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ÁREA DE CONCENTRAÇÃO ENDODONTIA

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**Eficácia de diferentes protocolos de irrigação final na
redução bacteriana em canais radiculares de raízes
mesiais de molares inferiores com istmo**

Curitiba

2019

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**Tese apresentada ao Programa de Pós-
Graduação em Odontologia da Pontifícia
Universidade Católica do Paraná, como
parte dos requisitos para obtenção do
título de Doutor em Odontologia, Área de
Concentração em Endodontia**

**Orientador: Prof. Dr. Ulisses Xavier da Silva
Neto**

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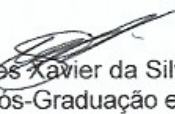
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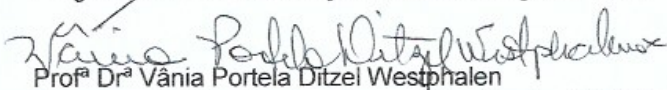
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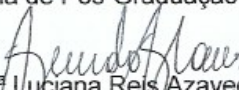
**EFICÁCIA DE DIFERENTES PROTOCOLOS DE IRRIGAÇÃO FINAL NA REDUÇÃO
BACTERIANA EM CANAIS RADICULARES DE RAÍZES MESIAIS DE MOLARES
INFERIORES COM ISTMO**

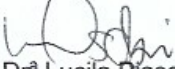
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SUMÁRIO

ARTIGO EM PORTUGUÊS.....	1
Página título.....	1
Resumo.....	2
Introdução.....	3
Material e Métodos.....	4
Resultados.....	11
Discussão.....	12
Referências.....	17
ARTIGO EM INGLÊS.....	20
Title page.....	20
Abstract.....	21
Introduction.....	22
Material and methods.....	23
Results.....	29
Discussion.....	30
References.....	34
Anexos.....	38
Aprovação do Comitê de Ética em Pesquisa.....	38
Normas para publicação.....	41

ARTIGO EM PORTUGUÊS

Página título

Eficácia de diferentes protocolos de irrigação final na redução bacteriana em canais radiculares de raízes mesiais de molares inferiores com istmo

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Resumo

Introdução: O estudo avaliou, *in vitro*, a eficácia dos sistemas de agitação mecânica da solução irrigadora Easy Clean (EC), EndoActivator® (EA), XP-endo® Finisher (XP) irrigação ultrassônica passiva (PUI) em eliminar *Enterococcus faecalis* em canais principais e túbulos dentinários de raízes mesiais de molares inferiores com istmo. **Métodos:** 80 espécimes tiveram os canais preparados com Wave One Gold Primary (Dentsply Sirona, Ballaigues, Suíça) e esterilizados. Os canais foram contaminados, utilizando suspensão contendo *Enterococcus faecalis* (ATCC29212) e incubados por 21 dias em BHI enriquecido com glucose 1%, renovado a cada 72 horas. Cada sistema foi utilizado 1 mm aquém do comprimento de trabalho durante 1 minuto para ativação do NaOCl 2,5% e 1 minuto para o EDTA 17%. A porcentagem de redução bacteriana em canais principais foi determinada pelo método colorimétrico MTT e em túbulos dentinários por meio de microscopia confocal de varredura a laser. **Resultados:** Não houve diferença entre os grupos tratados com o EC, EA e PUI quanto a redução de bactérias nos canais principais ($p>0,05$). Contudo, XP apresentou a maior porcentagem de redução bacteriana, diferindo dos grupos EC e EA ($p<0,05$). Todos os sistemas eliminaram bactérias dos túbulos dentinários na profundidade de 50 μm nos canais e no istmo ($p>0,05$). XP foi superior ao EC, nas profundidades de 100 e 150 μm , e ao PUI em 150 μm ($p<0,05$). **Conclusão:** Todos os sistemas foram capazes de reduzir a quantidade de bactérias no interior dos canais e nos túbulos dentinários; porém, o XP-endo® Finisher apresentou o melhor desempenho.

Palavras-chave: *Enterococcus faecalis*, Microscopia Confocal de Varredura a Laser, canal radicular

Introdução

A terapia endodôntica visa, entre outros objetivos, a redução da quantidade de microrganismos presentes no sistema de canais radiculares (SCR) para que, associado a um selamento adequado, possibilite a manutenção da saúde dos tecidos periapicais (1, 2). A infecção intrarradicular é apontada como uma das grandes causas do fracasso endodôntico, especialmente quando associada a presença de biofilme na porção apical do SCR (3, 4). Bactérias organizadas em biofilmes são capazes de adaptar-se genética e fisiologicamente para sobreviver às mudanças no SCR, dificultando a sua eliminação durante o tratamento (5). *Enterococcus faecalis* é um Gram positivo, anaeróbio facultativo com grande capacidade adaptativa, que forma biofilme, é capaz de sobreviver por longos períodos sem nutrientes, resiste em ambientes extremamente alcalinos, altera a resposta imune e invade túbulos dentinários. Está frequentemente associado a infecções endodônticas assintomáticas persistentes devido às suas características de sobrevivência e a seu papel de facilitadora da permanência de bactérias anaeróbias no canal radicular (6-8).

Variações anatômicas também são citadas como causas de insucesso e representam outro obstáculo a ser suplantado pelo tratamento endodôntico (9-11). Estudos de anatomia do SCR mostram que molares inferiores apresentam muitas variações, especialmente na raiz mesial, com prevalência alta de istmos, chegando a 64% (9, 12). Durante o preparo do canal, independente do sistema utilizado, grandes áreas podem permanecer intocadas pelos instrumentos, dependendo da morfologia do canal (13).

O desafio imposto pela microbiota aliada à complexidade morfológica gerou a busca por recursos que potencializassem a limpeza do SCR durante o tratamento endodôntico. A agitação mecânica da solução irrigadora tem sido proposta como forma de alcançar uma limpeza mais efetiva (14).

A irrigação ultrassônica passiva (PUI) consiste na aplicação de uma ponta metálica não cortante, acoplada a um aparelho de ultrassom, transmitindo energia acústica para a solução irrigadora contida no canal. É um método potencializador da limpeza dos canais radiculares, sendo mais eficaz que o uso da irrigação convencional com seringa (15). O EndoActivator® (Dentsply Tulsa Dental

Specialties, Tulsa, OK) é um sistema sônico que ativa a solução irrigadora, por meio de uma ponta flexível e não cortante, aumentando a eficácia da solução dentro do canal radicular, permitindo uma melhor limpeza do SCR (16). Existem também instrumentos desenvolvidos para uso no motor endodôntico com a mesma finalidade. Entre eles, o Easy Clean (Easy Equipamentos Odontológicos, Belo Horizonte, Brasil) é um instrumento plástico indicado para uso como auxiliar na limpeza dos canais radiculares. Atua fazendo a agitação mecânica das substâncias químicas e por meio do atrito de suas lâminas no interior do canal. É um instrumento indicado para uso em movimento reciprocante, podendo ser utilizado em movimento rotatório (17, 18). O XP-endo® Finisher (FKG Dentaire AS, La Chaux-de-Fonds, Suíça) é um instrumento rotatório, de diâmetro apical 0,25mm, sem conicidade (25/.00) que, em contato com a temperatura corporal, expande, devido a memória de forma de sua liga metálica (19). Esse instrumento tem se mostrado eficiente quanto à remoção de medicação intracanal, redução de debris e bactérias em dentes com morfologia complexa e em áreas de difícil acesso (20-22).

Diante da demanda por métodos que aumentem a eficácia da irrigação realizada durante o tratamento endodôntico, com intuito de reduzir o número de microrganismos presentes ao final do preparo do canal, especialmente em áreas de complexidade anatômica, este estudo se propõe a comparar os sistemas PUI, EndoActivator®, Easy Clean e XP-endo® Finisher quanto a capacidade de reduzir a quantidade de *E. faecalis* viáveis nos canais principais e em túbulos dentinários de istmos de raízes mesiais de molares inferiores. A hipótese nula é de que não há diferença entre os quatro sistemas quanto a capacidade de redução bacteriana.

Material e Métodos

Foram realizadas duas avaliações. A redução bacteriana nos canais principais foi avaliada por meio do método MTT e nos túbulos dentinários por meio de Microscopia Confocal de Varredura a Laser.

Seleção dos dentes:

Este estudo foi aprovado pelo Comitê de Ética em Pesquisa local, sob número 2.525.268. Para a seleção da amostra, 400 molares inferiores humano extraídos foram submetidos à tomografia computadorizada de feixe cônico a fim de avaliar e padronizar os parâmetros anatômicos. As tomografias foram realizadas, utilizando um tomógrafo Scanora 3D (Soredex Tuusula, Finlândia) em 120 kVp, 12.5 mA, FOV de 75x100mm e voxel de 0.2mm.

Foram selecionados 80 molares inferiores apresentando as seguintes características: ausência de tratamento endodôntico, formação radicular completa, ausência de trincas, fraturas ou reabsorções. As raízes mesiais apresentavam dois canais distintos, terminando em um mesmo forame (Vertucci II), presença de istmo, cuja porção mais cervical estava localizada de 3,5 a 6 mm do ápice. A curvatura dessas raízes era igual, ou inferior a 20 graus, e diâmetro apical igual ou inferior 0,25mm.

Preparo dos dentes:

As raízes foram separadas utilizando disco diamantado dupla face 7020 (KG Sorensen – São Paulo, Brasil) e a parede coronária distal da cavidade pulpar foi reconstruída com resina composta Opallis (FGM, Joinville, Brasil) com o intuito de criar um reservatório para a solução irrigadora. O tamanho dos espécimes foi padronizado em 18mm. A odontometria foi realizada com o auxílio de um microscópio clínico odontológico (DF Vasconcellos, Londrina, Brasil) em um aumento de oito vezes, utilizando uma lima tipo K #15 (Dentsply Sirona, Bellaigues, Suíça) a qual foi levada ao forame até que sua ponta pudesse ser visualizada na porção mais cervical do mesmo, estabelecendo o comprimento real do canal. Desta medida, diminuiu-se 1mm, determinando o comprimento de trabalho (CT). Os canais foram preparados utilizando o instrumento WaveOne® Gold Primary (Dentsply Sirona, Bellaigues, Suíça) em movimento recíprocante (WaveOne Gold ALL) do motor endodôntico X-Smart® Plus (Dentsply Sirona, Bellaigues Suíça) de acordo com as instruções do fabricante. Durante o preparo foi mantida a patência com uma lima tipo K #10, e a cada retirada do instrumento, os canais foram irrigados com 1 mL de hipoclorito de sódio (NaOCl) 2,5% (Farmácia Precisão, Curitiba, Brasil) utilizando uma agulha 30-G NaviTip (Ultradent, South Jordan, UT).

Para a irrigação final foi utilizado primeiramente 2mL de NaOCl, seguido de 2 mL de EDTA (Biodinâmica, Ibiporã, Brasil) e por fim, 2 mL de NaOCl. Após a irrigação final, foi feita a secagem do canal radicular com pontas de papel absorvente compatíveis com o sistema Wave One® Gold (Tanari, Manaus, Brasil) e as raízes foram cobertas com esmalte para unhas deixando apenas o forame livre, a fim de evitar contaminação (23).

Os dentes foram autoclavados, a 121 °C, imersos em solução tampão fosfato salino (PBS) pH 7,0.

Inoculação dos espécimes com *Enterococcus faecalis* (ATCC®29212™)

Os espécimes foram contaminados com *E. faecalis* cepa ATCC®29212™. As células foram colocadas em BHI (Brain Heart Infusion) para crescimento por 24 hs a 37°C. Foi preparada uma suspensão com *E. faecalis* 3x10⁸ UFC/mL (turbidez correspondente ao tubo #1 da escala Mc Farland), e a mesma foi introduzida no canal com uma seringa de insulina e uma agulha gauge 30 (Ultradent, South Jordan, UT). Após realizada a inoculação, os dentes foram colocados em tubos de centrifugação de 15 mL, preenchidos com 5 mL da solução de BHI acrescida de glucose a 1% (24). Os dentes permaneceram em estufa por 21 dias. Durante esse período, a cada 3 dias houve substituição do BHI, por um novo, contendo *E. faecalis* na mesma concentração utilizada na suspensão inicial de contaminação.

Avaliação por MTT

Foram utilizados para a avaliação da redução bacteriana em canais principais 44 espécimes. Dois espécimes foram levados ao microscópio eletrônico de varredura para confirmação da formação de biofilme e dois espécimes foram mantidos estéreis, sem inoculação bacteriana.

Em um fluxo laminar, os espécimes foram retirados dos tubos de centrifugação. O BHI foi aspirado dos canais radiculares e a superfície externa desinfetada com gaze embebida em NaOCl 2,5%. O ápice radicular de cada espécime foi selado com resina composta (FGM, Joinville, Brasil) e os mesmos

foram incluídos em silicona de condensação (Coltene Holding, Altstätten, Suíça), mantida em banho térmico a temperatura de 37° C. A temperatura foi verificada durante o experimento utilizando um termômetro infravermelho sem contato (LaserGrip 774, Etekcity, Anaheim, CA) e com termômetro espeto TP101 (BMax), inserido na silicona. Para a coleta da amostra inicial (S1), os canais foram preenchidos com PBS e, com uma lima Hedstroem #25 (Dentsply Sirona, Bellaigues, Suíça), foram feitos 20 movimentos de limagem contra as paredes do canal. Utilizando agulha gauge 24 e uma seringa de 3mL, o PBS contaminado foi coletado do interior dos canais radiculares e transferido para um microtubo de 1,5 mL (Eppendorf, Hamburgo, Alemanha) até completar 100 microlitros.

Para a aplicação dos protocolos de irrigação final foram utilizados 2 mL de NaOCl 2,5% (Farmácia Precisão, Curitiba, Brasil) e 2mL de EDTA 17% por canal. Após o completo preenchimento do canal com a solução, a mesma foi dispensada na câmara pulpar, durante 60 segundos, tempo de ativação por cada sistema empregado (EndoActivator®, XP-endo® Finisher, PUI e Easy Clean). A ativação foi realizada, levando o instrumento 1mm aquém do CT, em movimentos de introdução e tração, com amplitude de aproximadamente 3 mm. Finalmente, cada canal radicular foi irrigado com 1mL de NaOCl 2,5%, sem ativação, utilizando agulha gauge 30 a 1 mm do CT, seguido de 1 mL de tiosulfato de sódio a 10%, para neutralizar o NaOCl.

Os sistemas foram utilizados conforme descrito a seguir:

Easy Clean

O instrumento foi acoplado a um motor endodôntico (X-Smart® Plus), acionado em movimento recíprocante (WaveOne ALL).

EndoActivator®

Foi utilizada a ponta medium (25.04) e ativada a 10.000 ciclos por minuto.

PUI

Utilizou-se a ponta ultrassônica Irrisonic™ 20/01 (Helse, Santa Rosa de Viterbo, Brasil), acoplada ao aparelho Jet Sonic (Gnatus, São Paulo, Brasil), ajustado em potência 15%, no modo Endodontia.

XP-endo® Finisher

O instrumento foi resfriado antes da inserção no canal com um spray refrigerante (Endo-Frost, Roeko, Langenau, Alemanha) e ativado por meio de motor endodôntico X-Smart® Plus em movimento rotatório, configurado em 800 rotações por minuto (rpm) e 1 N.cm de torque.

As seringas, contendo as soluções, foram mantidas em banho térmico a 37° C até o momento da utilização. A coleta da amostra final (S2) foi executada da mesma forma que S1, após a aspiração do tiosulfato de sódio. Todo o instrumental utilizado foi previamente esterilizado.

A diminuição da carga bacteriana nos canais principais foi determinada pelo método colorimétrico de redução do brometo de 3-4,5-dimetil-tiazol-2-il-2,5-difeniltetrazólio (MTT). MTT é um sal tetrazolato que é reduzido a cristais de formazana na presença de células metabolicamente ativas. Formazana é um composto azul cuja intensidade é diretamente proporcional a quantidade de células viáveis (25).

Em microtubos de 1,5 mL, contendo as amostras (100 µL), foram adicionados 100 µL de MTT 1 mg/mL⁻¹ (Sigma-Aldrich, St. Louis, MN). Os tubos foram agitados em vórtex (1200 rpm, 30 s) e deixados no escuro a 37 °C, por 3 h. Esses tubos foram centrifugados em MiniSpin® com rotor F-45-12-11 (Eppendorf AG, Hamburgo, Alemanha) a 12100 ×g, por 60 s. Os sobrenadantes foram descartados e os *pellets* foram lavados com 500 µL de água destilada estéril seguido de centrifugação (12100 ×g, 60 s). Os sobrenadantes foram descartados e os *pellets* receberam 200 µL de álcool isopropílico, seguido de agitação em vórtex. A formazana, liberada pelas células, foi medida após centrifugação e transferência de alíquotas de 100 µL de sobrenadante para poços de placas de microtitulação com 96 poços de fundo chato. As densidades ópticas das amostras foram medidas em espectrofotômetro TP Reader (Thermo Plate, Brasil), a 540 nm. Para cada grupo experimental, dois poços foram preenchidos com PBS 100 mM (pH 7,0), que serviu como controle negativo.

A porcentagem de redução bacteriana foi calculada, seguindo a fórmula:

$$\text{Porcentagem de redução bacteriana} = 100 - [(100 \times S2) / S1]$$

Onde:

S1 = média aritmética entre valores da amostra inicial dos canais vestibular e lingual de cada espécime.

S2 = média aritmética entre valores da amostra final dos canais vestibular e lingual de cada espécime.

Avaliação por Microscopia Confocal de Varredura a Laser (MCVL):

Para a avaliação de redução bacteriana nos túbulos dentinários, foram utilizados 36 espécimes contendo istmo cuja porção mais cervical estivesse localizada entre 3,5 e 6 mm do ápice, para possibilitar a clivagem. Esses dentes foram igualmente distribuídos entre os grupos, de acordo com as dimensões do istmo.

Antes da esterilização e contaminação dos espécimes utilizados nessa avaliação, foram confeccionados, sulcos transversais ao longo eixo do dente, localizados na porção mais cervical do istmo, sem penetrar nos canais principais. Utilizou-se disco diamantado para essa etapa.

Os protocolos de irrigação foram aplicados exatamente como descrito na avaliação por MTT. Da mesma forma, o controle de temperatura foi mantido. Imediatamente após o uso do tiosulfato de sódio, os canais foram secos com pontas de papel absorvente estéreis e os espécimes clivados, empregando um cinzel.

Após a clivagem, foram corados utilizando LIVE/DEAD® BackLight™ Bacterial Viability Kit (Molecular Probes®, Invitrogen™, Eugene, OR). Esse contém os fluoróforos SYTO 9 (comprimento de onda de excitação 488 nm/ comprimento de onda de emissão 525 nm) que é capaz de corar todas as bactérias e o iodeto de propídeo (excitação 488 nm/emissão 560 nm), que marca apenas as bactérias que tiveram sua membrana plasmática rompida. Dessa forma, apresentam fluorescência em verde as bactérias vivas e em vermelho as mortas (26). As superfícies foram lavadas com 100 µL de PBS, e aplicou-se 10 µL de corante deixando por 5 minutos antes da análise.

Cada parte foi examinada em um microscópio confocal de varredura a laser Fluoview 1000 (Olympus, Tóquio, Japão) empregando aumento de 40X. As

imagens foram adquiridas utilizando o software Olympus FluoView Version 1.7 (Olympus, Tóquio, Japão) em resolução de 512 X 512 pixels. Foi utilizado passo de 1 μm e no mínimo 10 passos de profundidade foram adquiridos. A sobreposição dessas imagens (*stack*) gerou a imagem final para análise. Foram adquiridas quatro imagens de cada espécime localizadas em um ponto aleatório das paredes lingual, vestibular, mesial e distal de uma das partes (corte cervical ou apical do espécime). Ficou denominado como porcentagem de redução bacteriana em túbulos dentinários da região de canal principal os resultados correspondentes às paredes lingual e vestibular dos cortes e como porcentagem de redução bacteriana em túbulos dentinários da região de istmo, os das paredes mesial e distal dos cortes (figura 1). Cada imagem final foi dividida em três níveis (21), de acordo com a profundidade (50, 100 e 150 μm de profundidade nos túbulos dentinários a partir da parede dentinária) (figura 2).

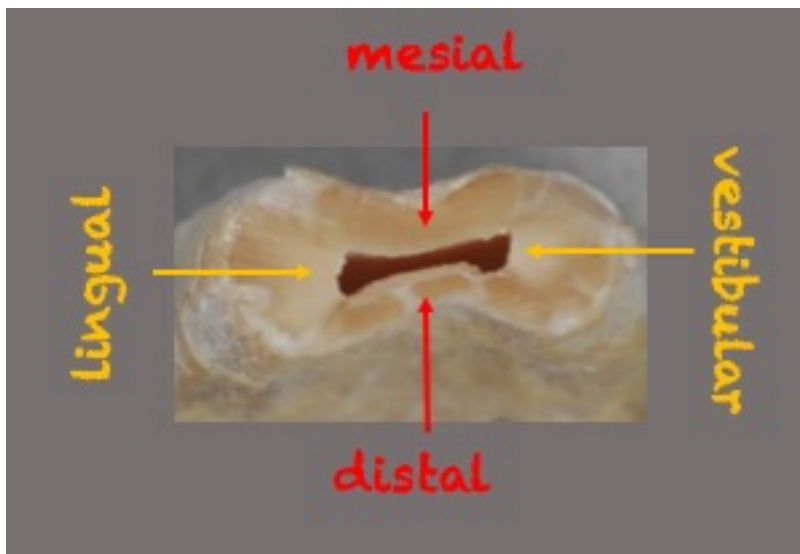


Figura 1: Corte transversal do espécime, mostrando região do canal, que contempla paredes lingual e vestibular do corte (em amarelo) e a região de istmo, representada pelas paredes mesial e distal (em vermelho).

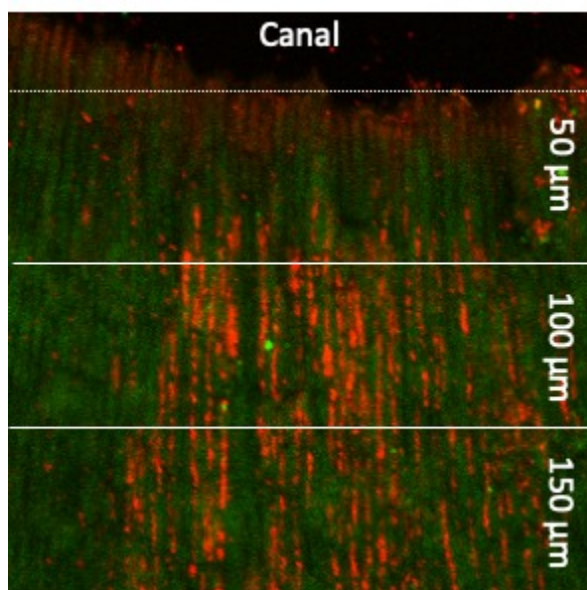


Figura 2: Imagem obtida em MCVL (40X), utilizando LIVE/DEAD kit, onde bactérias vivas (verde) e mortas (vermelho). Divisão da imagem adquirida para avaliação, conforme profundidade nos túbulos dentinários a partir da parede do canal radicular .

A avaliação de cada imagem final foi realizada empregando o software Bio Image_L[®] (The MathWorks Inc, Natick, MA). O software gera a informação da porcentagem de bactérias vivas e mortas em cada imagem (23).

Todos os dados foram tabulados e submetidos a análise estatística, utilizando o software IBM STATISTICS SPSS 25.0 (IBM Inc. Chicago, IL) com nível de significância estabelecido em 0,05. Em ambas avaliações a distribuição dos dados não apresentou normalidade, de acordo com o teste de Kolmogorov-Smirnov. Foi utilizado o teste não paramétrico Kruskal-Wallis, seguido do teste de Dunn. A análise do poder do teste mostrou que o tamanho da amostra foi adequado tanto para avaliação por MTT (90%) quanto para a avaliação por MCVL (99%).

Resultados

A tabela 1 mostra os resultados da redução bacteriana nos canais principais apresentados pela avaliação por MTT. Observou-se que não houve diferença estatisticamente significativa entre os protocolos de irrigação final, exceto quando se comparou o XP-endo[®] Finisher com o Easy Clean e com o EndoActivator[®], onde o primeiro foi capaz de eliminar mais bactérias nos canais radiculares ($p < 0,05$).

Tabela 1: Mediana, valores mínimo e máximo da porcentagem (%) de redução bacteriana nos canais principais por meio do método MTT.

Protocolo de irrigação final	n	Mediana (mín-máx)
Easy Clean	10	49,59 (25,77-92,27) ^A
EndoActivator [®]	10	69,47 (22,95-99,81) ^A
PUI	10	82,01 (25,64-100) ^{AB}
XP-endo [®] Finisher	10	94,73 (76,04-100) ^B

Diferentes letras maiúsculas indicam diferença estatisticamente significativa entre os grupos (teste de Dunn/ $p < 0,05$)

Não houve diferença entre os sistemas quanto a capacidade de reduzir bactérias localizadas dentro dos túbulos dentinários da região de canais principais e de istmo em 50 μm de profundidade a partir da parede dentinária ($p > 0,05$), conforme apresentado na tabela 2. Nas profundidades 100 e 150 μm o XP-endo[®] Finisher foi mais eficiente que o Easy Clean e demonstrou melhor desempenho que o PUI em 150 μm ($p < 0,05$) em ambas as regiões. Todos os protocolos de irrigação apresentaram performance semelhante, na mesma profundidade, se comparadas região de canal principal e de istmo ($p > 0,05$).

Tabela 2: Mediana, valores mínimo e máximo da porcentagem (%) de redução bacteriana em túbulos dentinários por meio de MCVL.

Área	n	Profundidade	Easy Clean	EndoActivator [®]	PUI	XP-endo [®] Finisher
Canal	16	50 μm	28,50 (1-100) ^A	85,50 (0-100) ^A	38,50 (3-99) ^A	75,00 (2-100) ^A
	16	100 μm	17,00 (0-52) ^A	22,50 (0-100) ^{AB}	24,00 (1-91) ^{AB}	59,50 (0-100) ^B
	16	150 μm	13,00 (0-63) ^A	16,50 (0-100) ^{AB}	14,00 (2-57) ^A	51,50 (0-100) ^B
Istmo	16	50 μm	31,50 (7-100) ^A	53,50 (4-100) ^A	53,00 (1-100) ^A	64,50 (3-96) ^A
	16	100 μm	11,00 (0-97) ^A	50,00 (1-100) ^B	23,00 (0-86) ^{AB}	42,00 (0-100) ^B
	16	150 μm	6,50 (0-99) ^A	23,00 (0-100) ^{AB}	8,50 (0-74) ^A	42,00 (0-99) ^B

Diferentes letras maiúsculas indicam diferença estatisticamente significativa entre os grupos na mesma linha (teste de Dunn $p < 0,05$)

Discussão

A proposta deste estudo foi comparar os quatro protocolos de irrigação final quanto a sua capacidade de reduzir bactérias nos canais principais e em túbulos dentinários do istmo e canais na região apical. A hipótese nula foi rejeitada, visto

que houve diferenças entre os protocolos nas duas avaliações. O XP-endo® Finisher apresentou desempenho superior quando comparado ao Easy Clean tanto para redução bacteriana nos canais principais, quanto mais profundamente (100 e 150 μm) nos túbulos dentinários dos canais e do istmo.

Os espécimes foram contaminados com *E. faecalis* (ATCC®29212™) para as duas avaliações. Embora biofilmes endodônticos sejam polimicrobianos, os formados apenas por *E. faecalis* são frequentemente utilizados para avaliação de ação antimicrobiana em Endodontia devido a capacidade de sobrevivência e adaptação desta bactéria e por sua associação a casos de insucesso na terapia endodôntica (7, 8). Para potencializar o crescimento bacteriano e a colonização dos túbulos dentinários, 1 % de glucose foi adicionada ao BHI (24). Além disso, o BHI, utilizado nas trocas do meio, continha nova cultura de *E. faecalis*, preparada 24 horas antes e ajustada em turbidez correspondente ao tubo n.1 da escala Mc Farland (27).

Para avaliar a redução bacteriana nos canais principais, optou-se por utilizar o método colorimétrico MTT. Este método foi proposto por Mosmann (25) para a detecção de células viáveis. Embora seja comumente utilizado para testes de citotoxicidade (28, 29), é aplicado também para viabilidade de células bacterianas em canais radiculares (21, 30). A capacidade dos protocolos testados de eliminar bactérias dentro dos túbulos dentinários foi avaliada por meio de MCVL. O método permite, a partir do uso de fluoróforos, diferenciar bactérias vivas e mortas (26). Semelhante a estudo realizado previamente (21), as imagens obtidas foram divididas, de acordo com a profundidade dos túbulos em relação a parede do canal radicular, com o intuito de detalhar melhor o desempenho alcançado pelos protocolos utilizados. A combinação dos dois métodos de avaliação resulta em uma visão mais completa do potencial de reduzir bactérias dos protocolos testados.

Os resultados em túbulos dentinários foram semelhantes para todos os sistemas na porção mais próxima à luz do canal (50 μm), mesmo na região de istmo. A eliminação dos microrganismos nos túbulos dentinários pode ser relacionada com a penetração do NaOCl nos mesmos. O NaOCl 1% penetra nos túbulos dentinários em torno de 77 μm , quando em temperatura ambiente, em um intervalo de dois minutos. A profundidade de penetração aumenta conforme o

aumento da concentração do mesmo (31). Em um estudo realizado em dentes bovinos contaminados com *E. faecalis*, o NaOCl 2,5%, sem agitação, eliminou 99% de células viáveis nos túbulos dentinários expostos à irrigação em intervalo de 5 minutos (23). É possível que a remoção de biofilme, debris e smear layer das paredes dentinárias facilite o acesso do hipoclorito aos túbulos. Portanto, o sistema que melhor desempenhar essas funções, permitirá maior eliminação bacteriana nos túbulos também.

O XP-endo[®] Finisher é um instrumento sem conicidade, fabricado com liga metálica NiTi MaxWire[®] (FKG Dentaire AS, La Chaux-de-Fonds, Suíça) cuja memória de forma permite a mudança da fase martensítica para a fase austenítica, durante o movimento rotatório, quando em contato com a temperatura corpórea. O instrumento sofre expansão sendo capaz de aumentar seu diâmetro em até 6 x (19). O presente experimento foi integralmente conduzido sob controle de temperatura, mantida a 37 °C, para atender a demanda do XP-endo[®] Finisher e manter as mesmas condições para todos os grupos. Embora o aquecimento do NaOCl aumente sua capacidade bactericida (32), a elevação da temperatura a 37 °C parece não exercer essa influência (33, 34). É importante salientar que, apesar da padronização, este é um estudo laboratorial, portanto não reproduz exatamente as condições de variação de temperatura *in vivo*. Esta diferença pode impactar na performance do instrumento em relação a quando o mesmo é utilizado clinicamente.

O PUI foi tão eficaz quanto o XP -endo[®] Finisher na redução bacteriana de canais principais e nos túbulos dentinários. Somente na porção mais profunda dos túbulos dentinários (150 µm) seu desempenho foi inferior. Esses sistemas apresentaram resultados semelhantes quando comparados quanto à redução de debris (22). Porém, quando avaliada remoção de biofilme em superfície do canal e em sulcos artificialmente produzidos, o XP-endo[®] apresentou desempenho superior (35). Por serem avaliações e protocolos de aplicação diferentes, não é possível comparar diretamente os resultados. Embora alguns dados apontem que o PUI tenha desempenho superior em redução de debris quando utilizado em três ciclos de 20 segundos (36), isso não é um padrão entre os estudos. Quando comparada a utilização de PUI em irrigação contínua e intermitente para remoção

de biofilme apical, não houve diferença (35). No presente estudo optou-se por padronizar a irrigação contínua e ativação por um minuto para todos os sistemas, baseando a escolha em achados da literatura (35, 37, 38) e na indicação do fabricante do XP-endo® Finisher do mesmo ser utilizado por esse tempo.

O sistema sônico EndoActivator®, na avaliação de redução bacteriana em canais principais, apresentou um desempenho inferior ao XP-endo® Finisher o que concorda com os resultados de estudo prévio que comparou os dois sistemas, utilizando a mesma metodologia, porém em raízes distais de molares inferiores (21). O uso de sistemas sônicos de agitação, como o EndoActivator®, é uma opção prática, já que são peças de mão portáteis, de fácil manipulação, uso e transporte, se comparado com PUI, cujo desempenho foi semelhante.

O Easy Clean é um instrumento de fácil aplicação na rotina da terapia endodôntica, por ser de uso em motor elétrico. A proposta do instrumento, apresentada pelo fabricante, é de que ele agite a solução irrigadora e toque as paredes do canal radicular (17). Por ser um instrumento 25.04, ele não fica completamente ajustado ao canal em preparos realizados com instrumentos 25.07, como no presente estudo. Entretanto, isso não impediu que ele apresentasse resultados favoráveis quanto a remoção de debris ou na remoção de medicação intracanal, mesmo em canais preparados com instrumentos 30.05, ou 40.06 respectivamente(17, 39). Embora tenha sido utilizado em movimento recíprocante, este instrumento pode ser utilizado em rotação contínua. Os autores desconhecem outro artigo avaliando este sistema quanto a redução de bactérias viáveis. No presente experimento, apesar de não ter sido tão eficaz quanto o XP-endo® Finisher, o Easy Clean apresentou resultados semelhantes aos outros sistemas em todas as avaliações. A diferença entre os resultados pode ser atribuída a velocidade de utilização. O primeiro foi aqui acionado a 800 rpm, enquanto o segundo foi utilizado em movimento recíprocante. O uso em movimento contínuo e recíprocante não influenciou de maneira significativa a remoção de debris para o Easy Clean (18). Porém, a influência do movimento na redução de bactérias não foi ainda avaliada.

Pesquisas *in vitro* têm sua importância na avaliação da eficácia de tratamentos devido a possibilidade maior do controle de variáveis, porém não

substituem ensaios clínicos (40). Seus resultados não podem ser extrapolados para a realidade clínica, mas podem ajudar a direcionar a escolha de tratamentos. A literatura carece de estudos clínicos que determinem se esses passos adicionais de limpeza dos canais radiculares realmente influenciam no sucesso clínico. PUI não foi capaz de aumentar a redução de *E. faecalis*, *in vivo*, quando avaliado em casos de retratamento. Contudo, a avaliação foi realizada por meio de coleta de amostras do canal principal de dentes com canal único (41). Seria importante poder avaliar as áreas de difícil acesso e fazer o acompanhamento clínico a longo prazo.

O XP-endo® Finisher teve um desempenho superior quanto a eliminação de *E. faecalis* no SCR, no entanto todos os sistemas produziram redução bacteriana e nenhum foi capaz de eliminar completamente as bactérias nos canais principais e nos túbulos dentinários. Todos os grupos apresentaram performance semelhante, na mesma profundidade, se comparadas região de canal e de istmo. Isso demonstra que, mesmo em áreas de difícil acesso, onde os instrumentos não tocarão diretamente a parede dentinária, a agitação mecânica das soluções irrigadoras utilizadas foi suficiente para diminuir a quantidade de bactérias viáveis nos túbulos dentinários. Seria de grande valia que futuros estudos *in vivo* avaliassem a importância destes passos adicionais no sucesso da terapia endodôntica e se as diferenças aqui apresentadas são reproduzidas clinicamente.

Referências

1. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int Endod J* 1997;30:297-306.
2. Sedgley CM, Lennan SL, Appelbe OK. Survival of *Enterococcus faecalis* in root canals *ex vivo*. *Int Endod J* 2005;38:735-42.
3. Siqueira Jr JF, Rôças IN, Ricucci D, Hülsmann M. Causes and management of post-treatment apical periodontitis. *Br Dent J* 2014;216:305-12.
4. Ricucci D, Candeiro GTM, Bugea C, Siqueira Jr JF. Complex apical intraradicular infection and extraradicular mineralized biofilms as the cause of wet canals and treatment failure: report of 2 cases. *J Endod* 2016;42:509-15.
5. Chavez de Paz LE. Redefining the persistent infection in root canals: possible role of biofilm communities. *J Endod* 2007;33:652-62.
6. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. *J Endod* 2006;32:93-8.
7. Chen L, Li X, Zhou X, et al. Inhibition of *Enterococcus faecalis* growth and biofilm formation by molecule targeting cyclic di-AMP synthetase activity. *J Endod* 2018;44:1381-8.
8. Möller AJR, Fabricius L, Dahlen G, et al. Apical periodontitis development and bacterial response to endodontic treatment. Experimental root canal infections in monkeys with selected bacterial strains. *Eur J Oral Sci* 2004;112:207-15.
9. Tahmasbi M, Jalali P, Nair MK, et al. Prevalence of middle mesial canals and isthmi in the mesial root of mandibular molars: an *in vivo* cone-beam computed tomographic study. *J Endod* 2017;43:1080-3.
10. Wolf TG, Paqué F, Zeller M, et al. Root canal morphology and configuration of 118 mandibular first molars by means of micro-computed tomography: an *ex vivo* study. *J Endod* 2016;42:610-4.
11. Estrela C, Rabelo LE, de Souza JB, et al. Frequency of root canal isthmi in human permanent teeth determined by cone-beam computed tomography. *J Endod* 2015;41:1535-9.
12. Jung I, Seo M, Fouad AF, et al. Apical anatomy in mesial and mesiobuccal roots of permanent first molars. *J Endod* 2005;31:364-8.
13. Paqué F, Ganahl D, Peters OA. Effects of root canal preparation on apical geometry assessed by micro-computed tomography. *J Endod* 2009;35:1056-9.
14. Bedier MM, Hashem AAR, Hassan YM. Improved dentin disinfection by combining different-geometry rotary nickel-titanium files in preparing root canals. *Restor Dent Endod* 2018;43:e46.
15. Van der Sluis LWM, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. *Int Endod J* 2007;40:415-26.

16. Bolles JA, He J, Svoboda KKH, et al. Comparison of Vibringe, EndoActivator, and needle irrigation on sealer penetration in extracted human teeth. *J Endod* 2013;39:708-11.
17. Kato AS, Cunha RS, Bueno CES, et al. Investigation of the efficacy of passive ultrasonic irrigation versus irrigation with reciprocating activation: an environmental scanning electron microscopic study. *J Endod* 2016;42:659-63.
18. Duque JA, Duarte MA, Canali LC, et al. Comparative effectiveness of new mechanical irrigant agitating devices for debris removal from the canal and isthmus of mesial roots of mandibular molars. *J Endod* 2017;43:326-31.
19. <https://www.fkg.ch/xpendo/finisher> [accessed on September 2, 2019].
20. Leoni GB, Versiani MA, Silva-Sousa YT, Bruniera JF, Pecora JD, Sousa-Neto MD. Ex vivo evaluation of four final irrigation protocols on the removal of hard-tissue debris from the mesial root canal system of mandibular first molars. *Int Endod J*. 2017;50(4):398-406.
21. Wigler R, Dvir R, Weisman A, et al. Efficacy of XP-endo finisher files in the removal of calcium hydroxide paste from artificial standardized grooves in the apical third of oval root canals. *Int Endod J* 2017;50:700-5.
22. Azim AA, Aksel A, Zhuang, MAT, et al. Efficacy of 4 irrigation protocols in killing bacteria colonized in dentinal tubules examined by a novel confocal laser scanning microscope analysis.
23. Rodrigues CT, Andrade FB, Vasconcelos LRSM, et al. Antibacterial properties of silver nanoparticles as a root canal irrigant against *Enterococcus faecalis* biofilm and infected dentinal tubules. *Int Endod J* 2018;51:901-11.
24. Bulacio ML, Galván LR, Gaudio C, et al. *Enterococcus faecalis* biofilm. Formation and development *in vitro* observed by scanning electron microscopy. *Acta Odontol. Latinoam* 2015;28:210-4.
25. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55-63.
26. Bottcher DE, Sehnem NT, Montagner et al. Evaluation of the effect of *Enterococcus faecalis* biofilm on the 2% chlorhexidine substantivity: an *in vitro* study. *J Endod* 2015;41:1364-70.
27. Saber Sel-D, El-Hady SA. Development of an intracanal mature *Enterococcus faecalis* biofilm and its susceptibility to some antimicrobial intracanal medications; an *in vitro* study. *Eur J Dent* 2012;6:43-50.
28. Meerloo J, Kaspers GJL, Cloos J. Cell sensitivity assays: the MTT assay. *Methods Mol Biol* 2011;731:237-45.
29. Nogales CG, Ferreira MB, Montemor AF, et al. Ozone therapy as an adjuvant for endodontic protocols: microbiological - *ex vivo* study and cytotoxicity analyses. *J Appl Oral Sci* 2016;24:607-13.
30. Zeng C, Willison J, Meghil MM, et al. Antibacterial efficacy of an endodontic sonic-powered irrigation system: An *in vitro* study. *J Dent* 2018;75:105-12.

31. Zou L, Shen Y, Li W, Haapasalo M. Penetration of Sodium Hypochlorite into Dentin. *Journal of Endodontics*. 2010;36(5):793-6.
32. Giardino L, Mohammadi Z, Beltrami R, Poggio C, et al. Influence of temperature on the antibacterial activity of sodium hypochlorite. *Braz Dent J*. 2016;27:32-6.
33. Del Carpio-Perochena A, Bramante CM, Duarte MH, et al. Effect of temperature, concentration and contact time of sodium hypochlorite on the treatment and revitalization of oral biofilms. *J Dent Res Dent Clin Dent Prospects*. 2015;9:209-15.
34. Gulsahi K, Tirali RE, Cehreli SB, et al. The effect of temperature and contact time of sodium hypochlorite on human roots infected with *Enterococcus faecalis* and *Candida albicans*. *Odontology*. 2014;102:36-41.
35. Bao P, Shen Y, Lin J, Haapasalo M. In Vitro Efficacy of XP-endo Finisher with 2 Different Protocols on Biofilm Removal from Apical Root Canals. *J Endod*. 2017;43(2):321-5.
36. Van der Sluis LWM, Vogels MP, Verhaagen B, et al. Study on the influence of refreshment/activation cycles and irrigants on mechanical cleaning efficiency during ultrasonic activation of the irrigant. *J Endod*. 2010;36:737-40.
37. Capar ID, Ozcan E, Arslan H, Ertas H, Aydinbelge HA. Effect of different final irrigation methods on the removal of calcium hydroxide from an artificial standardized groove in the apical third of root canals. *J Endod*. 2014;40:451-4.
38. Alves FR, Andrade-Junior CV, Marceliano-Alves MF, et al. Adjunctive steps for disinfection of the mandibular molar root canal system: a correlative bacteriologic, micro-computed tomography, and cryopulverization approach. *J Endod*. 2016;42:1667-72.
39. Oliveira RL, Guerisoli DMZ, Duque JA, et al. Computed microtomography evaluation of calcium hydroxide-based root canal dressing removal from oval root canals by different methods of irrigation. *Microsc Res Tech*. 2019;82: 232-7.
40. Neelakantan P, Romero M, Vera J, et al. Biofilms in Endodontics-current status and future directions. *Int J Mol Sci*. 2017;18:1748.
41. Rodrigues RC, Antunes HS, Neves MA, et al. Infection control in retreatment cases: *In vivo* antibacterial effects of 2 instrumentation systems. *J Endod*. 2015;41:1600-5.

ARTIGO EM INGLÊS

Title page

Efficacy of different final irrigation protocols in bacterial reduction in root canals of mesial roots of mandibular molars with isthmus

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Abstract

Introduction: The aim of this study was to evaluate the efficacy of mechanical irrigant agitating devices Easy Clean (EC), EndoActivator® (EA), XP-endo® Finisher (XP) and passive ultrasonic irrigation in eliminating *Enterococcus faecalis* of root canals and dentinal tubules of mesial roots of mandibular molars with isthmus.

Methods: 80 mesial roots were instrumented and sterilized. The root canals were contaminated with *Enterococcus faecalis* (ATCC29212) and incubated for 21 days in BHI supplemented with 1% glucose, renewed every 72 hours. Each device was used 1 mm from the working length for 1 minute for activation of NaOCl 2.5% and 1 minute for EDTA 17%. The percentage of bacterial reduction in main canals was determined by MTT assay and in the dentin tubules by means of confocal laser scanning microscopy. **Results:** There was no difference between the groups treated with EC, EA and PUI for the reduction of bacteria in the main canals ($p > 0.05$). However, XP presented the highest percentage of bacterial reduction, differing from the EC and EA groups ($p < 0.05$). All systems eliminated bacteria from dentinal tubules at 50 μm depth in the canals and in the isthmus area ($p > 0.05$). XP was more efficient than EC at 100 and 150 μm depth and PUI at 150 μm ($p < 0.05$).

Conclusion: All systems were able to reduce bacteria inside the root canals and in the dentinal tubules; however, the XP-endo® Finisher showed the best performance.

Keywords: Enterococcus faecalis, Confocal Laser Scanning Microscopy, root canal

Introduction

One of the goals of endodontic therapy is reducing the number of microorganisms present in the root canal system (RCS) that, together with a suitable sealing, allows for the maintenance of healthy periapical tissues (1, 2). Intraradicular infection is pointed as one of the major causes of endodontic failure, especially when associated with the presence of biofilm in the apical portion of SCR (3, 4). Bacteria organized in biofilms are able to adapt genetically and physiologically to survive changes in RCS, making it difficult to eliminate them during treatment (5). *Enterococcus faecalis* is Gram positive, facultative anaerobes with great adaptive capacity, which form biofilm, are able to survive for long periods without nutrients, resists extremely alkaline environments, alters the immune response and invaded tubules. It is frequently associated with persistent asymptomatic endodontic infections due to its survival characteristics and to its role of facilitating the presence of anaerobic bacteria in the root canal (6-8).

Anatomical variations are also cited as causes of failure and represent another obstacle to be supplanted by endodontic treatment (9-11). Studies of internal canal morphology show that mandibular molars have many variations, especially in the mesial root, with high prevalence of isthmus, reaching 64% (9, 12). During the instrumentation of the root canal, regardless of the system used, large areas may remain untouched by the instruments, depending on the morphology of the canal (13).

The challenge imposed by the microbiota allied to the morphological complexity generated the search for resources that would improve the SCR cleaning during the endodontic treatment. Mechanical agitation of the irrigation solution has been proposed as a way of achieving a more effective cleaning (14).

Ultrasonic irrigation (PUI) consists of the application of a non-cutting metallic tip, coupled to an ultrasound device, transmitting acoustic energy to the irrigator solution contained into the root canal. It is a method to enhance the root canal cleaning and it is more effective than the use of conventional syringe irrigation alone (15). The EndoActivator® system (Dentsply Tulsa Dental Specialties, Tulsa, OK) is a sonic device that activates the irrigation solution through a flexible and non-cutting tip, increasing the effectiveness of the solution within the root canal, allowing better

SCR cleaning (16). There are also instruments developed for use in the endodontic motor for the same purpose. Easy Clean (Dental Equipment, Belo Horizonte, Brazil) is a plastic instrument indicated for use as an aid in the cleaning of root canals. It acts by mechanical agitation of the chemical substances and by the friction of its blades inside the canal. It is an instrument suitable for use in reciprocating motion and can be used in a continuous rotation motion (17, 18). The XP-endo[®] Finisher (FKG Dentaire AS, La Chaux-Fonds, Switzerland) is a continuous rotation instrument with an apical diameter of 0.25mm without taper (25 / .00), which in contact with body temperature expands due to the shape memory of its NiTi alloy (19). This instrument has been shown to be efficient in the removal of intracanal medication, reduction of debris and bacteria in teeth with complex morphology and in areas of difficult access (20-22).

In view of the demand for methods that increase the efficiency of irrigation during endodontic treatment, in order to reduce the number of microorganisms present at the end of the root canal instrumentation, especially in areas of anatomical complexity, the aim of this study is to compare the PUI, EndoActivator[®], Easy Clean and XP-Endo[®] Finisher and the ability to reduce the amount of viable *E. Faecalis* in the main canals and into dentinal tubules of mesial root isthmus of mandibular molars. The null hypothesis is that there is no difference between the four systems regarding bacterial reduction ability.

Material and methods

Bacterial reduction was evaluated in the main canals by means of the MTT method, and into dentinal tubules, by means of Confocal Laser Scanning Microscopy.

Selection of teeth:

This study was approved by local ethical committee (No. 2.525,268). Four hundred extracted human mandibular molars were scanned using Cone Beam Computer Tomography (CBCT) to evaluate and to standardize anatomical

parameters, using a Scanora 3D scanner (Soredex Tuusula, Finland) at 120 kVp, 12.5 mA, 75x100mm FOV and 0.2mm voxel size.

Eighty mandibular molars were selected following these parameters: complete root formation, absence of endodontic treatment, cracks, fractures or any kind of resorptions. All teeth had medial root presenting two distinct canals ending in one foramen (Vertucci's type II) and isthmus located between 3,5 to 6 mm from apex. The curvature of medial roots was less than 20° and apical diameter was smaller than 0,25mm.

Specimens preparation:

The roots were separated using a double - sided diamond disk 7020 (KG Sorensen - São Paulo, Brazil) and the distal wall was reconstructed with Opallis composite resin (FGM, Joinville, Brazil). The size of the specimens was standardized at 18mm. Working length was established using a dental microscope (DF Vasconcellos, Londrina, Brazil) A # 15 K-file (Dentsply Sirona, Bellaigues, Switzerland) was taken to foramen until its tip could be visualized in the more cervical portion of the same, establishing the actual length of the canal. From this measurement, 1 mm was reduced, determining the working length (WL). The canals were shaped using WaveOne® Gold Primary (Dentsply Sirona, Bellaigues, Switzerland) in reciprocating motion (WaveOne Gold ALL) of the X-Smart® Plus endodontic motor (Dentsply Sirona, Bellaigues Switzerland) according to the manufacturer's instructions. Patency was maintained with a # 10 K-file, and with each withdrawal from the instrument, the canals were irrigated with 1 mL of sodium hypochlorite (NaOCl) 2.5% (Pharmacia Precision, Curitiba, Brazil) using a 30-G NaviTip needle (Ultradent, South Jordan, UT). For the final irrigation, 2mL of NaOCl was used, followed by 2mL of EDTA (Biodynamic, Ibiporã, Brazil) and finally, 2mL of NaOCl. After the final irrigation, the root canal was dried with paper points compatible with the Wave One® Gold system (Tanari, Manaus, Brazil) and the roots were covered with nail polish leaving only the foramen free, in order to avoid contamination (23).

The teeth were autoclaved at 121 ° C, immersed in phosphate buffered saline (PBS).

Specimens inoculation with *Enterococcus faecalis* (ATCC®29212™)

The specimens were contaminated with *E. faecalis* strain (ATCC®29212™). The cells were loaded into BHI (Brain Heart Infusion) for growth for 24 hours at 37 °C. A suspension was prepared with *E. faecalis* 3x10⁸ CFU / mL (turbidity corresponding to tube # 1 of the Mc Farland scale), and it was introduced into the canal with an insulin syringe and a gauge needle 30 (Ultradent, South Jordan, UT). After inoculation, the teeth were placed in 15 mL centrifuge tubes, filled with 5 mL of the BHI solution supplemented with 1% glucose (24). The teeth were incubated at 37 °C for 21 days. The BHI was replaced every 3 days with a new one containing *E. faecalis* at the same concentration used in the initial contamination suspension.

MTT Assay

Forty-four specimens were used to evaluate bacterial reduction in the main canal. Two specimens were evaluated in scanning electronic microscopy to confirm biofilm formation. Other two were kept sterilized, without bacterial inoculation.

In a laminar flow, the specimens were removed from the centrifuge tubes. The BHI was aspirated from the root canals and the external surface disinfected with gauze soaked in 2.5% NaOCl. The root apexes were sealed with composite resin (FGM, Joinville, Brazil) and set in condensation silicone (Coltene Holding, Altstätten, Switzerland), maintained in a 37 ° C bath. (LaserGrip 774, Etekcity, Anaheim, CA) The temperature was checked using a non-contact infrared thermometer (LaserGrip 774, Etekcity, Ch) and a stick thermometer TP101 (BMax) inserted into the silicone. For collection of the initial sample (S1), the canals were filled with PBS and, with a Hedstroem # 25 file (Dentsply Sirona, Bellaigues, Switzerland), 20 strokes were performed against the canal walls. Using gauge needle 24 and a 3mL syringe, contaminated PBS was collected and transferred to a 1.5 mL microtube (Eppendorf, Hamburg, Germany) until complete 100 microliters.

Two mL of NaOCl 2.5% (Precision Pharmacy, Curitiba, Brazil) and 2 mL of EDTA 17% per canal were used for the application of the final irrigation protocols. The canal was filled with the solution. The activation was performed for 60 seconds and during this time the solution was dispensed in a continuous flow into the pulp chamber (EndoActivator[®], XP-endo[®] Finisher, PUI and Easy Clean). Finally, each root canal was irrigated with 1mL of 2.5% NaOCl, without activation, using gauge needle 30 to 1 mm from the WL, followed by 1 mL of 10% sodium thiosulphate, to neutralize the NaOCl.

The protocols were applied as followed:

Easy Clean

The insert was coupled to an endodontic motor (X-Smart[®] Plus) in reciprocating motion (WaveOne ALL).

EndoActivator[®]

The medium tip (25.04) was used and activated at 10,000 cycles per minute.

PUI

The Ultrasonic tip Irrisonic[®] 20 / .01 (Helse, Santa Rosa de Viterbo, Brazil), was coupled to the Jet Sonic (Gnatus, São Paulo, Brazil) set at 15% power in endodontic mode.

XP-endo[®] Finisher

The instrument was cooled before insertion into the canal with refrigerant spray (Endo-Frost, Roeko, Langenau, Germany) and activated in continuous rotation motion (800 rpm and 1 N of torque).

Syringes containing solutions were kept in warm bath (37°C) until use. Final samples (S2) were collected in the same manner as S1 after aspiration of sodium thiosulphate. All the instruments used were previously sterilized.

Bacterial reduction in the root canals was determined by the colorimetric method of reduction of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). MTT is a tetrazolate salt which is reduced to formazan crystals in the presence of metabolically active cells. Formazana is a blue compound whose intensity is directly proportional to the amount of viable cells (25).

In 1.5 mL microtubes containing the samples (100 µL), 100 µL of 1 mg / mL-1 MTT (Sigma-Aldrich, St. Louis, MN) was added. The tubes were vortexed (1200 rpm, 30 s) and left in the dark at 37 °C for 3 h. These tubes were centrifuged in MiniSpin® with F-45-12-11 rotor (Eppendorf AG, Hamburg, Germany) at 12,100 × g for 60 sec. The supernatants were discarded, and the pellets were washed with 500 µL of sterile distilled water followed by centrifugation (12100 × g, 60 s). The supernatants were discarded, and the pellets received 200 µL of isopropyl alcohol, followed by vortexing. Cells released formazan, which was measured after centrifugation and aliquot transfer from 100 µL of supernatant to 96-well microtiter plates. The optical densities of the samples were measured in spectrophotometer TP Reader (Thermo Plate, Brazil) at 540 nm. For each experimental group, two blanks were included (PBS only), which served as a negative control.

The percentage of bacterial reduction was calculated, following the formula:

$$\text{Percentage of bacterial reduction} = 100 - [(100 \times S2) / S1]$$

Where:

S1 = arithmetic mean between initial sample values of the buccal and lingual canals of each specimen.

S2 = arithmetic mean between values of the final sample of the buccal and lingual canals of each specimen.

Confocal Laser Scanning Microscopy (MCVL) Evaluation:

For the evaluation of bacterial reduction into dentin tubules, 36 specimens containing isthmus, with cervical portion located between 3.5 and 6 mm from the apex, were used. These teeth were equally distributed between the groups, according to the dimensions of the isthmus.

Previously, a groove perpendicular to the long axis was cut, located in the most cervical portion of the isthmus, without penetrating the main canals. Diamond disc was used for this step.

Irrigation protocols were applied exactly as described in the MTT assay. Immediately after the use of sodium thiosulfate, the canals were dried with sterile a paper points and the specimens split using a chisel.

They were stained using LIVE / DEAD® BackLight® Bacterial Viability Kit (Molecular Probes®, Invitrogen®, Eugene, OR). It contains SYTO 9 fluorophores (excitation wavelength 488 nm / emission wavelength 525 nm) which is capable of staining all bacteria and propidium iodide (excitation 488 nm / emission 560 nm), which marks only the bacteria that had their plastic membrane ruptured. Live bacteria fluoresce green and dead organisms, red (26). Surfaces were washed with 100 µl of PBS, and 10 µl of dye was applied leaving for 5 minutes prior to analysis.

Each part was examined on a Fluoview 1000 laser scanning confocal microscope (Olympus, Tokyo, Japan) with 20X magnification and 2X zoom. Images were acquired using Olympus FluoView Version 1.7 software (Olympus, Tokyo, Japan) in 512 x 512 pixel resolution. A 1 µm step was used and at least 10 deep steps were acquired. The stack of these images generated the final image for analysis. It was denominated as “percentage of bacterial reduction in dentinal tubules of the main canal region” the results corresponding to the lingual and buccal walls of the cuts and as “percentage of bacterial reduction in dentinal tubules in the isthmus region” the results corresponding to the mesial and distal walls of the cuts (Figure 1). Each final image was divided into three levels (22), according to the depth (50, 100 and 150 µm deep in the dentinal tubules from the dentin wall) (Figure 2).

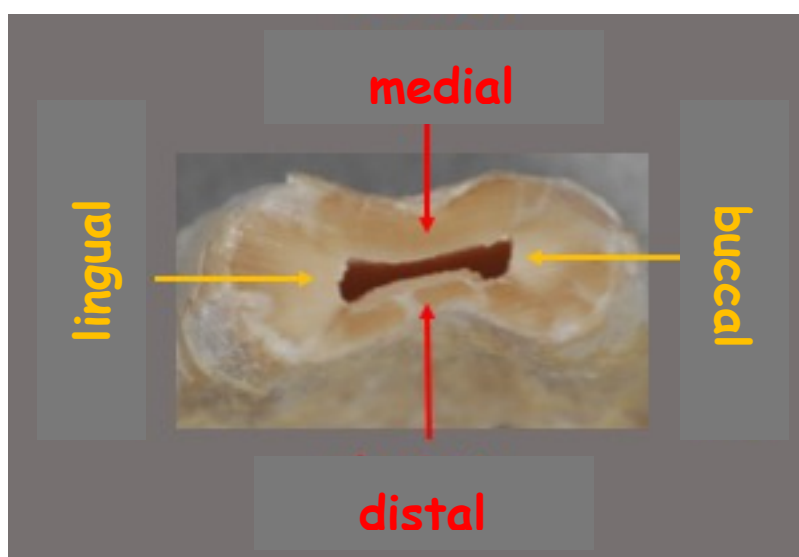


Figure 1: Cross section of the specimen, showing canal region, which includes lingual and vestibular walls of the cut (in yellow) and the isthmus region, represented by the mesial and distal walls (in red).

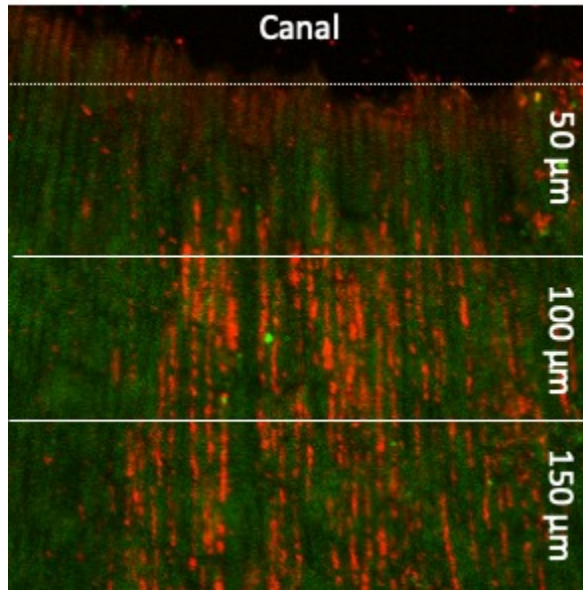


Figure 2: Image obtained in MCVL (40x) using LIVE / DEAD kit, revealing live bacteria (green) and dead (red) bacteria. Division of image for evaluation, according to depth into the dentinal tubules (50, 100 and 150 μm) from the root canal wall.

The evaluation of each image was performed using Bio Image_L[®] software (The MathWorks Inc, Natick, MA). The software generates information on the percentage of live and dead bacteria in each image (23).

Data were submitted to statistical analysis using the IBM STATISTICS SPSS 25.0 software (IBM Inc. Chicago, IL) with a significance level set at 0.05. In both evaluations, the data distribution did not present normality, according to the Kolmogorov-Smirnov test. The Kruskal-Wallis non-parametric test was used, followed by the Dunn test. Analysis of the power test showed that the sample size was adequate for both MTT (90%) and MCVL (99%) evaluation.

Results

Table 1 shows the results of bacterial reduction in the main canals presented by MTT evaluation. It was observed that there was no statistically significant difference between the final irrigation protocols, except when XP-endo[®] Finisher was compared with Easy Clean and EndoActivator[®], where the first one was able to eliminate more bacteria in the root canals ($p < 0.05$).

Table 1: Median, minimum e maximus values of percentage (%) of bacterial reduction in the main canals.

Final Irrigation Protocol	n	Median (min-max)
Easy Clean	10	49.59 (25,77-92,27) ^A
EndoActivator [®]	10	69.47 (22,95-99,81) ^A
PUI	10	82.01 (25,64-100) ^{AB}
XP-endo [®] Finisher	10	94.73 (76,04-100) ^B

Different capital letters indicate statically significant difference between groups (Dunn test/ $p < 0,05$)

There was no difference between the devices as to the ability to reduce bacteria located within the dentinal tubules of both the main canals and the isthmus region 50 μm deep from the dentin wall ($p > 0.05$), as shown in Table 2. XP-endo[®] Finisher was more efficient than Easy Clean at 100 and 150 μm levels and showed superior performance than PUI at 150 μm ($p < 0.05$) in both regions. All groups presented similar performance, at the same depth, when compared to the main and isthmus region ($p > 0.05$).

Table 2: Median, minimum and maximum values of percentage(%) of bacterial reduction in dentinal tubules

Area	n	Depth	Easy Clean	EndoActivator [®]	PUI	XP-endo [®] Finisher
Canal	16	50 μm	28.50 (1-100) ^A	85.50 (0-100) ^A	38.50 (3-99) ^A	75.00 (2-100) ^A
	16	100 μm	17.00 (0-52) ^A	22.50 (0-100) ^{AB}	24.00 (1-91) ^{AB}	59.50 (0-100) ^B
	16	150 μm	13.00 (0-63) ^{Aa}	16.50 (0-100) ^{AB}	14.00 (2-57) ^A	51.50 (0-100) ^B
Isthmus	16	50 μm	31.50 (7-100) ^A	53.50 (4-100) ^A	53.00 (1-100) ^A	64.50 (3-96) ^A
	16	100 μm	11.00 (0-97) ^A	50.00 (1-100) ^B	23.00 (0-86) ^{AB}	42.00 (0-100) ^B
	16	150 μm	6.50 (0-99) ^A	23.00 (0-100) ^{AB}	8.50 (0-74) ^A	42.00 (0-99) ^B

Different capital letters in the same line indicate statically significant difference between groups (Dunn test/ $p < 0,05$)

Discussion

The purpose of this study was to compare the four agitation devices for their ability to reduce bacteria in the main canals and in dentinal tubules of isthmus and canals in the apical region. The null hypothesis was rejected, since there were differences between the agitation devices in both evaluations. The XP-endo[®] Finisher presented superior performance when compared to Easy Clean for both bacterial reduction in the main canals, and deeper (100 and 150 μm) in the dentinal tubules of the canals and the isthmus.

The specimens were contaminated with *E. faecalis* (ATCC 29212) for the evaluations of both methods. Although endodontic biofilms are polymicrobial, *E. faecalis* alone are frequently used for evaluation of antimicrobial action in endodontics due to the survival and adaptation capacity of this bacterium and its association with cases of failure in endodontic therapy (7, 8). To enhance bacterial growth and colonization of dentin tubules, 1% glucose was added to the BHI (24). In addition, the BHI, used in medium replacement, contained a new culture of *E. faecalis*, prepared 24 hours earlier and adjusted to turbidity corresponding to No. 1 tube of the Mc Farland scale (27).

To evaluate the bacterial reduction in the the main canals we chose to use the MTT colorimetric method. This method was proposed by Mosmann (25) for the detection of viable cells. Although it is commonly used for cytotoxicity testing (28, 29), it is also applied for viability of bacterial cells in root canals (22, 30). The ability of the tested protocols to eliminate bacteria within the dentinal tubules was evaluated by means of MCVL. The method allows, from the use of fluorophores, to differentiate live and dead bacteria (26). Similar to the previous study (22), the images obtained were divided according to the depth of the tubules in relation to the root canal wall, in order to better detail the performance achieved by the protocols used. The combination of the two evaluation methods results in a more complete view of the potential to reduce bacteria from the tested protocols.

The results were similar for all groups into 50 µm deep dentinal tubules, even in the isthmus region. The elimination of the microorganisms in the dentinal tubules may be related to NaOCl penetration therein. NaOCl 1% penetrates 77 µm deep into dentinal tubules, when at room temperature, for two minutes. The depth of penetration increases as the concentration increases (31). In a study of bovine teeth infected with *E. faecalis*, 2.5% NaOCl, without agitation, eliminated 99% viable cells in the dentinal tubules exposed to irrigation at 5-minute intervals (23). It is possible that removal of biofilm, debris and smear layer from the dentinal walls facilitates hypochlorite access to the tubules. Therefore, the device that best perform these functions, will allow greater bacterial elimination in the tubules as well.

The XP-endo® Finisher is a non-tapered instrument made from a NiTi MaxWire® alloy (FKG Dentaire AS, La Chaux-Fonds, Switzerland). Its shape

memory allows the change of the martensitic phase to the austenitic phase during the rotational movement when in contact with body temperature. The instrument expands and is capable of increasing its diameter by up to 6 x (19). The present experiment was completely conducted under temperature control, maintained at 37 ° C, to meet the demand of XP-endo® Finisher and maintain the same conditions for all groups. Although heating of NaOCl increases its bactericidal capacity (32), raising the temperature to 37 ° C does not seem to exert this influence (33, 34). It is important to note that, despite standardization, this is a laboratory study, so it does not accurately reproduce the conditions of temperature variation *in vivo*. This difference may impact the performance of the instrument in relation to when it is used clinically.

PUI was as effective as XP-endo® Finisher in bacterial reduction of main canals and in the dentinal tubules. Only in the deepest part of the dentinal tubules (150 µm) XP-endo® Finisher was superior. These devices presented similar results when debris reduction was evaluated (20). However, when evaluating biofilm removal on canal surface and artificially produced grooves, XP-endo® Finisher presented superior performance (35). Since these studies methodology are different it is not possible direct comparison of results. Although some data point out that PUI has superior performance in debris reduction when used in three cycles of 20 seconds (36), this is not a standard among studies. When comparing the use of PUI in continuous and intermittent irrigation for apical biofilm removal, there was no difference (35). In the present study we opted to standardize continuous irrigation and activation for one minute for all devices, based on the choice of literature findings (35, 37, 38) and the manufacturer's indication of the XP-endo® Finisher for one-minute use.

The EndoActivator Sonic System presented inferior performance to XP-Endo Finisher in bacterial reduction in the main canal evaluation which agrees with the results of a previous study comparing the two systems, using the same methodology, but in distal roots of molars Inferior (21). The use of sonic agitation systems, such as EndoActivator, is a practical option, since they are handheld handpieces, easy to manipulate, use and transport when compared with PUI, since both had similar performance.

Easy Clean is an instrument that is easy to apply in the routine of endodontic therapy, because it is used in an electric motor. The manufacturer description indicates that it agitates the irrigation solution and touch the walls of the root canal (17). Because it is an instrument 25.04, it is not completely adjusted to the canals in preparations made with instruments 25.07, as in the present study. However, this did not prevent it from showing favorable results regarding the removal of debris or removal of intracanal medication, even in canals prepared with 30.05 instruments, or 40.06 respectively (17, 39). Although it has been used in reciprocating motion, this instrument can be used in continuous rotation. The authors are unaware of another article evaluating this instrument regarding the reduction of viable bacteria. In the present experiment, although not as effective as XP-endo® Finisher, Easy Clean presented similar results to other systems in all evaluations. The difference between the results can be attributed to speed of use. The first was driven here at 800 rpm, while the second was used in reciprocating motion. The use in continuous and reciprocating movement did not significantly affect the removal of debris for Easy Clean (18). However, the influence of motion on bacterial reduction has not yet been evaluated.

In vitro research has its importance in evaluating the efficacy of treatments because of the greater possibility of variable control, but it does not replace clinical trials (40). Their results should not be extrapolated to clinical reality but may help direct the choice of treatments. The literature lacks clinical studies that determine whether these additional root canal cleansing steps actually influence clinical success. PUI was not able to increase the reduction of *E. faecalis*, in vivo, when evaluated in cases of retreatment. However, the evaluation was performed by collecting samples from single canal teeth (41). It would be important to be able to assess areas of difficult access and to provide long-term clinical follow-up.

The XP-endo® Finisher had superior performance in eliminating *E. faecalis* in the SCR, however all devices produced bacterial reduction, and none were able to completely eliminate the bacteria in the main canals and in the dentinal tubules. All groups presented similar performance, at the same depth, when compared to the canal and isthmus region. This demonstrates that, even in areas of difficult access, where the instruments will not directly touch the dentin wall, the mechanical

agitation of the irrigating solutions used was sufficient to decrease the amount of viable bacteria in the dentin tubules. It would be of great value if future *in vivo* studies evaluated the importance of these additional steps in the success of endodontic therapy and whether the differences presented here are reproduced clinically.

References

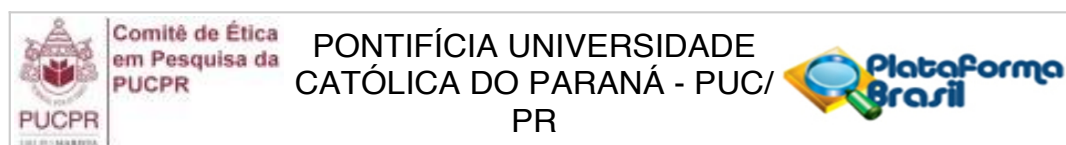
1. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int Endod J* 1997;30:297-306.
2. Sedgley CM, Lennan SL, Appelbe OK. Survival of *Enterococcus faecalis* in root canals *ex vivo*. *Int Endod J* 2005;38:735-42.
3. Siqueira Jr JF, Rôças IN, Ricucci D, Hülsmann M. Causes and management of post-treatment apical periodontitis. *Br Dent J* 2014;216:305-12.
4. Ricucci D, Candeiro GTM, Bugea C, Siqueira Jr JF. Complex apical intraradicular infection and extraradicular mineralized biofilms as the cause of wet canals and treatment failure: report of 2 cases. *J Endod* 2016;42:509-15.
5. Chavez de Paz LE. Redefining the persistent infection in root canals: possible role of biofilm communities. *J Endod* 2007;33:652-62.
6. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. *J Endod* 2006;32:93-8.
7. Chen L, Li X, Zhou X, et al. Inhibition of *Enterococcus faecalis* growth and biofilm formation by molecule targeting cyclic di-AMP synthetase activity. *J Endod* 2018;44:1381-8.
8. Möller AJR, Fabricius L, Dahlen G, et al. Apical periodontitis development and bacterial response to endodontic treatment. Experimental root canal infections in monkeys with selected bacterial strains. *Eur J Oral Sci* 2004;112:207-15.
9. Tahmasbi M, Jalali P, Nair MK, et al. Prevalence of middle mesial canals and isthmi in the mesial root of mandibular molars: an *in vivo* cone-beam computed tomographic study. *J Endod* 2017;43:1080-3.
10. Wolf TG, Paqué F, Zeller M, et al. Root canal morphology and configuration of 118 mandibular first molars by means of micro-computed tomography: an *ex vivo* study. *J Endod* 2016;42:610-4.
11. Estrela C, Rabelo LE, de Souza JB, et al. Frequency of root canal isthmi in human permanent teeth determined by cone-beam computed tomography. *J Endod* 2015;41:1535-9.
12. Jung I, Seo M, Fouad AF, et al. Apical anatomy in mesial and mesiobuccal roots of permanent first molars. *J Endod* 2005;31:364-8.

13. Paqué F, Ganahl D, Peters OA. Effects of root canal preparation on apical geometry assessed by micro-computed tomography. *J Endod* 2009;35:1056-9.
14. Bedier MM, Hashem AAR, Hassan YM. Improved dentin disinfection by combining different-geometry rotary nickel-titanium files in preparing root canals. *Restor Dent Endod* 2018;43:e46.
15. Van der Sluis LWM, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. *Int Endod J* 2007;40:415-26.
16. Bolles JA, He J, Svoboda KKH, et al. Comparison of Vibringe, EndoActivator, and needle irrigation on sealer penetration in extracted human teeth. *J Endod* 2013;39:708-11.
17. Kato AS, Cunha RS, Bueno CES, et al. Investigation of the efficacy of passive ultrasonic irrigation versus irrigation with reciprocating activation: an environmental scanning electron microscopic study. *J Endod* 2016;42:659-63.
18. Duque JA, Duarte MA, Canali LC, et al. Comparative effectiveness of new mechanical irrigant agitating devices for debris removal from the canal and isthmus of mesial roots of mandibular molars. *J Endod* 2017;43:326-31.
19. <https://www.fkg.ch/xpendo/finisher> [accessed on September 2, 2019].
20. Leoni GB, Versiani MA, Silva-Sousa YT, Bruniera JF, Pecora JD, Sousa-Neto MD. Ex vivo evaluation of four final irrigation protocols on the removal of hard-tissue debris from the mesial root canal system of mandibular first molars. *Int Endod J*. 2017;50(4):398-406.
21. Wigler R, Dvir R, Weisman A, et al. Efficacy of XP-endo finisher files in the removal of calcium hydroxide paste from artificial standardized grooves in the apical third of oval root canals. *Int Endod J* 2017;50:700-5.
22. Azim AA, Aksel A, Zhuang, MAT, et al. Efficacy of 4 irrigation protocols in killing bacteria colonized in dentinal tubules examined by a novel confocal laser scanning microscope analysis.
23. Rodrigues CT, Andrade FB, Vasconcelos LRSM, et al. Antibacterial properties of silver nanoparticles as a root canal irrigant against *Enterococcus faecalis* biofilm and infected dentinal tubules. *Int Endod J* 2018;51:901-11.
24. Bulacio ML, Galván LR, Gaudioso C, et al. *Enterococcus faecalis* biofilm. Formation and development *in vitro* observed by scanning electron microscopy. *Acta Odontol. Latinoam* 2015;28:210-4.
25. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:55-63.
26. Bottcher DE, Sehnem NT, Montagner et al. Evaluation of the effect of *Enterococcus faecalis* biofilm on the 2% chlorhexidine substantivity: an *in vitro* study. *J Endod* 2015;41:1364-70.

27. Saber Sel-D, El-Hady SA. Development of an intracanal mature *Enterococcus faecalis* biofilm and its susceptibility to some antimicrobial intracanal medications; an *in vitro* study. *Eur J Dent* 2012;6:43-50.
28. Meerloo J, Kaspers GJL, Cloos J. Cell sensitivity assays: the MTT assay. *Methods Mol Biol* 2011;731:237-45.
29. Nogales CG, Ferreira MB, Montemor AF, et al. Ozone therapy as an adjuvant for endodontic protocols: microbiological - *ex vivo* study and cytotoxicity analyses. *J Appl Oral Sci* 2016;24:607-13.
30. Zeng C, Willison J, Meghil MM, et al. Antibacterial efficacy of an endodontic sonic-powered irrigation system: An *in vitro* study. *J Dent* 2018;75:105-12.
31. Zou L, Shen Y, Li W, Haapasalo M. Penetration of Sodium Hypochlorite into Dentin. *Journal of Endodontics*. 2010;36(5):793-6.
32. Giardino L, Mohammadi Z, Beltrami R, Poggio C, et al. Influence of temperature on the antibacterial activity of sodium hypochlorite. *Braz Dent J*. 2016;27:32-6.
33. Del Carpio-Perochena A, Bramante CM, Duarte MH, et al. Effect of temperature, concentration and contact time of sodium hypochlorite on the treatment and revitalization of oral biofilms. *J Dent Res Dent Clin Dent Prospects*. 2015;9:209-15.
34. Gulsahi K, Tirali RE, Cehreli SB, et al. The effect of temperature and contact time of sodium hypochlorite on human roots infected with *Enterococcus faecalis* and *Candida albicans*. *Odontology*. 2014;102:36-41.
35. Bao P, Shen Y, Lin J, Haapasalo M. In Vitro Efficacy of XP-endo Finisher with 2 Different Protocols on Biofilm Removal from Apical Root Canals. *J Endod*. 2017;43(2):321-5.
36. Van der Sluis LWM, Vogels MP, Verhaagen B, et al. Study on the influence of refreshment/activation cycles and irrigants on mechanical cleaning efficiency during ultrasonic activation of the irrigant. *J Endod*. 2010;36:737-40.
37. Capar ID, Ozcan E, Arslan H, Ertas H, Aydinbelge HA. Effect of different final irrigation methods on the removal of calcium hydroxide from an artificial standardized groove in the apical third of root canals. *J Endod*. 2014;40:451-4.
38. Alves FR, Andrade-Junior CV, Marceliano-Alves MF, et al. Adjunctive steps for disinfection of the mandibular molar root canal system: a correlative bacteriologic, micro-computed tomography, and cryopulverization approach. *J Endod*. 2016;42:1667-72.
39. Oliveira RL, Guerisoli DMZ, Duque JA, et al. Computed microtomography evaluation of calcium hydroxide-based root canal dressing removal from oval root canals by different methods of irrigation. *Microsc Res Tech*. 2019;82: 232-7.
40. Neelakantan P, Romero M, Vera J, et al. Biofilms in Endodontics-current status and future directions. *Int J Mol Sci*. 2017;18:1748.
41. Rodrigues RC, Antunes HS, Neves MA, et al. Infection control in retreatment cases: *In vivo* antibacterial effects of 2 instrumentation systems. *J Endod*. 2015;41:1600-5.

Anexos

Aprovação do Comitê de Ética em Pesquisa



PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: AVALIAÇÃO DO EFEITO DE DIFERENTES PROTOCOLOS DE IRRIGAÇÃO NA REMOÇÃO DE DEBRIS E NA REDUÇÃO DE BACTÉRIAS NAS RAÍZES MESIAIS DE MOLARES INFERIORES

Pesquisador: ALESSANDRA TIMPONI GOES CRUZ

Área Temática:

Versão: 3

CAAE: 61195016.5.0000.0020

Instituição Proponente: Pontifícia Universidade Católica do Parana - PUCPR

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.525.268

Apresentação do Projeto:

O objetivo do tratamento endodôntico é remover restos pulpares, debris dentinários e microrganismos, dar forma ao canal e permitir uma adequada obturação dos canais radiculares. A permanência de bactérias no canal radicular após o preparo, muitas vezes abrigadas pelos debris, é um dos fatores diretamente relacionado ao fracasso da terapia endodôntica. O objetivo deste estudo é avaliar a capacidade de remover debris e reduzir a quantidade de bactérias viáveis no canal radicular dos seguintes protocolos de irrigação final: irrigação ultrassônica passiva (PUI), EndoActivator, EndoVac e EasyClean.

O objetivo deste trabalho é avaliar, in vitro, a redução de debris dentinários e de bactérias viáveis, em raízes mesiais de molares inferiores, produzida por quatro diferentes protocolos de irrigação.

Objetivo da Pesquisa:

O objetivo deste trabalho é avaliar, in vitro, a redução de debris dentinários e de bactérias viáveis, em raízes mesiais de molares inferiores, produzida por quatro diferentes protocolos de irrigação.

Avaliação dos Riscos e Benefícios:

Riscos e benefícios não se aplicam ao projeto e suas descrições estão adequadas à Res. CNS 466/12

Endereço: Rua Imaculada Conceição 1155
Bairro: Prado Velho **CEP:** 80.215-901
UF: PR **Município:** CURITIBA
Telefone: (41)3271-2103 **Fax:** (41)3271-2103 **E-mail:** nep@pucpr.br

Continuação do Parecer: 2.525.268

Comentários e Considerações sobre a Pesquisa:

Justificativa da Emenda:

Foi necessário alterar o projeto devido à inclusão de um novo grupo e necessidade de aumentar a amostra, tendo em vista que não seria viável utilizar os mesmos espécimes para as três avaliações propostas.

Emenda apresenta metodologia adequada aos resultados, estando dentro dos preceitos da Res. CNS 466/12.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos foram, neste versão, encaminhados em acordo com a Res. CNS 466/12.

Recomendações:

Não há.

Conclusões ou Pendências e Lista de Inadequações:

O projeto de pesquisa em questão, a partir de agora, atende os requisitos éticos da Resolução CNS nº 466/12 do sistema CEP/CONEP.

Considerações Finais a critério do CEP:

Lembramos aos senhores pesquisadores que, no cumprimento da Resolução 466/12, o Comitê de Ética em Pesquisa (CEP) deverá receber relatórios anuais sobre o andamento do estudo, bem como a qualquer tempo e a critério do pesquisador nos casos de relevância, além do envio dos relatos de eventos adversos, para conhecimento deste Comitê. Salientamos ainda, a necessidade de relatório completo ao final do estudo.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEPPUCPR de forma clara e sucinta, identificando a parte do protocolo a ser modificado e as suas justificativas.

Se a pesquisa, ou parte dela for realizada em outras instituições, cabe ao pesquisador não iniciá-la antes de receber a autorização formal para a sua realização. O documento que autoriza o início da pesquisa deve ser carimbado e assinado pelo responsável da instituição e deve ser mantido em poder do pesquisador responsável, podendo ser requerido por este CEP em qualquer tempo.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

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Continuação do Parecer: 2.525.268

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1027914_E1.pdf	28/11/2017 09:20:59		Aceito
Folha de Rosto	FOLHA_ROSTO.pdf	28/11/2017 09:09:07	ALESSANDRA TIMPONI GOES CRUZ	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Alessandra_ATUALIZADO.docx	28/11/2017 09:07:27	ALESSANDRA TIMPONI GOES CRUZ	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Termo_Banco_Dentes.pdf	28/11/2017 09:06:59	ALESSANDRA TIMPONI GOES CRUZ	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

CURITIBA, 05 de Março de 2018

**Assinado por:
NAIM AKEL FILHO
(Coordenador)**

Endereço: Rua Imaculada Conceição 1155		CEP: 80.215-901
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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 reviews 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.

[General Points on Composition](#)

[Organization of Original Research Manuscripts](#)

[Manuscripts Category Classifications and Requirements](#)

[Available Resources](#)

General Points on Composition

1. 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*:

2. 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.
3. 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.
4. 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.
5. 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.)

6. 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.
7. 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.”
8. 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.
9. 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.

[Top ^](#)

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.*

1. **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 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 order reprints when applicable. The contribution of each author should also be highlighted in the cover letter.

2. **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.
3. **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 the extensive review of the literature, and discuss the results of the study in this section.
4. **Materials and Methods:** The objective of the materials and methods section is to permit other investigators to repeat your experiments. The four components of 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.

5. **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. The 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).
6. **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 photos, 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 multi-panel, composite figure only counts as one figure when considering the total number of figures in a manuscript (see section 3, below, for the maximum number of allowable figures). The second type of figures is 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.

7. **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: 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).

% 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

8. **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 leadership. 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
9. **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
10. **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 to the number of references for review articles.

[Top ^](#)

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:

1. 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 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*.
2. Review Article-Manuscripts in this category is either narrative articles, or systematic reviews/meta-analyses. Case report/Clinical Technique articles even when followed by the extensive review of the literature will 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*.
3. 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*.
4. 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*.
5. 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 *.

6. 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 multi-panel figures must not exceed 1-page length. Manuscripts submitted with more than the allowed number of figures or tables will require the 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.

[Top ^](#)

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.

[Top ^](#)

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