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EFICÁCIA DA ATIVAÇÃO ULTRASSÔNICA PASSIVA DO SOLVENTE NA REMOÇÃO DE MATERIAL OBTURADOR DURANTE O RETRATAMENTO ENDODÔNTICO – ANÁLISE POR MICROTOMOGRAFIA COMPUTADORIZADA

DISSERTAÇÃO DE MESTRADO

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Santa Maria, RS, Brasil

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EFICÁCIA DA ATIVAÇÃO ULTRASSÔNICA PASSIVA DO SOLVENTE NA REMOÇÃO DE MATERIAL OBTURADOR DURANTE O RETRATAMENTO ENDODÔNTICO – ANÁLISE POR MICROTOMOGRAFIA COMPUTADORIZADA

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Orientador: Prof. Dr. Carlos Alexandre Souza Bier

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elaborada por
Carina Michelon

como requisito parcial para a obtenção do grau de
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Dedicatória

Dedico este trabalho especialmente a minha família, verdadeira razão do meu viver, base sólida sobre o qual edifico todos os meus sonhos...

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"Nesses anos todos, sob suas asas
Eu enriqueci
E tive a luz da vida
E os passos pra seguir
E como o vento, o tempo passa tão depressa
Eu cresci também
Eu não sou mais de vocês, agora é a minha vez
De ser alguém"

Falar dos meus pais - Roupa Nova

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Jerí Adríano,

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"Eu quero ser ao teu lado
Encontro inesperado
O arrepio de um beijo bom
Eu quero ser sua paz a melodia capaz
De fazer você dançar"

Pra você - Paula Fernandes/ Zezé di Camargo

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"Sua alegria é a minha alegria
Suas lágrimas choro também
não importa o que venha na vida,
irmãos, somos irmãos
Irmãos são feitos assim, tão diferentes
mas o Amor que corre nas veias
é maior do que tudo
Irmãos são feitos assim, Sorrindo e Chorando
mas o Amor que corre nas veias
é maior do que tudo"

Irmãos - Ana Paula Valadão

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minha sogra Maria Gorete e minha cunhada Elizandra,

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(Chico Xavier)

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"Que a inspiração chegue não depende de mim. A única coisa que posso fazer é garantir que ela me encontre trabalhando."
Pablo Picasso

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"A diferença entre um homem de sucesso e outro orientado para o fracasso é que um está aprendendo a errar, enquanto o outro está procurando aprender com seus próprios erros."

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De tudo ficam três coisas:
A certeza de que estamos sempre a começar...
A certeza de que precisamos continuar...
A certeza de que seremos interrompidos antes de terminar...
Portanto devemos:
Fazer da interrupção um caminho novo...
Da queda, um passo de dança...
Do medo, uma escada...
Do sonho, uma ponte...
Da procura, um encontro...
Fernando Pessoa

A todos que de alguma forma fizeram parte desta caminhada,
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"Não há transição que não implique um ponto de partida, um processo e um ponto de chegada. Todo amanhã se cria num ontem, através de um hoje. De modo que o nosso futuro baseia-se no passado e se corporifica no presente. Temos de saber o que fomos e o que somos, para sabermos o que seremos."

Paulo Freire

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Odontológicas
Universidade Federal de Santa Maria

EFICÁCIA DA ATIVAÇÃO ULTRASSÔNICA PASSIVA DO SOLVENTE NA REMOÇÃO DE MATERIAL OBTURADOR DURANTE O RETRATAMENTO ENDODÔNTICO – ANÁLISE POR MICROTOMOGRAFIA COMPUTADORIZADA

AUTORA: CARINA MICHELON
ORIENTADOR: PROFESSOR CARLOS ALEXANDRE SOUZA BIER
Local e Data da Defesa: Santa Maria, 02 de agosto de 2013.

A remoção do material obturador pré-existente é um requisito fundamental para a realização do retratamento endodôntico a fim de revelar restos de tecido necrótico e bactérias que podem ser responsáveis pela inflamação periapical e pela falha do tratamento. O objetivo deste estudo foi avaliar a eficácia da Ativação Ultrassônica Passiva (AUP) com uso de solvente orgânico, como um método auxiliar na remoção do material obturador durante o retratamento endodôntico e determinar um protocolo para sua utilização, através da Microtomografia Computadorizada (micro-CT). Trinta raízes mesiais curvas e com istmo de dentes molares inferiores foram instrumentadas e obturadas com guta percha e cimento EndoFill (Dentsply-Maillefer, Ballaigues, Suíça) através da técnica híbrida de Tagger. Após 30 dias, o material obturador foi removido com o sistema rotatório de desobturação ProTaper Retratamento (Dentsply-Maillefer, Ballaigues, Suíça) e o canal repreparado com os instrumentos F1-F4 do sistema rotatório ProTaper Universal (Dentsply-Maillefer, Ballaigues, Suíça). Os espécimes foram aleatoriamente divididos em dois grupos (n=15) de acordo com o protocolo de irrigação final. No Grupo Manual, os canais radiculares foram irrigados e agitados manualmente com solvente a base de Óleo de Laranja (Citrol, Biodinâmica, Ibiporã, Brasil), Hipoclorito de Sódio (NaOCl) 2,5% (Citrol, Biodinâmica, Ibiporã, Brasil) e Ácido Etilenodiaminotetracético (EDTA) 17% (Citrol, Biodinâmica, Ibiporã, Brasil) . No Grupo AUP, os espécimes foram submetidos à AUP com Óleo de Laranja, NaOCl 2,5% e EDTA 17%. A seleção da amostra, a análise da obturação e a mensuração do volume de material obturador remanescente após cada estágio do retratamento foram realizadas através da micro-CT. O volume de material obturador remanescente foi estabelecido e transformado em porcentagem em relação ao volume total de material obturador. Os dados foram submetidos à análise estatística com nível de significância fixado em $\alpha = 0.05$. O teste de Mann Whitney foi usado para comparar a porcentagem de material obturador remanescente entre os grupos, em cada fase do retratamento. O teste de Friedman foi usado para analisar diferenças entre o volume da obturação e os estágios do retratamento dentro de um mesmo grupo. Diferenças entre os terços cervical, médio e apical dentro do mesmo grupo foram analisadas com o teste de Friedman. O teste de Dunn foi usado como *post hoc* para múltiplas comparações. Não houve diferença estatisticamente significante entre os grupos quando a instrumentação rotatória (Estágio 1) foi considerada ($P > 0.05$). Nenhum dos protocolos de irrigação removeu completamente os remanescentes de material obturador em todos os espécimes. Tanto o protocolo da AUP quanto o Manual diminuíram a quantidade de remanescente de guta percha e cimento em relação ao Estágio 1 ($P = 0.001$). No grupo AUP, os canais radiculares mostraram significantemente menos porcentagem de remanescentes de material obturador que o grupo Manual, para todos os terços ($P < 0.05$). O protocolo da AUP deixou quantidade similar de material remanescente em todos os terços do canal radicular ($P > 0.05$). Nenhum dos protocolos de irrigação testados foi capaz de remover completamente a guta percha/cimento de canais radiculares curvos e com área de istmo. Contudo, os resultados obtidos neste estudo sugerem que em canais radiculares com anatomia complexa que necessitam de retratamento endodôntico, a AUP com óleo de laranja, NaOCl e EDTA pode ser um grande auxiliar na remoção dos remanescentes de material obturador deixados após a desobturação do canal radicular.

Palavras-Chaves: Retratamento. Ultrassom. Solventes. Micro-CT.

ABSTRACT

Masters Dissertation
Pos Graduate Program in Dental Sciences
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EFFICACY OF PASSIVE ULTRASONIC ACTIVATION OF THE SOLVENT IN REMOVAL OF ROOT FILLING MATERIAL DURING RETREATMENT ENDODONTIC – A MICRO-COMPUTED TOMOGRAPHY STUDY

AUTHOR: CARINA MICHELON
ADVISOR: PROFESSOR CARLOS ALEXANDRE SOUZA BIER
Place and Date of Defense: Santa Maria, august 02, 2013.

The removal of pre-existing root canal filling is basic requirement for endodontic retreatment in order to reveal necrotic tissue debris and bacteria which may be responsible for periapical inflammation and by the treatment failure. The aim of this study was to evaluate the efficacy of passive ultrasonic activation (PUA) with use of organic solvent as an assistant method in removal of filling material during endodontic retreatment and determine a protocol for its utilization, using high-resolution micro-Computed Tomography (micro-CT). Thirty curved mesial roots of mandibular molars with isthmus area were instrumented and filled with gutta-percha and EndoFill (Dentsply-Maillefer, Ballaigues, Switzerland) through Tagger's hybrid technique. After 30 days, the filling material was removed with the ProTaper Retreatment rotary system (Dentsply-Maillefer, Ballaigues, Switzerland) and the root canals were prepared with the F1-F4 ProTaper instruments (Dentsply-Maillefer, Ballaigues, Switzerland). The specimens will be randomized into two groups according to the final irrigation protocol. In the Manual Group, the root canals were irrigated and agitated manually with Orange Oil solvent (Citrol, Biodinâmica, Ibiporã, Brazil), 2,5% Sodium Hypochlorite (NaOCl) (Biodinâmica, Ibiporã, Brazil) and 17% Ethylenediaminetetraacetic Acid (EDTA) (Biodinâmica, Ibiporã, Brazil). In the PUA Group, the specimens were submitted to PUA with Orange Oil solvent, 2,5% NaOCl and 17% EDTA. High-resolution micro-CT scans was used to select the sample, analyze the root filling and measure the volume of the remaining root filling material after each stage of the retreatment. The volume of remaining filling material was established and transformed into percentage in relation to total volume of filling material. The dates were statistically analyzed with a significance level set at $\alpha = 0.05$. The Mann Whitney test was used to compare the percentage of remaining filling material between groups, for canal thirds in each retreatment step. The Friedman test was used to verify differences among root filling material and the successive retreatment stages at each canal segment in same group. The differences amongst the coronal, middle and apical third in the each group were analyzed with Friedman test. The Dunn test was performed as the post-hoc multiple comparison method. No significant differences between groups when rotary instrumentation (Stage 1) was considered ($P > 0.05$). None of the irrigation protocols removed completely the remains of the root canal filling. The use of Manual e PUA protocols decreases statistically the amount of remaining filling material compared to Stage 1 ($P = 0.000$). In the PUA Group, the root canals showed significantly less percentage remaining filling material compared with Manual Group, for all thirds ($P < 0.05$). The PUA protocol left similar amount of filling material among the apical, middle and cervical thirds ($P > 0.05$). None of the irrigation protocols tested was able to completely remove all gutta-percha/sealer from the curved canals and with isthmus area. However, the results of this study suggest that in root canals with complex anatomy that require endodontic retreatment, the PUA with orange oil, NaOCl and EDTA can be a great aid in the removal of the remaining root filling material left after removal of the bulk of filling material.

Key-words: Retreatment. Ultrasound. Solvents. Micro-CT.

LISTA DE SIGLAS, SÍMBOLOS E ABREVIATURAS

°	Grau
#	Diâmetro Apical do Instrumento
%	Porcentagem
°C	Grau Celsius
3D	Três dimensões
EDTA	Ácido Etilenodiaminotetracético
et al.	E outros ou e colaboradores
AUP	Ativação Ultrassônica Passiva
IUP	Irrigação Ultrassônica Passiva
kV	Quilowatt
Micro-CT	Microtomografia Computadorizada
Min	Minuto
Ml	Mililitro
Mm	Milímetro
NaOCl	Hipoclorito de Sódio
N/cm	Newton centímetro
Ni-Ti	Níquel-Titâneo
PUA	Passive Ultrasonic Activation
PUI	Passive Ultrasonic Irrigation
Rpm	Rotações por minuto
S	Segundo
SD	Standard Deviations
WL	Working Length
µA	Microampére
µL	Microlitro
µm	Micrometro

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SUMÁRIO

INTRODUÇÃO

O objetivo primordial do tratamento endodôntico é reduzir a infecção presente no sistema de canais radiculares (SIQUEIRA & RÔÇAS, 2008). No entanto, a completa limpeza e a adequada desinfecção do canal radicular são difíceis de serem alcançadas, principalmente, devido à sua complexidade anatômica (RICUCCI & BERGENHOLTZ, 2003; PETERS, 2004; NAIR et al., 2005, FAN et al., 2010; VILLAS-BÔAS et al., 2011). Assim, microrganismos podem sobreviver ao tratamento primário do canal radicular, sobretudo nas irregularidades anatômicas, e levar ao insucesso da intervenção (NAIR et al., 1990; NAIR et al., 1999; SIQUEIRA, 2001).

O retratamento endodôntico, também conhecido como reintervenção endodôntica não cirúrgica, é o procedimento de escolha para eliminar a infecção persistente (FRIEDMAN & STABHOLZ, 1986; HÜLSMANN, DREBENSTEDT, HOLSCHER, 2011). O retratamento consiste na reintervenção do conduto radicular e consequente remoção do material obturador pré-existente a fim de permitir uma efetiva desinfecção, instrumentação e obturação do sistema de canais radiculares (BERGENHOLTZ et al., 1979; STABHOLZ & FRIEDMAN, 1988; SCHIRRMEISTER et al., 2006a; SAAD, AL-HADLAQ, AL-KATHEERI, 2007). A remoção da guta percha e do cimento obturador são requisitos fundamentais para a realização do retratamento endodôntico, uma vez que podem abrigar restos de tecido necrótico e bactérias responsáveis pela inflamação periapical e, consequente falha do tratamento (SCHIRRMEISTER et al., 2006b).

Entretanto, a remoção completa do material obturador é difícil, sobretudo nos casos em que ele estiver bem condensado (SUNDQVIST & FIGDOR, 2003; RUDDLE, 2004). Várias técnicas são propostas para a sua remoção, incluindo o uso de brocas de aço inoxidável (GU et al., 2008; MOLLO et al., 2012), de instrumentos manuais (IMURA et al., 1996; BETTI & BRAMANTE, 2001) e rotatórios de Níquel-Titânio (Ni-Ti) (BARRIESHI-NUSAIR, 2002; GERGI & SABBAGH, 2007; RING et al., 2009; FARINIUK et al., 2011), além do calor (EZZIE et al., 2006) e dos solventes (FRIEDMAN, STABHOLZ, TAMSE, 1990; SCELZA et al., 2008). O uso de instrumentos ultrassônicos (DE MELLO JUNIOR et al., 2009; PIRANI et al., 2009) e da irradiação a laser (ANJO et al., 2004; TACHINAMI & KATSUUMI, 2010) também são propostos.

Atualmente, com o objetivo de aprimorar e facilitar a etapa de desobturação, instrumentos rotatórios de Ni-Ti estão sendo cada vez mais utilizados na remoção do material obturador (IMURA et al., 2000, BARATTO FILHO, FERREIRA, FARINIUK, 2002; MACIEL & SCELZA, 2006; GU et al., 2008; TASDEMIR et al., 2008). Alguns destes instrumentos foram criados especificamente para o retratamento endodôntico, como o Sistema ProTaper Retratamento (Dentsply-Maillefer, Ballaigues, Suiça), representado pelos instrumentos D1, D2 e D3. Além de serem eficientes na remoção do material obturador (GU et al., 2008; TASDEMIR et al., 2008), os instrumentos rotatórios de Ni-Ti para retratamento apresentam uma redução no tempo necessário para a desobturação quando comparados com instrumentos manuais (TAKAHASHI et al., 2009, KFIR et al., 2012).

Durante o retratamento endodôntico, os solventes orgânicos podem ser usados como auxiliares no processo de desobturação, com a proposta de diminuir a resistência do material obturador e facilitar à ação dos instrumentos (MARTOS et al., 2006; MAGALHÃES et al., 2007). Solventes como clorofórmio e xylol apresentam grande capacidade de dissolução na maioria dos cimentos (TAMSE et al., 1986; WENNBERG & ORSTAVIK, 1989; GÖRDUYSUS et al., 1997; WHITWORTH & BOURSIN, 2000; SCHÄFER & ZANDBIGLARI, 2002; MAGALHÃES et al., 2007), porém, apresentam elevado potencial carcinogênico (WARD et al., 2010) e toxicidade aos tecidos periapicais (BARBOSA, BURKARD, SPANGBERG, 1994; VAJRABHAYA et al., 2004). Já os óleos essenciais, eucaliptol e óleo de laranja, além de serem capazes de dissolver grande parte dos cimentos endodônticos, são seguros e adequados para esta finalidade (HUNTER et al., 1991; UEMURA et al., 1997; HANSEN 1998; RING et al., 2009). Além disso, o óleo de laranja tem grande capacidade de solubilização de cimentos a base de óxido de zinco e eugenol (PÉCORA et al., 1992).

Alcançar o sucesso do retratamento endodôntico pode ser um grande desafio, visto que uma quantidade substancial de remanescentes de material obturador, possivelmente infectados, permanece nas paredes do canal radicular, mesmo combinando técnicas de remoção (TAKAHASHI et al., 2009; KFIR et al., 2012). Com a finalidade de suprir esta lacuna, o uso da Irrigação Ultrassônica Passiva (IUP) pode ser um importante aliado. A IUP é descrita na literatura como um método auxiliar a limpeza do canal radicular durante o preparo biomecânico. Sua ação depende da transmissão de energia acústica por meio de ondas ultrassônicas que são geradas a partir de um instrumento oscilando na solução irrigadora. Esse mecanismo permite que as soluções alcancem áreas do sistema de canais radiculares inacessíveis à instrumentação e a irrigação convencional, como istmos, deltas, reentrâncias e

irregularidades nas paredes do canal, potencializando a remoção de resíduos dentinários, tecidos orgânicos, sujidades e microrganismos (METZLER & MONTGOMERY, 1989; WEBER et al., 2003; LEE, WU, WESSELINK, 2004; GUTARTS et al., 2005; VAN DER SLUIS, WU, WESSELINK, 2005; PASSARINHO-NETO et al., 2006; BURLESON et al., 2007; AL-JADAA et al., 2009; GRÜNDLING et al., 2011). O Hipoclorito de Sódio (NaOCl) e o Ácido Etilenodiaminotetracético (EDTA) são as soluções irrigadoras mais utilizadas para a ativação da IUP durante tratamento endodôntico. Contudo, estas soluções não tem capacidade de dissolver a guta percha (TOPÜZ et al., 2011). Deste modo, diante do exposto, questiona-se, se durante o retratamento endodôntico, a Ativação Ultrassônica Passiva (AUP) de um solvente poderia melhorar a remoção de material obturador.

Historicamente, várias metodologias podem ser usadas para quantificar a presença de material obturador remanescente durante o retratamento endodôntico, tais como, diafanização (SCHIRRMEISTER et al., 2006 a, c; TASDEMIR et al., 2008), avaliação radiográfica (FERREIRA, RHODES, FORD, 2001; MASIERO & BARLETTA, 2005; GERGI & SABBAGH, 2007), seccionamento longitudinal do dente para avaliação através de imagens e microscopia (HULSMANN & BLUHN, 2004; EZZIE et al., 2006; SÓ et al., 2008) e a microscopia eletrônica de varredura (KFIR et al., 2012). Todavia, a maioria dessas metodologias apresenta limitações, como por exemplo, a destruição da amostra e a impossibilidade de avaliar simultaneamente diferentes parâmetros do preparo, obturação e retratamento do canal radicular (SHEMESH et al., 2009). Estas dificuldades levaram a uma crescente busca por novas metodologias que possibilitem avaliações quantitativas e qualitativas do retratamento endodôntico, como a Microtomografia Computadorizada (micro-CT).

A micro-CT é um método não destrutivo que permite exames repetidos da amostra e, em alguns casos, a reutilização do espécime (NIELSEN et al., 1995). A sua principal vantagem em relação aos outros métodos de avaliação é a capacidade de demonstrar características morfológicas detalhadas do canal radicular, de maneira precisa e de forma não invasiva (DOWKER; DAVIS; ELLIOTT, 1997; RHODES et al., 2000). A sobreposição de imagens tridimensionais fornecidas pelo microtomógrafo, através de um software adequado, permite avaliar áreas de interesse dentro do lúmen e da estrutura do canal radicular, tais como, volume de dentina removido durante preparo dos canais radiculares (PETERS, SCHÖNENBERGUER, LAIB, 2001; PETERS et al., 2003; HÜBSCHER, BARBAKOW, PETERS, 2003; PAQUÉ, BARBAKOW, PETERS, 2005), espaços vazios dentro de obturações (JUNG, LOMMEL, KILMEK, 2005; HAMMAD, QUALTROUGH, SILIKAS,

2009) ou resíduos de material obturador durante o retratamento endodôntico (BARLETTA et al., 2007, 2008; ROGGENDORF et al., 2010). Além disso, esta metodologia oferece dados reprodutíveis em três dimensões, permitindo comparações antes e após a intervenção no conduto radicular (RHODES et al., 2000; PETERS et al., 2001; BERGMANS et al., 2003).

Nos estudos que avaliam a quantidade de material obturador remanescente, a micro-CT fornece imagens em que a dentina e o material obturador podem ser claramente distinguidos um da outro (JUNG et al., 2005; ROGGENDORF et al., 2010). Ademais, o volume de material obturador remanescente pode ser expresso como porcentagem do volume total da obturação, em cada secção da raiz, semelhante a outras metodologias (WILCOX et al.,1987; BETTI & BRAMANTE, 2001; MASIERO & BARLETTA, 2005; HASSANLO et al., 2007; TASDEMIR et al., 2008), porém de forma tridimensional.

Diante do exposto e da necessidade de encontrar um método capaz de remover completamente o material obturador do interior do sistema de canais radiculares, este estudo propôs-se a avaliar a eficácia da IUP com solvente orgânico e estabelecer um protocolo de uso para esta associação, quanto à remoção de remanescentes de guta percha e cimento durante o retratamento endodôntico, em raízes mesiais curvas e com área de istmo de dentes molares inferiores, por meio da micro-CT.

ARTIGO - EFFICACY OF PASSIVE ULTRASONIC ACTIVATION OF THE SOLVENT IN REMOVAL OF ROOT FILLING MATERIAL - A MICRO-COMPUTED TOMOGRAPHY STUDY

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EFFICACY OF PASSIVE ULTRASONIC ACTIVATION OF THE SOLVENT IN REMOVAL OF ROOT FILLING MATERIAL - A MICRO-COMPUTED TOMOGRAPHY STUDY

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ABSTRACT

Introduction: The aim of this study was to evaluate the efficacy of the passive ultrasonic activation (PUA) with organic solvent in removal of filling material during endodontic retreatment and to determine a protocol of use for this association, using high-resolution micro-computed tomography (micro-CT). **Methodos:** Thirty curved mesial roots of mandibular molars with isthmus area were instrumented and filled with gutta-percha and EndoFill through Tagger's hybrid technique. The root canal filling was removed with ProTaper retreatment instruments followed by F1-F4 ProTaper instruments. In the Manual Group, the specimens were irrigated and agitated manually with Orange Oil solvent, 2.5% NaOCl and 17% EDTA. In the PUA Group, the specimens were submitted to PUA with Orange Oil, 2.5% NaOCl and 17% EDTA. High-resolution micro-CT scans was used to select the sample, analyze the root filling and measure the volume of the remaining root filling material after each stage of the retreatment. Statistical analysis was performed using the Friedman and Mann Whitney tests. **Results:** Both irrigation protocols decreased the amount of remaining filling material compared to rotary instruments ($P < 0.05$). PUA Group showed significantly less percentage remaining filling material that Manual Group, for all thirds ($P < 0.05$). **Conclusions:** None of the irrigation protocols was able to completely remove the remains of the root canal filling from all curved canals with isthmus area. However, PUA protocol was more efficacious than Manual in removing of residual root filling material during endodontic retreatment.

Key-words: Retreatment. Ultrasound. Solvents. Micro-CT. Gutta-percha Removal.

INTRODUCTION

The root canal retreatment aims to eliminate or to substantially reduce the microbial load from the root canal system (1). Removing all root filling materials is a prerequisite of non-surgical retreatment, as it allows subsequent cleaning, shaping and filling of the root canal (2). Nevertheless, it is almost impossible to completely remove the root filling material from root canals, irrespective of the retreatment technique (3-5), especially in teeth with complex anatomy (6-8). Organic solvents can be used in this process to facilitate the removal of gutta-percha and sealer (9-11).

The passive ultrasonic irrigation (PUI) have been used as an effective auxiliary method in cleaning and disinfection the root canal, principally in areas inaccessible to instrumentation like isthmus, oval extensions and irregularities from the root canal wall (12-15). The PUI activates and potentiates the action of irrigant providing an effective removal of remnants of pulp tissue, dentine debris (12, 13, 16) and planktonic bacteria (17, 18). However, no studies have been published in the literature evaluating the passive ultrasonic activation (PUA) of organic solvents during the endodontic retreatment.

The high-resolution micro-computed tomography (micro-CT) has been used to evaluate the removal of root canal fillings (7, 19-23). This methodology allows differentiate the gutta-percha and sealer from the canal walls (24), and perform a three-dimensional quantitative evaluation of residual filling material (21). Moreover, the micro-CT enables nondestructive and stepwise assessment of the retreatment by repeated scans of the same specimen (21).

Considering the need to find a method to able removes the filling material from the root canal system, the aim of this study was to evaluate the efficacy of the PUA with organic solvent and to determine a protocol of use for this association, as an adjunct method in the removal of gutta-percha and sealer from curved mesial roots and with isthmus area of mandibular molars, using micro-CT. The null hypothesis tested was that there is no difference between the PUA and Manual protocols in the removal of residual filling material from root canals with complex anatomy.

MATERIAL AND METHODS

Selection of Teeth

Thirty curved mesial roots of mandibular molar teeth with isthmus area were selected. Canal curvatures and radius were measured in both directions by one calibrated operator using the methods described by Schneider (25) and Schäfer et al. (26), respectively. Root canals with curvature between 10° and 20° and maximum radius of curvature of 12 mm in buccolingual direction were included. The presence of the isthmus was evaluated using a high-resolution micro-CT system. Based on the 3D images obtained, the roots were classified according Vertucci (27). Specimens classified as type 1, 2, 3, 5, 6 e 7 of Vertucci were included in this study.

The pulp chambers and mesial root canals were accessed conventionally. Size 10 K-type files were inserted through the mesiobuccal and mesiolingual canals 1 mm beyond the apical foramen to establish apical patency. The working length (WL) was established 1 mm shorter than the length of the root canal. The clinical crown of the roots was not removed in order to allow that a greater amount of irrigant stayed in contact with the canal walls.

Root Canal Preparation and Filling

All root canals were prepared by the same operator with the ProTaper Universal rotary Nickel-Titanium (Ni-Ti) instruments (Dentsply Maillefer) according to manufacturer's instructions. The instruments S1, SX, S1, S2, F1 and F2 were used in this sequence and operated with electric motor (X-Smart; Dentsply Maillefer, Ballaigues, Switzerland) at a torque of 2 N/cm and a constant speed of 300 rpm. At each change of instrument, the canals were irrigated with 2 mL of 2,5% sodium hypochlorite (NaOCl) using a syringe and a 30-gauge irrigation needle (NaviTip, Ultradent, São Paulo, Brazil). After completed instrumentation, all the root canals were submitted the PUA with 2.5% NaOCl and 17% ethylenediaminetetraacetic acid (EDTA), with a piezoelectronic ultrasound (Gnatus, São Paulo, Brazil), at full power, with A90 adapter (Dabi Atlante, São Paulo, Brazil) equipped with instrument K 15, 1 mm short of the WL, and oscillating in the direction towards the isthmus areas (28). Later, the specimens were irrigated with 2 mL of saline solution and dried using paper points.

Root filling was performed with tapered gutta-percha cones and EndoFill (Dentsply-Maillefer, Ballaigues, Switzerland) through Tagger's hybrid technique. A standardized gutta-percha master cone size 25 was fitted with tug-back at WL. The sealer was manipulated according to manufacturer's instructions. This master cone was lightly covered with sealer and slowly inserted into the root canal until it reached WL. After insertion of the master cone, accessory gutta-percha cones (TP-FM, Dentsply Maillefer, Ballaigues, Switzerland) were

added with a #25 finger spreader (Dentsply Maillefer). The thermomechanical condensation of gutta-percha was performed with the McSpadden condenser 35. Next, the vertical condensation of the plasticized gutta-percha was performed with plugger in order to achieve a better adaptation inside the root canal system. The quality and apical extent of the root canal filling was assessed with radiographs in buccolingual and mesio-distal directions. Filling was determined adequate when it appeared to be homogeneous without any voids. Inadequate root fillings were recondensed. The access cavities were temporarily sealed with a cotton pellet and Coltosol (Coltène, Kostanz, Alemanha). Subsequently, the specimens were stored for 30 days at ambient temperature and 100% humidity to allow the complete setting of the sealer. All roots were subsequently scanned with micro-CT again, and the volume of root filling was determined.

Micro-CT Scanning Procedures and Evaluation

Specimens were scanned using SkyScan 1174 (SkyScan, Kontich, Bélgica) located at the Dental School of Bauru, in the University of São Paulo. The specimens were mounted in condensation silicone that allowed precise placement in the scanning unit without interference with the scanning process. Specimens were scanned at 50 kV, 800 µA, with rotational angle of 180° and with an isotropic resolution of 14.1µm, resulting in 700 to 900 slices per root. With the software NRecon 1.6.4.8 (SkyScan, Aartselaar, Bélgica), images obtained from the scan were reconstructed to show 2-D slices of the structure of the roots. The area of interest was selected extending from the apex of the root to the root canal orifice. The root canals were separated into three segments (apical, middle, coronal) of 3 mm each (21). The volume of root filling residue on the canal walls was quantified and reconstructed in 3D with the software CTAn 1.11.10 (Skyscan, Aartselaar, Bélgica). The pre- and postoperative volumes of root filling material were measured in mm^3 by one blinded observer. The percentage of residual filling material for each segment and each retreatment step was calculated in the all specimens. Three-dimensional reconstruction of the images was visualized using specific software CTVol 2.0 (Skyscan, Aartselaar, Bélgica).

Retreatment Techniques

Temporary restorations were removed, and one drop of orange oil was used for 2 min to soften the gutta-percha at the root canal orifice. The bulk of root filling material was removed using ProTaper retreatment system (Dentsply Maillefer). The instruments D1, D2 and D3 were used for cervical, middle and apical thirds, respectively, at 500 rpm and 3 N/cm.

One drop of orange oil was used among each instrument. Apical preparation was performed with ProTaper Universal instruments F1-F4 at 300rpm and 2 N/cm. ProTaper instruments were used in a torque-controlled motor according to the manufacturer's instructions. In all groups, irrigation was performed with 2 mL of 2.5% NaOCl, using a syringe and a NaviTip needle after each instrument. Removal was considered complete when no residual gutta-percha and sealer were observed in the instrument flutes or in the irrigation solution (Stage 1). Next, teeth were scanned with micro-CT again, and the volume of the remaining radiopaque residue was determined. The specimens were randomly divided into two groups according to the protocol of final irrigation (Stage 2) using Random Allocation software (Microsoft, Washington, USA). The conformation of the root canal established by Vertucci 1984 was used as stratum for randomization.

Manual Group (n = 15)

Solvent: Each root canal was filled with 1 drop of orange oil (25 µL) and with aid of the instrument 40 K-type the solvent was agitated during 3 min. Every minute the solvent present in the root canal was aspirated and a new solution was placed.

NaOCl: Each root canal was irrigated with 6 mL of 2.5% NaOCl during 1 min.

EDTA: The root canal was filled with 1 drop of 17% EDTA (25 µL) and the solution was agitated for 1 min with instrument 40 K-type.

The irrigation was performed using a syringe/Navitip needle and the aspiration was performed with Capillary Tips (Ultradent, São Paulo, Brazil) of medium sized, positioned at the entrance of the root canals.

PUA Group (n = 15)

PUA with Solvent: Each root canal was filled with 1 drop of orange oil (25 µL) and activated with PUA for 3 min. Every minute the solvent present in the root canal was aspirated and a new solution was placed.

PUA with NaOCl: The root canal was filled with 2.5% NaOCl and activated with PUA. Were performed three activations of the 20s each, totaling 1 min for canal. After each activation, the root canal was irrigated/aspirated with 2 mL of 2.5% NaOCl and inundated again.

PUA with EDTA: Each root canal was filled with 1 drop of 17% EDTA (25 µL). PUA was performed during 1 min continuous.

The PUA was realized with a piezoelectric ultrasound Ultra Sonic, at full power, with A90 adapter, equipped with instrument 15 K-file to 1 mm short of the WL, and oscillating in the direction towards the isthmus areas (28).

In both groups, each root canal was irrigated with 2 mL of saline solution, dried with absorbent paper points sterile and kept at ambient temperature, under humidity. All roots were scanned again, and the volume of remaining root filling was determined.

Statistical Analysis

The normal distribution of data was verified with the Shapiro-Wilk test. The Mann Whitney test was used to compare the percentage of remaining filling material between groups, for canal thirds in each retreatment step. The Friedman test was used to verify differences among root filling material and the successive retreatment stages at each canal segment in same group. The differences amongst the coronal, middle and apical third in the each group were analyzed with Friedman test. The Dunn test was performed as the post-hoc multiple comparison method. The alpha-type error was set at 0.05 and Prisma 5.0 (GraphPad Software Inc., La Jolla, CA, USA) was used as the analytical tool.

RESULTS

The means values for degree and radius of curvature of each group are shown in Table 1. No significant differences between the groups concerning angles of curvature ($P = 0.934$) and radius ($P = 0.575$) could be detected.

Measurements of the means of percentages of residual root canal filling material in each group, after Stage 1 and Stage 2 are shown in Table 2. No significant differences between groups when rotary instrumentation (Stage 1) was considered ($P > 0.05$). None of the irrigation protocols removed completely the remains of the root canal filling in all specimens. In both stages of the retreatment the percentage of remaining filling material decreased statistically compared to the root filling ($P < 0.05$). However, the use of Manual e PUA protocols showed less remaining filling material that stage 1 ($P < 0.05$). In the PUA Group, the root canals showed significantly less amount remaining filling material compared with Manual Group, for all thirds ($P < 0.05$). The PUA protocol left similar amount of filling material among the apical, middle and cervical thirds ($P > 0.05$).

Table 1 Characteristics of curved root canals (n=15 teeth per group)

Group	Curvature bl [°] (mean ± SD)	Radius bl [mm] (mean ± SD)
Manual	16,44 ± 3,22	9,46 ± 2,37
PUA	16,33 ± 3,68	9,02 ± 1,94
P	0.934	0.575

Statistical analysis for the equality of the mean values between the two groups was evaluated using Student *t* test ($P < 0.05$). bl, bucco-lingual direction.

Table 2 Means and standard deviations (SD) for residual root canal filling (in %), after Stage 1 (ProTaper instruments - before randomization) and after Stage 2 (Manual and PUA protocols).

	Canal Segment	Manual Group (mean ± SD)	PUA Group (mean ± SD)	*P* value*
Stage 1 (ProTaper)	Apical	35,41±17,26 ^{A}	30,59±15,66 ^{A}	.5069
	Middle	25,87±12,23 ^{AB}	26,03±7,41 ^{AB}	.7089
	Coronal	10,80±6,96 ^{C}	9,93±4,69 ^{C}	.9339
Stage 2	Apical	28,62±15,89 ^{A}	9,06±9,46 ^{A}	.0007
	Middle	20,52±10,18 ^{AB}	8,14±8,81 ^{A}	.0019
	Coronal	9,33±6,34 ^{B}	3,65±3,83 ^{A}	.0079

Capital letter: Comparison among thirds in the same group and Stage. Different letters indicate statistically significant difference ($P < 0.05$).
*Statistically significant difference if $P < 0.05$.

DISCUSSION

None of the irrigation protocols that were tested in the present study could render all canals completely free of root filling residues. Both groups showed a reduction in the amount of residual root filling material regarding the removal of the bulk of root filling material and instrumentation with rotary system. The first null hypothesis tested in this study was rejected since the PUA protocol left significantly less residue than Manual Group (Fig. 1). The best results obtained in the PUA Group can be assigned to the mechanism of action of the PUA with the solvent. A pilot study was conducted to evaluate the efficacy of the PUA with orange oil in dissolving of sealer and to verify the temperature increase in the orange oil generated by the ultrasound. The replacement of solvent avoided its saturation and the ultrasound caused an elevation in temperature of the solvent (2°C), increasing its ability to dissolve the sealer. Orange oil has been a good alternative for use in the endodontic retreatment because its capacity to dissolve the sealers is similar other solvents, but without the deleterious effects, justifying its choice in this study (9-11).

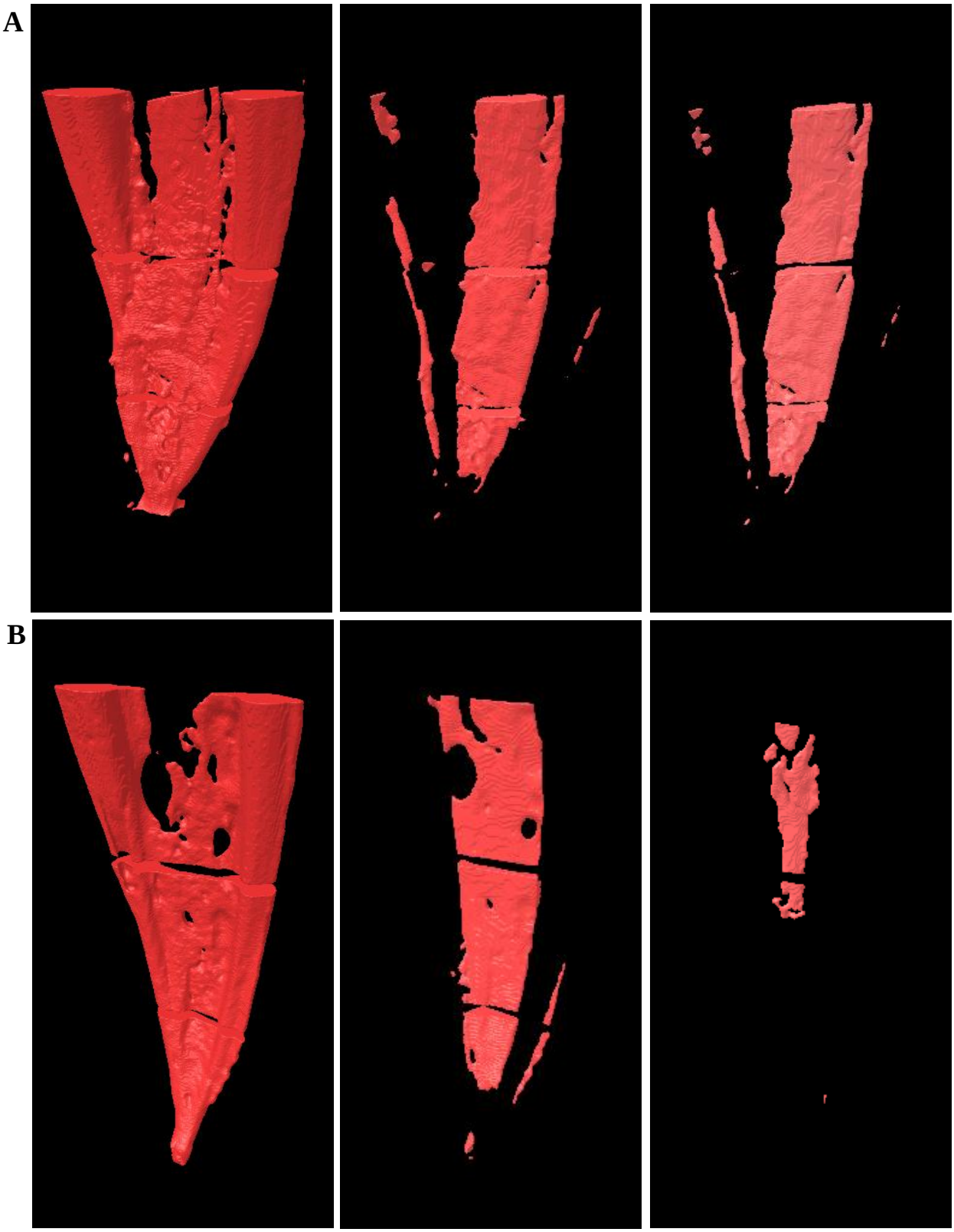

Figure 1. Representative cases of root filling removal in mesial canals in mandibular molars. 3D reconstructions of root filling material. (*A*) Root filling residue that remained in the canals after Manual Protocol. *Left*, the root filling after obturation; *center*, the root filling residue left in the canal after retreatment with ProTaper; *right*, the root filling residue left in the canal after protocol established for the Manual Group. (*B*) The root filling residue that was left in the canals after PUA Protocol. *Left*, the root filling after obturation; *center*, the root filling residue left in the canal after retreatment with ProTaper; *right*, the root filling residue left in the canal after protocol proposed for the PUA Group.

The PUA only with NaOCl and EDTA is unable of improve the amount of root filling material removed during endodontic retreatment (29). This result was expected because the NaOCl has no ability to dissolve root filling material (30). However, the combination these irrigants with orange oil and PUA shows good results. During the PUA it is essential that the file moves freely within the root canal to allow that the solution penetrate more easily into the root canals system (31, 32). According Boutsioukis et al. (33), the root canal size can affect the instrument oscillation and reduce the cleaning efficacy of the PUA. It is believed that the enlargement of canals performed by ProTaper system until F4 instrument (Size 40, 0.06 taper) allowed, in this study, that the oscillation amplitude of the file was minimally affected by previous contact with the root canal walls.

The use of the PUA during endodontic retreatment also was able of improve the cleaning apical. The results indicate a percentage similar of residual filling material among the thirds of the root canal after PUA protocol. However, the average percentage of filling material present in root canals after Stage 1 is consistent with Abramovitz et al. (34). When these authors used only ProTaper retreatment files in curved canals of mesial roots of mandibular molars, they found large amounts of residual root filling material in the apical third of the canals.

Although, in many cases, irrigation can effectively occur during the use of ultrasound (13, 35), the term 'passive ultrasonic irrigation' is confusing. In the present study, was not performed irrigation solvent during experimental protocol but, its activation through ultrasound. Therefore, is suggesting that the term 'passive ultrasonic activation' (PUA) is adopted to describe interventions where the solution is agitated/activated by ultrasound.

Based on our arbitrary definition, several cases in PUA group could be called “effectively cleaned” (ie, having less than 0.5% residue) than in the Manual Group. However, even the PUA Group presenting statistically the best results and, some root canals extremely clean, the average amount of remaining filling material was high compared to other studies in the literature (20-22, 36). A possible explanation for this fact is that the majority of these experimental studies have been performed using single straight root canals to simplify standardization of specimens. In the present study, curved mesial roots of mandibular molar and with isthmus area were selected. Although teeth with complex anatomy represent a common clinical situation (37), investigations of nonsurgical retreatment techniques for teeth with 2 curved canals and isthmus area are rare. Irregularities, isthmus, and reentrances (38) difficult the removal of the filling, cleaning, and instrumentation of the root canal, which may provide a refuge for microorganisms (39) influencing the success of endodontic retreatment.

In this study, the micro-CT analysis revealed that the most of the remaining filling material was found in the isthmus area.

In the present study, angles and radius of curvature were determined, and specimens were randomized with regard to homogenous mean values with no significant differences between the experimental groups ($P > 0.05$). To minimize variations amongst the specimens, teeth were submitted to micro-CT to verify the conformation of the root canal. The canals were classified according Vertucci et al. (27) and a stratified randomization was performed taking into account this classification. In addition, no statistical difference between the groups after the use of rotary instruments indicating that the specimens showed similarity in the presence of filling material before randomization in the experimental groups ($P > 0.05$).

The micro-CT methodology was used in current study because allows noninvasive analyzes of the specimens and the three-dimensional quantitative evaluation of residual filling material. Micro-CT imaging showed remnants of gutta-percha and sealer after stages retreatment, which was expressed as percentage of the preoperative volume of the filling material, similar to previous studies (7, 19, 20, 22, 23).

CONCLUSIONS

On the basis of the results of the present study, it can be concluded that in root canals with complex anatomy, PUA protocol was more efficacious than Manual irrigation protocol in removing residual filling material during endodontic retreatment. The amount the remaining filling material was similar among thirds in the PUA Group after protocol execution. Thus, the use of the PUA with solvent during endodontic retreatment seems to be an aid method promising in the removal of remaining filling material, however, further studies are necessary to confirm its clinical efficacy.

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CONCLUSÃO

Diante dos resultados obtidos e respeitando as limitações metodológicas empregadas no presente estudo conclui-se que o protocolo da AUP com óleo de laranja, NaOCl e EDTA foi mais eficaz que o protocolo manual na remoção de resíduos de material obturador durante o retratamento endodôntico. Deste modo, em canais radiculares com anatomia complexa, a AUP com solvente pode ser utilizada como um método auxiliar na remoção de material obturador durante a reintervenção do conduto radicular.

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ANEXOS

ANEXO A - Carta de Aprovação do Comitê de Ética em Pesquisa da Universidade Federal de Santa Maria.

ANEXO B – Normas da Revista *Journal of Endodontics*.

ANEXO A

UNIVERSIDADE FEDERAL DE SANTA MARIA/ PRÓ-REITORIA DE PÓS-GRADUAÇÃO E

PROJETO DE PESQUISA

Título: Efetividade da Irrigação Ultrassônica Passiva na Limpeza de Canais Radiculares Durante o Retratamento Endodôntico

Área Temática:

Área 9. A critério do CEP.

Versão: 2

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PARECER CONSUBSTANCIADO DO CEP

Número do Parecer: 109.809

Data da Relatoria: 20/09/2012

Apresentação do Projeto:

A remoção da gutta-percha e do cimento obturador infectados são requisitos fundamentais para a realização do retratamento endodôntico, a fim de revelar restos de tecido necrótico e bactérias que podem ser responsáveis pela inflamação periapical e pela falha do tratamento.

Objetivo da Pesquisa:

O objetivo deste estudo é a avaliar a efetividade da IUP com uso de solventes orgânicos, como um método auxiliar na remoção do material obturador, durante o retratamento endodôntico, através da micro-CT.

Avaliação dos Riscos e Benefícios:

os riscos e beneficios estao de acordo com a pesquisa proposta

Comentários e Considerações sobre a Pesquisa:

Trabalho de dissertação de mestrado do curso de odontologia. Estudo laboratorial, randomizado, duplo-cego. Calculo amostral será realizado após estudo piloto.

Considerações sobre os Termos de apresentação obrigatória:

Os termos estao adequados

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

aprovar o projeto

Situação do Parecer:

Aprovado

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UNIVERSIDADE FEDERAL DE SANTA MARIA/ PRÓ-REITORIA DE PÓS-GRADUAÇÃO E

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

SANTA MARIA, 27 de Setembro de 2012

Assinado por:
Félix Alexandre Antunes Soares

(Coordenador)

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ANEXO B

Guidelines for Publishing Papers in the JOE

Writing an effective article is a challenging assignment. The following guidelines are provided to assist authors in submitting manuscripts.

The *JOE* publishes original and review articles related to the scientific and applied aspects of endodontics. Moreover, the *JOE* has a diverse readership that includes full-time clinicians, full-time academicians, residents, students and scientists. Effective communication with this diverse readership requires careful attention to writing style.

1. **General Points on Composition**

a. Authors are strongly encouraged to analyze their final draft with both software (*e.g.*, spelling and grammar programs) and colleagues who have expertise in English grammar. References listed at the end of this section provide a more extensive review of rules of English grammar and guidelines for writing a scientific article. Always remember that clarity is the most important feature of scientific writing. Scientific articles must be clear and precise in their content and concise in their delivery since their purpose is to inform the reader. The Editor reserves the right to edit all manuscripts or to reject those manuscripts that lack clarity or precision, or have unacceptable grammar or syntax. The following list represents common errors in manuscripts submitted to the *JOE*:
b. The paragraph is the ideal unit of organization. Paragraphs typically start with an introductory sentence that is followed by sentences that describe additional detail or examples. The last sentence of the paragraph provides conclusions and forms a transition to the next paragraph. Common problems include one-sentence paragraphs, sentences that do not develop the theme of the paragraph (see also section "c" below), or sentences with little to no transition within a paragraph.
c. Keep to the point. The subject of the sentence should support the subject of the paragraph. For example, the introduction of authors' names in a sentence changes the subject and lengthens the text. In a paragraph on sodium hypochlorite, the sentence, "In 1983, Langeland et al., reported that sodium hypochlorite acts as a lubricating factor during instrumentation and helps to flush debris from the root canals" can be edited to: "Sodium hypochlorite acts as a lubricant during instrumentation and as a vehicle for flushing the generated debris (Langeland et al., 1983)." In this example, the paragraph's subject is sodium hypochlorite and sentences should focus on this subject.
d. Sentences are stronger when written in the active voice, *i.e.*, the subject performs the action. Passive sentences are identified by the use of passive verbs such as "was," "were," "could," etc. For example: "Dexamethasone was found in this study to be a factor that was associated with reduced inflammation," can be edited to: "Our results demonstrated that dexamethasone reduced inflammation." Sentences written in a direct and active voice are generally more powerful and shorter than sentences written in the passive voice.

e. Reduce verbiage. Short sentences are easier to understand. The inclusion of unnecessary words is often associated with the use of a passive voice, a lack of focus or run-on sentences. This is not to imply that all sentences need be short or even the same length. Indeed, variation in sentence structure and length often helps to maintain reader interest. However, make all words count. A more formal way of stating this point is that the use of subordinate clauses adds variety and information when constructing a paragraph. (This section was written deliberately with sentences of varying length to illustrate this point.)
f. Use parallel construction to express related ideas. For example, the sentence, "Formerly, endodontics was taught by hand instrumentation, while now rotary instrumentation is the common method," can be edited to "Formerly, endodontics was taught using hand instrumentation; now it is commonly taught using rotary instrumentation." The use of parallel construction in sentences simply means that similar ideas are expressed in similar ways, and this helps the reader recognize that the ideas are related.
g. Keep modifying phrases close to the word that they modify. This is a common problem in complex sentences that may confuse the reader. For example, the statement, "Accordingly, when conclusions are drawn from the results of this study, caution must be used," can be edited to "Caution must be used when conclusions are drawn from the results of this study."
h. To summarize these points, effective sentences are clear and precise, and often are short, simple and focused on one key point that supports the paragraph's theme.
i. Authors should be aware that the *JOE* uses iThenticate, plagiarism detection software, to assure originality and integrity of material published in the *Journal*. The use of copied sentences, even when present within quotation marks, is highly discouraged. Instead, the information of the original research should be expressed by new manuscript author's own words, and a proper citation given at the end of the sentence. Plagiarism will not be tolerated and manuscripts will be rejected, or papers withdrawn after publication based on unethical actions by the authors. In addition, authors may be sanctioned for future publication.

2. **Organization of Original Research Manuscripts**

Please Note: *All abstracts should be organized into sections that start with a one-word title (in bold),* i.e.*, Introduction, Methods, Results, Conclusions, etc., and should not exceed more than 250 words in length.*

a. **Title Page:** The title should describe the major emphasis of the paper. It should be as short as possible without loss of clarity. Remember that the title is your advertising billboard—it represents your major opportunity to solicit readers to spend the time to read your paper. It is best not to use abbreviations in the title since this may lead to imprecise coding by electronic citation programs such as PubMed (*e.g.*, use "sodium hypochlorite" rather than NaOCl). The author list must conform to published standards on authorship (see authorship criteria in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals at *www.icmje.org*). The manuscript title, name and address (including email) of one author designated as the corresponding author. This author will be responsible for editing proofs and ordering reprints when applicable. The contribution of each author should also be highlighted in the cover letter.

b. **Abstract:** The abstract should concisely describe the purpose of the study, the hypothesis, methods, major findings and conclusions. The abstract should describe the new contributions made by this study. The word limitations (250 words) and the wide distribution of the abstract (*e.g.*, PubMed) make this section challenging to write clearly. This section often is written last by many authors since they can draw on the rest of the manuscript. Write the abstract in past tense since the study has been completed. Three to ten keywords should be listed below the abstract.
c. **Introduction:** The introduction should briefly review the pertinent literature in order to identify the gap in knowledge that the study is intended to address and the limitations of previous studies in the area. The purpose of the study, the tested hypothesis and its scope should be clearly described. Authors should realize that this section of the paper is their primary opportunity to establish communication with the diverse readership of the *JOE*. Readers who are not expert in the topic of the manuscript are likely to skip the paper if the introduction fails to succinctly summarize the gap in knowledge that the study addresses. It is important to note that many successful manuscripts require no more than a few paragraphs to accomplish these goals. Therefore, authors should refrain from performing extensive review or the literature, and discussing the results of the study in this section.
d. **Materials and Methods:** The objective of the materials and methods section is to permit other investigators to repeat your experiments. The four components to this section are the detailed description of the materials used and their components, the experimental design, the procedures employed, and the statistical tests used to analyze the results. The vast majority of manuscripts should cite prior studies using similar methods and succinctly describe the essential aspects used in the present study. Thus, the reader should still be able to understand the method used in the experimental approach and concentration of the main reagents (*e.g.*, antibodies, drugs, etc.) even when citing a previously published method. The inclusion of a "methods figure" will be rejected unless the procedure is novel and requires an illustration for comprehension. If the method is novel, then the authors should carefully describe the method and include validation experiments. If the study utilized a **commercial product**, the manuscript must state that they either followed manufacturer's protocol *or* specify any changes made to the protocol. If the study used an ***in vitro*** **model** to simulate a clinical outcome, the authors must describe experiments made to validate the model, or previous literature that proved the clinical relevance of the model. Studies on **humans** must conform to the Helsinki Declaration of 1975 and state that the institutional IRB/equivalent committee(s) approved the protocol and that informed consent was obtained after the risks and benefits of participation were described to the subjects or patients recruited. Studies involving **animals** must state that the institutional animal care and use committee approved the protocol. The statistical analysis section should describe which tests were used to analyze which dependent measures; p-values should be specified. Additional details may include randomization scheme, stratification (if any), power analysis as a basis for sample size computation, drop-outs from clinical trials, the effects of important confounding variables, and bivariate versus multivariate analysis.
e. **Results:** Only experimental results are appropriate in this section (*i.e.*, neither methods, discussion, nor conclusions should be in this section). Include only those data that are critical for the study, as defined by the aim(s). Do not include all available data without justification; any repetitive findings will be rejected from publication. All Figures, Charts

and Tables should be described in their order of numbering with a brief description of the major findings. Author may consider the use of supplemental figures, tables or video clips that will be published online. Supplemental material is often used to provide additional information or control experiments that support the results section (*e.g.*, microarray data).

f. **Figures:** There are two general types of figures. The first type of figures includes photographs, radiographs or micrographs. Include only essential figures, and even if essential, the use of composite figures containing several panels of photographs is encouraged. For example, most photo-, radio- or micrographs take up one column-width, or about 185 mm wide X 185 mm tall. If instead, you construct a two columns-width figure (*i.e.*, about 175 mm wide X 125 mm high when published in the *JOE*), you would be able to place about 12 panels of photomicrographs (or radiographs, etc.) as an array of four columns across and three rows down (with each panel about 40 X 40 mm). This will require some editing to emphasize the most important feature of each photomicrograph, but it greatly increases the total number of illustrations that you can present in your paper. Remember that each panel must be clearly identified with a letter (*e.g.*, "A," "B," etc.), in order for the reader to understand each individual panel. Several nice examples of composite figures are seen in recent articles by Jeger et al (J Endod 2012;38:884–888); Olivieri et al., (J Endod 2012;38:1007 1011); Tsai et al (J Endod 2012;38:965–970). Please note that color figures may be published at no cost to the authors and authors are encouraged to use color to enhance the value of the illustration. Please note that a multipanel, composite figure only counts as one figure when considering the total number of figures in a manuscript (see section 3, below, for maximum number of allowable figures).

   The second type of figures are graphs (*i.e.*, line drawings including bar graphs) that plot a dependent measure (on the Y axis) as a function of an independent measure (usually plotted on the X axis). Examples include a graph depicting pain scores over time, etc. Graphs should be used when the overall trend of the results are more important than the exact numerical values of the results. For example, a graph is a convenient way of reporting that an ibuprofen-treated group reported less pain than a placebo group over the first 24 hours, but was the same as the placebo group for the next 96 hours. In this case, the trend of the results is the primary finding; the actual pain scores are not as critical as the relative differences between the NSAID and placebo groups.

g. **Tables:** Tables are appropriate when it is critical to present exact numerical values. However, not all results need be placed in either a table or figure. For example, the following table may not be necessary:

% NaOCl	**N/Group**	**% Inhibition of Growth**
0.001	5	0
0.003	5	0
0.01	5	0
0.03	5	0
0.1	5	100
0.3	5	100
1	5	100
3	5	100

h. Instead, the results could simply state that there was no inhibition of growth from 0.001-0.03% NaOCl, and a 100% inhibition of growth from 0.03-3% NaOCl (N=5/group). Similarly, if the results are not significant, then it is probably not necessary to include the results in either a table or as a figure. These and many other suggestions on figure and table construction are described in additional detail in Day (1998).
i. **Discussion:** This section should be used to interpret and explain the results. Both the strengths and weaknesses of the observations should be discussed. How do these findings compare to the published literature? What are the clinical implications? Although this last section might be tentative given the nature of a particular study, the authors should realize that even preliminary clinical implications might have value for the clinical readership. Ideally, a review of the potential clinical significance is the last section of the discussion. What are the major conclusions of the study? How does the data support these conclusions
j. **Acknowledgments:** All authors must affirm that they have no financial affiliation (*e.g.*, employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed in the past three years. Any other potential conflict of interest should be disclosed. Any author for whom this statement is not true must append a paragraph to the manuscript that fully discloses any financial or other interest that poses a conflict. Likewise the sources and correct attributions of all other grants, contracts or donations that funded the study must be disclosed
k. **References:** The reference style follows Index Medicus and can be easily learned from reading past issues of the *JOE*. The *JOE*uses the Vancouver reference style, which can be found in most citation management software products. Citations are placed in parentheses at the end of a sentence or at the end of a clause that requires a literature citation. Do not use superscript for references. Original reports are limited to 35 references. There are no limits in the number of references for review articles.

3. **Manuscripts Category Classifications and Requirements**

Manuscripts submitted to the *JOE* must fall into one of the following categories. The abstracts for all these categories would have a maximum word count of 250 words:

A. CONSORT Randomized Clinical Trial-Manuscripts in this category must strictly adhere to the Consolidated Standards of Reporting Trials-CONSORT- minimum guidelines for the publication of randomized clinical trials. These guidelines can be found at *www.consort-statement.org/*. These manuscripts have a limit of 3,500 words, [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.
B. Review Article-Manuscripts in this category are either narrative articles, or systematic reviews/meta-analyses. Case report/Clinical Technique articles even when followed by extensive review of the literature will should be categorized as “Case Report/Clinical Technique”. These manuscripts have a limit of 3,500 words, [including abstract, introduction, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.

C. Clinical Research (*e.g.*, prospective or retrospective studies on patients or patient records, or research on biopsies, excluding the use of human teeth for technique studies). These manuscripts have a limit of 3,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.

D. Basic Research Biology (animal or culture studies on biological research on physiology, development, stem cell differentiation, inflammation or pathology). Manuscripts that have a primary focus on biology should be submitted in this category while manuscripts that have a primary focus on materials should be submitted in the Basic Research Technology category. For example, a study on cytotoxicity of a material should be submitted in the Basic Research Technology category, even if it was performed in animals with histological analyses. These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or 4 tables*.

E. Basic Research Technology (Manuscripts submitted in this category focus primarily on research related to techniques and materials used, or with potential clinical use, in endodontics). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 3 figures and tables *.

F. Case Report/Clinical Technique (*e.g.*, report of an unusual clinical case or the use of cutting-edge technology in a clinical case). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or tables*.

* Figures, if submitted as multipanel figures must not exceed 1 page length. Manuscripts submitted with more than the allowed number of figures or tables will require approval of the *JOE* Editor or associate editors. If you are not sure whether your manuscript falls within one of the categories above, or would like to request preapproval for submission of additional figures please contact the Editor by email at *jendodontics@uthscsa.edu*.

Importantly, adhering to the general writing methods described in these guidelines (and in the resources listed below) will help to reduce the size of the manuscript while maintaining its focus and significance. Authors are encouraged to focus on only the essential aspects of the study and to avoid inclusion of extraneous text and figures. The Editor may reject manuscripts that exceed these limitations.

Available Resources:

Strunk W, White EB. The Elements of Style. Allyn & Bacon, 4th ed, 2000, ISBN 020530902X.

Day R. How to Write and Publish a Scientific Paper. Oryx Press, 5th ed. 1998. ISBN 1-57356-164-9.

Woods G. English Grammar for Dummies. Hungry Minds:NY, 2001 (an entertaining review of grammar).

Alley M. The Craft of Scientific Writing. Springer, 3rd edition 1996 SBN 0-387-94766-3.

Alley M. The Craft of Editing. Springer, 2000 SBN 0-387-98964-1.