**UNIVERSIDADE DE SÃO PAULO**
**FACULDADE DE ODONTOLOGIA DE BAURU**

**CLARISSA TELES RODRIGUES**

# Evaluation of methods for removal of filling material in endodontic retreatment and assessment of antimicrobial action of solutions used in final irrigation

# Avaliação de métodos de desobturação do canal radicular no retratamento endodôntico e avaliação da ação antimicrobiana de soluções utilizadas na irrigação final

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Tese constituída por artigos apresentada a Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Doutor em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Endodontia.

Orientador: Prof. Dr. Norberti Bernardineli

Coorientadora: Profa. Dra. Flaviana Bombarda de Andrade

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## DADOS CURRICULARES

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# RESUMO


**Avaliação de métodos de desobturação do canal radicular no retratamento endodôntico e avaliação da ação antimicrobiana de soluções utilizadas na irrigação final**

Em casos de insucesso do tratamento endodôntico, uma alternativa seria o retratamento do canal radicular. Durante este procedimento deve haver remoção de todo o material obturador para que seja realizada novo preparo biomecânico e nova obturação do sistema de canais radiculares. Bactérias são o principal fator etiológico em casos de fracasso da terapia endodôntica, e esta infecção persistente pode estar relacionada à capacidade dos microganismos em penetrar nos túbulos dentinários. Por este motivo é necessário o uso de soluções na irrigação do canal radicular com ação antimicrobiana e com boa tolerância tecidual. O objetivo deste trabalho foi avaliar o desempenho dos instrumentos de diferentes ligas metálicas na desobturação do canal radicular durante o retratamento endodôntico de incisivos laterais superiores com curvatura apical, por meio da microtomografia computadorizada e análise destas amostras no microscópio eletrônico de varredura após a ativação de irrigantes. Posteriormente foi avaliada a capacidade antimicrobiana de um irrigante do canal radicular contendo nanopartículas de prata, clorexidina a 2% e hipoclorito de sódio a 2,5% frente ao biofilme de *Enterococcus faecalis* e à dentina contaminada com este mesmo microrganismo. Foram selecionados trinta incisivos laterais superiores humanos extraídos que apresentavam curvatura apical. Os dentes foram instrumentados, obturados e divididos em três diferentes grupos de acordo com o protocolo de remoção do material obturador do canal radicular: no Grupo 1: a desobturação foi realizada com os instrumentos Reciproc R25, Mtwo 40 e ProDesign Logic 50.01; no Grupo 2: foram utilizados os instrumentos ProDesign R, ProDesign Logic 40 e ProDesign Logic 50.01; e no Grupo 3: a desobturação foi realizada com brocas de Gates-Glidden e instrumentos manuais tipo K e Hedstroem. Para a análise da remoção do material obturador, as amostras foram escaneadas em micrótomogafo SkyScan 1174 para que fossem comparadas as imagens antes e após a desobturação do canal radicular, e em cada amostra este volume foi calculado nos quatro níveis (apical 1, 2, médio e cervical). Estas amostras posteriormente foram clivadas e analisadas no

microscópio eletrônico de varredura, para a visualização de resíduos de material obturador antes e após a ativação de irrigantes com o ultrassom e com o sistema EasyClean utilizado em rotação contínua. Para a análise estatística dos resultados foram utilizados os testes de Kruskal-Wallis, Friedman, Wilcoxon e Dunn. Posteriormente, foi determinada a concentração inibitória mínima de uma solução irrigadora do canal contendo nanopartículas de prata frente a cepas de *Enterococcus faecalis*, através do método de diluição em caldo. Em seguida, foi testada a atividade antimicrobiana das soluções de nanopartículas de prata, da clorexidina a 2% e do hipoclorito de sódio a 2,5% sobre o biofilme de *Enterococcus faecalis in vitro*. Para isso, foram utilizados blocos de dentina bovina colocados em placas de 24 poços e biofilme de *Enterococcus faecalis* foi formado durante 21 dias. Os blocos de dentina foram divididos em 9 grupos experimentais com 5 blocos cada um, em função dos irrigantes avaliados e do tempo de exposição à solução irrigadora (5, 10 e 15 minutos). As amostras foram coradas com corante Live/Dead para posterior análise no microscópio confocal de varredura a laser (MCVL). Por fim, foi testada a atividade antimicrobiana destas soluções irrigadoras após a contaminação de túbulos dentinários com *Enterococcus faecalis*. Foram confeccionados tubos de dentina a partir de incisivos bovinos que foram levados à centrífuga e contaminados com *Enterococcus faecalis.* Os tubos de dentina receberam tratamento com a solução de nanopartículas de prata, com clorexidina a 2% e com hipoclorito de sódio a 2,5%, e foram analisados no MCVL para avaliar a atividade antimicrobiana das soluções sobre bactérias presentes nos túbulos dentinários. Os resultados demonstraram que resíduos de material obturador foram encontrados após a desobturação do canal em todos os grupos. Não houve diferença significante na remoção de material obturador entre os instrumentos reciprocantes Reciproc e ProDesign R e entre os instrumentos rotatórios Mtwo 40 e ProDesign Logic 40. O instrumento ProDesign Logic 50/.01 melhorou significantemente a remoção de material obturador comparado com o uso dos instrumentos Reciproc e ProDesign R. Os níveis apicais apresentaram uma maior quantidade de material obturador remanescente comparados com os níveis médio e cervical. Após a desobturação do canal radicular, a irrigação ultrassônica passiva e agitação dos irrigantes com o EasyClean melhoraram significantemente a remoção de resíduos de material obturador em todos os terços do canal radicular. Não houve diferença significante no desempenho do ultrassom e do EasyClean em relação à

remoção de resíduos de material obturador, assim como não foi observada diferença significante na remoção destes resíduos quando comparados terços apical, médio e cervical. No que diz respeito à ação das soluções irrigadoras sobre o *Enterococcus faecalis*, a concentração inibitória mínima da solução de nanopartículas de prata capaz de eliminar este microrganimo em meio de cultura e ágar foi de 94 ppm. Após a irrigação no biofilme de *Enterococcus faecalis*, a solução de nanopartículas de prata foi significantemente menos efetiva em matar bactérias comparada com a clorexidina quando utilizadas pelo tempo de 5 minutos. A solução de hipoclorito de sódio apresentou atividade antimicrobiana significantemente maior comparada com as soluções de nanopartículas de prata e clorexidina. Essa solução ainda apresentou maior capacidade de dissolução do biofilme em todos os tempos testados, enquanto que a solução de nanopartículas de prata apresentou maior capacidade de dissolver o biofilme comparada à clorexidina nos tempos de 5 e 15 minutos. Na dentina intratubular infectada com *Enterococcus faecalis*, a solução de hipoclorito de sódio apresentou efetividade significantemente maior que as soluções de nanopartículas de prata e clorexidina, principalmente no terço médio e na região profunda do canal radicular. Quando comparada a atividade antimicrobiana destas soluções no biofilme e na dentina intratubular infectada, verificou-se que quando a solução de nanopartículas de prata foi utilizada por um tempo mais curto, foi mais efetiva na dentina intratubular comparada com o biofilme. De maneira contrária, com tempo maior de 30 minutos, o número e bactérias viáveis foi maior na dentina intratubular do que no biofilme, o que ocorreu também com a solução de hipoclorito de sódio neste tempo de ação. Conclui-se que para se aumentar a chance de sucesso em tratamentos retratamentos endodônticos, a combinação do uso de instrumentos reciprocantes e rotatórios na desobturação do canal, agitação de irrigantes e uso de agentes com capacidade antimicrobiana podem ser utilizados na tentativa de se eliminar bactérias resistente ao tratamento endodôntico.

**Palavras-chave:** Biofilmes, Endodontia, Microtomografia por Raio-X, Nanopartículas, Retratamento.

# ABSTRACT

**Evaluation of methods for removal of filling material in endodontic retreatment and assessment of antimicrobial action of solutions used in final irrigation**

When endodontic treatment fails, an alternative could be root canal retreatment. During this procedure, all filling material should be removed to allow a new root canal preparation and new obturation of the root canal system. Bacteria are the main cause of endodontic treatment failure, and persistent infection may be related to microorganism ability to penetrate into dentinal tubules. Therefore, this requires the use of irrigating solutions with antimicrobial action and low toxicity. The aim of this study was to evaluate the performance of instruments made of different alloys in root canal re-instrumentation during endodontic retreatment of lateral incisors with apical curvature, using computed microtomography and assessment of these samples by scanning electron microscopy after irrigant activation. Furthermore, the antimicrobial action of a root canal irrigant containing silver nanoparticles, 2% chlorhexidine and 2.5% sodium hypochlorite was evaluated against *Enterococcus faecalis* biofilm and dentin infected with this microorganism. Thirty extracted maxillary lateral incisors with apical curvature were selected. The teeth were instrumented, filled and divided into three different groups according to the protocol for removal of filling material: Group 1: re-instrumentation with Reciproc R25 instruments, Mtwo 40 and ProDesign Logic 50.01; Group 2: re-instrumentation with ProDesign R, ProDesign Logic 40 and ProDesign Logic 50.01; Group 3: re-instrumentation with Gates-Glidden drills and manual instruments K-file and Hedstroem files. For filling material removal analysis, the samples were scanned in a microtomograph device SkyScan 1174, for comparison of images taken before and after removing the root canal filling, and in each sample the volume was calculated at four levels (apical 1, apical 2, middle and cervical). These samples were split and analyzed by scanning electron microscopy to visualize filling material residues before and after irrigant activation with an ultrasonic device and with the EasyClean system used in continuous rotary motion. Statistical analyses were performed using Kruskal-Wallis, Friedman, Wilcoxon and Dunn tests. Furthermore, the minimum inhibitory concentration of an irrigating solution containing silver nanoparticles was determined

against strains of *Enterococcus faecalis*, by using the microdilution method. Additionally, the antimicrobial activity of silver nanoparticle solution, 2% chlorhexidine and 2.5% sodium hypochlorite was tested against *Enterococcus faecalis* biofilm *in vitro*. For biofilm formation, bovine dentin blocks were placed in 24-well culture plates and *Enterococcus faecalis* biofilm was developed for 21 days. The dentin blocks were divided into 9 experimental groups of 5 blocks each, according to the irrigating solution used and the time in contact with the irrigant (5, 15 and 30 minutes). The samples were stained with Live/Dead reagent for analysis by confocal laser scanning microscopy (CLSM). Finally, the antimicrobial action of these solutions was tested after dentinal tubules were contaminated with *Enterococcus faecalis*. Dentin tubes were made from bovine incisors, taken to a centrifuge and infected with *Enterococcus faecalis*. The dentin tubes were treated with silver nanoparticle solution, 2% chlorhexidine and 2.5% sodium hypochlorite, and analyzed by CLSM to assess the antimicrobial activity of these solutions against bacteria in the dentinal tubules. The results showed that residues of filling material were found after root canal reinstrumentation in all groups. No significant difference was observed in removal of filling material between the reciprocating instruments Reciproc and ProDesign R and between rotary instruments Mtwo 40 and ProDesign Logic 40. The ProDesign Logic 50/.01 instrument significantly improved the removal of filling material compared with the use of Reciproc and ProDesign R instruments. The apical levels presented greater amount of remnant filling material compared with middle and cervical levels. After canal reinstrumentation, the passive ultrasonic irrigation and irrigant agitation with EasyClean significantly improved the removal of residual filling material in all root canal thirds. There was no significant difference between the performance of ultrasonics and EasyClean regarding the removal of residual filling material, as well no significant difference was observed in the removal of these residues when comparing apical, middle and cervical thirds. Regarding the action of the irrigating solutions against *Enterococcus faecalis*, the minimum inhibitory concentration of silver nanoparticle solution capable of eliminating this microorganism in broth and agar plates was 94 ppm. After the irrigation of *Enterococcus faecalis* biofilm, the silver nanoparticle solution was significantly less effective in killing bacteria compared with chlorhexidine when used for time of contact of 5 minutes. The sodium hypochlorite solution presented antimicrobial activity

significantly higher compared with the silver nanoparticle solution and chlorhexidine. This solution also presented higher ability to dissolve biofilm in all times tested, whereas the silver nanoparticle solution presented higher ability to dissolve biofilm compared with chlorhexidine in times of 5 and 15 minutes. In infected intratubular dentin with *Enterococcus faecalis*, the sodium hypochlorite solution presented significant higher effectiveness than the silver nanoparticle solutions and chlorhexidine, especially in middle third and deep areas of the root canal. When comparing the antimicrobial activity of these solutions in biofilm and infected intratubular dentin, it was shown that when the silver nanoparticle solution was used for shorter periods of time, it was more effective in intratubular dentin compared with biofilm. On the other hand, with longer time of 30 minutes, the number of viable bacteria was higher in intratubular dentin than in biofilm, which was also observed when using the sodium hypochlorite solution in this time of action. It was concluded that to increase the rate of success in endodontic retreatment, the combination of the use of reciprocating and rotary instruments in the removal of filling material, the agitation of irrigants and the use of antimicrobial agents could be used in an attempt to eliminate bacteria that resisted to endodontic treatment.

**Key-words:** Biofilm, Endodontics, X-Ray microcomputed tomography, Nanoparticles, Retreatment.

# LISTA DE ABREVIATURA E SIGLAS

| | |
|---|---|
| **%** | percentagem |
| **x** | vezes |
| **<** | menor |
| **>** | maior |
| **=** | igual |
| **º** | grau |
| **AgNp** | nanopartículas de prata |
| **BHI** | infusão de cérebro e coração |
| **CFU** | unidades formadoras de colônias |
| **CLSM** | microscopia confocal de varredura a laser |
| **CM** | memória controlada |
| **cm** | centímetro |
| **EDTA** | ácido etileno diaminotetracético |
| **KV** | quilovolts |
| **MA** | miliampère |
| **microCT** | microtomografia computadorizada |
| **min** | minutos |
| **mL** | mililitro |
| **mm** | milímetro |
| **$mm^3$** | milímetro cúbico |
| **n** | número |
| **NaOCL** | hipoclorito de sódio |
| **NiTi** | Níquel Titânio |
| **P** | significância estatística |
| **ppm** | partes por milhão |
| **PUI** | irrigação ultrassônica passiva |
| **rpm** | revoluções por minuto |
| **SEM** | microscopia eletrônica de varredura |
| **µL** | microlitro |
| **µm** | micrômetro |
| **$\mu m^2$** | micrômetro quadrado |

# SUMMARY

# 1 INTRODUCTION

# 1 INTRODUCTION

Nonsurgical endodontic retreatment is indicated in cases of failure after treatment because it is an additional alternative for attaining success before resorting to endodontic surgery (BERGENHOLTZ et al., 1979; ALLEN et al., 1989; KVIST; REIT, 1999; NUDERA et al., 2015; TORABINEJAD et al., 2009). In these cases of failure, the post-treatment disease may be attributed to persistent intraradicular infection in the complex apical root canal system and residual microbes in the apical portion of the root canal (NAIR, 2006). Incomplete removal of the filling material during retreatment may compromise thorough disinfection in some areas of the root canal system by restricting access of the antimicrobial agents to these regions (CAVENAGO et al., 2014). Irrigation solutions play an important role in cleaning the root canal, especially in the complex anatomy of root canal systems, because of their antimicrobial action, capacity for smear layer removal and dissolution of necrotic pulp remnants, thus preparing the root canal for filling with gutta-percha and a sealer (ZHENDER, 2006). Therefore, all filling material should be removed to promote effective action of the irrigants and adequate adaptation of the new filling material (CAVENAGO et al., 2014).

Several techniques have been proposed for removing filling material during endodontic retreatment, such as hand files, burs and rotary instruments (BERNARDES et al., 2016; BRAMANTE et al., 2010; CAVENAGO et al., 2014; FRUCHI et al., 2014; RIOS et al., 2014; RODIG et al., 2012; TAKAHASHI et al., 2009; ZUOLO et al., 2013; ZUOLO et al., 2016). The use of rotary NiTi instruments in retreatment has been shown to be safe, less time-consuming, efficient and reduced operator’s and patient’s fatigue (BERNARDES et al., 2016; BETTI et al., 2001; BRAMANTE et al., 2010; RODIG et al., 2012; RODIG et al., 2014; TAKAHASHI et al., 2009; ZUOLO et al., 2013). Nowadays, reciprocating instruments have been suggested for removal of filling material due to their efficacy, showing similar results when compared with those of rotary instruments, with the advantage of using a single-instrument (BERNARDES et al., 2016; FRUCHI et al., 2014; RIOS et al., 2014; YURUQUER et al., 2016; ZUOLO et al., 2016).

With regard to engine driven instruments, different types of alloys may be used during the instrument manufacturing process (GUTMANN et al., 2012; SHEN et al., 2013; ZHOU et al., 2012). Thermomechanical treatment of NiTi files has enhanced their performance in curved canals due to their increased flexibility and resistance to cyclic fatigue (SHEN et al., 2013). During the heating process, NiTi wire in the austenite phase is transformed into the R-phase, an intermediate phase formed during the transformation from martensite to austenite, and reverse transformation on cooling, resulting in M-wire instruments. Whereas, CM-wire is manufactured by a special thermomechanical process that controls the memory of the material, and is mainly in the martensite phase. CM-wire does not have shape memory as opposed to what is found with conventional NiTi files, however CM-wire files are extremely flexible, which reduces procedural errors and risk of instrument fracture during preparation of curved canals (COLTENEENDO, 2016; SHEN et al., 2013).

Irrespective of the technique or instrument used for re-instrumentation during retreatment, studies have shown that is not possible to achieve complete removal of the filling material (BERNARDES et al., 2016; BETTI et al., 2009; BRAMANTE et al., 2010; CAVENAGO et al., 2014; FRUCHI et al., 2014; HAMMAD et al., 2008; RIOS et al., 2014; RODIG et al., 2012; RODIG et al., 2014; TAKAHASHI et al., 2009; YURUKER et al., 2016; ZUOLO et al., 2013; ZUOLO et al., 2016), so that residues are frequently found, especially in the apical portion of the root canal (CAVENAGO et al., 2014; KELES et al., 2015; RODIG et al., 2012; UNAL et al., 2009). To overcome this clinical challenge, the association of techniques, such as the combination of reciprocating and rotary instruments or rotary instruments and hand files has been suggested (RODIG et al., 2012; YURUKER et al., 2016). The use of additional procedures, such as passive ultrasonic irrigation (PUI) and finishing instruments has also been used to improve removal of remnant filling material (ALVES et al., 2016; BERNARDES et al., 2016; CAVENAGO et al., 2014; GRISCHKE et al., 2014).

Conventional root canal irrigation promotes mechanical flushing and chemical action of the solution (VAN DER SLUIS et al., 2010). However, the flushing action from a syringe is unsatisfactory for removing debris from the root canal, and thus requires an irrigant activation technique to provide movement of the irrigants (ALVES et al., 2016; GRISCHKE et al., 2014; KATO et al., 2016; KLYN et al., 2010; MUNOZ; CAMACHO-CUADRA, 2012; PLOTINO et al., 2007; TOPCUOGLU et al., 2015;

TOWNSEND; MAKI, 2009; VAN DER SLUIS et al., 2007; VAN DER SLUIS et al., 2010). Passive ultrasonic irrigation is performed with the aid of an oscillating instrument, placed in the center of the root canal, for activating the irrigant and improving cleaning of the root canal space (VAN DER SLUIS *et al.*, 2007). This procedure increases disinfection, improves smear layer and debris removal, and has been shown to be useful in retreatment because of its ability to remove filling material residues (ALVES et al., 2011; BERNARDES et al., 2016; CAVENAGO et al., 2014; GRISCHKE et al., 2014; MUNOZ; CAMACHO-CUADRA, 2012; VAN DER SLUIS et al., 2007; VAN DER SLUIS et al., 2010). Recently, other activation system devices have also been studied with the purpose of overcoming some complications of PUI, such as the potential to cut canal walls during ultrasonic irrigation (RETSAS et al., 2016; VAN DER SLUIS et al., 2007). EasyClean is a new agitation device made of acrylonitrile butadiene styrene (ABS) plastic, with size 25/.04 and aircraft wing-shaped cross section; it presents minimum risk of deforming the canal walls, thus allowing its use up to the working length. A previous study showed that EasyClean used for irrigant agitation promoted cleaner walls when compared with PUI in the apical third of curved canals (KATO et al., 2016). Furthermore, when EasyClean was used in continuous rotation for irrigant agitation, more efficacy in cleaning isthmus area and root canal walls was achieved compared to its use in reciprocating motion (DUQUE et al., 2017).

When the filling material has not been completely removed, filling remnants may hinder the action of antimicrobial agents against bacteria, usually organized in the form of biofilm, located in some areas within the root canal system (CAVENAGO et al., 2014; RICUCCI, 2009). This persistent presence of bacteria in the root canal areas not affected by antimicrobial agents may lead to post-treatment apical periodontitis and failure of endodontic treatment (RICUCCI et al., 2009).

One microorganism frequently found in cases of endodontic treatment failure is *Enterococcus faecalis* (ROÇAS et al., 2004). This bacterium, a Gram-positive facultative anaerobe, is associated with root-filled teeth with persistent periradicular disease, because of its ability to form biofilms, invade dentinal tubules, and remain viable within the tubule (CHIVATXARANUKUL et al., 2008; GEORGE et al., 2005; Love, 2001). It can also adapt to environmental changes and persist in harsh environment conditions, which make this microorganism resistant to several intracanal medicaments and irrigating solutions used in endodontics (BASMACI et al., 2013; DISTEL et al., 2002; GEORGE et al., 2005; ORSTAVIK; HAAPASALO, 1990). Therefore, several disinfectant agents have been studied with the purpose of finding efficient antimicrobial agents capable of eliminating persistent microorganism in endodontic infections (HIRAISHI et al., 2010; SHRESTHA et al., 2009; SHRESTHA et al., 2010; WU et al., 2014).

At present, increasing interest in studying nanotechnology has emphasized the potential of using nanoparticles in endodontic therapy. Nanoparticles have unique physicochemical properties, such as ultrasmall sizes, large surface area/mass ratio and increased chemical reactivity, offering prospects of treating and preventing dental infections (SHRESHTA; KISHEN, 2016). Their application in endodontics includes forms of solutions for irrigation, intracanal medication and additives incorporated into sealers to improve root canal disinfection by disrupting biofilms and reducing adherence of *E. faecalis* (SHRESHTA; KISHEN, 2016). Silver nanoparticles are commonly used due to their capacity of adhering and penetrating into the bacterial cell wall, leading to disintegration of bacterial cell membrane and cell wall permeability and disturbing cell function by releasing silver ions (RAI et al., 2012). This compound has shown to be efficient in inhibiting biofilm formation and in the treatment and prevention of drug resistant microorganisms, with the advantage of showing biocompatibility, especially in lower concentrations (GOMES-FILHO et al., 2010; RAI et al., 2012; TAKAMIYA et al., 2016; WU et al., 2014).

Therefore, the aim of this study was to evaluate methods for the removal of filling during endodontic retreatment; and to assess the antimicrobial action of an irrigating solution containing silver nanoparticles. For this purpose, the study was performed in three steps:

1. Evaluation of instruments made of CM-wire, M-wire and NiTi, used in reciprocation and rotary motion sequentially, in the removal of filling material from curved canals, by using micro-computed tomography;
2. Scanning electron microscopy (SEM) evaluation of the performance of a new irrigant agitation device and passive ultrasonic irrigation in the removal of residual filling material after the re-instrumentation of curved

canals in endodontic retreatment;

3. Evaluation of the antimicrobial activity of a silver nanoparticle irrigant against *Enterococcus faecalis* biofilm, and against this microorganism when the irrigant was placed in the infected intratubular dentin, compared with NaOCl and chlorhexidine solutions, by means of confocal laser scanning microscopy (CLSM) in two different situations: in the first: the microorganism was protected from antimicrobial agents by the biofilm matrix; and in the second, the bacteria were sheltered by their location within the dentinal tubules.

# 2 ARTICLES

# 2 ARTICLES

## 2.1 Article 1 – **Efficacy of CM-Wire, M-Wire, and Nickel-Titanium Instruments for Removing Filling Material from Curved Root Canals: A Micro-Computed Tomography Study**

# Efficacy of CM-Wire, M-Wire, and Nickel-Titanium Instruments for Removing Filling Material from Curved Root Canals: A Micro–Computed Tomography Study

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## Abstract

**Introduction:** The aim of this *ex vivo* study was to evaluate the removal of filling material after using CM-wire, M-wire, and nickel-titanium instruments in both reciprocating and rotary motions in curved canals. **Methods:** Thirty maxillary lateral incisors were divided into 9 groups according to retreatment procedures: Reciproc R25 followed by Mtwo 40/.04 and ProDesign Logic 50/.01 files; ProDesign R 25/.06 followed by ProDesign Logic 40/.05 and ProDesign Logic 50/.01 files; and Gates-Glidden drills, Hedström files, and K-files up to apical size 30 followed by K-file 40 and K-file 50 up to the working length. Micro–computed tomography scans were performed before and after each reinstrumentation procedure to evaluate root canal filling removal. Statistical analysis was performed with Kruskal-Wallis, Friedman, and Wilcoxon tests ($P < .05$). **Results:** No significant differences in filling material removal were found in the 3 groups of teeth. The use of Mtwo and ProDesign Logic 40/.05 rotary files did not enhance filling material removal after the use of reciprocating files. The use of ProDesign Logic 50/.01 files significantly reduced the amount of filling material at the apical levels compared with the use of reciprocating files. **Conclusions:** Association of reciprocating and rotary files was capable of removing a large amount of filling material in the retreatment of curved canals, irrespective of the type of alloy of the instruments. The use of a ProDesign Logic 50/.01 file for apical preparation significantly reduced the amount of remnant material in the apical portion when compared with reciprocating instruments. *(J Endod 2016;42:1651–1655)*

## Key Words

Controlled memory wire, curved root canals, micro-computed tomography, reciprocating systems, root canal retreatment, rotary systems

### Significance

Complete removal of filling material is recommended to enable root canal disinfection in retreatment. Canal curvatures represent a challenging clinical situation, and reciprocating and rotary instruments have been used because they are safe and effective in removal of filling material.

When root canal treatment fails, nonsurgical retreatment should be considered a primary treatment approach because it offers a more favorable long-term outcome compared with endodontic surgery (1, 2). During retreatment procedures, the removal of all filling material is recommended to enable root canal disinfection and allow periradicular healing (2–4).

Several techniques have been used for removing root-filling materials, including hand files, burs, and rotary instruments (4–12). Nickel-titanium (NiTi) rotary systems have been used in endodontic retreatments because of their safety, efficiency, and ability to remove filling material faster than hand files (8, 13, 14). Reciprocating instruments have shown favorable results in retreatment procedures when used with a brushing motion against the root canal walls to remove filling material (8–12, 15).

Thermomechanical treatment of NiTi files provides significant benefits with regard to the efficacy and safety of endodontic instruments (16). Several thermal treatments of NiTi alloys such as M-wire and CM-wire have been used to optimize the microstructure of NiTi alloys because they have great influence on the reliability and mechanical properties of NiTi files (16–18). M-wire instruments were developed by transforming a NiTi wire in the austenite phase into the R-phase, an intermediate phase formed during the transformation from martensite to austenite on heating, and reverse transformation on cooling. CM-wire instruments are mainly in the martensite phase and have been manufactured by means of a special thermomechanical process that controls the memory of the material. This makes the files extremely flexible and resistant to cyclic fatigue and reduces procedural errors such as ledges and instrument fracture in curved canals (16, 19). Several properties of austenite NiTi and martensite NiTi are different.

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## Basic Research—Technology

CM-wire files do not have shape memory, as opposed to what is found with conventional forms of NiTi files (16).

Recently, reciprocating and rotary files, ProDesign R (25/.06) and ProDesign Logic 40/.05 (Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil), respectively, were introduced. According to the manufacturer, these files present a different hybrid design and CM heat treatment. Furthermore, the manufacturer introduced a new rotary file for apical preparation that has a CM-wire size 50 and taper 01. As far as we know, there are no studies about the use of CM-wire instruments for the removal of filling materials in retreatment procedures.

The aim of this study was to use micro–computed tomography (micro-CT) imaging to evaluate the percentage of remaining filling material in maxillary lateral incisors with apical curvature after retreatment with 3 different procedures performed sequentially. The null hypotheses were that ProDesign R and ProDesign Logic 40/.05 CM-wire files, which present more flexible alloy, would have the same ability to remove filling material during retreatment when compared with M-wire and NiTi files, and that ProDesign Logic 50/.01 file, which is a less tapered instrument made from CM-wire, would increase the removal of material in apical portion of the root canal.

## Materials and Methods

### Teeth Selection

The sample calculation was performed by using the G*Power v3.1 for Mac (Heinrich Heine, Universität Düsseldorf) by selecting the Wilcoxon–Mann-Whitney test of the *t* test family. The data of a previous study of retreatment that used uniradicular teeth (20) were used, and the effect size in the present study was established (=1.60). The alpha-type error of 0.05, a beta power of 0.95, and a ratio N2/N1 of 1 were also stipulated. A total of 8 samples per group were indicated as the ideal size required for noting significant differences. Ten samples per group were used because an additional 20% was calculated to compensate for possible accidents such as instrument separation that might lead to sample loss.

Maxillary lateral incisors with completely formed apices were used for this study. Sixty-five teeth were digitally radiographed in buccolingual and mesiodistal directions, and canal curvatures were measured in both directions according to the method of Schneider (21). Thirty teeth presenting apical curvature between 20° and 35° and 19–22 mm in length were selected.

### Root Canal Preparation and Filling

Access opening was performed by using diamond burs. The working length was established by introducing 10 K-file until its tip was visible at the apical foramen, and the working length was set 1.0 mm short of this measure. The root canals were prepared by using ProTaper Universal instruments (Dentsply Maillefer, Ballaigues, Switzerland) up to instrument F1 by using X-Smart electric motor (Dentsply Maillefer) at 300 rpm. The canals were irrigated with 1 mL 2.5% sodium hypochlorite, and a final rinse was performed with 5 mL 17% EDTA for 3 minutes. The canals were flushed with saline solution, dried with paper points, and obturated by the lateral compaction of gutta-percha cones and zinc oxide and eugenol–based sealer (Endofill; Dentsply Ind Com Ltda, Petrópolis, RJ, Brazil). Buccolingual and mesiodistal radiographs were taken to confirm that all specimens had well-compacted fillings extending to 1 mm short of the apex. Coronal accesses were sealed with temporary filling material (Coltosol; Coltene-Whaledent, Cuyahoga Falls, OH), and the teeth were stored at 37°C and 100% humidity for 30 days to allow complete setting of the sealer. After this, the samples were scanned by using the micro-CT system (SkyScan 1174v2; Bruker-microCT, Kontich, Belgium) with 50 kV, 800 mA, 1.0 step size rotation, and 19.7 $\mu$m voxel resolution. The data were elaborated by reconstruction software (NRecon v.1.6.3; Bruker-microCT), and the CTan software (Bruker-microCT) was used for measuring the volume (mm$^3$) of the radiopaque material.

### Retreatment

For retreatment procedures an operating microscope (M900; D. F. Vasconcellos, Valença, RJ, Brazil) at ×5 magnification was used. All specimens were prepared by the same operator. The teeth were divided into 3 groups, each with similar characteristics, to ensure homogeneity between the groups.

In the Reciproc group ($n$ = 10 teeth), root fillings were removed by using R25 Reciproc files (25/.08) (VDW, Munich, Germany) with the respective Reciproc program of the VDW Silver electric motor.

In the Mtwo group, reinstrumentation was performed with Mtwo rotary files size 40, 0.04 taper (VDW) by using the respective Mtwo 40/.04 program of the VDW Silver electric motor.

In the Logic 50/.01 group A, reinstrumentation was performed with ProDesign Logic 50/.01 rotary files (Easy Equipamentos Odontológicos) size 50, 0.01 taper used at 350 rpm and 1.5 N/cm torque.

In the ProDesign R group ($n$ = 10 teeth), root fillings were removed by using ProDesign R files (Easy Equipamentos Odontológicos) size 25, 0.06 taper by using the Reciproc program of the VDW Silver electric motor.

In the Logic 40/.05 group, reinstrumentation was performed with ProDesign Logic rotary files (Easy Equipamentos Odontológicos) size 40, 0.05 taper used at 500 rpm and 2.5 N/cm torque.

In the Logic 50/.01 group B, reinstrumentation was performed with ProDesign Logic 50/.01 rotary files (Easy Equipamentos Odontológicos) size 50, 0.01 taper used at 350 rpm and 1.5 N/cm torque.

In the 30 K-file group ($n$ = 10 teeth), root fillings were removed by using Gates-Glidden burs, sizes 2 and 3 (Dentsply Maillefer) in the cervical and middle thirds of the canals and with K-files size 30 (Dentsply Maillefer) up to the working length and Hedström files (Dentsply Maillefer) size 35, 40, and 45 by using the step-back technique.

In the 40 K-file group, reinstrumentation was performed with K-files size 40 (Dentsply Maillefer) up to the working length.

In the 50 K-file group, reinstrumentation was performed with K-files size 50 (Dentsply Maillefer) up to the working length.

For the Reciproc and ProDesign R instruments, reciprocating motion was used, and the files were introduced until resistance was felt and then used with 3 in-and-out pecking motions with light apical pressure. After reaching the working length, a brushing motion against the canal walls was used for both reciprocating and rotary files until no visual evidence of residual filling materials could be seen with the operating microscope and the canal walls were smooth.

An aliquot of 0.5 mL orange oil solvent (Citrol; Biodinâmica, Ibiporã, PR, Brazil) was put into the pulp chamber for 2 minutes to soften the gutta-percha at the cervical level before beginning with root-filling removal, and the same irrigation protocol was performed each time after the use of an instrument. The solvent was not used in the subsequent steps of instrumentation.

### Micro-CT Scanning Procedures

Each tooth was scanned 4 times: after the root canal filling and after the use of each instrument during the root canal retreatment. The teeth were placed in silicone molds to allow the sample to be scanned in the same position after each step and were mounted on a custom attachment in a micro-CT device. The same scanning parameters were used for all specimens. For reconstructions, the parameters



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used were exactly the same for each tooth after each scanning procedure. After binarization, the region of interest was determined as the circumferential area of the root canal. The measures of volume were performed manually in CTan software, and the end of the root apex was used as the landmark to determine the root canal areas analyzed. For each sample, the volume of filling material was calculated at 4 levels: apical 1, between 1.0 and 3.0 mm; apical 2, between 3.0 and 5.0 mm; middle third, between 5.0 and 7.0 mm; and cervical third, between 7.0 mm and up to the cementoenamel junction. The volumes were recorded, and the percentage of remnant filling material after each procedure was expressed in terms of percentage of the initial root-filling material volume.

### Statistical Analysis

Preliminary analysis of data normality was performed with the Shapiro-Wilk test, showing that the data were not normally distributed. Statistical analysis was performed with nonparametric tests. The Kruskal-Wallis test was used to compare removal of the filling material between the groups at the different levels, the Friedman test was used to compare the percentage reduction in filling material after each procedure, the Dunn test was used for post hoc analysis, and the Wilcoxon test was used to analyze the percentage reduction in material volume for intragroup comparison. The level of significance was set at $P < .05$.

## Results

Residual filling material was found in all groups after retreatment procedures. Three specimens presented complete removal of filling material: 1 in the Mtwo group, 1 in the Logic 50/.01 group B, and 1 in the 40 K-file group. Table 1 shows the median, minimum, and maximum percentage values of remaining material at the different root canal levels.

No significant difference was observed when comparing the removal of filling material by Reciproc and ProDesign R, by Mtwo and ProDesign Logic 40/.05, and by ProDesign Logic 50/.01 in the Logic 50/.01 group A and the Logic 50/.01 group B ($P > .05$) (Fig. 1).

The use of Mtwo and ProDesign Logic 40/.05 rotary files did not significantly enhance filling material removal after use of the reciprocating files at all root canal levels ($P > .05$).

The filling material remaining after the use of the ProDesign Logic 50/.01 file was significantly lower compared with the material remaining after Reciproc was used in apical 2 and ProDesign R in apical 1 and apical 2 ($P < .05$).

Apical 1 and apical 2 levels presented a larger amount of remaining filling material in comparison with the middle and cervical levels, irrespective of the group analyzed.

## Discussion

The aim of root canal retreatment is to remove as much filling material as possible in an attempt to eliminate bacterial remnants and improve the activity of irrigation solutions and intracanal medicaments on root canal dentin.

In this study, the authors used several retreatment procedures, including hand files, rotary files, and reciprocating instruments. Although the instruments were efficient in removing a large amount of gutta-percha, none of the techniques were able to completely remove the filling material from the root canal walls, which is in agreement with the literature (4–13, 15, 20, 22).

Recently, micro-CT has been used to evaluate the remaining filling material (4, 7, 9, 11, 12, 20, 22–25). Micro-CT imaging was chosen for this study because it is a nondestructive technique, enables three-dimensional assessment of the canal filling material before and after instrumentation, and provides precise quantitative evaluation of residual material, expressed in $mm^3$ (7, 11). Micro-CT analysis showed that all groups had remnants of filling material on root canal walls. Studies have shown that rotary (7, 14, 25–27) or reciprocating files (8, 11) remove more filling material, whereas some studies have reported hand files to be more effective (13, 20, 28). The present study indicated no significant difference between the use of reciprocating and rotary files compared with hand files, corroborating the findings of previous studies (5, 29). The hypothesis of the same efficacy of instruments with different CM-wire, M-wire, and NiTi alloys was confirmed. In this study, Reciproc, which is made from M-wire, had a similar performance in removing filling material compared with the ProDesign R CM-wire files. The Reciproc group showed slightly less remnant filling material compared with the ProDesign R group, but with no statistical difference. The Mtwo NiTi files and ProDesign Logic 40/.05 CM-wire files also showed no statistical difference in removing filling material, demonstrating that although CM-wire files are more flexible than M-wire and NiTi files, they showed the same ability to remove filling material during retreatment procedures.

A great advantage of using engine-driven instruments is that less time is required for filling material removal in retreatments (7–9, 13, 14, 22, 28). Furthermore, these instruments are safe and reduce both operator's and patient's fatigue (6, 9). In this investigation, Gates-Glidden burs were used in the 30 K-file group, and hand files were used in the 30 K-file, 40 K-file, and 50 K-file groups. Although these instruments were effective in removing filling material, the use of Gates-Glidden burs in a previously enlarged canal may be hazardous and often results in overextension of the preparation, weakening and thereby leading to the risk of vertical root fracture or perforation of the root canal. When re-treating curved root canals, ledges, perforations, and

**TABLE 1.** Median, Maximum, and Minimum Values of Percentage of Remaining Filling Material at Different Root Canal Levels

| Group | Apical 1 | Apical 2 | Middle | Cervical |
|---|---|---|---|---|
| Reciproc R25 | 18.42 (1.04–77.82)$^{aA}$ | 20.70 (0.25–80.00)$^{aA}$ | 1.99 (0.0–59.12)$^{aA}$ | 0.0 (0.0–15.01)$^{aA}$ |
| Mtwo 40/.04 | 2.48 (0.0–48.70)$^{aA}$ | 2.53 (0.0–19.88)$^{aAB}$ | 0.71 (0.0–12.06)$^{aA}$ | 0.0 (0.0–4.39)$^{aA}$ |
| ProDesign Logic 50/.01 | 1.48 (0.0–44.21)$^{aA}$ | 2.20 (0.0–16.76)$^{aB}$ | 0.24 (0.0–12.06)$^{aA}$ | 0.0 (0.0–4.39)$^{aA}$ |
| ProDesign R | 33.55 (0.0–77.62)$^{aA}$ | 16.83 (0.0–41.35)$^{abA}$ | 2.10 (0.0–59.54)$^{aA}$ | 0.20 (0.0–31.66)$^{aA}$ |
| ProDesign Logic 40/.05 | 4.51 (0.0–62.41)$^{aAB}$ | 0.0 (0.0–38.02)$^{aAB}$ | 0.0 (0.0–25.04)$^{aA}$ | 0.0 (0.0–16.05)$^{aA}$ |
| ProDesign Logic 50/.01 | 0.84 (0.0–36.82)$^{aB}$ | 0.0 (0.0–36.94)$^{abB}$ | 0.0 (0.0–24.41)$^{aA}$ | 0.0 (0.0–15.96)$^{aA}$ |
| Hand file 30 K | 32.84 (0.0–81.99)$^{aA}$ | 0.0 (0.0–47.72)$^{bA}$ | 0.0 (0.0–81.99)$^{aA}$ | 0.0 (0.0–1.95)$^{aA}$ |
| Hand file 40 K | 5.58 (0.0–57.95)$^{aA}$ | 0.0 (0.0–45.48)$^{aA}$ | 0.0 (0.0–34.61)$^{aA}$ | 0.0 (0.0–1.95)$^{aA}$ |
| Hand file 50 K | 5.29 (0.0–57.82)$^{aA}$ | 0.0 (0.0–41.82)$^{bA}$ | 0.0 (0.0–33.21)$^{aA}$ | 0.0 (0.0–1.95)$^{aA}$ |

Different superscript lower case letters in each column indicate statistical differences between groups ($P < .05$). Different superscript upper case letters in each column indicate statistical differences in the same group after different procedures ($P < .05$).



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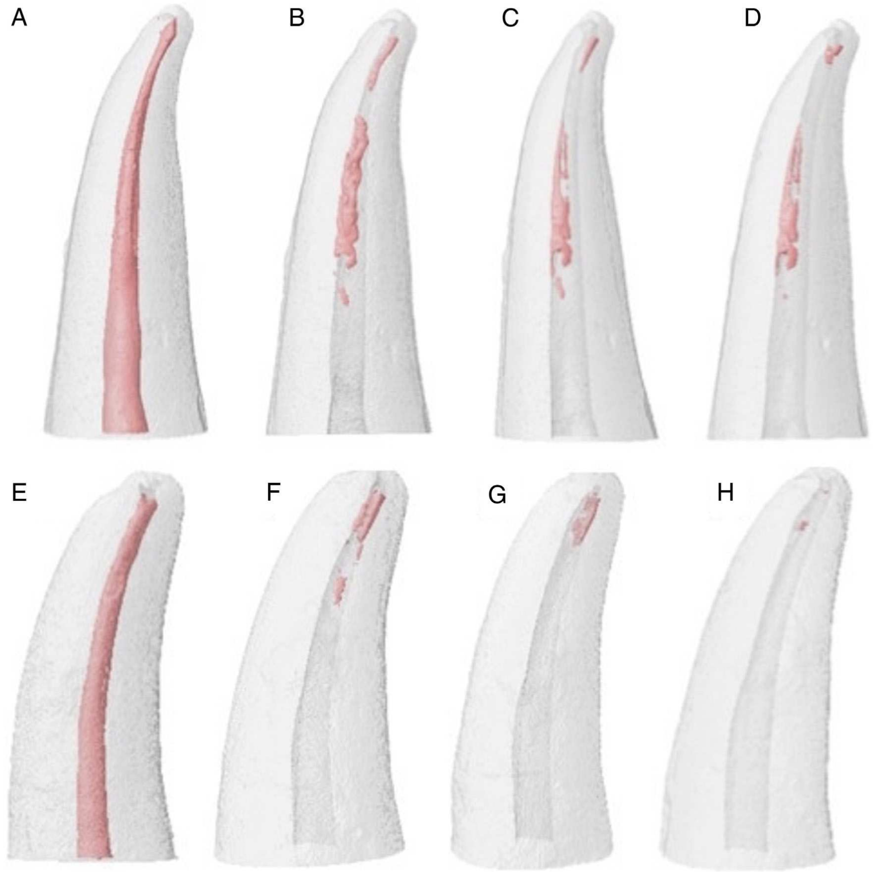


**Figure 1.** Micro-CT reconstructions of representative samples before and after each retreatment procedure. (*A* and *E*) Preoperative images, (*B*) Reciproc, (*C*) Mtwo, (*D* and *H*) ProDesign Logic 50/.01, (*F*) ProDesign R, and (*G*) ProDesign Logic 40/.05.

straightening of the inner side of the curve may occur because of the inflexibility of hand files (30).

Root canal anatomy is an important aspect to consider in nonsurgical retreatments. The majority of studies evaluating filling material removal have used straight roots to simplify specimen standardization (6, 8, 10–13). In the present study, maxillary lateral incisors with apical curvatures were used because this anatomic variation is frequently found in this group of teeth (31). Canal curvatures represent a challenging clinical situation in retreatment because alterations in root canal shape and instrument breakage may occur during reinstrumentation (30, 32). To overcome this difficulty, reciprocating and rotary instruments have been recommended because they are shown to be safe in maintaining the original canal shape in retreatment procedures when curvatures were present (9). The authors speculated that the CM-wire alloy used in the composition of some instruments used in this study may have contributed to the re-preparation of curved canals because of their characteristics such as fatigue resistance and flexibility.

In nonsurgical retreatment, the apical third is the critical zone in root canal re-preparation (30). Studies have demonstrated that the remnant filling material is more frequently found in this area (4, 7, 24, 32), which is in agreement with the present study. To achieve better cleaning up to the working length when performing reinstrumentation, procedures such as the clinical use of passive ultrasonic activation with NaOCl or solvent and the use of laser irradiation have been indicated (4, 9, 11, 24).

Because residual filling material is frequently observed, additional procedures have been used after initial reinstrumentation to improve filling removal. The combination of initial penetration with rotary instruments followed by the use of hand files for removing gutta-percha residues may be indicated because hand files provide superior tactile sense (7, 15). The use of Self-Adjusting Files also seems to improve removal of residual filling material after the use of rotary files, resulting in more effectively cleaned canals (23). In the present study, 3 specimens presented complete removal of filling material, probably because of the use of 2 additional instruments after the initial file. Under the



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experimental conditions of this study, the results demonstrated that the use of rotary systems, especially the ProDesign Logic 50/.01 file, improved the removal of filling material after the use of reciprocating instruments, suggesting that a hybrid technique associating these types of instruments is valuable in the retreatment of root canals. The hybridization of different techniques for removing filling material has shown favorable results, as demonstrated by Yuruker et al (15).

There is no consensus in literature with regard to the size to which it would be necessary to enlarge the root canal during retreatment (30). Studies have demonstrated that to obtain better apical cleaning, it is necessary to enlarge the diameter of the initial preparation with larger-sized instruments (6, 7, 11, 25). Rodig et al (7) instrumented the root canals to a final size 40 and concluded that this procedure promoted adequate enlargement, permitting conservation of tooth structure and prevention of procedural mishaps in curved canals. In addition, Alves et al (25) achieved up to 100% of filling material removal in a large number of samples after using Reciproc 40, compared with the use of Reciproc 25. In a previous study (11), a significant reduction in the amount of filling material was achieved by re-instrumenting the apical third 2 sizes beyond the initial preparation size, with final size 50. Zuolo et al (8) enlarged the root canal of maxillary central incisors to a final size 50 and observed better removal of filling material with Reciproc R50 when compared with hand and rotary files. However, Reciproc R50 presents a large taper 0.05, and it could lead to excessive removal of dentin. The ProDesign Logic 50/.01 instrument used in this investigation significantly reduced the amount of filling material in apical third compared with reciprocating files and seemed adequate for instrumenting and removing filling material in the apical portion without weakening the tooth structure. Moreover, this instrument was shown to be safe, because no instrument broke during root canal reinstrumentation in this study. Thus, the null hypothesis that ProDesign Logic 50/.01 files improved the removal of filling material in apical portion of the root canal was confirmed.

## Conclusions

The combination of reciprocating and rotary files in the retreatment of curved canals, irrespective of the type of alloy of the instruments, was efficient but did not remove root canal filling completely. The use of a ProDesign Logic 50/.01 CM-wire file significantly reduced the residual material at the apical levels compared with the use of reciprocating files.

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

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**2.2 Article 2 - Comparison of two methods of irrigant agitation in the removal of residual filling material in retreatment.**

*The article presented in this Thesis was written according to the Brazilian Oral Research instructions and guidelines for article submission*

**ABSTRACT**

The aim of this study was to compare the efficacy of passive ultrasonic irrigation and EasyClean for removing residual filling material in retreatment. Twenty-two maxillary lateral incisors with apical curvature were instrumented with ProTaper files and filled with Endofill using lateral compactation technique. Removal of filling material was performed with Reciproc, Mtwo and ProDesign Logic 50/.01 files. The teeth were inserted in a silicone mould, which was placed in a metal muffle, and split to visualize the residual filling material. The samples were divided into two groups ($n = 11$) according to the irrigation protocol: Passive ultrasonic irrigation (PUI group) with 3 activations of 20 seconds and EasyClean (Easy Equipamentos Odontológicos, Belo Horizonte, Brazil) (EC group) used in continuous rotation with 3 activations of 20 seconds, both using NaOCl and EDTA. Environmental scanning electron microscopic images were taken of apical, middle and cervical thirds before and after the irrigant activation. Kappa test was used to determine interexaminer agreement. Statistical analysis was performed using Kruskal-Wallis, Mann-Whitney and Wilcoxon tests ($P < 0.05$). PUI and EC improved the removal of remnant filling material in all root canal thirds ($P<0.05$). PUI and EC presented similar performance in the final step of retreatment ($P>0.05$). No significant difference was observed in the removal of filling material in apical, middle and cervical thirds in both groups ($P>0.05$). EasyClean in continuous rotary motion is useful in retreatment and showed to be as effective as ultrasonic activation in the removal of remnant filling material.

**Keywords**: Retreatment; Root canal Irrigants; Root canal obturation.

## INTRODUCTION

During endodontic retreatment, removal of filling material can be challenging, since remnants of filling material persist irrespective of the technique and instruments used in reinstrumentation[1-5]. Removal of filling remnants is even more difficult in the apical portion of the root canal or when curvatures are present[2-4,6-9]. However, the complete removal of filling material is ideal because it allows a better disinfection through the action of the irrigating solution and better adaptation of the new filling material to the canal walls[2].

Irrigation of the root canal provides both mechanical flush of debris and the chemical action of the solution[10]. Nevertheless, the flushing action from syringe irrigation is not sufficient to remove debris from the root canal[10-12]. Therefore, several irrigation activation techniques have been proposed to improve the efficacy of irrigating solutions by promoting continuous movement of the irrigants[4,10,13-18].

Passive ultrasonic irrigation (PUI) is the activation of an irrigant using an oscillating instrument placed in the center of the root canal, which induces acoustic streaming and/or cavitation of an irrigant. It has been used to improve the cleaning of the root canal space, by increasing disinfection, smear layer and debris removal, even in difficult anatomical areas[10,11,15,19].

Recently, a new agitation device called EasyClean (Easy Equipamentos Odontológicos, Belo Horizonte, Brazil) has been introduced. It consists of an acrylonitrile butadiene styrene (ABS) plastic instrument, which has a size of 25/.04 and an “aircraft wing” shaped cross section, and is recommended for use in reciprocating motion[18]. A previous study showed that irrigant agitation with EasyClean promoted cleaner walls compared to PUI by removing debris in the apical portion of curved canals[18].

It has been reported that the use of PUI in retreatments provides an enhancement in the removal of filling material remnants after the reinstrumentation[2,5,16]. However, there is no study regarding the efficacy of EasyClean in the removal of filling material from the root canals in retreatment.

The aim of this study was to compare the use of PUI and EasyClean used in a rotary movement in the removal of residual filling material from curved canals during retreatment. The null hypothesis was that there would be no significant difference in filling material removal between ultrasonic activation and the use of EasyClean.

## METHODOLOGY

### Teeth selection and root canal preparation and filling

The sample calculation was performed using the G*Power v3.1 for Mac (Heinrich Heine, Universität Düsseldorf) by selecting the Wilcoxon-Mann Whitney test of the T test family. The data of a previous study of retreatment that used uniradicular teeth[20] was used and the effect size in the present study was established (=1.60). The alpha type error of 0.05, a beta power of 0.95, and a ratio N2/N1 of 1 were also stipulated. A total of 08 samples per group were indicated as the ideal size required for noting significant differences. Eleven samples were used, considering a 30% risk of sample loss.

Ethical approval was granted by the local institutional ethics committee (protocol no. 075792/2016). Sixty-five maxillary lateral incisors with completely formed apices were used for this study. The teeth were digitally radiographed and canal curvatures were measured in buccolingual and mesiodistal directions according to Schneider's method[21], and twenty-two teeth presenting apical curvature between 20º and 35º were selected. Coronal access was performed using diamond burs. A 10 K-file was introduced until its tip was visible at the apical foramen and the working length was set 1.0 mm short of this measure. The canals were prepared with ProTaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) up to F1 instrument, by using X-Smart electric motor (Dentsply Maillefer) at 300 rpm. Irrigation was performed with 1 mL of 2.5% sodium hypochlorite and a final rinse with 5 mL of 17% EDTA for 3 minutes. The root canals were flushed with saline solution, dried with paper points and obturated by the lateral compaction of gutta-percha cones and Endofill sealer (Dentsply Ind Com Ltda, Petrópolis, RJ, Brazil). Buccolingual and mesiodistal radiographs were taken to confirm the quality of canal fillings. Coronal accesses were sealed with temporary filling material (Coltosol; Coltene-Whaledent, Cuyahoga Falls, OH), and the teeth were stored at 37ºC and 100% humidity for 30 days to allow complete setting of the sealer.

**Retreatment**

For retreatment procedures, all specimens were prepared by the same operator with the aid of an operating microscope (M900; D.F Vasconcellos, Valença, RJ, Brazil) at X5 magnification. For each sample, the removal of filling material was performed in three steps: initially, fillings were removed using R25 Reciproc files (25/.08) (VDW, Munich, Germany) with the respective Reciproc program of the VDW Silver electric motor; then, reinstrumentation was performed with Mtwo rotary files size 40, 0.04 taper (VDW) using the respective Mtwo 40/.04 program of the VDW Silver electric motor; finally, another reinstrumentation was carried out with ProDesign Logic 50/.01 rotary files (Easy Equipamentos Odontológicos) size 50, 0.01 taper, used at 350 rpm and 1.5N/cm torque. For both reciprocating and rotary files, a brushing motion against the canal walls was used after reaching the working length, until no visual evidence of residual filling materials could be seen and the canal walls were smooth. Before beginning root filling removal, an aliquot of 0.5 mL of orange oil solvent (Citrol, Biodinâmica, Ibiporã, PR, Brazil) was put into the pulp chamber for 2 minutes to soften the gutta-percha. The solvent was not used in the subsequent steps of instrumentation.

**Sample preparation for environmental scanning electron microscopy**

After retreatment procedures, the teeth were decoronated and two longitudinal grooves were made in buccal and palatal walls with a diamond disc in order to facilitate the root split. Before splitting the roots, the samples were embedded in a two-piece metal muffle containing silicone material (Figure 1). A silicone mould was made inside the muffle to allow the teeth to be inserted in the same position during the irrigation protocol, without extruding the irrigants. Thus, the samples could be analyzed before and after the irrigation protocol to compare the removal of filling material remnants. After the silicone set, the muffle was disassembled, the teeth were removed and the roots were split by applying a vertical force using a 24 spatula. Both halves were viewed under a stereomicroscope (Stemi 2000C; Carl Zeiss, Jena, Germany) at a magnification of 5.5X in order to choose the half with greater amount of remnant filling material. With a ¼ high-speed bur, three marks were made laterally to the root canal, in the selected half of the teeth, in order to define the position of apical, middle and cervical thirds during image acquisition. Preoperative images were taken of apical, middle and cervical thirds by environmental scanning electron microscopy (Aspex Express; Fei Europe, Eindhoven, Netherlands), at an accelerating voltage of 15-20 kV and a standard magnification of 750X. Statistical analysis using Mann-Whitney test were performed using all the preoperative images in order for the samples to be divided into two groups according to the amount of filling material. This procedure allowed the homogeneity of the samples between the groups, since no significant difference was observed regarding the amount of remaining filling material between the two groups.

**Irrigant activation**

The teeth were placed in the silicone moulds and mounted in the muffle, and the irrigation activation protocol was performed for the different experimental groups as follows:

**PUI Group** (*n*=11): passive ultrasonic irrigation using an ultrasonic E1-Irrisonic Tip with size 20, 0.01 taper and no cutting blades (Helse Dental Technology, Santa Rosa de Viterbo, SP, Brazil), mounted on an ultrasonic unit (NSK multi-task ultrasonic system; Nakanishi Inc., Tochigi, Japan) set to power 2. The ultrasonic activation was performed as described by van der Sluis et al.[10]. The ultrasonic tip was placed 1mm short of the working length and activated by 3 cycles of 20 seconds with each irrigating solution, which were 5 ml 2.5% NaOCl, 5 ml 17% EDTA and 1.0% NaOCl, respectively. All the irrigation solutions were renewed after each activation and a final flush with 5 ml of saline was performed.

**EasyClean Group** (*n*=11): continuous irrigant activation using EasyClean, coupled to a micromotor and a contra-angle, in low speed, at approximately 20000 rotations per minute (KaVo Kerr Group, Charlotte, NC, USA). The EasyClean tip was placed 1mm short of the working length and the same sequence of the irrigating solutions and irrigation times described for the PUI group were used.

The halves were removed from the moulds and taken to the environmental scanning electron microscope for postoperative images. All images were saved in digital file in TIFF format and loaded into the Microsoft PowerPoint software (Microsoft Corporation, Redmond, WA). In order to quantify the residual filling material, the images were divided into 100 squares by using a digital grid. Each square represented an area of 27.5 X 27.5 $\mu m^2$ (Figure 2). The number of squares with visible dentinal tubules, i.e., without residual filling material, was calculated by placing the digital grid over the images displayed in slide format on an LCD monitor. Two single examiners, previously calibrated and blind to the study, classified preirrigation and postirrigation images.

**Statistical analysis**

Kappa test was applied to determine the interexaminer agreement. Preliminary analysis of data normality obtained with the Shapiro-Wilk and D'Agostino & Person tests showed that the data were not normally distributed. The Wilcoxon test was used to analyze percentage of clean areas before and after irrigation procedures in each group; Mann-Whitney *U*-test was used to compare removal of the filling material between the groups at the different levels; Kruskal-Wallis was used to compare the removal of filling material between the three thirds in the same group. The level of significance was set at $P < 0.05$, and Prisma 5.0 software (GraphPad Software Inc, La Jolla, CA, USA) was used as the analytical tool.

**RESULTS**

The interexaminer agreement was very high (kappa=0.90).

Passive ultrasonic activation and continuous irrigant agitation with EasyClean increased removal of filling material significantly ($P<0.05$) in all thirds of the root canal. The median, minimum and maximum values of percentage of cleanliness of the root canal walls in the apical, middle and cervical thirds are shown in table 1.

No significant difference was observed in the performance of PUI and EC regarding the removal of residual filling material ($P>0.05$). There was no difference in the removal of remnant filling material when comparing the apical, middle and cervical thirds in both PUI and EC groups ($P>0.05$).

**DISCUSSION**

In nonsurgical retreatment, the complete removal of filling material is intended because when dentinal tubules are clean, intracanal medicaments and sealers may act more effectively in the root canal space[2,5].

Nevertheless, to date, none of the retreatment protocols studied has provided complete filling material removal from the root canal[1-5,8,9,16]. To overcome this clinical challenge, several supplementary treatments have been proposed to promote a better cleaning after reinstrumentation, such as the use of Self Adjusting File[1,22], laser irradiation[8], a NiTi instrument XP-endo Finisher[4] and the use of ultrasonics and solvents[2,23]. However, solvents tend to lead to more gutta-percha and sealer remnants on the root canal walls and inside dentinal tubules, and should only be recommended if the working length cannot be achieved without a solvent[24]. The use of PUI with NaOCl has also been recommended in retreatment as a final step because this procedure enhances the elimination of residual filling material[2,5,16], which is in agreement with the results of our study. In the present study, PUI was performed with NaOCl and EDTA because there was no intention of evaluating the chemical action of the solvent, only the mechanical action of the agitation devices. Despite these findings, no significant difference in removing filling residues after PUI has also been reported[3,25,26].

Some anatomical features may negatively influence the PUI performance, such as narrow and less tapered root canals[27,28]. In teeth with canal curvatures, PUI has a lower efficacy compared to straight canals because when a curvature is present, the ultrasonic tip may touch the canal walls, restricting its vibratory motion and reducing ultrasonic action[29].

Although mechanical activation of irrigants provides several benefits in root canal treatment, it has been reported that PUI promotes more effective cleaning of intermediate portions of the canal than of the last few millimeters[18]. On the other hand, a previous study[5] showed that PUI improved the filling material removal including the apical third, demonstrating that the clinical use of ultrasonic activation is useful for removing filling material in retreatment. In this study, the removal of filling material residues was analyzed in cervical, middle and apical third, and no significant difference was observed in the cleaning of the three thirds. The effective cleaning of the apical curvature area in this study could have been caused by the fact that the apical enlargement of the canals was performed up to a size 50, which created sufficient space for the ultrasonic tip (size 20) to vibrate freely in the irrigant. Previous studies also demonstrated better cleaning after PUI in wide and great tapered canals[27,28]. Therefore, the clinical use of ultrasonic activation in retreatment may be suggested after root canal reinstrumentation with a suitable apical enlargement.

During ultrasonic irrigation, the tip of the instrument has the potential to cut canal walls during its activation[11,30]. It may lead to accidents such as uncontrolled removal of dentin, canal deviation, apical zipping and even root perforation when an ultrasonic tip is activated in a curved canal[14,30]. To prevent this from happening, the use of smooth wires is preferable[11]. In this study, a non-cutting Irrisonic tip was used, placed in the center of the canal, avoiding touching root canal walls. Recently, different mechanical non-cutting devices have also been developed. EasyClean is made of ABS plastic and presents minimum risk of deforming the canal walls, thus allowing its introduction up to the working length[18]. The manufacturer recommends its use in reciprocating motion, in order to avoid the instrument threading and consequent fracture[31]. However, a recent study suggested the use EasyClean in continuous rotation at low speed, and greater efficacy in dentin debris removal from the root canal and isthmus area in mesial roots of mandibular molars was achieved compared to its use in reciprocation motion[32]. In this study, the authors incorporated a continuous rotary motion in EC in an attempt to promote a vigorous irrigant agitation and increase filling material removal that was attached to the root canal walls. EasyClean showed significant removal of filling material remnants after reinstrumentation, with no difference when compared to ultrasonic activation in all root canal thirds. Therefore, the null hypothesis was confirmed.

There is no consensus in literature about the irrigation protocol required for effective irrigant activation in removing smear layer and debris from the root canal. Several irrigation protocols were also described in retreatment procedures. Muller et al[25] used 60 seconds of PUI in teeth filled with AH Plus and gutta-percha, and reported that this time was insufficient to dislodge filling residues from the root canal walls. However, in the cited study, no replenish of solutions was performed during or after ultrasonic activation. In the present study, the solution was activated by 3 cycles of 20 seconds, with the renewal of the irrigant, totalizing 1 minute for each irrigant solution in both ultrasonics and EasyClean. The action of the 3 refreshment/activation cycles produces a cumulative effect and has shown to be effective in removing additional dentin debris[10,18]. Based on the results from the current and previous studies[5], this protocol seems to be useful in retreatment procedures as well, since it improved the removal of residual filling material after reinstrumentation.

The use of ESEM in this investigation allowed the same sample to be analyzed before and after the irrigant activation procedure, which eliminates interferences of anatomical variations and characteristics of the dentin present in different specimens, resulting in more consistent assessment of results. Due to the possibility of acquisition of pre- and postoperative images, no control group is required for comparing the amount of filling material between groups. Furthermore, ESME is a nondestructive method because the metallization process is not necessary such as in conventional SEM images, which allows it to be reused and evaluated in the same area[18,33].

**CONCLUSIONS**

None of the agitation methods completely removed the residual filling material. Although the ultrasonic agitation favored greater area of removed material, the difference was not significant in relation to the EasyClean agitation.

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## FIGURE LEGENDS

**Figure 1**

A: Two-piece metal muffle used to make the silicone mould. B: Sample inserted in the silicone mould to allow the two halves to be reassembled before and after the irrigant activation.

**Figure 2**

Representative preoperative image of a sample before irrigant activation with the digital grid overlapped used for quantification of clean areas. The picture shows that remnant filling material persisted in some areas after the reinstrumentation in retreatment procedure.

**Table 1**: Median, maximum and minimum values of the percentage of clean areas before and after irrigation procedures at different root canal levels.

| Group | Apical | Middle | Cervical |
|---|---|---|---|
| US pre | 1 (0 – 23)$^{A}$ | 7 (0 – 40)$^{A}$ | 18 (0 – 95)$^{A}$ |
| US pos | 73 (2 – 100)$^{B}$ | 87 (42 – 98)$^{B}$ | 94 (4 – 100)$^{B}$ |
| Difference pre-post US | 72 (2 – 96)$^{a1}$ | 74 (37 – 90)$^{a1}$ | 60 (4 – 91)$^{a1}$ |
| | | | |
| EC pre | 7 (0 – 24)$^{A}$ | 14 (0 – 78)$^{A}$ | 4 (0 – 95)$^{A}$ |
| EC pos | 47 (21 – 80)$^{B}$ | 78 (40 – 100)$^{B}$ | 80 (2 – 100)$^{B}$ |
| Difference pre-post EC | 36 (1 – 73)$^{a1}$ | 48 (19 – 100)$^{a1}$ | 59 (2 – 96)$^{a1}$ |

Different superscript capital letters in each column indicate statistical differences in the same group. ($P<0.05$)
Different superscript lowercase letters in each column indicate statistical differences between groups. ($P<0.05$)
Different superscript numbers in each row indicate statistical differences in each row in root canal thirds. ($P<0.05$)

**Figure 1**

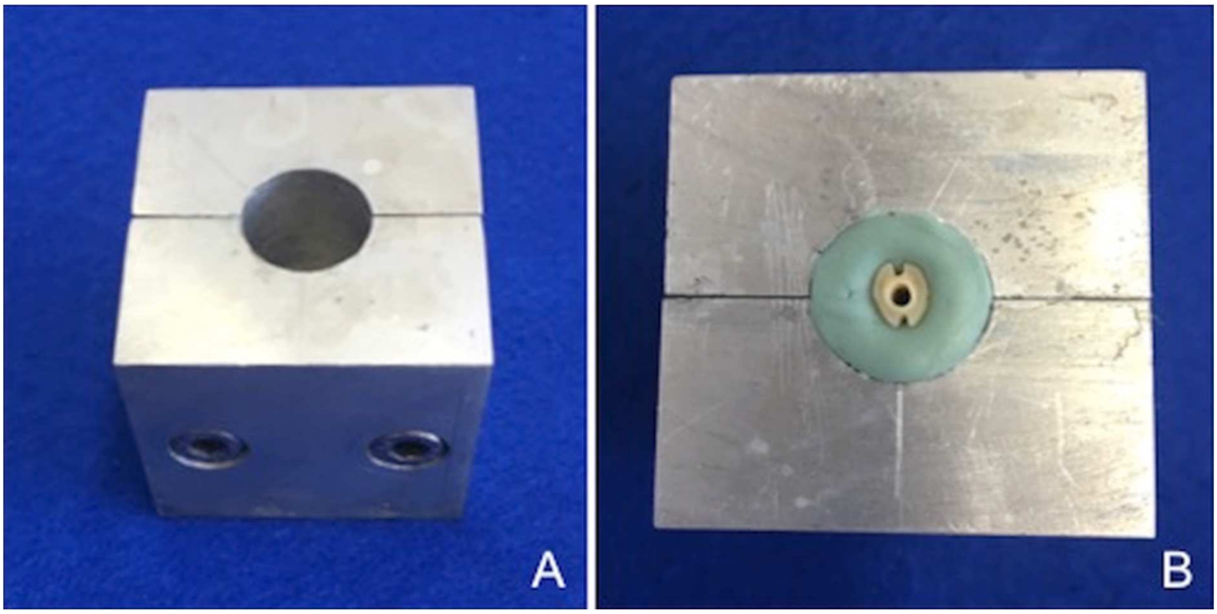
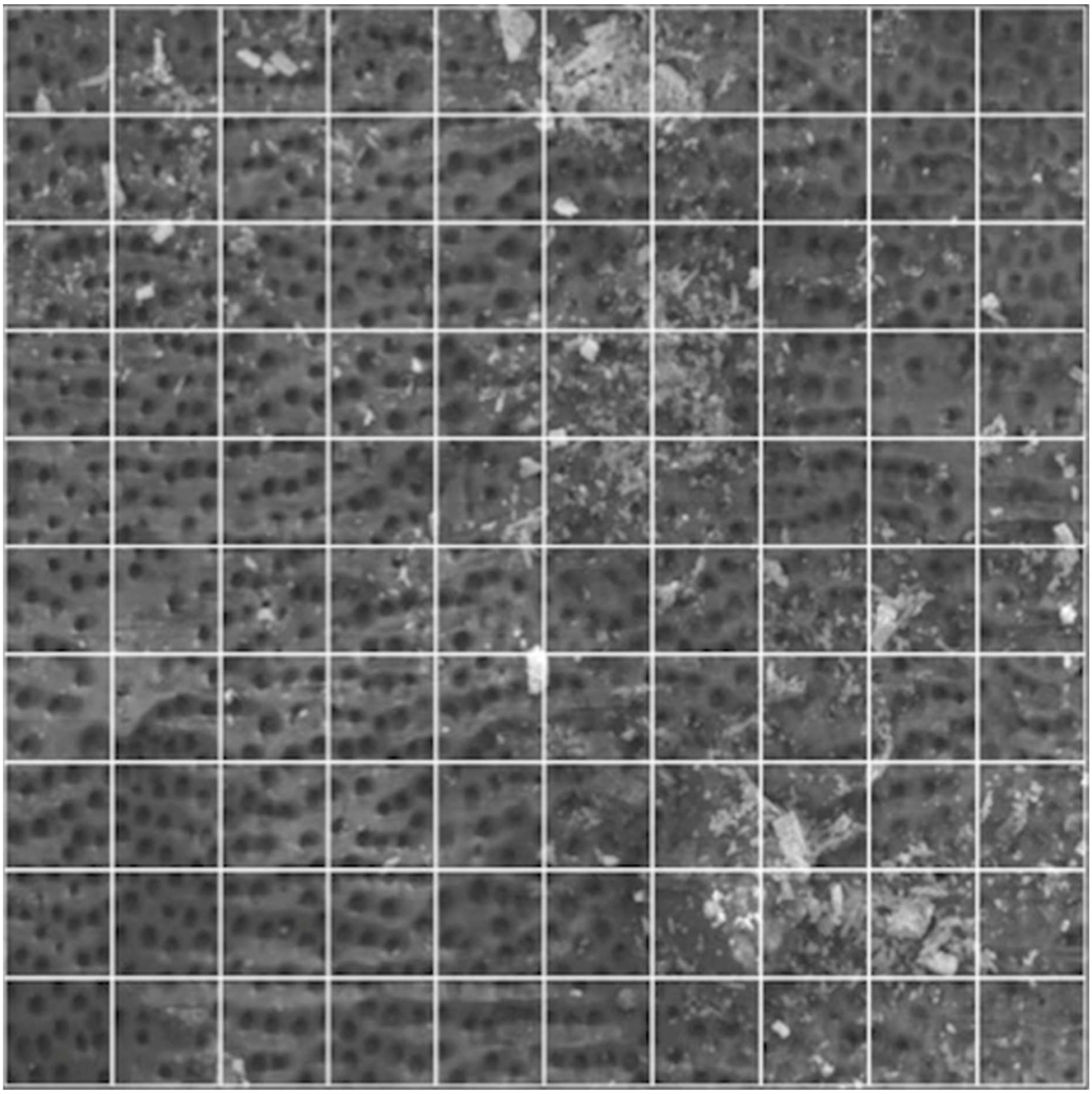


**Figure 2**



## 2.3 Article 3 - Antibacterial properties of silver nanoparticle as a root canal irrigant against *Enterococcus faecalis* biofilm and infected intratubular dentin.

*The article presented in this Thesis was written according to the International Endodontic Journal instructions and guidelines for article submission*


### ABSTRACT

**Aim** To evaluate the antimicrobial action of an irrigant containing silver nanoparticles (AgNp) against *Enterococcus faecalis* biofilm and infected intratubular dentin.

**Methodology** Dentin blocks were used for *E. faecalis* biofilm development for 21 days and irrigated with AgNp solution, 2.5% NaOCl and 2% chlorhexidine for 5, 15 and 30 minutes, analyzed by confocal laser scanning microscope (CLSM). For infection of intratubular dentin with *E. faecalis*, dentin tubules were made from bovine incisors and submitted to a contamination protocol performed for five days, with 8 centrifugation cycles on switched days. The infected dentin was irrigated with the same irrigation solutions and time intervals described for biofilm, and also analyzed by CLSM. Statistical analyses were performed using Kruskal-Wallis and Dunn tests and Mann-Whitney *U*-test ($P < 0.05$).

**Results** The AgNp solution eliminated fewer bacteria, but showed more ability to dissolve biofilm compared with chlorhexidine ($P < 0.05$). NaOCl presented the highest antimicrobial activity and biofilm dissolution capacity. AgNp solution showed less antimicrobial action in infected intratubular dentin compared with NaOCl ($P < 0.05$). AgNp solution for 5 minutes was more effective against planktonic bacteria in dentinal tubules than in biofilm, but in the time interval of 30 minutes fewer viable bacteria were observed in biofilm compared with intratubular dentin ($P < 0.05$).

**Conclusions** AgNp irrigant was not as effective *against E. faecalis* as solutions commonly used in endodontic therapy. Longer periods in contact with the AgNp solution are required to eliminate bacteria in biofilm and infected intratubular dentin.


**Keywords** Biofilm, *Enterococcus faecalis*, Infected dentin, Silver Nanoparticles.

## INTRODUCTION

Microorganisms and their by-products are the major cause of pulp and periapical pathogenesis (Kakehashi *et al.* 1965, Ricucci & Siqueira 2010). Therefore, bacteria need to be eliminated from root canal system for long-term success of root canal treatment (Siqueira & Roças 2008, Ma *et al.* 2011).

*Enterococcus faecalis* is the most frequently isolated bacteria from root canals with persistent periapical disease (Roças *et al.* 2004). This microorganism is a Gram-positive facultative anaerobe capable of adapting to harsh environmental changes. It is able to attach itself to dentin, invade dentinal tubules and remain viable within the tubules for prolonged periods of time (Love 2001, Chivatxaranukul *et al.* 2008, Arias-Moliz *et al.* 2009, Basmaci *et al.* 2013). An important characteristic of *E. faecalis* is biofilm formation (Afkhami *et al.* 2015), organized structures embedded in an exopolysaccharide matrix (Costertton 1999) that may contribute to bacterial resistance to several root canal irrigants and medicaments (Kishen 2012, Basmaci *et al.* 2013).

Root canal disinfection includes mechanical cleaning and irrigation using solutions with antimicrobial potential (Nair *et al.* 2005). Sodium hypochlorite is the most recommended root canal irrigant, because of its antimicrobial efficacy and tissue dissolution capacity (Haapasalo *et al.* 2010). However, direct application of sodium hypochlorite can be potentially harmful to the host (Bramante *et al.* 2015, Afkhami *et al.* 2017). As a root canal irrigant, Chlorhexidine has a wide range of activity against both Gram-positive and Gram-negative bacteria, antibacterial substantivity in dentin and acceptable biocompatibility. Nevertheless, it has a significantly lower ability to dissolve biofilms compared with sodium hypochlorite (Mohammadi *et al.* 2009).

To improve the characteristics of the antibacterial agents used in endodontic therapy, innovative antimicrobial delivery systems have been developed, such as nanoparticles (Samiei *et al.* 2016). Nanomaterial is defined as particles with external dimensions of 1-100 nm, presenting small sizes, large surface/area mass ratio and increased chemical reactivity (Rai *et al.* 2012, Shrestha *et al.* 2016). The higher surface area and charge density of nanoparticles enables them to interact to a greater extent with the negatively charged surface of bacterial cells, resulting in great antimicrobial activity (Shi *et al.* 2006, Kishen *et al.* 2008) thus, they have been applied in many health care fields (Silver *et al.* 2006, Chen *et al.* 2008). Silver nanoparticles are capable of attaching to and penetrating into the both Gram-positive and Gram-negative bacteria cell wall, disturbing cell function by releasing silver ions; thus they are used for the treatment and prevention of drug-resistant microorganisms and inhibition of the biofilm formation (Rai *et al.* 2012).

In dental practice, silver nanoparticles have been used in several forms: focused on their antimicrobial effects, they have been incorporated into bonding agents and restorative materials to prevent biofilm formation and reduce caries (Durner *et al.* 2011, Garcia Contreras *et al.* 2011, Cheng *et al.* 2012, Cheng *et al.* 2013); orthodontic adhesives (Ahn *et al.* 2009, Degrazia *et al.* 2016) and into implant materials (Sheik *et al.* 2010, Allaker *et al.* 2014). Nanoparticles have also been studied in the endodontic field in an attempt to reduce adherence of *E. faecalis* to dentin; eliminate biofilms (Kishen *et al.* 2008, Shrestha *et al.* 2010), and enhance root canal disinfection into dentinal tubules (Shrestha *et al.* 2009). Silver nanoparticles have been tested as endodontic irrigants and intracanal medication (Wu *et al.* 2014, Abbaszadegan *et al.* 2015); added to calcium hydroxide as a vehicle (Javidi *et al.* 2014, Afkhami *et al.* 2015); incorporated into endodontic filling material (Mohamed Hamouda 2012, Correa *et al.* 2015) and calcium silicate cements (Bahador *et al.* 2015, Vazquez-Garcia *et al.* 2016), and have shown lower levels of cytotoxicity (Gomes-Filho *et al.* 2010, Takamiya *et al.* 2016).

Considering current advances in nanotechnology, it is expected that nanoparticles will be incorporated into endodontic therapy as an additional resource for root canal disinfection (Abbaszadegan *et al.* 2009). The antimicrobial action of endodontic irrigants containing silver nanoparticles in biofilms has previously been described (Wu *et al.* 2014), however there is no study with regard to these solutions against *E. faecalis* in infected dentinal tubules. Both situations are challenging, although they involve different morphologies: while in the former, bacteria are protected by the biofilm matrix, in the latter, the microorganisms are sheltered by their position deep within dentinal tubule. Therefore, the aim of this study was to evaluate the antimicrobial action of a silver nanoparticle irrigant against *E. faecalis* biofilm and in infected dentinal tubules, compared with sodium hypochlorite and chlorhexidine. The null hypotheses were that there was no difference in the antimicrobial activity between the irrigation solutions tested in both biofilm and infected intratubular dentin.

## MATERIALS AND METHODS

### Minimum inhibitory concentration of a silver nanoparticle solution against *Enterococcus faecalis*

The silver nanoparticle solutions at concentrations of 23, 50 and 94 ppm (Khemia, IPEN, São Paulo, SP, Brazil) were obtained for minimum inhibitory concentration evaluation. To acquire solutions in other concentrations, the 94 ppm solution was diluted in distiller water.

All microbiological procedures were conducted under aseptic conditions in a laminar flow chamber (ESCO, Lobov Científica, São Paulo, SP, Brazil). A standard strain of *E. faecalis* (ATCC 29212) was reactivated in sterile brain-heart infusion (BHI) broth (BHI, Difco, Kansas City, MO, USA) and the strain purity was confirmed by Gram staining and colony morphology at several times during the experiments.

For the macrodilution test, screw-capped tubes containing BHI broth were used, and silver nanoparticle solutions in different concentrations were added to the tubes producing serial 2-fold dilutions. The inoculum was obtained after successive cultures in BHI broth at 37ºC for 24 hours. The cultures were read in a spectrophotometer (BEL Photonics 1105, Piracicaba, SP, Brazil) at 540 nm, compared with the 0.5 MacFarland standard, diluted to the concentration of $5 \times 10^5$ CFU/mL and distributed into each tube.

The tubes were agitated in a vortex (Vortex-mix VX200, Edison, NJ, USA) and their turbidity was evaluated in the spectrophotometer before and after incubation at 37ºC for 24 hours (Oven 502-421, Fanem, Guarulhos, SP, Brazil). Negative and positive controls of bacterial growth were performed. The minimum inhibitory concentration (MIC) for a silver nanoparticle solution was established by means of tube readouts.

To ascertain the minimum bactericidal concentration (MBC), after reading the final absorbance values, 100μL of all tubes were transferred to BHI agar plates. The plates were incubated at 37ºC for 48 hours. The MBC was considered to be the lowest concentration of the solution that inhibited bacterial growth on agar plates.

**_Enterococcus faecalis_ biofilm**

Bovine central incisors with fully developed roots were used to obtain dentin blocks. Radicular dentin was cut using trephine drills 4.0 mm in diameter under irrigation. The dentin blocks were treated with 2.5% sodium hypochlorite (NaOCl) (Rioquímica, São José do Rio Preto, SP, Brazil) for 15 minutes and 17% ethylenediaminetetraacetic acid (EDTA) (Biodinâmica, Ibiporã, PR, Brazil) for 3 minutes, and sterilized in a tube containing distilled water by autoclaving at 121ºC for 20 minutes.

The one of the specimen surfaces was marked with a pencil, and then specimens were placed in 24-well culture plates. The marked surface was placed facing toward the surface of the plate, and the other side was used for biofilm development. Biofilm formation was obtained by using *Enterococcus faecalis* (ATCC 29212) inoculum of 3 X $10^8$ CFU/mL, and verified by using a spectrophotometer in the same manner as previously described. The dentin blocks were kept submerged in 1.8 mL sterile BHI broth and 0.2 mL inoculum, in each well of the plate. The culture plates were placed in a bacteriological oven at 37ºC for 21 days. The BHI broth was refreshed every 48 hours without addition of new inoculum to ensure that the microorganisms had sufficient availability of nutrients. After the incubation period, the specimens covered with biofilm were washed twice in saline solution to remove traces of culture medium and nonadherent planktonic bacteria.

The dentin blocks were divided into 9 groups with 5 blocks each, according to the irrigation solution and the contact time: silver nanoparticle 94 ppm solution (5, 15 and 30 minutes), 2.5% NaOCl (5, 15 and 30 minutes), and 2% chlorhexidine (Maquira, Maringá, PR, Brazil) (5, 15 and 30 minutes). The volume of the irrigating solution was 1mL for all groups, each time the irrigation was performed. For all solutions, 15-minute and 30-minute groups were refreshed every 5 minutes to simulate a clinical condition. Specimens irrigated with NaOCl were treated with 100μL of 5% sodium thiosulfate (Pharmácia Specífica, Bauru, SP, Brazil). One additional dentin block in each group was treated with 1mL saline to serve as control.

The biofilm layer was stained with 30 μL Live/Dead reagent (Live/Dead BacLight Viability Kit; Molecular Probes, Eugene, OR) in a dark environment for 20 minutes to evaluate biofilm viability. The Live/Dead reagents stained live bacteria with a green pigment and dead bacteria with a red pigment, which enabled the identification of viable bacteria. Then, the specimens were examined under a confocal laser scanning microscope (Leica TCS-SPE; Leica Biosystems CMS, Mannheim, Germany). Four confocal images of random areas were obtained for each specimen with 40X oil lens. As there were 5 specimens per group, 20 images per group were acquired. The images were transferred to bioImage_L software (www.bioImageL.com) for quantification of total biovolume (volume of live cells in the biofilm) and percentage of green cells (viable cells) found after the antimicrobial treatment.

**Antimicrobial activity of irrigants in intratubular dentin infected by *E. faecalis***

Seventy-two bovine central incisors with fully developed roots were selected for dentinal tubule contamination, which was performed by using the microorganism *E. faecalis* (ATCC 29212) according to the methodology of Andrade *et al.* 2015. The extracted teeth were initially stored for 48 hours in 1% NaOCl solution for decontamination. The tooth crowns were removed and 5 apical millimeters were cut off using a diamond disk attached to a low-speed saw (Isomet 1000, Buehler Ltd, Lake Bluff, IL, USA), under irrigation. The dentin tubules were standardized at lengths of 12 mm, and the root canals were prepared with K files up to a size 120 (Dentsply, Maillefer, Ballaigues, Switzerland). The smear layer was removed by means of an ultrasonic bath with 1% NaOCl, 17% EDTA and saline solution for 10 minutes each. To avoid external microbial contamination, two layers of red nail varnish (L'Oréal Colorama, Rio de Janeiro, RJ, Brazil) were applied to cover the external surface of the dentin tubules. After 24 hours, the specimens were inserted into microtubes containing distilled water and were autoclaved at 121ºC. After sterilization, the water was removed and 1 mL of sterilized BHI was inserted into each microtube. The microtubes were submitted to an ultrasonic bath (Cristofoli Equipamentos de Biossegurança LTDA, Campo Mourão, PR, Brazil) for 15 minutes in order to allow the maximum penetration of the culture medium into the dentinal tubules before bacterial contamination. The inoculum of *E. faecalis* was transferred to another BHI flask and kept in an oven for further 24 hours to achieve exponential growth. This culture was adjusted to 3 X $10^8$ CFU/mL according to McFarland standard using a spectrophotometer. Exponential bacterial grown phase was achieved in 7 hours, as defined by the study of Andrade *et al.* 2015. After this period, 1 mL of the inoculum was inserted into the microtubes containing the specimens and taken to a centrifuge (Eppendorf 5424R, Eppendorf, Hamburg, Germany). The tubes were submitted to 8 centrifugation cycles in sequence at 1,400, 2,000, 3,600 and 5,600 g, at 25ºC, in two cycles of 5 minutes for each speed. Between every centrifugation cycle, the solution that had penetrated through the dentin specimen was discarded and a fresh solution of inoculum was added to the microtube. After the centrifugation procedures, sterilized BHI broth was inserted into the microtubes, agitated in a vortex and incubated at 37º under aerobic conditions for 24 hours. Intratubular dentin contamination was performed for five days, with centrifugation on alternative days according to Andrade *et al.* 2015. On the fifth day, the specimens were removed from the microtubes and prepared for CLSM analysis.

For root canal irrigation, the specimens were placed on a sterilized stainless steel table device to avoid hand contact with the specimens, and procedures were performed inside the laminar flow chamber. The dentin tubules were divided into 9 groups with 8 specimens each, according to the irrigant and contact time of the solution: silver nanoparticle 94 ppm solution (5, 15 and 30 minutes), 2.5% NaOCl (5, 15 and 30 minutes), and 2% chlorhexidine (5, 15 and 30 minutes). For 15-minute and 30-minute groups, the solution was refreshed every 5 minutes. NaOCl-treated specimens received a final wash with 100μL of 5% sodium thiosulfate. For each group one dentin tubule was irrigated with 1mL of sterile saline as control.

Before analysis by CLSM, the specimens were split by using a diamond disc fitted to an Isomet saw, under irrigation with sterilized saline. The halves were treated with 17% EDTA for 5 minutes for smear layer removal resulting from the sectioning process. The specimens were washed with sterilized saline solution; stained with 30μL of Live/Dead reagent for 20 minutes, and taken to an inverted Leica TCS-SPE confocal microscope. Eight sequential images were obtained from each specimen: four of the cervical third and four of the middle third. For each third, the images were taken in the most superficial area near the canal and in the dentinal depth area, totalizing 64 images per group. All the specimens were analyzed using 40 X oil lens in a 1μm step-size, and format of 1024 X 1024 pixels. The CLSM images were fragmented into a stack and converted into TIFF format by the LAS AF software. The images were exported to the bioImageL TM v21 software for quantification of green and red bacteria. Preliminary data normality analysis was performed with the Shapiro-Wilk test showing that the data were not normally distributed. Statistical analysis was performed with nonparametric tests by using Kruskal-Wallis and Dunn tests to compare the number of viable bacteria and total biovolume. The Mann-Whitney *U*-test was used to compare the antimicrobial action of each irrigant against biofim and in intratubular dentin. The level of significance was set at $P < 0.05$ and Prisma 5.0 software (GraphPad Software Inc, La Jolla, CA, USA) was used as the analytical tool.

## RESULTS

### Minimum inhibitory concentration determination

Bacterial grow was observed in agar plates containing silver nanoparticle solutions at concentrations of 23, 50, 75, 80, 85, 90, 91, 92 and 93 ppm. No bacterial growth was observed in the agar plates containing silver nanoparticle solutions at concentration of 94 ppm. The silver nanoparticle solution capable of inhibiting and eliminating *E. faecalis* in both broth and agar plates was the concentration of 94 ppm.

### *Enterococcus faecalis* biofilm

The results of viable bacteria and total biovolume of *E. faecalis* biofilm after irrigation are shown in Table 1.

The AgNp solution was significantly less effective in killing bacteria in biofilm than chlorhexidine when irrigated for 5 minutes, but no significant difference was observed between them in the time intervals of 15 and 30 minutes. Sodium hypochlorite presented significantly higher antimicrobial activity compared with AgNp and chlorhexidine solutions, showing a lower number of viable bacteria in all time intervals tested.

Significant difference was observed in the use of the silver nanoparticle between 5 and 15 minutes, 15 and 30 minutes and 5 and 30 minutes, with a lower number of viable bacteria in the longer time intervals. No significant difference was observed with the use of chlorhexidine for 5 and 15 minutes, but significant difference was found between 15 and 30 minutes and 5 and 30 minutes. There was no significant difference in the use of NaOCl in the time intervals of 5 and 15 minutes and 15 and 30 minutes, however significant difference was observed between 5 and 30 minutes with the use of this solution.

Regarding total biofilm volume, AgNp presented significantly more ability to dissolve biofilm compared with chlorhexidine in 5 and 15 minutes. Sodium hypochlorite eliminated significantly more biofilm compared with the other solutions tested in all time intervals tested.

**Antimicrobial activity of irrigants in intratubular dentin infected by *E. faecalis***

After analysis of images taken by CLSM, the silver nanoparticle solution showed significantly less effectiveness compared with sodium hypochlorite in both the cervical and middle thirds and in superficial and deep areas, in all time intervals tested. Chlorhexidine also showed less capability of eliminating bacteria in the middle third and deep area than sodium hypochlorite in 5, 15 and 30 minutes.

Table 2 shows the percentage of viable bacteria within the dentinal tubules in the cervical and middle thirds and in superficial and deep areas, after treatment with the irrigating solutions tested.

Comparison of the action of the solutions against the biofilm and in the infected intratubular dentin showed that when silver nanoparticles were used for 5 minutes, more viable bacteria were found in biofilm than in the intratubular dentin. However, when this solution was used for 30 minutes, the number of viable bacteria was higher in intratubular dentin compared with biofilm. The number of viable bacteria was also significantly higher in intratubular dentin when NaOCl was used for 30 minutes compared with biofilm.

**DISCUSSION**

Nanoparticle antimicrobial agents have been proposed as an alternative against intracanal infections due to their ability of disrupting biofilm and prevent bacterial adhesion to dentin (Kishen *et al.* 2008, Shrestha *et al.* 2010, Wu *et al.* 2014, Del Carpio-Perochena *et al.* 2015). Silver nanoparticles have been studied because they interact with the bacterial cell membrane, increase permeability and prevent DNA replication (Rai *et al.* 2012, Samiei *et al.* 2016, Shrestha & Kishen, 2016). Biofilms are made up of an extracellular polysaccharide matrix (Rai *et al.* 2012) and microoganisms in mature biofilms are notoriously difficult to eradicate with antimicrobial agents, so they can be extremely resistant (Mohamadi *et al.* 2009, Kishen 2012). Biofilms developed *in vitro* for short periods of time may not present the same resistance of a mature biofilm (Guerreiro-Tanomaru *et al.*, 2013). In this study *E. faecalis* were inoculated for 21 days, based on a previous study that observed *E. faecalis* mature biofilm formation after this period of time (Guerreiro-Tanomaru *et al.*, 2013). The 94 ppm silver nanoparticle solution tested was not effective in disrupting *E. faecalis* biofilm compared with NaOCl. A previous study also demonstrated that AgNp as irrigant had no capacity for disrupting biofilm (Wu *et al.* 2014). On the other hand, when irrigated with AgNp solution for 5 and 15 minutes, the total biovolume of biofilm was significantly lower compared with chlorhexidine irrigation. The inability of 2% chlorhexidine to eliminate biofilm has previously been demonstrated (Clegg *et al.* 2006, Mohammadi *et al.* 2009, Del Carpio-Perochena *et al.* 2011). So far, sodium hypochlorite in different concentrations has been the most efficient irrigating solution with biofilm dissolution properties (Clegg *et al.* 2006, Del Carpio-Perochena *et al.* 2011, Wu *et al.* 2014), which is in agreement with the results of this study. In endodontic therapy, biofilm dissolution is required because a significant area of the root canal system is untouched by instruments during root canal preparation (Del Carpio-Perochena *et al.* 2011). Although biofilm dissolution was less efficient in specimens irrigated with a silver nanoparticle solution compared with NaOCl, a large number of nonviable bacteria were observed, especially after 30 minutes irrigation.

Although the time of contact of the irrigation solutions ranged in this study, the variation of volume of the irrigant was not tested during the irrigation procedures. According to a previous study, (Del Carpio-Perochena *et al.* 2011) the variation between 500µl and 1mL of the solution was not significant on the antimicrobial action of sodium hypochlorite. In this study, 1mL of the solutions was used each time irrigation was performed, in order to simulate a clinical situation. Furthermore, a higher volume of the irrigating solution could promote a mechanical removal of the biofilm, which could influence in the results of this study.

Monospecies biofilms of *Enterococcus faecalis* were chosen for this study because the purpose was to evaluate the antimicrobial action of the irrigants against bacteria resistant to endodontic treatment. Multi-species biofilms contain more bacteria and expolymeric substances, which could hinder the action of the endodontic irrigants tested (Ozok et al, 2007).

The characteristics of nanoparticles influence their antimicrobial action against bacterial cells and mature biofilm, such as contact time, concentration, particle size and surface charge (Shrestha *et al.* 2010, Wu *et al.* 2014, Abbaszadegan *et al.* 2015). The extracellular polysaccharide matrix secreted by bacteria in biofilms prevents nanoparticle penetration and requires higher concentrations and a longer time of interaction to achieve biofilm elimination (Shrestha *et al.* 2010, Javidi *et al.* 2014, Shrestha & Kishen, 2016). In this study, 5 minutes of solution in contact with *E. faecalis* biofilm was less effective in killing bacteria compared with chlorhexidine and NaOCl in the same time of interaction, differing from the findings of a recent study (Afkhami *et al.* 2017) that showed that irrigation with 100 ppm AgNp had similar antimicrobial efficacy as that of 2.5% NaOCl. However, when the time of interaction was increased to 15 and 30 minutes, similar results were obtained compared with those of chlorhexidine. The resistance offered by the biofilm matrix, and the insufficient time for interaction between positively charged AgNps and negatively charged bacterial cells are possible explanations for these results (Wu *et al.* 2014). A previous study (Wu *et al.* 2014) used an irrigant containing silver nanoparticles for 2 minutes and AgNp gel as a medicament for 7 days, and found that only the AgNp gel was able to disrupt *E. faecalis* biofilm. Therefore, the use of silver nanoparticles as a medicament and not as an irrigant has been suggested to eliminate bacterial biofilms during root canal disinfection (Javidi *et al.* 2014, Wu *et al.* 2014, Samiei *et al.* 2016, Shrestha *et al.* 2016).

In this study, silver nanoparticles showed statistical difference in all time intervals tested, which meant that for an effective action of this solution against *E. faecalis* biofilm, a longer time of interaction between irrigating solution and biofilm was required, in this case, 30 minutes of contact. When chlorhexidine was used, significant difference was found between the use of this solution in 15 and 30 minutes, demonstrating that chlorhexidine used for 15 minutes was insufficient for eliminating bacteria in biofilm. Irrigation with NaOCl showed that a 30-minute action was more effective in killing bacteria compared with 5 minutes, but no difference was observed compared with irrigation for 15 minutes. If AgNp or chlorhexidine were chosen in a clinical situation, in which the root canal was infected with *E. faecalis* biofilm, a longer time of contact of 30 minutes of the irrigating solution would be needed. On the other hand, if the root canal were irrigated with NaOCl, 15 minutes irrigation time would be sufficient to eliminate the bacteria in biofilm. At present, with the use of single instruments and less time required for root canal preparation, based on the results of this study, irrigation with NaOCl seemed to be more suitable when compared with the use of AgNp or chlorhexidine, because of its efficient and quick antimicrobial action against endodontic biofilm.

Different formulations of nanoparticles have also been recommended for root canal treatment, such as endodontic sealers due to their prolonged contact time and sustained antimicrobial activity (Kishen *et al.* 2008, Del Carpio-Perochena *et al.* 2015, Samiei *et al.* 2016). Increased antimicrobial activity of agents containing nanoparticles can also be achieved when they are used in higher concentrations (Shrestha *et al.* 2010, Wu *et al.* 2014). Although solutions with higher concentrations present more antimicrobial effect, studies have demonstrated that toxicity increases when higher concentrations are used (Gomes-Filho *et al.* 2010, Abbaszadegan *et al.* 2015, Takamiyia *et al.* 2016), but AgNp was shown to be more biocompatible when compared with chlorhexidine and sodium hypochlorite solutions. The particle size was also related to the antimicrobial activity. The smaller the size, the larger the surface-area, and the higher the percentage of interaction with bacterial cells that would be obtained, when compared with bigger particles (Rai *et al.* 2012). Differences in antimicrobial activity of nanoparticles may be influenced by their surface charge. Abbaszadegan *et al.* 2015 suggested a positive charged nanoparticle as a root canal disinfectant because of its strong bactericidal potential, limited effect on dentin and cytocompatibility. A positive charge of AgNp could improve interaction between the negatively charged microorganism cells and lead to altered cell wall permeability.

*Enterococcus faecalis* has the ability to invade and remain viable within the dentinal tubules, even in previously filled root canals, reaching deeper penetrations when they grow in nutrient-rich conditions (Love 2001, George *et al.* 2005, Del Carpio-Perochena *et al.* 2015). The superficial bacteria on the canal wall can be killed more easily, but when microorganisms are deep within dentinal tubules they may be inaccessible to endodontic irrigants, medicaments and sealers because these agents have limited penetrability into this area (Shrestha *et al.* 2009, Del Carpio-Perochena *et al.* 2015).

Antimicrobial compounds containing silver, such as silver diamine fluoride may penetrate into the dentinal tubules and produce silver deposits that allow the gradual release of silver ions over time, and help to eliminate bacteria inside the dentinal tubules (Hiraishi *et al.* 2010). In this study, the variation of contact time showed no significant improvement in eliminating bacteria with any irrigating solution tested in the infected intratubular dentin. This meant that even if the solution had been in contact with root canal for a longer period of time, it would still not have been sufficient to act effectively against bacteria deep inside the dentinal tubules. In all time intervals tested, silver nanoparticle showed significantly less antimicrobial action compared with sodium hypochlorite, in deeper and superficial areas, and in the cervical and middle regions. As regards chlorhexidine, this solution presented significantly less bactericidal action in middle portions of the root canal and in deeper areas, compared with sodium hypochlorite, in all time intervals tested. Thus, the null hypotheses were rejected. These results demonstrated the difficulty in cleaning the apical portion of root canal and in eliminating bacteria found deep within the dentinal tubules. To overcome this problem, additional procedures may be suggested to induce the flow of antimicrobial agent into the dentinal tubules and improve the cleaning and disinfection of the root canal. Recently, Photodynamic therapy (PDT) has been associated with nanoparticles with potential for use as an adjunct to disinfect the root canal system (Shrestha *et al.* 2015, Afkhami *et al.* 2017). Ultrasonic devices have been widely used in endodontics because they induced acoustic streaming and/or cavitation of an irrigant and improved the cleaning and disinfection of the root canal space (van der Sluis *et al.* 2007). High-intensity focused ultrasound produced collapsing cavitation bubbles and improved root canal disinfection when associated with nanoparticles, because the diffusion of fluids alone was not enough to deliver antibacterial nanoparticles into the dentinal tubules, since the action of silver nanoparticles was shown to be significantly less efficient compared with the other solutions tested (Shrestha *et al.* 2009), in agreement with our study.

The irrigating solutions tested in this study were used with two different methods, and showed different antimicrobial action in both cases. While the formation of biofilm was performed in a period of 21 days, the contamination protocol was executed in 5 days. In this manner, it was possible to compare the action of the solutions against bacteria in a mature biofilm and in planktonic bacteria inside the dentinal tubules.  When a mature biofilm is formed inside dentinal tubules by longer times of contamination, increased resistance of *E. faecalis* to antimicrobial agents is observed (Yang *et al.*, 2016). In this study, when the silver nanoparticle solution was used for a period of 5 minutes, its action was significantly different against biofilm and in intratubular dentin. The number of viable bacteria in this case was higher in biofilm, which meant that when this solution was used for a short period of time, its action was more efficient against isolated cells when compared with the bacteria present in biofilm. This probably occurred because the biofilm matrix hindered the interaction between the silver nanoparticle solution and the bacteria. The silver nanoparticle solution required a longer time of contact to eliminate bacteria present in biofilm. The action of this irrigant used for 30 minutes also showed statistical difference, but in this time the number of viable bacteria was higher in intratubular dentin, i.e., its action was more efficient against biofilm. These results demonstrated that silver nanoparticles as a root canal irrigant presented more efficacy with a longer time in contact with biofilm. Furthermore, bacteria present in infected intratubular dentin were harder to eliminate in deeper areas and the middle portions because the access to the irrigant was more difficult in these areas. The action of the root canal irrigant was less efficient in intratubular dentin when compared with biofilm when sodium hypochlorite was used in a period of 30 minutes. This might have occurred because this time was sufficient for NaOCl to disrupt biofilm and consequently the bacteria became embedded in its matrix, while the bacteria present in deep areas in dentin tubules were protected from the action of irrigating solutions.

Based on the results of this study, the use of silver nanoparticle solutions might be used as an adjunct in root canal system disinfection, because it was not able to completely disrupt biofilm, but could have the ability to penetrate into the dentinal tubules and improve disinfection in root canals with persistent infection. Studies with the use of AgNp associated with ultrasonic activation are necessary to evaluate whether this procedure could improve its action without interfering in its cytotoxicity. Further studies are also required to evaluate whether the silver in this solution could cause discoloration, and to investigate the action of other formulations of nanoparticles, such as medicaments and sealers containing AgNp, on infected dentinal tubules.

## CONCLUSIONS

A longer time of interaction between silver nanoparticles was required for effective action on a monospecie biofilm. In cases when intratubular dentin was infected in deep areas, resistant bacteria such as *E. faecalis* were not easily eliminated by solutions containing silver nanoparticles.

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**FIGURE LEGENDS**

**Figure 1**: Representative images of *Enterococcus faecalis* biofilm after treatment with the irrigating solutions: (A, B and C) 94 ppm Silver nanoparticles in 5, 15 and 30 minutes respectively; (D, E and F) 2% Chlorhexidine in 5, 15 and 30 minutes respectively; and (G, H and I) 2.5% Sodium hypochlorite in 5, 15 and 30 minutes respectively.

**Figure 2:** Representative images of *Enterococcus faecalis* inside the dentinal tubules after treatment with the irrigating solutions: (A, B and C) 94 ppm Silver nanoparticles in 5, 15 and 30 minutes respectively; (D, E and F) 2% Chlorhexidine in 5, 15 and 30 minutes respectively; and (G, H and I) 2.5% Sodium hypochlorite in 5, 15 and 30 minutes respectively.

**Table 1.** Median, maximum and minimum values of viable cells and total volume of *E. faecalis* biofilm after irrigation with the solutions tested in 5, 15 and 30 minutes.

| **Groups** | 94 ppm AgNp | | | 2% CHX | | | 2.5% NaOCl | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 min Median (min-max) | 15 min Median (min-max) | 30 min Median (min-max) | 5 min Median (min-max) | 15 min Median (min-max) | 30 min Median (min-max) | 5 min Median (min-max) | 15 min Median (min-max) | 30 min Median (min-max) | Control Median (min-max) |
| **Viable cells (Green)** | 68,81 (11,86 - 93,30) Aa | 13,15 (0,063 - 9,16) Ab | 0,5001 (0,02797-20,08) ABc | 15,29 (1,629 - 65,63) Ba | 16,48 (0,0 - 84,42) Aa | 0,7131 (0,0 - 51,61) BCb | 0,6697 (0,0 - 39,35)Ba | 0,2493 (0,0 - 33,38) Bab | 0,05710 (0,0 - 1,244) Cb | 96,83 (91,43 - 97,95) A |
| **Total Biovolume** | 15771 (5719 - 121342) 2 a | 15983 (2337 - 94208)Ba | 36175 (11312 - 79161)*º a | 86368 (27053 -67453) 1a | 55020 (20807 - 82480)Aa | 60895 (12877 - 91083)* a | 1026 (34,00 - 30306) 3a | 873 (0,0 - 10977)Ca | 331 (0,0 - 1236)# b | 25872 (17571 - 28126) 2ABº |

Different capital letters in each row indicate statistical differences between groups of viable cells in the same time interval. (P<0.05)
Different small letters in each row indicate statistical differences in the same group of viable cells in the same irrigant, in different time intervals (P<0.05).
Different capital letters, numbers and symbols in the same row indicate statistical differences between groups of total biovolume in the same time interval, in different groups of irrigants. (P<0.05)

**Table 2.** Median, maximum and minimum values of the percentage of viable cells in intratubular dentin in cervical and middle thirds and in superficial and deep areas, after irrigation with the solutions tested in 5, 15 and 30 minutes (P<0.05).

| Groups | | 94 ppm AgNp | | | 2% CHX | | | 2.5% NaOCl | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 min Median (min-max) | 15 min Median (min-max) | 30 min Median (min-max) | 5 min Median (min-max) | 15 min Median (min-max) | 30 min Median (min-max) | 5 min Median (min-max) | 15 min Median (min-max) | 30 min Median (min-max) | Controle Median (min-max) |
| cervical | superficial | 7,314 (0,08121-70,13) | 17,46 (0,4238-49,53) | 18,85 (0,5933-48,14) | 1,012 (0,1172-50,28) | 1,110 (0,09245-14,17) | 2,110 (0,0-27,93) | 0,8453 (0,006628-24,40) | 0,5713 (0,009804-2,273) | 0,1543 (0,0-0,8143) | 81,51 (53,85-89,93) |
| | deep | 30,80 (0,0-4,55) | 35,41 (0,1285-98,00) | 45,68 (1,794-74,75) | 5,466 (0,2904-93,49) | 2,943 (0,05367-67,35) | 12,38 (0,03352-56,63) | 0,4421 (0,0-53,10) | 0,8854 (0,0-5,830) | 0,09698 (0,006935-8,523) | 76,86 (62,94-90,73) |
| middle | superficial | 18,37 (0,1542-72,88) | 29,28 (0,1169-81,59) | 16,05 (0,04970-56,06) | 4,862 (0,03844-52,93) | 2,034 (0,02453-67,03) | 3,427 (0,003296-25,32) | 0,8401 (0,0-8,387) | 0,9172 (0,06848-3,175) | 0,2783 (0,0-7,237) | 83,14 (65,91-91,52) |
| | deep | 33,89 (0,9306-94,20) | 51,28 (0,6962-98,04) | 22,80 (0,6138-69,76) | 21,12 (2,025-94,77) | 3,883 (0,01074-66,47) | 9,405 (0,03662-52,40) | 0,7365 (0,0-34,06) | 0,7675 (0,0-9,560) | 0,7742 (0,0-13,39) | 52,03 (3,511-95,08) |

**Figure 1**

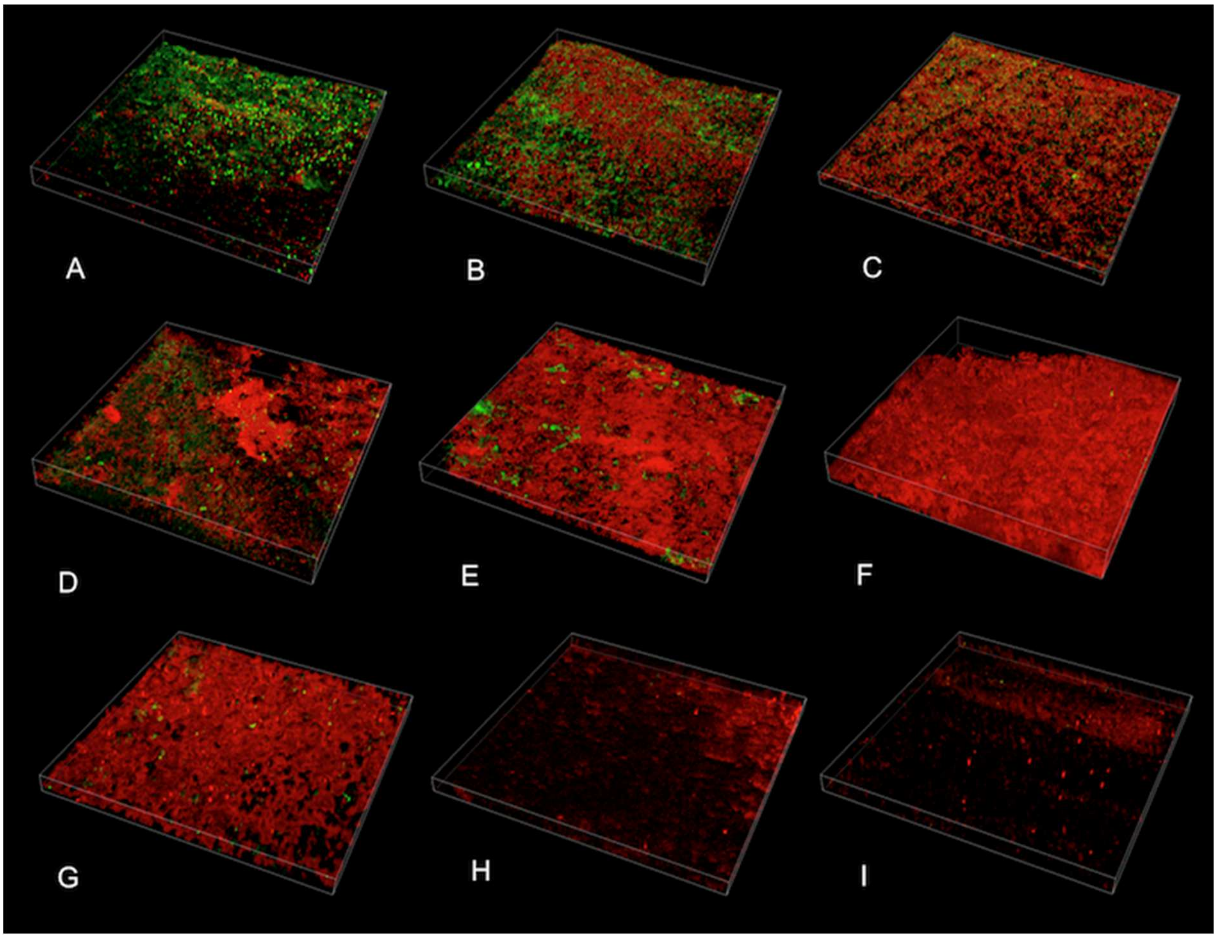
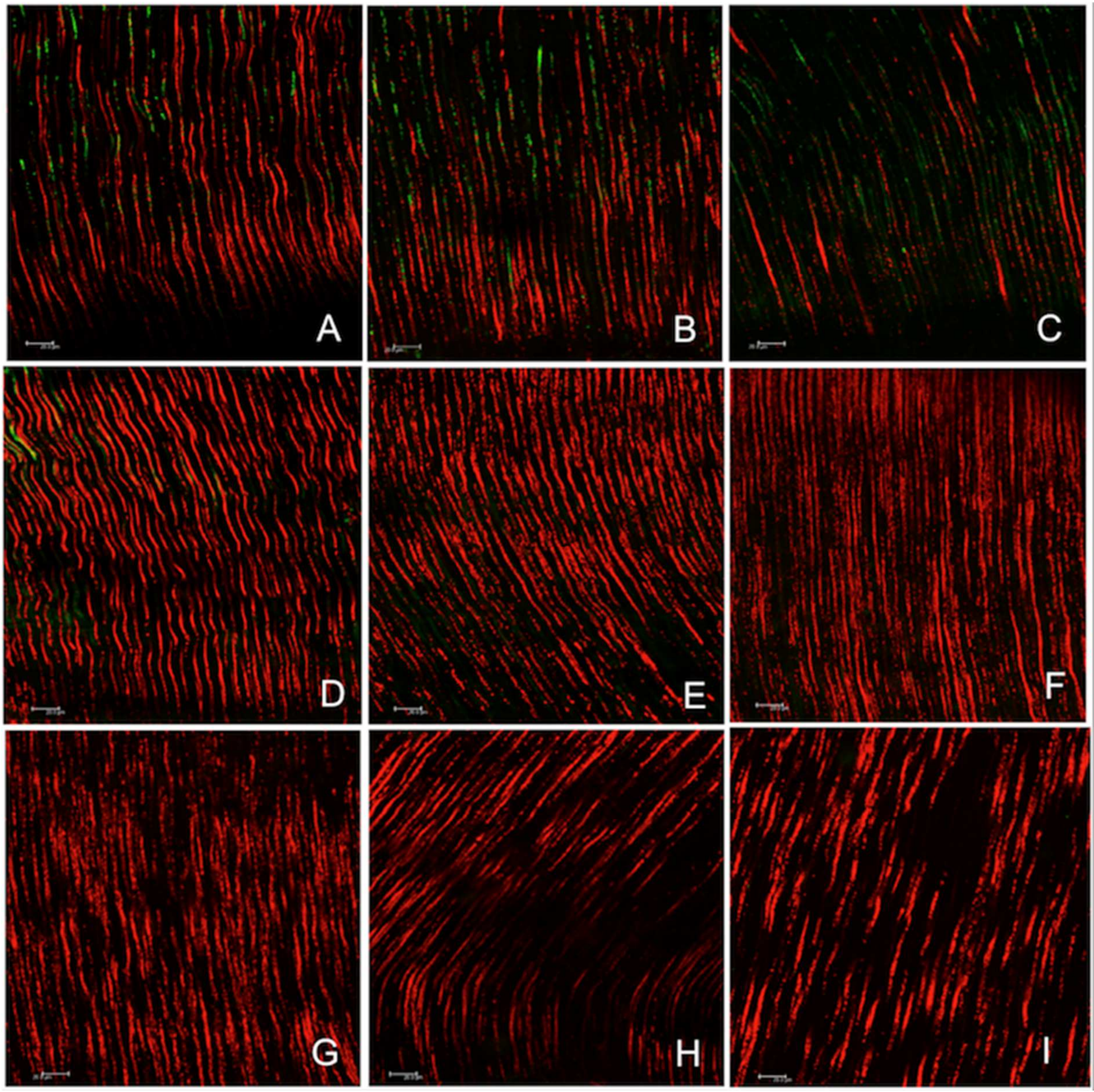


**Figure 2**



# 3 Discussion

# 3 DISCUSSION

In endodontic retreatment, there are some aims that need to be achieved, such as the removal of as much filling material as possible to obtain root canal walls free of material, to enable the antimicrobial agents to reach bacteria found inside the dentinal tubules. Thereby, the adequate action of irrigation solutions and medicaments can be achieved, providing effective disinfection for eliminating bacteria that persist after root canal therapy, which may be responsible for treatment failure (CAVENAGO et al., 2014; NAIR, 2006; RICUCCI 2009; SCHRRMEISTER et al., 2006). This process of filling removal represents a clinical challenge, especially in curved canals, because there is more probability of procedural errors, such as ledges, perforations, alterations in root canal shape and instrument fracture occurring when there are curvatures (HULSMANN et al., 2011; RODIG et al., 2012; RODIG et al., 2014; SCHIRRMEISTER et al., 2006).

During re-instrumentation, filling material can be removed by means of different materials and techniques. In the first part of this study, instruments such as sequentially reciprocating and rotary files made of different alloys, were used to assess their efficacy for removing filling material from curved canals. Although these instruments were made of different alloys, there were no significant differences between the groups that used M-wire and NiTi instruments and the group that used CM-wire instruments. CM-wire instruments were tested for retreatment purposes in this study because they offer several advantages, such as a higher level of flexibility and increased fatigue resistance during root canal preparation (COLTENEENDO, 2016; SHEN et al., 2013;), and also showed good performance in removing filling material from curved canals. However, although the tested protocols removed a large amount of filling material, they were unable to promote complete cleaning of the root canal walls. To date, no technique reported in the literature has been able to remove all filling material during root canal retreatment (BERNARDES et al., 2016; BETTI et al., 2009; BRAMANTE et al., 2010; CAVENAGO et al., 2014; FRUCHI et al., 2014; HAMMAD et al., 2008; RIOS et al., 2014; RODIG et al., 2012; RODIG et al., 2014; TAKAHASHI et al., 2009; YURUKER et al., 2016; ZUOLO et al., 2013; ZUOLO et al., 2016). Remnants of filling material have frequently been found after root canal reinstrumentation, irrespective of the instrument used, especially in the apical region (CAVENAGO et al., 2014; KELES et al., 2015; RODIG et al., 2012; UNAL et al., 2009). Due to the difficulty of cleaning the apical third, instruments with large size and small taper, designed for apical preparation, may be used. In this study, a final preparation was performed with a size 50 and taper 01 instrument, since the apical portion showed a larger amount of filling material after the use of reciprocating and rotary instruments. This instrument significantly improved the removal of filling material remnants from apical third compared with reciprocating instruments, with the advantage of not weakening tooth structure. Moreover, a more extensive apical enlargement allows more efficient irrigation and flushing of liquids in the apical portions of a curved canal, facilitating the use of agitation devices and increasing root canal disinfection (ALBRECHT et al., 2004; LEE et al., 2004; VAN DER SLUIS et al., 2005; WU; WESSELINK, 1995). Therefore, the present study suggests the association of instruments, such as reciprocating and rotary files, in a clinical situation, because this enhances the removal of filling material. In this study, the association of Gates-Glidden burs and hand files was as effective as the combination of reciprocating and rotary files, however the use of aggressive instruments such as Gates-Glidden burs may be hazardous to use in a previously enlarged root canal (HULSMANN et al., 2011).

In addition to using a hybrid technique with the association of instruments, other procedures have also been indicated for improving cleaning during root canal retreatment. Irrigant activation with ultrasonic devices has been recommended as a final step in retreatment, because this procedure improves residual filling material removal after re-instrumentation (BERNARDES et al., 2016; CAVENAGO et al., 2014; GRISCHKE et al., 2014). In the second part of this study, passive ultrasonic irrigation with an Irrisonic tip, and irrigant activation with EasyClean were performed to evaluate the removal of residual filling material. The manufacturer of EasyClean, a plastic agitation device, suggests its use in reciprocating motion to avoid instrument threading and fracture (EASYCLEAN, 2016), but EasyClean used in continuous rotation at low speed was shown to be more effective in dentinal debris removal (DUQUE et al., 2016). In this study, EasyClean was activated in continuous rotary motion in an attempt to promote vigorous irrigant agitation to remove residual filling material that remained after root canal re-instrumentation, and its performance was as effective as that of ultrasonic irrigation.

Both methods of irrigant agitation tested in this study were performed in 3 activation cycles lasting 20 seconds each, as a final irrigation protocol. These 3 activation cycles with renewal of the irrigant promoted improvement in cleaning the root canal, with a significant removal of residual filing material, irrespective of the device used. Therefore, this clinical protocol seems to be useful as a supplementary step after re-instrumentation during retreatment and should be indicated for complementary removal of filling material.

After irrigant activation, filling material remnants that persist on the root canal walls may contain bacteria and represent a physical barrier to antimicrobial agents in the infected dentin, which could compromise the long-term prognosis of endodontically treated teeth (CAVENAGO et al., 2014; SIQUEIRA; ROÇAS, 2008). Therefore, in addition to the mechanical and physical action of irrigant agitation, the chemical action of the irrigating solution is necessary to provide root canal disinfection (MOHAMMAD; ABBOTT, 2009; VAN DER SLUIS et al., 2010).

Microorganisms found in infected root canals may form colonies organized in biofilms or may penetrate into the dentinal tubules, which can make them inaccessible to antimicrobial agents (BASMACI et al., 2013; DEL CARPIO-PEROCHENA et al., 2015; KISHEN, 2012; SHRESTHA et al., 2009). Furthermore, some resistant bacteria, such as *Enterococcus faecalis*, have developed ways to survive after the use of endodontic irrigants and medications, which makes them more difficult to eliminate from root-filled teeth with persistent endodontic infections (ARIAS-MOLIZ et al., 2009; BASMACI et al., 2013; CHIVATXARANUKUL et al., 2008; LOVE, 2001).

In the third part of this study, an irrigating solution containing silver nanoparticles was tested, since nanotechnology has shown to be efficient in eliminating microorganisms, even when they are organized in the form of biofilms (JAVIDI et al., 2014; KISHEN et al., 2008; RAI et al., 2012; SHRESTHA et al., 2016; WU et al., 2014). This solution was chosen because it has antimicrobial action with the advantage of low toxicity (GOMES-FILHO et al., 2010; TAKAMIYA et al., 2016).

The 94 ppm silver nanoparticle irrigant was compared with 2.5% sodium hypochlorite and 2% chlorhexidine against *E. faecalis* biofilm and the intratubular dentin infected with these bacteria. The silver nanoparticle solution was shown to be more capable of disrupting biofilm compared with chlorhexidine, however it was not more effective in dissolving biofilm when compared with NaOCl, which is commonly used in endodontic therapy. Moreover, when the silver nanoparticle solution was used for a short period of time, it showed less ability to kill bacteria in biofilm, which is in agreement with previous studies (JAVIDI et al., 2014; WU et al., 2014). The use of antimicrobial agents containing nanoparticles are likely to be indicated against endodontic biofilms when they are maintained within the root canal, in forms of intracanal medication for longer periods of time, or added to sealers (DEL CARPIO-PEROCHENA et al., 2015; JAVIDI et al., 2014; KISHEN et al., 2008; SAMIEI et al., 2016; WU et al., 2014).

The silver nanoparticle solution behaved differently when in contact with biofilms, when compared with being in contact with isolated microorganisms in infected intratubular dentin. When this solution was used for short periods of time, its action was better against isolated bacteria in dentinal tubules compared with bacteria present in biofilm. In this study, the silver nanoparticle solution required a longer time of interaction with biofilms to eliminate bacteria, because in these cases, the bacteria are protected by the biofilm matrix. On the other hand, when this solution was left for longer periods of time within the root canal, nanoparticle solution acted more effectively against biofilm compared with planktonic bacteria in dentinal tubules. This emphasizes that a longer time of interaction of AgNp solution was required in endodontic infections for eliminating bacteria in biofilms, and in the infected dentin found in root filled canals with treatment failure, especially because bacteria found in middle portion and in deeper regions of the root canal were more difficult to eliminate, compared with bacteria located in the cervical portion and superficial area.

One possible clinical alternative for the use of nanoparticle solutions is in the final irrigation, which could complement the antimicrobial action of the main solution used during instrumentation. As sodium hypochlorite is the most commonly used irrigating solution in root canal preparation, studies are necessary to evaluate whether interactions could occur when silver nanoparticles are associated with sodium hypochlorite.

The use of ultrasonics associated with chitosan nanoparticles enabled the delivery of antimicrobial nanoparticles into the dentinal tubules and improved disinfection (SHRESTHA et al., 2009). Studies associating AgNp and ultrasonics are necessary to evaluate whether this activation would improve the action of the solution without interfering in its cytotoxicity.

Further studies are also required with other alternatives for root canal preparation during retreatment, such as the use of different instruments, other agitation devices and antimicrobial agents, with the purpose of helping to achieve success, especially in cases of treatment failure with persistent apical periodontitis.

# 4 Conclusions

# 4 CONCLUSIONS

- Although filling material residues persisted after instrumentation during retreatment of curved canals, the combination of reciprocating and rotary files was effective, irrespective of the type of alloy of the instruments.
- Irrigant agitation as a final irrigation protocol in re-treatment improved the removal of remnant filling material. Passive ultrasonic irrigation and irrigant agitation using EasyClean could be indicated as supplementary steps in retreatment.
- Irrigants containing silver nanoparticles were not as effective as the solutions, such as sodium hypochlorite and chlorhexidine, commonly used in endodontic therapy against *Enterococcus faecalis*.

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# APPENDIXES

## APENDIX A - DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS

We hereby declare that we are aware of the article ***Efficacy of CM-wire, M-wire and Nickel-Titanium instruments for removing filling material from curved root canals: a micro-computed tomography study*** will be included in the Thesis of the student (Clarissa Teles Rodrigues) and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, April 16th 2017.

Clarissa Teles Rodrigues

Clarissa Teles Rodrigues
Author

Signature

Marco A H Duarte

Marco Antonio Hungaro Duarte
Author

Signature

Marcela Milanezi de Almeida
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Flaviana Bombarda de Andrade
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## APENDIX B - DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS

We hereby declare that we are aware of the article ***Comparison of an agitation device and ultrasonic irrigation for removing filling material from curved canals*** will be included in the Thesis of the student (Clarissa Teles Rodrigues) and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, April 16th 2017.

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Rodrigo Ricci Vivan
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Norberti Bernardineli
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## APENDIX C - DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN THESIS

We hereby declare that we are aware of the article ***Antibacterial properties of silver nanoparticle as a root canal irrigant against Enterococcus faecalis biofilm and infected intratubular dentin*** will be included in the Thesis of the student (Clarissa Teles Rodrigues) and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, April 16th 2017.

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# ANNEXES

## ANNEX A – Ethics committee approval

FACULDADE DE ODONTOLOGIA DE BAURU-USP

**PARECER CONSUBSTANCIADO DO CEP**

**DADOS DO PROJETO DE PESQUISA**

**Título da Pesquisa:** Análise de métodos de desobturação do canal radicular no retratamento endodôntico e avaliação da ação antimicrobiana de soluções utilizadas na irrigação final.

**Pesquisador:** Clarissa Teles Rodrigues

**Área Temática:**

**Versão:** 1

**CAAE:** 58331116.0.0000.5417

**Instituição Proponente:** Universidade de Sao Paulo

**Patrocinador Principal:** Financiamento Próprio

**DADOS DO PARECER**

**Número do Parecer:** 1.698.505

**Apresentação do Projeto:**

Segundo a pesquisadora, em casos de insucesso do tratamento endodôntico, uma alternativa seria o retratamento do canal radicular. Durante este procedimento deve haver remoção de todo o material obturador para que seja realizada novo preparo biomecânico e nova obturação do sistema de canais radiculares. Bactérias são o principal fator etiológico em casos de fracasso da terapia endodôntica, e esta infecção persistente pode estar relacionada à capacidade dos microganismos em penetrar nos túbulos dentinários. Por este motivo é necessário o uso de soluções na irrigação do canal radicular com ação antimicrobiana e com boa tolerância tecidual. O objetivo deste trabalho será avaliar o desempenho dos instrumentos de diferentes ligas metálicas na desobstrução do canal radicular durante o retratamento endodôntico de incisivos laterais superiores com curvatura apical, por meio da microtomografia computadorizada e análise destas amostras no microscópio eletrônico de varredura após a ativação de irrigantes. Posteriormente
será avaliada a capacidade antimicrobiana de um irrigante contendo nanopartículas de prata, clorexidina a 2% e hipoclorito de sódio a 2,5% frente ao biofilme de Enterococcus faecalis e à dentina contaminada com este mesmo microrganismo. Serão selecionados trinta incisivos laterais humanos extraídos que apresentem curvatura apical. Os dentes serão instrumentados, obturados e divididos em três diferentes grupos de acordo com o protocolo de remoção do material

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obturador do canal radicular: no primeiro grupo a desobstrução será realizada com os instrumentos Reciproc, Mtwo 40 e Logic 50.01; no segundo grupo serão utilizados os instrumentos ProDesign R, Logic 40 e e Logic 50.01; e no terceiro grupo a desobstrução será realizada com instrumentos manuais. Para a análise da remoção do material obturador, as amostras serão escaneadas em micrótomogafo SkyScan 1174 para que sejam comparadas as imagens antes e após a desobstrução do canal radicular, e em cada amostra este volume será calculado nos quatro níveis (apical 1, 2, médio e cervical. Estas amostras posteriormente serão clivadas e analisadas no microscópio eletrônico de varredura, para a observação de resíduos de material obturador antes e após a ativação de irrigantes com o ultrassom e com o sistema EasyClean utilizado em rotação contínua. Para a análise estatística dos resultados serão utilizados os testes de Kruskal-Wallis,  Friedman, Wilcoxon e Dunn. Posteriormente, será determinada a concentração inibitória mínima da solução de hipoclorito de sódio a 2,5%, da solução de clorexidina a
2% e uma solução irrigadora do canal contendo nanopartículas de prata frente a cepas de Enterococcus faecalis, através do método de diluição em caldo. Em seguida, será testada a atividade antimicrobiana destas soluções irrigadoras sobre o biofilme de Enterococcus faecalis in vitro. Para isso, serão utilizados blocos de dentina bovina colocados em placas de 24 poços e biofilme de Enterococcus faecalis será formado durante 21 dias. Os
blocos de dentina serão divididos em 9 grupos experimentais com 5 blocos cada um, em função dos irrigantes avaliados e do tempo de exposição à solução irrigadora. As amostras serão coradas com corante Live/dead para posterior análise no microscópio confocal de varredura a laser (MCVL).
Por fim, será testada a atividade antimicrobiana das soluções irrigadoras após a contaminação de túbulos dentinários com Enterococcus faecalis, observada pelo MCVL. Serão confeccionados tubos de dentina a partir de incisivos bovinos que serão levados à centrífuga e contaminados com Enterococcus faecalis. Os tubos de dentina receberão tratamento com hipoclorito de sódio a 2,5%, com clorexidina a 2% e com a solução de
nanopartículas de prata e serão analisados no MCVL para avaliar descontaminação dos túbulos dentinários e pelo método de cultura microbiológica para posterior contagem de unidades formadoras de colônias por mililitro (UFC/mL).

**Objetivo da Pesquisa:**
- Avaliar a capacidade dos instrumentos de ligas metálicas CM-wire, M-wire e NiTi convencionais utilizando movimento reciprocante e rotatório contínuo, na remoção de material obturador de canais radiculares de incisivos laterais superiores com curvatura apical, durante o retratamento

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endodôntico, por meio da microtomografia computadorizada.
- Após o retratamento endodôntico, avaliar, por meio da microscopia eletrônica de varredura, a remoção de resíduos de material obturador por meio da ativação ultrassônica de irrigantes e da ativação de irrigantes com o EasyClean em rotação contínua.
- Determinar a concentração inibitória mínima da solução irrigadora de nanopartículas de prata sobre o microrganismo Enterococcus faecalis.
- Avaliar através do microscópio confocal de varredura a laser a ação das soluções de nanopartículas de prata, hipoclorito de sódio a 2,5% e clorexidina a 2% sobre o biofilme de Enterococcus faecalis in vitro.
- Avaliar através do microscópio confocal de varredura a laser e através de cultura microbiológica a atividade antimicrobiana das soluções de nanopartículas de prata, hipoclorito de sódio a 2,5% e clorexidina a 2% sobre a dentina radicular bovina após a contaminação dos túbulos dentinários com Enterococcus faecalis.

**Avaliação dos Riscos e Benefícios:**
Riscos:
A pesquisa não apresenta riscos diretos aos participantes da pesquisa, já que os dentes humanos que serão utilizados nos experimentos serão doados por uma cirurgiã-dentista que realizou a extração destes dentes por finalidade terapêutica, como consta no termo de cessão de dentes.
Benefícios:
Com esta pesquisa, espera-se que sejam determinadas melhores maneiras e materiais a serem utilizados durante o retratamento endodôntico. Isso inclui novas alternativas de irrigantes endodônticos, como soluções contendo nanopartículas de prata. Espera-se também observar a efetividade do uso sequencial de instrumentos reciprocantes e rotatórios na remoção de material obturador do canal radicular, assim como a agitação de irrigantes
no interior do canal radicular. Estes procedimentos poderiam trazer benefícios inclusive em dentes com complexidade anatômica como curvaturas radiculares, proporcionando menor possibilidade de trazer danos ao dente.

**Comentários e Considerações sobre a Pesquisa:**
Não há.

**Considerações sobre os Termos de apresentação obrigatória:**
Todos os documentos obrigatórios pertinentes foram apresentados.

**Endereço:** DOUTOR OCTAVIO PINHEIRO BRISOLLA 75 QUADRA 9
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Continuação do Parecer: 1.698.505

**Recomendações:**

Não há.

**Conclusões ou Pendências e Lista de Inadequações:**

Tendo em vista a apresentação de todos os documentos de forma correta, sou de parecer favorável a aprovação do respectivo projeto de pesquisa.

**Considerações Finais a critério do CEP:**

Esse projeto foi considerado APROVADO na reunião ordinária do CEP de 17.08.2016, com base nas normas éticas da Resolução CNS 466/12. Ao término da pesquisa o CEP-FOB/USP exige a apresentação de relatório final. Os relatórios parciais deverão estar de acordo com o cronograma e/ou parecer emitido pelo CEP. Alterações na metodologia, título, inclusão ou exclusão de autores, cronograma e quaisquer outras mudanças que sejam significativas deverão ser previamente comunicadas a este CEP sob risco de não aprovação do relatório final. Quando da apresentação deste, deverão ser incluídos todos os TCLEs e/ou termos de doação assinados e rubricados, se pertinentes.

**Este parecer foi elaborado baseado nos documentos abaixo relacionados:**

| Tipo Documento | Arquivo | Postagem | Autor | Situação |
|---|---|---|---|---|
| Informações Básicas do Projeto | PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_745810.pdf | 28/07/2016 10:29:58 | | Aceito |
| Outros | Termo_doacao_dentes.pdf | 28/07/2016 10:28:10 | Clarissa Teles Rodrigues | Aceito |
| Outros | Questionario.pdf | 28/07/2016 10:27:33 | Clarissa Teles Rodrigues | Aceito |
| Outros | Carta_de_encaminhamento.pdf | 28/07/2016 10:25:44 | Clarissa Teles Rodrigues | Aceito |
| Projeto Detalhado / Brochura Investigador | Projeto_de_pesquisa.pdf | 28/07/2016 10:18:51 | Clarissa Teles Rodrigues | Aceito |
| Declaração de Pesquisadores | Declaracao_de_compromisso.pdf | 28/07/2016 10:18:17 | Clarissa Teles Rodrigues | Aceito |
| Folha de Rosto | Folha_de_rosto.pdf | 28/07/2016 10:16:18 | Clarissa Teles Rodrigues | Aceito |

**Situação do Parecer:**

Aprovado

**Necessita Apreciação da CONEP:**

Não

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Continuação do Parecer: 1.698.505

BAURU, 26 de Agosto de 2016

**Assinado por:**
**Izabel Regina Fischer Rubira Bullen**
**(Coordenador)**

**Endereço:** DOUTOR OCTAVIO PINHEIRO BRISOLLA 75 QUADRA 9
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**ANNEX B** – Registration of research with animals

**Universidade de São Paulo**
**Faculdade de Odontologia de Bauru**

Comissão de Ética no Ensino e Pesquisa em Animais

Uso exclusivo da CEEPA/FOB/USP

Registro número: **003/2016**

Recebido em: 09/12/2016

Maristela Petenuci Ferrari

Secretária da CEEPA – SRTE 53052

**REGISTRO DE PESQUISA E/OU ENSINO, COM UTILIZAÇÃO DE CADÁVERES DE ANIMAIS, OU PARTE DELES**

| | |
|---|---|
| **Finalidade:** | Pesquisa |
| **Período:** | Novembro/2013 à Fevereiro/2017 |
| **Pesquisador Responsável:** | Prof. Dr. Norberti Bernardinelli |
| **Pesquisador Executor:** | Clarissa Teles Rodirgues |
| **Colaboradores:** | Não se aplica |
| **Dados Nota Fiscal/ Termo de Doação:** | Dentes bovinos adquiridos em 01/11/2013, sem nota fiscal |
| **Quantidade de Dentes Bovinos:** | 120 |

**ANNEX C** – Permission letter to include a published article from the Journal of Endodontics in this thesis

27/04/17 19:57

UOL mail

**RE: requesting permission to include article in my doctoral thesis**

**De:** Permissions Helpdesk
**Para:** clarit@uol.com.br
**Cópia:**
**Cópia oculta:**
**Assunto:** RE: requesting permission to include article in my doctoral thesis
**Data:** 20/04/2017 20:00

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Hop

**Hop Wechsler**

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Contact the Permissions Helpdesk

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**From:** Clarissa Teles [mailto:clarit@uol.com.br]
**Sent:** Sunday, April 16, 2017 9:59 PM
**To:** Permissions Helpdesk
**Subject:** requesting permission to include article in my doctoral thesis

***** External email: use caution *****

Good afternoon
I would like to know if I can attach in my Doctoral thesis my paper entitled "Efficacy of CM-wire, M-wire, and Nickel-Titanium instruments for removing filling material from curved root canals: a micro-computed tomography study.", published in the Journal of Endodontics, in November 2016, volume 42, number 11, pages 1651-1655.
Kind regards,

**Clarissa Teles Rodrigues**

https://mail.uol.com.br/main/print message?accountId=0&uid=ODYxODg&folder=INBOX Página 1 de 2

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Mar 21, 2017

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| Order Number | 501249449 |
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| Licensed Content Publisher | Elsevier |
| Licensed Content Publication | Journal of Endodontics |
| Licensed Content Title | Efficacy of CM-Wire, M-Wire, and Nickel-Titanium Instruments for Removing Filling Material from Curved Root Canals: A Micro–Computed Tomography Study |
| Licensed Content Author | Clarissa Teles Rodrigues,Marco Antonio Hungaro Duarte,Marcela Milanezi de Almeida,Flaviana Bombarda de Andrade,Norberti Bernardineli |
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| Expected completion date | Jun 2017 |
| Estimated size (number of pages) | 100 |
| Elsevier VAT number | GB 494 6272 12 |
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| Total | Not Available |

**ANNEX D** –Submission confirmation for Brazilian Oral Research Journal.

28/04/17 17:49

UOL mail

**Brazilian Oral Research - Manuscript ID BOR-2017-0223**

De: **Brazilian Oral Research**
Para: **clarit@uol.com.br**
Cópia: **clarit@uol.com.br ,mhungaro@fob.usp.br ,brunomgui@hotmail.com ,rodrigo.vivan@fob.usp.br ,norberti@fob.usp.br**
Cópia oculta:
Assunto: Brazilian Oral Research - Manuscript ID BOR-2017-0223
Data: 13/04/2017 17:10

13-Apr-2017

Dear Miss Rodrigues:

Your manuscript entitled "Comparison of an agitation device and ultrasonic irrigation for removing filling material from curved canals." has been successfully submitted online and is presently being given full consideration for publication in the Brazilian Oral Research.

Your manuscript ID is BOR-2017-0223.

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in to ScholarOne Manuscripts at https://mc04.manuscriptcentral.com/bor-scielo and edit your user information as appropriate.

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Thank you for submitting your manuscript to the Brazilian Oral Research.

Sincerely,
Brazilian Oral Research Editorial Office

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