PONTIFICIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM ENDODONTIA

ANÁLISE *IN VITRO* DA DESINFECÇÃO PROMOVIDA POR DIFERENTES PROTOCOLOS DE LIMPEZA DO CANAL RADICULAR COM O USO DO ULTRASSOM EM DENTES HUMANOS INFECTADOS POR *ENTEROCOCCUS FAECALIS*

Kathrein Tapia da Silva

PONTIFICIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM ENDODONTIA

ANÁLISE *IN VITRO* DA DESINFECÇÃO PROMOVIDA POR DIFERENTES PROTOCOLOS DE LIMPEZA DO CANAL RADICULAR COM O USO DO ULTRASSOM EM DENTES HUMANOS INFECTADOS POR *ENTEROCOCCUS FAECALIS*

KATHREIN TAPIA DA SILVA

Dissertação apresentada como parte dos requisitos obrigatórios para a obtenção do título de mestre em Odontologia, área de concentração em Endodontia, pela Pontifícia Universidade Católica do Rio Grande do Sul.

Linha de Pesquisa: Etiopatogênese e Tratamento das Doenças Periodontais e Periapicais

Orientador: Prof. Dr. José Antônio Poli de Figueiredo

Porto Alegre

2011

À minha mãe Rejane Tapia da Silva, pelo amor,
dedicação, estímulo e apoio recebido em todos os
momentos da minha vida e pelo seu exemplo
de mulher competente e vitoriosa.

“ Mestre não é quem sempre ensina
mas quem de repente aprende.”

João Guimarães Rosa

AGRADECIMENTOS

Aos meus pais Rejane Tapia da Silva e Moisés Ortiz da Silva, por seu amor e compreensão mas principalmente por acreditarem nos meus sonhos e se esforçarem para torná-los realidade.

Aos meus tios Rosane Tapia Schenhofen e Juarez Schenhofen por seu carinho, amor e apoio para que eu consiga concluir mais etapa.

A minha irmã Luciane Dias Beck e minha avó Gecy Pacheco Tapia por suas palavras amigas sempre prontas a me acolher e confortar.

A minha irmã e amiga Stéphani Tapia da Silva por seu empenho em me ajudar sempre que fosse preciso.

Aos meus amigos Ramiro Estacia da Silveira, Káren Guenter, Paula Lawal, Lia Ibaldo, Larissa Segabinazzi, Mônica Benatti, Daniel Longhi, Letícia Benin por vibrarem comigo a cada conquista e me darem coragem de enfrentar os obstáculos.

Ao meu namorado e amigo Renato Perlin de Cesaro por me apoiar a seguir em frente e me dar todo carinho necessário para que eu conseguisse concluir a pesquisa.

Aos meus colegas de mestrado pelo convívio maravilhoso que tivemos e pelas trocas de experiências vivenciadas dia a dia na PUCRS. Um agradecimento especial aos meus colegas e amigos Matheus Souza, Janaína Zechin e Grasiela Gundling por seu auxilio fundamental para a conclusão da pesquisa.

Aos professores do Programa de Pós – Graduação Strictu Sensu da Odontologia da PUCRS Profª Drª Maria Martha Campos, Profª Drª Fabiana Vier, Profº Drº João Batista Blessmann Weber, Profº Drº Eraldo Luiz Batista Júnior e Profº Drº José Antônio Poli de Figueiredo por contribuírem tanto para o meu crescimento profissional como para o meu crescimento pessoal.

Aos meus professores de graduação Profº Alexandre Azevedo Salles e Profº Luis Eduardo Irala pelos ensinamentos transmitidos e pela oportunidade de convívio e amizade ao longo da minha vida profissional.

A Profª Sílvia Dias do Laboratório de Imunologia e Microbiologia da PUCRS, por sua paciência e dedicação com a minha pesquisa.

Ao meu querido orientador Profº José Antônio Poli de Figueiredo pelo teu talento e competência, agradeço pela maneira simples e gentil de transmitir conhecimentos e mostrar o caminho a seguir.

RESUMO

Introdução: O objetivo deste estudo foi analisar, *in vitro*, a ação do ultrassom sobre os agentes antimicrobianos (hipoclorito de sódio 2,5% e clorexidina 2%) em dentes humanos infectados com *Enterococcus faecalis*. **Metodologia:** Foram utilizados 60 pré-molares humanos, os quais foram contaminados com uma cultura pura de *E. faecalis* com uma variável de 3.9 a 6.3 x 10^7(CFU/ml) por um período de 60 dias para formação do biofilme bacteriano. Os dentes foram divididos aleatoriamente em 8 grupos sendo que os grupos que fizeram uso de ultrassom tiveram um n= 10 e os grupos grupos controle (sem ultrassom) um n=5. Grupo 1- irrigação ultrassônica com hipoclorito de sódio 2,5%; grupo 2- irrigação ultrassônica com clorexidina líquida 2%; grupo 3- irrigação ultrassônica com clorexidina gel 2%; grupo 4- irrigação ultrassônica com água destilada; grupo 5- irrigação convencional com hipoclorito de sódio 2,5%; grupo 6- irrigação convencional com clorexidina líquida 2%; grupo 7- irrigação convencional com clorexidina gel 2% e grupo 8- irrigação convencional com água destilada. Foi realizada análise em Microscópio Eletrônico de Varredura (MEV). **Resultados:** Na análise em MEV em um aumento de 2000x em *backscattering*, analisou-se a remoção da *smear layer* e houve diferença significativa entre os grupos em todos os terços. Os grupos 1 e 2 apresentaram uma melhor capacidade de limpeza e foram similares em seus resultados. O grupo que apresentou um pior desempenho foi o grupo 7. No aumento de 10000X em *backscattering*, analisou- se a presença de bactéria no canal radicular e novamente houve diferença estatística entre os grupos em todos os terços. Os grupos que apresentaram menor biofilme bacteriano foram os grupos 1 e 2 e foram similares em seus resultados. O grupo que apresentou um pior desempenho no terço apical foi o grupo 8 e nos terços médio e cervical o grupo 7. **Conclusões:** Independente da solução, irrigação ultrassônica mostrou-se superior à irrigação convencional, indicando que o ultrassom deve ser utilizado como um excelente auxiliar na limpeza e desinfecção do canal radicular.

Palavras - chaves: *Enterococcus faecalis*, passive ultrasonic irrigation, scanning electron microscopy, sodium hypochlorite, chlorhexidine.

SUMÁRIO

INTRODUÇÃO GERAL

A permanência de microorganismos no sistema de canais é a principal causa de insucesso do tratamento endodôntico. Os patógenos mais encontrados nestes casos são as bactérias anaeróbias, se destacando o *Enterococcus faecalis*.

Devido à capacidade de sobrevivência na ausência de oxigênio e seu poder de recolonização em um curto período de tempo, o *E. faecalis*, se torna fonte de infecções persistentes. A necessidade de eliminar este microorganismo faz com que se busque um agente antimicrobiano capaz de combatê-lo.

Os agentes antimicrobianos utilizados com maior freqüência durante o preparo radicular são o hipoclorito de sódio e a clorexidina.

O hipoclorito de sódio é a solução mais utilizada durante o preparo químico - mecânico devido às suas propriedades de dissolver matéria orgânica e atividade antimicrobiana.

A clorexidina 2%, tanto na forma líquida como em gel, tem - se mostrado ser um antimicrobiano de amplo espectro além de ser compatível com os tecidos periapicais.

Devido à anatomia complexa do sistema de canais, a irrigação é uma etapa essencial do tratamento endodôntico, pois permite a limpeza do canal em locais onde o instrumento não atinge. A permanência de bactérias no interior do canal radicular, mesmo após o preparo, faz com que se busque um método de irrigação que potencialize a ação dos agentes antimicrobianos, como é o caso do ultrassom.

Os dispositivos ultrassônicos foram primeiramente introduzidos na endodontia por Richman em 1957 para ativação de instrumentos endodônticos. Por outro lado, o ultrassom tornou-se mais efetivo como um auxílio para irrigar e desinfectar o canal

radicular (Cheung & Stock, 1993; Huque et al., 1998; Spoleti et al.,2002; Van der Sluis et al., 2007; Jiang et al.,2010; Van der Sluis et al., 2010).

Na literatura, a irrigação ultrassônica tem sido descrita de duas formas: uma em que a irrigação é realizada simultaneamente com a instrumentação ultrassônica (UI) e outra sem a instrumentação simultânea, chamada de passiva (PUI). Durante a irrigação e instrumentação ultrassônica, o instrumento é levado de encontro a parede do canal sendo menos efetivo na remoção de tecidos pulpares e *smear layer* do canal radicular do que na irrigação passiva. A irrigação passiva foi primeiramente descrita por Weller et al., em 1980 e, consiste em ativar a solução irrigadora, com auxilio de um instrumento de pequeno calibre #15 ou #20 , após o preparo. Com o preparo concluído, o instrumento pode - se mover livremente permitindo que o irrigante penetre mais facilmente e se obtenha um maior saneamento (Weller et al., 1980; Van der Sluis et al., 2007).

Somente o preparo mecânico do canal radicular não é suficiente para a eliminação de microorganismos por ser um sistema com uma anatomia complexa, que faz com que algumas áreas não sejam atingidas durante a instrumentação. Isto faz com que se busquem soluções irrigadoras que além de atingir essas áreas consigam eliminar as bactérias impedindo que haja recolonização (Huque et al., 1998; Sedgley et al., 2006; Stuart et al., 2006; Dugan & Sedgley, 2007).

Este estudo busca testar a hipótese de que o ultrassom potencializa a ação de agentes antimicrobianos (NaOCl 2,5% e Clorexidina 2%) sobre o *E. faecalis*, comparado ao uso isolado desses irrigantes.

Artigo

IN VITRO ANALYSIS OF THE DISINFECTION PROMOTED BY DIFFERENT CLEANING PROTOCOLS OF THE ROOT CANAL USING ULTRASOUND IN HUMAN TEETH INFECTED BY *ENTEROCOCCUS FAECALIS*

Manuscrito enviado ao periódico Journal of Endodontics, fator de impacto 3.291

IN VITRO ANALYSIS OF THE DISINFECTION PROMOTED BY DIFFERENT CLEANING PROTOCOLS OF THE ROOT CANAL USING ULTRASOUND IN HUMAN TEETH INFECTED BY *ENTEROCOCCUS FAECALIS*

Kathrein Tapia da Silva[1]; Nathiele Boeno[2]; Sílvia Dias de Oliveira[2]; José Antônio Poli de Figueiredo[3]

1 Post-Graduate Program in Dentistry of the Pontifical Catholic University of Rio Grande do Sul, Master's Degree student in Endodontics, Pontifical Catholic University of Rio Grande do Sul - PUCRS, Porto Alegre, Brazil.

2 Immunology and Microbiology Laboratory, School of Biological Sciences, Pontifical Catholic University of Rio Grande do Sul - PUCRS, Porto Alegre, Brazil.

3 Post-Graduate Program in Dentistry Coordinator, Pontifical Catholic University of Rio Grande do Sul - PUCRS, Porto Alegre, Brazil.

Correspondence to:

José Antônio Poli de Figueiredo

Post-Graduate Program in Dentistry

Pontifical Catholic University of Rio Grande do Sul - PUCRS

Corresponding address:

Av. Ipiranga 6681 Prédio 6 sala 206

CEP 90619-900 Porto Alegre – RS – Brazil

Telephone: 55 51 3320 3500 ext. 4307

ABSTRACT

Introduction: This study aims at analyzing, *in vitro*, the ultrasonic action on microbial agents (sodium hypochlorite 2.5% and chlorhexidine 2%) in human teeth infected by *enterococcus faecalis*. **Methodology:** Sixty human premolars were used, which were contaminated with an *E. faecalis* pure culture with 3.9 to 6.3 x 10^7(CFU/ml) during a period of 60 days to form bacterial biofilm. Teeth were randomly divided in 8 groups, whereby those in which ultrasonic was used had a n= 10, the control groups (without ultrasound) had a n=5. Group 1- ultrasonic irrigation with sodium hypochlorite 2.5%; group 2- ultrasonic irrigation with chlorhexidine liquid 2%; group 3- ultrasonic irrigation with chlorhexidine gel 2%; group 4- ultrasonic irrigation with distilled water; group 5- conventional irrigation with sodium hypochlorite 2.5%; group 6- conventional irrigation with chlorhexidine liquid 2%; group 7- conventional irrigation with chlorhexidine gel 2% and group 8- conventional irrigation with distilled water. An analysis with a scanning electron microscopy (SEM) was performed. **Results:** In the SEM analysis with a 2000x magnification in *backscattering*, it was analyzed the removal of the *smear layer* and there was a significant difference among the groups in all the root portions. Groups 1 and 2 showed better cleaning capacity and were similar in their results. The group which showed the worst performance was group 7. In the 10000X magnfication in *backscattering* it was analyzed the presence of bacteria in the root canal and there was again statistic difference among the groups in all the root portions. The groups which showed the smallest bacterial biofilm were groups 1 and 2, and they were similar in their results. The group which showed the worst performance in the apical third was group 8, and in the medium and cervical third group 7. **Conclusion:** Regardless of the solution, ultrasonic irrigation proved to be superior to the conventional irrigation; we believe that ultrasonic can be used as an excellent auxiliary to the root canal cleaningness and disinfection.

Keywords: *Enterococcus faecalis,* passive ultrasonic irrigation, scanning electron microscopy, sodium hypochlorite, chlorhexidine.

INTRODUCTION

Endodontic treatment major goal in teeth with pulp necrosis is microorganism elimination; however, there are unsuccessful cases due to the permanence of canal system contamination. Even after treatment, failures can occur due to the presence of resistant bacteria, root canal anatomy and difficulty of instrumentation in the apical third (1,2,3).

Among the pathogens associated to treatment failure, the most frequent are anaerobic bacteria, especially *Enterococcus faecalis* (4). Enterococci are Gram - Positive cocci which can occur individually, in pairs, or in short chains. They are facultative anaerobic, usually found in the apical third, with the capacity of growing in presence or absence of oxygen (1, 5, 6, 7).

Enterococcus survival ability and its capacity of recolonization in a short period of time make it a source of persisting infections, which makes necessary to find antimicrobial agents capable of fighting it (2, 4, 8).

Sodium hypochlorite and chlorhexidine are the most studied antimicrobial agents and therefore used most frequently during root canal preparation (2, 9, 10, 11).

Sodium hypochlorite is the most frequently used solution in the chemical-mechanical preparation for being able to dissolve organic matter due to, its antimicrobial action, for helping lubrication in the canal, besides having low cytotoxicity when kept within the working length (10, 11, 12, 13, 14).

During a study carried out by Dunavant et al. 2006 (14) comparing the efficiency of irrigation solutions (NaOCl 6%, NaOCl 1%, Chlorhexidine 2%, REDTA, and Biopure MTDA) against *E. faecalis* biofilm, sodium hypochlorite was the most efficient, both in concentrations 6% of 1%, compared to the other solutions tested.

Chlorhexidine 2%, both liquid and gel, proved to be a broad spectrum antimicrobial which provides residual activity of up to 72 hours after instrumentation

(substantivity). In this concentration, it is used as a subgingival irrigator, being non-toxic to periodontum. It justifies its usage in endodontics, since it is compatible with periapical tissues (10, 11, 15, 16).

Viana et al. 2004 (17) demonstrated that chlorhexidine can inactivate resistant microorganisms within a contact period inferior to 15 seconds. Leonardo et al. 1999 (15) pointed out that chlorhexidine 2%, 48 hours after its use, continues active, performing a synergistic effect almost like an intracanal medication in areas inaccessible by instrumentation, or in areas with a possibility of reinfection or secondary infection, especially in dentinal tubules.

Due to the difficulty of bacteria elimination by root anatomy complexity, there have being carried out studies to come up with an irrigation method which potentizes antimicrobial agent action, as the case of ultrasonic. Other researchers have evaluated the efficiency of the irrigating solutions when used with syringe or activated by ultrasonics (18, 19, 20).

Equipments of ultrasonics origin work through an acoustic natural oscillation with frequency superior to 20.000Hz, inaudible to human ears; super sound. It activates the instruments through a continuous and transversal vibration. (21)

Ultrasonics usage in endodontics is widely spread both in endodontic instrument activation, suggested as a way to improve canal debridement and, becoming even more popular, and as an auxiliary to irrigate and disinfect the root canal (19, 20, 21, 22, 23, 24).

Ultrasonics can be used in irrigation in two ways described in literature: one in which irrigation is performed simultaneously with ultrasonics instrumentation, and another one without simultaneous instrumentation, called passive. During irrigation and ultrasonics instrumentation, the instrument is taken towards the canal wall, being less effective in the removal of pulp tissues and smear layer from the root canal than passive irrigation. In the passive irrigation, energy waves are transmitted through an instrument or even a nylon fiber to the irrigator present in the duct (24, 25).

It is known that only root duct mechanical preparation is not enough to eliminate microorganisms, since it is a system which present many accessory, secondary canals and a complex anatomy, causing some areas not to be reached during instrumentation. Thus, irrigating solutions have been searched which, besides reaching these areas, can also eliminate bacteria, preventing recolonization to happen (2, 4, 8, 19).

This study seeks to test the hypothesis that ultrasonics potentizes antimicrobial agent action (NaOCl 2.5% e Chlorhexidine 2%) on *E. faecalis*, compared to the isolated use of these irrigators.

MATERIALS AND METHODS

This study has been submitted to the Scientific and Ethics Committee of the School of Dentistry of the Pontifical Catholic University of Rio Grande do Sul – PUCRS, and approved under number CEP 10/05054.

Tooth acquisition and preparation

Sixty human mandibular premolars with only one root canal were used. After teeth acquisition, they were stored in distilled water until the beginning of the experiment, so as to preserve their properties. Teeth crowns were removed in a way that root remnant showed a length of about 15 to 18 mm; a file #10 and #15 (Dentsply, Maillefer - Ballaigues, Switzerland) was used to trespass apical foramen, which were stored overnight in sodium hypochlorite 2% (Biodynamics - Ibiporã, Brazil). Next day, root canal exploration with manual files K #8 or # 10 was performed; followed by, cervical preparation with LA Axxess burs (Sybron Endo - USA); working length was established from the introduction of a file in the canal until the tip trespassed the apical foramen, thereafter reducing 1 mm to set the real working length; the roots were instrumented with k-files (Dentsply Maileffer, Ballaigues - Switzerland) through crown-down technique and activated by an oscillating drill NSK TEP- E10R (Nakanishi inc., Japan); memory file was #35. Irrigation was performed with 1% sodium hypochlorite (Biodinâmica - Ibiporã, Brazil). Each tooth was fixed in an eppendorf tube 2ml (Genuine Axygen Quality – CA, USA) with cyanoacrylate (SuperBonder - SP, Brazil), so as it remained in vertical position, with the cervical portion facing up. A lateral

orifice was made in the eppendorf to allow culture medium renewal. Teeth were randomly divided in 8 groups, half in which ultrasonics was used (n= 10 for each gorup), and half served as control groups, without ultrasonics (n=5 for each group). A propylene box (Heathrow Scientific–Vernon hills, IL, USA) was used keep the samples of each group. The samples were sterilized in autoclave (Cristófoli – Campo Mourão, Paraná, Brazil) for 20 min.

Sterilization control

One tooth out of each group underwent sterilization control. After the sterilization of each propylene box containing the teeth, a cone of sterilized paper was introduced in the root canal of one of the tooth inside the box, and this cone was immediately inoculated in a tube with sterile saline solution 0.85%. The material was homogenized and, after 5 minutes, a 100 µL aliquot of the saline solution was plated, in duplicate, on the surface of blood agar, and incubated for 18 to 24 h, at a temperature of 37°C.

Cultivation and Preparation of Inoculum

The lineage used *E. faecalis* (ATCC 29212), was obtained and cultivated at the Immunology and Microbiology Laboratory of the School of Biological Sciences, at Pontifical Catholic University of Rio Grande do Sul - PUCRS, Porto Alegre, Brazil.

The bacteria were cultivated in BHI (*Brain Heart Infusion*) for 18 to 24 hours, at 37°C, in bacteriological incubator.

The number of the inoculum colony forming units (CFU/ml) was determined by counting the colonies in blood agar plates. For this prupose, *E. faecalis* cultivation was serially diluted until 10^{-9}, in saline solution 0.85%, and 100 µL of the dilutions 10^{-6}, 10^{-7} , 10^{-8} and 10^{-9} were plated on the surface of blood agar with the aid of a Drigalski loop, in duplicate. The plates were incubated at 37ºC for 24 hours and, following this period, it was performed CFU/mL counting of the plates which showed growth between 15 to 150 colonies. Results ranged from 3.9 to 6.3 x 10^{7}.

In each of the sixty previously sterilized samples, it was inoculated 100 µL of *E.*

faecalis for cultivation in the root canal. Following this procedure, it was added the sterile BHI in the micro tube, in a way that it became completely filled with the culture medium. *E. faecalis* cultivation was kept for 60 days for the biofilm to be formed, with the renewal of one third of the BHI volume each 48 hours. Once a week, one aliquot of the BHI taken from the teeth was submitted to Gram test and cultivated in blood agar, followed by catalase and aesculin tests, to verify the absence of external contamination. Each teeth manipulation was performed in aseptic conditions, in laminar flux hood.

Group classification

The roots were assembled on a utility wax (Wilson, Polidental – Cotia, SP) to prevent irrigating substance spill (Panvel PUCRS – Porto Alegre, RS). Group distribution was made as follows:

Group 1 (NaOCl-U) – the auxiliary chemical substance used in this group was sodium 2.5% hypochlorite, agitated with a k-file #15 (Dentsply Maileffer, Ballaigues - Switzerland) during 15 seconds. After that, root canal was filled again with the chemical substance and passive irrigation with ultrasonics was performed during 10 seconds; following this period, the solution was aspirated and renewed and a new irrigation cycle performed. Four irrigation cycles were performed altogether. A 5 ml disposable syringe (BD Brazil – São Paulo, SP) was used to its insertion with 25x0.6 hypodermic needles (Injex Indústria Cirúrgica – Ourinhos, SP).

Group 2 (CHXL-U) – the same root canal mechanical preparation protocol performed in group 1 was adopted for this group, changing the auxiliary chemical substance (2% liquid Chlorhexidine). A 5 ml disposable syringe (BD Brazil – São Paulo, SP) was used to its insertion with 25x0.6 hypodermic needles (Injex Indústria Cirúrgica – Ourinhos, SP).

Group 3 (CHXG-U) - the same root canal mechanical preparation protocol performed in group 1 was adopted for this group, changing the auxiliary chemical substance (2% Chlorhexidine gel). A 3 ml disposable syringe (BD Brazil – São Paulo, SP) was used to its insertion with hypodermic needles and, for its removal, it was

performed irrigation with saline 0.9% using 5 ml disposable syringe (BD Brazil – São Paulo, SP) with 25x0.6 hypodermic needles (Injex Indústria Cirúrgica – Ourinhos, SP).

Group 4 (DW-U) - the same root canal mechanical preparation protocol performed in group 1 was adopted for this group, changing the auxiliary chemical substance (distilled water). A 5 ml disposable syringe (BD Brazil – São Paulo, SP) was used to its insertion with 25x0.6 hypodermic needles(Injex Indústria Cirúrgica – Ourinhos, SP).

Group 5 (NaOCl) – the same mechanical preparation protocol performed in group 1 was adopted for this group, however without the use of ultrasonics, and the auxiliary chemical substance used was sodium hypochlorite 2.5%. A 5 ml disposable syringe (BD Brazil – São Paulo, SP) was used to its insertion with 25x0.6 hypodermic needles (Injex Indústria Cirúrgica – Ourinhos, SP).

Group 6 (CHXL) - the same mechanical preparation protocol performed in group 1 was adopted for this group, however without the use of ultrasonics, and the auxiliary chemical substance used was chlorhexidine liquid 2%. A 5 ml disposable syringe (BD Brazil – São Paulo, SP) was used to its insertion with 25x0.6 hypodermic needles (Injex Indústria Cirúrgica – Ourinhos, SP).

Group 7 (CHXG) - the same mechanical preparation protocol performed in group 1 was adopted for this group, however without the use of ultrasonics, and the auxiliary chemical substance used was chlorhexidine gel 2%. A 3 ml disposable syringe (BD Brazil – São Paulo, SP) was used to its insertion with 25x0.6 hypodermic needles (Injex Indústria Cirúrgica – Ourinhos, SP) and, for its removal, it was performed irrigation with saline 0.9% using 5 ml disposable syringe (BD Brazil – São Paulo, SP) with hypodermic needle.

Group 8 (DW) - the same mechanical preparation protocol performed in group 1 was adopted for this group, however without the use of ultrasonics, and the auxiliary chemical substance used was distilled water. A 5 ml disposable syringe (BD Brazil – São Paulo, SP) was used to its insertion with 25x0.6 hypodermic needle (Injex Indústria Cirúrgica – Ourinhos, SP).

Table 1 summarizes the group distribution.

Preparation for SEM

The scanning electron microscopy (SEM) was performed at the Electron Microscopy and Microanalysis Center of Pontifical Catholic University of Rio Grande do Sul.

The roots were fixed during a week on glutaraldehyde 2.5% and, after that underwent 3 washes of 30 minutes each in buffer solution phosphate 0.2 M and distilled water proportionally 1:1. Following that, the samples were dehydrated through immersion in ascending concentrations of acetone (30, 50, 70, 90 and 100%). Longitudinal grooves were made on the free surfaces of the roots with a diamond disk (Dhpro, Rhadartrade – Paranaguá, PR), taking care to not trespass into the root canal. The complete fracture was achieved with chisel and hammer, providing two halves of each sample, which were put in *stubs* with the portion of the root canal facing up. Then, the samples were palladium-gold sputtered for electron conduction.

The evaluation was performed on scanning electron microscopy (Philips XL 30, Eindhoven, Holland) 500, 2000, 5000 and 10000x magnifications, assessing the roots in thirds, from the least to the higher magnification; 2000X was selected to verify the root canal cleaning capacity and 10000X to differentiate smear layer from bacterial biofilm and thus verify root canal decontamination.

Images were obtained using BSE to verify the presence of bacteria and smear in the root canal.

A single observer, having the images and unaware of the experimental groups, rated them at 2000x regarding the presence of smear layer through a position rank scoring and, the same way, with images at 10000x, using the rank score to rate bacterial presence. Using the software PowerPoint, each image occupied a slide, which was further transferred to a computer screen in the form of a presentation. After that, the images had their positions modified according to the contamination level found, so as position number 1 was the most contaminated and position 60 the least

contaminated. This rating was performed to each third (cervical, medium, and apical). Then, for each third of the image, it was calculated the position mean of the group.

Statistical Analysis

The data obtained with electron microscopy were ranked by thirds. It was applied one-way ANOVA followed by Tukey post hoc (α=0.05). Data were analyzed using the software SPSS version 17.0.

RESULTS

Table 2 and Figures 1 and 2 show results according to position rank. Figure 3 illustrates scanning electron photomicrograph demonstrating the effect of the treatment.

Under 2000x in backscattering, the groups with ultrasonics showed very similar results concerning cleaningness and showed superior results when compared to control, except in the cervical and apical thirds, in which group 3 showed similar results to group 5 (figure 1). There were significant differences among all groups in all thirds. Groups 1 and 2 were statistically similar, showing better cleaning capacity. Group 7 displayed the worst performance.

10000X samples in backscattering showed that groups with ultrasonics in the cervical and medium thirds had similar results concerning root canal decontamination. In the apical third, however, group 6 showed superior results compared to groups 3 and 4 (figure 2). The groups with the lowest bacterial load were groups 1 and 2 and were similar in their results. There was statistical difference among the groups in all thirds. The group with the worst performance in the apical third was group 8 and in the medium and cervical third, group 7.

In root canal decontamination, group 3 was similar to group 8, and in cleaningness showed the worst result.

DISCUSSION

The formation of bacterial biofilm used in this research was based on other studies using similar antimicrobial strategies. There is not a consensus about the time needed to biofilm formation, varying from 24h up to 6 weeks; however, it is known that the longest the time for bacterial formation the more difficult it becomes for bacterial elimination. It was adopted a 60-day period for it is believed that, this way, the biofilm would be more structured, better illustrating a clinical situation (26).

Root canal preparation was performed previously to bacterial inoculation to enable uniform biofilm formation, once human teeth do not show root canals as wide as bovine teeth.

SEM was used in this study for it allows the preview of bacteria quantity and display along the bacterial biofilm (3, 19, 27).

Passive irrigation ultrasonics has been described in literature as an auxiliary to endodontic disinfection as it allows greater smear layer removal from root canal system. (20, 21, 23, 24, 28). It is considered passive irrigation when the instrument does not touch root canal walls, so a file #20 was used for ultrasonics irrigation.

In this study, under 2000X in backscattering it was not possible to differentiate smear layer from bacterial biofilm, thus, it was used as evaluation criterion root canal cleaningness and the groups with ultrasonics showed similar results among themselves and superior when compared to their controls. Groups 1 (sodium hypochlorite 2.5% + ultrasonics) and 2 (chlorhexidine liquid 2% + ultrasonics) showed higher cleaning capacity and were similar in their results.

Under 10000X in backscattering it was observed bacterial presence and the groups with ultrasonics showed again superior results when compared to controls. The groups with the lowest bacterial biofilm were groups 1 and 2 and were similar in their results.

The results obtained in this study showed that ultrasonics use during root canal irrigation provides better root canal cleaningness and disinfection, which confirms the results obtained by Spoleti et al., 2003(20); Van der Sluis et al., 2007(21); Jiang et al., 2010(23) and Van der Sluis et al., 2010(24). Sodium hypochlorite together with ultrasonics was superior both in debris and bacteria removal than the other solutions (19, 21, 24).

The results of this study diverge from Grundling et al 2011(29), who did not find differences between the use of ultrasonics with sodium hypochlorite compared to its isolated use. It must be emphasized that, although the *in vitro* infection model was similar, the present study used human teeth, while Grundling et al used bovine teeth. It is possible to speculate that the greater diameter of dentinal tubules in bovine teeth represents an advantage to the irrigating solution used, while the cleaning obstacles in human teeth are more difficult to overcome. It seems that the human tooth model represents better the clinical situation of what the professional is going to face. In such cases, ultrasonics use contributed to a better performance of the root canal cleaning substances.

Based on the results obtained, regardless of the solution, ultrasonics irrigation proved to be superior to conventional irrigation. We believe that ultrasonics can be used as an auxiliary to the root canal cleaningness and disinfection.

REFERENCES

1. Kayaoglu G, Orstavik D. Virulence factors of *Enterococcus faecalis*: Relationship to endodontic disease. Crit Rev Oral Biol Med. 2004; 15(5):308-320.

2. Stuart CH, Schwartz AS, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. JOE 2006; 32(2): 93-98

3. Estrela C, Sydney GB, Figueiredo JAP, Estrela CRA. A model system to study antimicrobial strategies in endodontics biofilm. J Appl Oral Sci. 2009;17(2):87-91.

4. Duggan JM, Sedgley CM. Biofilm formation of oral and endodontic *Enterococcus faecalis*. JOE 2007; 33(7): 815-818.

5. Peciuliene V, Balciuniene I, Eriksen HM, Haapasalo M. Isolation of *Enterococcus faecalis* in previously root-filled canals in Lithuanian population. JOE 2000; 26(10):593-595.

6. Love RM. *Enterococcus faecalis* – a mechanism for its role in endodontic failure. IEJ.2001; 34:399-405.

7. Chivantxaranukul P, Dashper SG, Messer HH. Dentinal tubule invasion and aderence by *Enterococcus faecalis*. IEJ 2008; 41: 873-882.

8. Sedgley C, Nagel A, Dahlén G, Reit C, Molander A. Real-time quantitative polymerase chain reaction and culture analyses of Enterococcus faecalis in root canals. JOE 2006; 32(3): 173-177.

9. Vahdaty A, Pitt Ford TR, Wilson RF. Efficacy of chlorhexidine in disinfecting dentinal tubules in vitro. Endod. Dent. Traumatol. 1993; 9:243-248.

10. Davis JM, Maki J, Bahcall JK. An in vitro comparison of the antimicrobial effects of various endodontic medicaments on *Enterococcus faecalis*. JOE 2007; 33(5): 567-569.

11. Estrela C, Silva JA, Alencar AHG, Leles CR, Decurcio DA. Eficaccy of Sodium Hypochlorite and Chlorexidine against *Enterococcus faecalis* – A systematic review. J Appl Oral Sci. 2008;16(6):364-368.

12. Siqueira Júnior JF, Machado AG, Silveira RM, Lopes HP, De Uzeda M. Evaluation of the effectiveness of Sodium Hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal, in vitro. IEJ.1997; 30: 279-282.

13 . Berber VB, Gomes BPFA, Sena NT, Vianna ME, Ferraz CCR, Zaia AA, Souza-Filho FJ. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing *Enterococcus faecalis* within root canals and dentinal tubules. IEJ. 2006; 39:10-17.

14. Dunavant TR, Regan JD, Glickman, GN, Solomon ES, Honeyman AL. Comparative evaluation of endodonics irrigants against *Enterococcus faecalis* biofilms. JOE 2006; 32(6): 527-531.

15. Leonardo MR, Tanomaru Filho M, Silva LAB, Nelson Filho P, Bonifácio KC, Ito Y. In vivo antimicrobial activity of 2% Clhorhexidine used as a root canal irrigating solution. JOE 1999; 25(3): 167-171.

16. Dametto FR, Ferraz CCR, Gomes BPFA, Zaia AA, Teixeira FB, Souza-Filho FJ. In vitro assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against *Enterococcus faecalis*. Oral surg Oral Med Oral Pathol Oral Radiol Endod 2005;99(6): 768 – 772.

17. Vianna ME, Gomes BP, Berber VB, et al. In Vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral surg Oral Med Oral Pathol Oral Radiol Endod 2004; 97: 79-84.

18. Abbot PV, Heijkooop PS, Cardaci SC, Hume WR, Heithersay GS. An SEM study of the effects of different irrigation sequences and ultrasonics. IEJ.1991; 24:308-316.

19. Huque J, Kota K, Yamaga M, Iwaku M, Hoshino E. Bacterial eradication from root dentine by uktrasonic irrigation with Sodium Hypochlorite. IEJ.1998; 31:242-250.

20. Spoleti P, Siragusa M, Spoleti MJ. Bacteriological evaluation of passive ultrasonic activation. JOE 2003; 29(1): 12-14.

21. Van der Sluis LWM, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. IEJ 2007; 40: 415-426.

22. Cheung GS, Stock CJR. In vitro cleaning ability of root canal irrigants with and without endosonics. IEJ. 1993; 26:334-343.

23. Jiang LM, Verhaagen B, Versluis M, Zangrillo C, Cuchovic D, Van der Sluis LWM. An Evaluation of the effect of pulsed ultrasound on the cleaning efficacy of passive ultrasonic irrigation.JOE 2010; 36(11): 1887-1891.

24. Van der Sluis LWM, Vogels MPJM, Verhaagen B, Macedo R, Wesselink PR. Study on the Influence of Refreshment/Activation Cycles and Irrigants on Mechanical Cleaning Efficiency During Ultrasonic Activation of the Irrigant. JOE 2010; 36(4) :737-740.

25. Weller RN, Brady JM, Bemier WE. Efficacy of ultrasonic cleaning. JOE 1980; 6: 740-743.

26.Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG. Evaluation of antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTDA, and 5% sodium hypochlorite against *Enterococcus faecalis* biofilm formed on tooth substrate: An *in vitro* study. JOE 2010; 36(1): 83-86.

27. Liu H, Wei X, Ling J, Wang W, Huang X. Biofilm Formation capability of *Enterococcus faecalis* cells in starvation phase and its susceptibility to sodium hypochlorite. JOE 2010; 36(4): 630-635.

28.Gregorio C, Estevez R, Cisneros R, Heilborn C, Cohenca N. Effect of EDTA,Sonic and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals : An *In vitro* study. JOE 2009:35(6):891-895.

29. Gründling GL, Zechin JG, Jardim WM, Oliveira SD, Figueiredo JAP. Effect of ultrasonics on *Enterococcus faecalis* biofilm in a bovine tooth model. JOE 2011; 37(8): 1128-1133.

ANEXOS

Tables and figures

Table 1 – Group distribution according to the irrigating solution and use of ultrasonics.

GROUP	IRRIGATING SOLUTION	ULTRASONICS
1 (n=10)	SODIUM HYPOCHLORITE 2.5%	X
2 (n=10)	CHLORHEXIDINE LIQUID 2%	X
3 (n=10)	CHLORHEXIDINE GEL 2%	X
4 (n=5)	DISTILLED WATER	X
5 (n=5)	SODIUM HYPOCHLORITE 2.5%	-
6 (n=5)	CHLORHEXIDINE LIQUID 2%	-
7 (n=5)	CHLORHEXIDINE GEL 2%	-
8 (n=5)	DISTILLED WATER	-

Table 2 – Comparison of contamination levels between different cleaning treatments applied on human root canal

Variable	NaOCl+US n=10	CHX+US n=10	CHXg+US n=10	H_2O+US n=10	NaOCl n=5	CHX n=5	CHXg n=5	H_2O n=5	P
Canal Space BS (2,000 x MEV)									
Apical third	42.2±12.9^{c}	42.4±11.0^{c}	26.2±13.8^{a,b,c}	41.1±13.9^{b,c}	19.8±15.1^{a,b}	18.0±13.1^{a}	5.2±6.1^{a}	19.2±14.9^{a,b}	<0.001
Medium third	47.0±13.1^{c}	38.4±14.1^{b,c}	35.5±11.6^{b,c}	34.6±11.2^{b,c}	20.0±13.8^{a,b}	9.8±4.2^{a}	4.2±3.1^{a}	21.0±16.2^{a,b}	<0.001
Coronal third	39.9±17.2^{c,d}	39.0±16.3^{c,d}	27.0±11.2^{b,c,d}	43.1±9.7^{d}	31.4±15.4^{b,c,d}	20.2±11.3^{a,b,c}	4.0±2.7^{a}	12.4±9.9^{a,b}	<0.001
Canal Space BS (10,000 x MEV)									
Apical third	49.8±6.3^{e}	45.9±15.1^{d,e}	26.8±10.4^{a,b,c}	28.2±11.7^{b,c,d}	11.2±6.9^{a,b}	30.4±10.1^{c,d}	14.4±10.8^{a,b,c}	8.6±10.4^{a}	<0.001
Medium third	47.3±10.8^{c}	42.8±10.5^{c}	31.3±13.0^{b,c}	33.8±13.4^{b,c}	18.8±9.4^{a,b}	9.4±6.5^{a}	8.4±2.7^{a}	19.0±20.9^{a,b}	<0.001
Coronal third	43.9±11.8^{d}	43.3±12.0^{d}	38.4±15.1^{c,d}	32.5±9.7^{b,c,d}	18.6±14.9^{a,b,c}	11.0±8.8^{a}	5.8±4.7^{a}	14.4±5.9^{a,b}	<0.001

Data are presented as mean ranks ± standard deviation within thirds.H_2O: Destilled water, H2O+US: Ultrasonics and destilled water, NaOCl: Sodium hypochlorite, US+NaOCl: Ultrasonics and sodium hypochlorite, CHXg: Chlorexidine gel, CHXg +US: Chlorexidine gel and ultrasonics, CHX:Chlorexidine, CHX+US: Chlorexidine and Ultrasonics. P: significance using ANOVA on ranks. Different index letters represent statistical significant different at the post-hoc procedure (Tukey test).

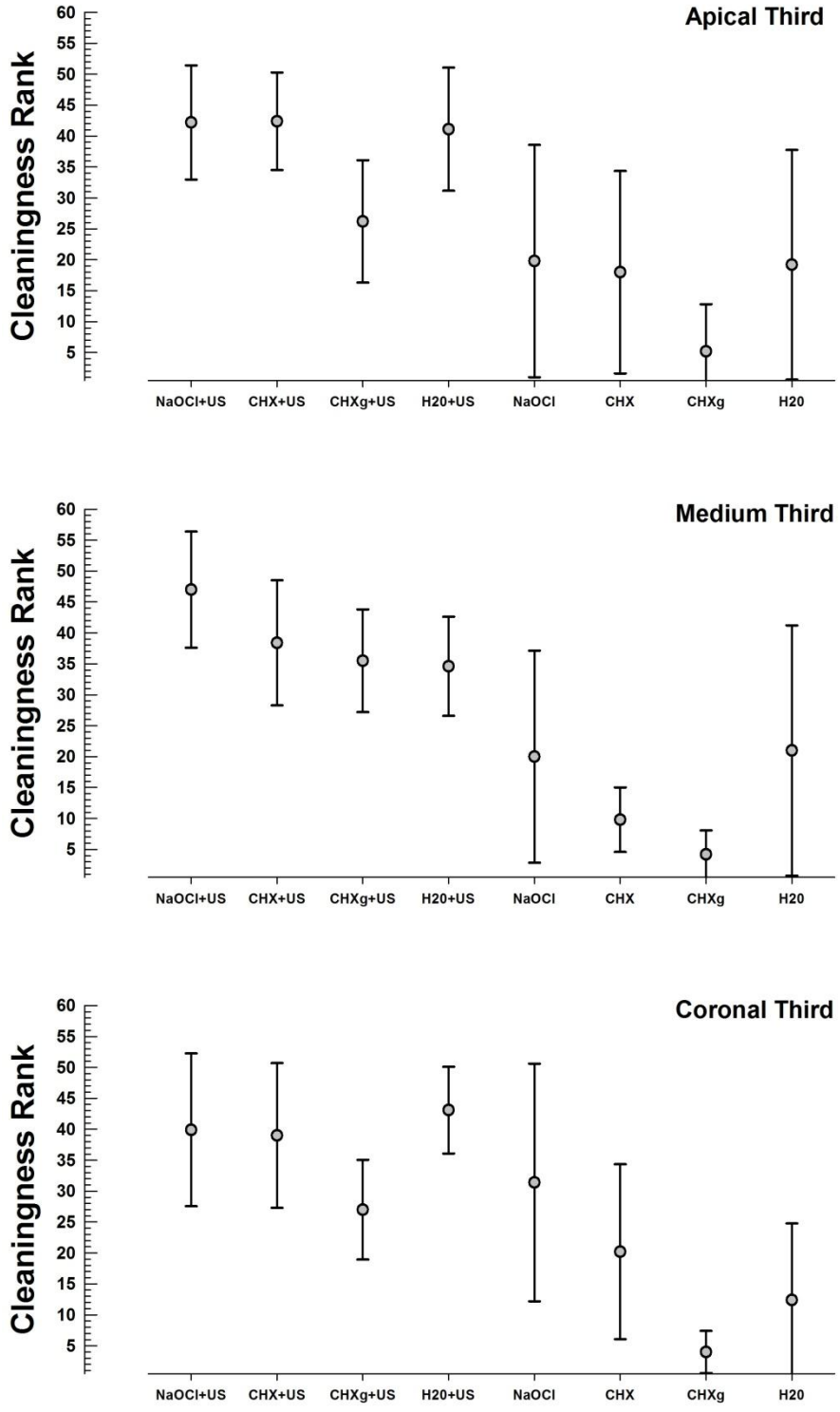

Figure 1 – Each point represents 2000X in BSE: H_2O: Distilled water, H2O+US: Ultrasound and distilled water, NaOCl: Sodium hypochlorite, US+NaOCl: Ultrasound and sodium hypochlorite, CHXg: Chlorexidine gel, CHXg +US: Chlorexidine gel and ultrasound, CHX:Chlorexidine, CHX+US: Chlorexidine and Ultrasound.

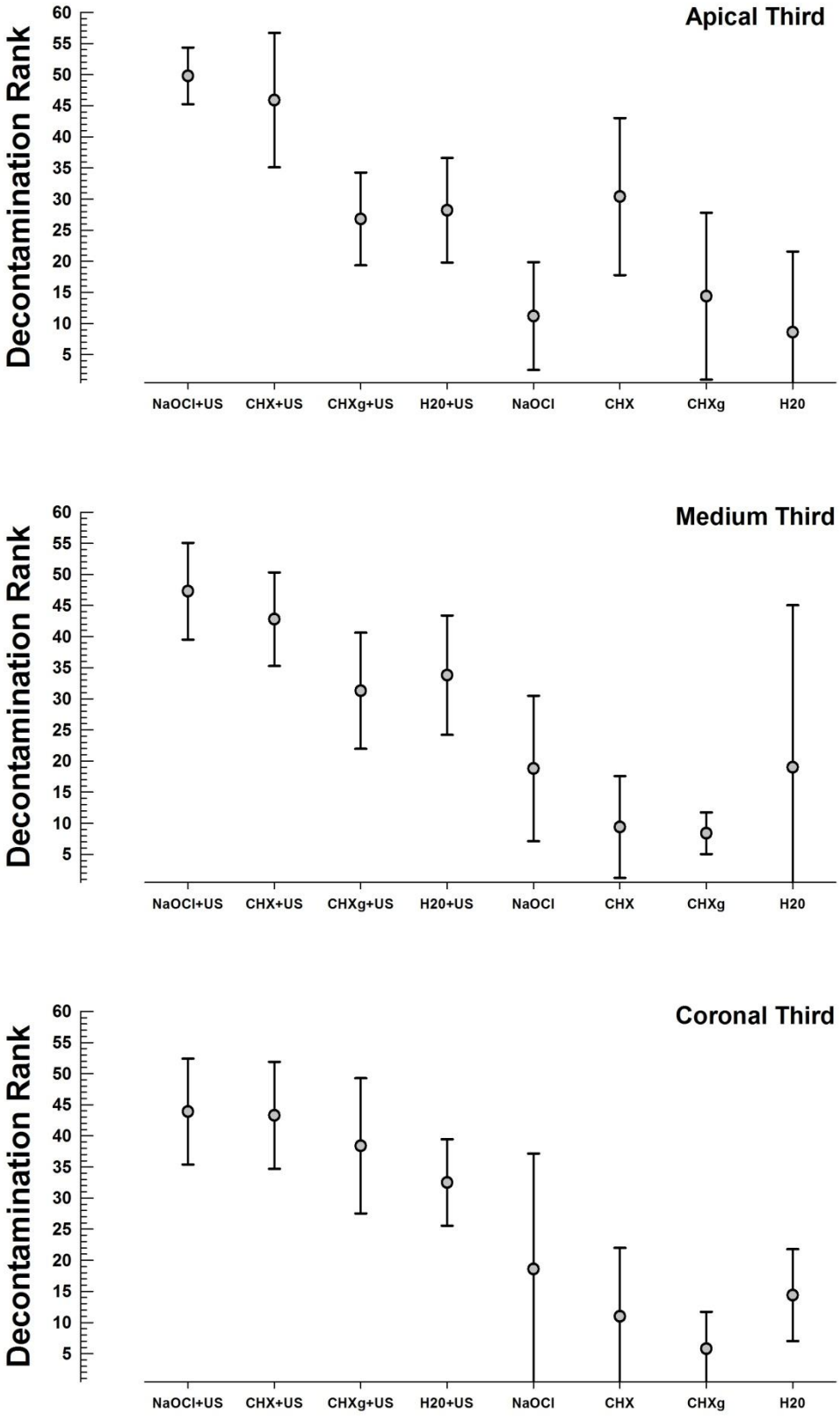

Figure 2 - Each poit represents 10000X magnification in BSE: H_2O: Distilled water, H2O+US: Ultrasound and distilled water, NaOCl: Sodium hypochlorite, US+NaOCl: Ultrasound and sodium hypochlorite, CHXg: Chlorexidine gel, CHXg +US: Chlorexidine gel and ultrasound, CHX:Chlorexidine, CHX+US: Chlorexidine and Ultrasound.

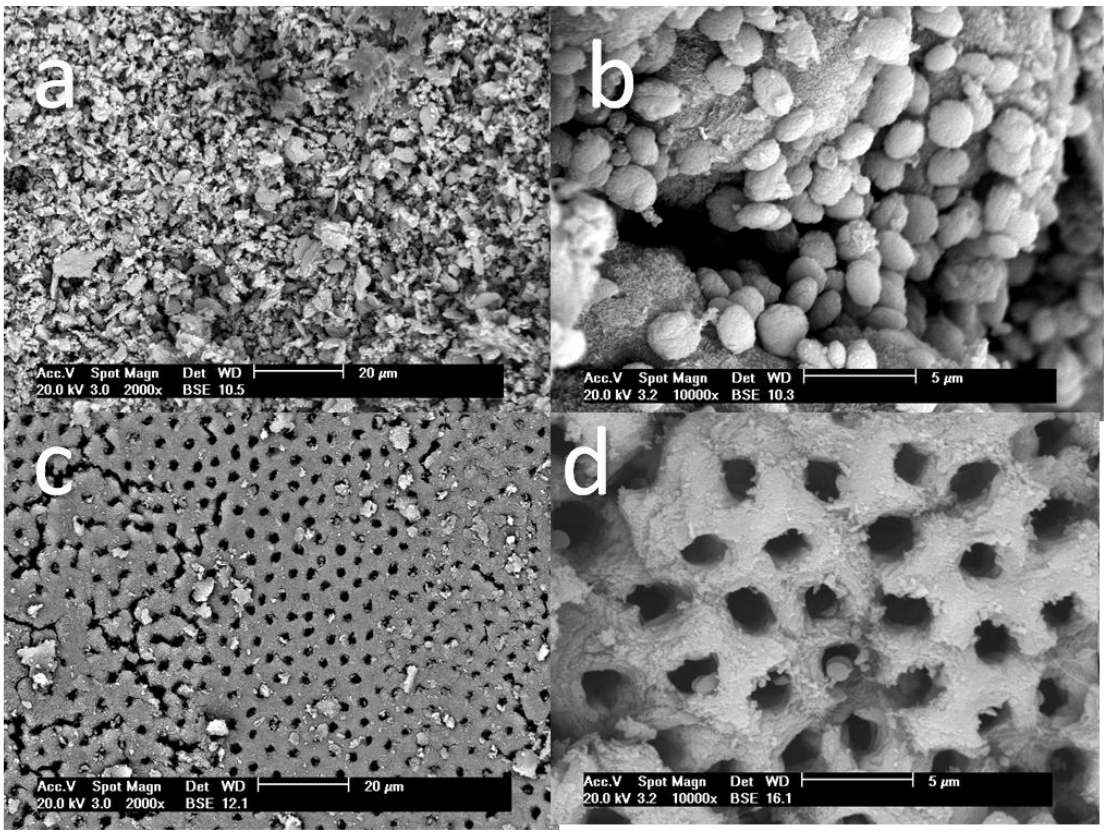

Figure 3 MEV of groups displaying cleaningness and decontamination features presenting the group that got better and worse results in the respective magnification. a. 2% Chlorhexidine gel (2000x); b. Distilled water (10000X); c. 2.5% sodium hypochlorite + ultrasonics (2000x); d. 2.5% sodium hypochlorite + ultrasonics (10000x).

DISCUSSÃO GERAL

Por não existir um consenso na literatura em relação ao tempo necessário para a formação do biofilme variando entre 24h até 6 semanas optou-se por um período de 60 dias por acreditar-se que desta maneira o biofilme estaria mais estruturado exemplificando melhor a situação clínica. Sabe-se que quanto maior o tempo de formação bacteriana menor foi a eliminação bacteriana (Prabhakar et al.,2010).

Previamente à inoculação foi realizado o preparo do canal radicular para propiciar a formação de um biofilme uniforme já que os dentes humanos não apresentam um canal radicular tão amplo quanto os dentes bovinos e também para permitir que o meio de cultura BHI atingisse todos os terços do canal radicular.

O uso do ultrassom em endodontia vem sido descrito na literatura de duas formas uma em que realizada a instrumentação e irrigação simultaneamente e outra sem instrumentação, também denominada passiva, pois o instrumento não toca as paredes do canal radicular. Dessa forma foi utilizada uma lima #20 para irrigação ultrassônica. De acordo com os estudos prévios, o ultrassom permitiu uma maior desinfecção e remoção de *smear layer* do sistema de canais radiculares quando utilizado de forma passiva do que simultaneamente com a instrumentação (Spoleti et al.,2003; Van der Sluis et al., 2007; Gregorio et al., 2009; Jiang et al., 2010; Van der Sluis et al., 2010).

Para termos uma visualização da quantidade e distribuição das bactérias ao longo do biofilme bacteriano utilizou-se a microscopia eletrônica de varredura (MEV) que também que já foi utilizada em diversos estudos como nos estudos de Huque et al., 1998; Estrela et al., 2009 e Liu et al.,2010.

Neste estudo, tanto em um aumento de 2000X em *backscattering* como no aumento de 10000X em *backscattering*, os grupos com ultrassom mostraram resultados superiores quando comparado ao controle. Independente do aumento, os grupos 1 (hipoclorito de sódio 2,5% + ultrassom) e grupo 2 (clorexidina 2% líquida + ultrassom) apresentaram uma melhor capacidade de limpeza e um menor biofilme bacteriano.

Para permitir uma detecção de pelo menos 1,5 unidades de desvio-padrão entre as medias observadas nos grupos, alcançando poder estatístico de 90% e nível de significância de 5%, foi estimada a necessidade de 10 unidades experimentais por grupo teste (ultrassom) e 5 unidades experimentais para seus respectivos controles (sem ultrassom). Com a utlização de escores, o teste Anova de uma via sobre escores seguido do post-hoc de Tukey foi aplicado, para $\alpha=0.05$.

Os resultados obtidos neste estudo demonstram que a utilização do ultrassom durante a irrigação do canal radicular proporciona uma maior limpeza e desinfecção indo de encontro aos resultados obtidos por Spoleti et al.,2003; Van der Sluis et al.,2007; Jiang et al., 2010 e Van der Sluis et al.,2010. Porém, estes resultados divergem dos de Gründling et al 2011, que não encontraram diferenças entre o uso do ultrassom com hipoclorito de sódio comparado ao seu uso isolado. Contudo, o estudo de Gründling et al utilizou dentes bovinos, o que provavelmente favoreceu a solução irrigadora utilizada devido ao maior diâmetro dos túbulos dentinários, enquanto que em dentes humanos as dificuldades anatômicas são maiores. No presente estudo, o uso do ultrassom contribuiu para um melhor desempenho das soluções irrigadoras.

Frente aos resultados obtidos, independente da solução, a irrigação ultrassônica mostrou-se superior à irrigação convencional, fazendo crer que o ultrassom deve ser utilizado como um excelente auxiliar na limpeza e desinfecção do canal radicular potencializando a ação dos irrigantes.

REFERÊNCIAS BIBLIOGRÁFICAS

1. Abbot PV, Heijkooop PS, Cardaci SC, Hume WR, Heithersay GS. An SEM study of the effects of different irrigation sequences and ultrasonics. IEJ.1991; 24:308-316.

2. Berber VB, Gomes BPFA, Sena NT, Vianna ME, Ferraz CCR, Zaia AA, Souza-Filho FJ. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing *Enterococcus faecalis* within root canals and dentinal tubules. IEJ. 2006; 39:10-17.

3. Cheung GS, Stock CJR. In vitro cleaning ability of root canal irrigants with and without endosonics. IEJ. 1993; 26:334-343.

4. Chivantxaranukul P, Dashper SG, Messer HH. Dentinal tubule invasion and aderence by *Enterococcus faecalis*. IEJ 2008; 41: 873-882.

5. Dametto FR, Ferraz CCR, Gomes BPFA, Zaia AA, Teixeira FB, Souza-Filho FJ. In vitro assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against *Enterococcus faecalis*. Oral surg Oral Med Oral Pathol Oral Radiol Endod 2005;99(6): 768 – 772.

6. Davis JM, Maki J, Bahcall JK. An in vitro comparison of the antimicrobial effects of various endodontic medicaments on *Enterococcus faecalis*. JOE 2007; 33(5): 567-569.

7. Duggan JM, Sedgley CM. Biofilm formation of oral and endodontic *Enterococcus faecalis*. JOE 2007; 33(7): 815-818.

8. Dunavant TR, Regan JD, Glickman, GN, Solomon ES, Honeyman AL. Comparative evaluation of endodonics irrigants against *Enterococcus faecalis* biofilms. JOE 2006; 32(6): 527-531.

9. . Estrela C, Silva JA, Alencar AHG, Leles CR, Decurcio DA. Eficaccy of Sodium Hypochlorite and Chlorexidine against *Enterococcus faecalis* – A systematic review. J Appl Oral Sci. 2008;16(6):364-368.

10. Estrela C, Sydney GB, Figueiredo JAP, Estrela CRA. A model system to study antimicrobial strategies in endodontics biofilm. J Appl Oral Sci. 2009;17(2):87-91.

11. Gregorio C, Estevez R, Cisneros R, Heilborn C, Cohenca N. Effect of EDTA,Sonic and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals : An *In vitro* study. JOE 2009:35(6):891-895.

12. Gründling GL, Zechin JG, Jardim WM, Oliveira SD, Figueiredo JAP. Effect of ultrasonics on *Enterococcus faecalis* biofilm in a bovine tooth model. JOE 2011; 37(8): 1128-1133.

13. Huque J, Kota K, Yamaga M, Iwaku M, Hoshino E. Bacterial eradication from root dentine by uktrasonic irrigation with Sodium Hypochlorite. IEJ.1998; 31:242-250.

14. Jiang LM, Verhaagen B, Versluis M, Zangrillo C, Cuchovic D, Van der Sluis LWM. An Evaluation of the effect of pulsed ultrasound on the cleaning efficacy of passive ultrasonic irrigation.JOE 2010; 36(11): 1887-1891.

15. Kayaoglu G, Orstavik D. Virulence factors of *Enterococcus faecalis*: Relationship to endodontic disease. Crit Rev Oral Biol Med. 2004; 15(5):308-320.

16. Leonardo MR, Tanomaru Filho M, Silva LAB, Nelson Filho P, Bonifácio KC, Ito Y. In vivo antimicrobial activity of 2% Clhorhexidine used as a root canal irrigating solution. JOE 1999; 25(3): 167-171.

17. Liu H, Wei X, Ling J, Wang W, Huang X. Biofilm Formation capability of *Enterococcus faecalis* cells in starvation phase and its susceptibility to sodium hypochlorite. JOE 2010; 36(4): 630-635.

18. Love RM. *Enterococcus faecalis* – a mechanism for its role in endodontic failure. IEJ.2001; 34:399-405.

19. Peciuliene V, Balciuniene I, Eriksen HM, Haapasalo M. Isolation of *Enterococcus faecalis* in previously root-filled canals in Lithuanian population. JOE 2000; 26(10):593-595.

20. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG. Evaluation of antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTDA, and 5% sodium hypochlorite against

Enterococcus faecalis biofilm formed on tooth substrate: An *in vitro* study. JOE 2010; 36(1): 83-86.

21. Sedgley C, Nagel A, Dahlén G, Reit C, Molander A. Real-time quantitative polymerase chain reaction and culture analyses of Enterococcus faecalis in root canals. JOE 2006; 32(3): 173-177.

22. Siqueira Júnior JF, Machado AG, Silveira RM, Lopes HP, De Uzeda M. Evaluation of the effectiveness of Sodium Hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal, in vitro. IEJ.1997; 30: 279-282.

23. Spoleti P, Siragusa M, Spoleti MJ. Bacteriological evaluation of passive ultrasonic activation. JOE 2003; 29(1): 12-14.

24. Stuart CH, Schwartz AS, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. JOE 2006; 32(2): 93-98

25. Vahdaty A, Pitt Ford TR, Wilson RF. Efficacy of chlorhexidine in disinfecting dentinal tubules in vitro. Endod. Dent. Traumatol. 1993; 9:243-248.

26. Van der Sluis LWM, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. IEJ 2007; 40: 415-426.

27. Van der Sluis LWM, Vogels MPJM, Verhaagen B, Macedo R, Wesselink PR. Study on the Influence of Refreshment/Activation Cycles and Irrigants on Mechanical Cleaning Efficiency During Ultrasonic Activation of the Irrigant. JOE 2010; 36(4) :737-740.

28. Vianna ME, Gomes BP, Berber VB, et al. In Vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral surg Oral Med Oral Pathol Oral Radiol Endod 2004; 97: 79-84.

29. Weller RN, Brady JM, Bemier WE. Efficacy of ultrasonic cleaning. JOE 1980; 6: 740-743.