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QMIX: AÇÃO SOBRE ENDOTOXINA BACTERIANA E EFEITO DA COMBINAÇÃO COM HIPOCLORITO DE SÓDIO SOBRE AS PAREDES DO CANAL RADICULAR

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RESUMO

Este trabalho teve o objetivo de avaliar a ação de um novo irrigante, o QMix, sobre endotoxinas bacterianas, bem como o efeito da sua combinação com hipoclorito de sódio sobre as paredes do canal radicular. Com a finalidade de avaliar sua ação sobre endotoxinas bacterianas, 50 dentes humanos extraídos foram contaminados *in vitro* com endotoxinas, e preenchidos com QMix, EDTA, clorexidina (CHX) e hipoclorito de sódio (NaOCl). Foi utilizado um teste de Lisado de Amebócito de *Limulus* (LAL) para quantificação de endotoxinas remanescentes após a irrigação. Os resultados deste primeiro estudo demonstraram que o QMix reduziu os níveis de endotoxinas quando comparado com as outras soluções irrigadoras testadas. O segundo estudo da presente tese teve o propósito de observar em Microscopia Eletrônica de Varredura (MEV) a formação de precipitado sobre as paredes do canal decorrente do uso concomitante de NaOCl e QMix. Os resultados deste estudo demonstraram que, apesar da presença de CHX na composição do QMix, seu uso após o preparo químico mecânico do canal com NaOCl não resultou na formação de precipitado sobre as paredes do canal. Muito pelo contrário, as imagens em MEV apresentaram paredes limpas com túbulos dentinários expostos. Por se tratar de um produto novo, ainda existem poucos estudos sobre o QMix. Porém, de acordo com os resultados apresentados neste trabalho, pode-se concluir que o QMix é eficaz na eliminação de endotoxinas bacterianas do interior do canal radicular, e seu uso concomitante com NaOCl não resulta na formação de precipitado sobre as paredes do canal.

Palavras-chaves: Clorexidina. EDTA. Endotoxinas. QMix. Hipoclorito de Sódio. Microscopia Eletrônica de Varredura.

ABSTRACT

This thesis had the purpose to evaluate the action of a new irrigant, QMix, on bacterial endotoxins as well as the effect of its combination with sodium hypochlorite on the root canal walls. Having the main purpose to evaluate its action on bacterial endotoxins, 50 extracted human teeth were contaminated *in vitro* with endotoxins and irrigated with QMix, EDTA, chlorhexidine (CHX) and sodium hypochlorite (NaOCl). It was used a *Limulus* Amebocyte Lysate (LAL) assay to quantify the remaining endotoxins after the irrigation. The results of this first study showed that QMix reduced the endotoxins level when compared to the other irrigant solutions that were tested. The second study from this thesis had the purpose to observe in scanning electron microscopy (SEM) the formation of precipitate on the root canal walls due to the concomitant use of NaOCl and QMix. The results from this study showed that despite the presence of CHX in the composition of QMix, its use after the chemical mechanical preparation of the canal with NaOCl did not result in the formation of a precipitate on the root canal walls. On the contrary, the images in SEM showed clean walls with exposed dentinal tubules. Once it is a new product there are still few studies about QMix. Although, according to the results presented on this study, it can be concluded that QMix is effective on the elimination of bacterial endotoxins from the root canal, not showing any precipitate formation when used concomitantly with NaOCl.

Keywords: Chlorhexidine. EDTA. Endotoxins. QMix. Sodium Hypochlorite. Scanning Electron Microscopy.

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1 INTRODUÇÃO

Os microrganismos e seus produtos têm papel fundamental na indução e perpetuação de lesões periapicais (SUNDQVIST 1976; KAKEHASHI et al., 1965). A terapia endodôntica visa à eliminação destes patógenos do canal radicular, com consequente reparo da região periapical.

Com esta finalidade, uma das ferramentas utilizadas no combate aos microrganismos é a solução irrigadora. Diversas soluções irrigadoras têm sido recomendadas para a eliminação de microrganismos, dissolução de tecidos e remoção de debris e *smear layer* do canal radicular. Porém, nenhuma solução, quando utilizada sozinha, é capaz de preencher todos estes requisitos, sendo necessária sua associação.

O hipoclorito de sódio (NaOCl) vem sendo amplamente utilizado desde a sua introdução na Endodontia por Walker em 1936. Além da sua ação alvejante, desodorizante e de dissolução de tecidos, o hipoclorito tem se mostrado um efetivo agente desinfetante (SIQUEIRA et al., 1997), e por isso é o irrigante de primeira escolha da maioria dos profissionais.

Por outro lado, a clorexidina (CHX), devido a sua baixa toxicidade, vem sendo reconhecida como um irrigante alternativo, principalmente em casos de dentes com ápices muito abertos, uma vez que o extravasamento de NaOCl através do ápice poderia provocar uma reação inflamatória considerável (JEANSONNE e WHITE, 1994). Além disso, apresenta atividade antibacteriana satisfatória (GOMES et al., 2001) e substantividade, podendo ter sua ação antimicrobiana prolongada quando utilizada como irrigante (WHITE et al., 1997). De outro modo, o detergente tem capacidade de reduzir a tensão superficial, aumentando o escoamento das soluções irrigadoras (ABOU-RASS e PATONAI, 1982), além de apresentar efeito antibacteriano (WANG et al., 2012).

O EDTA é um agente quelante que complementa a ação da solução irrigadora, facilitando a instrumentação do canal. O uso de um agente quelante é importante para preparar a superfície do canal radicular, promovendo a remoção de *smear layer* (KISHEN et al., 2008) para que o irrigante exerça sua ação em profundidade, nos canais acessórios e no interior dos túbulos dentinários (BERUTTI et al., 1997). Recentes estudos têm avaliado a ação de um novo produto para irrigação final do canal, o QMix (Dentsply Tulsa Dental, Tulsa, EUA). De acordo com o fabricante, este irrigante é uma solução aquosa que inclui EDTA, correspondendo a aproximadamente 0,5 a 20% do peso, Clorexidina, correspondendo a 0,01 a 5% do peso, e um detergente (cetrimida), correspondendo a 0,001 a 3% do peso, visando associar as vantagens de cada uma dessas substâncias (QMIX BROCHURE; US PATENT PUBLICATION).

Alguns estudos já comprovaram a efetividade do QMix frente ao *Enterococcus faecalis* (STOJICIC et al., 2011; WANG et al., 2012). Porém, bactérias gram-negativas são comumente encontradas no interior do canal radicular de dentes com polpa necrosada. Estas bactérias possuem em sua parede a endotoxina, um lipopolissacarídeo (LPS) liberado durante a multiplicação e morte bacteriana capaz de desencadear respostas biológicas importantes no desenvolvimento e manutenção da reação inflamatória periapical (SHEIN e SCHILDER, 1975) e reabsorção óssea (PITTS et al. 1982, GOMES et al. 2012). Além disso, existe uma correlação positiva entre a concentração de LPS e o desenvolvimento de infecções sintomáticas (JACINTO et al. 2005, MARTINHO et al. 2011). Sendo assim, é de grande relevância a avaliação da ação do QMix frente a endotoxinas bacterianas.

É consenso na literatura que o uso concomitante de algumas substâncias irrigadoras pode provocar reações químicas indesejadas. Quando se mistura clorexidina com EDTA, é difícil a obtenção de uma solução homogênea, devido à formação de um precipitado. Este precipitado é um sal formado pela neutralização da clorexidina (catiônica) pelo EDTA

(aniônico) (GONZÁLEZ-LOPEZ et al., 2006). Por outro lado, a combinação de hipoclorito de sódio com clorexidina causa uma reação ácido-base, onde a CHX (ácido) doa prótons para o NaOCl (base). Esta troca de prótons resulta na formação de um precipitado de coloração marrom (BASRANI et al., 2007) que, além de recobrir a superfície do canal ocluindo os túbulos dentinários (BUI et al., 2008), pode conter paracloroanilina (BASRANI et al., 2007; BASRANI et al., 2010), uma substância tóxica e carcinogênica (CHHABRA et al., 1991). Uma vez que o QMix, apesar de conter clorexidina, é indicado para irrigação final do canal, mesmo após o preparo químico mecânico com hipoclorito de sódio, algumas investigações sobre essa combinação merecem ser conduzidas.

Frente a essas questões, levando-se em consideração que o QMix é um produto novo no mercado e que ainda merece uma série de investigações antes de seu amplo uso na Endodontia, torna-se justificável a realização deste estudo, com o intuito de avaliar sua ação sobre endotoxinas bacterianas, bem como o efeito da sua combinação com hipoclorito de sódio sobre a parede do canal radicular.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar a ação de um novo irrigante, QMix, sobre endotoxinas bacterianas, bem como o efeito da sua combinação com hipoclorito de sódio sobre as paredes do canal radicular.

2.2 OBJETIVOS ESPECÍFICOS

2.2.1 Investigar, *in vitro*, a capacidade do QMix de reduzir endotoxinas de canais radiculares de dentes humanos extraídos, através de um teste de Lisado de Amebócito de *Limulus* (LAL).

2.2.2 Avaliar em microscopia eletrônica de varredura o efeito da combinação do QMix com hipoclorito de sódio 2,5%, observando a formação de precipitado nas paredes de canais radiculares de dentes humanos extraídos.

3 ARTIGO 1

QMix® irrigant reduces lipopolysaccharide (LPS) levels in an *in vitro* model

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Abstract

Objectives To determine the effect of QMix® and other three root canal irrigants on *Escherichia coli* LPS.

Materials and methods Root canals of single-rooted teeth were prepared. Samples were detoxified with Co-60 irradiation and inoculated with *E. coli* LPS (24h, at 37°C). After that period, samples were divided into 4 groups, according to the irrigation solution tested: QMix®, 17% EDTA, 2% chlorhexidine solution (CHX), and 3% sodium hypochlorite (NaOCl). LPS quantification was determined by Limulus Amebocyte Lysate (LAL) assay. The initial counting of endotoxins for all samples, and the determination of LPS levels in non-contaminated teeth and in contaminated teeth exposed only to non-pyrogenic water were used as controls.

Results QMix® reduced LPS levels, with a median value of 1.11 endotoxins units (EU)/mL ($P<0.001$). NaOCl (25.50 EU/mL), chlorhexidine (44.10 EU/mL) and positive control group (26.80 EU/mL) samples had similar results. Higher levels were found with EDTA (176.00 EU/mL) when compared to positive control ($P<0.001$). There was no significant difference among EDTA, NaOCl and CHX groups. Negative control group (0.005 EU/mL) had statistically significant lower levels of endotoxins when compared to all test groups ($P<0.001$).

Conclusion QMix® decreased LPS levels when compared to the other groups ($P<0.001$). NaOCl, CHX and EDTA were not able to significantly reduce the root canal endotoxins load.

Clinical relevance The presence of endotoxin within the root canal was associated with periapical inflammation, bone resorption and symptomatic infections. Its removal/neutralization from infected root canals during endodontic treatment seems to be important for the healing process of periapical tissues.

Keywords Chlorhexidine. EDTA. Endotoxins. QMix. Sodium hypochlorite.

Introduction

Microorganisms play an important role in the induction and maintenance of periapical

diseases [1, 2]. They have unique virulence factors, such as fimbriae, pilli, membrane receptors and endotoxins. Lipopolysacharide (LPS) is an endotoxin that is present in the outer layers of Gram-negative bacteria cell walls, usually detected in root canal infections, promoting biological responses associated with periapical inflammation [3] and bone resorption [4, 5]. There is a positive correlation between LPS concentration in root canals and the development of symptomatic infections [6, 7].

Endodontic therapy aims the infection control, allowing the periapical healing. Several chemical substances have been used as adjuvant to the root canal mechanical preparation. Sodium hypochlorite (NaOCl) has been the most widely used root canal irrigant. NaOCl dissolves organic tissues and has a strong antimicrobial activity [8]. On the other hand, Chlorhexidine (CHX) is a biocompatible agent that has the antimicrobial action associated with substantivity [9, 10]. Ethylenediaminetetraacetic acid (EDTA) is a chelating agent, allowing the smear layer removal [11]. EDTA favors the action of other irrigants into the dentinal tubules and root canal ramifications [12].

QMix® (Dentsply Tulsa Dental, Tulsa, USA) is a novel irrigant to be used as a final rinse. It is supposed to combine the antimicrobial and substantivity properties of CHX with smear layer removing properties of EDTA [13]. Moreover, QMix® contains a detergent that decreases surface tension and increases wettability in solutions [14]. Recent studies demonstrated that QMix® is effective against *Enterococcus faecalis* [15, 16].

Previous studies showed that root canal mechanical preparation plays an important role in reducing endotoxin load [17-19]. The effect of endodontic irrigants has also been established when in direct contact with LPS [20]. Nevertheless, it is not known if this effect is similar when endotoxins are within the root canal system. Therefore, the present *in vitro* study investigated the effects of auxiliary chemical and QMix ® substances on endotoxins within the root canal space.

Materials and methods

This study was approved by the Research Board and Ethics Committed for Research from the Pontifical Catholic University of Rio Grande do Sul (PUCRS) (protocol numbers 0017/13 and

310.698, respectively).

Sample selection and root canals preparation

Fifty (50) single-rooted teeth were selected. Roots were sectioned at cementum-enamel junction, with a diamond disc (Dhpro, Rhadartrade, Paranaguá, PR, Brazil) under water-cooling. The working length (WL) was visually established, with a #10 hand instrument (Dentsply-Maillefer, Ballaigues, Switzerland) that was inserted into the canal until its tip reached the apical foramen. The WL was determined 1 mm shorter to the apex. The root canals were prepared using the standardized K-files series (Maillefer, Michigan, EUA), from a #10 K-file to a #60 K-file in the entire WL, to facilitate the LPS introduction and collection. At each change of instrument, the canals were flushed with 2 mL of NaOCl 1% (Biodinamica, Ibiporã, Brazil). After preparation, canals were filled with EDTA 17% (Biodinamica, Ibiporã, Brazil) for 3 minutes and irrigated with 5 mL of sterile saline solution. Canals were dried with paper points (Dentsply, Rio de Janeiro, RJ, Brazil). Each sample was fixed with epoxy resin (Loctite, São Paulo, Brazil) in a well, in 12-well cell culture plates (Kasvi, Curitiba, PR, Brazil).

Sterilization and detoxification

The specimens were irradiated with 60-Co gamma rays (EMBRARAD; Empresa Brasileira de Radiações, Cotia, SP, Brazil) for degradation of preexisting LPS [21]. The sterilization and detoxification of the instruments used in the experiment were performed in an oven at 250°C, for 30 minutes [22].

Contamination with endotoxin

The specimens contamination with endotoxins was performed as previously described [21]. Briefly, inside a laminar flow chamber, 20 μL (1.000.000 UE/mL) of a solution containing *Escherichia coli* 055:B5 endotoxin (Lonza, Walkersville, MD, USA) was inoculated into the root canals of 45 specimens. Five teeth were not contaminated (control samples). Pyrogen-free cotton pellets were placed in the cervical portion of the canals in all samples. The plates containing the specimens were closed and incubated at 37^{O}C under a humidified atmosphere

for 24 hours.

Experimental Groups

After the incubation period, samples were divided into the following groups, according to the irrigation solution: QMix® (Dentsply Tulsa Dental, Tulsa, USA), 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil), 2% CHX (Maquira, Maringá, PR, Brazil), 3% NaOCl (Farmácia Marcela, Porto Alegre, RS, Brazil) (n=9 per group). As control groups, the initial count (ICo) of endotoxins was determined after contamination (n=4), non-contaminated teeth (negative control NCtrl, n=5) and contaminated samples rinsed with non-pyrogenic water (the flushing control – positive control PCtrl, n=5).

With disposable syringes (Descapack, São Paulo, SP, Brazil), the root canals were filled with each solution. After 3 minutes [15], canal content was aspirated with a new disposable plastic syringe. Then, the root canal was filled with non-pyrogenic water. Root canal content was collected with three #45 paper points, which were immediately transferred to glass tubes, closed, and kept at -20°C until the analysis. For all control groups, canals were filled with non-pyrogenic water and the sample was collected. In order to certify the accuracy of LPS counting, the quantification of endotoxins levels in non-pyrogenic water and in the paper points used for sampling was determined.

The tubes containing the paper points were filled with 1 mL of non-pyrogenic water, warmed (37°C ± 1°C) for 1 hour and vortexed (Phoenix, Araraquara, SP, Brazil) for 1 minute. The protocol previously described [23] was applied for endotoxins quantification. Briefly, the PYROGENT 5000 (Lonza, Walkersville, MD, USA) is a quantitative, kinetic assay for the detection of Gram-negative bacterial endotoxin. The sample is mixed with the reconstituted LAL reagent, placed in the photometer, and automatically monitored over time for the appearance of turbidity. The concentration of endotoxin in unknown samples can be calculated from a standard curve [7]. LAL reagent water (blank) was used as a negative control. All reactions were accomplished in duplicate to validate the test. 100 µL of the LAL Reagent Water blank, endotoxin standards, product samples, positive product controls (PPC: an aliquot of test sample spiked with a known amount of endotoxin) were carefully dispensed into the appropriate wells of a 96-well microplate (Corning Costar, Tewksbury, MA, USA). The filled plate was placed in the microplate Kinetic-QCL Reader and pre-incubated for ≥10

minutes at 37°C ± 1°C. After incubation period, 100 µL of the PYROGENT - 5000 Reagent was dispensed into all wells of the microplate and the test was initiated.

According to the positive product control (PPC), the test groups' samples need a 100-fold dilution to avoid the interference of the irrigants for the quantification assay. The ICo and PCtrl group samples need a 10-fold dilution. No dilution was required for the NCtrl group samples.

Statistical analysis

Data collected were log transformed and one-way analysis of variance was applied on these data followed by the Tukey post hoc. The level of significance was set to $P < 0.001$. Data were analyzed using SPSS version 17.0 (SPSS, Chicago, IL).

Results

Endotoxins in the water, as well as in the paper points used during the experiment were quantified as < 0.01 EU/mL.

According to LAL assay manufacturer´s recommendations, the endotoxin recovered at PPC should equal the known concentration of the spike within 50% to 200%.

Table 1 and Fig. 1 show the endotoxin load for both control and test groups. QMix® reached the lowest levels of endotoxins amongst all test solutions ($P<0.001$). There was no significant difference among NaOCl, CHX and PCtrl group samples. Higher levels were found with EDTA, when compared to the PCtrl ($P<0.001$). There was no statistically significant difference among the endotoxin content for the EDTA, NaOCl and CHX groups. The NCtrl group presented statistically significant lower levels of endotoxins compared to all test groups ($P<0.001$).

Discussion

The presence of endotoxin within the root canal was associated with periapical inflammation [3], bone resorption [4, 5] and symptomatic infections [6, 7]. Acute endodontic infections harbor diverse and complex microbial communities, formed especially by Gram-negative anaerobic species [24]. It can be postulated that the endotoxic content inside root canals is heterogeneous. Each Gram-negative bacteria species have a unique LPS molecule, with diverse immunogenic activity [25]. It can also be observed that each root canal harbors a specific load of endotoxins [6]. It is hard to reproduce this complex and rich environment in the laboratory. Standardization for the LPS load and characteristics can be achieved *in vitro* through the inoculation of isolated *Escherichia coli* endotoxin. Recently, these *in vitro* protocols have been employed to assess the effect of irrigants in direct contact to LPS [20], and also the effect of chemomechanical preparation [19] and intracanal medicaments [21] on the root canal endotoxic load. However, no study assessed the isolated effect of auxiliary chemical substances in root canals infected with endotoxins, especially for final rinsing substances such as EDTA and QMix®.

The LAL assay is a biological test system that quantifies endotoxins with extremely high sensitivity. This test utilizes a preparation of Limulus Amebocyte Lysate, in combination with an incubating photometer and appropriate software, to detect endotoxin photometrically. Gram-negative bacterial endotoxin catalyzes the activation of a proenzyme in the Limulus Amebocyte Lysate. The initial rate of activation is determined by the concentration of endotoxin present. The activated enzyme (coagulase) hydrolyzes specific bonds within a clotting protein (Coagulogen) also present in Limulus Amebocyte Lysate. Once hydrolyzed, the resultant coagulin self-associates and forms a gelatinous clot. The turbidimetric LAL assay measures the increase in turbidity (optical density) that precedes the formation of the gel clot.

LPS from most bacterial species is composed of three distinct regions: the O-antigen region, a core oligosaccharide, and Lipid A [26]. In an aqueous environment, amphiphilic molecules like lipid A form supramolecular aggregate structures, changing the physical structure of LPS from monomeric molecules to multimeric aggregates. Mueller et al. [27] have demonstrated that LPS in an aggregate structure had a higher biological activity than monomerized LPS. The LAL assay is not able to detect monomerized LPS [27]. Therefore, no detection of LPS through the assay should not be considered as the absence of endotoxin. It

could be associated with the presence of the low toxic monomerized LPS.

According to Dawson [28], some solutions, like NaOCl and EDTA, can interfere in the LAL reaction due to pH variations and chelating activity. In the present study, the samples were diluted to avoid the interference of the irrigants on the analytic procedures. Furthermore, absence of interferences was determined through the results observed in the PPC, which detects inhibition or enhancement of LAL through the addition of a known concentration of *E. coli* endotoxins to its sample, as recommended by the manufacturer (spike procedure). To inactivate the LPS from teeth before its contamination was employed irradiation with Co-60. The irradiation reduces LPS toxicity keeping dentin characteristics [22]. Further material employed in the experiment was detoxified through dry heat (250°C, for 30 minutes) [22]. The absence of endotoxins was determined before the analysis, and all materials showed negative results for the presence of endotoxins at the control procedures.

Extracted human teeth were employed to simulate the clinical conditions and to obtain a proper substrate for endotoxin contamination. It is known that over time, dentinal tubules can become completely occluded with age, even under natural physiological conditions [29]. Therefore, it should be emphasized that it is difficult to standardize the donor's age and the presence of variability among samples.

Sampling methods to study the root canal microbial communities have been discussed in the current literature [30-32]. Their limitations can affect also endotoxin's sampling procedures. In the present study, root canal sampling was performed with paper points, as previously reported by Jacinto et al. [6], Martinho et al. [23] and Gomes et al. [5]. Signoretti et al. [21] washed the root canals with apirogen water to collect the content of endotoxins in calcium hydroxide medicated root canals. The authors employed this method because the endotoxic content might be removed during flushing for removal of the medicaments. On the other hand, Alves et al. [30] determined the bacterial communities in segments of infected root canals in grinded samples from human extracted teeth. Grinded samples allowed obtaining a more comprehensive sample, however it can only be applied to extracted teeth. In the present study, the paper point method was employed to simulate the clinical limitations that are imposed during sampling. It should be emphasized that paper points were not able to entirely remove the endotoxins that are in the dentinal tubules and root canal irregularities. In this study, it was inoculated 20μL of a 1.000.000 UE/mL solution containing *Escherichia coli* endotoxin into the root canals. It was observed that a median value of 33.75 EU/mL was

recovered in the initial sampling (ICo). Therefore, endotoxins can be distributed in the entire root canal system. It might be suggested that endotoxins can be trapped in the deep dentin layers, isthmus and irregularities. Similar and broad-covering sampling methods should be developed to allow a broad endotoxin recovery from root canals, for both *in vitro* and *in vivo* studies.

Because of the high toxicity of endotoxin, some substances have been tested to obtain its inactivation. Sodium hypochlorite has been widely used as an auxiliary chemical substance. Besides its bleaching, deodorant and tissue dissolution effects, sodium hypochlorite has been proven to be an effective disinfectant [8]. On the other hand, chlorhexidine is a biocompatible agent that has the antimicrobial action associated with substantivity [9, 10]. In a clinical study, Gomes et al. [33] compared the efficacy of chemomechanical preparation with 2.5% NaOCl and 2% CHX gel on eliminating LPS in teeth with pulp necrosis and apical periodontitis. They concluded that NaOCl and CHX have no detoxifying effect on endotoxins, and that the removal of more than 47% of the LPS content was related to the mechanical action of the instruments in dentin walls accomplished by the flow and backflow of the irrigants. In the same way, the results of the present study demonstrated that sodium hypochlorite and chlorhexidine are not able to detoxify root canals infected with LPS. Buttler & Crawford [34] and Buck et al. [20] evaluated different irrigants in direct contact with LPS and the same behavior was observed.

Ethylenediaminetetraacetic acid is a chelating agent that promotes the smear layer removal [11]. It favors the action of other irrigants into the dentinal tubules and root canal ramifications [12]. According to Buck et al. [20], when an aqueous solution of LPS was mixed with EDTA, there was little breakdown of LPS. On the other hand, in the present study, there was a high level of endotoxins for the EDTA group when compared to the positive control group. Burton & Carter [35] observed that EDTA can exert a chelating action in the calcium present in the lipid A portion of the endotoxin molecules. Therefore, the action of EDTA on exposing the deep layers of contaminated dentin may improve LPS release by dentin, increasing its recovery rates. Furthermore, Leive & Shovlin [36] reported that EDTA can enhance the endotoxin release by *E. coli* cells after a brief exposure, without changing its biological activity. In the present study, isolated endotoxin was employed to contaminate the samples. Therefore, this effect may be determinant especially for clinical studies that evaluate the content of endotoxins inside root canals infected with free LPS and bacteria.

QMix® has been employed after root canal preparation as a final rinse to improve root canal cleaning and disinfection [15]. It comprises an aqueous solution of EDTA, chlorhexidine and N-cetyl-N,N,N-trimethylammonium bromide [37]. Stojicic et al. [15] employed a 3-minute period to evaluate the antimicrobial effect of QMix® in a direct exposure test. Morgental et al. [38] demonstrated that QMix® promoted additional antimicrobial action, especially in longer periods (> 1 minute). There is no study that reported the effect of QMix® over the endotoxic content within root canals. According to the results, QMix® had the potential to reduce LPS content from the root canal when compared to the other irrigants. Guerreiro-Tanomaru et al. [39] reported that the presence of a high amount of surfactant and EDTA in QMix® composition may explain the great ability of this solution to remove biofilm cells from a substrate. Thus, the presence of EDTA can enhance the LPS removal from the samples, due to its ability to expose the infected inner dentin and to its potential to bind to the calcium present in the lipid A. Additionally, Jang et al. [40] and Nasser & Moghazy [41] demonstrated that tensioactive agents may favor LPS removal, emulsifying endotoxins, and favoring the physical action of irrigant solutions on its removal.

It is possible to conclude that the chemical action of NaOCl, CHX and EDTA was not able to reduce LPS load inside the root canal system. The physical action of irrigants associated with mechanical instrumentation may be necessary to reach LPS reduction. QMix® seemed to reduce LPS load inside the root canal system. Further studies should be performed to determine if this QMix® property may enhance the ability of the chemomechanical preparation on reducing the total endotoxic load from root canals.

Conflict of interest The authors deny any conflict of interest related to this study.

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Table 1 – Endotoxin Values

EU/mL	ICo[c] n=4	NCtrl[a] n=5	PCtrl[c] n=5	QMix[b] n=9	EDTA[d] n=9	CHX[c,d] n=9	NaOCl[c,d] n=9
Median	33.75	0.005	26.80	1.11	176.00	44.10	25.50
Minimum	6.48	0.005	12.50	0.50	26.70	3.25	9.26
Maximum	60.70	0.064	31.70	4.68	370.00	147.00	106.00

ICo: Initial count, NCtrl: Negative control, PCtrl: Positive control. $P < 0.001$: significance using ANOVA. Different index letters represent statistical significant different at the post-hoc procedure (Tukey test).

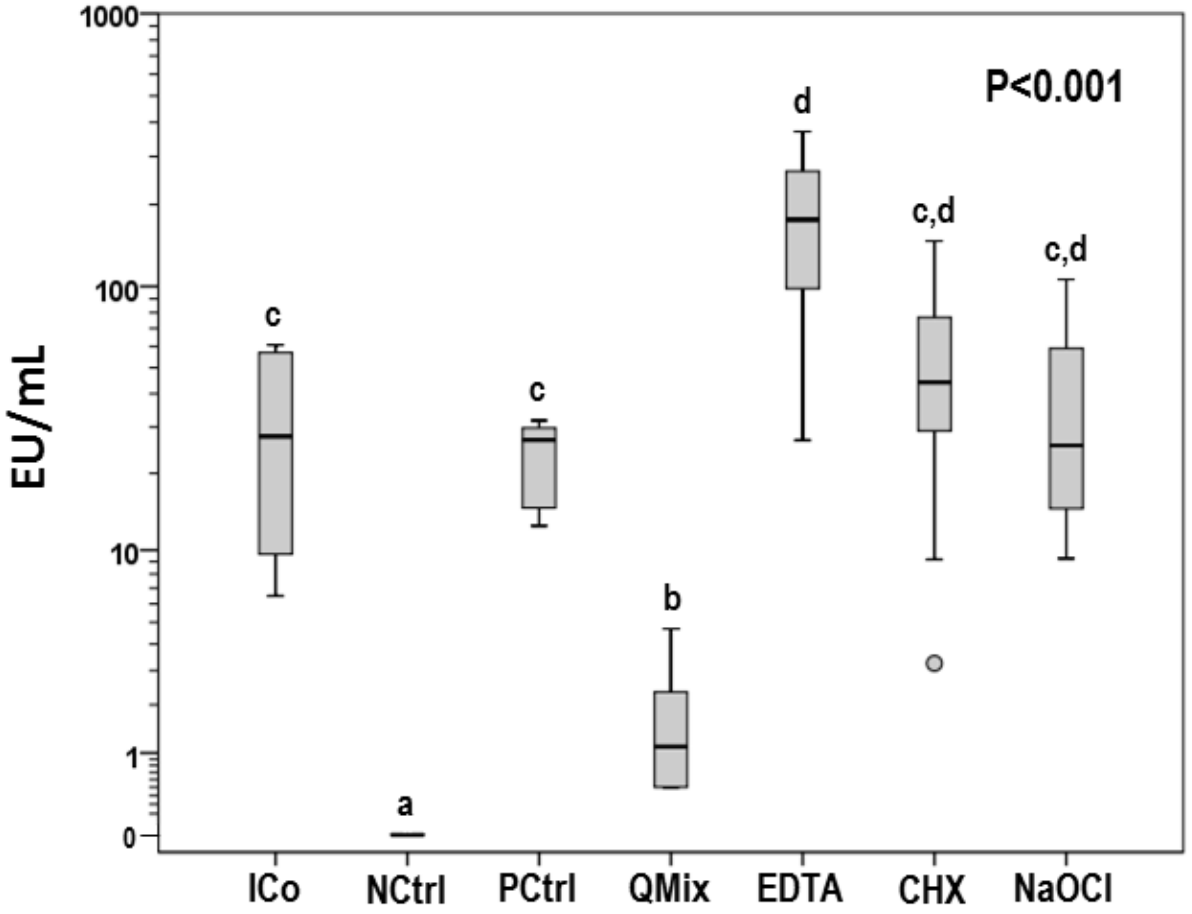

Fig. 1 Endotoxin values. ICo: Initial count, PCtrl: Positive control, NCtrl: Negative control. Different index letters represent statistical significant different at the post-hoc procedure (Tukey test).

4 ARTIGO 2

Effect of the Combination of Sodium Hypochlorite and QMix®: Scanning Electron Microscopy Precipitate Observation

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Abstract

Introduction: The purpose of this study was to observe, under scanning electron microscopy (SEM), the potential formation of a precipitate on the root canals walls due to the combined use of sodium hypochlorite (NaOCl) and QMix®. **Methods:** Sixteen single-rooted human teeth were selected. After instrumentation, the root canal surfaces were conditioned for smear layer removal using 15% citric acid solution under ultrasonic activation and a final wash with distilled water. The specimens were randomly divided into four groups as follows: negative control (no irrigation); positive control (2.5% NaOCl + 2% CHX); 2.5% NaOCl + QMix®; 2.5% NaOCl + distilled water + QMix®. All roots were split in half and each third was evaluated by SEM at 1,000X, 5,000X and 15,000X. **Results:** There was no precipitate formation when QMix® was used after NaOCl, regardless of the use of distilled water between the two solutions. The positive control group showed precipitate formation occluding dentinal tubules, especially at the cervical third, while the negative control showed a clean surface with permeable dentinal tubules. **Conclusions:** The use of QMix® for removing smear layer after chemicomechanical preparation with NaOCl causes no precipitate formation on the root canal walls.

Keywords: chlorhexidine, QMix, SEM, sodium hypochlorite.

INTRODUCTION

Microorganisms and their by-products play a key role in the induction and maintenance of periapical lesions (1, 2). Endodontic therapy aims to eliminate these pathogens from the root canal system, with subsequent periapical healing. To achieve this goal, along with an appropriate mechanical preparation, the irrigant is critical in disinfecting the root canal space (3).

Sodium hypochlorite (NaOCl) is an effective antimicrobial agent, and also has bleaching, deodorant and tissue-dissolving activities (4). Therefore, it has been widely used since its introduction in Endodontics by Walker in 1936 (5). On the other hand, chlorhexidine (CHX) has satisfactory antibacterial activity (6) and substantivity, promoting a prolonged antimicrobial effect when used as irrigant (7).

The use of a chelating agent is important to prepare the root canal surface, enabling smear layer removal (8) so that the irrigant exert its action in depth, reaching accessory canals and dentinal tubules (9). Recent studies have evaluated the action of a new product proposed as a final endodontic irrigant, named QMix® (Dentsply Tulsa Dental, Tulsa, USA). According

to the manufacturer, the irrigant is an aqueous solution that includes EDTA in an amount from about 0.5 to about 20 percent by weight, chlorhexidine in an amount from about 0.01 to about 5.0 percent by weight, and N-cetyl-N,N,N-trimethyllammonium bromide in an amount from about 0.001 to about 3.0 percent by weight, aiming at bringing together, in a single product, the antimicrobial properties and substantivity achieved by chlorhexidine, the smear layer removal capacity of EDTA and the low surface tension of the detergent (10).

Nevertheless, it is well known that the concurrent use of NaOCl and CHX leads to the formation of a brown precipitate that covers the root canal surface, occluding dentinal tubules (11). Once QMix®, despite containing CHX, is indicated for the final irrigation of root canals even after the use of NaOCl during chemomechanical preparation, some investigations about this combination deserves to be conducted. Thus, the purpose of this study was to observe, under SEM, the potential formation of precipitate on the root canal walls, due to the combined use of NaOCl and QMix®.

MATERIALS AND METHODS

This study was approved by the Research Board and Ethics Committee for Research of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), under protocol numbers 0017/13 and 310.698, respectively.

Sample selection and root canals preparation

Sixteen (16) single-rooted human teeth were selected. Roots were sectioned at the cementum-enamel junction, with a diamond disc (Dhpro, Rhadartrade, Paranaguá, PR, Brazil) under water-cooling. The working length (WL) was visually established, with a size #10 hand instrument (Dentsply-Maillefer, Ballaigues, Switzerland) that was inserted into the canal until its tip reached the apical foramen. The WL was determined 1 mm shorter to the apex. The root canals were prepared as previously described (12), using Gates-Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland) in a descending order from #5 to #2 to shape the middle and cervical thirds. Root canal instrumentation was carried out with stainless steel K-files up to a size #30 (Dentsply Maillefer, Ballaigues, Switzerland) in a crown-down technique. Irrigation was performed with 1 mL of distilled water at each change of instrument. The root canal surfaces were conditioned for smear layer removal using 15% citric acid solution under ultrasonic activation during 5 minutes in a Schuster L-100 unit (Schuster, Santa Maria, RS, Brazil), followed by a final flush with distilled water to remove any trace of the demineralizing solution (13). The root canals were dried with absorbent paper points

(Dentsply, Rio de Janeiro, RJ, Brazil). Afterwards, roots were mounted on a base of utility wax (Wilson Polidental, Cotia, SP, Brazil) in order to avoid the extrusion of irrigants, and samples were divided into four (4) groups (n=4 per group): negative control (no irrigation); positive control [2.5% NaOCl (CIENTEC, Porto Alegre, RS, Brazil) + 2% CHX (Maquira, Maringá, PR, Brazil)]; 2.5% NaOCl + QMix® (Dentsply Tulsa Dental, Tulsa, USA); 2.5% NaOCl + distilled water + QMix®.

The specimens were filled with 2.5% NaOCl solution using a disposable syringe (Descapack, São Paulo, SP, Brazil) and a 30G irrigation needle (Ultradent Products Inc., South Jordan, UT, USA). NaOCl remained in the root canal for 30 minutes to simulate the time a tooth remains in contact with the irrigant during chemomechanical preparation. Then, the irrigant was aspirated with a syringe and the canal was filled with the second solution, remaining during 90 seconds, which is the period of time recommended by the manufacturer of QMix®.

In the negative control group, no irrigation protocol was used, in order to ensure the smear layer produced during root canal preparation was removed, and could not be confused with the precipitate formed by the combination of substances.

At the end of these procedures, the canals were dried with absorbent paper cones and prepared for SEM analysis.

Preparation for Scanning Electron Microscopy

Longitudinal grooves were carved on the free surfaces of the roots with a diamond saw (Dhpro; Rhadartrade, Paranaguá, PR, Brazil) before sample preparation, taking care not to invade the inner part of the root canal. After the irrigation protocol, the complete fracture was accomplished with a chisel and hammer, providing two halves of each sample. The best one was chosen for SEM analysis. Samples were dehydrated by immersion in 70%, 90%, and 100% acetone and placed on stubs with the root canal portion positioned upward. Then, samples were coated with gold-palladium for conducting electrons. The evaluation was made in a scanning electron microscope (XL 30; Philips, Eindhoven, Netherlands) along to the medium line, dividing the root into thirds. Starting with a smaller magnification (1,000X), when a precipitate was observed, 5,000X and 15,000X images were obtained (13).

Data was described qualitatively by the presence or absence of a precipitate.

RESULTS

There was no precipitate formation when QMix® was used after NaOCl, regardless of the use of distilled water between the two solutions (Figs. 1 and 2).

Figure 3 shows precipitate formation occluding dentinal tubules, especially at the cervical third when CHX was used after NaOCl (positive control).

The protocol for removing smear layer prior to irrigation protocols proved to be efficient (negative control), promoting a clean surface with permeable dentinal tubules.

DISCUSSION

Endodontic treatment aims to eliminate pathogens from the root canal system and one of the tools used for this purpose is the irrigating solution. A clinical protocol widely used for smear layer removal prior to root canal filling is the use of EDTA after chemomechanical preparation with NaOCl (14). Recently a new auxiliary chemical substance named QMix® has been studied for final irrigation. It is supposed to combine the antimicrobial and substantivity properties of CHX with smear layer removing properties of EDTA (10). Moreover, QMix® contains a detergent (cetrimide) that decreases surface tension and increases wettability in solutions (15). Recent studies demonstrated that QMix® is effective against *Enterococcus faecalis* (16, 17). In a previous study, QMix® proved as effective as EDTA in removing smear layer from the root canal walls (18).

Although the combination of irrigants favors its action, possible chemical reactions must be considered. It is well-established that CHX cannot be combined with high concentration EDTA without precipitation formation. When mixing CHX with EDTA, it is difficult to obtain a homogeneous solution; a precipitate composed chiefly of the original components forms. It was suggested that the precipitate was most likely a salt formed by neutralization of the cationic CHX by anionic EDTA (19). QMix® overcomes the problem of maintaining effective amounts of EDTA and CHX in solution. In the method for making the composition of QMix®, CHX is first mixed with cetrimide before EDTA is added. CHX and cetrimide in water appear to form a micelle formulation that protects the combination from precipitation (10).

However, it is well-established that the concomitant use of NaOCl and CHX forms a precipitate which, in addition to obliterate dentinal tubules, reduces dentin permeability (13), may be toxic (20), and also may cause color changes in dentin and enamel (21).

The combination of NaOCl and CHX causes an acid-base reaction. CHX is a dicationic acid (pH 5.5 - 6.0) that has the ability to donate protons. Alkaline NaOCl is able to accept protons from the dicationic CHX. This proton exchange results in the formation of a

neutral and insoluble substance referred as precipitate (22). It has been reported by previous researchers that this precipitate contains a cytotoxin so-called parachloroaniline (PCA) (22, 23), which is carcinogenic and toxic (24). However, recent studies (20, 25) showed that PCA was not produced in any measurable quantity. Regardless of the presence of PCA, there is a consensus in the literature that this precipitate formation must be avoided, since it can present detrimental consequences for endodontic treatment, including a risk of discoloration and potential leaching of unidentified chemicals into the periradicular tissues (26).

The antimicrobial activity (16, 27), the smear layer removal capacity (16, 28), as well as the biocompatibility (29) of QMix® have been tested. However, no study has evaluated the effect of its use combined with NaOCl on root canal surface. The manufacturer recommends irrigation with distilled water or saline for a complete removal of NaOCl before using QMix®. This study demonstrated no precipitate formation on the root canal walls due to the concomitant use of the two irrigating solutions, independent of the use of distilled water. Our results confirm for the first time the hypothesis proposed by Stojicic et al. (16) that, despite the presence of CHX, mixing QMix® with NaOCl produces no precipitate or color change, although they have not yet present a specific study.

By allowing the display of the amount and distribution of smear layer on the root canal walls, the use of SEM in the observation of this smear layer is usual in the literature (12, 13). SEM images of the positive control group (2.5% NaOCl + 2% CHX) showed a larger precipitate formation at the coronal third. These results are in agreement with the findings of Bui et al. (11), which found no significant difference between control and experimental groups in the obliteration of apical dentinal tubules. This fact can be explained by the greater difficulty of irrigation at the apical third, which perhaps causes the irrigating solutions do not mix at this region.

According to the results, it can be concluded that QMix® is an alternative to assist in disinfection and smear layer removal from the root canal walls and its concomitant use with NaOCl should not cause concern.

Acknowledgements: The authors deny any conflicts of interest related to this study.

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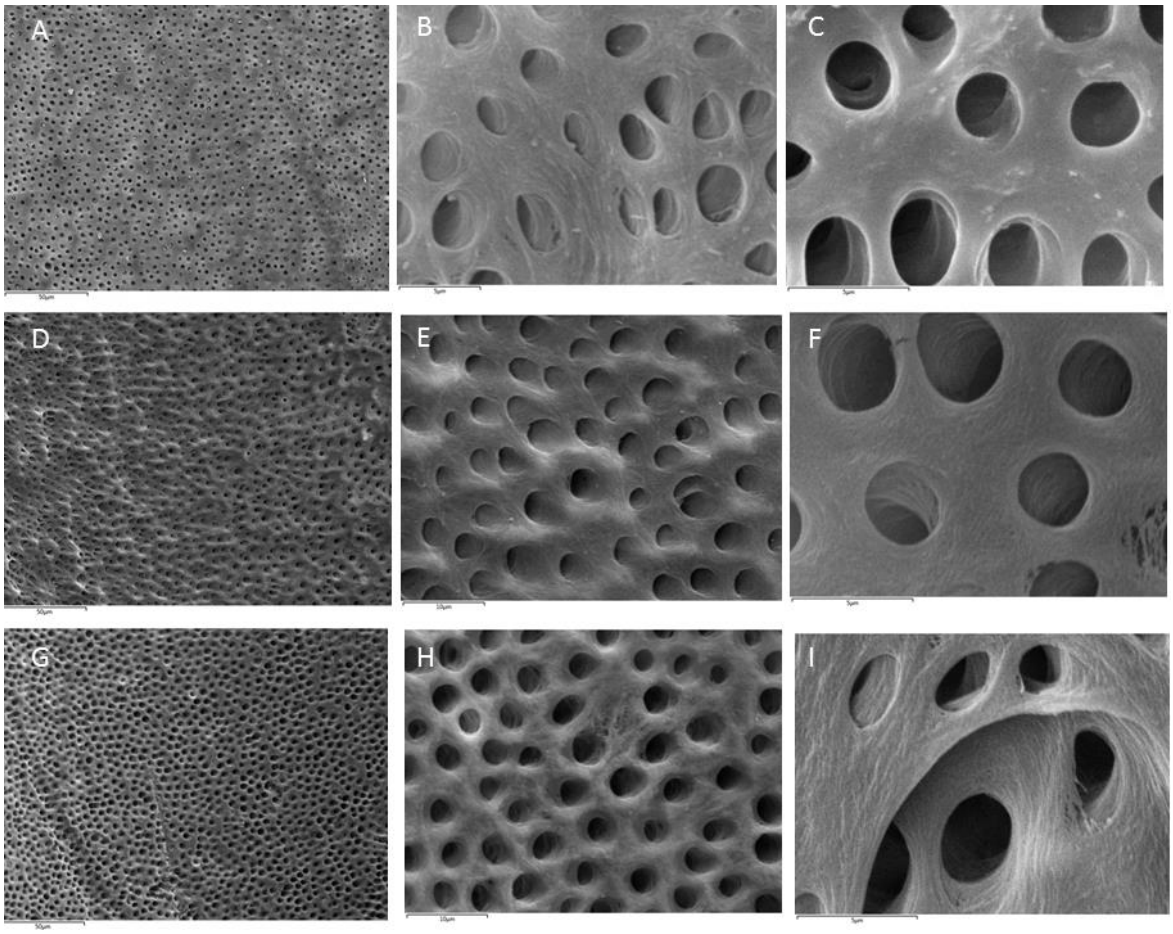

Figure 1. SEM images of samples irrigated with NaOCl and QMix®: no precipitate formation. A, B and C: cervical third at 1,000X, 5,000X and 15,000X respectively; D, E and F: medium third at 1,000X, 5,000X and 15,000X respectively; G, H and I: apical third at 1,000X, 5,000X and 15,000X respectively.

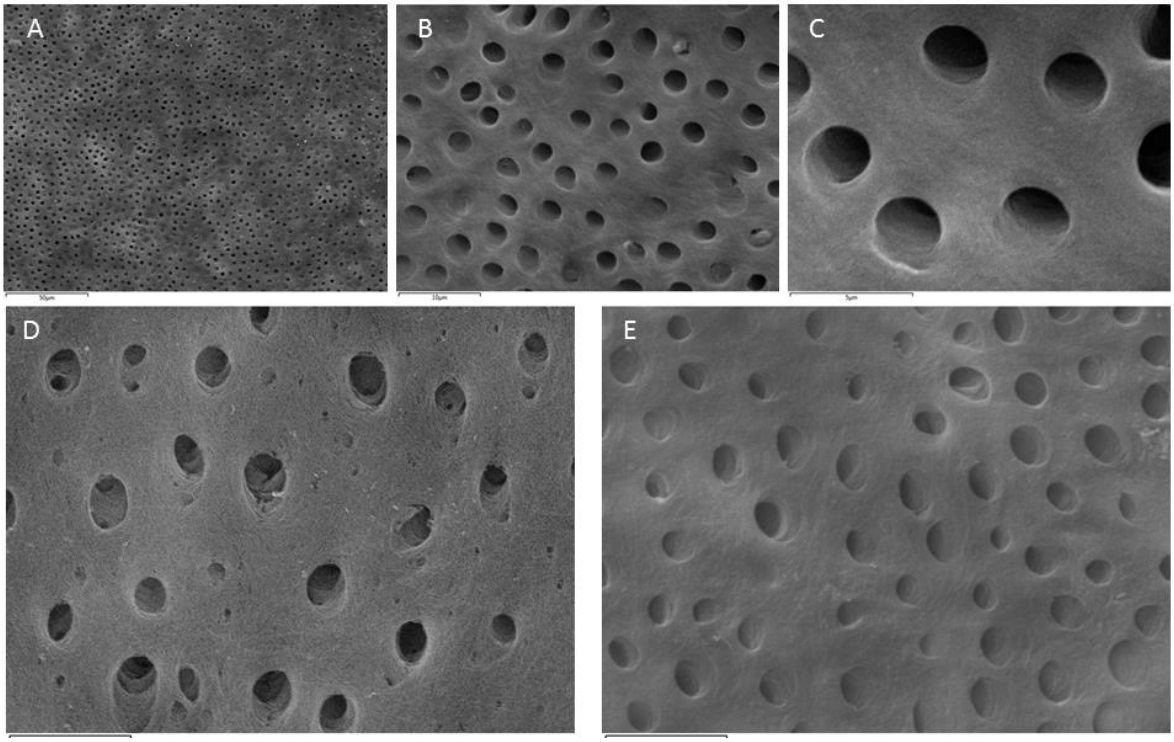

Figure 2. SEM images of samples irrigated with NaOCl, distilled water and QMix®: no precipitate formation. A, B and C: cervical third at 1,000X, 5,000X and 15,000X respectively; D: medium third at 5,000X; E: apical third at 5,000X.

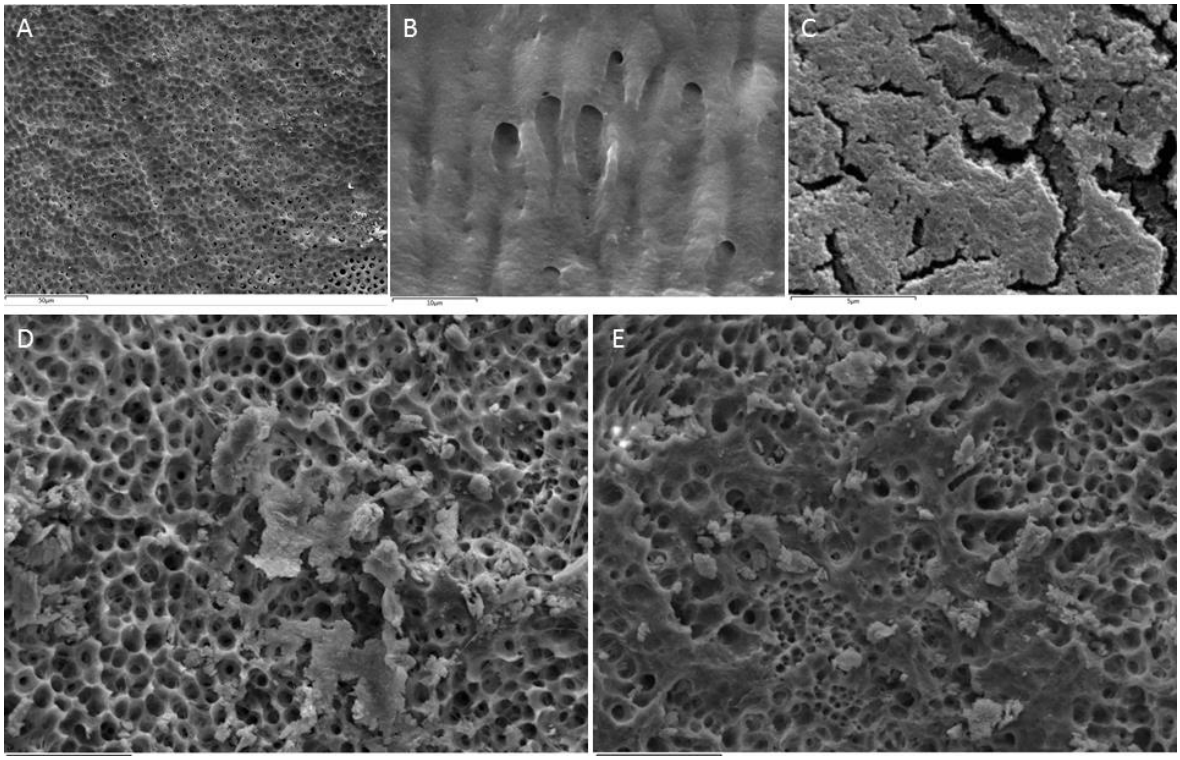

Figure 3. SEM images of samples irrigated with NaOCl and CHX: precipitate formation occluding dentinal tubules, especially at the cervical third. A, B and C: cervical third at 1,000X, 5,000X and 15,000X respectively; D: medium third at 1,000X; E: apical third at 1,000X.

5 DISCUSSÃO GERAL

O tratamento endodôntico visa à eliminação de patógenos do interior do canal radicular. Apesar da significativa evolução das técnicas e instrumentos endodônticos nos últimos anos, devido ao pequeno diâmetro do canal e suas ramificações, existe uma grande dificuldade de alcançar uma completa desinfecção do sistema de canais radiculares. Dentre os procedimentos utilizados no controle da infecção endodôntica, a irrigação pode exercer um papel fundamental na eliminação destes microrganismos do canal radicular. Os procedimentos de limpeza e desinfecção intracanal são dependentes dos efeitos químicos (JEANSONNE e WHITE, 1994) e mecânicos (GOMES et al., 2009) da solução irrigadora. Porém, é sabido que nenhuma solução irrigadora preenche todos os requisitos necessários para um adequado tratamento endodôntico. Frente a este apontamento, algumas associações começam a ser testadas com o intuito de preencher esta lacuna. Neste sentido, o QMix surge como uma alternativa para limpeza e desinfecção do sistema de canais radiculares, auxiliando na irrigação final do canal. A sua composição química promete unir, em um só produto, a capacidade antimicrobiana e a substantividade alcançadas com a clorexidina, a remoção de *smear layer* do EDTA e a baixa tensão superficial do detergente. Em estudo prévio (STOJICIC et al., 2011), a habilidade de remoção de *smear layer* do QMix foi comparável à do EDTA.

Apesar da combinação de irrigantes favorecer sua ação, possíveis reações químicas merecem ser consideradas. Sabe-se que a clorexidina não pode ser combinada com altas concentrações de EDTA sem que ocorra a formação de precipitado. De acordo com o fabricante, o QMix superou este problema, mantendo uma efetividade satisfatória de ambas as soluções. Isto pode ser explicado pelo método como o produto é fabricado. A clorexidina é primeiramente misturada ao detergente, para posteriormente ser adicionado o EDTA. Este método protegeria a solução da formação do precipitado (US PATENT PUBLICATION).

Estudos avaliando a microbiota de canais radiculares com polpa necrosada comprovam a presença de bactérias gram-negativas (SCHEIN e SCHILDER, 1975). Estas, durante a multiplicação e morte bacteriana, liberam endotoxinas (lipopolissacarídeos – LPS), substâncias importantes na indução e perpetuação da inflamação periapical. Sendo assim, a eliminação destas endotoxinas do interior do canal radicular é de fundamental importância para o sucesso da terapia endodôntica. Entretanto, torna-se difícil reproduzir este ambiente complexo e rico em laboratório. Assim, a HIMA (Health Industry Manufacturers Association) nos Estados Unidos escolheu *E. coli* 055:B5 como padrão de referência de endotoxina após conduzir um estudo colaborativo entre os laboratórios que realizavam teste de pirogênio em coelhos nos Estados Unidos (FUKUMORI, 2008).

O procedimento utilizado para a detecção de endotoxinas foi o Lisado de Amebócito de *Limulus* (LAL). Este teste é referenciado como o mais recomendado para quantificar a presença de endotoxinas. O princípio biológico do teste do LAL decorre da coagulação do sangue isolado de um caranguejo denominado *Limulus polyphemus* (figura 5 – Anexo A) (LOPES, 2010). Esta coagulação é causada pela ativação, na presença de LPS, de uma série de enzimas localizadas em células sanguíneas (amebócitos) do *Limulus* (SIGNORETTI, 2009).

O teste cinético turbidimétrico utilizando LAL é um teste quantitativo na determinação da concentração de LPS. É capaz de medir por espectrofotometria o aumento da turvação (densidade ótica) que precede a formação do coágulo gelatinoso quando o LAL encontra-se na presença de LPS. As concentrações de LPS presentes nas amostras são calculadas a partir do tempo de reação de cada amostra, por comparação ao tempo de reação de soluções contendo quantidades conhecidas de padrão de LPS, utilizadas na construção da curva padrão. Este tempo de reação é inversamente proporcional à quantidade de endotoxina presente (LOPES, 2010; LONZA MANUAL PRODUCT INSTRUCTIONS).

Alguns produtos podem provocar a inibição ou potencialização da reação. Como uma maneira de controlar possíveis interferências nos resultados, no presente estudo foi incluído um controle positivo do produto (PPC), ou seja, cada amostra foi contaminada com uma quantidade conhecida de LPS. A amostra contaminada (PPC) foi testada juntamente com a amostra não contaminada. De acordo com os protocolos recomendados pelo fabricante, a endotoxina recuperada deve ser igual à concentração conhecida no PPC dentro de uma faixa de 50% a 200% (LONZA MANUAL PRODUCT INSTRUCTIONS), sendo tal recomendação seguida no estudo. Quando o resultado demonstrou interferência na reação, a amostra foi diluída até o ponto em que não ocorressem mais interferências.

Estudos têm demonstrado que tanto o NaOCl quanto a CHX não são efetivos na remoção de endotoxinas do interior do canal radicular (GOMES et al., 2009), o que está de acordo com os achados do primeiro estudo da presente tese. Por outro lado, tais achados demonstraram que o QMix reduziu os níveis de LPS quando comparado às outras soluções irrigadoras testadas. Este efeito pode ser explicado pela presença de detergente e EDTA. O EDTA expõe as camadas mais internas de dentina contaminada, além da possibilidade de ligar-se ao cálcio presente no lipídio A da estrutura da endotoxina (BURTON e CARTER, 1964), liberando-a das paredes dentinárias. O detergente por sua vez, parece emulsificar a endotoxina, favorecendo a ação mecânica de remoção exercida pelo irrigante (NASSER e MOGHAZY, 2011). Além disso, a cetrimida, por ser um detergente catiônico, interage com a endotoxina, que possui carga negativa (MAGALHÃES et al., 2007). Ainda nesse sentido, ligações apolares ocorrem entre o Lipídio A da endotoxina e o detergente (MAGALHÃES et al., 2007). Desta forma, as características de carga e a interação do detergente com a endotoxina parecem ser importantes na redução dos níveis de LPS.

Por outro lado, o uso combinado de diversas substâncias deve pressupor a ausência de formação de produtos tóxicos ou que interfiram negativamente no resultado do tratamento

endodôntico. O QMix, por se tratar de um produto para irrigação final do canal, na maioria das vezes, será utilizado após o preparo químico mecânico com hipoclorito de sódio, irrigante amplamente utilizado pela maioria dos profissionais. Porém, é sabido que a utilização concomitante do hipoclorito de sódio com a clorexidina, um dos componentes do QMix, causa uma reação ácido-base a qual resulta na formação de um precipitado que, além de ser tóxico, pode obliterar os túbulos dentinários, reduzindo a permeabilidade dentinária. Até o momento, a avaliação do resultado da associação entre QMix e NaOCl não havia sido explorado.

A análise das paredes do canal em Microscopia Eletrônica de Varredura (MEV) permite a avaliação ultraestrutural da limpeza da superfície de dentina após diferentes protocolos de instrumentação ou irrigação. Além disso, possibilita a visualização da quantidade e distribuição da *smear layer* nas paredes do canal, e por isso, tornou-se um método comumente utilizado na literatura para este fim (AKISUE et al., 2010; GASIC et al., 2012; PRADO et al., 2013).

Após análise em MEV, os resultados do segundo estudo da presente tese demonstraram que, apesar da presença de CHX na composição do QMix, seu uso após o preparo químico mecânico do canal com NaOCl, não resultou na formação de precipitado sobre as paredes do canal. Muito pelo contrário, as imagens apresentaram paredes limpas com túbulos dentinários expostos. Nossos resultados confirmam pela primeira vez a afirmação feita por STOJICIC et al. (2011) de que, apesar da presença de clorexidina na formulação do QMix, sua combinação com NaOCl não resulta na formação de precipitado, apesar de não terem apresentado nenhum estudo específico até então.

Por se tratar de um produto novo, ainda existem poucos estudos sobre o QMix. Porém, de acordo com os resultados apresentados na presente tese, pode-se concluir que o QMix é

eficaz na eliminação de endotoxinas do interior do canal radicular, e seu uso concomitante com NaOCl não resulta na formação de precipitado sobre as paredes do canal.

Frente aos resultados encontrados nos presentes estudos, levando-se em consideração também os resultados descritos na literatura apresentada, a presente tese sugere que o QMix surge como uma alternativa para otimizar a irrigação do canal radicular, podendo ser utilizado concomitantemente com o hipoclorito de sódio, contribuindo para que se obtenha uma maior limpeza e descontaminação do canal, favorecendo o sucesso.

6 CONCLUSÕES

A partir dos resultados do presente estudo pode-se concluir:

1. A ação química do NaOCl, CHX e EDTA não foi capaz de reduzir os níveis de LPS do sistema de canais radiculares.

2. O QMix foi eficaz na eliminação de endotoxinas do interior do canal radicular.

3. O uso concomitante de NaOCl e QMix não resulta na formação de precipitado sobre as paredes do canal.

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8 ANEXOS

8.1 ANEXO A – Figuras

Terço cervical Terço médio Terço apical

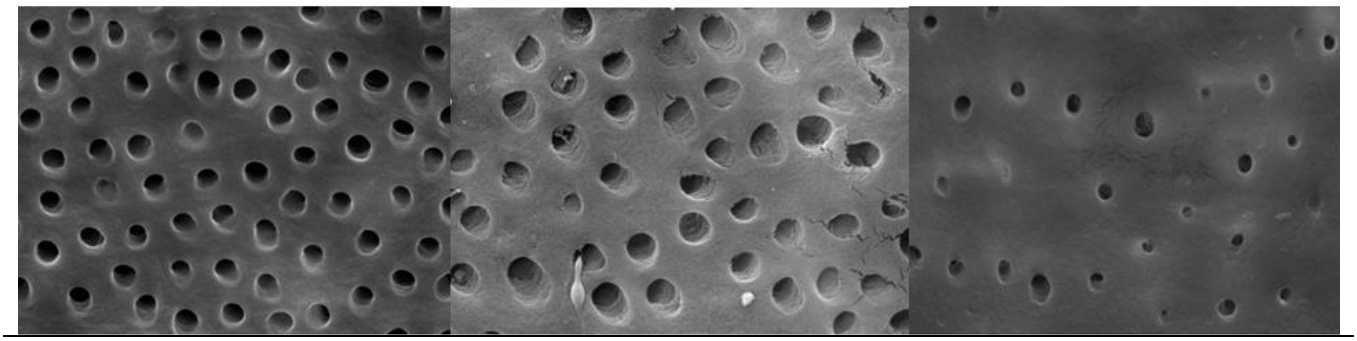

Figura 1 – Grupo controle negativo (5000x).

Terço cervical Terço médio Terço apical

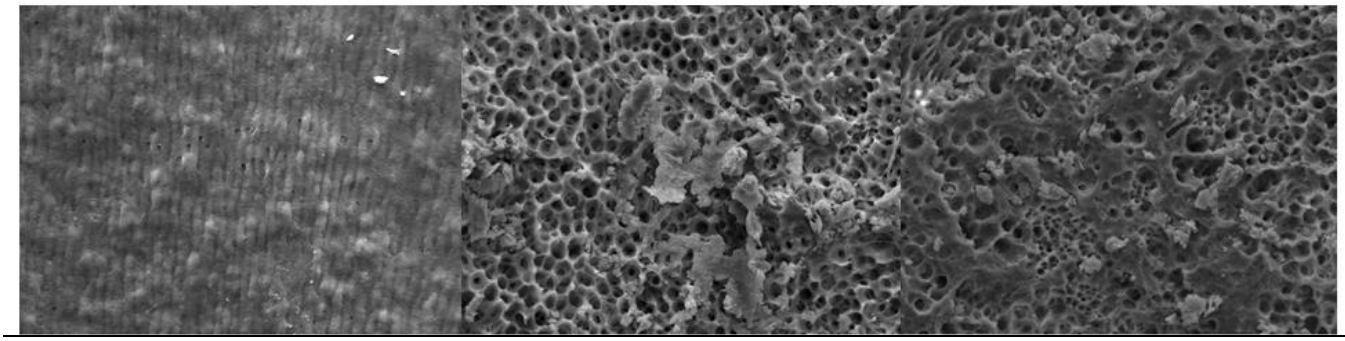

Figura 2 – Grupo controle positivo (1000x).

Terço cervical Terço médio Terço apical

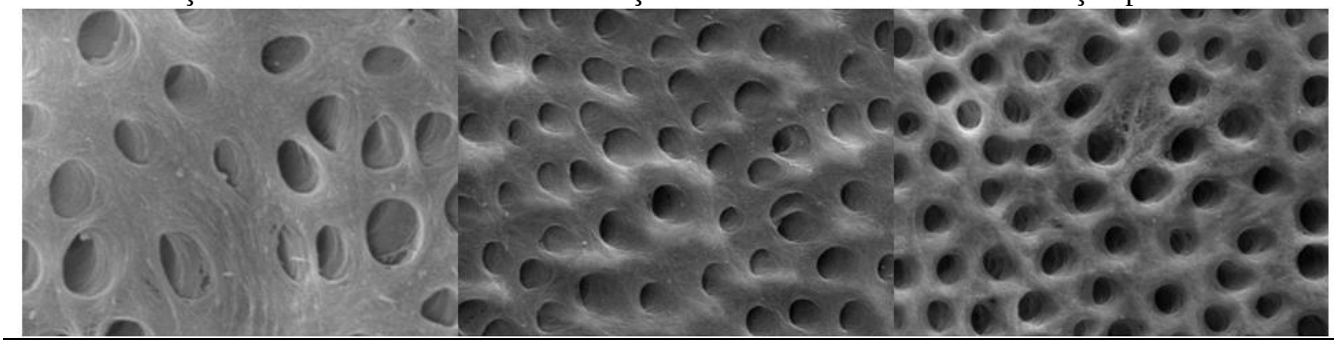

Figura 3 – NaOCl + QMix (5000x).

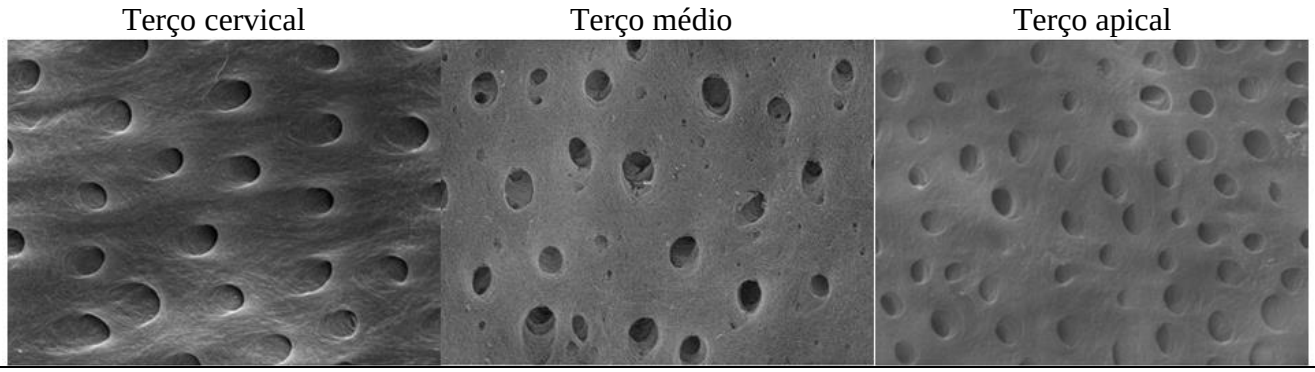

Figura 4 – NaOCl + H_2O + QMix (5000x).

Figura 5: Caranguejo Ferradura (*Limulus polyphemus*): normalmente encontrado no Golfo do México e ao longo das costas do Atlântico Norte (LOPES, 2010).

8.2 ANEXO B - Cartas de submissão dos artigos

CLOI: Submission Confirmation for QMix® irrigant reduces lipopolysacharide (LPS) levels in an in vitro model

Entrada x

Clinical Oral Investigations <no-reply@editorialmanager.com> 14:26 (Há 1 hora)

para mim

inglês › português Traduzir mensagem Desativar para: inglês ×

Dear Mrs Grundling,

Your submission entitled "QMix® irrigant reduces lipopolysacharide (LPS) levels in an in vitro model" has been received by Clinical Oral Investigations

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is http://cloi.edmgr.com/. Alternatively, please call us at 001-630-468-7784 (outside the US)/(630)-468-7784 (within the US) anytime from Monday to Friday.

Your manuscript will be given a reference number once an Editor has been assigned.

Thank you for submitting your work to our journal.

Kind regards,

Editorial Office
Clinical Oral Investigations

Elsevier Editorial System(tm) for Journal of Endodontics
Manuscript Draft

Manuscript Number:

Title: Effect of the Combination of Sodium Hypochlorite and QMix®: Scanning Electron Microscopy Precipitate Observation

Article Type: Basic Research - Technology

Keywords: chlorhexidine; QMix; SEM; sodium hypochlorite.

Corresponding Author: Roberta Scarparo, MD; PhD

Corresponding Author's Institution: PUCRS

First Author: Grasiela L Grundling, MsC

Order of Authors: Grasiela L Grundling, MsC; Renata D Morgental, PhD; Roberta Scarparo, MD; PhD; Fabiana V Vier-Pelisser, PhD

Manuscript Region of Origin: Latin & South America

Abstract: Introduction: The purpose of this study was to observe, under scanning electron microscopy (SEM), the potential formation of a precipitate on the root canals walls due to the combined use of sodium hypochlorite (NaOCl) and QMix®. Methods: Sixteen single-rooted human teeth were selected. After instrumentation, the root canal surfaces were conditioned for smear layer removal using 15% citric acid solution under ultrasonic activation and a final wash with distilled water. The specimens were randomly divided into four groups as follows: negative control (no irrigation); positive control (2.5% NaOCl + 2% CHX); 2.5% NaOCl + QMix®; 2.5% NaOCl + distilled water + QMix®. All roots were split in half and each third was evaluated by SEM at 1,000X, 5,000X and 15,000X. Results: There was no precipitate formation when QMix® was used after NaOCl, regardless of the use of distilled water between the two solutions. The positive control group showed precipitate formation occluding dentinal tubules, especially at the cervical third, while the negative control showed a clean surface with permeable dentinal tubules. Conclusions: The use of QMix® for removing smear layer after chemicomechanical preparation with NaOCl causes no precipitate formation on the root canal walls.

8.3 ANEXO C - Cartas de aprovação dos comitês

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL - PUC/RS

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: QMIX: AÇÃO SOBRE ENDOTOXINA BACTERIANA E EFEITO DA COMBINAÇÃO COM HIPOCLORITO DE SÓDIO SOBRE AS PAREDES DO CANAL RADICULAR

Pesquisador: FABIANA VIEIRA VIER PELISSER

Área Temática: Área 3. Fármacos, medicamentos, vacinas e testes diagnósticos novos (fases I, II e III) ou não registrados no país (ainda que fase IV), ou quando a pesquisa for referente a seu uso com modalidades, indicações, doses ou vias de administração diferentes daquelas estabelecidas, incluindo seu emprego em combinações.

Versão: 2

CAAE: 16664813.7.0000.5336

Instituição Proponente: UNIAO BRASILEIRA DE EDUCACAO E ASSISTENCIA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 310.698

Data da Relatoria: 20/06/2013

Apresentação do Projeto:

vide considerações finais

Objetivo da Pesquisa:

vide considerações finais

Avaliação dos Riscos e Benefícios:

vide considerações finais

Comentários e Considerações sobre a Pesquisa:

vide considerações finais

Considerações sobre os Termos de apresentação obrigatória:

vide considerações finais

Recomendações:

vide considerações finais

Conclusões ou Pendências e Lista de Inadequações:

pendência atendida. Pela aprovação

Endereço: Av.Ipiranga, 6681
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UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3320-3345 **Fax:** (51)3320-3345 **E-mail:** cep@pucrs.br

Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 24 de abril de 2013

O Projeto de: Tese

Protocolado sob nº: 0017/13

Intitulado: Qmix: ação sobre endotoxina bacteriana e efeito da combinação com hipoclorito de sódio sobre as paredes do canal radicular.

Pesquisador Responsável: Profa. Dra. Fabiana Vieira Vier Pelisser

Pesquisadores Associados: Grasiela Sabrina Longhi Gründling; Francisco Montagner; Roberta Scarparo

Nível: Tese / Doutorado

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *24 de abril de 2013.*

Este projeto deverá ser imediatamente encaminhado ao CEP/PUCRS.

Profa. Dra. Luciane Macedo de Menezes
Coordenadora da Comissão Científica e de Ética da
Faculdade de Odontologia da PUCRS

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