzidos de microinfiltração coronária. Em relação à resistência de união à dentina, no sistema obturador guta-percha/AH Plus, os grupos em que se utilizou as associações NaOCl/ácido fosfórico e clorexidina/EDTA apresentaram maiores valores de resistência de união, ao passo que no sistema Resilon/Real Seal SE, os maiores valores foram encontrados nos grupos clorexidina/ácido fosfórico. A utilização da clorexidina como irrigante final não afetou negativamente os sistemas obturadores avaliados. **Conclusões:** A interação entre as substâncias químicas auxiliares pode levar à formação de precipitados. As diferentes substâncias químicas auxiliares, quando associadas, possuem atividade antimicrobiana. Irrigações intermediárias entre as diferentes substâncias químicas auxiliares são

necessárias para reduzir ou até mesmo impedir a formação de precipitados, visualizados na superfície dentinária como uma smear layer química. Durante o preparo químico-mecânico, as diferentes substâncias químicas auxiliares geram modificações na superfície dentinária que influenciam na microinfiltração coronária e na resistência de união dos sistemas obturadores guta-percha/AH Plus e Resilon/Real Seal SE.

Palavras Chave: irrigantes do canal radicular, análise química, resistência microbiana, obturação do canal radicular, infiltração dentária, adesividade.

ABSTRACT

Introduction: The aim of the present study was to evaluate the interaction among different chemical auxiliary substances used in endodontics and their effects on different steps of endodontic treatment. **Methods:** 0.16%, 1%, 2.5% and 5.25% sodium hypochlorite solutions (NaOCl), 2% chlorhexidine solution and gel, 17% EDTA, 10% citric acid, 37% phosphoric acid, distilled water, and saline solution were used both associated with each other (1:1 ratio) and not associated. The solutions were chemically examined with mass spectrometry. They were microbiologically examined using the Agar Diffusion Method, against different pathogens. In addition, eleven protocols were analyzed in regards to chemical smear layer with scanning electron microscopy. Finally, nine irrigation protocols (associated with two filling systems, i.e. gutta-percha/AH Plus and Resilon / Real Seal SE) were examinated regarding coronal microleakage and were examined as well regarding their bond strength with dentin. **Results:** NaOCl, at all concentrations, associated with both chlorhexidine formulations, led to precipitate formation, similar to the association between chlorhexidine/EDTA, and the chlorhexidine/saline solution association. All associations had some degree of inhibition against the evaluated pathogens. Intermediate flush, with 10 mL of distilled water, between NaOCl and chlorhexidine did not inhibit chemical smear layer formation, similar to intermediate flushes with EDTA and citric acid. Among the different irrigation protocols that were evaluated, when final flush with 2% chlorhexidine was present, the lowest levels of coronal microleakage were found. Regarding the bond strength to dentin, in the gutta-percha/AH Plus system, the groups with NaOCl/phosphoric acid and chlorhexidine/EDTA associations showed higher bond strength values. In the Resilon/Real Seal SE system, the highest values were found in the chlorhexidine/phosphoric acid groups. The use of chlorhexidine as a final flush did not negatively affect the filling systems evaluated. **Conclusions:** The interaction among the auxiliary chemical substances may lead to precipitate formation. The association between the different substances has antimicrobial activity. Intermediate flushes are necessary to reduce or even avoid the formation of chemical smear layer on the dentin surface. During the chemo-mechanical preparation, the various auxiliary chemical substances used do

modify the dentine surface. These modifications have influence in the coronal microleakage and in the bond strength of the filling systems gutta-percha/AH Plus and Resilon / Real Seal SE.

Keywords: root canal irrigants, chemical analysis, microbial resistance, root canal filling, dentine infiltration, bond strength

SUMÁRIO

INTRODUÇÃO

O objetivo principal da terapia endodôntica é eliminar, ou pelo menos reduzir o número de microorganismos, e ainda remover o tecido pulpar inflamado ou necrótico, presente no interior do sistema de canais radiculares (Thomas & Sem, 2010). Para isso, instrumentos manuais e/ou rotatórios são utilizados concomitante ao uso de substâncias químicas auxiliares e irrigantes.

Algumas características são requeridas a essas substâncias para que sejam qualificadas como auxiliares à instrumentação. Essas características incluem: baixa tensão superficial, atividade de dissolução tecidual, atividade antimicrobiana, lubrificante, suspensão de detritos e biocompatibilidade (Lopes & Siqueira JR, 2004). Diversas substâncias químicas auxiliares são encontradas no mercado odontológico, entre elas podemos destacar o hipoclorito de sódio em concentrações variando entre 0,5% e 6%, a clorexidina 2% (em formas de apresentação solução e gel), o EDTA 17%, o ácido cítrico 10%, entre outras.

Das substâncias químicas auxiliares, o hipoclorito de sódio é, mundialmente, a mais utilizada durante o preparo químico-mecânico por associar capacidade de dissolução tecidual (Senia et al., 1971; Koskinen *et al*., 1980; Zehnder et al., 2002; Beltz et al., 2003) com atividade antimicrobiana (Vianna et al., 2004; Sena et al., 2006).

A clorexidina 2% tem sido utilizada como substância química auxiliar em endodontia, nas formulações líquida e gel, por possuir atividade antimicrobiana de amplo espectro (Ferraz et al., 2001; 2007; Gomes et al., 2001; Vianna et al., 2004; Sena et al., 2006), substantividade (Basrani et al., 2002; Dametto et al., 2005, Carrilho et al., 2010; Baca et al., 2012) e baixa toxicidade (Yesilsoy et al., 1995; Lee et al., 2010; Trevino et al., 2011). Na formulação gel, a clorexidina ainda facilita a instrumentação, lubrificando o canal radicular, o que diminui o atrito entre as paredes e os instrumentos no interior do sistema de canais radiculares. Além disso, a utilização de clorexidina gel durante a instrumentação cria uma superfície radicular mais limpa, devido a menor formação de smear layer, quando comparada ao hipoclorito de sódio e à solução de clorexidina (Ferraz et al., 2001). Entretanto, devido a sua incapacidade de dissolução de tecido orgânico, a

clorexidina tem sido sugerida como um irrigante final (Zehnder, 2006) ou também como medicação intra-canal (Gomes et al. 2003; 2006).

Em relação à utilização da clorexidina como irrigante final, De Assis et al. (2011) analisaram a influência das soluções irrigadoras empregadas em Endodontia nas propriedades adesivas da superfície dentinária em contato com os cimentos endodônticos AH Plus e Real Seal SE e observaram que a utilização da solução clorexidina 2% como irrigante final favoreceu ao escoamento desses cimentos na superfície dentinária. Hashem et al. (2009) avaliaram a resistência de união dos sistemas ActiV GP e guta-percha/AH Plus em dentes após diferentes regimes de irrigação e observaram que a utilização de clorexidina 2% como irrigante final, após irrigação com EDTA 17%, aumentou os valores de resistência de união do sistema ActiV GP. Ainda em relação a irrigação final com clorexidina, estudos mostram que esta solução aumenta a longevidade da adesão de cimentos resinosos à dentina (Carrilho et al., 2007; Cecchin et al., 2011; Ricci et al., 2010).

Durante o preparo químico-mecânico devido ao atrito dos instrumentos com as paredes do canal, uma massa composta por materiais inorgânicos, como raspas de dentina, e orgânicos, como restos de tecido pulpar e bactérias, é formada. Essa massa, que se adere a superfície dentinária, é denominada smear layer (Lopes & Siqueira Jr, 2004). A smear layer age como uma barreira física reduzindo a ação das substâncias químicas auxiliares e medicação nos túbulos dentinários, diminuindo a penetração dos cimentos endodônticos nos túbulos, como também dificultando a adesão do cimento à superfície dentinária (Calas et al., 1994; Love et al.,1996; Buck et al., 2001; Kokkas et al., 2004). Observa-se que, ao remover essa camada há um aumento da permeabilidade dentinária, uma diminuição da microinfiltração (Sen et al., 1995; Vivacqua-Gomes et al., 2002) e um aumento nos níveis de força de adesão dos cimentos endodônticos à superfície dentinária (De Assis, 2011).

As soluções comumente utilizadas durante o preparo químico-mecânico, hipoclorito de sódio e clorexidina, não são capazes de agir de forma eficaz sobre essa camada e removê-la. Dessa forma, agentes quelantes e ácidos, como o EDTA 17% e ácido cítrico 10% são utilizados para esse fim. Em 2011, Prado et al. propuseram a utilização do ácido fosfórico 37% para remoção da smear layer radicular. Os autores

observaram que esta solução foi eficaz na remoção da smear layer e que em 3 minutos apresentou uma efetividade maior que o EDTA 17% e ácido cítrico 10% no terço apical.

Em geral, um irrigante não é completamente removido do interior do sistema de canais radiculares quando um subsequente é utilizado. Dessa forma, os irrigantes rotineiramente entram em contato entre si no interior do sistema de canais radiculares. Nesse contexto, a literatura mostra que essas substâncias podem reagir quimicamente. De acordo com estudos de Basrani e colaboradores (Basrani et al., 2007; Basrani et al., 2009; Basrani et al., 2010) a utilização do hipoclorito de sódio em combinação com a clorexidina produz um precipitado marrom-alaranjado, identificado por esses autores como para-cloroanilina. Esse precipitado leva a uma alteração na coloração dentária, é citotóxico e carcinogênico (Burkhardt-Holm et al., 1999; Basrani et al., 2007). Entretanto, Thomas & Sem (2010) e Nowicki & Sem (2011) não observaram a formação de para-cloroanilina na combinação entre hipoclorito de sódio e clorexidina.

Ainda em relação à associação hipoclorito de sódio e clorexidina, de acordo com Bui et al. (2008) e Akisue et al. (2010) essa interação leva à formação de uma smear layer química que recobre os túbulos dentinários. Vivacqua-Gomes et al. (2002) observaram que o protocolo de irrigação associando clorexidina 2% e NaOCl 1% influenciou negativamente no selamento de dentes obturados com guta-percha e cimento Endomethasone. Com o objetivo de reduzir ou mesmo impedir a formação desse precipitado, Krishnamurthy & Sudhakaran (2010) sugerem a utilização de irrigações intermediárias, entre as soluções de hipoclorito de sódio e clorexidina, com álcool absoluto, solução salina ou água destilada. Ainda, Mortenson et al. (2012) sugeriram o uso de ácido cítrico 50% ou EDTA 14% como irrigante intermediário.

Outras associações relacionadas à formação de precipitado são encontradas na literatura. De acordo com Rasimick et al. (2008) a associação entre o EDTA e a clorexidina leva à formação de um precipitado branco-leitoso. Akisue et al. (2010) observaram que, ao misturar ácido cítrico à clorexidina, há também a formação de um precipitado branco-leitoso. Cecchin (2010) observou que ao associar a clorexidina 2% com álcool absoluto (etanol 100%) havia a precipitação de sais. Nas associações entre hipoclorito de sódio com EDTA e hipoclorito de sódio com ácido cítrico há a liberação de gás cloro (Baumgartner & Ibay, 1987).

A terapia endodôntica visa a remoção de tecido pulpar e a máxima redução de microrganismos, durante o preparo químico-mecânico, e a obturação tridimensional através de um selamento hermético do sistema de canais radiculares. Para se conseguir este fim, durante o preparo químico-mecânico e previamente à obturação diferentes substâncias químicas são utilizadas, exercendo atividades antimicrobiana, solvente de tecido, lubrificação e auxiliando na remoção da smear layer. Pode ser ainda utilizada com o objetivo de aumentar os índices de resistência de união e reduzir os níveis de microinfiltração coronária.

Estas substâncias são utilizadas de forma seqüencial e mesmo com a sua remoção através da irrigação/aspiração, entram em contato entre si no interio do sistema de canais radiculares. Embora a literatura reporte que tais associações estão relacionadas a formação de subprodutos, estes ainda não foram completamente elucidados. Além disso, tais produtos podem ser tóxicos, corrosivos, insolúveis e interferirem não apenas nos processos de adesão, como também aumentar os níveis de microinfiltração coronária.

Desta forma, o presente estudo objetivou avaliar as associações entre as diferentes substâncias químicas auxiliares durante o tratamento endodôntico (hipoclorito de sódio, clorexidina, ácido cítrico, EDTA, ácido fosfórico), identificando quimicamente os produtos dessas associações e ainda a ação antimicrobiana de tais associações.

Ainda, com o objetivo de avaliar o potencial das diferentes substâncias químicas auxiliares na resistência de união de diferentes sistemas de obturação à base de resina (guta-percha/AH Plus e Resilon/ Real Seal SE) e na microinfiltração coronária, o presente estudo comparou diferentes protocolos de irrigação. Tais protocolos consistiram no uso do hipoclorito de sódio durante a instrumentação, seguido do uso de EDTA ou ácido fosfórico para remoção de smear layer e finalmente a influência da solução de clorexidina como irrigante final. Ainda, a utilização de clorexidina gel 2% durante a instrumentação seguido do uso de EDTA ou ácido fosfórico para remoção de smear layer e finalmente a influência da solução clorexidina como irrigante final. Tal estudo foi realizado procurando aumentar os índices de sucesso do tratamento endodôntico, visto que a utilização das substâncias químicas auxiliares associadas ao preparo mecânico é fundamental para a eliminação de microrganismos e tecidos pulpares e podem influenciar na adesão e na microinfiltração coronária.

CAPÍTULO 1

Interactions between Irrigants Commonly Used in Endodontic Practice: A Chemical Analysis

Maíra do Prado[1], Helvécio M. Santos Júnior[2], Claudia M. Rezende[3], Angelo C. Pinto[4], Roberto B. Faria[4], Renata A. Simão[5], Brenda P.F.A. Gomes[6]

[1]DDS, MSc – PhD Student, Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.

[2]DSc, MSc, PhD – Postdoctoral Student, Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

[3]DSc, MSc, PhD – Adjunct Professor, Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

[4]DSc, MSc, PhD – Full Professor, Institute of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

[5]DSc, MSc, PhD – Adjunct Professor, Department of Metallurgic and Materials Engineering, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

[6]DDS, MSc, PhD – Full Professor, Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.

Correspondence: Dr Brenda Paula F. A. Gomes - Department of Restorative Dentistry - Piracicaba Dental School, State University of Campinas
Avenida Limeira, 901, Piracicaba, SP – Brazil 13414-018 Phone: (0055) 19 2106-5215 Fax: (0055) 19 2106-5218 E-mail: bpgomes@fop.unicamp.br

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ABSTRACT

Introduction: The aim of this work is to describe the chemical nature of the interactions between two common irrigants used in root canal therapy, as sodium hypochlorite and chlorhexidine. **Methods:** Sodium hypochlorite (NaOCl, 0.16%, 1%, 2.5% and 5.25%) was associated with 2% chlorhexidine (CHX) solution and gel, 17% EDTA, 10% citric acid (CA), 37% phosphoric acid (PA), saline solution (SS), ethanol, and distilled water (DW). CHX was also associated with all above mentioned irrigants, except NaOCl. The solutions were mixed in a 1:1 ratio and electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-QTOF-MS) was used to characterize the precipitates when formed. **Results:** CHX produced an orange-brown precipitate when associated with NaOCl from 1% to 5.25% and an orange-white precipitate when associated with 0.16% NaOCl. When associated with EDTA, CHX produced a white-milky precipitate and when associated with SS and ethanol, a salt precipitation was produced. No precipitation was observed when CHX was associated with CA, PA or DW. In the NaOCl associations, precipitation occurred only when CHX was present. **Conclusion:** The precipitate observed in the association between CHX and NaOCl occurs due the oxidation caused by the latter substance, leading to chlorination of the guanidino nitrogens of the CHX, assigned using ESI-QTOF-MS technique, and thus resulting in the orange-brown precipitate. The precipitate formed between CHX and EDTA, saline solution, and ethanol was associated with acid-base reactions, salting-out process and reduced solubility, respectively. NaOCl associated with EDTA, citric or phosphoric acid leads mainly to chlorine gas formation.

INTRODUCTION

The aim of root canal therapy is to eliminate or at least reduce the number of microorganisms and remove inflamed or necrotic pulpar tissue (1,2). Because of the anatomical complexity of the root canal system, mechanical instrumentation cannot remove all the infected tissue and bacteria present in isthmuses and ramifications. Therefore, the use of irrigant solutions in association with mechanical instrumentation is required. Different irrigants have been proposed and used, such as: sodium hypochlorite (NaOCl), chlorhexidine (CHX), ethylenediamine tetraacetic acid (EDTA), citric acid,

MTAD (doxycycline isomers, citric acid and Tween 80) and phosphoric acid solutions (3-5).

In general, an irrigant solution is not completely flushed out from the root canal before applying the next irrigant. As a result, endodontic irrigants routinely come into contact with each other inside the root canal (6). This can produce toxic byproducts and precipitates forming a chemical smear layer, which occludes the dentinal tubules and may interfere with the seal of the root filling (7-9).

Although different associations have already been studied, there is a scarce literature regarding these interactions. Baumgartner & Ibay (10) studied the interaction between NaOCl/EDTA and NaOCl/citric acid, whereas other authors studied the interaction between NaOCl and CHX. However, the chemical composition of the orange-brown precipitate observed is not completely elucidated. Basrani et al. (8,11) and Krishnamurthy & Sudhakaran (12) detected the presence of *para*-chloroaniline (PCA). On the other hand, Thomas and Sem (2) failed to detect this compound. Other interactions between EDTA and CHX as well as citric acid and CHX were also evaluated (6,7), but the interaction between phosphoric acid, an efficient solution for smear layer removal, and NaOCl or CHX was never described. In this context, the aim of the present study was to characterize the interactions between the most commonly used irrigants in the endodontic practice, through electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-QTOF-MS) analyses.

MATERIALS AND METHODS

Chemical substances

The irrigants used in the present work were: aqueous NaOCl at 0.16%, 1%, 2.5% and 5.25% (Drogal, Piracicaba, SP, Brazil), 2% chlorhexidine (CHX) solution and gel (Drogal, Piracicaba, SP, Brazil), 17% EDTA (Drogal, Piracicaba, SP, Brazil), 10% citric acid (Drogal, Piracicaba, SP, Brazil), 37% phosphoric acid (Drogal, Piracicaba, SP, Brazil), saline solution (0.9% sodium chloride), ethanol and distilled water.

Associations

NaOCl solutions were associated with CHX solution and gel, EDTA, citric acid, phosphoric acid, saline solution, ethanol, and distilled water. In addition, CHX solution

and gel were mixed with EDTA, citric acid, phosphoric acid, saline solution, ethanol, and distilled water. The solutions were mixed in a 1:1 ratio on flat-top 1.5-mL polypropylene microtubes, 0.5 mL of each, at room temperature, as summarized in Table 1. The study was performed in triplicate. The microtubes were evaluated qualitatively for color changes in the solutions after being mixed, presence of bubbles, and precipitate formation. They were observed every 15 minutes for 2 hours.

Characterization of the Precipitate

After mixing the solutions and observing the precipitate formation (entries 1-5, 9-15, 19 and 20; Table 1), ESI-QTOF-MS was used to determine their chemical composition. For this purpose, the samples were centrifuged, the supernatant removed, and 0.5 mg of the precipitate solubilized in 1.0 mL of deionized water (Type I, 18 mΩ.cm), acetonitrile (HPLC/Spectro, Tedia, Rio de Janeiro, RJ, Brazil), methanol (HPLC/Spectro, Tedia, Rio de Janeiro, RJ, Brazil) or combinations of these, and then acidified with 0.1% of formic acid (to aid protonation in the positive ion mode; ACS, Tedia, Rio de Janeiro, RJ, Brazil). After solubilization of the precipitate, ESI-QTOF-MS analyses were performed on QTOF Micro mass spectrometer (Waters, Wythenshawe, Manchester, UK). The mass was described as mass-to-charge ratio (*m/z*) and the analyses were recorded between *m/z* 90 to 1000 in positive ion mode [ESI(+)-MS], with mass spectrometer parameters being the following: nebulization gas was set to 500 L/h at 120 °C, cone gas set to 50 L/h, and source temperature set to 100 °C. Capillary and cone voltage were set to 3000 V and 30 V, respectively. The QTOF acquisition rate was set to 1.0 s, with a 0.4 s inter-scan delay and data processed on the MassLynx 4.0 software (Waters, Wythenshawe, Manchester, UK). The analyses were carried out by direct infusion using a syringe pump at flow ratio of 5.0 μL/min.

RESULTS

Table 1 describes the experimental groups and the organoleptic characteristics of the associations.

Table 1: Association of irrigants and visual characteristic of the products.

Entry	Solution 1	Solution 2	Appearance of the resultant association[a]
1	2% CHX gel	5.25% NaOCl	Orange-brown precipitate
2	2% CHX gel	2.5% NaOCl	Orange-brown precipitate
3	2% CHX gel	1% NaOCl	Orange-brown precipitate
4	2% CHX gel	0.16% NaOCl	Orange-white precipitate
5	2% CHX gel	17% EDTA	White milky precipitate
6	2% CHX gel	10% Citric acid	Unchanged
7	2% CHX gel	37% Phosphoric acid	Unchanged
8	2% CHX gel	Distilled water	Unchanged
9	2% CHX gel	Saline solution	Salt precipitation
10	2% CHX gel	Ethanol	Salt precipitation
11	2% CHX solution	5.25% NaOCl	Orange-brown precipitate
12	2% CHX solution	2.5% NaOCl	Orange-brown precipitate
13	2% CHX solution	1% NaOCl	Orange-brown precipitate
14	2% CHX solution	0.16% NaOCl	Orange-white precipitate
15	2% CHX solution	17% EDTA	White milky precipitate
16	2% CHX solution	10% Citric acid	Unchanged
17	2% CHX solution	37% Phosphoric acid	Unchanged
18	2% CHX solution	Distilled water	Unchanged
19	2% CHX solution	Saline solution	Salt precipitation
20	2% CHX solution	Ethanol	Salt precipitation
21	5.25% NaOCl	17% EDTA	Bubble formation
22	5.25% NaOCl	10% Citric acid	Bubble formation
23	5.25% NaOCl	37% Phosphoric acid	Yellow solution with bubble formation
24	5.25% NaOCl	Distilled water	Unchanged
25	5.25% NaOCl	Saline solution	Unchanged
26	5.25% NaOCl	Ethanol	Unchanged
27	2.5% NaOCl	17% EDTA	Bubble formation
28	2.5% NaOCl	10% Citric acid	Bubble formation
29	2.5% NaOCl	37% Phosphoric acid	Yellow solution with bubble formation
30	2.5% NaOCl	Distilled water	Unchanged
31	2.5% NaOCl	Saline solution	Unchanged
32	2.5% NaOCl	Ethanol	Unchanged
33	1% NaOCl	17% EDTA	Bubble formation
34	1% NaOCl	10% Citric acid	Bubble formation
35	1% NaOCl	37% Phosphoric acid	Slight yellow solution with bubble formation
36	1% NaOCl	Distilled water	Unchanged
37	1% NaOCl	Saline solution	Unchanged
38	1% NaOCl	Ethanol	Unchanged
39	0.16% NaOCl	17% EDTA	Unchanged
40	0.16% NaOCl	10% Citric acid	Unchanged
41	0.16% NaOCl	37% Phosphoric acid	Unchanged
42	0.16% NaOCl	Distilled water	Unchanged
43	0.16% NaOCl	Saline solution	Unchanged
44	0.16% NaOCl	Ethanol	Unchanged

[a] solution 1 and solution 2 mixed in a 1:1 ratio.

Two-percent CHX gel and solution produced an orange-brown precipitate (Figure 1A) when associated with 1, 2.5 and 5.25% NaOCl solutions, and an orange-white precipitate (Figure 1B) was observed for 0.16% NaOCl. When CHX was associated with 17% EDTA, a white milky precipitate (Figure 1C) was observed, whereas the association with saline solution and ethanol produced a salt precipitation (Figures 1D,E). No precipitation was observed when CHX was associated with 10% citric acid, phosphoric acid or distilled water.

When NaOCl, at the different concentrations, was associated with 17% EDTA, 10% citric acid, 37% phosphoric acid, distilled water, saline solution and ethanol, no precipitate was found. The association of NaOCl with 17% EDTA (Figure 1F), 10% citric acid (Figure 1G) and 37% phosphoric acid leads to the formation of bubbles and yellow precipitate in those cases involving phosphoric acid (Figure 1H).

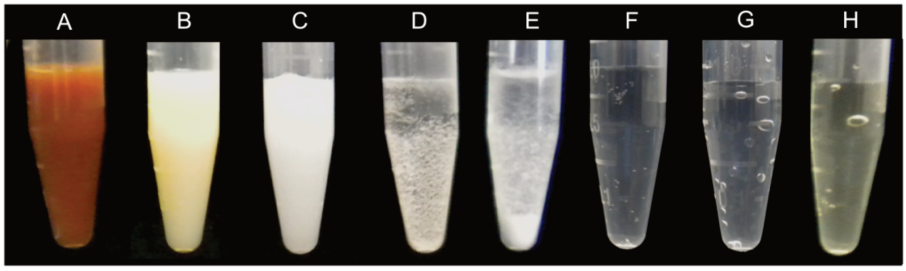

Figure 1: Visual aspect of the interactions between 5.25% NaOCl and 2% CHX (A), 0.16% NaOCl and 2% CHX (B), 17% EDTA and 2% CHX (C), saline solution and 2% CHX (D), ethanol and 2% CHX (E), 5.25% NaOCl and 17% EDTA (F), 5.25% NaOCl and 10% citric acid (G), 5.25% NaOCl and 37% phosphoric acid (H).

Among the cases showing precipitate formation, redox-type reactions were observed for the precipitate formed in the reaction between NaOCl solutions and CHX, assigned by mass spectrometry analyses, since the NaOCl is known to be an oxidizing agent. Figure 2 illustrates the ESI(+)-MS for the different experimental groups of CHX. The first spectrum for 2% CHX (Figure 2A) appears as control; the second (Figure 2B) and third (Figure 2C) spectra show the association between NaOCl and CHX, with development of orange-brown and orange-white precipitates, respectively (relative to entries 1, 4, 11 and 14 in Table 1). The main signals attributed to these groups were:

Figure 2A - *m*/z 253 $[M+2H]^{2+}$ / $C_{22}H_{32}Cl_2N_{10}^{2+}$, *m*/z 505 $[M+H]^+$ $C_{22}H_{31}Cl_2N_{10}^+$ (see example in this Figure), *m*/z 701 $[M+H]^+$ $C_{28}H_{43}Cl_2N_{10}O_7^+$ (chlorhexidine gluconate); Figures 2B and 2C - *m*/z 270 $[M+2H]^{2+}$ $C_{22}H_{31}Cl_3N_{10}^{2+}$ / *m*/z 539 $[M+H]^+$ $C_{22}H_{30}Cl_3N_{10}^+$, *m*/z 287 $[M+2H]^{2+}$ $C_{22}H_{30}Cl_4N_{10}^{2+}$ / *m*/z 573 $[M+H]^+$ $C_{22}H_{29}Cl_4N_{10}^+$, *m*/z 304 $[M+2H]^{2+}$ $C_{22}H_{29}Cl_5N_{10}^{2+}$ / *m*/z 607 $[M+H]^+$ $C_{22}H_{28}Cl_5N_{10}^+$, *m*/z 321 $[M+2H]^{2+}$ $C_{22}H_{28}Cl_6N_{10}^{2+}$ / *m*/z 641 $[M+H]^+$ $C_{22}H_{27}Cl_6N_{10}^+$, *m*/z 338 $[M+2H]^{2+}$ $C_{22}H_{27}Cl_7N_{10}^{2+}$ / *m*/z 675 $[M+H]^+$ $C_{22}H_{26}Cl_7N_{10}^+$, *m*/z 355 $[M+2H]^{2+}$ $C_{22}H_{26}Cl_8N_{10}^{2+}$ / *m*/z 709 $[M+H]^+$ $C_{22}H_{25}Cl_8N_{10}^+$. Due to the presence of two chlorine atoms in the molecule of CHX and to others chlorines introduced in the oxidation step with NaOCl (Figures 2A,D), the isotopic profile of the observed *m/z* signals were evaluated and found to be in accordance with the profile obtained using the Isotope Model tool of the MassLynx 4.0 software (Waters, Wythenshawe, Manchester, UK) and literature as well (13-15) (see example in Figure 2B). The probable sites of chlorine addition to chlorhexidine ($C_{22}H_{30}Cl_2N_{10}$) occur at the guanidino nitrogens (1-3 and 1´-3´), according to the proposed mechanism showed in Figure 2D.

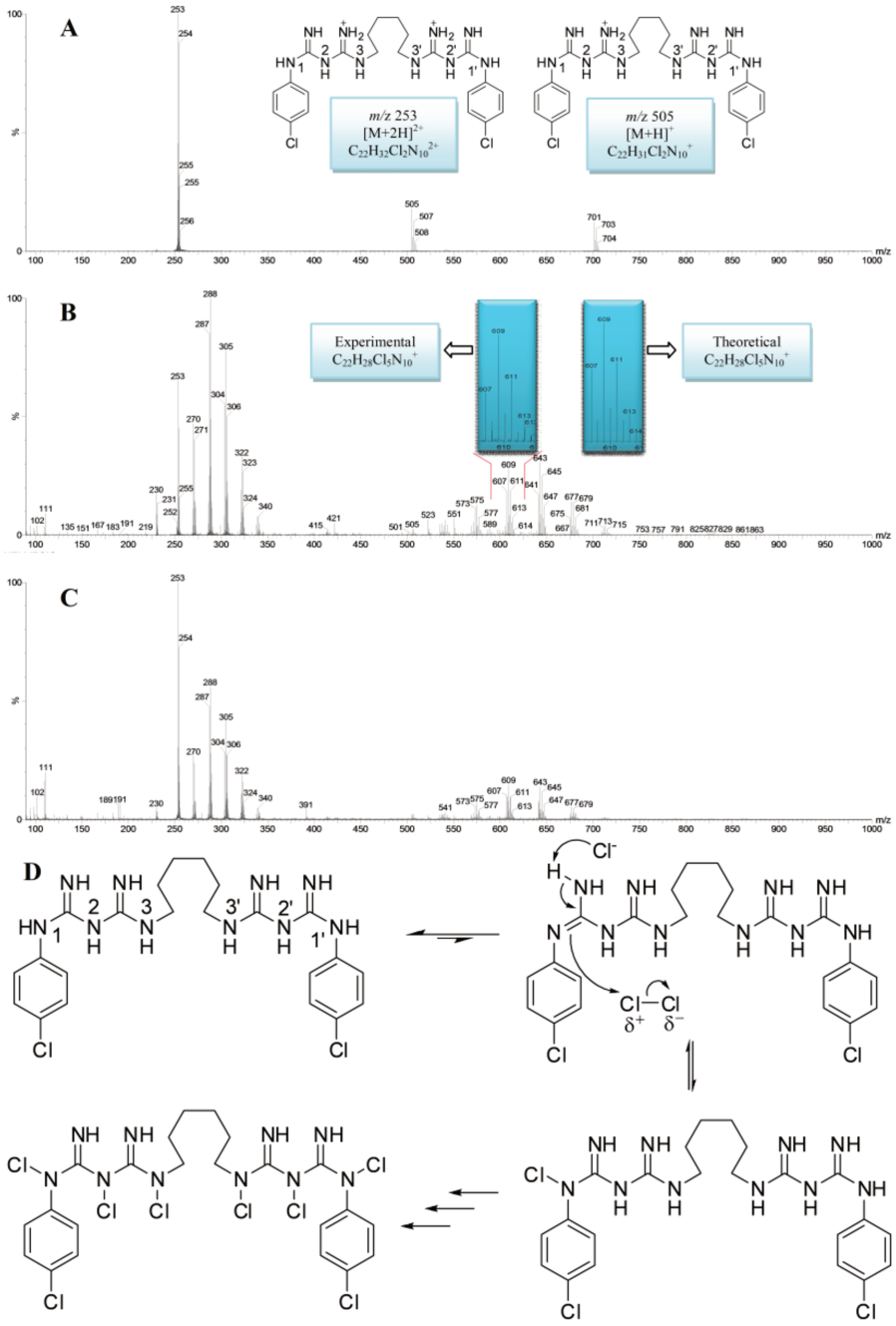

Figure 2: Electrospray ionization mass spectrum in positive ion mode [ESI(+)-MS] for the different experimental groups of chlorhexidine digluconate (A- 2% CHX, B- 5.25% NaOCl + 2% CHX, C- 0.16% NaOCl + 2% CHX) and the proposed mechanism (D) of chlorination of the guanidino nitrogens (1-3 and 1´-3´).

The observed signals in the ESI(+)-MS spectra of the precipitate of associations between CHX and EDTA were related only to the isolated compounds, which allowed to associate the milky precipitate formation with acid-base reactions. The same occurs for the precipitates formed between CHX with saline solution and ethanol, attributed to the salting-out process (19) and to the reduced solubility of the CHX salt in ethanol solution.

Finally, the bubble formation observed in the reactions between NaOCl and 17% EDTA, 10% citric acid or 37% phosphoric acid can be explained mainly by the formation of chlorine gas (Cl_2) resulting from an increase in proton (H^+) concentration in the presence of chloride ions (Cl^-), which is the usual impurity of NaOCl solutions, shifting the equilibrium towards the formation of Cl_2, according to the reactions (Eqs. 1, 2 and 3) (10,20-23):

$$HOCl(aq) + NaOH(aq) \leftrightharpoons NaOCl(aq) + H_2O(l) \qquad K = 3.20 \times 10^6 \qquad (1)$$

$$Cl_2(g) + H_2O(l) \leftrightharpoons H^+(aq) + HOCl(aq) + Cl^-(aq) \qquad K = 1.04 \times 10^{-3} \qquad (2)$$

$$HOCl(aq) \leftrightharpoons H^+(aq) + OCl^-(aq) \qquad K_a = 3.20 \times 10^{-8},\ pK_a = 7.50 \qquad (3)$$

K equilibrium constant; K_a = acid-dissociation equilibrium constant; $pK_a = -\log K_a$

In addition, Cl_2 can also be produced by the oxidation of EDTA or citric acid by HOCl (Eq. 4) (24):

$$HOCl(aq) + 2H^+ + 2e^- \text{ ® } Cl_2(g) + 2H_2O \qquad E° = 1.63\ V \qquad (4)$$

$E°$ = standard reduction potential

DISCUSSION

The association between different irrigants enhances their antimicrobial effect (25,26), allowing an effective removal of organic and inorganic debris (smear layer) (27,28) and favoring the wettability of sealers in the dentin surface (29). However, some interactions can lead to the formation of precipitates, resulting in a chemical smear layer which occludes the dentinal tubules and may interfere with the seal of the root filling while generating toxic byproducts (30). For this reason, the knowledge of these interactions is indispensable.

In the present study, as reported previously by Basrani et al. (8), the association of NaOCl at concentrations of 1 to 5.25% with 2% CHX solution and gel results in an orange-brown precipitate. The mass spectrometry analyses confirms the presence of several products of chlorination from the oxidizing agent NaOCl, which occurs at one to six guanidino nitrogens of the chlorhexidine (2,16-18) and the color change can be associated with the guanidine oxidation (31). The signal at *m/z* 128 of PCA ($[M+H]^+$, $C_6H_7ClN^+$) was not detected (Figures 2B,C), in accordance with Thomas and Sem (2). The orange-white precipitate observed when 0.16% NaOCl was associated with CHX was attributed to the lower concentration of NaOCl. Regarding the attributed *m/z* signals (Figures 2B,C), only the chemical structure of the chlorhexidine chlorinated at 1, 2 and 3 guanidino nitrogens (*m*/z 304 $[M+2H]^{2+}$ $C_{22}H_{29}Cl_5N_{10}^{2+}$ / *m*/z 607 $[M+H]^+$ $C_{22}H_{28}Cl_5N_{10}^+$) was found in the literature, but none on its toxicity (32). This compound is a very potent inhibitor of the human enzyme soluble epoxide hydrolase (human sEH, IC_{50} = 1.05 ± 0.03), which can be used to selectively inhibit epoxide hydrolase in therapeutic applications (e.g. inflammation treatment, affinity separations of the epoxide hydrolases) and in conjunction with cancer therapy (32).

When NaOCl was mixed with EDTA, citric acid or phosphoric acid, an exothermic reaction with formation of bubbles was observed. The presence of bubbles was more intense for phosphoric acid, followed by citric acid and finally less intense for EDTA. These bubbles are mainly chlorine gas (20), a toxic product (33). The yellow color observed in the cases of phosphoric acid is associated with the high formation of Cl_2 due to the lower pK_a = 2.15 (more acidic character) of the phosphoric acid. With the dilution of NaOCl less undesirable products are formed, so when phosphoric acid was chosen for smear layer removal, intermediate flushes with distilled water are important to remove or at least reduce the concentration of NaOCl present in the root canal.

ESI(+)-MS analyses of the white milky precipitate observed in the association of EDTA with CHX solution and gel allowed to associate the precipitate formation with acid-base reactions. Our data are in accordance with Rasimick et al. (6), who analyzed the precipitate formed after mixing 17% EDTA with 2% or 20% CHX by using reversed-phase high-performance liquid chromatography and observed that over 90% of the precipitate mass was either EDTA or CHX salt. When CHX was associated with saline

solution and ethanol, the problem of solubility was observed. When CHX gel and solution was mixed with citric and phosphoric acids, no precipitate formation was observed, being the latter more effective for smear layer removal (5). This can be an alternative after the use of CHX during chemo-mechanical preparation for this purpose.

NaOCl, at different concentrations, and 2% CHX gel and solution, when associated with distilled water, did not form any precipitate. According to the present study, distilled water is the most indicated solution to be employed as irrigant during intermediate rinse.

In conclusion, the orange-brown precipitate observed in the association between CHX and NaOCl occurs due the presence of NaOCl, an oxidizing agent causing chlorination of the guanidino nitrogens of the CHX. The precipitates formed in the reaction of CHX with EDTA, saline solution and ethanol were associated with acid-base reactions, salting-out process and lower solubility, respectively. NaOCl associated with EDTA, citric acid and phosphoric acid leads mainly to chlorine gas formation.

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CAPÍTULO 2

Interaction between irrigants commonly used in endodontic practice: An antimicrobial analysis

Maíra do Prado[1], Thais Mageste Duque[2], Renata A. Simão[3], Brenda P.F.A. Gomes[4]

[1]DDS, MSc – PhD Student, Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.
[2]DDS – MSc Student, Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.[3]DSc, MSc, PhD
[3]DSc, MSc, PhD – Adjunct Professor, Department of Metallurgic and Materials Engineering, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
[4]DDS, MSc, PhD – Titular Professor, Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.

Correspondence: Dr Brenda Paula F. A. Gomes - Department of Restorative Dentistry - Piracicaba Dental School State University of Campinas
Avenida Limeira, 901, Piracicaba, SP – Brazil 13414-018 Phone: (0055) 19 2106-5215 Fax: (0055) 19 2106-5218 E-mail: bpgomes@fop.unicamp.br

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ABSTRACT

Introduction: In general, an irrigant is not completely flushed out from the root canal before applying the next irrigant. As a result, endodontic irrigants routinely come into contact with each other inside the root canal. The aim of the present study was to evaluate the interaction between irrigants commonly used in endodontic practice in relation to its antimicrobial activity using the Agar Diffusion Method. **Method:** 0.16%, 1%, 2.5% and 5.25% NaOCl, 2% CHX solution and gel, 17% EDTA, 10% citric acid and 37% phosphoric acid were associated in 1:1 ratio. The pure solutions were used as control. The bacterial activity was tested against *Candida albicans*, *Staphylococcus aureus*, *Actinomyces naeslundii, Enterococcus faecalis*, *Escherichia coli, Lactobacillus casei, Actinomices meyeri*, *Parvimonas micra, Porphyromonas gingivalis* and *Porphyromonas nigrescens*. Zones of inhibition of microbial growth around the cylinder containing the tested substances were measured and recorded. Data were statistically evaluated. **Results:** Zone of bacterial inhibition was verified in all associations. **Conclusion:** The present study revealed that all associations presented antimicrobial activity and this activity was related with bacterial strain.

INTRODUCTION

Bacteria and their by-products play an essential role in the establishment and development of pulpal and periapical diseases (1). Once bacteria are established within the root canal system, they cannot be eliminated by host defense mechanisms, therefore pulpal infections must be treated by chemo-mechanical procedures (2). For this purpose, different instruments associated with a combination of irrigants are used. The mechanical preparation of the root canal system removes a large amount of irritants, but complete debridement is hampered by its complex anatomy, and irrigation (chemical preparation) of the root canal is essential for cleaning and disinfection during endodontic preparation (3-7).

Sodium hypochlorite (NaOCl) is the most widely used endodontic irrigant, because of its bactericidal activity and ability to dissolve vital and necrotic organic tissue (8). Chlorhexidine (CHX) has been suggested as an irrigant during root canal therapy based on its antibacterial effects, substantivity, and relative absence of cytotoxicity (9).

However, these two solutions are not capable of removing the smear layer (10). Therefore, the adjunctive use of chelating agents such as EDTA, citric acid or phosphoric acid is suggested in order to remove the smear layer formed during the root canal instrumentation (11).

In general, an irrigant is not completely flushed out from the root canal before applying the next irrigant. As a result, endodontic irrigants routinely come into contact with each other inside the root canal. In this aspect, Kuruvilla and Kamath (12) observed that the antimicrobial effect of 2.5% NaOCl and 0.2% CHX used in combination was better than that of either component. However, Vianna and Gomes (13) observed that the association of NaOCl and CHX did not improve the antimicrobial activity of CHX alone. Heling and Chandler (4) investigated sodium hypochlorite and chlorhexidine, with and without EDTA, when used in combination as endodontic irrigants against *Enterococcus faecalis* and verified that combining EDTA with NaOCl or CHX was more effective than using EDTA alone.

Some interactions, as NaOCl associated with chlorhexidine and EDTA associated with chlorhexidine, leads to the formation of precipitates, resulting in a chemical smear layer that covers the dentinal tubules. As some irrigant protocols did not remove completely this layer and this layer remains in the dentin surface, the knowledgment of the smear layer's antimicrobial effect is important. Additionally, reganding other associations, such as NaOCl and phosphoric acid, chlorhexidine and citric acid or chlorhexidine and phosphoric acid, no antimicrobial activity were found in the endodontic literature. Therefore, the aim of the present study was to evaluate the interaction between irrigants commonly used in endodontic practice in relation to its antimicrobial activity using the Agar Diffusion Method.

MATERIALS AND METHODS

Chemical auxiliary substances

The substances evaluated in the present study were: 0.16%, 1%, 2.5% and 5.25% NaOCl (Drogal, Piracicaba, SP, Brazil), 2% CHX solution and gel (Drogal, Piracicaba, SP, Brazil), 17% EDTA (Drogal, Piracicaba, SP, Brazil), 10% citric acid (Drogal, Piracicaba, SP, Brazil) and 37% phosphoric acid (Drogal, Piracicaba, SP, Brazil). NaOCl

solutions were associated with CHX solution and gel, EDTA, citric acid (CA) and phosphoric acid (PA). As well as, CHX solution and gel were mixed with EDTA, CA and PA. The solutions were tested individually (control) and in association as specified above.

Microorganisms

The species of microorganisms used in this experiment were two aerobes, four facultative and four strict anaerobic microorganisms commonly isolated from infected root canals, as follows: *Candida albicans* (ATCC 10556), *Staphylococcus aureus* (ATCC 25923), *Actinomyces naeslundii* (ATCC 19039), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Lactobacillus casei* (ATCC L324M), were grown on Brain Heart Infusion Agar plates (BHI, Lab M, Bury, UK); and *Actinomices meyeri* (isolated from the root canal infections and identified by using conventional biochemical tests), *Parvimonas micra* (ATCC 33270), *Porphyromonas gingivalis* (ATCC 49417) and *Porphyromonas nigrescens* (ATCC 33563) were grown on Fastidious Anaerobe Agar plates (FAA, Lab M, Bury, UK). In both media, 5% defibrinated sheep blood was added.

Agar diffusion method

The methodology used was adapted from Gomes et al (14,15). All microorganisms were previously subcultured in appropriate culture media and under gaseous conditions to confirm their purity.

The aerobes and the facultative anaerobic strains were individually inoculated into tubes containing 5 mL of sterile saline solution. The suspension was adjusted spectrophotometrically at 800 nm (Optical Density800) to match the turbidity of 1.5 X 10^8 $CFUmL^{-1}$ (equivalent to 0.5 McFarland standard). Five-hundred mL of each test microorganism suspension were used to inoculate glass bottles containing 50 mL of BHI Agar (Lab M, Bury, UK) at 46°C, mixed and poured onto 150 mm plates containing a previously set layer of Mueller Hinton agar (Oxoid, Unipath Ltd, Basingstoke, UK).

The anaerobic microorganisms were suspended spectrophotometrically at 800 nm to match the turbidity of 3.0 X 10^8 $CFUmL^{-1}$ (equivalent to 1 McFarland standard). Sterile swabs were dipped into the bacterial suspension and were used to inoculate pre-reduced 150 mm plates containing 5% sheep-blood- Fastidious Anaerobe Agar (FAA -

Lab-M, Bury, UK). The inoculum procedures used were appropriate to provide a semi-confluent growth of the tested microorganisms (16).

Three sterilized stainless steel tubes of 8.0 X 1.0 X 10.0 mm (inner diameter of 6 mm) were added to the surfaces of the media and filled with 0.2 mL of each pure or composite substance. The plates were maintained for 2 hours at room temperature in the appropriate gaseous conditions to allow the diffusion of the agents through the agar and then incubated at 37°C again under the appropriate gaseous conditions for an appropriate period of time: aerobes, 24 hours; facultative, 24-48 hours in a CO_2 incubator (Jouan, Saint Herblain, France), in an atmosphere of 10% CO_2 and anaerobes in the anaerobic workstation (Don Whitley Scientific, Bradford, UK) in an atmosphere of 10% H_2, 10% CO_2, 80% N_2 for 7 days.

Zones of inhibition of microbial growth around the cylinder containing the tested substances were measured and recorded after the incubation period. The inhibitory zone was considered to be the shortest distance (mm) between the outer margin of the cylinder and the initial point of the microbial growth. Three replicates were made for each microorganism. Data were statistically analyzed using non-parametric tests: Kruskal-Wallis and Mann- Whitney.

RESULTS

Figure 1 shows the average values against the ten bacterial strains tested. Regarding the pure solutions, the NaOCl solutions values of growth inhibition decreased with the concentration, CHX gel showed bigger zones of inhibition than CHX solutions and the three agents used for smear layer removal showed antimicrobial activity. PA showed bigger zones of inhibition when compared with other pure solutions.

The associations of 2.5% NaOCl/ EDTA, 1% NaOCl/ EDTA and 0.16% NaOCl/ EDTA showed bigger values of growth inhibition zones than the pure solutions. In the cases of 5.25% NaOCl, CHX gel and solutions associated with EDTA, the pure solutions values of growth inhibition zones were bigger than the associations. The associations between CA and NaOCl solutions, CA and CHX liquid or CA and CHX gel remained equal or decreased the zones of inhibition when compared with the pure solutions. When NaOCl solutions were associated with CHX gel or CHX solution the zones of inhibition

of the association were smaller than the pure solutions, excluding the group 0.16% NaOCl/CHX solution.

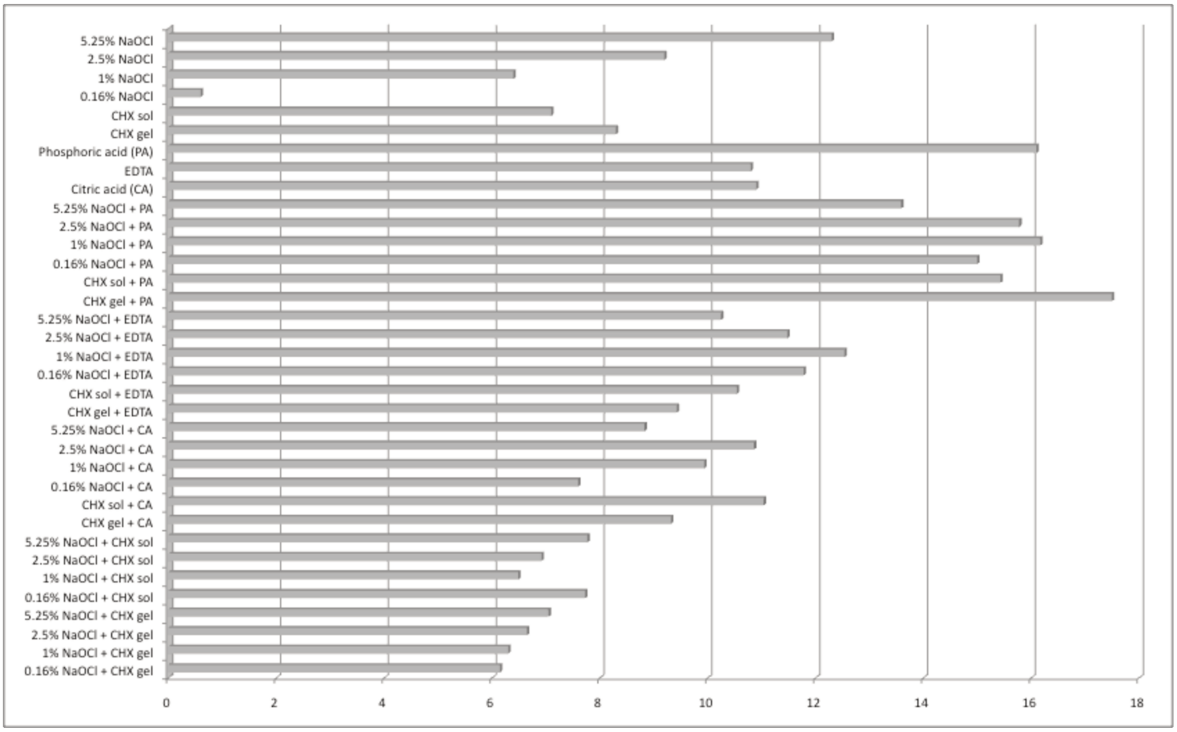

Figure 1: Average values of growth inhibition zones (in mm) produced by the solutions against the ten bacterial strains tested (in millimeters).

Regarding the microorganisms tested (Figure 2), *Enterococcus faecalis* was the more resistent microorganism, as can be observed by the lesser values of growth inhibition zones.

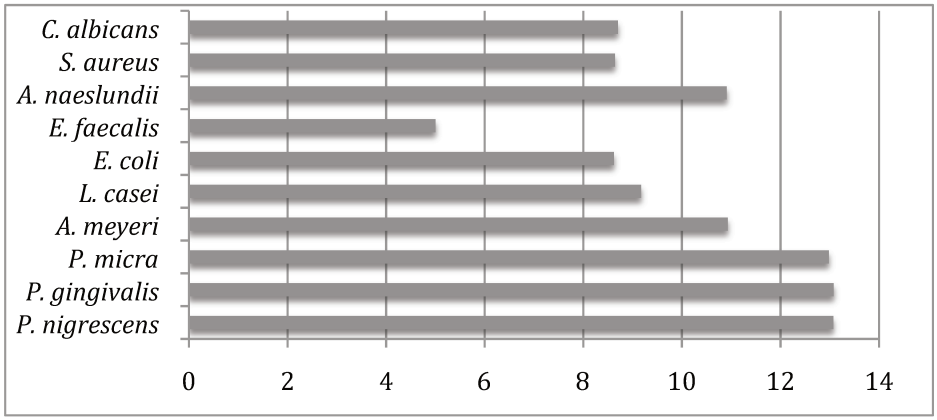

Figure 2. Average values of growth inhibition zones (in mm) by the solution and its associations against all microorganisms tested.

DISCUSSION

Various methods have been described for the analysis of the antimicrobial activity of endodontic irrigants. Agar diffusion test is the generally accepted procedure worldwide for determining in vitro sensitivity under routine laboratory conditions. Several studies of irrigants have used the agar diffusion method (4,17,18). This method is simple, standard, and reproducible. Even though the agar method is one of the popular tests, it may have some limitations (19,20). Some factors such as pH of the substrate, incubation period, toxicity, sensitivity, and diffusion capacity of the substance may have an effect on the antimicrobial activity of the test materials in the plates. However, there is also evidence that agar diffusion tests show good correlation with other antimicrobial susceptibility tests (21,22).

Because irrigant solutions are used in succession, they come into contact with each other inside the endodontic space. In this aspect the literature reports that it might impact treatment due to modifications of tissue dissolution, antimicrobial and cleaning efficacy, sealing, the risk of discoloration, and most importantly the potential adverse effects to a patient's general health as a consequence of leaching chemicals in the periradicular tissues (10). The present study evaluated the associations between irrigants commonly used in endodontic practice and compared with the antimicrobial effect of the pure irrigants. Furthermore the 0.16% NaOCl was used because when this solution is associated with 2% CHX, the orange-brown precipitate is not verified.

When the pure solutions were evaluated against the ten bacterial strains tested, the greatest zone of inhibition values were found with 37% PA, followed by 5.25% NaOCl, 10% CA, 17% EDTA, 2.5% NaOCl, 2% CHX gel, 2% CHX solution, 1% NaOCl and 0.16% NaOCl. In this aspect, Siqueira et al. (17) evaluated the zones of inhibition produced by the irrigant solutions against the eight bacterial strains tested and observed greatest zone of inhibition values for 4% NaOCl, followed by 2% CHX, 0.2% CHX, 17% EDTA, 10% CA and 0.5% NaOCl. Additionaly Ferraz et al. (18) observed that the largest growth inhibition zones were produced when the test bacteria were in contact with 2% chlorhexidine gel, being significantly different from the growth inhibition zones produced by NaOCl concentrations (0.5%, 1%, 2.5%, 4%, 5.25%). The difference in the

results of thepresent study and the other authors can be associated with the different number and strains of bacteria tested.

The association between NaOCl (5.25%, 2.5% and 1%) and 2% CHX (solution and gel) produces an orange-brown precipitate that forms a chemical smear layer that occludes dentinal tubules and changes the tooth color (23,24). According to the findings of the present study, independent of the bacterial strain, this precipitate has antimicrobial activity. In 1998, Kuruvilla & Kamath (12) compared the effect of 2.5% NaOCl, CHX 0.2% and their combination and observed that the use of sodium hypochlorite and chlorhexidine combined within the root canal resulted in the greatest percentage reduction of post irrigant positive cultures. They found that this reduction was significant, compared to sodium hypochlorite alone but not significant compared to chlorhexidine gluconate alone. Vianna & Gomes (13), using the agar diffusion test, compared the efficacy of the combination of sodium hypochlorite (1%, 2.5% and 5.25%) and 2% chlorhexidine (liquid and gel) against *Enterococcus faecalis* with the antimicrobial activity of the same irrigating substances when applied alone. The results of the present study are in accordance with Vianna & Gomes, which found larger mean microbial growth zones for CHX when compared with NaOCl and that the association of NaOCl and CHX did not improve the antimicrobial activity of these solutions alone.

As NaOCl is not able to remove smear layer produced during chemo-mechanical preparation, after its use, other solution must be used. For this purpose PA, CA or EDTA are used (11). In the present study when these solutions were associated, they presented some antimicrobial activity. It was dependent of bacteria strain. This activity was equal or smaller than the pure solution. In this context, Grawehr et al. (25) analyzed the effects on antimicrobial ability, related to the interactions between EDTA and NaOCl, using an agar diffusion test against *Enterococcus faecalis* and *Candida albicans*. They observed that NaOCl alone produced smaller zones of inhibition when compared with EDTA or the mixture of EDTA/NaOCl. Also, Zehnder et al. (26) tested the impact of CA and EDTA on NaOCl's antimicrobial action against *Enterococcus faecalis* and found that CA and EDTA eliminated NaOCl's antimicrobial action because bacterial growth was observed. No study associating NaOCl and phosphoric acid was found.

When CHX gel and solution were associated with PA, CA and EDTA, antimicrobial activity was observed. Concerning these associations, Heling and Chandler (4) evaluated the antimicrobial effect of irrigant combinations within dentinal tubules observing that the effect of EDTA in combination with chlorhexidine was more effective than EDTA alone. Studies regarding the antimicrobial activity of the association between citric acid with CHX and phosphoric acid with CHX were not found.

In conclusion, the present study revealed that all associations present antimicrobial activity and this activity is related with the bacterial strain.

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CAPÍTULO 3

Evaluation of Different Irrigation Protocols Concerning the Formation of Chemical Smear Layer

Maíra do Prado[1], Renata A. Simão [2], Brenda P. F. A. Gomes[3]

[1] Post Graduate Student- Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.

[2] Adjunct Professor, Department of Metallurgic and Materials Engineering, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

[3] Titular Professor, Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.

Running title: Formation of Chemical Smear Layer

Correspondence: Dr Brenda P. F. A. Gomes

Department of Restorative Dentistry - Piracicaba Dental School State University of Campinas.

Avenida Limeira, 901, Piracicaba, SP – Brazil 13414-018 Phone: (0055) 19 2106-5215 Fax: (0055) 19 2106-5218 E-mail: bpgomes@fop.unicamp.br

Keywords: chlorhexidine, citric acid, EDTA, phosphoric acid, smear layer, sodium hypochlorite.

ABSTRACT

The aim of this work was to evaluate, by means of scanning electron microscopy (SEM) and electron dispersive spectroscopy (EDS), the different irrigation protocols concerning the formation of chemical smear layer (CSL). Fifty-five teeth were instrumented by using distilled water (DW) as irrigant. Next, the teeth were submitted to a protocol for removal of the mechanical smear layer produced. The teeth were divided into eleven groups, according to irrigation regimen. Then, the teeth were prepared, and analyzed with scanning electron microscopy and electron dispersive spectroscopy. Only in Group 1 (1 mL NaOCl + 10 mL DW + 1 mL chlorhexidine solution), Group 2 (1 mL NaOCl + 10 mL DW + 1 mL EDTA + 10 mL DW + 1 mL chlorhexidine solution) and Group 3 (1 mL NaOCl + 10 mL DW + 1 mL citric acid + 10 mL DW + 1 mL chlorhexidine solution) the formation of chemical smear layer was observed. In the groups where chlorhexidine gel was used alone or in association with EDTA, citric acid, phosphoric acid and chlorhexidine solution, no formation of CSL was observed. According to the results found in the cases in which one wants to associate NaOCl with chlorhexidine, the protocol using phosphoric acid (1 mL NaOCl + 10 mL DW + 1 mL phosphoric acid + 10 mL DW + 1 mL chlorhexidine solution) did not induce formation of chemical smear layer.

INTRODUCTION

The aim of root canal therapy is to eliminate, or at least reduce the number of microorganisms, and remove inflamed or necrotic pulpar tissue (Tomas and Sem, 2010). During the therapy, various solutions are used for different purposes. The most common irrigant used during chemo-mechanical preparation is the sodium hypochlorite (NaOCl). This solution has the ability to dissolve organic tissue (Beltz et al., 2003; Koskinen et al., 1980; Senia et al., 1971; Zehnder et al., 2002) and it is an effective antimicrobial agent (Sena et al., 2006; Vianna et al., 2004). Chlorhexidine has been used during endodontic therapy because it has lower toxicity (Yesilsoy et al., 1995) and is an effective antimicrobial agent (Ferraz et al., 2001; Sena et al., 2006; Vianna et al., 2004) with substantivity (Basrani et al., 2002; Dametto et al., 2005). Because CHX lacks the tissue-dissolving capabilities of the NaOCl, it has been suggested the use of CHX as additional

irrigant for intracanal medication or final irrigation (Zehnder, 2006). A combination of NaOCl and CHX has been reported by Kuruvilla & Kamath (1998) and Vianna & Gomes (2009).

Furthermore, solutions like EDTA, citric acid (CA) and phosphoric acid (PA) are used to finish the chemo-mechanical preparation by removing the mechanical smear layer formed during the root canal preparation (Prado et al., 2011).

In general, an irrigant is not completely flushed out from the root canal before applying the next irrigant. As a result, endodontic irrigants routinely come into contact with each other inside the root canal. In this aspect, the literature shows that when NaOCl interacts with CHX inside the root canal, it is produced an orange-brown precipitate. This precipitate forms a chemical smear layer in dentin surface, which occludes the dentinal tubules. This layer may affect close adaptation between root canal filling materials and the root canal walls, interfering in the seal of the obturation. In addition, this precipitate changes the color of the tooth and is cytotoxic (Akisue et al., 2010; Basrani et al., 2007; Bui et al., 2008; Burkhardt-Holm et al., 1999). Other interactions between EDTA and CHX as well as between citric acid and CHX are also observed (Akisue et al., 2010; Rasimick et al., 2008).

In this context, the aim of this work was to evaluate, by means of scanning electron microscopy (SEM) and electron dispersive spectroscopy (EDS), the different irrigation protocols concerning the formation of chemical smear layer.

MATERIALS AND METHODS

Fifty-five uniradicular pre-molars with straight root canal, mature root apices and similar anatomical characteristics were selected in this study. The crowns were removed with carborundum discs (KG Sorensen, Barueri, SP, Brazil) at the level of the amelo-dentinal junction, thus facilitating cervical access. All teeth were instrumented by using a hybrid technique (Berber et al., 2006). This technique consists in the use of Gates-Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland) in descending order from 5 to 2 to prepare the middle-cervical third. Canals were instrumented with stainless steel K-files up to #30 (Dentsply Maillefer, Ballaigues, Vallorbe, Switzerland) at the apex in a crown-down technique. Irrigation was performed with 1 mL of distilled water at each

change of instrument. All teeth were submitted to ultrasonic bath for 10 minutes in 17% EDTA, followed by 10 minutes in 5.25% NaOCl bath. Next, they were submitted to tampon phosphate bath for 10 minutes to eliminate EDTA and hypochlorite residues, followed by immersion in distilled water bath for equal period of time, according to Perez et al. (1993), in order to eliminate the smear layer produced during the initial preparation.

To prevent extrusion of the irrigants out of the apex, all teeth had their apices sealed with utility wax (Technew, Rio de Janeiro, RJ, Brazil). After that, the teeth were randomly divided into groups of five according to the irrigation regimen, as described in Table 1. The substances used were: 5.25% NaOCl (Drogal, Piracicaba, SP, Brazil), 2% CHX solution and gel (Drogal, Piracicaba, SP, Brazil), 17% EDTA solution (Drogal, Piracicaba, SP, Brazil), 10% citric acid solution (Drogal, Piracicaba, SP, Brazil), 37% phosphoric acid solution (Drogal, Piracicaba, SP, Brazil), and distilled water (DW). The canals were irrigated 3 mm from the apex by using a 26-gauge hypodermic needle.

Table 1: Protocols for irrigation

Group	**Protocol for irrigation**
1	1 mL 5.25% NaOCl + 10 mL distilled water + 1 mL 2% chlorhexidine (CHX) solution
2	1 mL 5.25% NaOCl + 10 mL distilled water + 1 mL 17% EDTA + 10 mL distilled water + 1 mL 2% CHX solution
3	1 mL 5.25% NaOCl + 10 mL distilled water + 1 mL 10% citric acid + 10 mL distilled water + 1 mL 2% CHX solution
4	1 mL 5.25% NaOCl + 10 mL distilled water + 1 mL 37% phosphoric acid + 10 mL distilled water + 1 mL 2% CHX solution
5	1 mL 2% CHX gel + 10 mL distilled water + 1mL 17% EDTA + 1 mL distilled water

6	1 mL 2% CHX gel + 10 mL distilled water + 1mL 10% citric acid + 1 mL distilled water
7	1 mL 2% CHX gel + 10 mL distilled water + 1mL 37% phosphoric acid + 1 mL distilled water
8	1 mL 2% CHX gel + 10 mL distilled water + 1mL 17% EDTA + 10 mL distilled water + 1 mL 2% CHX solution
9	1 mL 2% CHX gel + 10 mL distilled water + 1mL 10% citric acid + 10 mL distilled water + 1 mL 2% CHX solution
10	1 mL 2% CHX gel + 10 mL distilled water + 1mL 37% phosphoric acid + 10 mL distilled water + 1 mL 2% CHX solution
11	Control (10 mL distilled water)

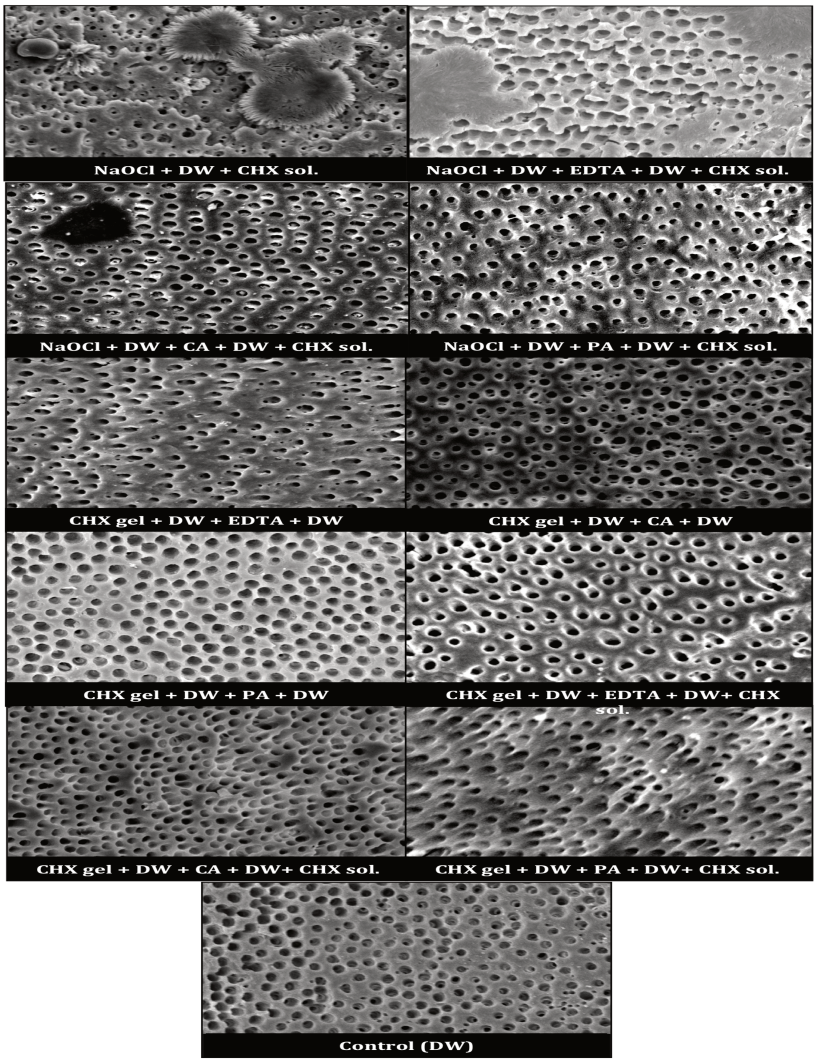
Next, the teeth were dried with medium-sized paper points (Endopoints, Paraiba do Sul, RJ, Brazil). Finally, two longitudinal grooves were prepared on both buccal and lingual surfaces by using a diamond disc without penetrating the canal. The roots were then splited into two halves with a hammer and chisel. For each root, the half containing the most visible part of the apex was used for study. The samples were prepared and analyzed with both scanning electron microscope (JEOL JSM 6460 LV, Tokyo, Japan) and electron dispersive spectroscopy (EDS).

All samples were numbered and photomicrographs were performed without knowing the group being tested. Three photomicrographs (1000x) of each third were obtained for each tooth, totalizing 9 images per tooth and 45 images per group (n=5). In the end, each group had 15 images for the three thirds.

Data were evaluated qualitatively in terms of presence or absence of chemical smear layer. EDS spectrum was obtained from the chemical smear layer in order to compare the chemical composition of this layer with mechanical smear layer and control group (without smear layer).

RESULTS

Figure 1 shows a representative photomicrograph of all groups evaluated. Only in Groups 1, 2 and 3 the presence of chemical smear layer was observed. The results showed that 10 mL of distilled water were not enough to inhibit the formation of precipitate in all groups. In Group 1, three (60%) of the five samples exhibited chemical smear layer formation in all roots. Formation of chemical smear layer was observed in all thirds of one (20%) sample treated with 17% EDTA (Group 2) and in two (40%) samples treated with 10% citric acid (Group 3). On the other hand, when phosphoric acid was used, there was no formation of chemical smear layer.



Note: NaOCl – 5.25% NaOCl; CHX – 2% Chlorhexidine; DW- distilled water; EDTA – 17% EDTA; CA – 10% citric acid; PA – 37% phosphoric acid.

Figure 1: Representative photomicrographies of the groups evaluated.

Figure 2 shows three images and the EDS related to them. Image (a) represents a control image, without smear layer; (b) represents a mechanical smear layer, and (c) chemical smear layer. It was clearly noticed the morphological difference between chemical and mechanical smear layers and the chemical composition of them. Additionally, the chemical composition shown in the images of control and mechanical smear layer samples is equal.

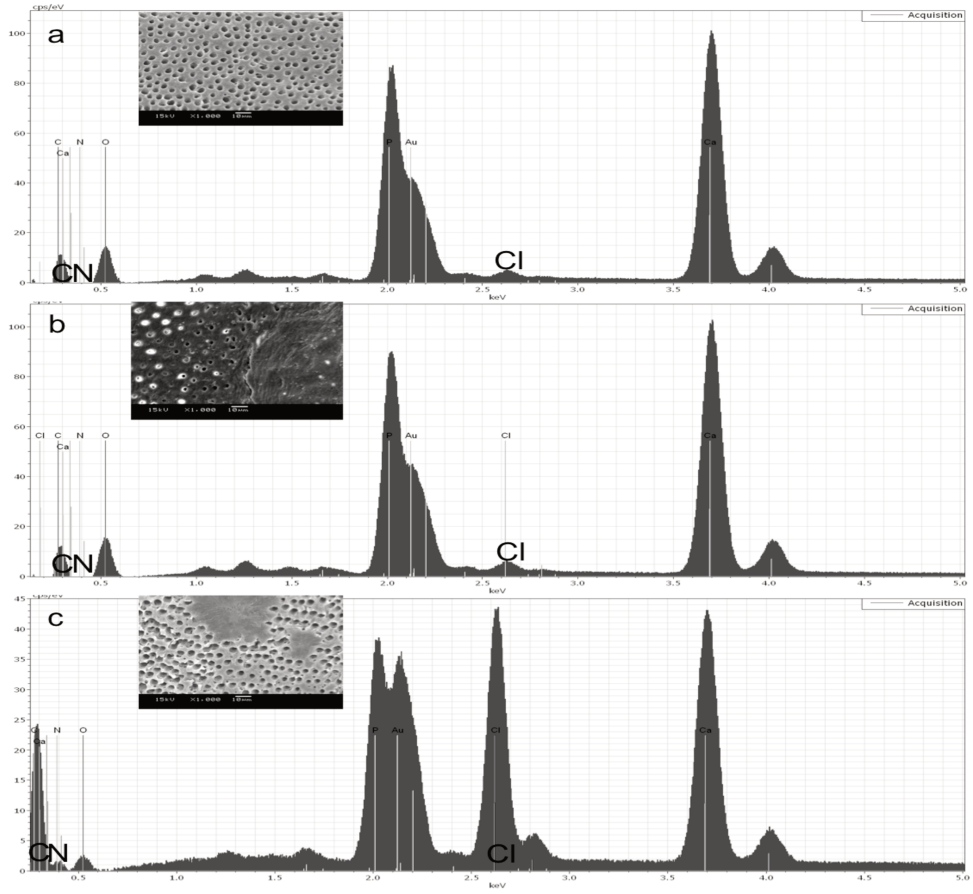


Figure 2: EDS spectra of (a) dentine without smear layer, (b) mechanical smear layer, and (c) chemical smear layer.

DISCUSSION

NaOCl is the most common irrigant used during chemo-mechanical preparation because it has the ability to dissolve organic tissue and is an effective antimicrobial agent (Beltz et al., 2003; Koskinen et al., 1980; Sena et al., 2006; Senia et al., 1971; Vianna et al., 2004; Zehnder et al., 2002). Chlorhexidine can be alternatively used because of its antimicrobial action, substantivity, and easy removal from the root canal system (Gomes

et al., 2006; Krithikadatta et al., 2007; Nandini et al., 2006). Additionally, the use of chlorhexidine as final irrigant has been shown to be favorable because it increases the levels of adhesion to dentin (Lindblad et al., 2010; Ricci et al., 2010).

The association between NaOCl and CHX, however, produces a precipitate which compromises the obturation seal and changes the color of the tooth, besides being cytotoxic (Akisue et al., 2010; Bui et al., 2008; Burkhardt-Holm et al., 1999). Thus, the use of CHX as intracanal medication or final irrigant would require the removal of NaOCl present inside the canal through intermediate flushes. Accordingly, the present study has evaluated several protocols for irrigation, with different intermediate flushes, concerning the chemical smear layer formation.

Firstly, before coating the samples for SEM analysis, they were observed by means of stereomicroscopy as reported by Krishnamurthy and Sudhakaran, who verified the presence of orange-brown precipitate when 5 mL of distilled water was used between irrigations with NaOCl and CHX. In the present study, this orange-brown precipitate was not observed in any of the tested groups after using 10 mL of distilled water. Comparing our findings with those of Krishnamurthy and Sudhakaran (2010), the difference in the results can be associated with the volume of the irrigant employed.

In Group 1, 10 mL of distilled water was used between irrigations with NaOCl and CHX. Although no precipitate was detected by stereomicroscopy, the SEM images showed formation of a precipitate – the so-called chemical smear layer. In Groups 2 and 3, where 10 mL of 17% EDTA and 10% citric acid were respectively used in order to simulate the removal of mechanical smear layer, the formation of precipitate also occurred. During the irrigation procedure in these three groups, a white ‘‘milky’’ solution was observed in some samples, indicating the presence of chemical smear layer. Consequently, we believe in a possible association between this white ‘‘milky’’ solution and the precipitate found.

In the present study, Figure 2c shows the EDS of chemical smear layer (found in Groups 1, 2 and 3). EDS of these groups showed the following chemical elements: N, C and high levels of Cl. As these chemical elements are compounds of chlorhexidine ($C_{22}H_{30}Cl_2N_{10}$), the precipitate found can be associated with the degradation of chlorhexidine by the oxidative action of NaOCl, in accordance with Nowicki and Sem

(2011). With regard to the mechanical smear layer (Figure 2b), EDS showed low levels of Cl but no presence of N. The same behavior was observed in the controls. Morphologically, it was clear the difference between chemical and mechanical smear layers. The chemical smear layer is more irregular than the mechanical smear layer, as observed in Figures 2b and 2c.

When CHX gel was used to simulate the chemo-mechanical preparation (Groups 5 to 10), 10 mL of distilled water was found to be enough to inhibit the formation of precipitate in all groups, with no white ''milky'' solution observed. Thus, it is believed that the white ''milky'' solution, observed in Groups 1, 2 and 3, is related to the presence of NaOCl and not to the association between EDTA and CHX or between CA and CHX (Akisue et al., 2010; Rasimick et al., 2008). This explains the fact that when EDTA or CA were used, less samples presented chemical smear layer, probably because NaOCl had not been completely inhibited.

According to the findings of this work, the protocols using CHX and distilled water followed by EDTA, citric acid or phosphoric acid did not exhibit chemical smear layer formation. With regard to the use of NaOCl with CHX, 10 mL of distilled water in association or not with 17% EDTA and 10% citric acid was not enough to inhibit the formation of chemical smear layer. In the cases where one wants to associate these solutions, the protocol using phosphoric acid to remove the smear layer did not induce formation of chemical smear layer.

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CAPÍTULO 4

Effects of chlorhexidine on root canal filling: A coronal microleakage study.

Maíra do Prado [1], Renata A. Simão [2], Brenda P. F. A. Gomes [3]

[1] Post Graduate Student- Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.

[2] Adjunct Professor, Department of Metallurgic and Materials Engineering, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

[3] Titular Professor, Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.

Correspondence: Dr Brenda P. F. A. Gomes

Department of Restorative Dentistry - Piracicaba Dental School, State University of Campinas.

Avenida Limeira, 901, Piracicaba, SP – Brazil 13414-018 Phone: (0055) 19 2106-5215 Fax: (0055) 19 2106-5218 E-mail: bpgomes@fop.unicamp.br

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ABSTRACT

A final flush with 2% chlorhexidine favors the wettability of resin-based sealers and improves the bond strength of glass ionomer sealer. The objective of the present study was to evaluate the effect of 2% chlorhexidine, during chemo-mechanical preparation and final flush, on coronal microleakage of resin-based sealers. One hundred ninety pre-molars were used. The teeth were divided into 18 experimental groups, according to the irrigation protocols and filling materials used. The protocols used were: distilled water; sodium hypochlorite (NaOCl)+EDTA; NaOCl+H_3PO_4; NaOCl+EDTA+chlorhexidine(CHX); NaOCl+H_3PO_4+CHX; CHX+EDTA; CHX+ H_3PO_4; CHX+EDTA+CHX and CHX+H_3PO_4+CHX. Gutta-percha/AH Plus or Resilon/Real Seal SE were used as root filling materials. The coronal microleakage was evaluated for 90 days against *Enterococcus faecalis*. Data were statistically analyzed using Kaplan-Meier survival test, Kruskal-Wallis and Mann-Whitney tests. No significant difference was verified in the groups using chlorhexidine or sodium hypochlorite during the chemo-mechanical preparation followed by EDTA or phosphoric acid for smear layer removal. The same results were found for filling materials. However, the statistic analyses revealed that a final flush with 2% chlorhexidine reduced significantly the coronal microleakage. A final flush with 2% chlorhexidine after smear layer removal reduces coronal microleakage of teeth filled with gutta-percha/ AH Plus or Resilon/Real Seal SE.

INTRODUCTION

The major aim of root canal therapy is to prevent and treat periradicular inflammation by eliminating microorganisms from the root canal system. The methods commonly used for this purpose include the root canal preparation by using different instruments and irrigants, adequate filling, and coronal restoration (Adamo et al. 1999, Jacobovitz et al. 2009).

Chemical irrigants are essential for successful debridement of root canals during cleaning and shaping procedures (Hashem et al. 2009). They are used during 'the dentinal walls, flush out debris and dissolve organic and inorganic components of the smear layer, thus cleaning the dentin surface (Akisue et al. 2010, Neelakantan et al. 2011). Different

irrigants were proposed and used, among them: 5.25% sodium hypochlorite, 2% chlorhexidine, 17% EDTA, 10% citric acid, MTAD and 37% phosphoric acid solution (Park et al. 2004, Neelakantan et al. 2011, Prado et al. 2011b).

Chlorhexidine has been used as irrigant during root canal therapy because of its antibacterial effects, substantivity, and relative absence of cytotoxicity, even though this solution is unable to dissolve the tissue. Additionally, chlorhexidine has been suggested as a final irrigant (Zehnder, 2006). In this respect, De Assis et al. (2011) observed that a final flush with 2% chlorhexidine favors the wettability of AH Plus and Real Seal SE sealers on the dentin surface. Additionally, Hashem et al. (2009) verified that the bond strength of ActiV GP was improved by using 2% chlorhexidine in the final irrigation after 17% EDTA.

The development and maintenance of the sealing of the root canal system is the key to the success of root-canal treatment. The resin-based adhesive material has the potential to reduce the microleakage of the root canal because of its adhesive properties and penetration into dentinal walls (Shokouhinejad et al. 2010). Moreover, the irrigation protocols may have an influence on the adhesiveness of resin-based sealers to root dentin (De-Deus et al. 2008).

A variety of experimental models are used to detect and measure any leakage along endodontic fillings, such as dye penetration, clearing of the teeth, radioisotope tests, bacterial penetration, electrochemical tests, fluid filtration, and glucose penetration model (Shemesh et al. 2006, Hirai et al. 2010).

The aim of the present study was to evaluate the effect of 2% chlorhexidine, during the chemo-mechanical preparation and final flush, on coronal microleakage of resin-based sealers.

MATERIALS AND METHODS

Sample Preparation

One hundred ninety single-rooted pre-molars with straight roots, mature root apices and similar anatomical characteristics were used in this study. Conventional access was performed using high-speed diamond burns. A size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was used to verify the patency of the canals and to determine the

total length of the root canal, i.e. the work length. This was observed when the instrument reached the apical foramen. Next, the foramina were standardized by using a size 20 K-file and root canals were shaped by using a MTwo NiTi rotary system (VDW, Munich, Germany). The sequence employed was the following: 10/.04, 15/.05, 20/.06, 25/.06, 30/.05, 35/.04, 40/.04, and 25/.07. The teeth were divided into groups of ten, according to the irrigation regimen (Table 1) and root canal filling.

Table 1: Protocols for irrigation.

Groups	Chemical-auxiliary substance	Intermediate flush	Smear layer removal	Final flush
G1 and G10	6 mL DW	10 mL DW	3 mL DW	10 mL DW
G2 and G11	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 17% EDTA	10 mL DW
G3 and G12	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	10 mL DW
G4 and G13	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 17% EDTA	5 mL DW + 5 mL 2% CHX solution
G5 and G14	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	5 mL DW + 5 mL 2% CHX solution
G6 and G15	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 17% EDTA	10 mL DW
G7 and G16	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	10 mL DW
G8 and G17	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 17% EDTA	5 mL DW + 5 mL 2% CHX solution
G9 and G18	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	5 mL DW + 5 mL 2% CHX solution

* DW - Distilled Water, CHX - chlorhexidine

Before the insertion of each file, 5.25% sodium hypochlorite (NaOCl) (Drogal, Piracicaba, SP, Brazil) or 2% Chlorhexidine (CHX) gel (Drogal, Piracicaba, SP, Brazil) were used as chemical-auxiliary substance. After the use of each file, 5 mL of distilled

water (DW) were used to remove the chemical-auxiliary substance used. Once the preparation was finished, 10 mL of DW was used to remove the chemical-auxiliary substance. Next, 17% EDTA or 37% phosphoric acid solution (Drogal, Piracicaba, SP, Brazil) was used for 3 minutes to remove the smear layer, with changes every 1-minute (1 mL *per* minute). Again, DW was used to remove the remaining solution. Finally, 2% chlorhexidine solution (Drogal, Piracicaba, SP, Brazil) was used for final flush. During the chemo-mechanical preparation, all teeth had their apices sealed with utility wax (Technew, Rio de Janeiro, RJ, Brazil) to prevent flow through them.

The root canals were dried with medium-sized paper points (Endopoints, Paraiba do Sul, RJ, Brazil). Groups 1 to 9 had the canals filled with gutta-percha cones (Odous, Belo Horizonte, MG, Brazil) associated with AH Plus sealer (Dentsply, Petropolis, RJ, Brazil), whereas Groups 10 to 18 had the canals filled with Resilon associated with Real Seal SE (SybronEndo, Orange, CA, USA). A System-B endodontic heat source unit (SybronEndo, Orange, CA, USA) was used to down-pack and Obtura System (J Morita, São Paulo, SP, Brazil) to repack. All teeth were radiographed mesiodistally and buccolingually to assess the quality of the filling. To allow the materials to set properly, all roots were kept on sponges at 37°C and 100% humidity for 2 weeks before leakage measurement.

Next, the external root surface of all specimens was sealed with two layers of red nail varnish (Revlon, Nova York, NY, USA), except the last 1 mm of the apex.

Analysis of Coronal Bacterial Microleakage

The apparatus used to evaluate coronal leakage was previously described by Gomes *et al*. (2003), with modifications (Figure 1a). Glass vials with rubber stoppers were adjusted for use. By using a shear, a hole was made at the centre of each rubber stopper (Figure 1b) through which each tooth was inserted under pressure up to the cemento-enamel junction, so that its crown was outside the vial and its root inside (Figure 1c). Cyanoacrylate glue (CG) was applied at the interface between tooth and stopper for sealing (Imura *et al*. 1997). Cylinders prepared from 10 mL plastic syringes were adapted to the outer surface of the stoppers to create a chamber around the crown of the tooth (Figure 1d). Again, CG was used at the interface between syringes and stoppers, followed

by a Parafilm layer (American National CanTM, Menasha, WI, USA) to help in the sealing. The syringe/stopper/tooth sets were submitted to sterilization by gamma-rays (Embrarad, São Paulo, SP, Brazil). The glass flasks were autoclaved at 121°C for 15 minutes.

The sterilized glass flasks were then filled with sterile Brain Heart Infusion broth (BHI; Oxid, Basingstoke, UK) so that a 2-mm length of root apex was immersed in the broth. CG and Parafilm were used to seal the interface between stopper and flask (Figure 1e). In all samples, in order to ensure the efficiency of the seal, 2 mL of 1% sterile methylene blue dye was placed into the tube until the coronal portion of the sample was reached (Malone *et al*. 1997). The flasks were then incubated at 37°C for 3 days to ensure sterilization. After the third day, the syringe chambers were removed so that methylene blue could be removed with sterile distilled water, whereas pipettes were used to insert the microbial medium. A green medium meant that seal was defective and the specimen was then discarded.

For preparation of microbial medium, *Enterococcus faecalis* ATCC 29212 was grown on BHI agar plates (Brain Heart Infusion agar, Oxoid, Basingstoke, UK) and supplemented with 5% sheep blood for 24 hours at 37°C in CO_2. Then, the *Enterococcus faecalis* was inoculated into tubes containing 5 mL sterile BHI suspension, which were adjusted spectrophotometrically at 800 nm (OD800) to a turbidity of 1.5 X 10^8 colony-forming units (CFU)/mL^{-1} (Vianna *et al*. 2005). Subsequently, 5 mL of these suspensions were placed into the syringe apparatuses, which were left at 37°C for 90 days in CO_2 and checked daily for turbidity in the BHI broth. When turbidity (Figure 1f) was observed, the day was recorded.

Every 2 days, 3 mL of the suspension (BHI + microorganisms) was removed from the chamber and replaced by 3 mL of BHI to avoid saturation and to confirm the growth of *Enterococcus faecalis* (Berber *et al*. 2006).

After this period, all apparatuses were opened to evaluate the sterile hood. Positive cultures were confirmed by using Gram staining (gram-positive), colony morphology on blood agar plates (cocci) and biochemical identification kits (Rapid ID 32 Strep, BioMérieux SA, Marcyl'Etoile,France).

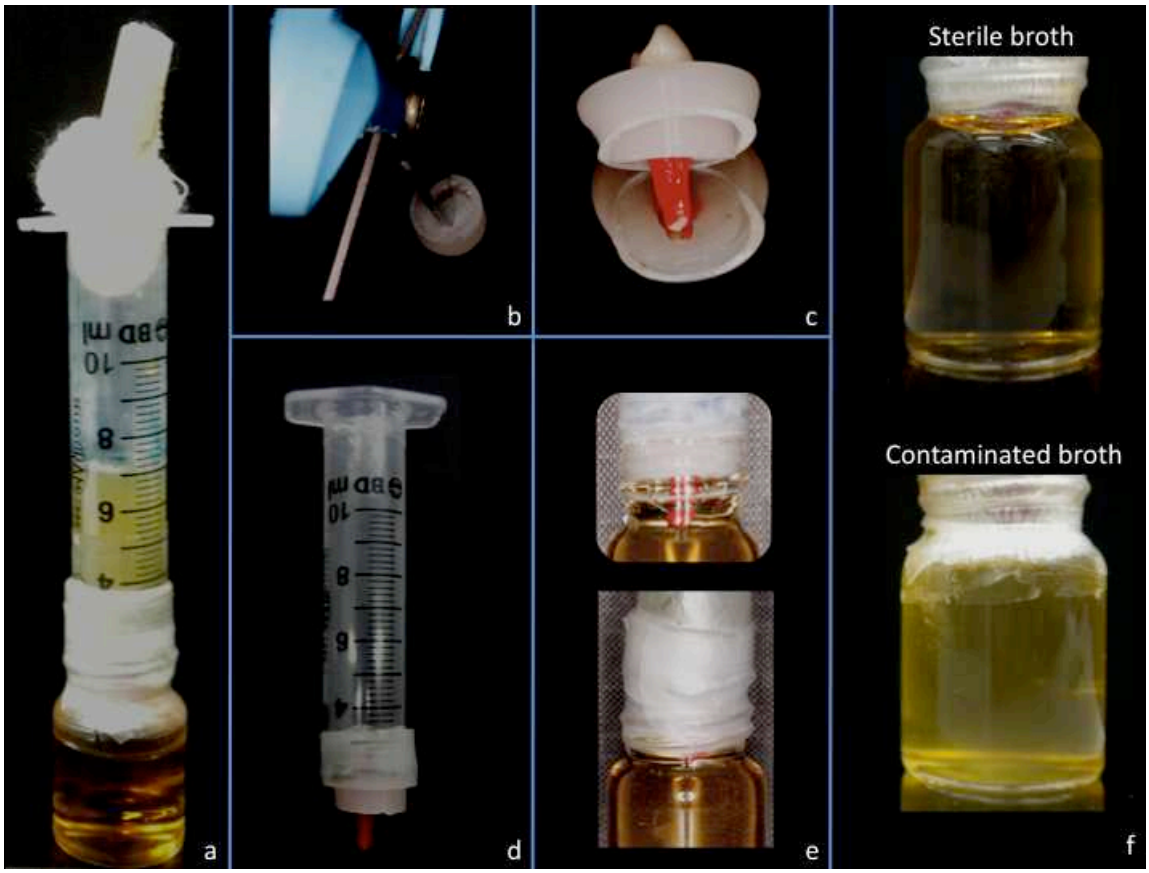

Figure 1: The apparatus used to evaluate coronal leakage (a), its development (b-e) and evaluation (f).

Ten samples were used as positive (n=5) and negative (n=5) controls. The positive controls consisted of instrumented teeth without obturation, while negative controls consisted of sound teeth.

The results were analyzed with Kaplan-Meier survival test, Kruskal-Wallis and Mann-Whitney tests ($p < 0.05$).

RESULTS

Statistic analysis revealed no difference in relation to the root canal filling systems. Also, no differences were found following the use of chlorhexidine or NaOCl associated with EDTA or phosphoric acid for smear layer removal. Graph 1 shows the number of contaminated samples according to the irrigation protocol. It was clearly

observed that groups receiving a final flush with chlorhexidine showed a lower number of contaminated samples.

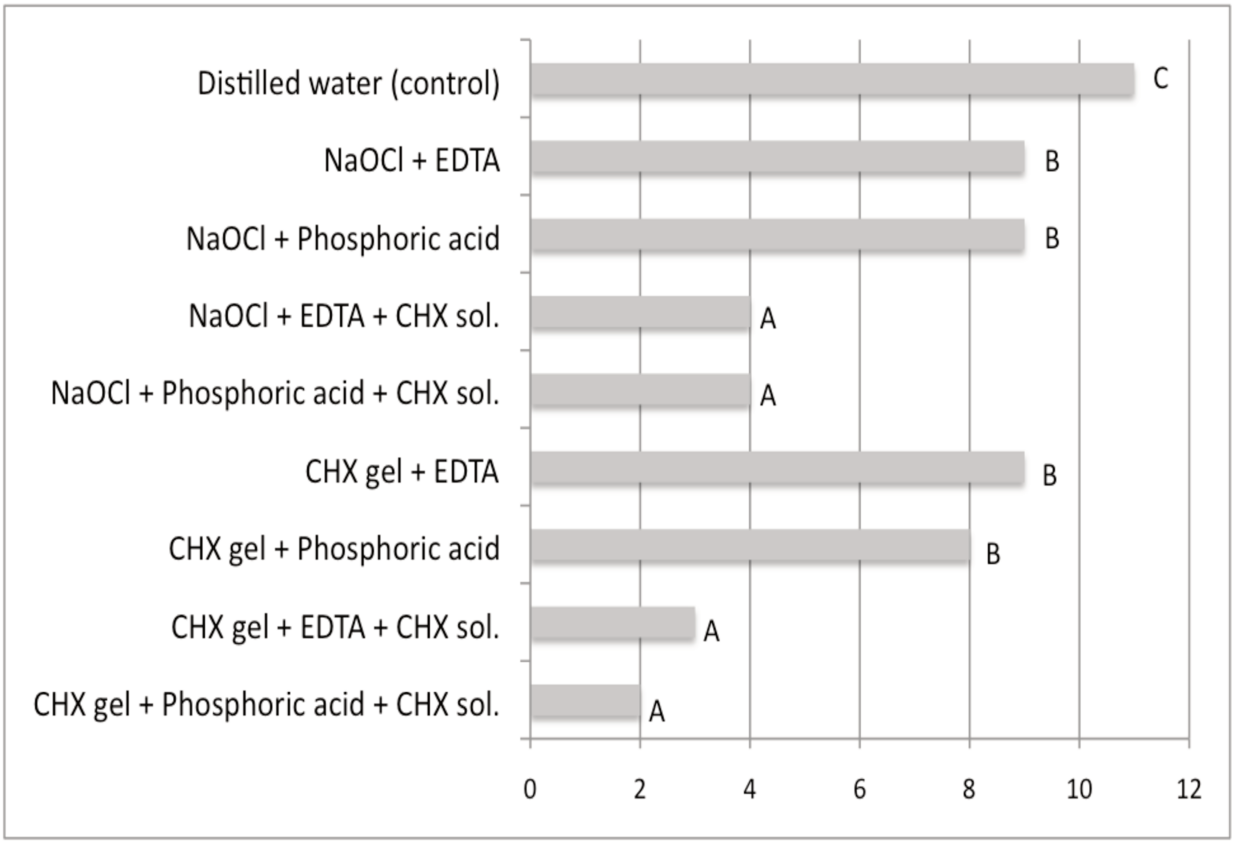

Figure 2: Graph showing the number of contaminated samples according to the irrigation protocol.

Table 2 shows the number of contaminated samples in relation to time. Statistic analysis of the contamination days revealed difference in the groups receiving the final flush with chlorhexidine. Chlorhexidine groups start to contaminate only in the 6th week, while in the others, the microbial growth was verified in the 1st or 2nd week. The control apparatuses showed broth turbidity within 1 day of incubation in all samples, whereas no microbial growth was found in the negative control throughout the experiment.

Table 2: Number of contaminated samples per week.

GROUPS	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	WEEK 7	WEEK 8	WEEK 9	WEEK 10	WEEK 11	WEEK 12	WEEK 13
DW	0/10	2/10	0/10	2/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
N+E+GA	0/10	1/10	1/10	0/10	0/10	1/10	0/10	0/10	1/10	0/10	0/10	0/10	1/10
N+PA+GA	0/10	2/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
N+E+CS+GA	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	1/10
N+PA+CS+GA	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
CG+E+GA	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10	2/10
CG+PA+GA	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	1/10
CG+E+CS+GA	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10
CG+PA+CS+GA	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
DW	2/10	2/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	1/10
N+E+RR	1/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10
N+PA+RR	1/10	1/10	0/10	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
N+E+CS+RR	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
N+PA+CS+RR	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	0/10	1/10
CG+E+RR	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10
CG+PA+RR	0/10	1/10	1/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10	1/10	0/10	1/10
CG+E+CS+RR	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
CG+PA+CS+RR	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10

* DW- distilled water; N- 5.25% NaOCl; E- EDTA, PA- phosphoric acid; CG- 2% chlorhexidine gel; CS- 2% chlorhexidine solution; GA- gutta-percha+AH Plus; RR- Resilon/Real Seal SE.

DISCUSSION

The present study has compared the use of 5.25% NaOCl and 2% CHX gel followed by 17% EDTA or 37% phosphoric acid for coronal microleakage. In our findings, no significant difference was found between the groups. According to Vivacqua-Gomes et al. (2002), the coronal leakage after using irrigation protocol with NaOCl alone was higher than that with NaOCl plus EDTA. The latter protocol had the same behavior than that with chlorhexidine alone. The protocol associating NaOCl plus chlorhexidine had the worse results due chemical smear layer formation. Our pilot study

detected no chemical smear layer formation in all groups by using scanning electron microscopy.

Prado et al. (2011b), in a SEM study of smear layer removal, have suggested the use of phosphoric acid for this purpose. According to the authors, in three minutes this solution showed better results than EDTA in the apical third. According to the present study, the better performance of phosphoric acid described by Prado et al (2011b) did not influence the coronal leakage.

In the present study, 2% chlorhexidine was the only solution used for final flush after smear layer removal. This procedure was made, because according to Neelakantan *et al*. (2011), a final flush with NaOCl decreases the bond strength between epoxy resin and dentin and increased the leakage. According to the literature, the use of chlorhexidine as a final flush improves the integrity of the hybrid layer (Carrilho *et al*. 2007), the wettability of endodontic sealers on dentin and gutta-percha (De Assis *et al*. 2011, Prado *et al*. 2011a), and resin-dentin bond stability (Sharifian *et al*. 2010). In this context, the results of the preset study showed that a final flush with chlorhexidine reduced significantly the coronal microleakage when compared to other experimental groups. These results are in disagreement with Stratton *et al*. (2006), who observed no statistically significant difference in leakage after using 2% chlorhexidine and NaOCl. However, Neelakantan *et al*. (2011) did the same comparison and observed that NaOCl increased the leakage compared to chlorhexidine. These authors suggest that the final flush with 2% chlorhexidine has no effect on microleakage. The differences in the results can be associated to the methodological approach as in our study bacterial microleakage was measured, whereas other authors (Stratton *et al*. 2006, Neelakantan *et al*. 2011) used fluid-filtration method. Another fact that can explain the results is that chlorhexidine increases the time required for recontamination of root filled (Sharifian *et al*. 2010). According to Rosenthal *et al*. (2004), root canals irrigated with 2% chlorhexidine maintained its antimicrobial action for up to 12 weeks, due to its substantivity.

In the present study, no difference was found in the use of gutta-percha/AH Plus and Resilon/Real Seal SE regarding the different irrigation protocols. Our data are in accordance with Baumgartner *et al*. *(*2007), Fransen *et al*. (2008) and Shokouhinejad *et al*. (2010), who found no significant difference in leakage when they compared root

canals filled with either gutta-percha/AH Plus or Resilon/Epiphany. Alternatively, other studies have reported that the use of Resilon/Epiphany sealer was be more efficient than gutta-percha/AH Plus (Wedding *et al*. 2007, Hirai *et al*. 2010), whereas others reported the opposite [Stratton *et al*. 2006, Saleh *et al*. 2008).

With regard to the methodology employed, the bacterial leakage is reproducible and has clinical relevance, presetting reliable data and simulating clinical conditions (Chailertvanitkul *et al*. 1997, Nair *et al*. 2011). This methodology permits that the number of days for sample contamination can be verified in relation to the broth turbidity.

In conclusion, the findings of the present study reveal that a final flush with 2% chlorhexidine after smear layer removal decreases the coronal microleakage of teeth filled with gutta-percha/ AH Plus and Resilon/ Real Seal SE due the to chlorhexidine substantivity. This behavior only was possible to be verified because a bacterial leakage methodology was used.

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CAPÍTULO 5

Effect of different irrigation protocols on resin sealer bond strength to dentin.

Maíra do Prado[1], Renata A. Simão[2], Brenda P. F. A. Gomes[3]

[1] Post Graduate Student- Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.

[2] Adjunct Professor, Department of Metallurgic and Materials Engineering, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

[3] Titular Professor, Department of Restorative Dentistry, Endodontic Division, State University of Campinas- UNICAMP, Piracicaba, SP, Brazil.

Correspondence: Dr Brenda P. F. A. Gomes

Department of Restorative Dentistry - Piracicaba Dental School, State University of Campinas.

Avenida Limeira, 901, Piracicaba, SP – Brazil 13414-018 Phone: (0055) 19 2106-5215 Fax: (0055) 19 2106-5218 E-mail: bpgomes@fop.unicamp.br

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ABSTRACT

Introduction: The use of different irrigants is essential for a successful debridement of root canals, although they can affect the root canal filling. The aim of the present study was to evaluate different irrigation protocols concerning the resin-based sealer bond strength to dentin. **Methods:** One hundred eighty single-rooted pre-molars were used. The roots were divided into eighteen groups according to the irrigation protocol adopted. They were instrumented using 5.25% NaOCl or 2% chlorhexidine gel as chemical-auxiliary substances, and 17% EDTA or 37% phosphoric acid for smear layer removal. Finally, CHX solution was used as a final irrigant in some groups. The root canals were filled with gutta-percha and AH Plus (GP/AH) or with Resilon/Real Seal SE (R/RSSE). The bond strength was measured by using the push-out test at a cross-head speed of 1 mm/min. Data were statistically analyzed by using Kruskal–Wallis and Mann-Whitney tests. **Results:** In the GP/AH groups, high values of bond strength were obtained when NaOCl was associated with phosphoric acid. However, when chlorhexidine gel was used, the association with EDTA presented better results. Evaluation of R/RSSE showed that higher values of bond strength were reached when chlorhexidine gel was used. In this case, the association of chlorhexidine/phosphoric acid was better than chlorhexidine/EDTA. **Conclusion:** Irrigation protocols influenced the bond strength of resin sealers to dentin.

INTRODUCTION

The use of different chemical irrigants is essential for a successful debridement of root canals during cleaning and shaping procedures (1). Sodium hypochlorite has a long history of successful usage in endodontics (2). Three key features make sodium hypochlorite solutions popular among clinicians: their antimicrobial effect and ability to dissolve biofilms (3), their capacity to solubilize tissues (4), and their reasonable price combined with availability from many commercial sources (5). However, the use of sodium hypochlorite could affect penetration of the resin sealer into the dentin as well as its polymerization. Additionally, this solution is a deproteinizing agent that can degenerate dentin by collagen dissolution (6).

Chlorhexidine has been used as irrigant during root canal therapy because of its antibacterial effects, substantivity, and relative absence of cytotoxicity (7,8), even though

this solution is unable to dissolve the tissue (9). Additionally, chlorhexidine has been suggested as a final irrigant (10). In this respect, De Assis et al. (11) observed that a final flush with 2% chlorhexidine favors the wettability of AH Plus and Real Seal SE sealers on the dentin surface. Additionally, Hashem et al. (12) verified that the bond strength of ActiV GP was improved by using 2% chlorhexidine in the final irrigation after 17% EDTA.

Cleaning and shaping of the root canals produce a smear layer that covers the instrumented walls. This layer contains inorganic and organic substances that include fragments of odontoblastic processes, microorganisms, and necrotic materials. This layer may impede penetration of intracanal medications into the dentinal tubules and may affect a close adaptation between root canal filling materials and root canal walls. The smear layer removal improves the bonding ability of resin-based sealers and reduces the coronal microleakage (13). As sodium hypochlorite and chlorhexidine are not capable of removing the smear layer, the adjunctive use of chelating agent or acids (e.g., EDTA and citric acid) is suggested. In 2011, Prado et al. (14) suggested that 37% phosphoric acid might be used for smear layer removal. According to the authors, a 3-minute application of this solution to the apical third was more effective than 17% EDTA and 10% citric acid for smear layer removal

Having in mind that the substances used during chemo-mechanical preparation can have an effect on the dentin surface, the aim of the present study was to evaluate the bond strength of resin-based sealers to dentin that has been submitted to different irrigant protocols as follows: 5.25% sodium hypochlorite or 2% chlorhexidine gel for chemo-mechanical preparation, associated with 17% EDTA (an usual solution employed for smear layer removal) or 37% phosphoric acid. Additionally, a final flush with 2% chlorhexidine solution was performed in some of these groups.

MATERIALS AND METHODS

Sample preparation

One hundred eighty single-rooted pre-molars with straight roots, mature root apices, and similar anatomical characteristics were used in this study. The crowns were removed at the cement-enamel junction by using a high-speed carbide bur with water

coolant. A size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was used to verify the patency of the canals and to determinate the total length of the root canal, i.e. the work length. This was observed when the instrument reached the apical foramen. Next, the foramina were standardized by using a size 20 K-file.

The teeth were divided into groups of ten, according to the irrigation regimen (Table 1) and root canal filling. The root canals were shaped by using the MTwo NiTi rotary system (VDW, Munich, Germany). The sequence employed was the following: 10/.04, 15/.05, 20/.06, 25/.06, 30/.05, 35/.04, 40/.04, and 25/.07. During the preparation, before insertion of each file, 1 mL of 5.25% sodium hypochlorite (NaOCl) (Drogal, Piracicaba, SP, Brazil) or 2% Chlorhexidine gel (Drogal, Piracicaba, SP, Brazil) was used as chemical-auxiliary substance. After the use of the files, 5 mL of distilled water were used to remove the chemical-auxiliary substance used.

Table 1: Protocols for irrigation.

Groups	Chemical-auxiliary substance	Intermediate flush	Smear layer removal	Final flush
G1 and G10	6 mL DW	10 mL DW	3 mL DW	10 mL DW
G2 and G11	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 17% EDTA	10 mL DW
G3 and G12	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	10 mL DW
G4 and G13	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 17% EDTA	5 mL DW + 5 mL 2% CHX solution
G5 and G14	1 mL 5.25% NaOCl + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	5 mL DW + 5 mL 2% CHX solution
G6 and G15	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 17% EDTA	10 mL DW
G7 and G16	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	10 mL DW
G8 and G17	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 17% EDTA	5 mL DW + 5 mL 2% CHX solution
G9 and G18	1 mL 2% CHX gel + 5 mL DW	10 mL DW	3 mL 37% phosphoric acid	5 mL DW + 5 mL 2% CHX solution

* DW - Distilled Water, CHX - chlorhexidine

Once the preparation was finished, a #5 Gates-Glidden bur was used to standardize the preparation and modify its taper throughout the work length, in order to

standardize the incidence of forces inside the filling materials. Next, 10 mL of distilled water was used before application of either 17% EDTA or 37% phosphoric acid solution (Drogal, Piracicaba, SP, Brazil), for 3 minutes in order to remove the smear layer, with changes every 1 minute (1 mL *per* minute). Once again, distilled water was used to remove the remaining solution. Finally, in some groups, 2% chlorhexidine solution (Drogal, Piracicaba, SP, Brazil) was used for final flush. During the steps above, all teeth had their apices sealed with utility wax (Technew, Rio de Janeiro, RJ, Brazil) to prevent flow through them.

The root canals were dried with paper points (Endopoints, Paraíba do Sul, RJ, Brazil). Groups 1 to 9 had the canals filled with gutta-percha cones (Odous, Belo Horizonte, MG, Brazil) associated with AH Plus sealer (Dentsply, Petropolis, RJ, Brazil), whereas Groups 10 to 18 had the canals filled with Resilon associated with Real Seal SE (SybronEndo, Orange, CA, USA). A System-B endodontic heat source unit (SybronEndo, Orange, CA, USA) was used to pack down the material and Obtura System (J Morita, São Paulo, SP, Brazil) to re-pack it. All teeth were radiographed mesiodistally and buccolingually to assess the quality of the filling. All specimens of the Resilon/Real Seal SE groups were light-cured for 40 seconds with an Optilight LD MAX LED curing light device (Gnatus, Ribeirão Preto, SP, Brazil). To allow the materials to set properly, all roots were kept on sponges at 37°C and 100% relative humidity for 2 weeks.

Push-out Assessment

Each root was horizontally sectioned with a slow-speed, water-cooled diamond saw (Buehler Isomet 2000, Lake Bluff, IL, USA) to produce two slices of approximately 1 mm thick for each root region (apical, middle, and coronal). The first (coronal) and last (apical) slices were discarded. Six slices from each root canal were evaluated, resulting in 60 slices per group (20 slices per third in each group).

The filling material was loaded with a 1.30-mm diameter cylindrical stainless steel plunger, which provided the most extended coverage over the filling material without touching the canal wall. The teeth were marked in order to ensure that the plunger push was from apical to coronal direction to avoid any interference of the root canal taper. Gates-Glidden bur were also used to eliminate the root canal taper.

Loading was performed by using a universal testing machine (EMIC DL200MF, São José dos Pinhais, PR, Brazil) at a cross-head speed of 1 mm/min until bond failure occurred. Debonding values (maximum load) were used to calculate the push-out strength in megapascals (MPa), according to the following formula:

$$\text{Push-out bond strength (MPa)} = \frac{\text{Maximum load (N)}}{\text{Adhesion area (mm}^2\text{)}}$$

The Adhesion area was calculated by using the following formula:

$$\pi(R + r)[(h^2+(R-r)2]^{0.5}$$

where π=3.14, R is the coronal radius, r is the apical radius, and h is the thickness of the slice.

The thickness of each slice was measured by using a digital caliper (Vonder, Curitiba, PR, Brazil), and the total bonding area for each root canal segment was measured with a magnifying stereoscope (Leika MZ75, Meyer Instruments, Houston, TX, USA) and IM50 software (Leika IM50 Image manager). Data were analyzed with Kruskal–Wallis and Mann-Whitney tests ($p < 0.05$).

Next, the specimens were buccolingually divided, coated with gold by using a Denton Vacuum Desk II Sputtering device (Denton Vacuum, Cherry Hill, NJ, USA), and then observed with a scanning electron microscope (JSM– 5600LV, JEOL Ltd., Tokyo, Japan) at 1000X magnification to classify the failure pattern into three types, based on the percentage of substrate-free material (15): adhesive failure: > 75% of substrate-free material; mixed failure: > 25 to < 75% of substrate-free material, and cohesive failure: < 25% of substrate-free of material.

RESULTS

When the bond strength values of gutta-percha/AH Plus and Resilon Real Seal SE were compared, independent of the irrigant protocols used, it was observed that the bond strength of the former was significantly higher than the latter (Figure 2). In contrast, when comparing the different thirds, no statistical difference was found.

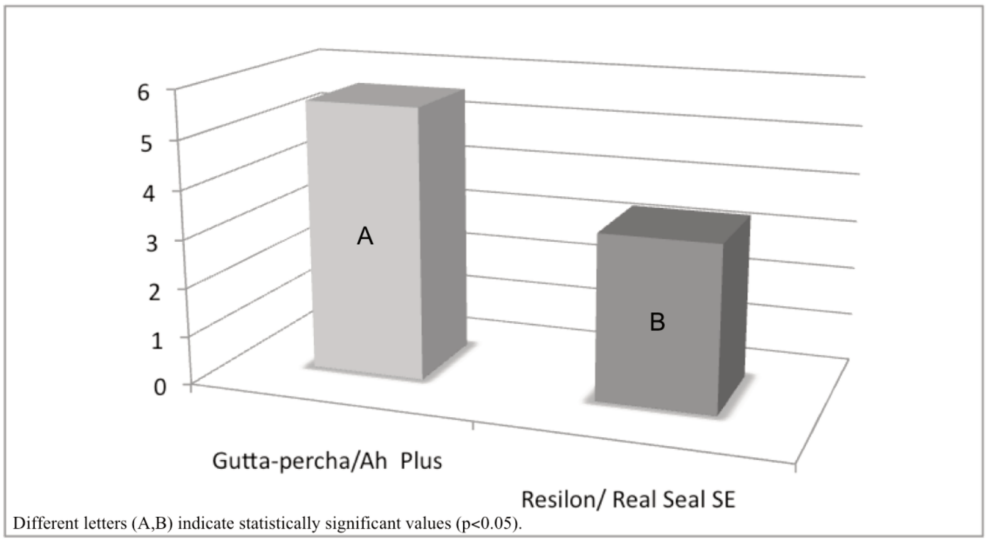

Figure 1: Bond strength mean values of gutta-percha/AH Plus and Resilon/ Real Seal SE

Table 2 shows the mean and standard deviation values of bond strength regarding the experimental groups based on their failure pattern. Concerning Gutta-percha/AH Plus groups, when NaOCl was used as irrigant during chemo-mechanical preparation, the higher bond strength values were obtained when phosphoric acid was used for smear layer removal. However, when CHX was used during chemo-mechanical preparation, the use of EDTA associated with this irrigant presented better bond strength values. By evaluating the Resilon/ Real Seal SE groups, higher values of bond strength were reached when CHX was used as irrigant. In this case, the association of CHX with phosphoric acid was better than CHX with EDTA. The use of chlorhexidine as final irrigant, in both cases (gutta-percha/AH Plus and Resilon/Real Seal SE), did not affect negatively the bond strength. With regard to the failure pattern results, gutta-percha/AH Plus system was found to have mainly a cohesive pattern (i.e. < 25% of substrate-free sealer), whereas the Resilon/ Epiphany SE system had an adhesive pattern (i.e. > 75% of substrate-free sealer).

Table 2: Bond strength values and failure pattern of the experimental groups.

Irrigant Protocol	Gutta-percha/ AH Plus				Resilon/ Real Seal SE			
	Bond strength MPa (Mean ±SD)	Failure pattern (n)			Bond strength MPa (Mean ±SD)	Failure pattern (n)		
		Adhesive	Cohesive	Mixed		Adhesive	Cohesive	Mixed
Distilled water	2.1 ± 2.8[c]	6	36	18	1.1 ± 1.6[d]	40	0	20
NaOCl+EDTA	5.2 ± 2.6[b]	0	36	24	3.2 ± 1.9[c]	32	0	28
NaOCl+PA	7.4 ± 3.8[a]	0	49	11	3.7 ± 2.4[b]	26	0	34
NaOCl+EDTA+CHX	5.2 ± 1.6[b]	0	37	23	2.8 ± 1.2[c]	38	0	22
NaOCl+PA+CHX	6.9 ± 3.9[ab]	0	45	15	3.6 ± 1.9[b]	30	1	29
CHX+EDTA	6.0 ± 2.3[ab]	0	40	20	3.7 ± 2.7[b]	24	2	34
CHX+PA	5.3 ± 1.8[b]	0	34	26	4.2 ± 2.0[ab]	20	3	37
CHX+EDTA+CHX	7.5 ± 3.2[a]	0	51	9	3.1 ± 1.4[b]	32	1	27
CHX+PA+CHX	5.4 ± 2.1[b]	0	37	23	5.2 ± 3.0[a]	13	6	41

Different letters [(a,b,c)] in the column indicate statistically significant values ($p<0.05$).

DISCUSSION

The use of irrigants with cleaning and demineralizing capacities is effective to improve the retention mechanisms of resin-based sealers to root canal walls (16). In the present study, the resin-based sealers were associated with the highest bond strength values in the groups where smear layer was removed by using 17% EDTA and 37% phosphoric acid. The control groups, where smear layer remained, showed the worst results.

In the current study, the Resilon/Real Seal SE system was compared to gutta-percha/AH Plus. The highest bond strength values were verified in the gutta-percha/ AH Plus system. These results might be attributed to the fact that AH Plus is based on the polymerization reaction of epoxy resin–amines, having excellent physical properties and less volumetric polymerization shrinkage (1). Methacrylate-based sealers, such as Real Seal SE or Ephiphany SE, undergo incomplete polymerization inside the canal and significant volumetric shrinkage during polymerization. The higher C-factor value of the canal causes accentuated interfacial gap formation (1,17). By comparing our data to the literature, De Deus et al. (17) and Haragushiku et al. (18) equally verified that gutta-percha/AH Plus showed higher bond strength results than those of Resilon/ Epiphany SE.

The bond strength values of the present study were higher than those reported by other studies (12,13,17) evaluating the same systems. This finding may be explained by the root canal preparation associated with the high plunger diameter used in the present study (1.30 mm). According to Nagas et al. (19), different plunger diameters are associated with significantly different intra-radicular push-out bond strengths of root filling systems, where the 1.25-mm plunger generated higher debonding values compared to those obtained with diameters of 0.75 mm and 1.0 mm.

The failure pattern may be also associated with the plunger diameter, since it is similar to the diameter of the root canal being prepared. In this way, the plunger has full contact with the filling material (Gutta-percha/ Resilon) on the surface, promoting its expulsion and leaving only the sealer. With regard to the failure pattern results, gutta-percha/AH Plus system was found to have, predominantly, a cohesive pattern (i.e. < 25% of substrate-free sealer), whereas the Resilon/ Epiphany SE system had an adhesive pattern (i.e. > 75% of substrate-free sealer). This can be explained by the fact that gutta-percha does not present adhesive characteristics, and the sealers remained on the dentin surface after the experiment. Additionally, in the Resilon/Epiphany SE system, the necessary force to rupture the sealer/dentin interface was lower than that at the Resilon/sealer interface.

The effect of different irrigants on the bond strength of resin-based sealers to dentin has already been studied. However, few works have evaluated the association of chlorhexidine gel with chelant agents (6) and of NaOCl with 37% phosphoric acid. The protocols associating CHX with chelant agents were suggested because, according to Ferraz et al. (20), 2% chlorhexidine gel produced a cleaner root canal surface compared to NaOCl. These authors did not report that the use of chlorhexidine gel produced no smear layer. Therefore, an agent for smear layer removal is necessary. In this respect, Vilanova et al. (1) compared the effect of 1% NaOCl, 1% NaOCl + 17% EDTA, and 2% chlorhexidine gel and observed that the association of 1% NaOCl with 17% EDTA had bond strength values higher than that using 2% CHX. Neelakantan et al. (13) observed that the association of 3% NaOCl with 17% EDTA showed bond strength values higher than that using 2% chlorhexidine.

In the present study, the evaluation of the gutta-percha/AH Plus system showed

higher bond strength values using 5.25% NaOCl and 37% phosphoric acid or 2% chlorhexidine gel with 17% EDTA, when compared to either 5.25% NaOCl with 17% EDTA or 2% chlorhexidine gel with 37% phosphoric acid. The use of chlorhexidine as final irrigant did not affect the results. The bond strength of AH Plus to dentine is associated with the sealer penetration into the dentinal tubules and with the covalent bond formation between the sealer's epoxide rings and exposed amino groups in the collagen network (1,20). The best results of the phosphoric acid might be related to the higher demineralization degree. Phosphoric acid can remove the collagen layer damaged by NaOCl and expose the healthy one to monomer infiltration. Also, this solution causes further exposure of the dentinal tubules (14,21), allowing greater sealer penetration into them. However, its effects on the periapical tissues should be evaluated. The use of chlorhexidine gel during chemo-mechanical preparation is associated with less smear layer formation compared to the use of NaOCl. Moreover, chlorhexidine gel does not affect collagen layer (21). The use of phosphoric acid, a strong acid, associated with chlorhexidine can remove completely the inorganic elements of the smear layer and attack the dentin, thus affecting its mechanical properties. It explains the best results for the chlorhexidine/EDTA association (21,22).

With regard to the Resilon/Real Seal SE group, the protocols associated with the use of NaOCl showed lower bond strength values compared to the chlorhexidine groups. Sodium hypochlorite is a strong oxidizing agent. It leaves an oxygen-rich layer on the dentin surface, which reduces the bond strength due to the strong inhibition of the interfacial polymerization of methacrylate-based resins (23,24). Additionally, reduction of the bond strength may occur due to the removal of collagen fibrils from the dentin surface by sodium hypochlorite, which may inhibit the formation of a consistent hybrid layer (21). Our data is in disagreement with Wachlarowicz et al. (26), who found higher bond strength values when 6% NaOCl was used, when compared to 2% chlorhexidine. However, when Vilanova et al. (1) compared 1% NaOCl to 2% CHX, higher values were found in the chlorhexidine group.

For the Resilon/Real Seal SE system, the best results found in the chlorhexidine groups can be explained by the hydrophilic characteristics of methacrylate-based sealers associated with the absence of proteolytic action of chlorhexidine, which makes the

dentinal surface more hydrophilic (26,27). Additionally, chlorhexidine can incorporate adhesive particles of the methacrylate-based sealers, resulting in greater adhesion (28).

In the cases of self-etch adhesive systems, such as Real Seal SE, dentinal tubules have only a minor contribution to the dentin adhesion process. The major retention is provided by micromechanical interactions between bonding agent, collagen matrix and the underlying mineralized zone in the intertubular dentin (21). As chlorhexidine did not affect the organic matrix, the use of strong acid, such as phosphoric acid, can completely remove the inorganic elements of the smear layer, exposing the collagen matrix. The collagen fibers exposed offers the possibility of dentin hybridization with hydrophilic materials (29).

In conclusion, the irrigation protocols influenced the bond strength of the resin sealers to dentin. In the gutta-percha/AH Plus groups, the bond strength values were higher when associating NaOCl with phosphoric acid or chlorhexidine with EDTA. In the Resilon/Real Seal SE groups, the protocol associating chlorhexidine with phosphoric acid showed better results. The use of chlorhexidine as final irrigant did not affect negatively the bond strength.

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CONSIDERAÇÕES GERAIS

Tendo em vista que durante o preparo químico-mecânico, diferentes substâncias são utilizadas e que estas frequentemente entram em contato entre si no interior do sistema de canais radiculares, o presente estudo teve por finalidade avaliar essas diferentes interações em relação à formação de subprodutos (capítulo 1), em relação à ação antimicrobiana dessas interações (capítulo 2) e ao efeito dessas interações no tratamento endodôntico (capítulos 3, 4 e 5).

No capítulo 1, avaliou-se a interação entre as substâncias comumente empregadas durante o preparo químico-mecânico. Embora estudos da associação de substâncias sempre tenham sido encontrados na literatura endodôntica, principalmente em relação à associação NaOCl/agente quelante-ácidos (Bystrom & Sundqvist, 1985; Baumgartner & Ibay, 1987; Grawehr et al., 2003) esse tema começou a ser mais difundido em 2007 quando Basrani et al., ao avaliarem a interação entre o NaOCl e a CHX, concluíram que essa associação levava à formação de um subproduto tóxico e cancerígeno, marrom-alaranjado, denominado para-cloroanilina. Esses pesquisadores realizaram diferentes estudos, com diferentes técnicas de análise, chegando sempre à conclusão da existência desse subproduto. Além disso, outros estudos avaliaram tal interação na superfície dentinária e observaram que a interação entre o NaOCl e a CHX levava à formação de uma smear layer química, que recobria os túbulos dentinários, o que poderia interferir na obturação do sistema de canais radiculares (Bui et al., 2008; Akisue et al., 2010).

Devido à formação de um componente tóxico no interior do sistema de canais radiculares, associada à formação de uma smear layer química, estudos começaram a ser desenvolvidos com o objetivo de tentar eliminar esse subproduto. Para isso diferentes protocolos de irrigação foram propostos, tanto para evitar a formação desse composto como para removê-lo (Bui et al., 2008; Akisue et al., 2010; Krishnamurthy & Sudhakaran, 2010; Mortenson et al., 2012).

Em 2010, Thomas & Sem, em nova análise da interação NaOCl/CHX, não encontraram a formação de para-cloroanilina. Entretanto, apenas em 2011, Nowicki & Sem propõem como produtos desta associação: clorofenilguanidil-1,6-diguanidil hexano (PCGH) e para-clorofenil-ureia (PCU). No presente estudo, quando avaliada a associação

entre NaOCl e CHX, assim como nos estudos de Sem e colaboradores (Thomas & Sem, 2010; Nowicki & Sem, 2011), a formação de para-cloroanilina não foi observada.

Em 2007, Basrani et al. associaram a presença de para-cloroanilina à presença do pico 127, pico característico desse composto, a coloração marrom-alaranjada, também característica desse composto, e a razão N/Cl. No presente estudo e nos de Thomas & Sem (2010) e Nowicki & Sem (2011), em contraste, o pico 127 da para-cloroanilina não foi encontrado. Entretanto, a coloração marrom-alaranjada, sim, foi encontrada. Como discutido no capítulo 1, a coloração marrom-alaranjada estaria associada ao forte poder oxidativo do hipoclorito de sódio. Este oxidaria os nitrogênios guanidinos da clorexidina, e levaria a esta coloração. Ainda em relação à razão N/Cl, esta sempre ocorrerá em maior ou menor grau, visto que estes elementos são componentes estruturais da clorexidina. Adicionalmente, é importante ressaltar que, devido ao forte poder oxidativo do hipoclorito de sódio, diferentes subprodutos são formados, como pode se observar na Figura 2B do capítulo 1, com vários picos encontrados.

Em relação a outras interações como clorexidina/EDTA, clorexidina/solução salina e clorexidina/álcool, estas associações estão relacionadas, respectivamente, a uma reação ácido-base (EDTA + clorexidina), a uma reação de salting-out (Solução salina + clorexidina) e a uma diminuição de solubilidade (álcool + clorexidina). No primeiro caso, ocorre precipitação de sais de EDTA e clorexidina, enquanto nos demais há precipitação de sais de clorexidina.

Por fim, nas associações entre NaOCl/EDTA, NaOCl/ácido cítrico e NaOCl/ácido fosfórico houve a formação de gás cloro, um subproduto tóxico de forte odor. A alta concentração de gás cloro levou a forte coloração amarelada na interação NaOCl/ácido fosfórico. De acordo com os resultados do presente trabalho, quando irrigarmos os canais com NaOCl, o ideal é que, previamente à utilização de ácidos ou quelantes para remoção da smear layer, seja feita uma irrigação intermediária com água destilada ou soro fisiológico, para tentar eliminar ou pelo menos reduzir a concentração de hipoclorito existente. Pois, como verificado neste trabalho, quanto menor a concentração de NaOCl, menor a formação de gás cloro.

Durante o preparo químico-mecânico, substâncias são utilizadas como agentes antimicrobianos para tentar eliminar, ou pelo menos reduzir os microorganismos

presentes no sistema de canais radiculares. No capítulo 2 foi avaliada a capacidade antimicrobiana das diferentes substâncias químicas após interagirem (NaOCl, clorexidina, EDTA, ácido cítrico e ácido fosfórico). Embora muitas das soluções avaliadas apresentem capacidade antimicrobiana quando isoladas, até o presente momento não havia sido encontrado na literatura a atividade antimicrobiana de todas as interações avaliadas no presente estudo. Com os resultados encontrados, foi possível observar que todas as associações apresentaram atividade antimicrobiana e esta atividade está relacionada ao microorganismo avaliado. Estudos investigando a citotoxicidade dessas associações estão sendo realizados no Laboratório de Endodontia da FOP-Unicamp.

O capítulo 3 foi um estudo piloto para os capítulos 4 e 5. Nele diferentes protocolos de irrigação foram avaliados em relação à formação de smear layer química. Krishnamurthi & Sudhakaran (2010) mostraram que a irrigação com EDTA, seguido de NaOCl e finalmente CHX líquida levam à formação de smear layer química. Também observaram que a utilização de 5 mL de água destilada e solução salina entre o NaOCl e a clorexidina diminuiu o grau de precipitação. Entretanto, no presente estudo verificou-se que utilizando 10 mL de água destilada entre cada solução, ainda houve a formação de smear layer química. Por isso, se propôs o protocolo com utilização de irrigações intermediárias com água destilada em todas as etapas (entre cada irrigação com NaOCl e após o término da instrumentação), com o objetivo de diminuir a formação de smear layer química, pois esta seria uma variável a mais que poderia influenciar nos resultados dos capítulos 4 e 5.

Tendo em vista que nos grupos em que a CHX foi utilizada como substância química auxiliar empregou-se 1 mL de CHX 2% gel seguido de 5 mL de água destilada entre cada instrumento, terminando o preparo com 10 mL de água destilada, previamente ao uso de 3 mL de ácidos para a remoção da smear layer, seguida de uma nova irrigação com 10 mL de água destilada, decidiu-se fazer o mesmo regime de irrigação com o NaOCl. Normalmente o hipoclorito de sódio é empregado sem as irrigações intermediárias com água destilada. Entretanto, visando igualar os volumes das substâncias, optou-se pelo protocolo utilizado para o grupo da clorexidina.

Adicionalmente, observou-se que, utilizando tal protocolo, não houve a formação de smear layer química.

Como o objetivo do capítulo 3 foi comparar protocolos clínicos amplamente utilizados na prática endodôntica e que estes protocolos estão associados ao uso de NaOCl sem posterior irrigação com uma solução inerte e da CHX associada a um irrigante (água destilada), os grupos NaOCl + água destilada não foram incluídos no artigo. De acordo com os resultados do capítulo 3, quando o NaOCl 5,25% for a solução de escolha, o ideal é utilizar ácido fosfórico para remoção de smear layer, pois nessa associação não se verificou a presença de smear layer química. Esse resultado pode estar associado a dois fatores, primeiro, por se tratar de um ácido forte, o ácido fosfórico inibiu a formação de smear layer química, uma vez que interagiu com o hipoclorito de sódio, reduzindo seu poder oxidativo. Outra justificativa é que, por se tratar de um ácido forte, o ácido fosfórico tem a capacidade de resolubilizar o precipitado formado e dessa forma eliminá-lo. Adicionalmente, é importante lembrar que entre a irrigação de NaOCl e ácido fosfórico é ideal que se faça uma lavagem intermediária com uma solução inerte como água destilada ou soro fisiológico, pois, como visto no capítulo 1, a associação NaOCl/ ácido fosfórico leva à formação de um gás tóxico.

Quando a CHX for utilizada como substância química auxiliar, as soluções de EDTA, ácido cítrico ou ácido fosfórico podem ser utilizadas para remoção de smear layer. Lembrando que, no caso do EDTA, é ideal que uma solução inerte como a água destilada seja utilizada como irrigante intermediário, já que essas soluções (EDTA/CHX) reagem quimicamente levando à formação de subprodutos.

Nos capítulos 4 e 5, diferentes protocolos de irrigação foram avaliados para remoção de smear layer, adotando o regime de irrigações intermediárias com 10 mL de água destilada. Foram utilizados NaOCl 5,25% ou CHX 2% gel como substância química auxiliar, seguido da utilização de 17% EDTA ou 37% ácido fosfórico para remoção de smear layer. Investigou-se ainda, se a utilização da solução de clorexidina 2% como irrigante final afetaria os níveis de microinfiltração coronária e de resistência de união de materiais obturadores "adesivos" à dentina. Em relação aos resultados encontrados no presente trabalho, a utilização de NaOCl ou CHX gel e EDTA ou ácido fosfórico não foi significativa nos valores de microinfiltração coronária. Entretanto, uma irrigação final

com CHX levou à diminuição dos valores de microinfiltração coronária de dentes obturados com os sistemas guta-percha/ AH Plus e Resilon/ Real Seal SE.

As diferentes substâncias químicas auxiliares utilizadas durante o preparo químico-mecânico e na remoção da smear layer afetaram significativamente os níveis de resistência de união. Entretanto, a irrigação final com CHX não afetou estes níveis. Os resultados conflitantes podem ser inerentes às metodologias empregadas (microinfiltração coronária e resistência de união). Na microinfiltração coronária avalia-se os gaps existentes ao longo da obturação, ao passo que na metodologia de resistência de união, o alvo da avaliação é o material obturador aderido à superfície dentinária. Ainda, os melhores resultados da microinfiltração coronária com irrigação final com a clorexidina pode ser devido à substantividade desta solução, evitando que os *Enterococcus faecalis* atravessassem toda a superfície radicular e atingissem a região apical.

Em relação aos resultados propriamente ditos, quando comparados os sistemas guta-percha/ AH Plus e Resilon/ Real Seal SE, melhores valores de resistência de união foram conseguidos no primeiro sistema. Essas dados estão de acordo com os estudos prévios de De Deus et al. (2009) e Haragushiku et al. (2012), que utilizaram a mesma metodologia. Esses resultados podem ser explicados pelos diferentes mecanismos de polimerização dos cimentos avaliados. Adicionalmente, De Assis et al. (2011) e De Assis (2011) verificaram um melhor escoamento do cimento AH Plus quando comparado ao Real Seal SE na superfície dentinária, verificaram também uma maior força de adesão do cimento AH Plus à superfície dentinária, quando comparado ao cimento Real Seal SE.

Em relação aos sistemas avaliados, nas obturações com o sistema guta-percha/ AH Plus, quando o NaOCl for escolhido como substância química auxiliar, a utilização de ácido fosfórico é preferível para remoção da smear layer. Entretanto, quando a CHX for utilizada, esta deve ser associada ao EDTA. No caso do sistema Resilon/Real Seal SE, protocolos de irrigação associando CHX ao ácido fosfórico estiveram associados a maiores valores de resistência de união. Estes resultados podem estar associados a um maior ou menor grau de desmineralização associado ao efeito deletério do hipoclorito de sódio na estrutura de colágeno da dentina, como discutido no capítulo 5. Além disso, em ambos os sistemas obturadores, uma irrigação final com CHX solução deve ser

empregada pois influenciará positivamente em termos de redução de microinfiltração coronária.

CONCLUSÃO

De acordo com os resultados obtidos e dentro das limitações dos estudos realizados foi possível concluir que:

1. Em relação à interação entre as substâncias químicas auxiliares comumente utilizadas no tratamento endodôntico, essas interações podem estar associada a formação de novos produtos sólidos, a diminuição da solubilidade das substâncias avaliadas e ainda a liberação de gás cloro.

2. Durante o tratamento endodôntico, irrigações intermediárias com solução inerte, como água destilada, devem ser realizadas com o objetivo de prevenir ou pelo menos reduzir a formação de subprodutos.

3. A interação entre as diferentes substâncias químicas auxiliares comumente utilizadas no tratamento endodôntico apresentou atividade antimicrobiana, e esta atividade é dependente do microrganismo avaliado.

4. Quando o NaOCl for utilizado como substância química auxiliar, durante o preparo químico-mecânico, e a clorexidina 2% como irrigante final, a utilização de ácido fosfórico para remoção de smear layer é preferível, visto que este protocolo está relacionado com paredes mais limpas, sem a presença de smear layer química.

5. A irrigação final com solução de clorexidina 2% levou a redução dos níveis de microinfiltração coronária dos sistemas obturadores guta-percha/AH Plus e Resilon/Real Seal SE;

6. Em termos de resistência de união, protocolos de irrigação associando NaOCl/ ácido fosfórico ou Clorexidina/ EDTA nos casos de obturação com o sistema

guta-percha/AH Plus, Clorexidina /ácido fosfórico no sistema Resilon/Real Seal SE estão relacionados a maiores valores de resistência de união.

7. A irrigação final com solução de clorexidina 2% não afetou negativamente os níveis de resistência de união dos sistemas obturadores, guta-percha/AH Plus e Resilon/Real Seal SE, à dentina.

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DETALHAMENTO DAS METODOLOGIAS

Capítulo 1 – Análise química das interações entre os irrigantes comumente utilizados na prática endodôntica

Este estudo foi realizado em parceria entre a FOP-UNICAMP e o Instituto de Química da Universidade Federal do Rio de Janeiro. Nele foram realizadas análises de espectrometria de massa com o objetivo de identificar os subprodutos formados na interação entre diferentes irrigantes endodônticos.

Interação entre as substâncias

No presente trabalho foram utilizadas as seguintes substâncias: EDTA 17% (Drogal, Piracicaba, SP, Brasil), ácido cítrico 10% (Drogal, Piracicaba, SP, Brasil), ácido fosfórico 37% (Drogal, Piracicaba, SP, Brasil), clorexidina (CHX) líquida 2% (Drogal, Piracicaba, SP, Brasil), CHX gel 2% (Drogal, Piracicaba, SP, Brasil), NaOCl (Drogal, Piracicaba, SP, Brasil) nas concentrações de 5,25%, 2,5%, 1% e 0,16%, água destilada, etanol e soro fisiológico.

A concentração de NaOCl 0,16% foi incluída no presente estudo pois, ao reagir com a clorexidina 2%, em ambas as formulações, não se observou a formação de precipitado marrom-alaranjado e, sim, de um precipitado branco-alaranjado (Figura 1).

As substâncias foram misturadas na proporção de 1:1 (0,5 mL: 0,5mL) em tubos eppendorfs, como descrito na Tabela 1.

Tabela 1: Grupos experimentais

Solução 1	**Solução 2**
CHX 2% gel	NaOCl 5,25%
CHX 2% gel	NaOCl 2,5%
CHX 2% gel	NaOCl 1%

CHX 2% gel	NaOCl 0,16%
CHX 2% gel	EDTA 17%
CHX 2% gel	Ácido cítrico 10%
CHX 2% gel	Ácido fosfórico 37%
CHX 2% gel	Água destilada
CHX 2% gel	Soro fisiológico
CHX 2% gel	Etanol
CHX 2% solução	NaOCl 5,25%
CHX 2% solução	NaOCl 2,5%
CHX 2% solução	NaOCl 1%
CHX 2% solução	NaOCl 0,16%
CHX 2% solução	EDTA 17%
CHX 2% solução	Ácido cítrico 10%
CHX 2% solução	Ácido fosfórico 37%
CHX 2% solução	Água destilada
CHX 2% solução	Soro fisiológico
CHX 2% solução	Etanol
NaOCl 5,25%	EDTA 17%
NaOCl 5,25%	Ácido cítrico 10%
NaOCl 5,25%	Ácido fosfórico 37%
NaOCl 5,25%	Água destilada
NaOCl 5,25%	Soro fisiológico
NaOCl 5,25%	Etanol
NaOCl 2,5%	EDTA 17%
NaOCl 2,5%	Ácido cítrico 10%
NaOCl 2,5%	Ácido fosfórico 37%
NaOCl 2,5%	Água destilada
NaOCl 2,5%	Soro fisiológico
NaOCl 2,5%	Etanol
NaOCl 1%	EDTA 17%

NaOCl 1%	Ácido cítrico 10%
NaOCl 1%	Ácido fosfórico 37%
NaOCl 1%	Água destilada
NaOCl 1%	Soro fisiológico
NaOCl 1%	Etanol
NaOCl 0,16%	EDTA 17%
NaOCl 0,16%	Ácido cítrico 10%
NaOCl 0,16%	Ácido fosfórico 37%
NaOCl 0,16%	Água destilada
NaOCl 0,16%	Soro fisiológico
NaOCl 0,16%	Etanol

Análise por espectrometria de massa

A espectrometria de massa é um método utilizado para identificar os diferentes átomos que compõem uma substância. Um espectrômetro de massa bombardeia uma substância com elétrons para produzir íons, ou átomos eletricamente carregados. Os íons atravessam um campo magnético que curva suas trajetórias de modos diferentes, dependendo de suas massas. O campo separa os íons em um padrão chamado espectro de massa.

No presente estudo, após as reações, onde houve a formação de precipitado. A espectrometria de massas com ionização por electrospray com analisador do tipo quadrupolo time-of-flight (IES-QTOF-EM) foi utilizada para caracterização química destes precipitados. Para tanto, as amostras foram centrifugadas, o sobrenadante removido e 0,5 mg do precipitado solubilizado em 1 mL de água deionizada (Tipo I, 18 mΩ.cm), 1 mL de metanol (HPLC/Spectro, Tedia, Rio de Janeiro, RJ, Brasil) e 1 mL de acetonitrila (HPLC/Spectro, Tedia, Rio de Janeiro, RJ, Brasil), ou a combinação destes. Em seguida, a solução obtida foi acidificada com 0,20 μL de ácido fórmico 0,1% (substância utilizada para auxiliar a protonação para análises em modo de ionização positiva) (HPLC/Spectro, Tedia, Rio de Janeiro, RJ, Brasil). As análises por IES-QTOF-EM foram realizadas no espectrômetro Waters QTOF Micro (Wythenshawe, Manchester, UK), mostrado na Figura 1. As massas foram descritas como relação massa/carga (m/z) e

as análises realizadas entre m/z 90 a 1000 em modo de ionização positiva [IES(+)-EM]. Foram utilizados os seguintes parâmetros: gás de nebulização nitrogênio com fluxo de 500 L/h a 120°C e fluxo no cone de amostragem de 50 L/h, temperatura na fonte de 100°C e voltagens do capilar e cone de 3000 V e 30 V, respectivamente. Os dados foram obtidos e processados, utilizando o programa MassLynx 4.0 (Waters, Wythenshawe, Manchester, UK), com aquisição a cada 1,0 s e atraso entre aquisições de 0,4 s. As análises foram realizadas por infusão direta na fonte de ionização com fluxo de 5,0 μL/min.

Figura 1: Espectrômetro de massa Walters QTOF Micro.

***Capítulo 2* - Análise antimicrobiana das interações entre os irrigantes comumente utilizados na prática endodôntica**

A análise antimicrobiana do produto das interações entre os irrigantes comumente utilizados na prática endodôntica foi realizada pelo método clássico de difusão radial em ágar com algumas modificações (método da camada dupla para os microrganismos aeróbios e facultativos e plaqueamento direto dos microrganismos anaeróbios estritos) e posterior leitura dos halos de inibição de crescimento microbiano.

Substâncias avaliadas:

Como controle foram utilizadas as seguintes substâncias: NaOCl 5,25%, NaOCl 2,5%, NaOCl 1%, NaOCl 0,16%, CHX solução 2%, CHX gel 2%, EDTA 17%, ácido cítrico 10% e ácido fosfórico 37%. As interações testadas estão descritas na Tabela 2.

Tabela 2: Associações testadas

Solução 1	Solução 2
NaOCl 5,25%	CHX sol 2%
NaOCl 5,25%	CHX gel 2%
NaOCl 5,25%	EDTA 17%
NaOCl 5,25%	Ácido cítrico 10%
NaOCl 5,25%	Ácido fosfórico 37%
NaOCl 2,5%	CHX sol 2%
NaOCl 2,5%	CHX gel 2%
NaOCl 2,5%	EDTA 17%
NaOCl 2,5%	Ácido cítrico 10%
NaOCl 2,5%	Ácido fosfórico 37%
NaOCl 1%	CHX sol 2%
NaOCl 1%	CHX gel 2%
NaOCl 1%	EDTA 17%
NaOCl 1%	Ácido cítrico 10%

NaOCl 1%	Ácido fosfórico 37%
NaOCl 0,16%	CHX sol 2%
NaOCl 0,16%	CHX gel 2%
NaOCl 0,16%	EDTA 17%
NaOCl 0,16%	Ácido cítrico 10%
NaOCl 0,16%	Ácido fosfórico 37%
CHX sol 2%	EDTA 17%
CHX sol 2%	Ácido cítrico 10%
CHX sol 2%	Ácido fosfórico 37%
CHX gel 2%	EDTA 17%
CHX gel 2%	Ácido cítrico 10%
CHX gel 2%	Ácido fosfórico 37%

Microrganismos

Os microrganismos avaliados foram:

Aeróbios e anaeróbios facultativos: *Candida albicans* (ATCC 10556), *Staphylococcus aureus* (ATCC 25923), *Actinomyces naeslundii* (ATCC 19039), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) e *Lactobacillus casei* (ATCC L324M).

Anaeróbios estritos: *Actinomices meyeri* (isolado do canal radicular e identificado através de testes bioquímicos), *Parvimonas micra* (ATCC 33270), *Porphyromonas gingivalis* (ATCC 49417) e *Porphyromonas nigrescens* (ATCC 33563).

Cultivo dos microorganismos

Os organismos aeróbios e facultativos foram subcultivados em placas de BHI Ágar sangue (BHIA) e incubados por 18-24 h a 37ºC em 10% CO_2 (estufa de CO_2, Jouan, Saint-Herblain, França). Os organismos anaeróbios estritos foram cultivados em placas de FAA + sangue de carneiro desfibrinado 5% (EBEFARMA, Araras, SP) e incubados em câmara de anaerobiose (Don Whitley Scientific, Bradford, UK) em atmosfera anaeróbia de 80% N_2, 10% CO_2, 10% H_2 por 48h.

Após crescimento em meio sólido, colônias isoladas de aeróbios e facultativos foram suspensas em tubos contendo BHI. Após agitação mecânica, a suspensão foi ajustada em espectrofotômetro com transmitância de 800 nm, até atingir a concentração equivalente a 0,5 da escala de Mc Farland (1,5 x 10^8 bactéria/mL). Tal concentração de inóculos foi utilizada por promover crescimento semi-confluente de todos os microrganismos testados.

Para as bactérias estritamente anaeróbias, colônias foram suspensas em solução estéril de NaCl a 0,85% até atingir a concentração equivalente a 1 da escala de McFarland. Tais inóculos foram utilizados por promoverem crescimento semi-confluente de todos os microrganismos testados.

Análise pelo Método de Difusão em Ágar

Em todo o experimento foram utilizadas placas de 150x20 mm. Os testes foram realizados em triplicata.

Para microrganismos aeróbios/ facultativos:

Inicialmente foram preparadas placas contendo MHA (Figura 2A) que serviram de base para a camada de inóculo. Em seguida, BHIA foi preparado e autoclavado em frascos de vidro com tampas rosqueáveis. Durante o processo de resfriamento, quando o BHIA atingia 45ºC, ainda em estado líquido, adicionou-se o inóculo microbiano, promovendo agitação uniforme do conjunto. O BHIA contendo 1% de inóculo microbiano foi então distribuído sobre a camada sólida de MHA (Figura 2B).

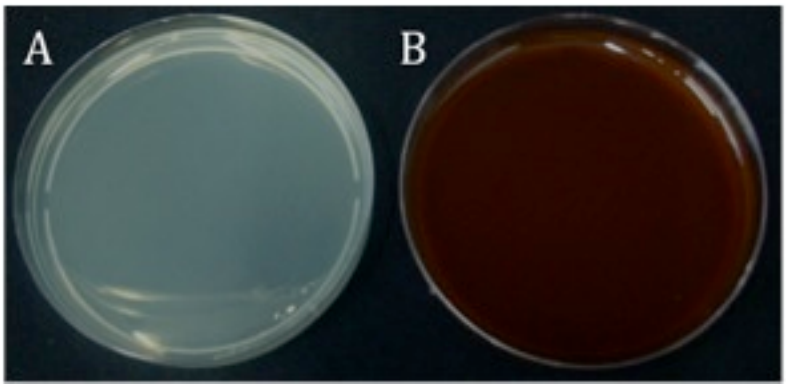

Figura 2: Meios utilizados para crescimento de microrganismos aeróbios/ facultativos: MHA (A) e BHIA (B) sobre MHA.

Para microrganismos anaeróbios estritos:

Para as bactérias anaeróbias estritas foi utilizada somente uma camada do meio de cultura de FAA + sangue de carneiro desfibrinado 5% (Figura 3A), na qual o inóculo bacteriano foi plaqueado diretamente (Figura 3B). Após a solidificação do ágar, as placas foram colocadas em atmosfera de anaerobiose por 24 horas para serem pré-reduzidas. A seguir, o inoculo bacteriano foi plaqueado, de forma uniforme, diretamente sobre o meio de cultura com o uso de um *swab* estéril.

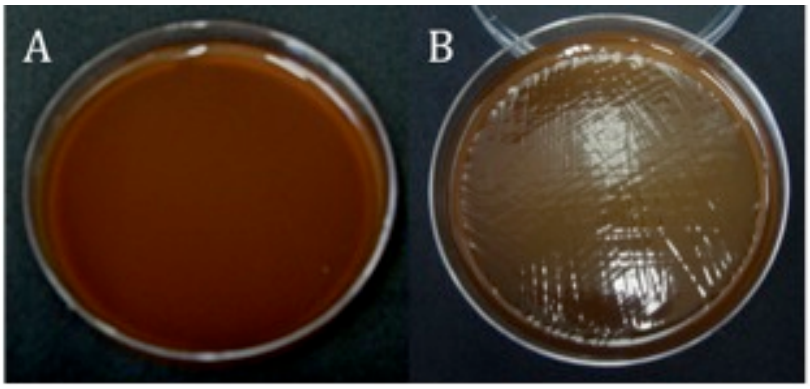

Figura 3: Meio utilizado para crescimento de microrganismos anaeróbios estritos: FAA (A) e meio após o plaqueamento (B).

Após a solidificação dos meios de cultura, cilindros de inox estéreis foram dispostos sobre a superfície do ágar (Figura 4 A e B) e foram preenchidos com 0,2 mL das substâncias/misturas a serem testadas**.** Foram colocados 3 cilindros em cada placa.

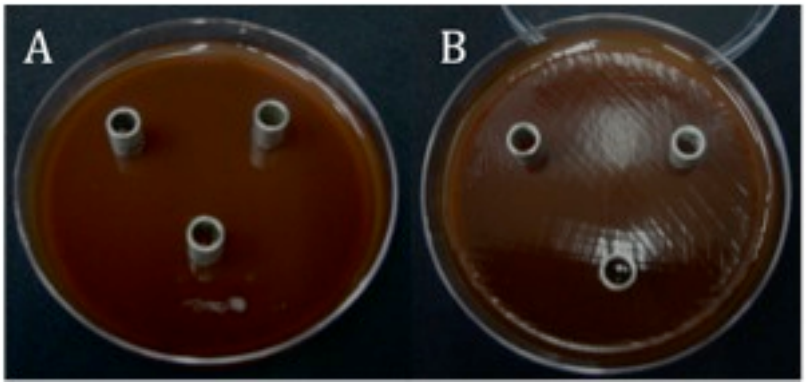

Figura 4: Disposição dos cilindros nas placas de BHIA (A) e FAA (B).

As placas foram mantidas a 37ºC em condições gasosas apropriadas: aeróbios em estufa de CO_2 (Figura 5A) a 37ºC por 24 horas e facultativos em estufa por 24-48 horas. Placas com microrganismos anaeróbios estritos foram incubadas em câmara de anaerobiose (Don Whitley Scientific, Bradford, UK) (Figura 5B) em atmosfera anaeróbia por 7 dias.

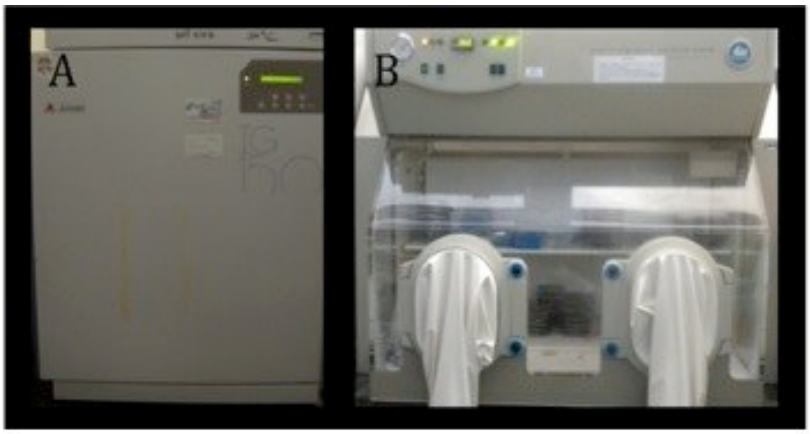

Figura 5: Estufa de CO_2 (A) e câmara de anaerobiose (B).

A leitura para os organismos aeróbios foi feita após 24 h de incubação. Enquanto a leitura para anaeróbios facultativos foi realizada após 48 horas de incubação. Finalmente, a leitura para anaeróbias estritas foi feita após 7 dias de incubação em atmosfera anaeróbia.

A leitura foi realizada pela medida dos raios das zonas de inibição microbiana. Essa medida correspondeu à menor distância entre a superfície externa do cilindro e o início da região de crescimento microbiano, os quais foram medidos com o auxílio de paquímetro digital. Esta medida está representada na Figura 6 por uma linha. Os dados foram avaliados estatisticamente pelos testes de Kruskal-Wallis e Mann-Whitney ($p<0,05$).

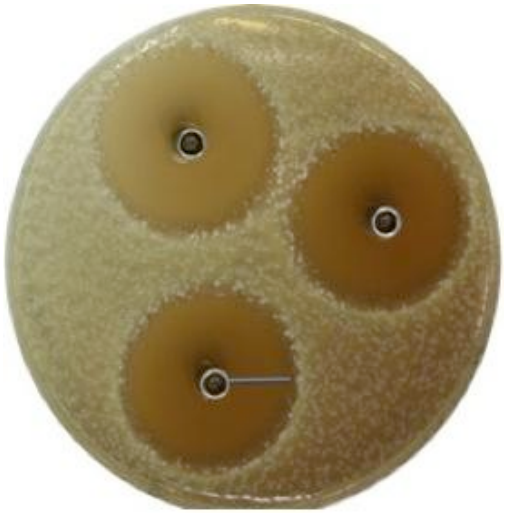

Figura 6: Leitura dos halos de inibição.

***Capítulo 3* - Avaliação de diferentes protocolos de irrigação em relação à formação de smear layer química**

Seleção dos dentes

No presente estudo foram utilizados 55 pré-molares unirradiculares com completa formação radicular e características anatômicas similares. As coroas foram removidas ao nível da junção amelo-cementária (Figura 7) utilizando-se disco de carborundum (KG Sorensen, Barueri, SP, Brasil).

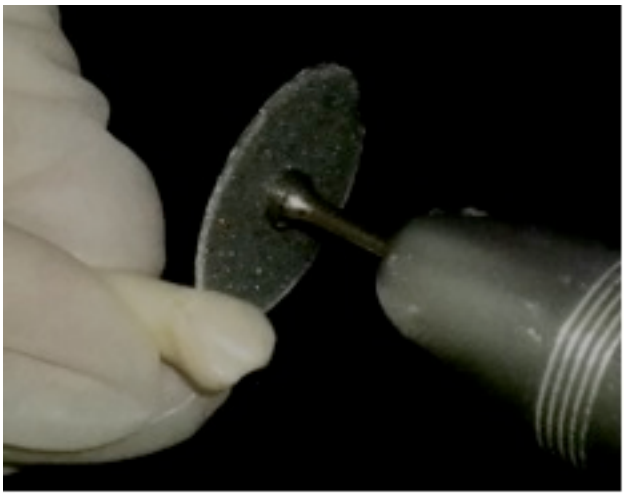

Figura 7: Remoção das coroas com disco de carborundum.

Limas tipo K #10 foram utilizadas para verificar a patência dos dentes e limas tipo K #20 para padronização do forame apical. Em seguida, as raízes foram instrumentadas, com o emprego de brocas de Gates-glidden (Dentsply Maillefer, Ballaigues, Suíça) em ordem decrescente (5 a 2) para o preparo do terço médio-cervical. Em seguida, o terço apical foi instrumentado até a lima K #30 (Dentsply Maillefer, Ballaigues, Vallorbe, Suíça), no ápice. A Figura 8 ilustra a técnica empregada no preparo mecânico. A cada troca de instrumento 1 mL de água destilada foi utilizada como solução irrigadora.

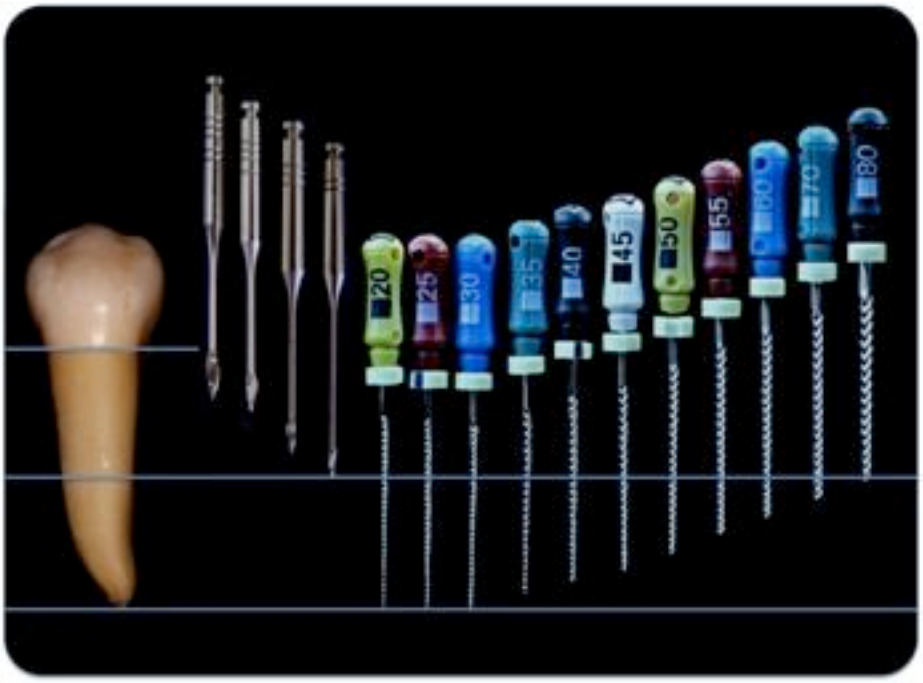

Figura 8: Seqüência do preparo mecânico.

Terminada a instrumentação, com o objetivo de remover a smear layer produzida durante essa etapa, os dentes foram submetidos ao protocolo proposto por Perez et al. (1993), que consiste em uma lavagem em ultrassom (Branson 1210- Lab Extreme, Kent City, MI, EUA) por 10 minutos com EDTA 17%, seguida de uma lavagem em NaOCl 5,25% durante 10 minutos e uma lavagem em fosfato tamponado por 10 minutos para eliminar os resíduos de EDTA e NaOCl. Em seguida, foi realizada uma lavagem em água destilada por igual período de tempo. Finalmente, os dentes permaneceram em água corrente por 1 hora para remover os possíveis resíduos ainda existentes das soluções utilizadas.

As raízes tiveram seus ápices selados com cera utilidade, para evitar a extrusão de irrigante durante o experimento. As raízes foram divididas em grupos de 5 (n=5) de acordo com o protocolo de irrigação, como descrito na Tabela 3. As substâncias químicas auxiliares utilizadas no presente trabalho foram: Soluções de EDTA 17% (Drogal, Piracicaba, SP, Brasil), ácido cítrico 10% (Drogal, Piracicaba, SP, Brasil), ácido fosfórico 37% (Drogal, Piracicaba, SP, Brasil), NaOCl 5.25% (Drogal, Piracicaba, SP, Brasil),

CHX 2%, água destilada (DW) e CHX gel 2% (Drogal, Piracicaba, SP, Brasil). Os canais foram irrigados a 3 mm do ápice com uma agulha hipodérmica 26-gauge.

Tabela 3: Protocolos de irrigação

GRUPO	PROTOCOLO DE IRRIGAÇÃO
1	1 mL NaOCl + 10 mL DW + 1 mL CHX solução
2	1 mL NaOCl + 10 mL DW + 1 mL EDTA + 10 mL DW + 1 mL CHX solução
3	1 mL NaOCl + 10 mL DW + 1 mL CA + 10 mL DW + 1 mL CHX solução
4	1 mL NaOCl + 10 mL DW + 1 mL PA + 10 mL DW + 1 mL CHX solução
5	1 mL CHX gel + 10 mL DW + 1mL EDTA + 1 mL DW
6	1 mL CHX gel + 10 mL DW + 1mL CA + 1 mL DW
7	1 mL CHX gel + 10 mL DW + 1mL PA + 1 mL DW
8	1 mL CHX gel + 10 mL DW + 1mL EDTA + 10 mL DW + 1 mL CHX solução
9	1 mL CHX gel + 10 mL DW + 1mL CA + 10 mL DW + 1 mL CHX solução
10	1 mL CHX gel + 10 mL DW + 1mL PA + 10 mL DW + 1 mL CHX solução
11	10 mL DW

DW- água destilada/ CHX – clorexidina 2%/ NaOCl – hipoclorito de sódio 5,25%/ EDTA- EDTA 17%/ CA- ácido cítrico 10%/ PA- ácido fosfórico 37%

Em seguida, os canais foram secos com cones de papel absorvente medium (Endopoints, Paraíba do Sul, RJ, Brasil).

Dois sulcos longitudinais foram realizados nas superfícies vestibular e lingual de todas as raízes com o auxílio de um disco diamantado de dupla face (KG Sorensen, Barueri, SP, Brasil), sem penetrar no canal radicular (Figura 9). Em seguida, com o auxílio de um cinzel e martelo, as raízes foram divididas em 2 segmentos. O segmento com a parte apical mais preservada foi selecionado e preparado para análise em microscopia eletrônica de varredura e espectroscopia de energia dispersiva (Microscópio JEOL JSM 6460 LV, Tokyo, Japão).

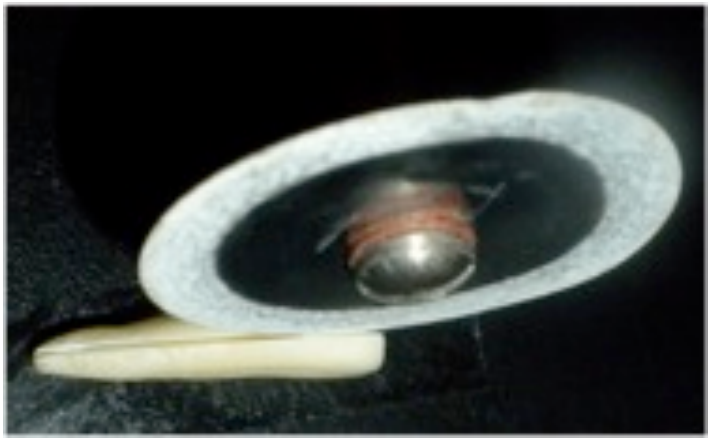

Figura 9: Sulco longitudinal com disco diamantado de dupla face.

Em cada amostra, 3 fotomicrografias (1000x) de cada terço foram realizadas, totalizando 9 fotomicrografias por amostra e 45 fotomicrografias por grupo.

As amostras foram analisadas em relação à presença ou ausência de smear layer química.

***Capítulo 4* - Efeitos da clorexidina na obturação: Estudo de microinfiltração coronária**

Foram utilizados 190 pré-molares inferiores, com um único canal e ápices completos, extraídos por indicação ortodôntica. Os dentes foram acessados com o auxilio de brocas diamantadas nº 1012-1014 HL (KG Sorensen Ind. Com. Ltda., Barueri, SP, Brasil) e instrumentados utilizando-se o motor VDW (VDW Endodontic Synergy, Munich, Alemanha) e o sistema MTwo (Figura 10), na seqüência:10.04; 15.05; 20.06; 25.06; 30.05; 35.04; 40.04; 25.07, sendo todas levadas ao comprimento de trabalho, comprimento zero, isto é, ao ápice radicular.

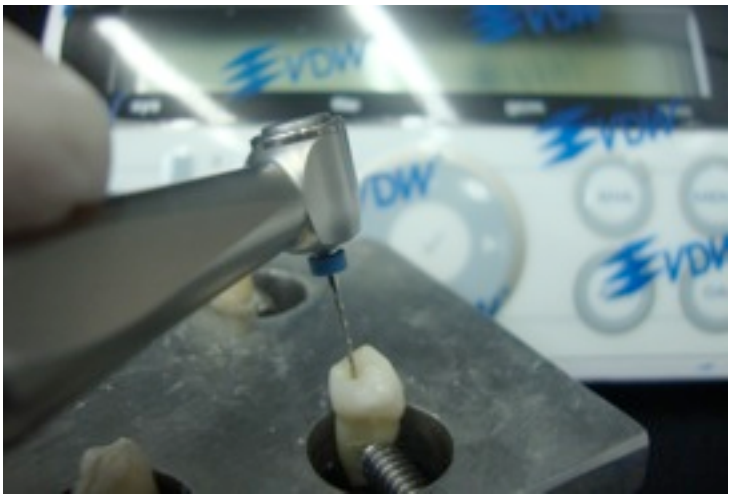

Figura 10: Instrumentação dos dentes com motor VDW.

A cada troca de lima, a solução irrigadora, descrita na Tabela 4 como substância química auxiliar, foi utilizada.

Tabela 4: Regimes de irrigação.

Grupos	Subst. Química Aux.	Lavagem	Rem. Smear layer	Lavagem final	Obturação
G1 (n=10)	6 ml água destilada	10 ml água destilada	3ml água destilada	10ml água destilada	Guta-percha + AH Plus
G2 (n=10)	1ml NaOCl 5,25% + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	10ml água destilada	Guta-percha + AH Plus
G3 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	10ml água destilada	Guta-percha + AH Plus
G4 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml EDTA 17%	5ml água destil + 5ml Clorex 2%	Guta-percha + AH Plus
G5 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	5ml água destil + 5ml Clorex 2%	Guta-percha + AH Plus
G6 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	10ml água destilada	Guta-percha + AH Plus
G7 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	10ml água destilada	Guta-percha + AH Plus
G8 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	5ml água destil + 5ml Clorex 2%	Guta-percha + AH Plus
G9 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	5ml água destil + 5ml Clorex 2%	Guta-percha + AH Plus
G10 (n=10)	6 ml água destilada	10 ml água destilada	3ml água destilada	10ml água destilada	Resilon + Real Seal SE
G11 (n=10)	1ml NaOCl 5,25% + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	10ml água destilada	Resilon + Real Seal SE
G12 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	10ml água destilada	Resilon + Real Seal SE
G13 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml EDTA 17%	5ml água destil + 5ml Clorex 2%	Resilon + Real Seal SE
G14 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	5ml água destil + 5ml Clorex 2%	Resilon + Real Seal SE
G15 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	10ml água destilada	Resilon + Real Seal SE
G16 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	10ml água destilada	Resilon + Real Seal SE
G17 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	5ml água destil + 5ml Clorex 2%	Resilon + Real Seal SE
18 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	5ml água destil + 5ml Clorex 2%	Resilon + Real Seal SE

Terminada a instrumentação, foi feita uma lavagem com 10 mL de água destilada. Em seguida foi utilizado 3 mL de solução para remoção de smear layer - 1mL a cada minuto, totalizando 3 mL em 3 minutos. Por fim foi realizada uma lavagem final. Em cada grupo foram utilizados dez dentes.

Terminado o preparo químico-mecânico, os canais foram secos com cone de papel absorvente medium (Endopoints, Paraíba do Sul, RJ, Brasil), Figura 11.

Figura 11: Secagem do canal

A obturação foi realizada com dois diferentes materiais obturadores resinosos:

a) Sistema 1 (Figura 13): Foi utilizado o cimento AH Plus (Dentsply, Petrópolis, RJ, Brasil) em conjunto com cone de guta-percha (Odous, Belo Horizonte, MG, Brasil) sendo aplicada a técnica de Schilder. Após a seleção e adaptação do cone de guta-percha "Medium", o cimento obturador foi manipulado de acordo com as recomendações do fabricante e levado ao interior do canal radicular com o cone de guta-percha. Para a técnica de obturação, utilizou-se o termocompactador Easy-endo (Easy, Belo Horizonte, MG, Brasil) para o down pack e o Obtura II (J Morita, São Paulo, SP, Brasil) para o repack ou back-fill. Terminada a obturação, os dentes foram radiografados para análise da qualidade da obturação.

Figura 12: Obturação com o sistema guta-percha/AH Plus.

b) Sistema 2 (Figura 14): Foi utilizado o sistema Resilon/Real Seal SE, que é composto pelo cimento Real Seal self-eaching e pelo cone Resilon, sendo aplicada a mesma técnica de obturação descrita no sistema 1. Terminada a obturação, assim como no sistema 1, os dentes foram radiografados com o mesmo fim. Em seguida foi realizada polimerização do sistema obturador na região coronária por 40 segundos com o auxílio de um LED (Gnatus, Ribeirão Preto, SP, Brasil) conforme recomendações do fabricante.

Figura 13: Obturação com o sistema Resilon/Real Seal SE.

Ao término da obturação, os dentes foram envolvidos em gazes umedecidas e mantidos no interior de placas de cultura estéreis tampadas, colocadas na câmara de anaerobiose, na temperatura de 37°C, num período de 14 dias para permitir a presa do cimento.

Infiltração coronária bacteriana

Uma camada de esmalte na cor vermelha Revlon foi utilizada para impermeabilizar os dentes, mantendo os ápices ausentes de impermeabilização para permitir a infiltração microbiana durante o experimento.

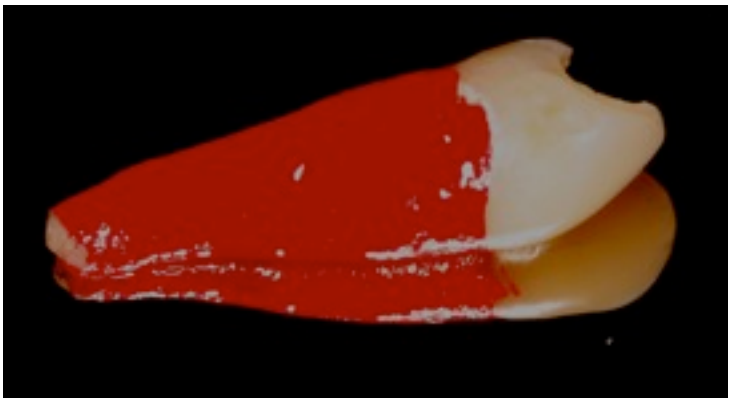

Figura 14: Impermeabilização das raízes.

Dez dentes foram utilizados como controles positivos e negativos, conforme descrito a seguir:

a) Controle negativo (5 dentes). Dentes hígidos foram colocados no aparato em contato com o inóculo bacteriano para comprovar a ausência de microinfiltração.

b) Controle positivo (5 dentes). Dentes instrumentados e não obturados foram colocados no aparato em contato com o inóculo bacteriano.

Um modelo similar ao de Gomes *et al*. (2003) foi utilizado. Os aparatos foram compostos por frascos de vidro acoplados a "stoppers" (tampas) de borracha (Sama Vidros, São Paulo, SP, Brasil). Acima destes foram adaptados cilindros de seringas plásticas de 10 mL devidamente preparados, de modo que se ajustem à superfície externa dos "stoppers", criando uma câmara para depósito do inóculo bacteriano.

Uma tesoura foi utilizada para a confecção de orifícios circulares no centro dos "stoppers" de borracha (Figura 15).

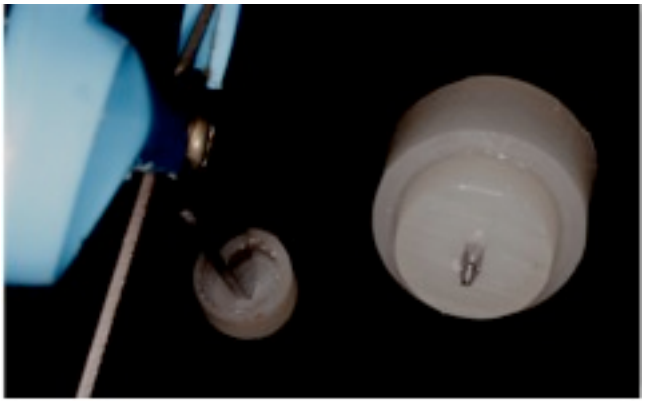

Figura 15: Confecção de orifício no centro do "stopper" de borracha com tesoura.

Nesta abertura os dentes foram inseridos, pressionando-os até a junção cemento–esmalte (Figura 16) de modo que a porção coronária estivesse em contato com o interior da seringa. A interface dente-stopper foi selada com cimento de cianocrilato (Super Bonder; Loctite, Itapevi, SP, Brasil) (IMURA *et al*., 1997).

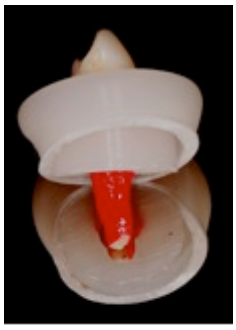

Figura 16: Dente inserido no "stopper" de borracha

O conjunto constituído de dente + stopper de borracha + seringa foram enviados para esterilização em óxido de etileno (Embrarad, Cotia, SP, Brasil). Os frascos utilizados para a colocação do BHI caldo foram esterilizados em autoclave. Em câmara de fluxo laminar, foi adicionado BHI caldo estéril no interior dos frascos e a seguir o conjunto dente + stopper de borracha + seringa estéril acoplado ao frasco, ocorrendo a imersão das raízes em BHI (Figura 17).

Figura 17: Imersão da raiz em BHI.

Na interface entre o frasco e o "stopper" foi colocado parafilm (Parafilmâ M, Marienfeld Laboratory Glassware, Lauda-Königshofen, Alemanha). Com o auxílio de uma pipeta, foi colocado 1mL de azul de metileno no interior dos "stoppers" para verificar se o vedamento na interface dente–stopper foi eficiente. Os aparatos foram incubados a 37ºC por 3 dias para assegurar a esterilização.

Após este período, o azul de metileno foi aspirado com ponteiras de plástico estéreis de 200 mL, o interior da seringa foi irrigado com soro fisiológico estéril para remoção de qualquer traço de azul de metileno e novamente o conteúdo aspirado com as ponteiras. Foi então depositado no interior da seringa 5 mL do inóculo bacteriano. Este foi feito a partir de culturas puras de *E. faecalis* (ATCC 29.212), que, após crescimento em meio sólido, foram suspensas em tubos contendo 5 mL de BHI estéril. Após agitação mecânica em vórtex, a suspensão foi ajustada no espectrofotômetro com absorbância de 800 nm, até atingir a concentração equivalente a 0,5 para aeróbios e anaeróbios facultativos (transmitância 90) da escala de McFarland (1,5 x 10^8 UFC). O conjunto foi incubado a 37°C em câmara de CO_2 (Figura 18) e o turvamento do meio foi verificado diariamente.

Figura 18: Aparatos em câmara de CO_2.

O turvamento foi verificado por 90 dias, com posterior plaqueamento para confirmação da presença do *E. faecalis*. A constatação do crescimento bacteriano durante o período de incubação foi dado pela presença de turbidez do meio (Figura 19).

A  B

Figura 19: Imagem demonstrando meio límpido, sem crescimento bacteriano (A) e meio turvo, com crescimento bacteriano (B).

Alíquotas de 100 μL do frasco com crescimento positivo foram plaqueadas em placas de petri contendo BHI Agar com a adição de 5% de sangue de carneiro desfibrinado, que foram incubadas na estufa de CO_2 por 48 horas. A pureza das culturas foi observada através da morfologia das colônias plaqueadas em BHI ágar-sangue e a confirmação foi realizada utilizando-se teste de coloração de Gram, teste de Catalase e

método bioquímico de identificação bacteriana (Api 20 Strep, bioMérieux SA, Marcy-l'Etoile, França).

No final do período de 90 dias de observação, foram coletadas alíquotas de 100 μL de todos os frascos que não apresentaram turbidez do meio de cultura, as quais foram plaqueadas em placas de petri contendo BHI Agar com a adição de 5% de sangue de carneiro desfibrinado, que foram incubadas em estufa de CO_2 à 37ºC por 48 horas, para a comprovação da ausência de crescimento microbiano.

O dia em que foi verificada a turbidez do meio foi computado e os dados avaliados estatisticamente pelos testes de Kaplan-Meier, Kruskal-Wallis e Mann-Whitney ($p< 0,05$).

***Capítulo 5* - Efeito de diferentes protocolos de irrigação na força de adesão de cimentos endodônticos resinosos à dentina**

Foram utilizados 180 pré-molares, com um único canal e ápices completos, extraídos por indicação ortodôntica.

As coroas foram removidas na junção cemento-esmalte com o auxílio de um disco diamantado de dupla face (KG Sorensen Ind. Com. Ltda., Barueri, SP, Brasil).

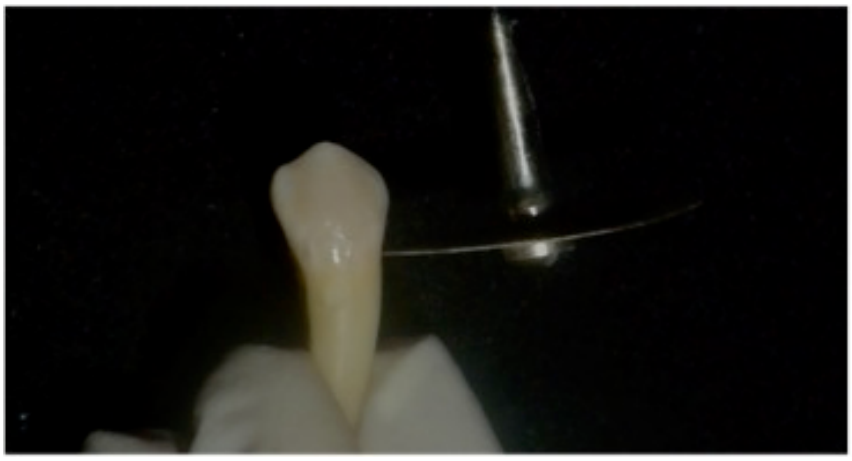

Figura 20: Remoção das coroas com disco diamantado dupla face.

Os canais foram instrumentados na sequência mostrada na Figura 21. Inicialmente foi utilizado o sistema MTwo, na seqüência: 10.04; 15.05; 20.06; 25.06; 30.05; 35.04; 40.04; 25.07, sendo todas levadas ao comprimento de trabalho, comprimento zero, isto é, ao ápice radicular. A cada troca de lima, a solução irrigadora, descrita na Tabela 5 como substância química auxiliar, foi utilizada.

Tabela 5: Regimes de irrigação.

Grupos	Subst. Química Aux.	Lavagem	Rem. Smear layer	Lavagem final	Obturação
G1 (n=10)	6 ml água destilada	10 ml água destilada	3ml água destilada	10ml água destilada	Guta-percha + AH Plus
G2 (n=10)	1ml NaOCl 5,25% + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	10ml água destilada	Guta-percha + AH Plus
G3 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	10ml água destilada	Guta-percha + AH Plus
G4 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml EDTA 17%	5ml água destil + 5ml Clorex 2%	Guta-percha + AH Plus
G5 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	5ml água destil + 5ml Clorex 2%	Guta-percha + AH Plus
G6 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	10ml água destilada	Guta-percha + AH Plus
G7 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	10ml água destilada	Guta-percha + AH Plus
G8 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	5ml água destil + 5ml Clorex 2%	Guta-percha + AH Plus
G9 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	5ml água destil + 5ml Clorex 2%	Guta-percha + AH Plus
G10 (n=10)	6 ml água destilada	10 ml água destilada	3ml água destilada	10ml água destilada	Resilon + Real Seal SE
G11 (n=10)	1ml NaOCl 5,25% + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	10ml água destilada	Resilon + Real Seal SE
G12 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	10ml água destilada	Resilon + Real Seal SE
G13 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml EDTA 17%	5ml água destil + 5ml Clorex 2%	Resilon + Real Seal SE
G14 (n=10)	1ml NaOCl 5,25%+ 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	5ml água destil + 5ml Clorex 2%	Resilon + Real Seal SE
G15 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	10ml água destilada	Resilon + Real Seal SE
G16 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	10ml água destilada	Resilon + Real Seal SE
G17 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml EDTA 17%	5ml água destil + 5ml Clorex 2%	Resilon + Real Seal SE
18 (n=10)	1ml CHX 2% gel + 5ml água destilada	10 ml água destilada	3ml Ácido fosfórico 37%	5ml água destil + 5ml Clorex 2%	Resilon + Real Seal SE

Em seguida uma broca de Gates-glidden 5 foi utilizada a fim de eliminar ou pelo menos reduzir a conicidade existente.

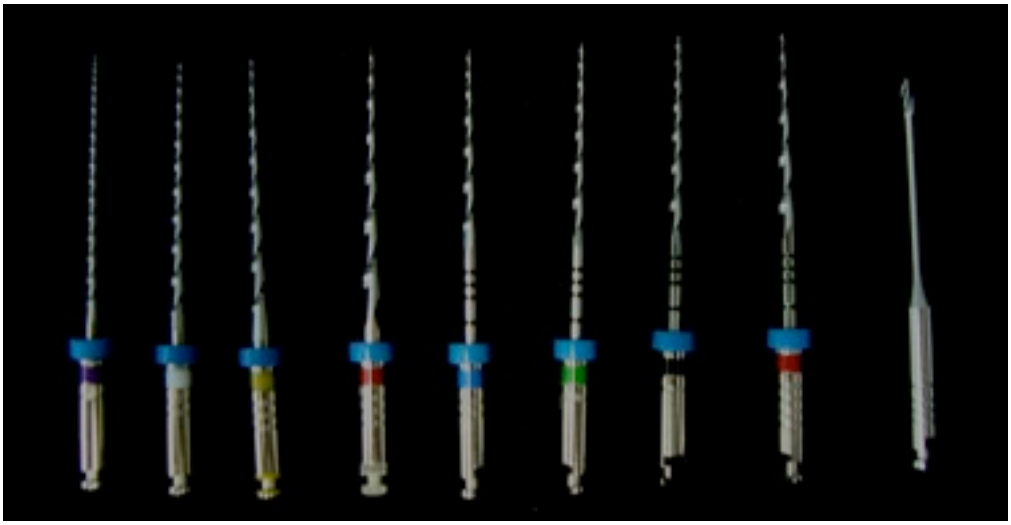

Figura 21: Seqüência empregada no preparo mecânico

Posteriormente, foi feita uma lavagem com 10 mL de água destilada e 3 mL de solução para remoção de smear layer. Foi utilizado 1 mL a cada minuto, totalizando 3 mL em 3 minutos. Por fim foi realizada uma lavagem final. Em cada grupo foram utilizados dez dentes.

Terminado o preparo químico-mecânico, os canais foram secos com cone de papel absorvente (Endopoints, Paraíba do Sul, RJ, Brasil).

A obturação foi realizada com dois diferentes materiais obturadores: cimento AH Plus (Dentsply, Petrópolis, RJ, Brasil) em conjunto com cone de guta-percha (Odous, Belo Horizonte, MG, Brasil) e sistema Resilon/ Real Seal SE como descrito na metodologia do capítulo 4.

Ao término da obturação, os dentes foram envolvidos em gazes umedecidas e mantidos no interior de placas de cultura estéreis tampadas, colocadas na câmara de anaerobiose, na temperatura de 37°C, num período de 14 dias para permitir a presa do cimento.

Terminados os 14 dias, as raízes foram fixadas em placas de resina acrílica com cera pegajosa e em seguida foram adaptadas a uma cortadora metalográfica (Isomet 1000,

Buehler, Lake Bluff, Illinois, EUA) com disco diamantado de dupla face (Buehler, Lake Bluff, Illinois, EUA), acionado a uma velocidade de 200 rpm sob refrigeração (Figura 22). Foram realizadas fatias com espessura de aproximadamente 1 mm. A primeira e a última fatia foram descartadas. Duas fatias foram avaliadas, por terço, em cada amostra.

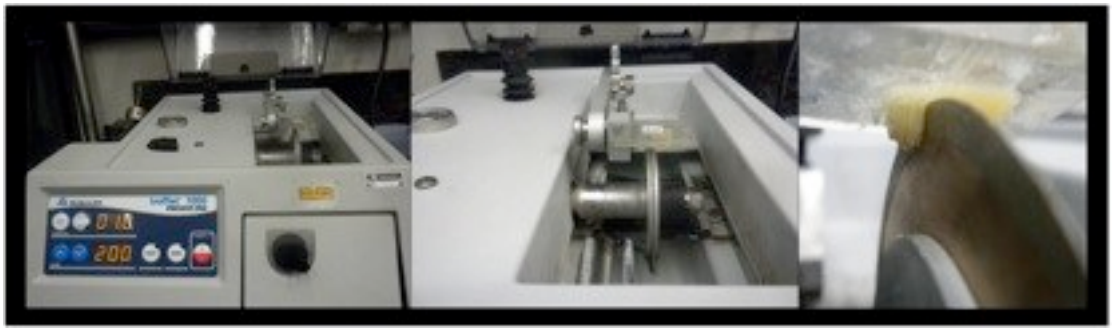

Figura 22: Corte das amostras na Isomet.

As fatias foram posicionadas em uma base, de tal maneira que o material obturador ficasse desapoiado da superfície metálica, na área indicada pela seta.

Além disso, mais dois cuidados foram tomados: que a força fosse aplicada no sentido ápico-coronal e que a ponta utilizada não tocasse a superfície dentinária.

Para o teste de push-out utilizou-se uma máquina de Ensaios Universal (EMIC DL 2000, São José dos Pinhais, PR, Brasil) a uma velocidade de 1 mm/min. Um aparato com uma ponta de 1.3 mm de diâmetro foi confeccionada para se ajustar ao preparo dos canais radiculares (Figura 23). A força máxima até o momento da ruptura foi computada.

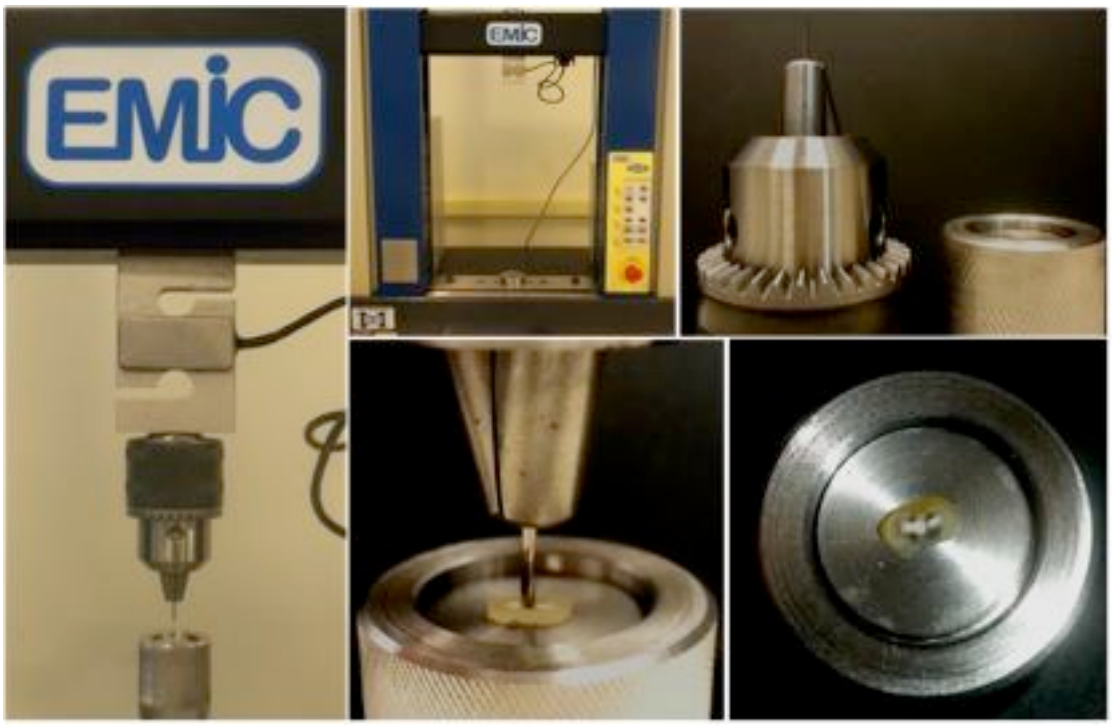

Figura 23: Máquina de ensaios Universal e aparato utilizado para ensaios de push-out.

Para calcular a resistência de união em Mega Pascal (MPa), transformou-se a carga no momento da extrusão, obtida em Newton (N), dividindo pela área adesiva (AA).

$$MPa = \frac{\text{Carga (N)}}{AA\ (mm^2)}$$

A área adesiva foi encontrada pela fórmula:

$$AA = \pi(R+r)[(h^2+(R-r)^2]^{0,5}$$

Na fórmula acima "π" representa a constante 3,14; "R" o maior raio do canal radicular na porção cervical; "r", o menor raio do canal radicular na porção apical. Estes valores foram medidos com o auxílio de uma lupa esteoroscópica (Leika MZ75, Meyer Instruments, Houston, TX, EUA) associada ao uso do "software" IM50 (Leika IM50 Image Manager), "h" é o valor da altura do segmento e foi mensurada por meio de um paquímetro digital (Vonder Paquímetro Eletrônico Digital, Curitiba, PR, Brasil). Os

dados de resistência de união foram avaliados utilizando-se os testes de Kruskal-Wallis e Mann-Whitney ($p< 0,05$).

Posteriormente, as amostras foram analisadas em microscopia eletrônica de varredura para classificar o padrão de fratura. Fotomicrografias de 1000x foram realizadas em todos os segmentos (Carvalho, 2010) e os padrões de fratura classificados de acordo com percentual de superfície livre de material obturador (Fowler et al., 1992): fratura adesiva (Figura 24A) – mais de 75% da superfície livre de material obturador, fratura mista (Figura 24B) – mais de 25% e menos de 75% de substrato livre de material obturador e fratura coesiva (Figura 24C) – menos de 25% da superfície livre de material obturador.

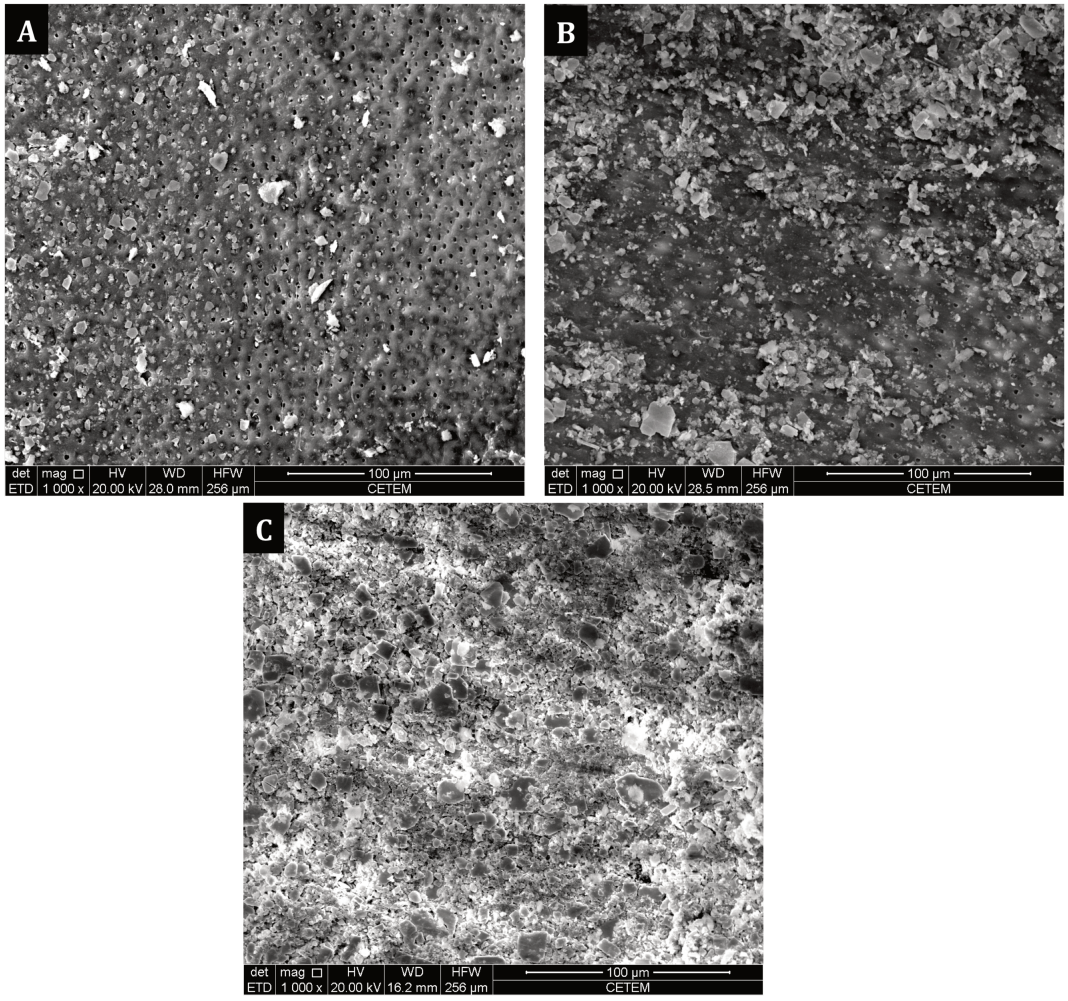

Figura 24: Padrões de fratura: Adesiva (A), mista (B) e coesiva (C).

ANEXO

Carta de aprovação do Comitê de Ética

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Avaliação da ação de diferentes soluções irrigadoras na adesão entre o complexo material obturador- dentina: Proposição de aprimoramento nos métodos de avaliação de força de adesão e associação adesão- infiltração bacteriana"**, protocolo nº 149/2009, dos pesquisadores Brenda Paula Figueiredo de Almeida Gomes e Maira do Prado, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 25/11/2009.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Evaluation of the different irrigating solutions action on the adhesion between the complex root canal filling-dentin: Purpose of improvement in measurement of bond strength and association bond strength -bacterial infiltration"**, register number 149/2009, of Brenda Paula Figueiredo de Almeida Gomes and Maira do Prado, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 11/25/2009.

Prof. Dr. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo apareoe como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.