UNIVERSIDADE ESTADUAL PAULISTA
"Júlio de Mesquita Filho"

Carlos Roberto Emerenciano Bueno

TESE DE DOUTORADO

Estudo de novos dispositivos, instrumentos e materiais empregados no tratamento endodôntico

ARAÇATUBA-SP
2019

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Tese de doutorado apresentada à Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP como parte dos requisitos para obtenção do título de Doutor em Ciência Odontológica, área de concentração em Endodontia.

Orientador: Prof. Assoc. Eloi Dezan-Junior

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Nascido em **01 de junho de 1986** em **C**ampinas (SP), casado com **V**anessa **M**anzini **D**reibi **B**ueno, filho de **C**arlos **R**oberto **B**ueno e **N**orma **P**ignataro **E**merenciano **B**ueno.

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2017 – ESTÁGIO NO EXTERIOR

Período sanduíche em ***Ultradent Products, Inc.*** *(Utah, Salt Lake City, USA)***,** sob co-orientação do professor **C**arlos **A**lberto **S**pironelli **R**amos.

Bueno CRE. **Estudo de novos dispositivos, instrumentos e materiais empregados no tratamento endodôntico**. [Tese]. Faculdade de Odontologia de Araçatuba, UNESP - Universidade Estadual Paulista, Araçatuba, Brasil, 2019.

Resumo

Introdução: O tratamento endodôntico visa manter ou restabelecer a saúde do periodonto através do preparo biomecânico, utilizando instrumentos de níquel-titânio automatizados e associados com insertos ultrassônicos para potencializar a ação dos irrigantes. Os materiais empregados na endodontia que permanecerão em contato com tecidos devem apresentar biocompatibilidade e preferencialmente ser biomineralizador, a fim de selar comunicações (fisiológicas/iatrogênicas), isolando o sistema de canais radiculares.

Objetivos:

1- Avaliar a resistência à fadiga cíclica das limas reciprocantes Genius e EdgeFile X1 Small, comparados à lima WaveOne Gold Primary;

2- Avaliar a capacidade de limpeza de um inserto ultrassônico de níquel titânio em ativação da irrigação de forma contínua e passiva;

3- Avaliar a biocompatiblidade e biomineralização do cimento reparador MTA Flow.

Material e Métodos: Para avaliar a resistência à fadiga cíclica dos sistemas Genius, EdgeFile e WaveOne Gold, 80 instrumentos foram utilizados (n=20), divididos em 4 grupos experimentais: Genius 25.04, Genius 30.04; EdgeFile X1 Small e Wave One Gold Primary, acionados em um canal artificial de aço inoxidável com ângulo de curvatura de 60^{o} e raio de curvatura de 5 mm. O tempo decorrido da ativação do motor foi gravado em um cronômetro digital e parado assim que a fratura foi detectada. O número de ciclos até a falha (NCF) e o tempo para fratura (TF) foram calculados e os comprimentos dos segmentos fraturados medidos. Para avaliar a capacidade de limpeza do inserto ultrassônico, 45 pré-molares inferiores, padronizados em 16 mm foram utilizados. A instrumentação foi realizada até lima 50.04 sob irrigação com hipoclorito de sódio e foram divididos em 3 grupos (n = 15) de acordo com a técnica de ativação final da solução irrigadora: irrigação convencional (IC) (passiva com pressão positiva/sucção concomitante) como controle; ativação ultrassônica passiva (PUI) e ativação ultrassônica contínua (CUI) do irrigante. Os três grupos tiveram os protocolos de ativação/irrigação final em soluções de hipoclorito de sódio e EDTA. As amostras foram clivadas, as imagens obtidas com microscopia eletrônica de varredura (MEV) e avaliadas quanto à capacidade de remoção da camada de *smear layer* no terço cervical, médio e apical, por um sistema de escores. Para análise biológica do material reparador MTA Flow, os materiais MTA Angelus e ProRoot MTA foram utilizados para comparação. Quarenta ratos receberam implantes subcutâneo de tubos de polietileno contendo os 3 materiais e tubo vazio como controle ($n=10$). Após 7, 15, 30 e 60 dias, os animais foram eutanasiados e os tubos removidos com o tecido conjuntivo circundantes. Infiltrado inflamatório e espessura da cápsula fibrosa foram avaliados histologicamente e indução da mineralização analisada por Von Kossa e sob luz polarizada. Os dados foram analisados com testes estatísticos específicos ($p<5\%$).

Resultados: Quanto à fadiga cíclica, os instrumentos EdgeFile X1 Small apresentaram maior resistência (NCF: 6175.74 ± 1608.99; TF: 1058.7 ± 275.82), seguido por ambos instrumentos Genius ($p<0.05$), de forma significativa comparados ao Waveone Gold Primary. O inserto de NiTi aumentou a limpeza, comparado com IC, principalmente no terço apical e CUI mostrou os melhores resultados ($p<0.05$). Nos resultados biológicos, MTA Angelus induziu a reação mais leve após 15 dias ($p<0.05$), seguido do MTA Flow. ProRoot MTA induziu uma inflamação severa no dia 7, reduzindo após 15 dias. Nenhuma diferença foi observada entre os materiais após 30 ou 60 dias ($p>0.05$). Estruturas coradas por Von Kossa e birrefringentes foram positivas para todos os materiais.

Conclusões:

1- Os instrumentos EdgeFile X1 Small demonstraram maior resistência à fadiga cíclica que Genius e WaveOne Gold Primary. Ambos instrumentos Genius apresentaram resistência superior à WaveOne Gold Primary.

2- A ativação final do irrigante com o inserto NiTi melhorou a remoção de smear layer, com protocolo CUI superior à PUI ou IC.

3- O MTA Flow mostrou biocompatibilidade e induziu a biomineralização em todos os períodos observados.

Palavras-Chave: endodontia, inflamação, ultrassom, teste de materiais.

Bueno CRE. **Evaluation of new devices, instruments and materials used in endodontic treatment**. [Thesis, PhD]. Araçatuba School of Dentistry, UNESP – São Paulo State University, Araçatuba, Brazil, 2019.

Abstract

Introduction: The endodontic treatment aims to maintain or restore periodontal health through biomechanical instrumentation with nickel-titanium automatized instruments, associated with an ultrasonic tip to activate the irrigant, enhancing cleanness. Materials used in endodontics that will remain in contact with tissues, should demonstrate properties as biocompatibility and biomineralization ability, in order to seal communications (physiological/iatrogenic) isolating the root canal system.

Objectives:

1- Evaluate the cyclic fatigue resistance of reciprocating Genius and EdgeFile X1 Small instruments compared to Waveone Gold Primary instruments;

2- Evaluate the cleaning effectiveness of a nickel-titanium ultrasonic tip in continuous and passive irrigant activation;

3-Evaluate the biocompatibility and biomineralization of MTA Flow repair cement.

Materials and Methods: To evaluate the cyclic fatigue resistance of Genius, EdgeFile and WaveOne Gold, 80 instruments were used (n = 20), divided into 4 experimental groups: Genius 25.04, Genius 30.04; EdgeFile X1 Small and Wave One Gold Primary, reciprocating in a stainless steel artificial canal with a 60° angle of curvature and 5mm radius of curvature. The elapsed time of the motor activation was recorded in a digital timer and stopped as soon as the fracture was detected. The number of cycles to fracture (NCF) and time to fracture (TF) were calculated and the fractured segments lengths measured. To evaluate the ultrasonic tip cleanness capacity, forty-five mandibular premolars, standardized at 16 mm were used. The instrumentation was performed until file 50.04 under irrigation with sodium hypochlorite. The specimens were divided into 3 groups (n = 15) according to the final irrigant activation protocol: conventional irrigation (CI) (passive, with positive pressure/simultaneous aspiration) as control; passive ultrasonic irrigant activation (PUI) and continuous ultrasonic irrigant activation (CUI). All groups had the final activation / irrigation protocols with solutions of sodium hypochlorite and EDTA, standardized with same volume. Samples were sodium hypochlorite and EDTA, standardized with same volume. Samples were cleaved and images obtained through scanning electron microscopy (SEM) to assess smear layer removal in the cervical, middle and apical thirds, via a score system. To biologically analyze MTA Flow repair cement, MTA Angelus and ProRoot MTA cements were used as comparison. Forty rats received subcutaneous implants of polyethylene tubes containing the 3 cements and empty tube as control (n = 10). After days 7, 15, 30 and 60, the animals were euthanized and the tubes removed with the surrounding tissues. Inflammatory infiltrate and fibrous capsule thickness were histologically evaluated and mineralization induction assessed by Von Kossa staining and under polarized light. The data were analyzed with specific statistical tests ($p<5\%$).

Results: The cyclic fatigue test showed EdgeFile X1 Small with the highest resistance (NCF: 6175.74 ± 1608.99; TF: 1058.7 ± 275.82), followed by both Genius instruments ($p<0.05$). The nickel-titanium tip enhanced cleanness, compared to CI, mainly in the apical area, whereas CUI showed the best results ($p<0.05$). In the biological results, MTA Angelus induced the mildest reaction after 7 ($p>0.05$) and 15 days ($p<0.05$), followed by MTA Flow. ProRoot MTA induced severe inflammation on day 7, reducing after day 15 ($p>0.05$). No difference was observed after 30 or 60 days ($p>0.05$). Von Kossa staining and birefringents structures were positive for all materials.

Conclusion:

1- EdgeFile X1 Small instruments demonstrated superior cyclic fatigue resistance than Genius and WaveOne Gold Primary. In addition, both Genius instruments showed superior resistance than WaveOne Gold Primary.

2- The final irrigant activation with the NiTi tip improved smear layer removal, with CUI protocol superior to PUI or CI.

3 - MTA Flow showed biocompatibility and induced biomineralization in all observed periods.

Keywords endodontics, inflammation, ultrasonics, material testing

Dedicatória

O título de Doutor faz parte de uma das conquistas mais significativas que terei. Portanto, dedico esta tese às pessoas mais importantes da minha vida.

À minha amada esposa **Vanessa Manzini Dreibi Bueno**

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Por trás de todo sucesso do homem há uma grande mulher. Este é um dos meus grandes sucessos, obrigado por ser minha grande mulher. Te amo!!

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"A fé é a certeza daquilo que esperamos e a convicção de fatos que não vemos"

Hebreus 11:1

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Bruna, Vinicius & Vininho

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"Cuide bem dos seus irmãos... Eles são a melhor ponte com o seu passado e possivelmente quem vai sempre te apoiar no futuro"

Baz Luhrmann

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"O mestre é um caminho para seu aprendiz chegar à sabedoria e o verdadeiro mestre se orgulha de ter sido um degrau na vida do aprendiz que venceu na vida"

Içami Tiba

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Às Secretárias da Seção de Pós-Graduação

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Obrigado por toda a ajuda com papelada e burocracia. Sempre dispostas a ajudar, independente de quão em cima do prazo entregamos os documentos.

À Faculdade de Odontologia de Araçatuba - **FOA**

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Ao Exército Brasileiro

Foi durante minha vida na fronteira que li "**Caminhos da Polpa**". Após a faculdade, esse período foi fundamental para sedimentar meu interesse na Endodontia.

"Todo mundo já passou por algo que nos modificou de tal modo que não foi mais possível voltar a ser a pessoa que éramos Antes. "

-Autor desconhecido

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – **CAPES**

Pela bolsa concedida nesses **6 anos** de mestrado, doutorado e doutorado sanduíche.

"Quando você quer alguma coisa, todo o universo conspira para que você realize o seu desejo. "

Paulo Coelho

"Um dia, quando olhares para trás, verás que os dias mais belos foram aqueles em que lutaste. "

Sigmund Freud

"Tudo o que não puder contar como fez, não faça! *Se há razões para não contar*, essas *são* as *razões para não fazer.* "

Immanuel Kant

" O que vale na vida não é o ponto de partida e sim a caminhada."

Cora Coralina

"FAÇA AS COISAS MAIS DIFÍCEIS ENQUANTO SÃO FÁCEIS E FAÇA AS GRANDES ENQUANTO SÃO PEQUENAS. "

LAO TSE

"E aqueles que foram vistos dançando foram julgados insanos por aqueles que não podiam escutar a música. "

Friedrich Nietzsche

"...não tenha medo de falhar; se falhar, não tenha medo de chorar; se chorar, avalie seus erros, mas não desista, dê sempre uma nova chance para si mesmo. "

Augusto Cury

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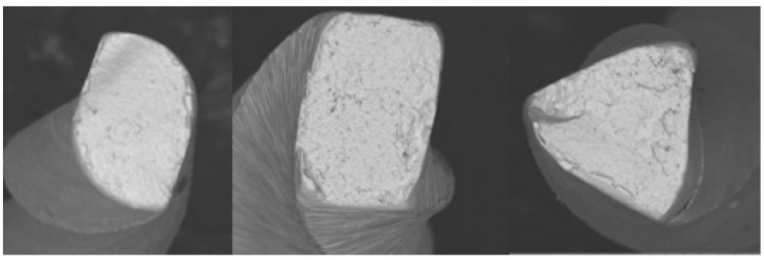
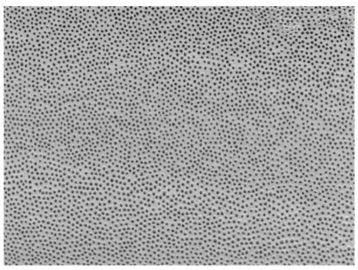
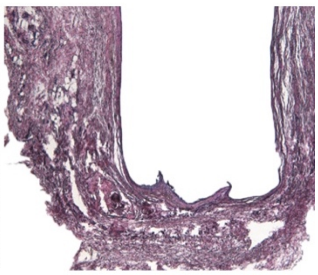


I. Introdução e justificativa

A limpeza e modelagem do sistema de canais radiculares é essencial para alcançar os objetivos mecânicos e biológicos do tratamento endodôntico. Essa etapa envolve a remoção do tecido pulpar vital ou necrótico, assim como microrganismos e subprodutos, enquanto modela a forma cônica apropriada para subsequente obturação do canal radicular (Schilder 2006). Tal modelagem e antissepsia dos condutos radiculares é realizada durante a etapa de instrumentação, caracterizada como biomecânica, uma vez que emprega substâncias químicas auxiliares que reduzem a carga bacteriana, e utiliza-se instrumentos para limagem das paredes dos canais, respeitando os princípios biológicos.

Os instrumentos de endodontia fabricados com níquel-titânio (NiTi) foram introduzidos por Walia e colaboradores (1988) para melhorar a instrumentação de canais curvos, uma vez que os instrumentos NiTi são mais flexíveis e apresentam resistência superior à fratura por torção em comparação com a rigidez dos instrumentos de aço inoxidável, diminuindo erros durante instrumentação (Peters 2004, Gergi *et al.* 2010). A introdução da instrumentação automatizada representou um avanço importante, aprimorando a qualidade e previsibilidade do preparo do canal radicular. Além disso, reduz erros de procedimento, fadiga do operador e a duração do tratamento, aperfeiçoando o tratamento endodôntico tanto para o profissional como para o paciente (Gavini *et al*. 2018). Além disso, reduz a fadiga do operador, os erros no procedimento e a duração do tratamento do canal radicular, melhorando a qualidade da terapia endodôntica tanto para o profissional como para o paciente.

Apesar das vantagens do NiTi, a incidência da fratura é a falha mais comum que ocorre durante o uso (Spanaki-Voreadi *et al*. 2006) como resultado da flexão, torção ou mesmo de uma combinação de ambos (Wei *et al.* 2007), sem qualquer indicação visível de deformação plástica (Tripi *et al.* 2001). Portanto, os fabricantes recomendam o uso limitado ou único de instrumentos NiTi especialmente em canais curvos.

A fadiga do metal causada pelo stress em rotação repetitiva é um importante mecanismo de falha (Pruett *et al.* 1997). Neste tipo de fratura, também conhecido como fadiga cíclica, os instrumentos NiTi estão sob tensão e forças de compressão na área de curvatura máxima do canal, até a fratura (Cheung & Darvell 2007). Estudos anteriores mostraram que o movimento reciprocante aumentou a resistência à fadiga cíclica em relação ao movimento de rotação contínua (Lopes *et al.* 2013, Dagna *et al.* 2014, Pedullà *et al.* 2014), tornando a cinemática reciprocante mais segura do que a rotatória para instrumentar o canal radicular.

O WaveOne Gold (Dentsply Maillefer, OK, USA) foi introduzido no mercado como técnica de instrumento único, fabricada com tratamento térmico aquecendo o instrumento e depois esfriando lentamente, em contraste com o tratamento térmico pré-fabricação M-Wire. Pode ser encontrado com diferentes diâmetros iniciais como Small (20.07), Primary (25.07), Medium (35.06) e Large (45.05), além de conicidade variada ao longo do instrumento. O WaveOne Gold Primary possui conicidade inicial .07, reduzindo para .06 após 4mm e para .03 após mais 4mm. O movimento do WaveOne anterior foi mantido, com uma ação de corte em 170^o no sentido anti-horário (counter clockwise, CCW) e liberação em 50^o no sentido horário (clockwise, CW), porém com dimensões e geometria modificadas, apresentando o centro de massa descentralizado e secção transversal em formato de paralelogramo quando comparado ao seu antecessor WaveOne (Dentsply Syrona WaveOne Gold 2019, Webber 2015).

O sistema Genius (Ultradent, South Jordan, UT) foi desenvolvido para associar técnicas rotatórias e reciprocantes, com liga NiTi tratada termicamente e conicidade .04. Segundo o fabricante, o movimento rotatório é indicado para ampliar o terço cervical, e 2 limas na cinemática reciprocantes são utilizadas para preparar o canal radicular, com 90^o de ação de corte (CW) e 30^o de liberação (CCW). Os instrumentos têm um design de secção transversal em “S” com ação de corte positiva para direita e a instrumentação é finalizada com a configuração rotatória, removendo os detritos do canal (Endo-Eze Genius Brochure 2018, Gavini *et al.* 2018).

O EdgeFile (EdgeEndo, Albuquerque, EUA) é apresentado como um instrumento reciprocante de secção transversal triangular e conicidade constante .06 em uma liga de níquel-titânio tratada termicamente com tecnologia patenteada (Fire-Wire™). De acordo com o fabricante, as limas devem ser usadas na configuração WaveOne®, com a mesma cinemática de corte em 170^o

(CCW) e 50^{o} para liberação (CW) do instrumento (EdgeEndo EdgeFile X-1 Brochure 2019).

Independente do sistema de instrumentação utilizado no preparo biomecânico, é imprescindível a utilização de irrigantes concomitantes à ação das limas, uma vez que, além de ação neutralizante de toxinas pelo poder antibacteriano (Hasselgren *et al.* 1988, Baumgartner e Cuenin 1992), o irrigante age diminuindo o atrito do instrumento com as paredes do canal radicular (Haapasalo *et al.* 2014). A instrumentação biomecânica produz uma camada de *smear layer* que cobre as paredes dentinárias, composta por material orgânico e inorgânico, formado principalmente por dentina, remanescente pulpar e, em alguns casos, bactérias (MacComb & Smith 1975, Mader *et al*. 1984). A presença desta *smear layer*, dificulta a penetração de agentes antimicrobianos e cimentos endodônticos nos túbulos dentinários (Goldberg & Abramovich 1977), possivelmente comprometendo a vedação entre o material obturador e a parede do canal radicular, o que pode interferir no sucesso do tratamento (Zehnder 2006).

O hipoclorito de sódio (NaOCl) é um irrigante amplamente utilizado para a desinfecção do canal radicular, mas, quando usado exclusivamente, é ineficaz na remoção da *smear layer* (Economides *et al.* 1999, Peters e Barbakow 2000). A adição de ácido etilenodiaminotetraacetico (EDTA) em concentração de 17% ao protocolo de irrigação, alternando com NaOCl, é recomendada para aumentar a efetividade na remoção da camada de *smear layer* (Haapasalo *et al.* 2005), uma vez que essa alternância tem ação tanto na camada orgânica quanto inorgânica da *smear layer*. Portanto, a irrigação desempenha um papel essencial na remoção de tecido pulpar, detritos e microorganismos (Uzunoglu *et al.* 2015).

Na técnica de irrigação convencional, a ponta da agulha deve ser posicionada 1-2 mm aquém do comprimento de trabalho (Lui *et al.* 2007) e, mesmo assim, a limpeza da região apical permanece incerta sem o auxílio de técnicas de agitação do irrigante. Para aumentar a eficácia e a dinâmica dessas soluções, vários protocolos de irrigação foram investigados em associação com ultrassom (Weller *et al.* 1980, van der Sluis *et al.* 2010, Grischke et al. 2014). A irrigação ultrassônica passiva (PUI) foi descrita por Weller *et al.* (1980). O protocolo baseia-se na inserção passiva de uma ponta metálica anexada a um dispositivo ultrassônico, no canal preenchido com o irrigante (van der Sluis *et al.*

2010, Beus *et al.* 2012). Após a ativação, o instrumento é cercado pela transmissão acústica, agitando a solução, aumentando a remoção de detritos (van der Sluis *et al.* 2007, Nagendrababu *et al.* 2018).

Outro protocolo de ativação ultrassônica foi proposto por Gutarts *et al.* (2005), no qual a ponta ultrassônica é colocada no canal e a solução irrigante flui continuamente, permitindo sua constante substituição. Quando é utilizado o hipoclorito de sódio como solução irrigante, fornece um suprimento ininterrupto de cloro nascente para a dissolução do tecido orgânico (Gutarts *et al.* 2005, Zehnder 2006). Essa irrigação ultrassônica contínua (CUI) não requer a substituição manual do irrigante entre ativações (van der Sluis *et al.* 2007), demonstrando penetração maior da solução irrigante quando utilizada no protocolo final de irrigação (Castelo-Baz *et al.* 2012).

Os insertos ultrassônicos convencionais utilizados para ativação da solução irrigante são fabricados a partir de uma liga de aço inoxidável (Hui-min *et al.* 2010) e devem ser posicionados a 1 ou 2 mm aquém do comprimento de trabalho (van der Sluis *et al.* 2010). Entretanto, foi introduzida no mercado uma ponta de níquel-titânio (NiTi) de 23 mm (NiTiSonic®, Ultradent Products Inc, South Jordan, Utah) com ponta 20.02 que, de acordo com o fabricante, pode ser usada até o comprimento do trabalho, mesmo em canais curvos (Ultradent Products UltraWave NiTiSonic Tip 2019).

Apesar da evolução nos materiais para otimizar e aprimorar o tratamento endodôntico, perfurações podem ocorrer de forma iatrogênica (fase de acesso, preparo biomecânico e preparo para pino) ou patológica (processo carioso ou reabsorções), durante a rotina clínica endodôntica (Tsesis & Fuss 2006, Roda & Gettleman 2016). Essa perfuração é caracterizada como uma comunicação entre o sistema de canais radiculares e a superfície dentária externa (American Association of Endodontists 2016), suscetível a ocorrer em qualquer fase do tratamento e pode levar ao insucesso (Estrela *et al.* 2017). Portanto, perfurações devem ser seladas com cimentos reparadores biocompatíveis e que induzam a formação de tecido mineralizado, uma vez que estes estarão em contato permanente com tecidos (Mori *et al.* 2014).

O agregado de trióxido mineral (MTA), um material hidráulico à base de silicato de cálcio derivado do cimento Portland (Hinata *et al.* 2017), foi desenvolvido para selar a comunicação entre os dentes e o periodonto, em perfurações de raízes patológicas ou iatrogênicas (Lee *et al*. 1993, Torabinejad *et al.* 1993). Estudos prévios confirmaram sua eficácia como material biocompatível, induzindo a formação de tecido mineralizado, além da atividade antimicrobiana (Holland *et al.* 1999, Camilleri & Pitt Ford 2006). Essas propriedades biológicas são atribuídas ao seu pH alcalino e capacidade de liberação de íons cálcio, mantendo um pH alto (Sarkar *et al.* 2005, Zarrabi *et al.* 2010, Tanomaru-Filho *et al.* 2017). A natureza hidrofílica das partículas de MTA permite seu uso mesmo na presença de umidade, uma vez que os componentes principais são silicato de tricálcio e dicálcico, aluminato tricálcico, óxido tricálcico e agentes radiopacificantes, como o óxido de bismuto (Parikoh e Torabinejad 2010).

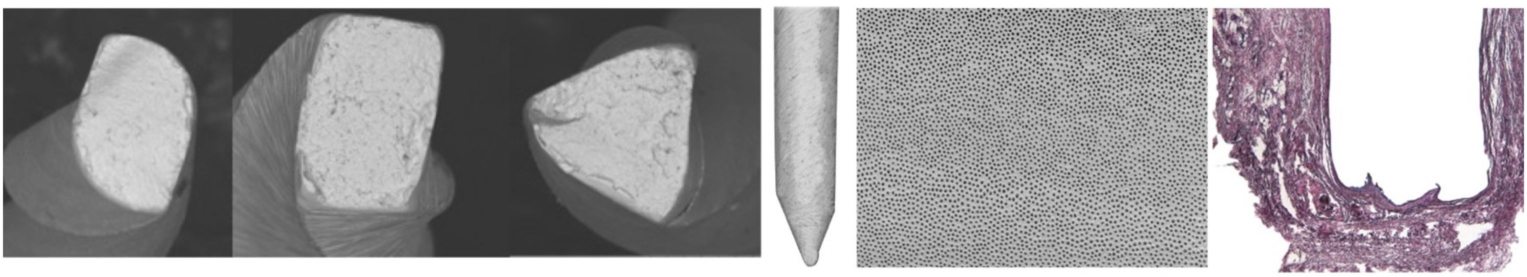
O MTA Angelus (MTA-Ang) (Angelus Indústria de Produtos Odontologicos S/A, Londrina, PR, Brasil) e ProRoot MTA (Tulsa Dental Products, Tulsa, OK) foram introduzidos no mercado com composições e indicações similares e são amplamente utilizados, sendo estudado ao longo dos anos, evidenciando suas propriedades biológicas aprimoradas (Camilleri 2008, Cintra *et al. 2017*).

No entanto, a consistência final arenosa dos materiais semelhantes ao MTA resulta em difícil espatulação e inserção (Canadas *et al.* 2014). Considerando a importância da capacidade de manipulação que os cimentos reparadores devem apresentar (Duarte *et al.* 2010), foram desenvolvidos cimentos de alta plasticidade com o objetivo de melhorar essas características.

O MTA Flow (Ultradent Products Inc, South Jordan, UT) consiste em um material reparador composto por silicato tricálcico e dicálcico, óxido de bismuto como radiopacificante e um veículo líquido composto por um gel à base de água. De acordo com o fabricante, o gel proporciona uma maior plasticidade, melhorando o manuseio e inserção, o que pode facilitar o uso em diversos procedimentos: uma consistência espessa para pulpotomia ou perfuração da câmara pulpar; uma consistência fina para reabsorção, apicificação e plug apical; ou uma consistência de massa para o preenchimento da porção apical da raiz em rizogênese incompleta ou retrobturações (EndoEze MTA Flow Gel Safety Data Sheet 2015, Ultradent Products and Procedures Manual 2017). Estudos físico-químicos com MTA Flow mostraram radiopacidade adequada, baixa solubilidade, capacidade de alcalinização e de formar fosfato de cálcio (Guimarães *et al.* 2017).

A introdução de instrumentos e materiais para uso odontológico devem ser acompanhadas de pesquisas para assegurar e indicar seu uso, além de apontar possíveis melhorias. Instrumentos endodônticos para utilização automatizada devem passar por diversos testes, principalmente envolvendo testes de fadiga do metal (cíclica e torcional), com a finalidade de se obter dados adicionais para uso clínico seguro. A fabricação em níquel-titânio de insertos ultrassônicos tende a aumentar sua resistência frente a fraturas, porém estudos são necessários para avaliar se a liga de NiTi também potencializa a ação dos irrigantes, uma vez que os insertos convencionais são fabricados em aço inoxidável. Quanto à resposta biológica de materiais reparadores, a apresentação em diferentes consistências do MTA Flow, associado às propriedades físico-químicos favoráveis, indicam esse material como alternativa clínica. Porém, ainda há a necessidade de estudos *in vivo* que forneçam dados sobre a reação tecidual após contato com esse material.

II. Objetivos



II. Objetivos

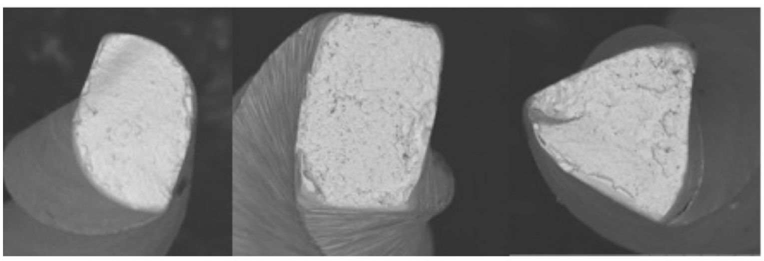
Os objetivos deste trabalho foram divididos em três capítulos que resultaram em artigos distintos:

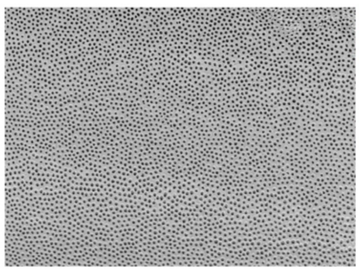
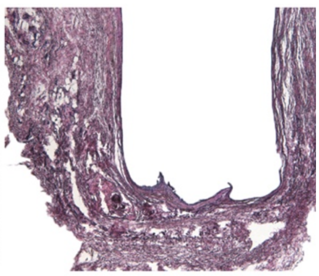
1- Avaliar a resistência à fadiga cíclica dos instrumentos reciprocantes de níquel titânio Genius 25.04, 30.04 e EdgeFile X1 Small comparados com WaveOne Gold Primary;
2- Avaliar em MEV a capacidade de limpeza, por meio da remoção da *smear layer*, de um inserto ultrassônico de níquel-titânio em ativação ultrassônica passiva e contínua da irrigação (PUI e CUI), comparados com irrigação convencional.
3- Avaliar *in vivo* a biocompatibilidade e capacidade de biomineralização do Agregado de Trióxido Mineral Flow (MTA Flow), utilizando como controle positivo o ProRoot MTA e o MTA Angelus, analisando:

- Infiltrado inflamatório e cápsula fibrosa (coloração de HE);
- Estruturas mineralizadas (coloração de Von Kossa);
- Estruturas birrefrigentes sob a luz polarizada (sem coloração).

III. Artigo 1

Avaliação da resistência à fadiga cíclica dos instrumentos reciprocantes de níquel-titânio Genius e EdgeFile



Cyclic fatigue resistance of novel Genius and Edgefile nickel-titanium reciprocating instruments

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Abstract: This study aimed to assess the cyclic fatigue resistance of Genius and EdgeFile X1 reciprocating instruments compared with WaveOne Gold Primary. Twenty Genius (Ultradent) 25.04, 20 Genius 30.04, 20 EdgeFile X1 (EdgeEndo) and 20 WaveOne Gold Primary (Dentsply Maillefer) instruments were included in this study and tested in a static cyclic fatigue testing device, which has an artificial stainless steel canal with a 60° angle of curvature and a 5-mm radius of curvature. All instruments were operated in reciprocation mode until fracture occurred. The number of cycles to failure (NCF) was calculated and time to fracture (TF) was recorded in seconds using a digital chronometer. The mean and standard deviations of NCF and TF were calculated for each reciprocating system and the data were subjected to Kruskal-Wallis one-way analysis of variance and to Dunn's test ($p < .05$) using SigmaPlot software (Systat software, CA, USA). The fractured surfaces of five instruments from each brand were randomly examined and microphotographed by a low-vacuum environmental scanning electron microscopy – SEM (Tabletop Microscope TM3030, Hitachi, Japan) to confirm the cyclic fatigue fracture. EdgeFile exhibited the highest cyclic fatigue resistance, followed by both Genius files ($p < .05$). Within the limitations of this *in vitro* study, EdgeFile X1 instruments had significantly higher cyclic fatigue resistance than did Genius and WaveOne Gold Primary instruments. The cyclic fatigue resistance of both Genius files was higher than that of WaveOne Gold Primary.

Keywords: Dental Instruments; Endodontics; Root Canal Preparation.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Introduction

Endodontic instruments manufactured with nickel-titanium (NiTi) were introduced by Walia et al.[1] to improve the instrumentation of curved canals, once NiTi instruments are more flexible and have superior resistance to torsional fracture compared with the rigidity of stainless steel instruments, reducing canal transportation.[2,3] In addition, operator fatigue, procedural errors, and length of root canal treatment[4] are reduced, improving the quality of endodontic therapy for both the professional and the patient.

Despite the advantages of NiTi, fracture incidence is the most common failure that occurs during its use[5] as a result of flexural or torsional strength, or even a combination of both,[6] without any visible indication of plastic deformation.[7] Therefore, the manufacturers recommend limited or even single-time use of NiTi instruments, especially in curved canals.

The metal fatigue caused by repetitive stressing cycling is an important failure mechanism.[8] In this fracture type, also known as cyclic fatigue, NiTi instruments undergo tension and compression forces in the area of maximum canal curvature, until fracture.[9] Several studies have shown that reciprocating motion enhances cyclic fatigue resistance compared to rotation motion,[10,11,12] making reciprocating instruments safer than rotary ones for root canal shaping.

WaveOne Gold (Dentsply Maillefer, USA) is available in the market as a single-file technique, with Small (20.07), Primary (25.07), Medium (35.06), and Large (45.05) instruments as recent versions of WaveOne (Dentsply Maillefer). It is manufactured with a gold heat treatment procedure, which is performed by heating the file and then cooling it slowly, in contrast to the pre-manufacturing heat treatment of M-Wire technology. The reciprocating motion of WaveOne was maintained, with a cutting action at 150° CCW and disengagement at 30° in the CW direction, but the file dimensions and geometry differ from the original WaveOne, featuring an off-center design with cutting edges and alternate 1-point contact in a parallelogram design. Also, Wave One Gold instruments have a different taper in the same instrument, as WOG Primary starts with a 25.07 tip, decreasing to .06 after 4 mm and to .03 after another 4 mm.[13,14]

The Genius system (Ultradent, South Jordan, UT) has been recently developed to associate rotary and reciprocating techniques, with thermally treated NiTi alloy and a .04 taper, improving resistance and flexibility, as announced by the manufacturer. Rotary motion is indicated to enlarge the cervical third, and two reciprocating files are used to prepare the root canal, with 90° of cutting action (CW) and 30° of release (CCW). The instrumentation finishes with a 360° rotation, removing the debris from the canal. The files have an S-shaped cross-section design with double right positive cutting action and, according to the manufacturer, the progressive pitch of the file during instrumentation prevents the "screw-in" effect.[15]

The EdgeEndo company (Albuquerque, USA) has launched several automated instruments such as reciprocating (X1), rotary (X3, X5 and X7), or retreatment (XR) endodontic files. EdgeFile X1 (EdgeEndo) features a constant .06 tapered reciprocating instrument with a triangular cross-section and annealed heat-treated nickel-titanium alloy (Fire-Wire™), which according to manufacturer, increases flexural strength, enhancing durability and flexibility. The files must be used in the WaveOne® motor setting, therefore with the same motion of 150° (CCW) cutting action and 30° (CW) release, as recommended by the manufacturer.[16]

To date, there have been no studies on the cyclic fatigue resistance of reciprocating Genius and EdgeFile systems in curved canals. Therefore, the present study aimed to compare the cyclic fatigue resistance of Genius and EdgeFile reciprocating files with WaveOne Gold Primary instruments. The null hypothesis was that there would be no significant difference in the cyclic fatigue resistance of the instruments.

Methodology

In this study, 80 files were selected (n=20), as previously used for cyclic fatigue tests,[17,18,19,20] as follows: 20 Genius (25.04), 20 Genius (30.04), 20 WaveOne Gold Primary (25.07), and 20 EdgeFile X1 (20.06) files. To measure the cyclic fatigue resistance of the files, an artificial canal, made of stainless steel with an inner diameter of 1.5 mm, a 60° angle of curvature, and a curvature radius of 5 mm, was used. The curvature of the artificial canal was located at the 5-mm coronal end of the canal.[21,22] To reduce the friction of the files as they contacted the artificial walls of the canal, a synthetic oil (WD-40 Company, Milton Keynes, UK) was used for lubrication.[22] All cyclic tests were performed at room temperature (20° ± 1°C).

The files were divided into four experimental groups (n = 20) and underwent the following procedures:

a. Group 1: Genius 25.04: The files were used with the Genius reciprocating & rotary motor (Ultradent Products, Inc, South Jordan, USA) connected to a cyclic fatigue testing instrument and operated at 350 rpm, set to the genius files in reciprocation mode, with 90° of cutting action (CW) and 30° of release (CCW), until they fractured.

b. Group 2: Genius 30.04: The files were used with the Genius reciprocating & rotary motor (Ultradent Produtcs, Inc, South Jordan, USA) connected to a cyclic fatigue testing instrument and operated at 350 rpm, set to the genius files in reciprocation mode, with 90º of cutting action (CW) and 30º of release (CCW), until they fractured.

c. Group 3: WaveOne Gold Primary: The files were used with the E3 Torque Control Motor (Dentsply Tulsa Dental Specialties, USA) connected to the cyclic fatigue testing instrument and operated at 350 rpm with the "WaveOne ALL" program until they fractured.

d. Group 4: EdgeFile X1: The files were used with the E3 Torque Control Motor (Dentsply Tulsa Dental Specialties, USA) connected to the cyclic fatigue testing instrument and operated at 350 rpm with the "WaveOne ALL" program, as recommended by the manufacturer, until they fractured.

All the instruments were reciprocating and time from motor activation was recorded and stopped as soon as a fracture was detected visually and/or audibly on a digital chronometer. The number of cycles to failure (NCF) of each file was then calculated using the following formula: NCF = revolution per minute (rpm) X time (seconds)/60. The lengths of the fractured segments were measured by a digital caliper (Mitutoyo, Absolute Digimatic, Japan).

The fractured surfaces of five instruments from each brand, randomly selected after the cyclic fatigue test, were examined by a low-vacuum environmental scanning electron microscopy – SEM (Tabletop Microscope TM3030, Hitachi, Japan), in order to confirm that the files fractured because of the cyclic fatigue,[17,21] and photomicrographs of the fractured surfaces were taken, as shown in Figure 1. Before SEM evaluation, the instruments were ultrasonically cleaned to remove any debris.[22]

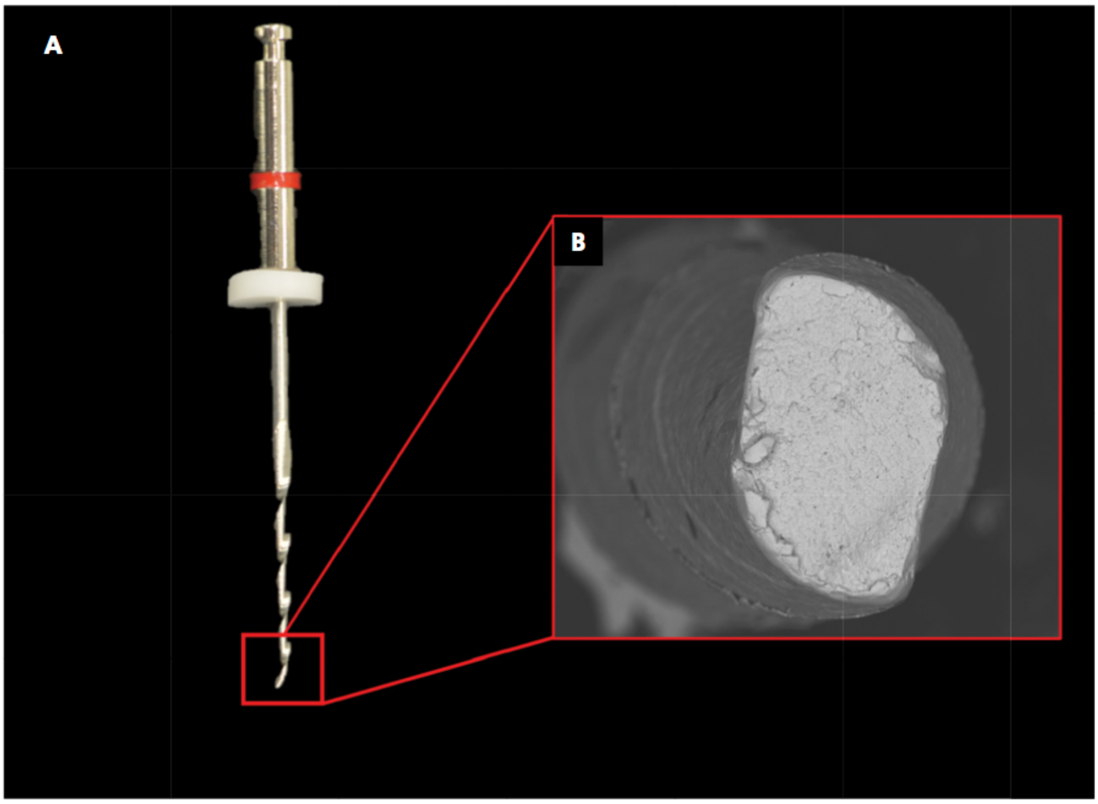

Figure 1. Illustrative example of a Genius 25.04 broken file after reciprocation inside the simulated canal, evidencing the area of analysis (A) and a SEM image (B), showing the analyzed inspection area of a cyclic fatigue ductile fracture.

Statistical analyses

The data were first analyzed using the Shapiro-Wilk test to verify the assumption of normality. The Kruskal-Wallis and Dunn's tests were performed using SigmaPlot software (Systat Software, San Jose, USA) to statistically analyze the data. The statistical significance level was set at $p < .05$.

Results

The means and standard deviations of the NCF, time to fracture, and the lengths of fractured segments are shown in Table. EdgeFile X1 had the highest fatigue resistance (NCF: 6175.74 ± 1608.99; TF: 1058.7 ± 275.82) while Wave One Gold Primary had the lowest fatigue resistance (NCF: 881.7 ± 108.04; TF: 151.15 ± 18.52) with a statistical difference ($p < .05$). The fatigue resistance of both Genius 25.04 (NCF: 1217.12 ± 230.36; TF: 208.65 ± 39.49) and 30.04 (NCF: 1365.58 ± 224.81; TF: 234.10 ± 38.56) was statistically higher than that of Wave One Gold Primary (881.7 ± 108.04) ($p < 0.05$), but presented no significant difference between the Genius instruments ($p > 0.05$).

The mean lengths of fractured segments were recorded in order to evaluate the correct positioning of the tested files inside the canal curvature, since each group maintained the approximate segment length. There was a statistically significant difference ($p < .05$) in the mean length of the fractured fragments among all instruments, except for Genius 25.04 and EdgeFile X1 (Table).

SEM evaluation

Scanning electron microscopy images of the fractured surface showed typical features of cyclic fatigue failure for all instruments. All the instruments presented fractured surfaces with microvoids, a mechanical characteristic of ductile fracture (Figure 2).

Discussion

Since the introduction of NiTi instruments in endodontics by Walia et al.[1] in the 1980s, manufacturers have been improving the manufacturing process, aiming to enhance the mechanical properties of the alloy and decreasing the failures (broken files) observed during the endodontic treatment.[23] Nowadays, NiTi endodontic files are classified into instruments containing mainly the austenite phase, such as conventional NiTi, M-Wire, or R-Phase, and those containing mainly the martensite phase, such as CM Wire, Gold, and Blue (thermally treated). The instruments in the austenite phase show superelastic properties and high torque values, indicated for shaping straight or slightly curved canals or even as pathfinding instruments because of the smaller diameter. Martensitic instruments are more flexible, used in root canals with severe or double curvature, in addition to being prebendable.[24]

However, several factors can affect the resistance of NiTi endodontic instruments, such as the manufacturing process, metallurgical design, instrument size, taper, helix angle, cross-sectional design, core diameter, file kinematics, heat treatment applied to the file,[25,26,27] or even the temperature to which the instrument is subjected.[20,28,29]

Many cyclic fatigue test devices may be used with static or dynamic test models. In this study, the model used for the cyclic fatigue test was static, in which an instrument-fixed working length is bended and then rotated until fracture occurs with no axial movement,

Table. Mean and standard deviations of the number of cycles to failure (NCF), time to fracture (seconds) and the length of the fractured fragment (mm) of the tested files

Group	NFC	TF (s)	Fractured Length (mm)
Genius 25.04	1217.12 ± 230.36 [a]	208.65 ± 39.49 [a]	3.14 ± 0.18 [a]
Genius 30.04	1365.58 ± 224.81 [a]	234.10 ± 38.56 [a]	3.86 ± 0.54 [b]
Wave One Gold Primary	881.7 ± 108.04 [b]	151.15 ± 18.52 [b]	2.37 ± 0.50 [c]
EdgeFile X1	6175.74 ± 1608.99 [c]	1058.7 ± 275.82 [c]	3.02 ± 0.45 [a]

Different superscript letters indicate statistical significance ($p < .05$).

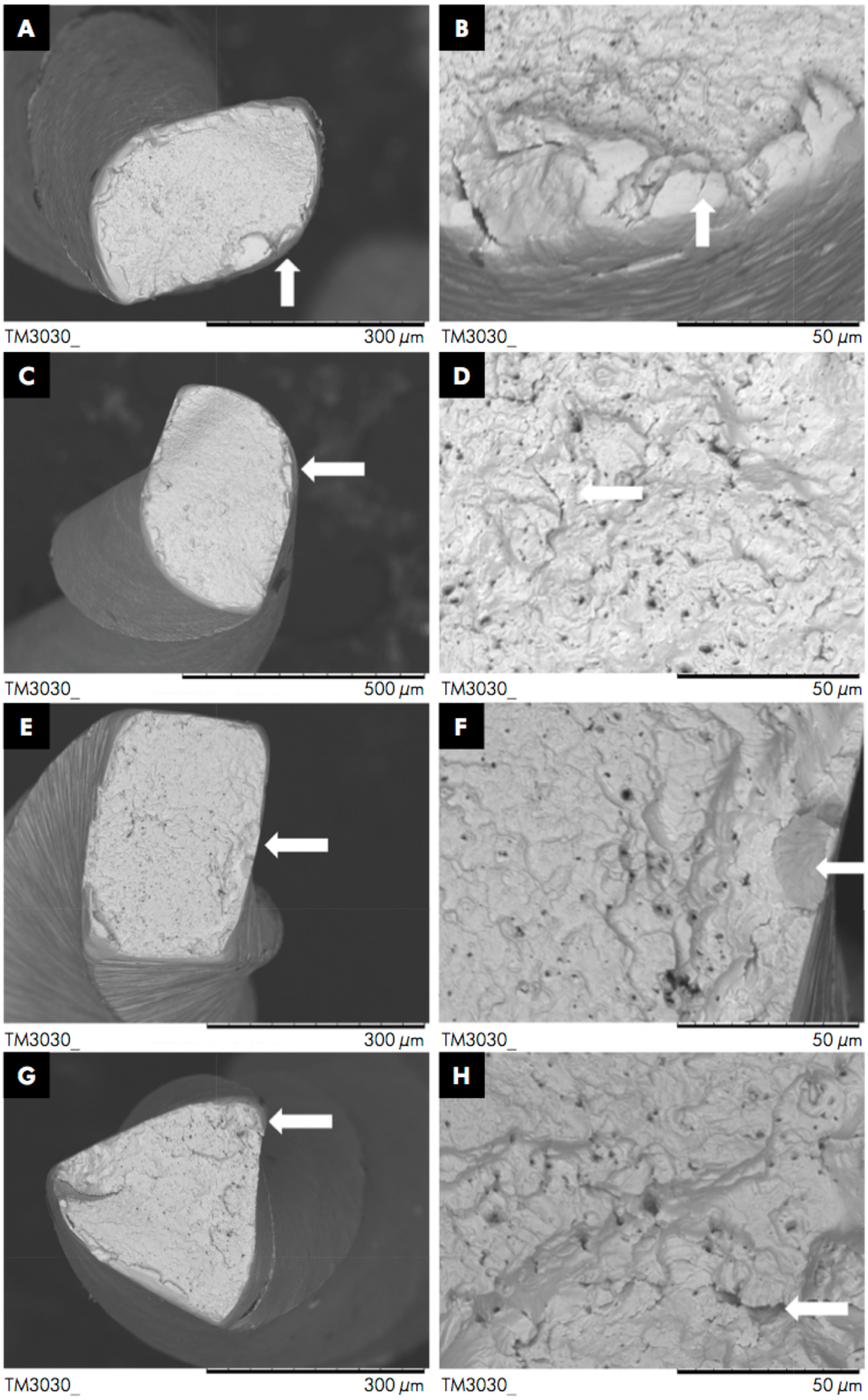

Figure 2. SEM Images after the fatigue cyclic test of Genius, WaveOne Gold Primary, and EdgeFile X1 instruments. General view of (A) Genius 25.04, (C) Genius 30.04, (E) WaveOne Gold Primary, and (G) EdgeFile X1 and high-magnification view of (B) Genius 25.04, (D) Genius 30.04, (F) WaveOne Gold Primary, and (H) EdgeFile X1 instruments, showing numerous dimples and fatigue striations, typical features of cyclic fatigue (white arrows).

allowing for a precise trajectory in the simulated artificial canal in a stainless steel block, as used in previous studies.[10,17,22,30,31] The advantages of the static model consist mainly in reducing variables, such as the amplitude of axial movements.[10,20,31]

With respect to the temperature of the *in vitro* test, the room temperature (20° ± 1 °C) was previously used to perform the cyclic fatigue test.[19,21,32,33] However, recent findings have shown that NiTi martensite instruments operating at body temperature (37°C) show a significant decrease in flexural resistance and consequently in NCF, since heating may induce a transition to a stiffer austenite phase, which is more susceptible to fatigue failure.[20,28,29,34]

The present study compared the cyclic fatigue resistance of the reciprocating Genius File and EdgeFile with WaveOne Gold. According to our results, the cyclic fatigue resistance of both Genius and EdgeFile systems was higher than that of WaveOne Gold, evidencing the highest fatigue resistance for EdgeFile. Thus, the null hypothesis of the present study was rejected.

This is the first study on the cyclic fatigue of a reciprocating EdgeFile instrument. A recent study has shown that reciprocating EdgeFile X1 induces low transportation, has a high centering ability,[35] and shows similar shaping ability to iRaCe and XP-endo Shaper.[36] According to the manufacturer, the annealed heat-treated Firewire NiTi increases flexibility, along with its 6% taper and a hyperbolic cross section.[16] The association of a thermally treated alloy with a low core mass (taper .06) may explain the results obtained in this study. The elevated values for cyclic fatigue is in accordance with a previous study on a rotary EdgeFile X7, which presented the highest NCF values at different temperatures.[27] Additionally, another research study on rotary EdgeFiles X7 demonstrated that CM heat treatment has higher fatigue resistance than instruments without thermal or Fire-wire treatment.[37]

Supporting the findings of the present study, previous research studies have reported that the resistance to cyclic fatigue of S-shaped endodontic files with two cutting edges (*e.g.*, Reciproc files) was greater than that of WaveOne files,[38,39,40] attributing their findings to the different cross sections of the files. Cheung et al.[41] also reported, through finite elemental analysis, that NiTi instruments with an S-shaped or triangular cross-section present better fatigue resistance than does a square or rectangular cross section.

Grande et al.[42] compared the cyclic fatigue resistance of two different file cross sections: S-shaped Mtwo NiTi files (VDW) and the convex triangular ProTaper Universal files (Dentsply Maillefer). They found that Mtwo files, which have a smaller core mass, were more resistant to cyclic fatigue, in accordance with later findings, showing that files with a greater metal core mass have lower fracture resistance.[29] There is no consensus about cross-sectional shape on cyclic fatigue resistance, but previous studies have indicated the dimension of the cross-sectional area as the most important factor in cyclic fatigue resistance than the type of alloy.[8,40,41]

The curvature of the artificial canal was located 5-mm from the coronal end. Considering the taper of each file, the diameter at 5 mm from the tip (D5), which would be at the center of the curvature, shows different values. Wave One Gold has diameter 59 at D5, whereas Genius 25 is a 45, Genius 30 is a 50 and EdgeFile is also a 50. Therefore, the greater taper and, consequently, the large core mass of Wave One Gold at D5, associated with the parallelogram cross section, may have influenced the low values on the cyclic fatigue test. Corroborating previous results and the findings of the present study, we suggest that, although the Genius File does not undergo heat treatment in the martensite phase, its higher cyclic fatigue resistance compared with WaveOne Gold might be because of the reduced taper and therefore a low core mass, explaining its superior performance, since cyclic fatigue decreases when file diameters are increased.[45] In recent years, manufacturers have tended to produce lower taper files to ensure a more conservatively shaped canal. Lower taper files could enhance the fatigue resistance of NiTi endodontic files.[45]

Another major aspect is the difference in the reciprocating angle recommended by each manufacturer, which might explain the high resistance, since decreasing the reciprocation range of instruments results in an increased cyclic fatigue resistance.[46] A previous study regarding different reciprocation angles found that 90º CW-30º CCW was safer for enhancing instrument resistance to fatigue failure, when compared to instruments operating at greater angles.[47] Additionally, the present study also corroborates the findings of Ozyurek et al.,[48] which showed the highest cyclic and bending resistance in double (S-shaped) curved canal for Genius files, when compared to WOG Primary and Reciproc Blue.

The fractured length of each file was at the center of the curvature or just above this point, confirming the positioning of the instruments. The SEM images showed regular fractographic appearance of cyclic fatigue with crack initiation areas and overload zones, which was similar in the four experimental groups.

Conclusions

Within the limitations of the present study, our results showed that EdgeFile X1 had significantly higher cyclic fatigue resistance. In addition, both Genius files showed higher cyclic fatigue resistance than WaveOne Gold Primary. Further research is necessary to analyze fatigue at different temperatures and with different tapers.

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References

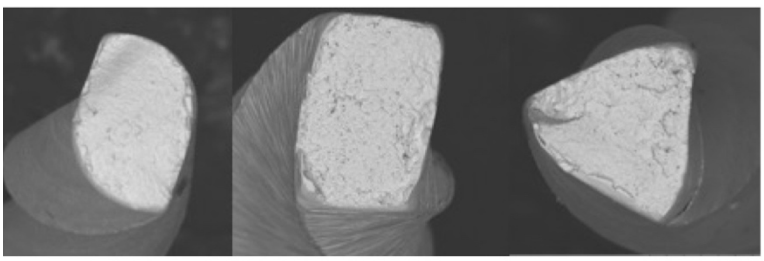
1. Walia HM, Brantley WA, Gerstein H. An initial investigation of the bending and torsional properties of Nitinol root canal files. J Endod. 1988 Jul;14(7):346-51. https://doi.org/10.1016/S0099-2399(88)80196-1
2. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod. 2004 Aug;30(8):559-67. https://doi.org/10.1097/01.DON.0000129039.59003.9D
3. Gergi R, Rjeily JA, Sader J, Naaman A. Comparison of canal transportation and centering ability of twisted files, Pathfile-ProTaper system, and stainless steel hand K-files by using computed tomography. J Endod. 2010 May;36(5):904-7. https://doi.org/10.1016/j.joen.2009.12.038
4. El Batouty KM, Elmallah WE. Comparison of canal transportation and changes in canal curvature of two nickel-titanium rotary instruments. J Endod. 2011 Sep;37(9):1290-2. https://doi.org/10.1016/j.joen.2011.05.024
5. Spanaki-Voreadi AP, Kerezoudis NP, Zinelis S. Failure mechanism of ProTaper Ni-Ti rotary instruments during clinical use: fractographic analysis. Int Endod J. 2006 Mar;39(3):171-8. https://doi.org/10.1111/j.1365-2591.2006.01065.x
6. Wei X, Ling J, Jiang J, Huang X, Liu L. Modes of failure of ProTaper nickel-titanium rotary instruments after clinical use. J Endod. 2007 Mar;33(3):276-9. https://doi.org/10.1016/j.joen.2006.10.012
7. Tripi TR, Bonaccorso A, Tripi V, Condorelli GG, Rapisarda E. Defects in GT rotary instruments after use: an SEM study. J Endod. 2001 Dec;27(12):782-5. https://doi.org/10.1097/00004770-200112000-00018 PMID:11771591
8. Pruett JP, Clement DJ, Carnes DL Jr. Cyclic fatigue testing of nickel-titanium endodontic instruments. J Endod. 1997 Feb;23(2):77-85. https://doi.org/10.1016/S0099-2399(97)80250-6
9. Cheung GS, Darvell BW. Fatigue testing of a NiTi rotary instrument. Part 2: fractographic analysis. Int Endod J. 2007 Aug;40(8):619-25. https://doi.org/10.1111/j.1365-2591.2007.01256.x
10. Lopes HP, Elias CN, Vieira MV, Siqueira JF Jr, Mangelli M, Lopes WS, et al. Fatigue Life of Reciproc and Mtwo instruments subjected to static and dynamic tests. J Endod. 2013 May;39(5):693-6. https://doi.org/10.1016/j.joen.2012.11.048
11. Pedullà E, Franciosi G, Ounsi HF, Tricarico M, Rapisarda E, Grandini S. Cyclic fatigue resistance of nickel-titanium instruments after immersion in irrigant solutions with or without surfactants. J Endod. 2014 Aug;40(8):1245-9. https://doi.org/10.1016/j.joen.2014.02.005
12. Dagna A, Poggio C, Beltrami R, Colombo M, Chiesa M, Bianchi S. Cyclic fatigue resistance of OneShape, Reciproc, and WaveOne: an in vitro comparative study. J Conserv Dent. 2014 May;17(3):250-4. https://doi.org/10.4103/0972-0707.131788
13. Webber J. Shaping canals with confidence: WaveOne GOLD single-file reciprocating system. Int Dent African Ed. 2015;6(3):6-17.
14. Dentsply Sirona. WaveOne Gold. York, 2018 [cited 2018 Nov]. Available from: https://www.dentsplysirona.com/en-us/products/endodontics/waveone-gold.html#waveonegoldfiles
15. Ultradent Products. Endo-Eze™ Genius® System. South Jordan, 2018 [cited 2018 Nov]. Avaliable from. https://www.ultradent.com/en-us/Dental-Products-Supplies/Endodontics/Instrumentation/endo-eze-genius-motor-contra-angle-and-files-system/Pages/default.aspx

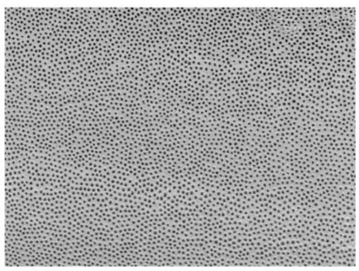
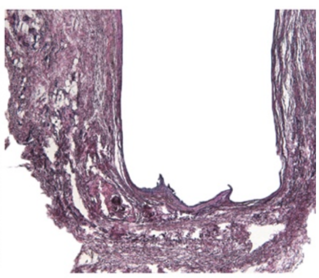
16. EdgeEndo™. EdgeFile® X-1 Heat Treated Fire-Wire™ NiTi Reciprocating Files. Albuquerque, 2018 [cited 2018 Nov]. Avaliable from: https://edgeendo.com/wp-content/uploads/2015/08/DFU-EdgeFile-x1.pdf
17. Özyürek T. Cyclic fatigue resistance of reciproc, waveone, and waveone gold nickel-titanium instruments. J Endod. 2016 Oct;42(10):1536-9. https://doi.org/10.1016/j.joen.2016.06.019
18. Özyürek T, Uslu G, İnan U. A Comparison of the cyclic fatigue resistance of used and new glide path files. J Endod. 2017 Mar;43(3):477-80. https://doi.org/10.1016/j.joen.2016.10.044
19. Alcalde MP, Duarte MA, Bramante CM, de Vasconselos BC, Tanomaru-Filho M, Guerreiro-Tanomaru JM, et al. Cyclic fatigue and torsional strength of three different thermally treated reciprocating nickel-titanium instruments. Clin Oral Investig. 2018 May;22(4):1865-71. https://doi.org/10.1007/s00784-017-2295-8
20. Klymus ME, Alcalde MP, Vivan RR, Só MV, Vasconselos BC, Duarte MA. Effect of temperature on the cyclic fatigue resistance of thermally treated reciprocating instruments. Clin Oral Investig. 2018 Nov;(Nov):1-6. https://doi.org/10.1007/s00784-018-2718-1
21. Gündoğar M, Özyürek T. Cyclic fatigue resistance of oneshape, HyFlex EDM, WaveOne Gold, and reciproc blue nickel-titanium instruments. J Endod. 2017 Jul;43(7):1192-6. https://doi.org/10.1016/j.joen.2017.03.009
22. Alcalde MP, Tanomaru-Filho M, Bramante CM, Duarte MA, Guerreiro-Tanomaru JM, Camilo-Pinto J, et al. Cyclic and torsional fatigue resistance of reciprocating single files manufactured by different nickel-titanium alloys. J Endod. 2017 Jul;43(7):1186-91. https://doi.org/10.1016/j.joen.2017.03.008
23. Gambarini G, Grande NM, Plotino G, Somma F, Garala M, De Luca M, et al. Fatigue resistance of engine-driven rotary nickel-titanium instruments produced by new manufacturing methods. J Endod. 2008 Aug;34(8):1003-5. https://doi.org/10.1016/j.joen.2008.05.007
24. Zupanc J, Vahdat-Pajouh N, Schäfer E. New thermomechanically treated NiTi alloys - a review. Int Endod J. 2018 Oct;51(10):1088-103. https://doi.org/10.1111/iej.12924
25. Lopes HP, Gambarra-Soares T, Elias CN, Siqueira JF Jr, Inojosa IF, Lopes WS, et al. Comparison of the mechanical properties of rotary instruments made of conventional nickel-titanium wire, M-wire, or nickel-titanium alloy in R-phase. J Endod. 2013 Apr;39(4):516-20. https://doi.org/10.1016/j.joen.2012.12.006
26. Pereira ES, Peixoto IF, Viana AC, Oliveira II, Gonzalez BM, Buono VT, et al. Physical and mechanical properties of a thermomechanically treated NiTi wire used in the manufacture of rotary endodontic instruments. Int Endod J. 2012 May;45(5):469-74. https://doi.org/10.1111/j.1365-2591.2011.01998.x
27. Turpin YL, Chagneau F, Vulcain JM. Impact of two theoretical cross-sections on torsional and bending stresses of nickel-titanium root canal instrument models. J Endod. 2000 Jul;26(7):414-7. https://doi.org/10.1097/00004770-200007000-00009
28. Dosanjh A, Paurazas S, Askar M. The effect of temperature on cyclic fatigue of nickel-titanium rotary endodontic instruments. J Endod. 2017 May;43(5):823-6. https://doi.org/10.1016/j.joen.2016.12.026
29. Jamleh A, Yahata Y, Ebihara A, Atmeh AR, Bakhsh T, Suda H. Performance of NiTi endodontic instrument under different temperatures. Odontology. 2016 Sep;104(3):324-8. https://doi.org/10.1007/s10266-015-0214-5
30. Almeida-Gomes F, de Matos HR, Nunes RF, Arrais AM, Ferreira-Maniglia C, de Morais Vitoriano M, et al. Cyclic fatigue resistance of different continuous rotation and reciprocating endodontic systems. Indian J Dent Res. 2016 May-Jun;27(3):278-82. https://doi.org/10.4103/0970-9290.186244
31. Wan J, Rasimick BJ, Musikant BL, Deutsch AS. A comparison of cyclic fatigue resistance in reciprocating and rotary nickel-titanium instruments. Aust Endod J. 2011 Dec;37(3):122-7. https://doi.org/10.1111/j.1747-4477.2010.00222.x
32. De-Deus G, Silva EJ, Vieira VT, Belladonna FG, Elias CN, Plotino G, et al. Blue thermomechanical treatment optimizes fatigue resistance and flexibility of the Reciproc files. J Endod. 2017 Mar;43(3):462-6. https://doi.org/10.1016/j.joen.2016.10.039
33. Topçuoğlu HS, Düzgün S, Aktı A, Topçuoğlu G. Laboratory comparison of cyclic fatigue resistance of WaveOne Gold, Reciproc and WaveOne files in canals with a double curvature. Int Endod J. 2017 Jul;50(7):713-7. https://doi.org/10.1111/iej.12674
34. Vasconcelos RA, Murphy S, Carvalho CA, Govindjee RG, Govindjee S, Peters OA. Evidence for reduced fatigue resistance of contemporary rotary instruments exposed to body temperature. J Endod. 2016 May;42(5):782-7. https://doi.org/10.1016/j.joen.2016.01.025
35. Hasheminia SM, Farhad A, Sheikhi M, Soltani P, Hendi SS, Ahmadi M. Cone-beam computed tomographic analysis of canal transportation and centering ability of single-file systems. J Endod. 2018 Dec;44(12):1788-91.
36. Versiani MA, Carvalho KK, Mazzi-Chaves JF, Sousa-Neto MD. Micro-computed tomographic evaluation of the shaping ability of XP-endo Shaper, iRaCe, and EdgeFile Systems in long oval-shaped canals. J Endod. 2018 Mar;44(3):489-95. https://doi.org/10.1016/j.joen.2017.09.008 PMID:29273492
37. Tanomaru-Filho M, Galletti Espir C, Carolina Venção A, Macedo-Serrano N, Camilo-Pinto J, Guerreiro-Tanomaru J. Cyclic fatigue resistance of heat-treated nickel-titanium instruments. Iran Endod J. 2018;13(3):312-7.
38. Plotino G, Grande NM, Testarelli L, Gambarini G. Cyclic fatigue of Reciproc and WaveOne reciprocating instruments. Int Endod J. 2012 Jul;45(7):614-8. https://doi.org/10.1111/j.1365-2591.2012.02015.x

39. Arias A, Perez-Higueras JJ, Macorra JC. Differences in cyclic fatigue resistance at apical and coronal levels of Reciproc and WaveOne new files. J Endod. 2012 Sep;38(9):1244-8. https://doi.org/10.1016/j.joen.2012.05.022
40. Kim HC, Kwak SW, Cheung GS, Ko DH, Chung SM, Lee W. Cyclic fatigue and torsional resistance of two new nickel-titanium instruments used in reciprocation motion: reciproc versus WaveOne. J Endod. 2012 Apr;38(4):541-4. https://doi.org/10.1016/j.joen.2011.11.014
41. Cheung GS, Zhang EW, Zheng YF. A numerical method for predicting the bending fatigue life of NiTi and stainless steel root canal instruments. Int Endod J. 2011 Apr;44(4):357-61. https://doi.org/10.1111/j.1365-2591.2010.01838.x
42. Grande NM, Plotino G, Pecci R, Bedini R, Malagnino VA, Somma F. Cyclic fatigue resistance and three-dimensional analysis of instruments from two nickel-titanium rotary systems. Int Endod J. 2006 Oct;39(10):755-63. https://doi.org/10.1111/j.1365-2591.2006.01143.x
43. Pedullà E, Grande NM, Plotino G, Gambarini G, Rapisarda E. Influence of continuous or reciprocating motion on cyclic fatigue resistance of 4 different nickel-titanium rotary instruments. J Endod. 2013 Feb;39(2):258-61. https://doi.org/10.1016/j.joen.2012.10.025
44. Capar ID, Kaval ME, Ertas H, Sen BH. Comparison of the cyclic fatigue resistance of 5 different rotary pathfinding instruments made of conventional nickel-titanium wire, M-wire, and controlled memory wire. J Endod. 2015 Apr;41(4):535-8. https://doi.org/10.1016/j.joen.2014.11.008
45. Adıgüzel M, Capar ID. Comparison of cyclic fatigue resistance of WaveOne and WaveOne Gold small, primary, and large instruments. J Endod. 2017 Apr;43(4):623-7. https://doi.org/10.1016/j.joen.2016.11.021
46. Saber SD, Abu El Sadat SM. Effect of altering the reciprocation range on the fatigue life and the shaping ability of WaveOne nickel-titanium instruments. J Endod. 2013 May;39(5):685-8. https://doi.org/10.1016/j.joen.2012.12.007
47. Gambarini G, Rubini AG, Al Sudani D, Gergi R, Culla A, De Angelis F, et al. Influence of different angles of reciprocation on the cyclic fatigue of nickel-titanium endodontic instruments. J Endod. 2012 Oct;38(10):1408-11. https://doi.org/10.1016/j.joen.2012.05.019
48. Özyürek T, Gündoğar M, Yılmaz K, Uslu G. Bending resistance and cyclic fatigue life of Reciproc Blue, WaveOne Gold, and Genius files in a double (S-shaped) curved canal. J Dent Res Dent Clin Dent Prospects. 2017;11(4):241-6.

IV. Artigo 2

Avaliação da capacidade de limpeza de um inserto ultrassônico de níquel-titânio em irrigação ativada por ultrassom: Um estudo em MEV



Cleaning effectiveness of a nickel-titanium ultrasonic tip in ultrasonically activated irrigation: a SEM study

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Abstract: In endodontic treatment, regardless of the instrumentation technique, the presence of a smear layer covering contaminated dentin walls is always a concern. Thus, irrigation plays an essential role in reducing bacterial load. To enhance irrigation effectiveness, different ultrasonic activation methods and the use of different tips have been studied. This study assessed the cleaning capacity of the novel NiTi ultrasonic tip for smear layer removal using ultrasonically activated irrigation (UAI) with passive or continuous ultrasonic irrigation (PUI or CUI, respectively), compared with conventional irrigation. Forty-five single-rooted human mandibular premolars were decoronated to a standardized length of 16 mm. Instrumentation was performed using the Genius system up to size 50.04 and irrigated with 3% NaOCl. The specimens were divided into three groups (n = 15) according to the final irrigation activation technique: conventional irrigation (CI), as control group; PUI; and CUI, following the manufacturer's protocol. The samples were longitudinally cleaved and analyzed under a scanning electron microscope for smear layer removal according to a cleanliness score for the cervical, middle, and apical thirds. Data were evaluated by means of the Kruskal-Wallis and Tukey's tests, with a 5% level of significance. UAI enhanced cleaning compared to conventional irrigation, mainly at the apical third. CUI showed the best results, with statistically significant lower scores than PUI and CI ($p < 0.05$). Final irrigant activation with the NiTi tip showed better cleaning capacity than conventional irrigation. In addition, CUI resulted in better smear layer removal than PUI.

Keywords: Endodontics; Instrumentation; Dental Pulp Cavity.

Introduction

Root canal instrumentation produces a smear layer, consisting of organic and inorganic material, mainly formed by dentin, pulp tissue remnants, odontoblast processes and, in a contaminated canal, there are also bacteria on dentin walls.[1,2] The presence of a smear layer hinders penetration of antimicrobial agents and endodontic sealers into dentinal tubules,[3,4] possibly compromising the sealing between the filling material and the root canal wall, which could interfere in treatment success.[5]

Sodium hypochlorite (NaOCl) is a widely used irrigant for root canal disinfection but, when used alone, it is ineffective in smear layer removal.[6,7] The addition of 17% ethylenediaminetetraacetic acid (EDTA) to the irrigation protocol, alternating with NaOCl, has been recommended for increasing the effectiveness in smear layer removal.[7,8]

Therefore, irrigation plays an essential role in the removal of pulp tissue, debris, and microorganisms.[9,10] In the conventional irrigation technique, the needle tip should be 1–2 mm from the working length (WL);[11,12] nonetheless, the cleaning of the apical region remains uncertain without the assistance of agitation techniques.

To increase the effectiveness and dynamics of these solutions, different irrigation protocols have been investigated in association with ultrasonically activated irrigation (UAI).[13,14]

Passive ultrasonic irrigation (PUI) was described by Weller et al.[15] The protocol is based on passive insertion of a metal tip/file attached to an ultrasonic device oscillating at a frequency of 30 kHz into the canal filled with irrigant.[15,16] After activation, the instrument is surrounded by acoustic streaming, agitating the solution and enhancing debris removal from the apical region, dissociating the bacterial biofilm.[17,18]

Another ultrasonic protocol was proposed by Gutarts et al.[19] The ultrasonic tip is placed in the canal and the NaOCl keeps flowing, enabling the irrigant to be continually replaced, providing an uninterrupted supply of nascent chlorine for organic tissue dissolution.[6,19] This continuous ultrasonic irrigation (CUI) does not require irrigant replacement between ultrasonic file activations[20] and has demonstrated a significantly greater penetration of irrigant solution when used as a final rinse.[14]

A recent systematic review of activation of irrigants with ultrasonic agitation was conducted by Nagendrababu et al.,[21] emphasizing the use of the term UAI to encompass all ultrasonic activation techniques, whether they are passive or continuous.

The 23-mm nickel-titanium (NiTi) ultrasonic tips (NiTiSonic®, Ultradent Products Inc, South Jordan, Utah), recently put on the market, present a 20.02 tip made of NiTi, which, according to the manufacturer, can be used all the way along the working length, even in curved canals. This ultrasonic tip releases irrigant from its base, allowing it to flow to the tip.

Therefore, the aim of this study was to evaluate the *ex vivo* cleaning effectiveness of the new NiTi ultrasonic tip, comparing the PUI and CUI protocols with conventional syringe irrigation. The null hypothesis was that there would be no difference in cleaning effectiveness of NiTi tips between UAI and conventional irrigation.

Methodology

Root canal preparation

Forty-five single-rooted human mandibular premolars with single straight canals and fully formed roots, free from caries, cracks, endodontic treatments, restorations, and curvatures less than 30°, according to Schneider's classification,[22] were selected from donations made from a dental clinic. Mesiodistal and buccolingual radiographs were taken to confirm a single straight canal and canal space, excluding calcified root canals. The root lengths were standardized to 16 mm by decoronation, using a high-speed, water-cooled diamond disc.[14] The tooth length was obtained by introducing #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal to the point of displaying its tip at the apical foramen. The WL was obtained by subtracting 1 mm from the tooth length. To simulate clinical conditions, the apical region of each root was sealed with a layer of OpalDam Green gingival barrier (Ultradent Products Inc, South Jordan, USA), avoiding extravasation of the irrigating solutions.[23] To prevent the gingival barrier from entering the canal, a #10 K-file was inserted before the layer was applied.

Specimens were randomly divided into control group (conventional irrigation) and two experimental groups, PUI and CUI (n = 15). Root canal instrumentation was performed by the same operator, an endodontic specialist, with Genius reciprocating system (Ultradent Products Inc, South Jordan, USA), according to the manufacturer's instructions. After cervical instrumentation with orifice shaper 30.08, the canals were prepared with a shaping instrument 25.04, followed by an apical finishing file 50.04, until the working length was reached. Before cervical preparation with the orifice shaper, the canals were rinsed with 1 mL of 3% NaOCl (ChlorCid, Ultradent Products Inc, South Jordan, USA). At each file change (25.04 and 50.04), the canals were irrigated with 2 mL of 3% NaOCl by using a 5-mL syringe and 31-gauge double-side port NaviTip (Ultradent Products Inc, South Jordan, USA) calibrated to stop -2 mm from the WL, totalizing 5 mL per sample during instrumentation before the final irrigation protocol. Ultrawave XS LED piezoelectric ultrasonic device (Ultradent Products Inc, South Jordan, Utah) was used, promoting the CUI protocol by changing the water supply for the specific NaOCl bottle and the specific 18% EDTA (Ultradent Products Inc, South Jordan, USA). For PUI and CUI, the noncutting NiTi ultrasonic tip #20.02 (Figure 1) (Ultradent Products Inc, South Jordan, USA) was used all the way along the working length. Simultaneously, suction was accomplished by using a plastic cannula. Apical patency was maintained at each change of instrument by inserting #10 K-file up to the apical foramen. The groups were divided according to the final irrigation protocol.

Control group

After insertion of the last instrument, conventional irrigation with 5 mL of 3% NaOCl was performed using positive pressure irrigation. The canal was then washed and filled with 18% EDTA for 3 minutes, without agitation, totaling 5 mL; the 18% EDTA was washed out with 5 mL of 3% NaOCl. The solutions were delivered with a 31-gauge double-side port NaviTip (Ultradent Products Inc, South Jordan, USA).

PUI Group

After insertion of the last instrument, the ultrasonic device was set to power #4, as recommended by the manufacturer, and the PUI protocol was followed according to van der Sluis et al.[18] The canal was rinsed and filled with 1 mL of 3% NaOCl. The ultrasonic NiTi tip was placed 1.0 mm short

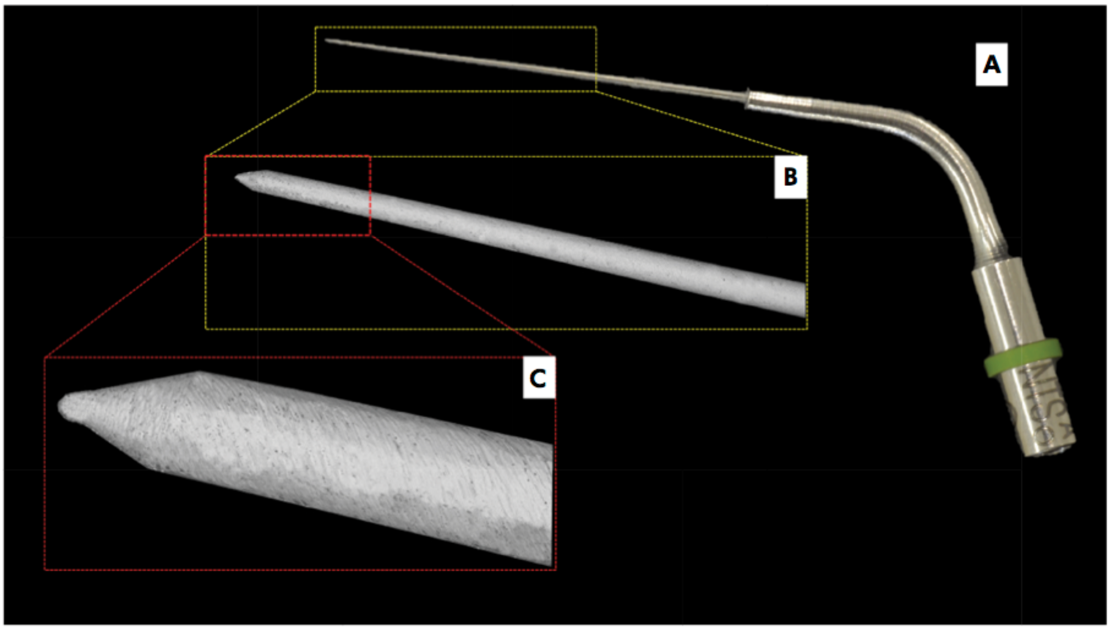

Figure 1. The ultrasonic nickel-titanium tip NiTiSonic® (A); SEM images at 30x magnification (B) and at 150x (C), evidencing the smooth surface and the noncutting tip.

of the WL and first activated with NaOCl with three cycles of 20 seconds with 2 mL of irrigant replacement before each cycle; Then, the canal was rinsed and filled with 1 mL of 18% EDTA and the activation protocol was repeated with 2 mL of solution replacement before each cycle. Lastly, a final agitation with 3% NaOCl was repeated, totaling 5 mL of irrigant during each activation.

CUI Group

After insertion of the last instrument, the water supply in the ultrasonic device was replaced with the 3% NaOCl bottle and set to power #4, as recommended by the manufacturer. The ultrasonic device was adjusted to release 5 mL for 30 seconds of activation, and CUI was performed for 30 seconds, with the tip inserted 1 mm from the WL. Then, the 3% NaOCl supply bottle was replaced with the 18% EDTA bottle, adjusted to release 5 mL for 30 seconds, and another activation was performed for 30 seconds. The 3% NaOCl bottle was reinserted for a final CUI of 5 mL for 30 seconds, totaling 5 mL of irrigant during each activation.

At the end of all procedures, all canals were irrigated with 5 mL of distilled water to remove possible salt residues from the irrigation solutions,[23] aspirated, and dried with absorbent paper points.

Specimen preparation

A low-vacuum environmental scanning electron microscope – ESEM (Tabletop Microscope TM3030, Hitachi, Japan) was used to evaluate endodontic smear layer removal from the dentin walls, avoiding the gold coating requirement, which could hinder the analysis. A gutta-percha cone with the same size of the last instrument (Genius gutta percha 50.04, Ultradent Products Inc, South Jordan, USA) was introduced into the canal.[23,24] In order to facilitate the fractures into halves, longitudinal grooves were made on the buccal and lingual external surfaces of each tooth by using double-sided diamond discs (22 mm in diameter and 0.1 mm in thickness), operated at low speed, and until the presence of the pink gutta-percha cone was seen, avoiding accidental contamination and invasion of the canal by cutting debris.[24]

After the teeth were cleaved with the aid of a chisel, one out of the two halves was selected for evaluation by SEM. Three external markings were made on this half with a fine-tip pen on the external root surface, perpendicularly to the long axis, to divide it into cervical, middle, and apical thirds of the same length. After locating the markings on the canals, the most well-defined area was chosen at a magnification of 50x.

The images were obtained at 500x using a software (TM3030 Hitachi software) and were used to evaluate smear layer removal.

SEM evaluation

Cleanliness was evaluated according to a 5-score index system, previously used[23] and codified by Hulsmann et al.,[25] which assesses the presence, quantity, and distribution of the smear layer as follows: score 1, smear layer is completely absent; most tubules are patent and debris-free; score 2, smear layer covering > 25% of the canal wall and dentinal tubules; score 3, smear layer evident in 25%–50% of the canal surface and tubules; score 4, smear layer evident in 50%–75% of the canal surface and tubules; and score 5, smear layer covering 75%–100% of the canal surface and tubules.

Statistical evaluation

The images were blindly evaluated by two observers after a calibration exercise. The kappa test was used to verify intra- and interexaminer reproducibility, in order to validate the subjective findings. The Kruskal-Wallis test was used to compare the results obtained by the scores for cleansing efficacy. Tukey's test was used for pairwise multiple comparison. All statistical calculations were performed using the SigmaPlot Software (version 12.0), with a level of significance of 5%.

Results

Kappa values of 0.90 and above were obtained, demonstrating excellent intra- and interexaminer agreement for the scores. In the SEM analysis, cleanliness was evaluated by scores for all samples

Table. Percentage of samples in each group categorized according to the 1-5 smear layer score and the median at the three analyzed canal thirds.

Variable	Score (%)					Median
Cervical	1	2	3	4	5	
Control	50	40	10	0	0	1
PUI	60	30	10	0	0	1
CUI	100*	0	0	0	0	1
Middle						
Control	10	30	25	25	10	2
PUI	45	25	10	10	10	1
CUI	75	25	0	0	0	1
Apical						
Control	10	20	10	35	25	4
PUI	10	45	10	30	5	2
CUI	60*	30	0	10	0	1

*Statistical difference.

(n = 15), and the results for the various groups are reported in Table as median score.

All groups achieved median score 1 in the cervical area. The control group had the highest scores for the middle (score 2) and apical (score 4) thirds (Table). Although the PUI group had lower smear scores than the control group, there was no statistical difference between the two groups ($p > 0.05$). The CUI group showed the lowest scores, achieving median 1 at all thirds (Table 1), with statistical difference from both control and PUI groups ($p < 0.05$). There was also a statistical difference between cleanliness at the apical and cervical thirds ($p < 0.05$) and between the middle and cervical thirds ($p < 0.05$). A representative sample from each group is shown in Figure 2.

Discussion

The present study evaluated the cleaning effectiveness of the new NiTi ultrasonic tips in extracted mandibular premolars from the apical to the cervical thirds. Based on the results, the null hypothesis was rejected because the NiTi ultrasonic tip enhanced smear layer removal during the final irrigation in both UAI protocols. The premolars used had a closed-end canal, to simulate *in vivo* conditions, in which there is possible gas entrapment inside the root canal and the foramen is sealed by the periodontal ligament and embedded in alveolar bone.[26]

According to a previous report,[27] the minimum instrumentation size needed for penetration of irrigants into the apical third of the root canal is #30; however, enlarging the canals to sizes over #40 is recommended to achieve better cleaning.[28,29] In order to standardize instrumentation, the protocol was the same for all three groups (CI, PUI, and CUI). The apical third was initially instrumented with a reciprocating file #25.04 and extended to a #50.04, in order to allow for adequate penetration of irrigants, since larger canals permit better flow of solutions.[30,31]

In the literature, there is no consensus about the efficiency of PUI in smear layer removal over conventional irrigation. Previous studies have reported that PUI enhanced canal cleaning,[23,32] but other studies have shown no difference between those methods.[24,33] It is important to observe that the PUI protocol used in those studies was different from the one used in the present research as the instrumentation protocol.

PUI followed van der Sluis protocol, according to which three agitation cycles of 20 seconds and replacement of the irrigant solution promote better cleaning, indicating the presence of a cumulative effect.[18] The efficacy of a three-step protocol with renewal of the irrigants has also been recently

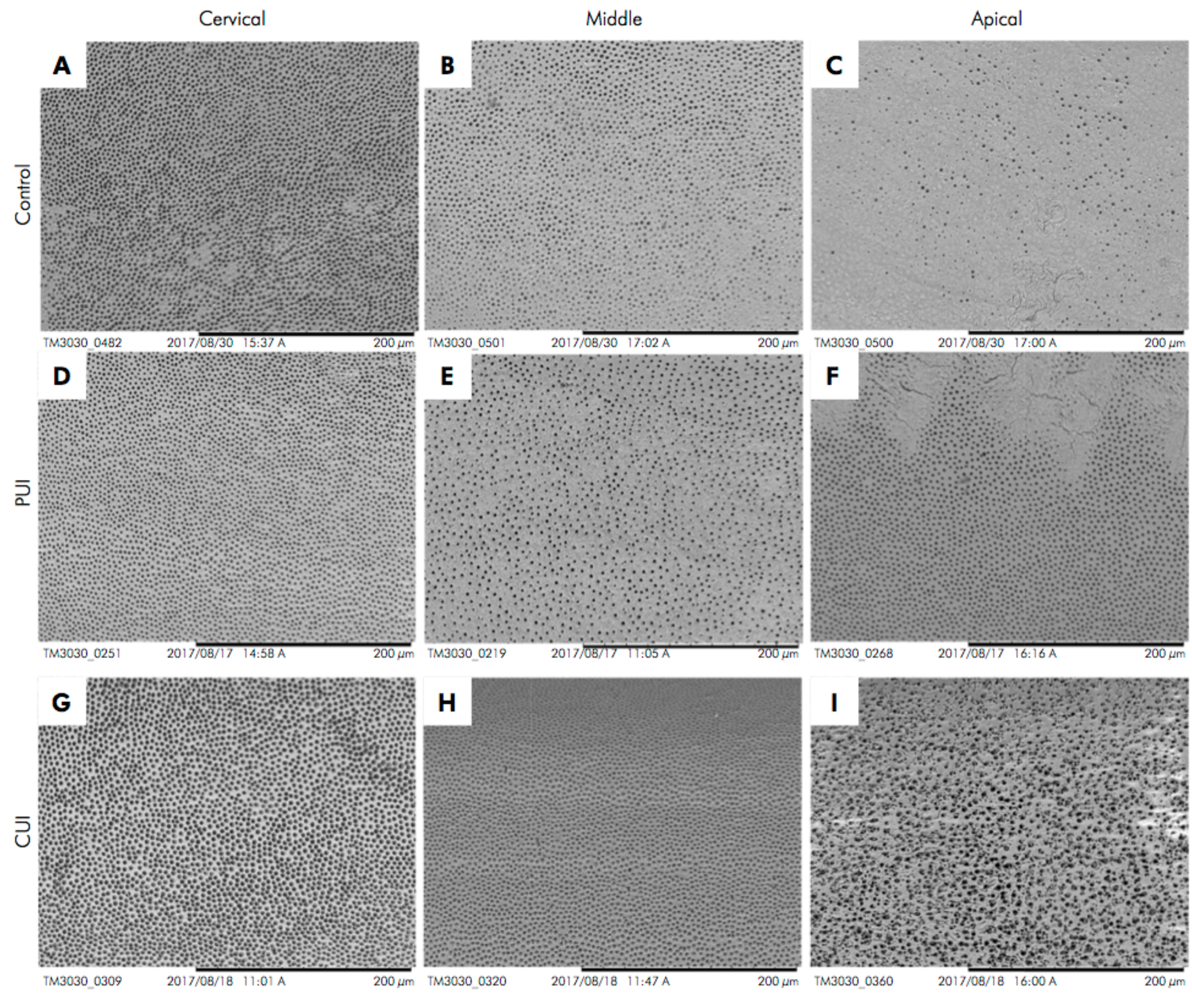

Figure 2. SEM images (500x) of representative samples from the apical, middle, and coronal thirds, illustrating the different irrigant activation techniques. Conventional irrigation (control) group (A-C) showing a clean wall with few smear plugs (arrows) at the coronal third, less open tubules with more smear layer at the middle third, and a thicker smear layer with few open tubules at the apical third, with smear layer evident in 50%–75% of the canal surface and tubules. PUI group (D–F) showing a clean wall at the coronal third, less open tubules with more smear layer plugs at the middle third, and a smear layer covering >25% of the canal wall and dentinal tubules at the apical third; CUI group (G-I) showing a highly clean canal surface with open dentinal tubules at the coronal, middle, and apical thirds, where most tubules are open and debris-free.

confirmed by Bao et al.,[34] with PUI showing a better cleaning effect than that of conventional needle irrigation. Our results showed an efficient cleaning when this protocol was used, corroborating previous findings.[34,35] These results might be ascribed to the acoustic microstreaming produced along the instrument by the ultrasonic device, leading to formation of irrigating jets, directed towards the root canal wall.[36,37,38]

CUI showed the best results regarding cleaning effectiveness. This is in accordance with previous findings reporting that CUI used as a final rinse significantly increases the penetration of the irrigating solution.[14] Ultrawave XS allows connecting bottles containing EDTA or NaOCl directly to the ultrasonic device and using the irrigant immediately for PUI or CUI. In addition, it permits a final flush with water, which cleans the equipment internally, preventing any damage to it. The EDTA concentration that is commercially available with Ultradent® bottles is 18%. This method allows the continuous replenishment of the solution and its activation by the ultrasonically energized tip, which may explain our results. The release of the irrigant in CUI was set to 5 mL in 30 seconds, using 5 mL as standard for all irrigating protocols. Therefore, to adopt the manufacturer's protocol for the NiTi tip, 18% EDTA was used in the ultrasonic protocol and in the conventional positive pressure irrigation group.

A study concerning dentin erosion found that 17% EDTA should not remain in contact with root canal walls for more than 1 minute.[39] However, Mancini et al.[23] reported that 17% EDTA for 1 minute was insufficient to completely remove the smear layer from the apical third. In our study, the time for the EDTA protocol was 3 minutes, as used in previous studies.[23, 24, 34]

Both UAI techniques were compared with each other and also with conventional irrigation. The analyzed data show that both ultrasonic techniques improved cleanliness, but the CUI technique was more effective, as evidenced by the statistical difference. A systematic review has recently demonstrated that UAI provides better root canal disinfection than other irrigation systems.[21] Our results corroborate those of previous studies, demonstrating that the use of ultrasonic irrigation improves smear layer removal in PUI,[13,23,32] but mainly in CUI.[14] Overall, the new NiTi ultrasonic tip enhanced smear layer removal in both UAI techniques, showing better results than the CI technique, as assessed by the cleanliness score.

Conclusions

Despite the limitations of this study, the new NiTi ultrasonic tip seems to improve smear layer removal, promoting better cleaning than conventional irrigation. In addition, the CUI protocol showed better results than PUI. Further studies are necessary to analyze the behavior of this tip along the WL, as far as irrigant extrusion, dentin removal, or its effectiveness in isthmuses, lateral canals, or curved roots are concerned.

Acknowledgments

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References

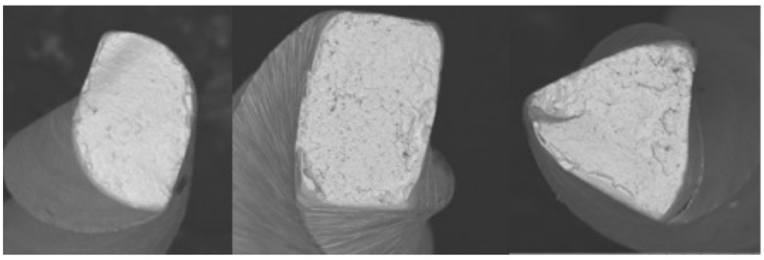
1. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod. 1975 Jul;1(7):238-42. https://doi.org/10.1016/S0099-2399(75)80226-3
2. Mader CL, Baumgartner JC, Peters DD. Scanning electron microscopic investigation of the smeared layer on root canal walls. J Endod. 1984 Oct;10(10):477-83. https://doi.org/10.1016/S0099-2399(84)80204-6
3. Goldberg F, Abramovich A. Analysis of the effect of EDTAC on the dentinal walls of the root canal. J Endod. 1977 Mar;3(3):101-5. https://doi.org/10.1016/S0099-2399(77)80203-3
4. Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol. 1990 Aug;6(4):142-9. https://doi.org/10.1111/j.1600-9657.1990.tb00409.x
5. Shahravan A, Haghdoost AA, Adl A, Rahimi H, Shadifar F. Effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis. J Endod. 2007 Feb;33(2):96-105. https://doi.org/10.1016/j.joen.2006.10.007
6. Zehnder M. Root canal irrigants. J Endod. 2006 May;32(5):389-98. https://doi.org/10.1016/j.joen.2005.09.014
7. Peters OA, Barbakow F. Effects of irrigation on debris and smear layer on canal walls prepared by two rotary techniques: a scanning electron microscopic study. J Endod. 2000 Jan;26(1):6-10. https://doi.org/10.1097/00004770-200001000-00002
8. Economides N, Liolios E, Kolokuris I, Beltes P. Long-term evaluation of the influence of smear layer removal on the sealing ability of different sealers. J Endod. 1999 Feb;25(2):123-5. https://doi.org/10.1016/S0099-2399(99)80010-7

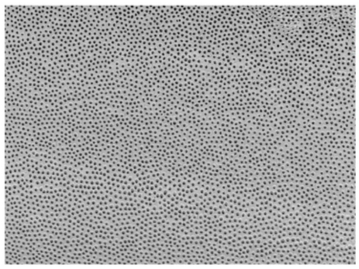
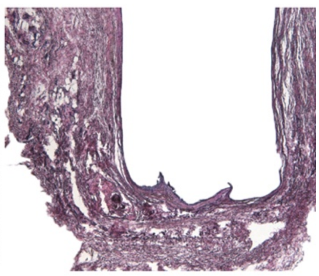
9. Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. Endod Top. 2005 Aug;10(1):77-102. https://doi.org/10.1111/j.1601-1546.2005.00135.x
10. Guerreiro-Tanomaru JM, Loiola LE, Morgental RD, Leonardo RT, Tanomaru-Filho M. Efficacy of four irrigation needles in cleaning the apical third of root canals. Braz Dent J. 2013;24(1):21-4. https://doi.org/10.1590/0103-6440201302153
11. Helvacıoğlu Kıvanç B, Deniz Arısu H, Yanar NO, Silah HM, İnam R, Görgül G. Apical extrusion of sodium hypochlorite activated with two laser systems and ultrasonics: a spectrophotometric analysis. BMC Oral Health. 2015 Jun;15(1):71. https://doi.org/10.1186/s12903-015-0056-0
12. Uzunoglu E, Görduysus M, Görduysus Ö. A comparison of different irrigation systems and gravitational effect on final extrusion of the irrigant. J Clin Exp Dent. 2015 Apr;7(2):e218-23. https://doi.org/10.4317/jced.52158
13. Lui JN, Kuah HG, Chen NN. Effect of EDTA with and without surfactants or ultrasonics on removal of smear layer. J Endod. 2007 Apr;33(4):472-5. https://doi.org/10.1016/j.joen.2006.12.007
14. Castelo-Baz P, Martín-Biedma B, Cantatore G, Ruíz-Piñón M, Bahillo J, Rivas-Mundiña B, et al. In vitro comparison of passive and continuous ultrasonic irrigation in simulated lateral canals of extracted teeth. J Endod. 2012 May;38(5):688-91. https://doi.org/10.1016/j.joen.2011.12.032
15. Weller RN, Brady JM, Bernier WE. Efficacy of ultrasonic cleaning. J Endod. 1980 Sep;6(9):740-3. https://doi.org/10.1016/S0099-2399(80)80185-3
16. Grischke J, Müller-Heine A, Hülsmann M. The effect of four different irrigation systems in the removal of a root canal sealer. Clin Oral Investig. 2014 Sep;18(7):1845-51. https://doi.org/10.1007/s00784-013-1161-6
17. Beus C, Safavi K, Stratton J, Kaufman B. Comparison of the effect of two endodontic irrigation protocols on the elimination of bacteria from root canal system: a prospective, randomized clinical trial. J Endod. 2012 Nov;38(11):1479-83. https://doi.org/10.1016/j.joen.2012.07.005
18. Sluis LW, Vogels MP, Verhaagen B, Macedo R, Wesselink PR. Study on the influence of refreshment/activation cycles and irrigants on mechanical cleaning efficiency during ultrasonic activation of the irrigant. J Endod. 2010 Apr;36(4):737-40. https://doi.org/10.1016/j.joen.2009.12.004
19. Gutarts R, Nusstein J, Reader A, Beck M. In vivo debridement efficacy of ultrasonic irrigation following hand-rotary instrumentation in human mandibular molars. J Endod. 2005 Mar;31(3):166-70. https://doi.org/10.1097/01.don.0000137651.01496.48
20. Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J. 2007 Jun;40(6):415-26. https://doi.org/10.1111/j.1365-2591.2007.01243.x
21. Nagendrababu V, Jayaraman J, Suresh A, Kalyanasundaram S, Neelakantan P. Effectiveness of ultrasonically activated irrigation on root canal disinfection: a systematic review of in vitro studies. Clin Oral Investig. 2018 Mar;22(2):655-70. https://doi.org/10.1007/s00784-018-2345-x
22. Schneider SW. A comparison of canal preparations in straight and curved root canals. Oral Surg Oral Med Oral Pathol. 1971 Aug;32(2):271-5. https://doi.org/10.1016/0030-4220(71)90230-1
23. Mancini M, Cerroni L, Iorio L, Armellin E, Conte G, Cianconi L. Smear layer removal and canal cleanliness using different irrigation systems (EndoActivator, EndoVac, and passive ultrasonic irrigation): field emission scanning electron microscopic evaluation in an in vitro study. J Endod. 2013 Nov;39(11):1456-60. https://doi.org/10.1016/j.joen.2013.07.028
24. Schmidt TF, Teixeira CS, Felippe MC, Felippe WT, Pashley DH, Bortoluzzi EA. Effect of Ultrasonic Activation of Irrigants on Smear Layer Removal. J Endod. 2015 Aug;41(8):1359-63. https://doi.org/10.1016/j.joen.2015.03.023
25. Hülsmann M, Rümmelin C, Schäfers F. Root canal cleanliness after preparation with different endodontic handpieces and hand instruments: a comparative SEM investigation. J Endod. 1997 May;23(5):301-6. https://doi.org/10.1016/S0099-2399(97)80410-4
26. Tay FR, Gu LS, Schoeffel GJ, Wimmer C, Susin L, Zhang K, et al. Effect of vapor lock on root canal debridement by using a side-vented needle for positive-pressure irrigant delivery. J Endod. 2010 Apr;36(4):745-50. https://doi.org/10.1016/j.joen.2009.11.022
27. Khademi A, Yazdizadeh M, Feizianfard M. Determination of the minimum instrumentation size for penetration of irrigants to the apical third of root canal systems. J Endod. 2006 May;32(5):417-20. https://doi.org/10.1016/j.joen.2005.11.008
28. Wu MK, Wesselink PR. Efficacy of three techniques in cleaning the apical portion of curved root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1995 Apr;79(4):492-6. https://doi.org/10.1016/S1079-2104(05)80134-9
29. Usman N, Baumgartner JC, Marshall JG. Influence of instrument size on root canal debridement. J Endod. 2004 Feb;30(2):110-2. https://doi.org/10.1097/00004770-200402000-00012
30. Khademi A, Saatchi M, Shokouhi MM, Baghaei B. Scanning electron microscopic evaluation of residual smear layer following preparation of curved root canals using hand instrumentation or two engine-driven systems. Iran Endod J. 2015;10(4):236-9.
31. Caron G, Nham K, Bronnec F, Machtou P. Effectiveness of different final irrigant activation protocols on smear layer removal in curved canals. J Endod. 2010 Aug;36(8):1361-6. https://doi.org/10.1016/j.joen.2010.03.037

32. Leoni GB, Versiani MA, Silva-Sousa YT, Bruniera JF, Pécora 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 Apr;50(4):398-406. https://doi.org/10.1111/iej.12630
33. Saber SD, Hashem AA. Efficacy of different final irrigation activation techniques on smear layer removal. J Endod. 2011 Sep;37(9):1272-5. https://doi.org/10.1016/j.joen.2011.06.007
34. 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 Feb;43(2):321-5. https://doi.org/10.1016/j.joen.2016.09.021
35. Duque JA, Duarte MA, Canali LC, Zancan RF, Vivan RR, Bernardes RA, 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 Feb;43(2):326-31. https://doi.org/10.1016/j.joen.2016.10.009
36. Jiang LM, Verhaagen B, Versluis M, Langedijk J, Wesselink P, Sluis LW. The influence of the ultrasonic intensity on the cleaning efficacy of passive ultrasonic irrigation. J Endod. 2011 May;37(5):688-92. https://doi.org/10.1016/j.joen.2011.02.004
37. Malki M, Verhaagen B, Jiang LM, Nehme W, Naaman A, Versluis M, et al. Irrigant flow beyond the insertion depth of an ultrasonically oscillating file in straight and curved root canals: visualization and cleaning efficacy. J Endod. 2012 May;38(5):657-61. https://doi.org/10.1016/j.joen.2012.02.001
38. Wagner MH, da Rosa RA, de Figueiredo JA, Duarte MA, Pereira JR, Só MV. Final irrigation protocols may affect intraradicular dentin ultrastructure. Clin Oral Investig. 2017 Sep;21(7):2173-82. https://doi.org/10.1007/s00784-016-2006-x
39. Çalt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endod. 2002 Jan;28(1):17-9. https://doi.org/10.1097/00004770-200201000-00004

V. Artigo 3

Avaliação da biocompatibilidade e biomineralização do agregado de trióxido mineral Flow.



Biocompatibility and biomineralization assessment of mineral trioxide aggregate flow

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Abstract

Objective Evaluate, in vivo, the biocompatibility via subcutaneous inflammatory tissue response and mineralization ability of the new MTA Flow compared to MTA Angelus and ProRoot MTA.

Materials and methods Forty male Wistar rats were assigned and received subcutaneous polyethylene tube implants containing the test materials and a control group with empty tube ($n = 10$ animals/group). After days 7, 15, 30, and 60, the animals were euthanized and the polyethylene tubes were removed with the surrounding tissues. Inflammatory infiltrate and thickness of the fibrous capsule were histologically evaluated. Mineralization was analyzed by Von Kossa staining and under polarized light. Data were analyzed via Kruskal-Wallis and Dunn's test with a significance level of 5%.

Results MTA Angelus induced the mildest reaction after 7 ($P > .05$) and 15 days ($P < .05$) followed by MTA Flow, both cements achieving mild inflammatory reaction after 15 days. ProRoot MTA induced a severe inflammation on day 7 and was reducing after day 15 ($P > .05$). No difference was observed after days 30 or 60 ($P > .05$). Von Kossa staining and birefringent structures were positive to all materials.

Conclusions At the end of the experiment, the novel MTA Flow showed biocompatibility and induced biomineralization in all time periods.

Clinical relevance The final consistence obtained in MTA Flow may facilitate several procedures, indicating that the MTA Flow has a promising application in endodontics.

Keywords Biocompatibility · Biomineralization · Inflammation · Mineral trioxide aggregate

Introduction

Biocompatibility and capacity to induce biomineralized tissue formation are important requirements for sealing materials that will be in contact with tissue [1].

The biocompatibility evaluation in rat model may be assessed by different methodologies such as pulpotomy [2], subcutaneous implantation [3], cell viability [4], or even in dental alveolus [5]. The implantation of these materials into the subcutaneous tissue of rats is considered a standardized [6], common, and valid test for biocompatibility [7], analyzing the inflammatory tissue response through HE staining [3–5, 8, 9] within time periods of the recommended standard practices for biological evaluation of dental materials [10].

The mineral trioxide aggregate (MTA), a Portland cement-derived calcium silicate-based hydraulic material [11], was developed to seal communication between teeth and the periodontium, in pathologic or iatrogenic root perforations [12, 13]. Numerous studies confirmed its effectiveness as a biocompatible material, inducing the formation of mineralized tissue, besides the antimicrobial activity. These excellent biological properties are attributed to its alkaline pH and calcium ion release capacity, maintaining a high pH [14–16]. The hydrophilic nature of the MTA particles allows its use even in the presence of moisture, once the main components are tricalcium and dicalcium silicate, tricalcium aluminate, tricalcium oxide, and radiopacifying agents, such as bismuth oxide [17].

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MTA Angelus (Angelus Indústria de Produtos Odontológicos S/A, Londrina, PR, Brazil) and ProRoot MTA (Tulsa Dental Products, Tulsa, OK, USA) are MTA cements with proven biological and physical-chemical properties, both known for their favorable tissue response and capability of forming mineralized tissue [8, 18].

However, the poor working properties of MTA-like cements results in a paste hard to manipulate and to deliver [19]. Considering the importance of the ideal flow ability that endodontic cements should present reducing the difficulty of handling [20], high plasticity cements were developed with the aim of improving these characteristic.

Recently released, the MTA Flow (Ultradent Products Inc., South Jordan, UT) consists of a gray powder of di- and tricalcium silicate, bismuth oxide as radiopacifier, and a liquid vehicle composed of a water soluble silicone-based gel [21]. According to the manufacturer, the gel provides a higher plasticity, improving handling and its insertion, which may facilitate the use in various procedures: a thick consistency for pulp capping, pulp chamber perforation, and pulpotomy; a thin consistency for resorption, apexification, and apical plug; or a putty consistency for root end filling [22]. The manufacturer also claims that unlike other MTA cements, which use water and have a coarse sandy consistency, MTA Flow cement has a creamy and homogeneous characteristic after mixing. The presentation of this material seems to be innovative in relation to the other cements available. However, as a new cement, there are no published studies concerning its biological properties or ability to induce the formation of hard tissue.

Therefore, the aim of the present study was to evaluate MTA Flow, analyzing the in vivo reaction of subcutaneous tissue of rats, and its ability to induce mineralization, assessed by von Kossa staining and presence of structures birefringent under polarized light. MTA Angelus and ProRoot MTA were used for comparison.

Materials and methods

This study was approved by the institutional Ethics Committee on the Use of Animals at UNESP-Universidade Estadual Paulista (São Paulo, Brazil) and conducted in accordance with relevant guidelines (CEUA protocol 00225-2017).

In vivo study

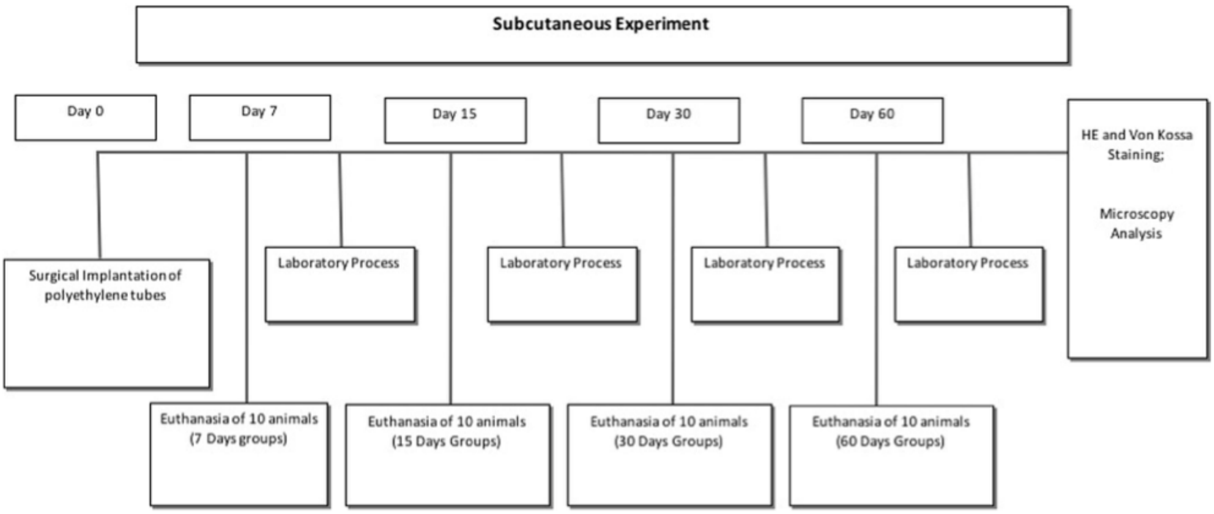
Subcutaneous implant Forty male 4 to 6-month-old Wistar rats, weighing 250–280 g, were used in the study. The animal sample size was based on previous studies that used the same methodology to assess biocompatibility and biomineralization in subcutaneous tissue of rats [3–5, 8, 23]. The animals were housed in temperature-controlled rooms and were provided with water and food ad libitum.

One hundred and sixty polyethylene tubes (Abbott Laboratories of Brazil, Sao Paulo, SP, Brazil) were prepared according to previous studies [8, 24] and filled with the tested cements. ProRoot MTA and MTA Angelus were spatulated according to the manufacturer's specification and inserted into the tubes with the aid of a lentullo spiral. MTA Flow was manipulated and inserted into a proper syringe and directly inserted into the polyethylene tubes. Therefore, all polyethylene tubes were completely filled, standardizing the same amount of cement for all experimental groups. Forty extra polyethylene empty tubes were used as controls, totaling 160 tubes in the experiment.

The surgical procedure was performed following previous studies [3, 8, 23, 24]. After administration of xylazine (10 mg/kg Rhobifarma Indústria Farmacêutica Ltda, Hortolândia, Brazil) and ketamine (25 mg/kg União Química Farmacêutica Nacional S/A, São Paulo, Brazil) intramuscular anesthesias, the backs of the animals were shaved, antisepsis was obtained with 5% iodine solution, and a 2-cm incision was formed in a head-tail orientation with no. 15 Bard-Parker blade (BD, Franklin Lakes, NJ). The skin was reflected to create two pockets on the right side (upper and lower) and another two pockets on the left side of the incision (upper and lower), totalizing four experimental sites. Three polyethylene tubes, containing the cements, and an empty tube, as the control, were implanted in the dorsal region of each animal in the created pockets in opposite directions (upper right, upper left, lower right, and lower left), and the skin was closed with a 4/0 silk suture (Johnson & Johnson Produtos Prossionais Ltda, São José dos Campos, Brazil).

After 7, 15, 30, and 60 days, the animals were killed by an anesthetic overdose. Polyethylene tubes, with the surrounding tissues, were removed and fixed in 10% formalin solution at a pH 7.0. The fixed specimens were processed and embedded in paraffin and serially sectioned into 5-μm cuts for staining with hematoxylin-eosin and 10-μm cuts for staining with the von Kossa technique, used to observe mineralization as it darkly stains mineralized structures. Some slices were kept unstained for examination under polarized light to observe the presence of birefringent structures (Graph 1).

Histologic analysis was performed by a single calibrated operator in a blinded manner under × 400 light microscopy (DM 4000 B; Leica, Wetzlar, Germany). Tissue reactions at the open end of the tubes were scored according to previous studies [3–5, 8, 23] as follows: 0, few inflammatory cells or no reaction; 1, less than 25 cells and mild reaction; 2, between 25 and 125 inflammatory cells and moderate reaction; and 3, 125 or more inflammatory cells and severe reaction. Fibrous capsules were considered thin when < 150 μm and thick when > 150 μm, as exemplified in Fig. 1. The Hematoxylin-Eosin staining allowed the evaluation of the contact area between



Graph 1 Study graphical outline, showing the experiment protocol at day 0 (subcutaneous implantation), days 7, 15, 30, and 60 (euthanasia, followed by the laboratory process: tube removal with surrounding tissue and paraffin embedding), samples staining and microscopy analysis.

the subcutaneous tissue and the tested material (at the lower center of the polyethylene tube) and one of the scores was attributed, classifying the tissue response as mild, moderate, or severe for each experimental time period of 7, 15, 30, and 60 days.

Calcification was recorded as positive or negative by Von Kossa staining and present or absent under PL [3, 8].

Statistical analysis Data were submitted to statistical analysis using the Sigma Plot (version 12.0, Systat Software Inc., CA) software program. The Kruskal-Wallis test followed by the Dunn test was performed for the nonparametric data; $P < .05$ was considered significant.

Results

The control group

A moderate chronic inflammatory infiltrate (median score 2) was observed at the first two experimental periods (Table 1), on days 7 and 15 (Fig. 2(A,a, B,b)). The inflammatory reaction, with lymphocytes and macrophages, was present in the thick fibrous capsule. After 30 and 60 days (Fig. 2(C,c, D,d)), the fibrous capsule became thin, with few inflammatory cells (median score 1). The mineralization was negative for Von Kossa and no birefringent structures were present under PL at all time periods, as observed in Table 2 and Fig. 3(A,a–D,d).

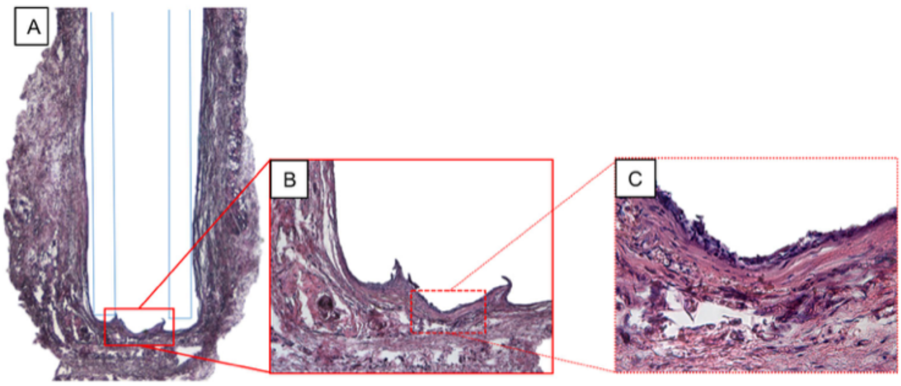


Fig. 1 Example of subcutaneous tissue in the MTA Flow group (60 days after implantation) situated around the polyethylene tube, after the tube removal for the paraffin embedding process, evidencing the area of analysis in the tube opening in × 25 magnification (A). The contact area in the center of the tube opening in × 100 (B); and the inflammatory response in the tube opening in × 400 (C), where it is possible to observe fibroblasts and a few inflammatory cells, mainly composed by lymphocytes (less than 25), characterizing a mild inflammatory reaction (score 1)

Table 1 Percentage of samples in each group categorized according to the inflammatory score, the median and the thickness of fibrous capsule in the four experimental periods

	Score (%)				Median	Capsule
7 days	0	1	2	3		
Control	0	0	100	0	2	Thick
MTA Flow	0	20	80	0	2	Thick
MTA Angelus	0	40	60	0	2	Thick
ProRoot MTA	0	30	20	50	3	Thick
15 days						
Control	0	0	100	0	2	Thick
MTA Flow	0	70	30	0	1	Thin
MTA Angelus	0	100	0	0	1	Thin
ProRoot MTA	0	30	40	30	2	Thick
30 days						
Control	0	100	0	0	1	Thin
MTA Flow	0	100	0	0	1	Thin
MTA Angelus	0	100	0	0	1	Thin
ProRoot MTA	0	80	20	0	1	Thin
60 days						
Control	20	80	0	0	1	Thin
MTA Flow	0	100	0	0	1	Thin
MTA Angelus	0	100	0	0	1	Thin
ProRoot MTA	0	100	0	0	1	Thin

MTA Angelus

The inflammatory infiltrate was considered moderate after 7 days (median score 2) and reduced to a mild inflammation (median score 1) from day 15 ($P<.05$) to 60, as observed in Fig. 2(E,e–H,h) and Table 1. The fibrous capsule near the tube opening was thick only on day 7 (Fig. 2(E,e)) and became thin after 15 days and continued until the end of the experimental time periods (Fig. 2(F,f–H,h)). Granulations birefringent to PL and Von Kossa-positive staining were observed near the tube opening at all time periods (Fig. 3(E,e–H,h) and Table 2).

MTA Flow

Only on day 7, a moderate inflammatory cell infiltration (median score 2), comprising lymphocytes and macrophages, was present in the thick fibrous capsule (Fig. 2(I,i); Table 1). After 15, 30, and 60 days, the intensity of the inflammation was reduced (median score 1; $P>.05$) and the fibrous capsule was thin, similar to the control group (Fig. 2(J,j–L,l). The presence of granulations birefringent under PL and Von Kossa-positive stains was observed in all time periods (Fig. 3(I,i–L,l) and Table 2).

ProRoot MTA

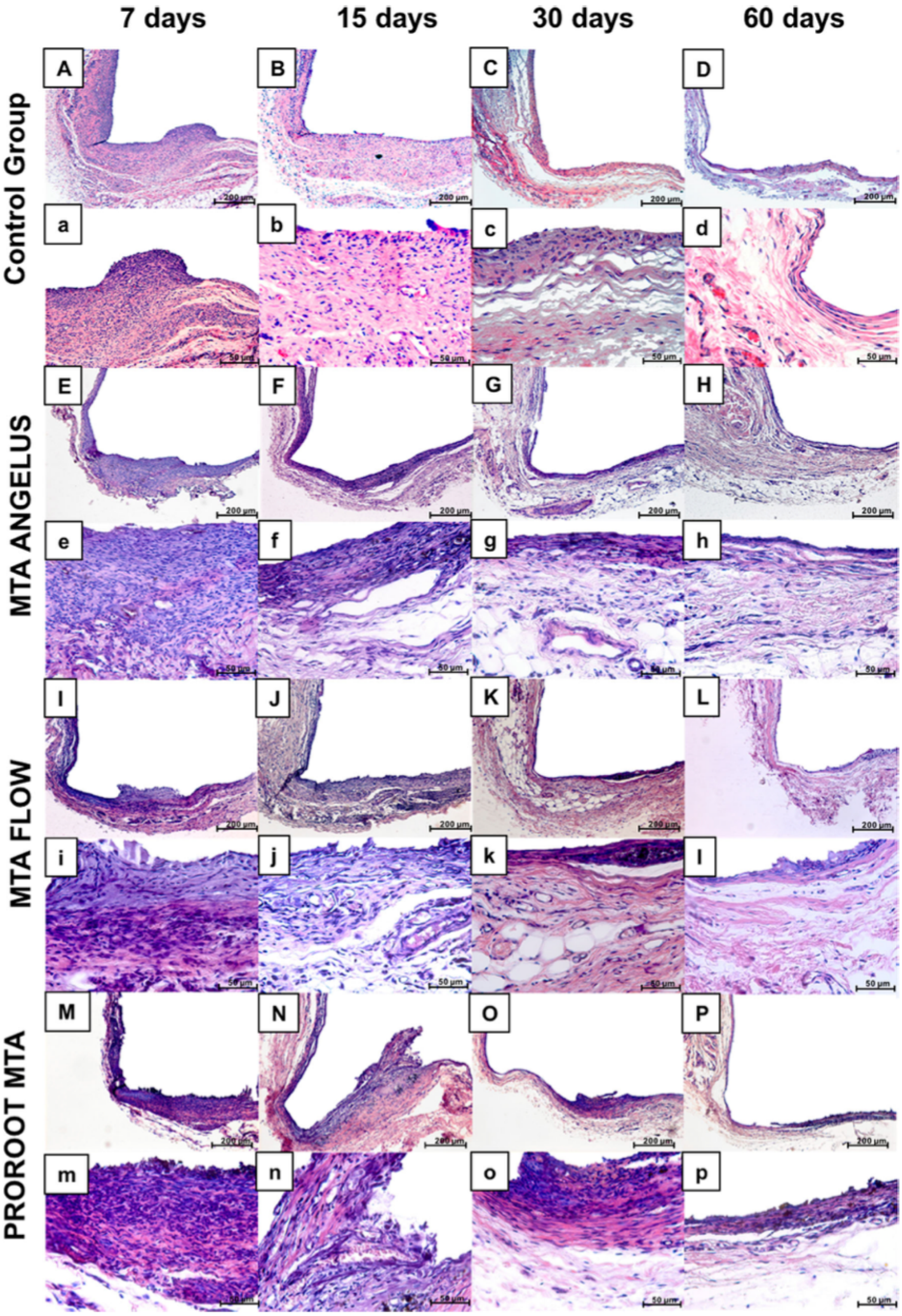
Although a severe inflammatory infiltrate was observed (median score 3) after 7 days ($P>.05$), it was notable that almost half of the specimens achieved a moderate and mild inflammation (median score 2 and 1, respectively), both in a thick fibrous capsule (Fig. 2(M,m) and Table 1). After 15 days, the inflammation was reduced (median score 2) but the fibrous capsule remained thick (Table 1). Only after 30 and 60 days, the inflammatory infiltrate was considered mild (median score 1; $P>.05$) and with a thin fibrous capsule in both periods (Fig. 2(O,o, P,p) and Table 1), same result as the control group. The biomineralization ability was observed in all time periods, with structures darkly stained by the Von Kossa technique and the presence of birefringent structures under PL was also observed (Fig. 3(M,m–P,p) and Table 2).

Comparison among groups

Data were compared for each time period (Tables 1 and 2). After 7 days, the control group showed a median score 2, with a thick fibrous capsule. ProRoot MTA showed a severe inflammation (median score 3) after 7 days, with a thick fibrous capsule. After 15 days, it was possible to observe a reduction on the inflammatory infiltrate regarding MTA Angelus ($P<.05$) and MTA Flow, which induced a mild inflammation (median score 1) in a thin fibrous capsule. The control group remained with a thick fibrous capsule and the moderate inflammation after 15 days, same result as the ProRoot MTA. After 30 and 60 days, all groups presented a mild inflammatory infiltrate (median score 1), restricted to a thin fibrous capsule, without statistical difference ($P>.05$).

Regarding the biomineralization, with exception of the control group, all cements induced the formation of mineralized tissue in all experimental periods, confirmed by the presence of structures darkly stained by the Von Kossa technique (Fig. 3(A–P); Table 2) and the birefringent structures observed under polarized light (Fig. 3(a–p); Table 2).

Fig. 2 Representative images of subcutaneous tissue reactions in the experimental groups. The control group (A,a–D,d), at 7 and 15 days: thick fibrous capsule and moderate inflammatory reaction (A,a, B,b); after 30 days: reduction in the thickness of the fibrous capsule and mild inflammatory reaction (C,c) and (D,d) thin fibrous capsule and mild inflammatory reaction (day 60). MTA Angelus (E,e–H,h); at 7 days: (E,e) thick fibrous capsule formation and moderate inflammatory cell infiltration; after 15, 30, and 60 days: (F,f–H,h) reduction in the thickness of the fibrous capsule formation and mild inflammatory cell infiltration. MTA Flow (I,i–L,l), at 7 days: (I,i) moderate cell infiltration in a thick fibrous capsule; after 15, 30, and 60 days: (J,j–L,l) mild inflammatory reaction in a thin fibrous capsule. ProRoot MTA (M,m–P,p), at 7 days: (M,m) severe inflammatory infiltrate in a thick fibrous capsule; at 15 days: (N,n) moderate inflammation in a thick fibrous capsule; after 30 and 60 days: (O,o, P,p) mild inflammatory cell infiltration and a thin fibrous capsule. Hematoxylin-eosin staining. Original magnification: (A–P) × 100, (a–p) × 400 ▶


7 days
15 days
30 days
60 days
Control Group
MTA ANGELUS
MTA FLOW
PROROOT MTA
A
B
C
D
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L
i
j
k
l
M
N
O
P
m
n
o
p
200 µm
50 µm

Table 2 Percentage of samples in each group categorized according to Von Kossa-positive to mineralization and presence of birefringents crystals under polarized light

	Von Kossa (%)	Polarized light (%)
7 days		
Control	0	0
MTA Flow	100	100
MTA Angelus	100	100
ProRoot MTA	100	100
15 days		
Control	0	0
MTA Flow	100	100
MTA Angelus	100	100
ProRoot MTA	100	100
30 days		
Control	0	0
MTA Flow	100	100
MTA Angelus	100	100
ProRoot MTA	100	100
60 days		
Control	0	0
MTA Flow	100	100
MTA Angelus	100	100
ProRoot MTA	100	100

Discussion

This research showed the MTA Flow was biocompatible and induced biomineralization, as well as the MTA Angelus and the ProRoot MTA. According to the American Association of Endodontists, a new material should be biologically and clinically studied before its use [25], since materials that will be in close contact with the periapical tissues should exhibit biocompatibility and the ability to induce the formation of mineralized tissue [26]. The rat model for subcutaneous implantation is widely used as one of the pre-clinical research tests for biocompatibility, when facing new materials [6, 7, 27].

The choice of using MTA Angelus and the ProRoot MTA as a positive control was due to several studies concerning biocompatibility and biomineralization. In the present study, the groups showed a moderate initial inflammatory response, reducing over time as the fibrous capsule became thin, corroborating previous findings [9, 24, 28–31]. This initial inflammatory reaction is due to the surgical procedure, and the normal reaction form the tissue when facing a subcutaneous implant, as well as for the MTA Angelus and the MTA Flow group. It is normal to observe an initial inflammatory reaction when using MTA-like cements, because the alkalinity damage provoked on tissues but it is also important to state that the biocompatibility of a material is achieved if the initial inflammatory reaction reduces over time, to a not significant level [32, 33].

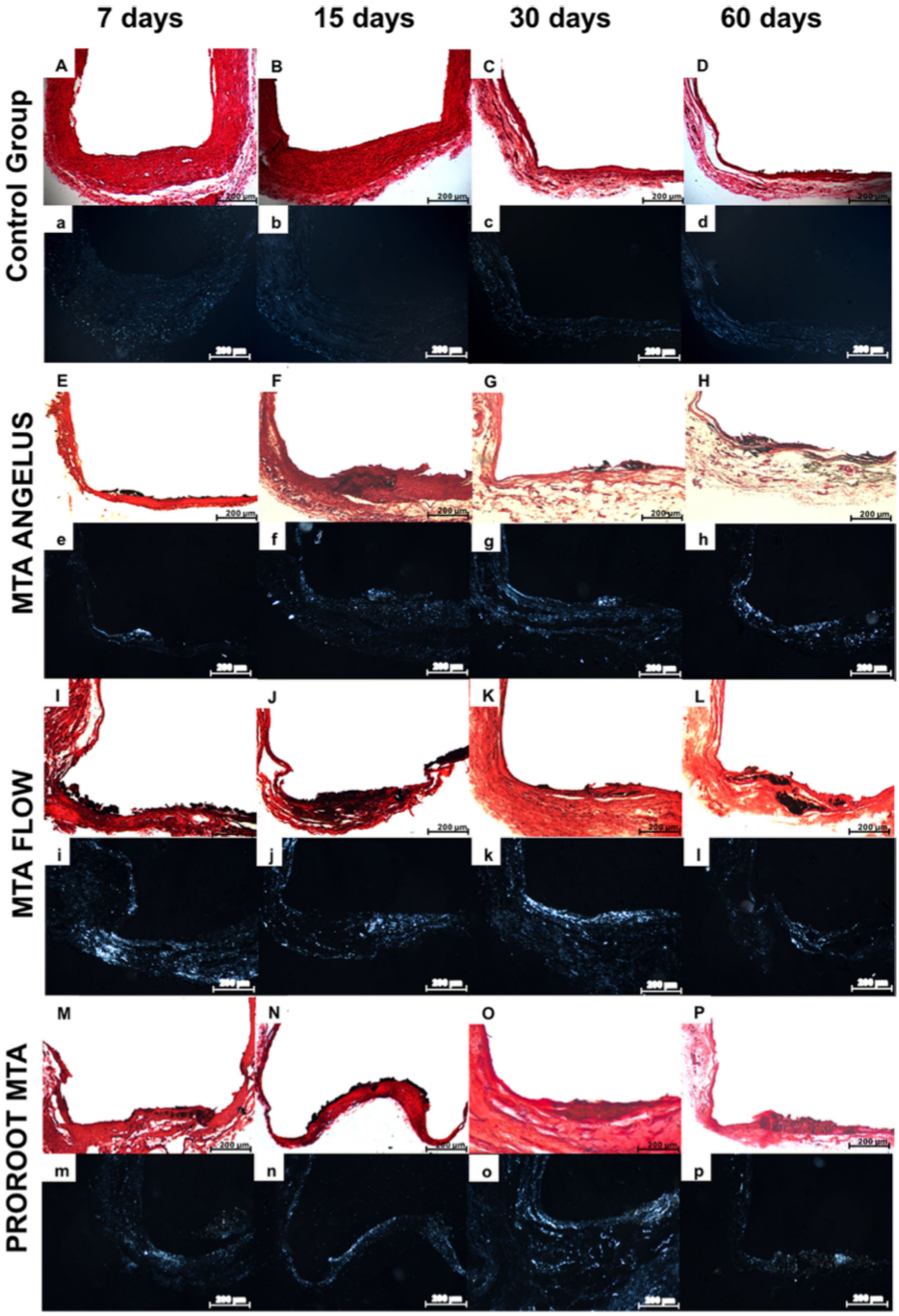
The biocompatibility and bioactivity of MTA begins with its setting reaction. The hydration of mineral oxide compounds produces calcium silicate hydrate and calcium hydroxide, which after dissociation, continuously releases Ca ions, providing a high alkalinity environment and inducing the formation of mineralized tissue [34]. The ions Ca, originating from the MTA dissociation, interacts with P ions in body fluids resulting in the formation of calcium phosphate crystalline structures on the materials' interfacial surfaces, which is an amorphous form of calcium phosphate and/or carbonate apatite [35–38].

The earliest MTA cements exhibited a difficult handling and consequently difficult delivering [39] leading to the constant search of new formulations to improve flowability, combining enhanced handling, mechanical and biological properties [40, 41]. This innovation in the vehicle used to achieve a higher plasticity type of MTA has been recently studied, showing biocompatibility and inducing biomineralization [8].

A recent study regarding the chemical-physical properties and the apatite-forming ability of the MTA Flow reported alkalinizing capability, low solubility, good radiopacity and the ability to form calcium phosphate deposits [42]. Until now, there are no studies in the literature concerning the biological properties of the MTA Flow. The manufacturer claims that this novel MTA-repair cement combined with the water silicone-based gel improves the handling of the cement and can be adapted depending on the procedure, since after mixing, the MTA Flow achieves a consistency that passes through a syringe, facilitating insertion [22].

Previous reports that used the implantation of bioactive materials in subcutaneous tissue of rats described the formation of a mineralized layer, darkly stained by the Von Kossa technique [3, 34]. In the present study, the biomineralization ability of MTA Flow was detected by the presence of structures birefringent to polarized light and von Kossa-positive staining, also observed in the MTA Angelus and ProRoot MTA. According to Hinata [11], the presence of Von Kossa-positive structures is in accordance with a production of Ca and P surface precipitates and a Ca- and P-rich layer at the interface between material and tissue, supporting the ability of calcium silicate-based materials to promote biomineralization after contact with connective tissue. Reinforcing these findings, Guimaraes et al. [42] reported through an energy-dispersive X-ray (EDX) analysis that the calcium (Ca) and phosporous (P) ratios of MTA Flow were higher in

Fig. 3 Biomineralization in the experimental groups in Von Kossa (A–P) ▶ and under polarized light (a–p) under × 100 magnification; control at 7, 15, 30, and 60 days: absence of dystrophic calcification (A–D) and absence of birefringent structures under polarized light (a–d) in all time periods; MTA Angelus at 7, 15, 30, and 60 days: Von Kossa-positive staining (E–H) and granulations birefringent to PL (e–h) were observed near the tube opening at all time periods; MTA Flow at 7, 15, 30, and 60 days: presence of Von Kossa-positive staining (I–L) and birefringent structures under PL (i–l) in all periods; ProRoot MTA at 7, 15, 30, and 60 days: Von Kossa-positive staining (M–P) and birefringent structures under polarized light (m–p) in all experimental periods


7 days
15 days
30 days
60 days
Control Group
MTA ANGELUS
MTA FLOW
PROROOT MTA
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M
N
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P
m
n
o
p
200 µm

comparison with MTA Angelus, possibly related to the gel vehicle, which increases the resistance of particles to external water.

Another important factor observed in our laboratory experience was the different consistency between the types of MTA used in the study during the mixing. Although the particle size was not analyzed in this research, the granulations in MTA Angelus and ProRoot MTA were apparently more thick, hindering the cement handling, while the MTA Flow showed a thinner composition of the powder, which, in addition of the water-based gel, facilitated the procedure, corroborating previous findings [42]. The manufacturer claims that the smooth consistency achieved with MTA Flow cement is due not only to the proprietary gel medium, but mainly also to the ultrafine-fined powder, which differs from others MTA cements available in the market [22]. The potential influence of particle size might increase the reactivity of the dicalcium and tricalcium silicate particles, since the geometry of small MTA particles enables entering into open dentin tubules, which could be an important mechanism to enhance the sealing ability and even improving the handling of MTA-like cements [43].

Within the limits of this study in the rat model, the novel MTA Flow showed biocompatibility and the ability to form biomineralizated tissue, representing an alternative to the conventional MTA, with a final consistency that could facilitate its clinical use. Since this is the first study evaluating biological properties, additional research is necessary to confirm the present findings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Before any procedure, the project was approved by Faculdade de Odontologia de Araçatuba, UNESP, Animal Ethical Committee (CEUA protocol 00225-2017).

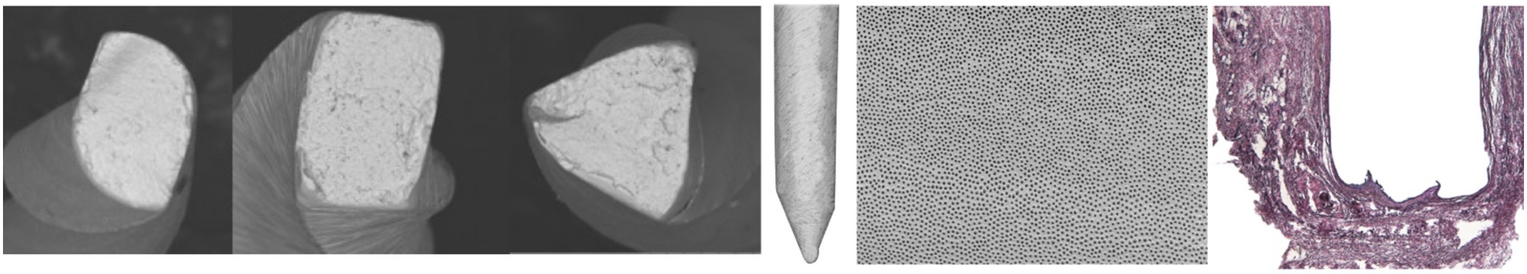
Informed consent For this type of study, formal consent is not required.

References

1. Mori GG, Teixeira LM, de Oliveira DL et al (2014) Biocompatibility evaluation of biodentine in subcutaneous tissue of rats. J Endod 40:1485–1488
2. Edanami N, Yoshiba N, Ohkura N, Takeuchi R, Tohma A, Noiri Y, Yoshiba K (2017) Characterization of dental pulp myofibroblasts in rat molars after pulpotomy. J Endod 43:1116–1121
3. Bueno CRE, Valentim D, Marques VAS, Gomes-Filho JE, Cintra LT, Jacinto RC et al (2016) Biocompatibility and biomineralization assessment of bioceramic-, epoxy-, and calcium hydroxide-based sealers. Braz Oral Res 30(1):81
4. Cintra LTA, Benetti F, Queiroz IOA, Ferreira LL, Massunari L, Bueno CRE, Oliveira SHPO, Gomes-Filho JE (2017) Evaluation of the cytotoxicity and biocompatibility of new resin epoxy-based endodontic sealer containing calcium hydroxide. J Endod 43:2088–2092
5. Bueno CRE, Lopes GA, Valentim D, Marques VAS, Vasques AMV, Cury MT, Sivieri-Araujo G, Jacinto RDC, Cintra LTA, Dezan-Junior E (2017) Mixing failures of endodontic sealers: an in vivo biocompatibility study. Braz Dent Sci 20(4):85–92
6. ISO 10993-6. Biological evaluation of medical devices. Part 6: Tests for local effects after implantation International Standard Norm, Porto, Portugal (2007)
7. Olsson B, Sliwkowsky A, Langeland K (1981) Subcutaneous implantation for the biological evaluation of endodontic materials. J Endod 7:355–369
8. Cintra LTA, Benetti F, Queiroz IOA, Lopes JMA, Sandra Oliveira SHP, Sivieri Araujo G, Gomes-Filho JE (2017) Cytotoxicity, biocompatibility, and biomineralization of the new high-plasticity MTA material. J Endod 43:774–778
9. Cintra LT, Ribeiro TA, Gomes-Filho JE et al (2013) Biocompatibility and biomineralization assessment of a new root canal sealer and root-end filling material. Dent Traumatol 29:145–150
10. Federation Dentaire Internationale (1980) Recommended standard practices for the biological evaluation of dental materials. Int Dent J 30:174–176
11. Hinata G, Yoshiba K, Han L, Edanami N, Yoshiba N, Okiji T (2017) Bioactivity and biomineralization ability of calcium silicate-based pulp-capping materials after subcutaneous implantation. Int Endod J 50:40–45
12. Torabinejad M, Watson TF, PittFord TR (1993) Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod 19:591–595
13. Lee SJ, Monse FM, Torabinejad M (1993) Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod 19:541–544
14. Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I (2005) Physicochemical basis of the biologic properties of mineral trioxide aggregate. J Endod 31(2):97–100
15. Tanomaru-Filho M, Torres FFE, Bosso-Martelo R, Chávez-Andrade GM, Bonetti-Filho I, Guerreiro-Tanomaru JM (2017) A novel model for evaluating the flow of endodontic materials using micro-computed tomography. J Endod 43:796–800
16. Zarrabi MH, Javidi M, Jafarian AH, Joushan B (2010) Histologic assessment of human pulp response to capping with mineral trioxide aggregate and a novel endodontic cement. J Endod 11:1778–81. 12
17. Parirokh M, Torabinejad M (2010) Mineral trioxide aggregate: a comprehensive literature review—part I: chemical, physical, and antibacterial properties. J Endod 36:16–27
18. Camilleri J (2008) The chemical composition of mineral trioxide aggregate. J Conserv Dent 11:141–143
19. Canadas PS, Berastegui E, Gaton-Hernandez P et al (2014) Physicochemical properties and interfacial adaptation of root canal sealers. Braz Dent J 25:435–441
20. Duarte MA, Ordinola-Zapata R, Bernardes RA et al (2010) Influence of calcium hydroxide association on the physical properties of AH Plus. J Endod 36:1048–1051
21. Endo-Eze MTA FLOW Gel Safety Data Sheet (2017). Avaliable at: www.ultradent.com/en-us/Dental-Products-Supplies/Endodontics/MTA-repair-cement/endo-eze-MTAflow-mineral-trioxide-aggregate-repair-cement. Acessed in: February/2018
22. Ultradent Products, Inc. Products and Procedures Manual (2017) Repair Material: MTA Flow p. 54–57

23. Valentim D, Bueno CRE, Marques VAS, Vasques AMV, Cury MTS, Cintra LTA, Dezan-Junior E (2017) Calcium hydroxide associated with a new vehicle: Psidium cattleianum leaf extracts. Tissue response evaluation. Braz Oral Res 31(43):1-8
24. Gomes-Filho JE, de Azevedo Queiroz IO, Watanabe S, da Silva Santos LM, Lodi CS, Okamoto R, Ervolino E, Dezan E Jr, Cintra LTA (2015) Influence of diabetes mellitus on tissue response to MTA and its ability to stimulate mineralization. Dent Traumatol 31:67–72
25. American Association of Endodontists (2013) New materials/technologies position paper. American Association of Endodontists, Chicago
26. Chhabra A, Teja TS, Jindal V, Singla MG, Warring K (2011) Fate of extruded sealer: a matter of concern. J Oral Health Comm Dent 5(3):168–172
27. International Organization for Standardization. Dentistry—preclinical evaluation of biocompatibility of medical devices used in dentistry. Test methods for dental materials: ISO/TR 7405-1997(E). Switzerland: ISO, 1997
28. Gomes-Filho JE, de Moraes Costa MT, Cintra LT et al (2010) Evaluation of alveolar socket response to Angelus MTA and experimental light-cure MTA. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 110:93–97
29. Holland R, Souza V, Nery MJ, Otoboni-Filho JA, Bernabé PFE, Dezan-Júnior E (1999) Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. J Endod 25(3):161–166
30. Cintra LT, de Moraes IG, Estrada BP et al (2006) Evaluation of the tissue response to MTA and MBPC: microscopic analysis of implants in alveolar bone of rats. J Endod 32:556–559
31. Gomes-Filho JE, de Moraes Costa MM, Cintra LT et al (2011) Evaluation of rat alveolar bone response to Angelus MTA or experimental light-cured mineral trioxide aggregate using fluorochromes. J Endod 37:250–254
32. Gomes-Filho JE, Watanabe S, Lodi CS, Cintra LT, Nery MJ, Otobon Filho JA et al (2012) Rat tissue reaction to MTA FILLAPEX. Dent Traumatol 28(6):452–456
33. Silveira CM, Pinto SC, Zedebski Rde A et al (2011) Biocompatibility of four root canal sealers: a histopathological evaluation in rat subcutaneous connective tissue. Braz Dent J 22:21–27
34. Camilleri J (2008) Characterization of hydration products of mineral trioxide aggregate. Int Endod J 41:408–417
35. Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I (2005) Physicochemical basis of the biologic properties of mineral trioxide aggregate. J Endod 31:97–100
36. Tay FR, Pashley DH, Rueggeberg FA, Loushine RJ, Weller RN (2007) Calcium phosphate phase transformation produced by the interaction of the Portland cement component of white mineral trioxide aggregate with a phosphate-containing fluid. J Endod 33:1347–1351
37. Gandolfi MG, Taddei P, Tinti A, Prati C (2010) Apatite-forming ability (bioactivity) of ProRoot MTA. Int Endod J 43:917–929
38. Camilleri J (2015) Mineral trioxide aggregate: present and future developments. Endod Top 32:31–46
39. Parirokh M, Torabinejad M (2010) Mineral trioxide aggregate: a comprehensive literature review—part III: clinical applications, drawbacks, and mechanism of action. J Endod 36:400–413
40. Marciano MA, Guimarães BM, Amoroso-Silva P, Camilleri J, Hungaro Duarte MA (2016) Physical and chemical properties and subcutaneous implantation of mineral trioxide aggregate mixed with propylene glycol. J Endod 42:474–479
41. Hsieh SC, Teng NC, Lin YC, Lee PY, Ji DY, Chen CC, Ke ES, Lee SY, Yang JC (2009) A novel accelerator for improving the handling properties of dental filling materials. J Endod 35:1292–1295
42. Guimarães BM, Vivan RR, Piazza B, Alcalde MP, Bramante CM, Duarte MAH (2017) Chemical-physical properties and apatite-forming ability of mineral trioxide aggregate flow. J Endod 43(10):1692–1696
43. Komabayashi T, Spångberg LSW (2008) Comparative analysis of the particle size and shape of commercially available mineral trioxide aggregates and Portland cement: a study with a flow particle image analyzer. J Endod 34:94–98

VI. Considerações Finais



VI. Considerações Finais

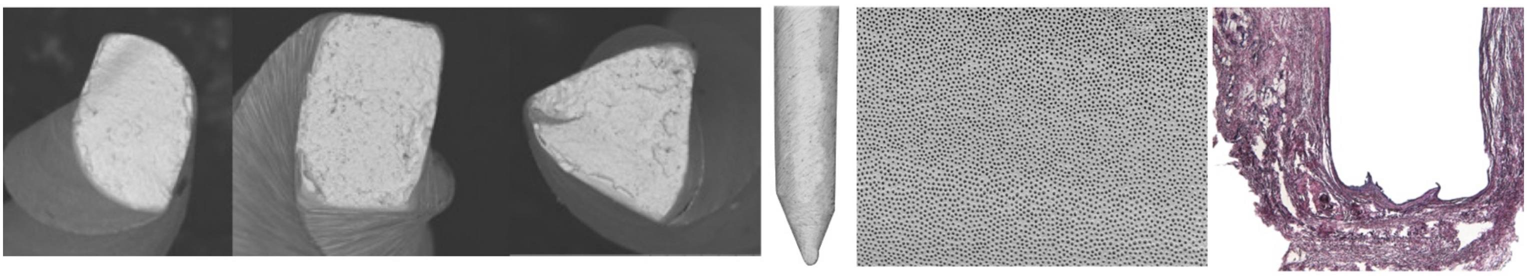
A instrumentação dos canais radiculares tem evoluído, desde a introdução de instrumentos em níquel-titânio na década de 80 até os dias de hoje, com diferentes ligas, tratamentos térmicos e cinemática proporcionadas por motores endodônticos para automatização das limas. A evolução das metodologias de pesquisas leva a uma consequente evolução dos materiais, possivelmente contornando desvantagens. Logo, novos materiais, devem continuar passando por pesquisas, objetivando se aproximar de um material ideal.

O teste de fadiga cíclica fornece dados sobre diferentes resultados relativos às resistências das secções transversais, conicidade, cinemática e tipo de liga utilizada no instrumento. Apesar de existir aparatos que permitam testes de fadiga torcional ou cíclica, de forma estática ou dinâmica, o presente estudo utilizou o teste estático cíclico. Os resultados mostraram desempenho satisfatório das novas limas, porém testes adicionais são recomendados, para avaliar diferentes situações de estresse do instrumento, tanto em cinemáticas quanto em curvaturas diferentes e temperatura corporal.

No que diz respeito ao inserto ultrassônico, o protocolo final de ativação da solução irrigante já é considerado de primordial necessidade em tratamentos convencionais, mas principalmente em retratamentos. A importância da ativação e potencialização da solução irrigante tornou-se fundamental após automatização das limas e sua evolução para instrumento único, uma vez que volume, frequência e renovação do irrigante tende a diminuir com a redução do número de limas utilizadas no tratamento, considerando o protocolo de irrigação entre troca de instrumentos. Até o momento, não há uma única solução a ser utilizada, devendo haver alternância entre irrigantes. O hipoclorito de sódio, amplamente utilizado e considerado por muitos autores como padrão ouro, deve ser alternado com o EDTA para remoção eficaz da *smear layer*. As filosofias que preconizavam a permanência da *smear layer* cobrindo os túbulos dentinários, advogavam uma menor permeabilidade entre o canal radicular e o meio externo. Porém, sabe-se que a permanência de bactérias no interior dos túbulos está diretamente associada com maior insucesso no tratamento. Sua remoção influencia tanto em uma melhor ação da medicação intracanal, quando maior penetrabilidade dos cimentos endodônticos na fase de obturação. Comumente, pontas utilizadas para ativar solução irrigante são feitas de uma liga de aço inox. O inserto de níquel-titânio tem a premissa de ser mais resistente, porém, o presente trabalho avaliou a eficácia do inserto fabricado com liga de NiTi em ativar a solução irrigante. Nossos resultados mostraram que o inserto de NITI pode ser utilizado para protocolo final de irrigação, sendo eficaz e melhorando a remoção da *smear layer*, em ambos os protocolos de ativação. Esses achados corroboram resultados anteriores com insertos convencionais, indicando que tanto a ativação passiva quanto a contínua são eficazes em aumentar a remoção de *smear layer*.

O agregado de trióxido mineral (MTA), apesar de todos os aspectos positivos de biocompatibilidade e biomineralização ao longo de anos (desde sua introdução na endodontia na década de 90), sempre foi apresentado com uma característica negativa relativa à sua manipulação. A consistência final arenosa obtida no MTA dificulta a manipulação e, principalmente, sua inserção. Tendo em vista essa peculiaridade, os fabricantes têm voltado atenção para contornar essa dificuldade e formulações recentes foram apresentadas como Alta Plasticidade (HP) ou Flow. Para alterar essa consistência, mudanças foram feitas tanto no veículo, quanto no próprio pó, com utilização de micropartículas. Quando ocorre alterações nas formulações, os produtos devem ser sempre pesquisados para avaliar alterações nas propriedades físico-químicas e biológicas do material. O presente estudo utilizou um modelo animal em rato, frequentemente utilizado na literatura, para análise da resposta subcutânea do MTA Flow, quanto à inflamação e biomineralização. O MTA flow reduziu a inflamação inicial e induziu a deposição de estruturas mineralizadas. Os achados biológicos do presente trabalho e físico-químicos previamente demonstrados, associados à consistência de fácil manipulação em alta plasticidade observada em laboratório, sugere este cimento como uma alternativa entre os materiais reparadores à base de MTA presentes no mercado.

VII. Referências



VII. Referências[1]

American Association of Endodontists. Glossary of endodontic terms. 9th ed. Chicago: American Association of Endodontists; 2016.

Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J Endod. 1992;18(12):605-12.

Beus C, Safavi K, Stratton J, Kaufman B. Comparison of the effect of two endodontic irrigation protocols on the elimination of bacteria from root canal system: a prospective, randomized clinical trial. J Endod. 2012 Nov;38(11):1479-83. https://doi.org/10.1016/j.joen.2012.07.005.

Camilleri J, Pitt Ford TR. Mineral trioxide aggregate: a review of the constituents and biological properties of the material. Int Endod J. 2006;39:747–54.

Camilleri J. The chemical composition of mineral trioxide aggregate. J Conserv Dent. 2008; 11:141–143.

Canadas PS, Berastegui E, Gaton-Hernandez P *etal*. Physicochemical properties and interfacial adaptation of root canal sealers. Braz Dent J. 2014; 25:435–441.

Castelo-Baz P, Martín-Biedma B, Cantatore G, Ruíz-Piñón M, Bahillo J, Rivas-Mundiña B, *et al*. In vitro comparison of passive and continuous ultrasonic irrigation in simulated lateral canals of extracted teeth. J Endod. 2012 May;38(5):688-91. https://doi.org/10.1016/j.joen.2011.12.032.

Cheung GS, Darvell BW. Fatigue testing of a NiTi rotary instrument. Part 2: fractographic analysis. Int Endod J. 2007 Aug;40(8):619-25. https://doi.org/10.1111/j.1365-2591.2007.01256.x.

Cintra LTA, Benetti F, Queiroz IOA, Lopes JMA, Sandra Oliveira SHP, Sivieri Araujo G, Gomes-Filho JE. Cytotoxicity, biocompatibility, and biomineralization of the new high-plasticity MTA material. J Endod. 2017; 43:774–778.

Dagna A, Poggio C, Beltrami R, Colombo M, Chiesa M, Bianchi S. Cyclic fatigue resistance of OneShape, Reciproc, and WaveOne: an in vitro comparative study. J Conserv Dent. 2014 May;17(3):250-4. https://doi.org/10.4103/0972-0707.131788.

Dentsply Sirona WaveOne Gold, 2019. Disponível em: https://www.dentsplysirona.com/en-us/products/endodontics/waveone-gold.html Acesso: Abril/2019.

Duarte MA, Ordinola-Zapata R, Bernardes RA *et al*. Influence of calcium hydroxide association on the physical properties of AH Plus. J Endod. 2010;36:1048–1051.

[1] Referencias utilizadas para introdução geral

Economides N, Liolios E, Kolokuris I, Beltes P. Long-term evaluation of the influence of smear layer removal on the sealing ability of different sealers. J Endod. 1999 Feb;25(2):123-5. https://doi.org/10.1016/S0099-2399(99)80010-7.

EdgeEndo EdgeFile X-1 Brochure, 2019. Heat Treated Fire-WireTM NiTi Reciprocating Files. Albuquerque, Usa. Disponível em: https://edgeendo.com/wp-content/uploads/2015/08/DFU-EdgeFile-x1.pdf. Acesso: Abril/2019.

Endo-Eze Genius System Brochure, 2018. South Jordan, Utah, USA. Disponível em:https://assets.ctfassets.net/wfptrcrbtkd0/5k3KKb8xii60QzdhBGlOea/3716974a6451949cfceb5f4d955b0acc/Genius-Files-Brochure_1003834.6.pdf. Acesso: Abril/2019.

Endo-Eze MTA Flow Gel Safety Data Sheet, 2015. Disponível em: https://www.ultradent.com/products/categories/endodontics/mta-repair/mta-flow. Acesso: Abril/2019.

Estrela C, Pécora JD, Estrela CRA et al. Common operative procedural errors and clinical factors associated with root canal treatment. Braz Dent J. 2017 Jan-Apr;28(2):179-90. https://doi.org/10.1590/0103-6440201702451.

Gavini G, *et al*. Nickel–titanium instruments in endodontics: a concise review of the state of the art. Braz Oral Res. 2018;32(1):e67.

Gergi R, Rjeily JA, Sader J, Naaman A. Comparison of canal transportation and centering ability of twisted files, Pathfile- ProTaper system, and stainless steel hand K-files by using computed tomography. J Endod. 2010 May;36(5):904-7. https://doi.org/10.1016/j.joen.2009.12.038.

Goldberg F, Abramovich A. Analysis of the effect of EDTAC on the dentinal walls of the root canal. J Endod. 1977 Mar;3(3):101-5. https://doi.org/10.1016/S0099-2399(77)80203-3.

Grischke J, Müller-Heine A, Hülsmann M. The effect of four different irrigation systems in the removal of a root canal sealer. Clin Oral Investig. 2014 Sep;18(7):1845-51. https://doi.org/10.1007/s00784-013-1161-6.

Guimarães BM, Vivan RR, Piazza B, Alcalde MP, Bramante CM, Duarte MAH. Chemical-physical properties and apatite-forming ability of mineral trioxide aggregate flow. J Endod. 2017;43(10):1692–1696.

Gutarts R, Nusstein J, Reader A, Beck M. In vivo debridement efficacy of ultrasonic irrigation following hand-rotary instrumentation in human mandibular molars. J Endod. 2005 Mar;31(3):166-70. https://doi.org/10.1097/01.don.0000137651.01496.48.

Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. Endod Top. 2005 Aug;10(1):77-102. https://doi.org/10.1111/j.1601-1546.2005.00135.x.

Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J. 2014;216(6):299-303.

Hasselgren G, Olsson B, Cvek M. Effects of calcium hydroxide and sodium hypochlorite on the dissolution of necrotic porcine muscle tissue. J Endod. 1988 Mar;14(3):125-7.

Hinata G, Yoshiba K, Han L, Edanami N, Yoshiba N, Okiji T. Bioactivity and biomineralization ability of calcium silicate-based pulp-capping materials after subcutaneous implantation. Int Endod J. 2017; 50:40–45.

Holland R, de Souza V, Nery MJ, *et al*. Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. J Endod. 1999;25:161–6.

Hui-min Z, *et al*. Fatigue Behavior of Ni-Ti Alloy Endodontic Files under Ultrasonic Unconstrained Condition. Key Eng Mat. 2010;417-418: 77-80. doi:10.4028/www.scientific.net/KEM.417-418.77

Lee SJ, Monse FM, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod. 1993;19:541–544.

Lopes HP, Elias CN, Vieira MV, Siqueira JF Jr, Mangelli M, Lopes WS, *et al*. Fatigue Life of Reciproc and Mtwo instruments subjected to static and dynamic tests. J Endod. 2013 May;39(5):693-6. https://doi.org/10.1016/j.joen.2012.11.048

Lui JN, Kuah HG, Chen NN. Effect of EDTA with and without surfactants or ultrasonics on removal of smear layer. J Endod. 2007 Apr;33(4):472-5. https://doi.org/10.1016/j.joen.2006.12.007.

Mader CL, Baumgartner JC, Peters DD. Scanning electron microscopic investigation of the smeared layer on root canal walls. J Endod. 1984 Oct;10(10):477-83. https://doi.org/10.1016/S0099-2399(84)80204-6.

McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod. 1975 Jul;1(7):238-42. https://doi.org/10.1016/S0099-2399(75)80226-3.

Mori GG, Teixeira LM, de Oliveira DL *et al*. Biocompatibility evaluation of biodentine in subcutaneous tissue of rats. J Endod. 2014;40: 1485–1488.

Nagendrababu V, Jayaraman J, Suresh A, Kalyanasundaram S, Neelakantan P. Effectiveness of ultrasonically activated irrigation on root canal disinfection: a systematic review of in vitro studies. Clin Oral Investig. 2018 Mar;22(2):655-70. https://doi.org/10.1007/s00784-018-2345-x.

Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review—part I: chemical, physical, and antibacterial properties. J

Endod. 2010; 36:16–27.

Pedullà E, Franciosi G, Ounsi HF, Tricarico M, Rapisarda E, Grandini S. Cyclic fatigue resistance of nickel-titanium instruments after immersion in irrigant solutions with or without surfactants. J Endod. 2014 Aug;40(8):1245-9. https://doi.org/10.1016/j.joen.2014.02.005.

Peters OA, Barbakow F. Effects of irrigation on debris and smear layer on canal walls prepared by two rotary techniques: a scanning electron microscopic study. J Endod. 2000 Jan;26(1):6-10. https://doi.org/10.1097/00004770-200001000-00002.

Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod. 2004 Aug;30(8):559-67. https://doi.org/10.1097/01.DON.0000129039.59003.9D.

Pruett JP, Clement DJ, Carnes DL Jr. Cyclic fatigue testing of nickel-titanium endodontic instruments. J Endod. 1997 Feb;23(2):77-85. https://doi.org/10.1016/S0099-2399(97)80250-6.

Roda RS, Gettleman BH. Nonsurgical retreatment. In: Hargreaves KM, Berman LH, editors. Cohen's Pathways of the pulp. 11th ed. St. Louis: Elservier; 2016. p. 324-86.

Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I. Physicochemical basis of the biologic properties of mineral trioxide aggregate. J Endod. 2005; 31(2):97–100.

Schilder H. Filing root canals in three dimensions. J Endod. 2006;32(4):281-90. doi:10.1016/j.joen.2006.02.007.

Spanaki-Voreadi AP, Kerezoudis NP, Zinelis S. Failure mechanism of ProTaper Ni-Ti rotary instruments during clinical use: fractographic analysis. Int Endod J. 2006 Mar;39(3):171-8. https://doi.org/10.1111/j.1365-2591.2006.01065.x

Tanomaru-Filho M, Torres FFE, Bosso-Martelo R, Chávez- Andrade GM, Bonetti-Filho I, Guerreiro-Tanomaru JM. A novel model for evaluating the flow of endodontic materials using micro-computed tomography. J Endod. 2017;43:796–800.

Torabinejad M, Watson TF, PittFord TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod. 1993;19:591–595

Tripi TR, Bonaccorso A, Tripi V, Condorelli GG, Rapisarda E. Defects in GT rotary instruments after use: an SEM study. J Endod. 2001 Dec;27(12):782-5. https://doi.org/10.1097/00004770-200112000-00018 PMID:11771591.

Tsesis I, Fuss Z. Diagnosis and treatment of acidental root perforations. Endod Topics. 2006 Mar;13(1):95-107. https://doi.org/10.1111/j.1601-

1546.2006.00213.x

Ultradent Products and Procedures Manual. Repair Material: MTA Flow 2017; p. 54–57.

Ultradent Products UltraWave NiTiSonic Tip, 2019. South Jordan, Utah, USA. Disponível em: https://www.ultradent.com/products/categories/endodontics/endodontic-equipment/ultrawave-xs-tip-and-bottle-connector. Acesso: Abril/2019.

Uzunoglu E, Görduysus M, Görduysus Ö. A comparison of different irrigation systems and gravitational effect on final extrusion of the irrigant. J Clin Exp Dent. 2015 Apr;7(2):e218-23. https://doi.org/10.4317/jced.52158

van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J. 2007 Jun;40(6):415-26. https://doi.org/10.1111/j.1365-2591.2007.01243.x.

van der Sluis LW, Vogels MP, Verhaagen B, Macedo R, Wesselink PR. Study on the influence of refreshment/activation cycles and irrigants on mechanical cleaning efficiency during ultrasonic activation of the irrigant. J Endod. 2010 Apr;36(4):737-40. https://doi.org/10.1016/j.joen.2009.12.004.

Walia HM, Brantley WA, Gerstein H. An initial investigation of the bending and torsional properties of Nitinol root canal files. J Endod. 1988 Jul;14(7):346-51. https://doi.org/10.1016/S0099-2399(88)80196-1.

Webber J. Shaping canals with confidence: WaveOne GOLD single-file reciprocating system. Int Dent African Ed. 2015;6(3):6-17.

Wei X, Ling J, Jiang J, Huang X, Liu L. Modes of failure of ProTaper nickel-titanium rotary instruments after clinical use. J Endod. 2007 Mar;33(3):276-9. https://doi.org/10.1016/j.joen.2006.10.012.

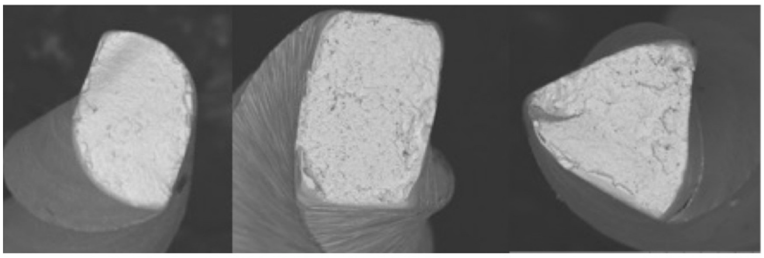
Weller RN, Brady JM, Bernier WE. Efficacy of ultrasonic cleaning. J Endod. 1980 Sep;6(9):740-3. https://doi.org/10.1016/S0099-2399(80)80185-3.

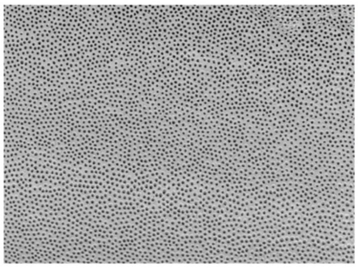
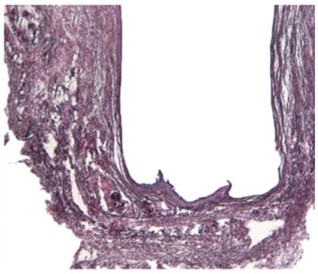
Zarrabi MH, Javidi M, Jafarian AH, Joushan B. Histologic assessment of human pulp response to capping with mineral trioxide aggregate and a novel endodontic cement. J Endod. 2010;11:1778–81. 12

Zehnder M. Root canal irrigants. J Endod. 2006 May;32(5):389-98. https://doi.org/10.1016/j.joen.2005.09.014.

VIII. Anexo 1

Instrução Normativa N.o014-CPPGCO, de 14 de março de 2013



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

INSTRUÇÃO NORMATIVA N.º 014-CPPGCO, de 14 de março de 2013.

Dispõe sobre as Normas para Redação de Dissertações e Teses do Programa de Pós-Graduação em CIÊNCIA ODONTOLÓGICA, de acordo com a Resolução UNESP-24, DE 24/02/2012.

O Coordenador do Programa de Pós-Graduação em Ciência Odontológica desta Faculdade, considerando a decisão do Conselho do Programa, por ocasião de sua reunião, levada a efeito em 08/03/2013, baixa a seguinte instrução normativa:

Artigo 1º - A redação do trabalho de Dissertação ou Tese do aluno do Programa de Pós-Graduação em Ciência Odontológica poderá ser realizada pela forma tradicional ou em formato de artigo.
Artigo 2º - O trabalho em formato de artigo deverá conter os seguintes elementos em sua estrutura:
I – Trabalho resultando em somente um artigo
1. Capa;
2. Folha de Rosto;
3. Ficha catalográfica (no verso da folha de rosto);
4. Dados Curriculares (facultativo);
5. Dedicatória (facultativo);
6. Agradecimentos (facultativo);
7. Epígrafe (facultativo);
8. Título e Resumo em Português;
9. Título e Resumo em Inglês (Abstract);
10. Lista de figuras (facultativo);
11. Lista de tabelas (facultativo);
12. Lista de abreviaturas (facultativo);
13. Sumário;
14. Artigo na íntegra (redigido nas normas do periódico escolhido)
15. Anexos

II – Trabalho resultando em dois artigos ou mais
1. Capa;
2. Folha de Rosto;
3. Ficha catalográfica (no verso da folha de rosto);
4. Dados Curriculares (facultativo);
5. Dedicatória (facultativo);
6. Agradecimentos (facultativo);
7. Epígrafe (facultativo);
8. Título e Resumo Geral em Português;
9. Título e Resumo Geral em Inglês (Abstract);
10. Lista de figuras (facultativo);
11. Lista de tabelas (facultativo);
12. Lista de abreviaturas (facultativo);
13. Sumário;
14. Introdução Geral (com referências inseridas como notas de rodapé);
15. Artigos na íntegra (redigido nas normas do periódico escolhido)
16. Anexos

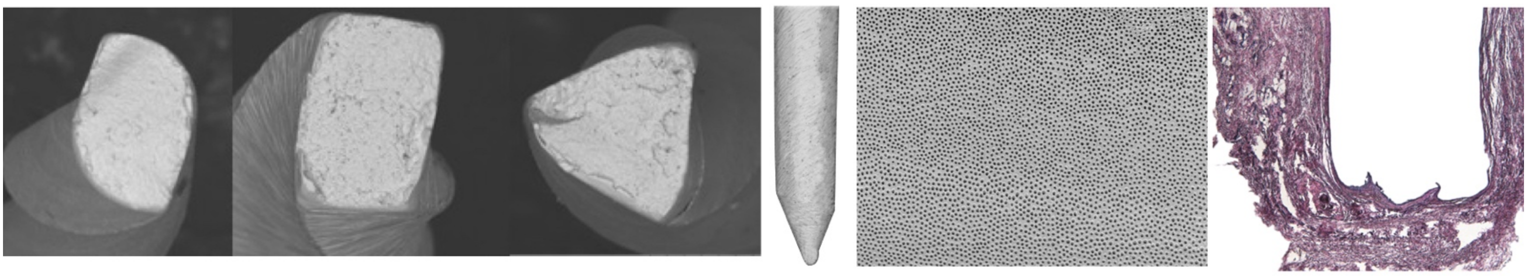
Parágrafo único – Os anexos deverão obrigatoriamente conter as normas dos periódicos nos quais os artigos foram redigidos. Além disso, toda a informação que vier a complementar o trabalho e que não constar no texto do artigo deverá ser colocada sob a forma de anexos. Estes poderão incluir, mas não se limitam a, imagens, fluxogramas, protocolos laboratoriais, tabelas, etc.
Artigo 3º - Esta instrução normativa entra em vigor na data de sua publicação.

Conselho do Programa, 14 de março de 2013.

Prof. Adj. ALBERTO CARLOS BOTAZZO DELBEM
Coordenador do Programa

IX. Anexo 2

Certificado do Comitê de Ética no Uso de Animais - CEUA



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Projeto de Pesquisa intitulado **"Avaliação da resposta tecidual e capacidade de biomineralização de materiais endodônticos bioceramicos"**, Processo FOA nº 00225-2017, sob responsabilidade de Eloi Dezan Júnior apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 19 de Abril de 2017.

VALIDADE DESTE CERTIFICADO: 19 de Abril de 2018.
DATA DA SUBMISSÃO DO RELATÓRIO FINAL: até 19 de Maio de 2018.

CERTIFICATE

We certify that the study entitled **"Biocompatibility and biomineralization assessment of bioceramic endodontic materials"**, Protocol FOA nº 00225-2017, under the supervision of Eloi Dezan Júnior presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on April 19, 2017.

VALIDITY OF THIS CERTIFICATE: April 19, 2018.
DATE OF SUBMISSION OF THE FINAL REPORT: May 19, 2018.

Prof. Ass. Dr. Leonardo Perez Faverani
Coordenador da CEUA
CEUA Coordinator

CEUA - Comissão de Ética no Uso de Animais
Faculdade de Odontologia de Araçatuba
Faculdade de Medicina Veterinária de Araçatuba
Rua José Bonifácio, 1193 – Vila Mendonça - CEP: 16015-050 – ARAÇATUBA – SP
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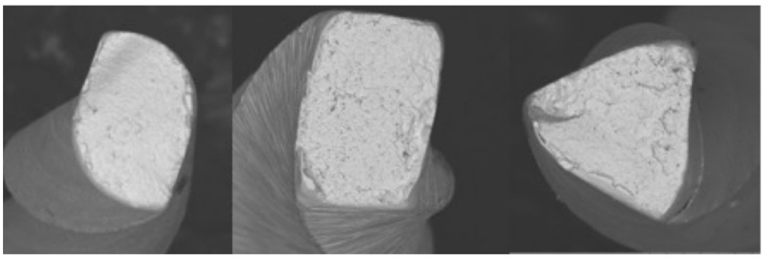
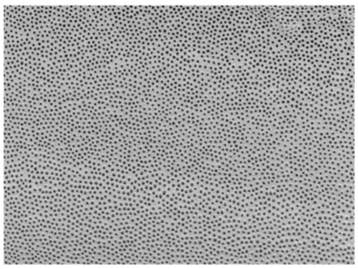
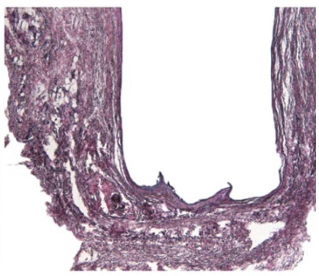
X. Anexo 3

Guia para submissão de Artigos



Guidelines for publishing papers in Brazilian Oral Research

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The manuscript text should be written in English and provided in a digital file compatible with "Microsoft Word" (in DOC, DOCX, or RTF format).

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Bhutta ZA, Darmstadt GL, Hasan BS, Haws RA. Community-based interventions for improving perinatal and neonatal health outcomes in developing countries: a review of the evidence. Pediatrics. 2005;115(2 Suppl):519-617. doi:10.1542/peds.2004-1441.

Usunoff KG, Itzev DE, Rolfs A, Schmitt O, Wree A. Nitric oxide synthase-containing neurons in the amygdaloid nuclear complex of the rat. Anat Embryol (Berl). 2006 Oct 27. Epub ahead of print. doi: 10.1007/s00429-006-0134-9

Walsh B, Steiner A, Pickering RM, Ward-Basu J. Economic evaluation of nurse led intermediate care versus standard care for post-acute medical patients: cost minimisation analysis of data from a randomised controlled trial. BMJ. 2005 Mar 26;330(7493):699. Epub 2005 Mar 9.

Papers with Title and Text in Languages Other Than English
Li YJ, He X, Liu LN, Lan YY, Wang AM, Wang YL. [Studies on chemical constituents in herb of Polygonum orientale]. Zhongguo Ahong Yao Za Zhi. 2005 Mar;30(6):444-6. Chinese.

Supplements or Special Editions
Pucca Junior GA, Lucena EHG, Cawahisa PT. Financing national policy on oral health in Brazil in the context of the Unified Health System. Braz Oral Res. 2010 Aug;24 Spec Iss 1:26-32.

Online Journals
Barata RB, Ribeiro MCSA, De Sordi M. Desigualdades sociais e homicídios na cidade de São Paulo, 1998. Rev Bras Epidemiol. 2008;11(1):3-13 [cited 2008 Feb 23]. Available from: http://www.scielosp.org/pdf/rbepid/v11n1/01.pdf.

Books
Stedman TL. Stedman's medical dictionary: a vocabulary of medicine and its allied sciences, with pronunciations and derivations. 20th ed. Baltimore: Williams & Wilkins; 1961. 259 p.

Books Online
Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

Websites
Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000 [cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Instituto Brasileiro de Geografia e Estatística [homepage]. Brasília (DF): Instituto Brasileiro de Geografia e Estatística; 2010 [cited 2010 Nov 27]. Available from: http://www.ibge.gov.br/home/default.php.

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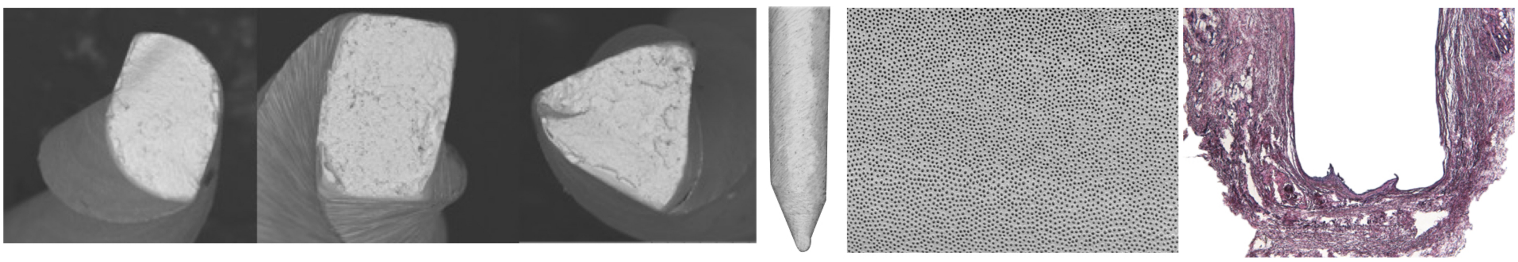
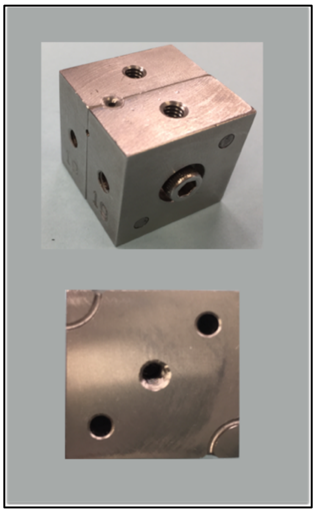
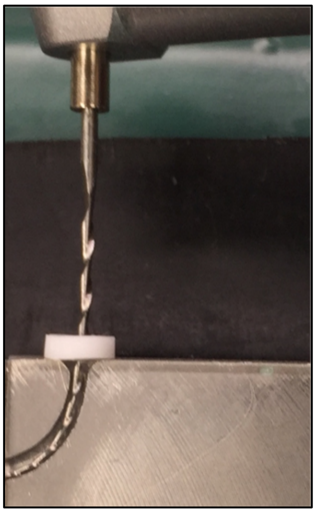
XI. Anexo 4

Figuras metodologias



Figuras metodologia Artigo 1: Avaliação da resistência à fadiga cíclica dos instrumentos reciprocantes de níquel-titânio Genius e EdgeFile

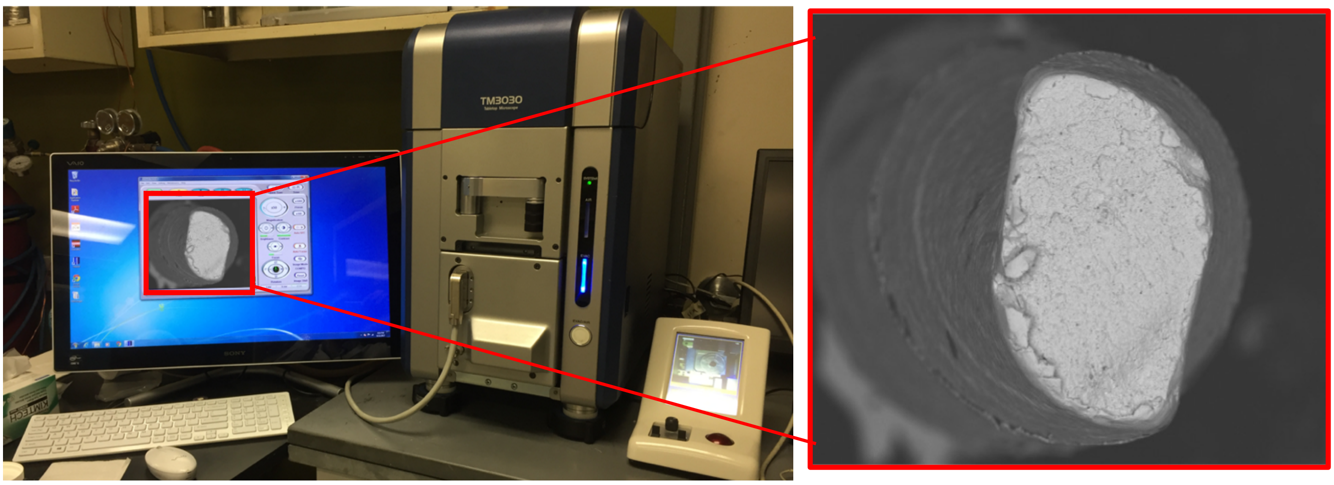




Bloco metálico com canal simulado: 60° de curvatura e 5mm de raio de curvatura;
Instrumento Genius 30.04 em cinemática reciprocante.

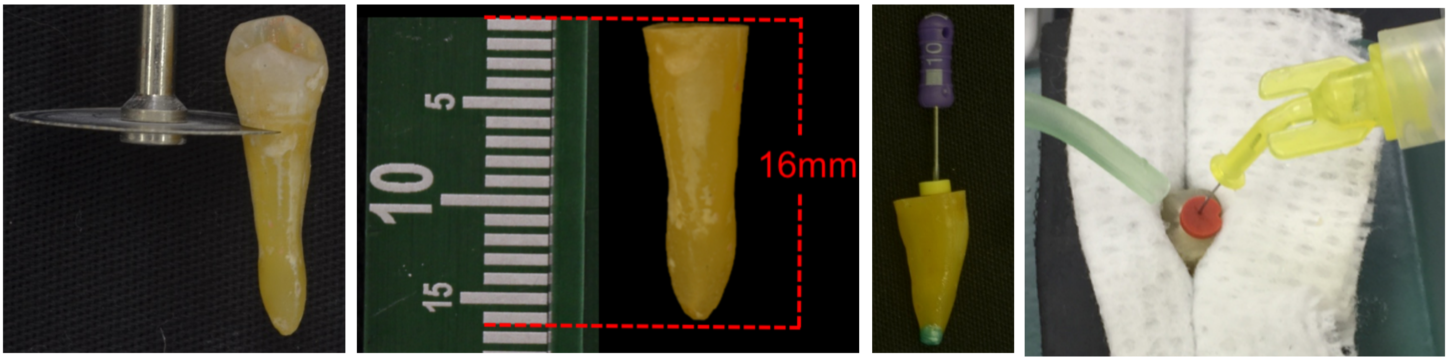
Modelo do cronômetro digital utilizado na pesquisa;
Paquímetro digital utilizado para medição dos fragmentos após fratura, exemplificado na figura acima.

Motores utilizados na pesquisa: à esquerda, motor Genius (Ultradent), utilizado para instrumentos Genius;
À direita: motor E3 torque control (Dentsply), utilizado para os instrumentos Waveone Gold e EdgeFile.

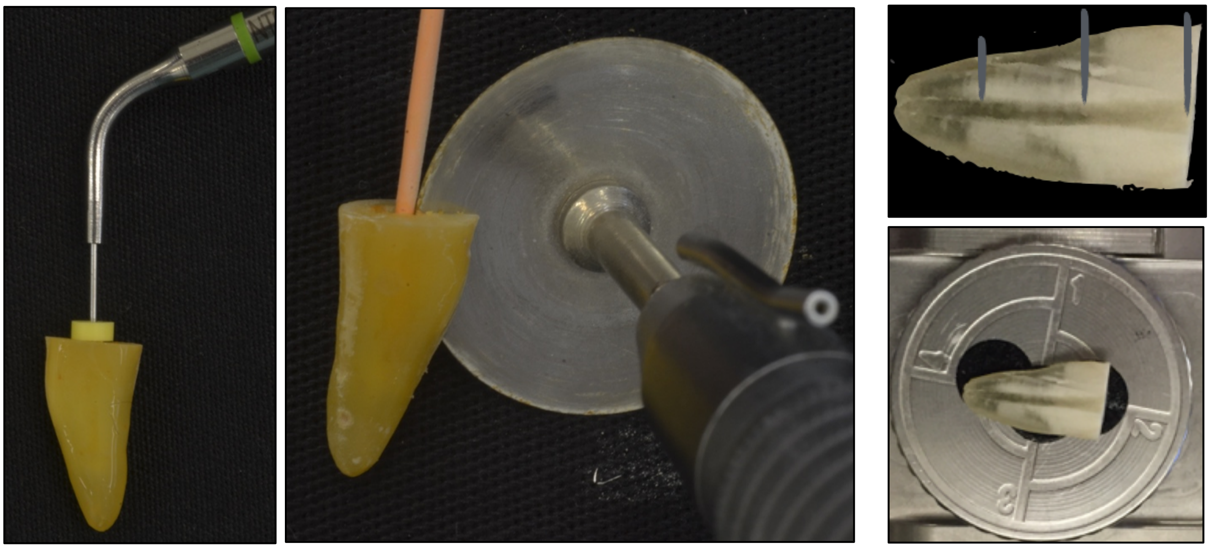


Microscópio eletrônico de varredura (MEV) de baixo vácuo (TableTop Microscope TM3030 Hitachi) utilizado para análise do tipo de fratura (torcional ou dúctil); Imagem capturada em 500x.

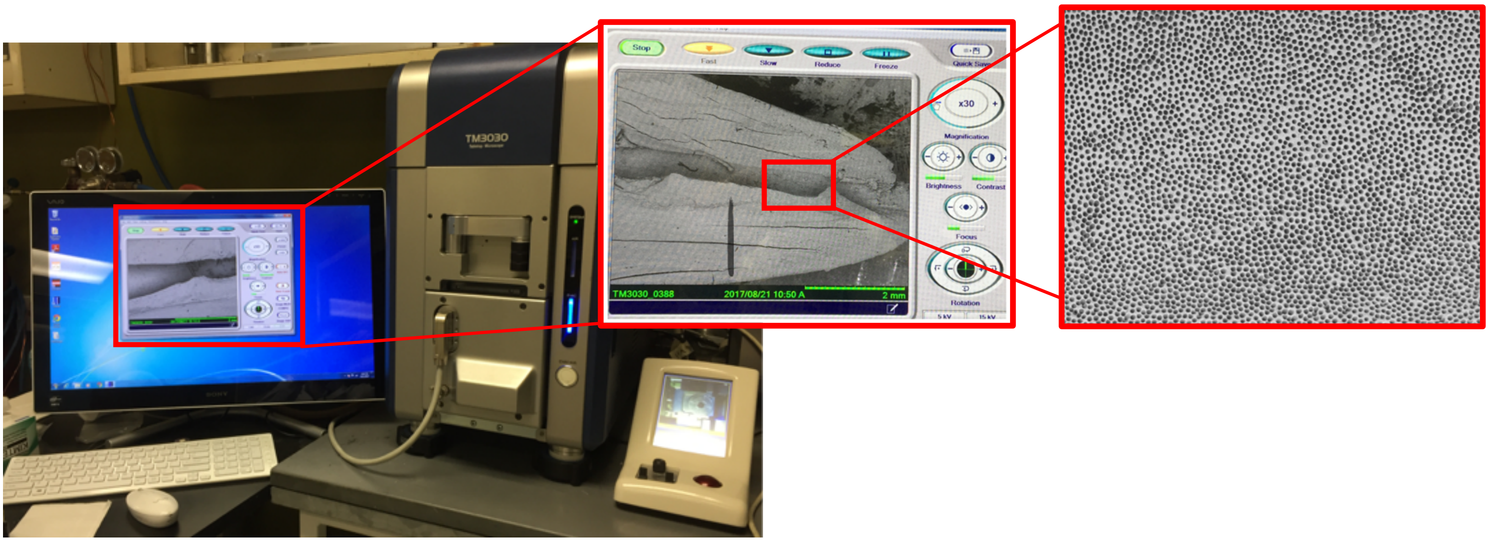
Figuras metodologia Artigo 2: Avaliação da capacidade de limpeza de um inserto ultrassônico de níquel-titânio em irrigação ativada por ultrassom: Um estudo em MEV



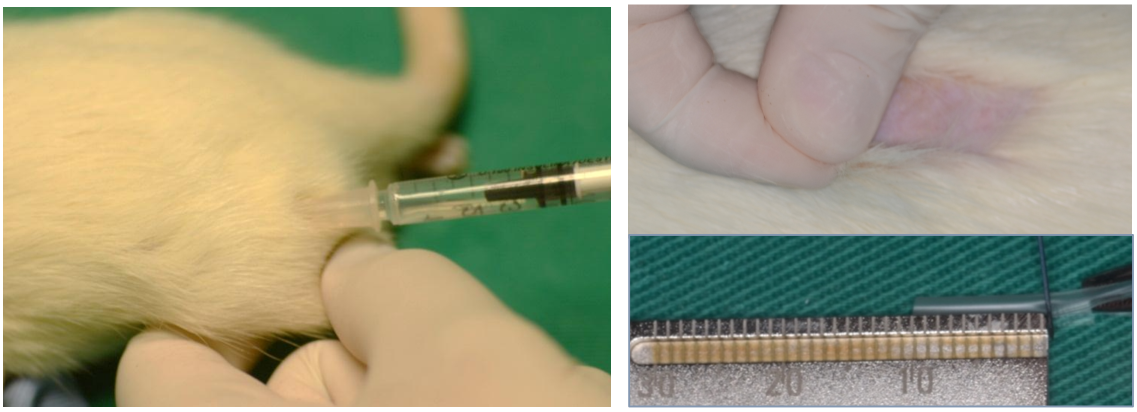
Decoronação de pré-molares inferiores para padronização em 16mm; Lima manual K#10 para obter comprimento real do dente (CRD) e comprimento real de trabalho (CRT= CRD-1mm); Selamento apical com barreira gengival; Instrumentação até lima 50.04 com irrigação/aspiração convencional.



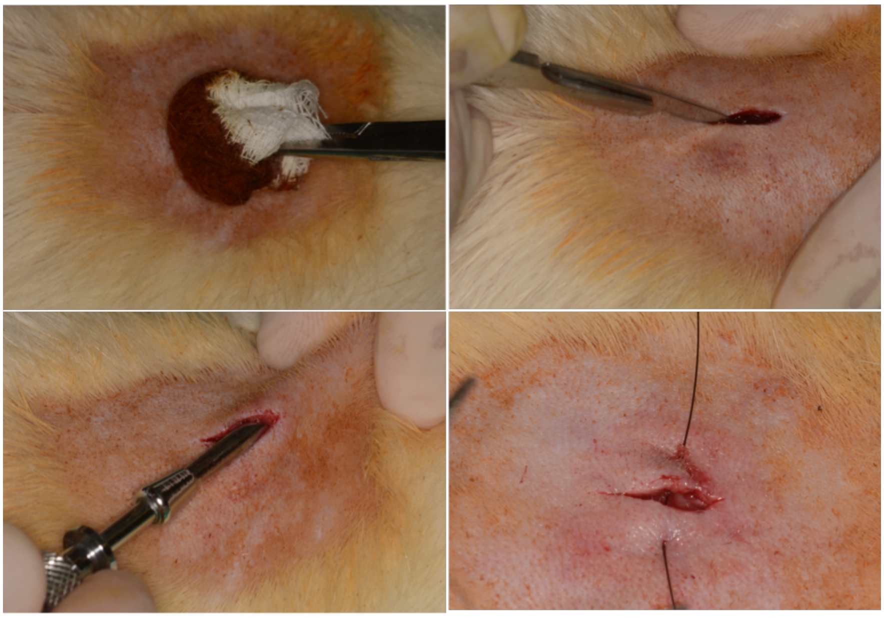
Ativação do irrigante em grupo PUI; clivagem da amostra com cone de guta percha para proteção do canal; marcação com caneta para retroprojetor; posicionamento no microscópio em discos de carbono.

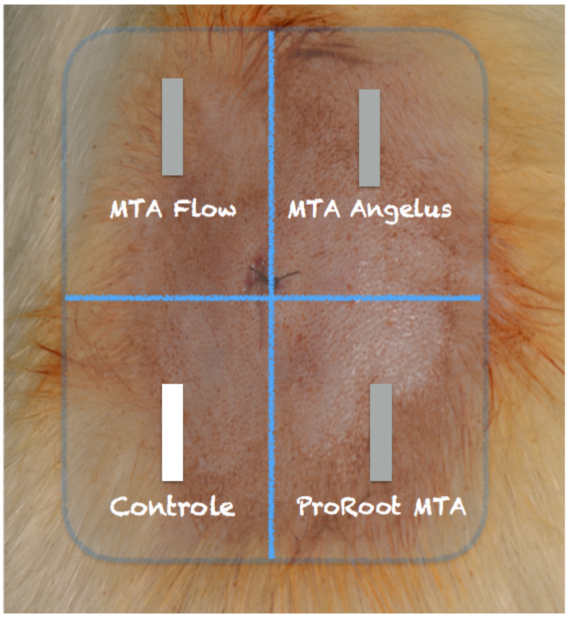


Microscópio eletrônico de varredura (MEV) environmental (Tabletop Microscope TM3030 Hitachi); análise da abertura de túbulos dentinários da amostra em terço apical, observada pela marcação com caneta, em aumento de 500x

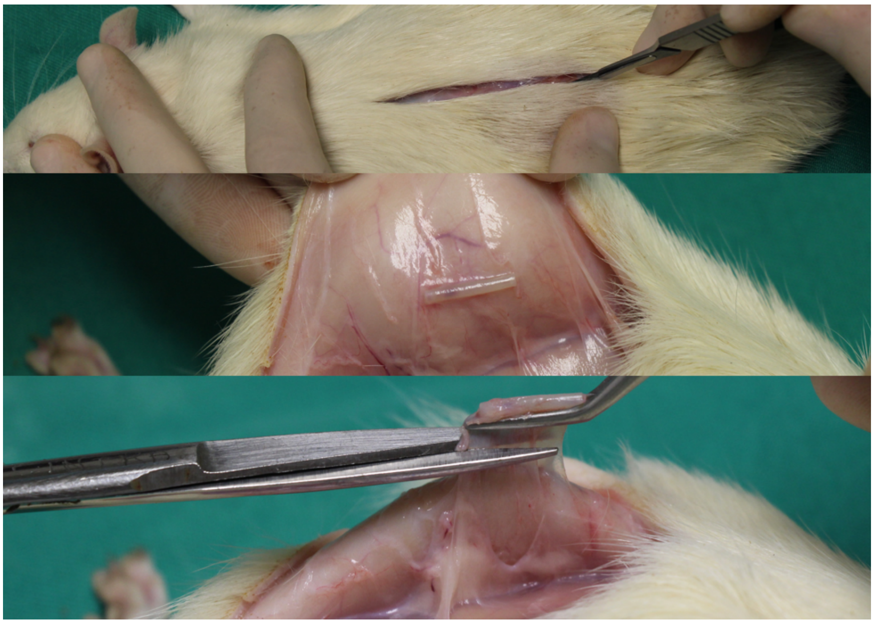


À esquerda: Anestesia intramuscular (xilazina/ketamina 25mg/kg); à direita: tricotomia manual no dorso do animal (área a ser operada); Confecção dos tubos de polietileno em 1 cm a partir de sonda uretral N 4.





Implante subcutâneo: Figura à esquerda: antissepsia da área com iodo; incisão com bisturi (aprox. 1 cm); inserção dos tubos com cimento com o auxílio de um trocarte; sutura simples com fio Nylon 4-0; Figura à direita: esquema das posições dos tubos (modificações das posições após rodizio em alguns ratos).



Após eutanásia (overdose anestésica): remoção dos tubos de acordo com período experimental (7,15,30 e 60 dias); exposição do tubo e remoção com tecido adjacente, seguida da imersão em formol e processamento laboratorial para análise histológica.