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EDIUILSON ILO LISBÔA

AVALIAÇÃO DA INFILTRAÇÃO BACTERIANA E PENETRAÇÃO INTRATUBULAR DE MTA FILLAPEX E AH PLUS EM DENTES HUMANOS OBTURADOS – ESTUDO *IN VITRO*.

Curitiba
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SUMÁRIO

ARTIGO EM PORTUGUÊS

Página título

AVALIAÇÃO DA INFILTRAÇÃO BACTERIANA E PENETRAÇÃO INTRATUBULAR DE MTA FILLAPEX E AH PLUS EM DENTES HUMANOS OBTURADOS – ESTUDO *IN VITRO*.

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Resumo

Objetivo: Avaliar in vitro a profundidade de penetração de dois diferentes cimentos endodônticos nos túbulos dentinários e avaliar a infiltração bacteriana em obturações com os cimentos AH Plus e MTA Fillapex.

Materiais e Métodos: 34 pré-molares monorradiculados humanos foram selecionados. As coroas removidas e as raízes padronizadas com 16 mm, divididos aleatoriamente em dois grupos de 15 espécimes, instrumentados com o sistema rotatório Pro Taper, um grupo obturado com o cimento AH Plus (Grupo AH) e outro com MTA Fillapex (Grupo FI) pela técnica de condensação lateral. 4 espécimes foram utilizados como controle negativo e positivo. As raizes foram mantidos em estufa a 37 °C, esterilizados em gás óxido de etileno e montados em eppendorf para avaliação da infiltração coronária de bactérias para *Enterococcus Faecalis*. As amostras foram observadas diariamente durante 30 dias para constatação ou não da turvação no meio de cultura em contato com a porção apical. Após o primeiro teste, os mesmos espécimes foram seccionados em 5 e 7 mm a partir do ápice, formando uma secção de 2mm de espessura, as faces das secções de 5mm do ápice foram então, metalizadas e analisadas em MEV, para se avaliar a profundidade de penetração nos túbulos dentinários (aumento de 1000x).

Resultados: Para a infiltração bacteriana, considerando o número de amostras infiltradas para AH e MTA Fillapex, usou-se o teste exato de Fisher (p=0,0341) onde houve diferença estatística significativa. Na avaliação da penetração intratubular, quando aplicado o teste-t, a diferença entre os dois grupos de cimentos foi considerada estatisticamente significativa (P=0,0006).

Conclusões: Ambos os cimentos apresentaram infiltração bacteriana sendo que o MTA Fillapex teve pior desempenho, a capacidade de penetração nos túbulos dentinários do AH Plus foi significativamente maior que o MTA Fillapex.

Palavras-chave: cimentos endodônticos, penetração intratubular, infiltração bacteriana, AH Plus, MTA Fillapex.

Introdução

O cimento MTA Fillapex (Angelus, Londrina, PR, Brasil) tem em sua formulação uma base resinosa o que o torna apropriado para o uso com cones obturadores, tem boas características de escoamento, tempo de trabalho, e pH alcalino, características estas importantes na obturação e manutenção da esterilidade do sistema de canais radiculares (1, 2), além disso, é um dos primeiros cimentos endodônticos que tem em sua composição o MTA, que é comparável ao hidróxido de cálcio em termos de reparação tecidual (3), quando aplicado diretamente aos tecidos induz o reparo, sendo um material bioativo (4, 5).

O MTA Fillapex demonstrou ter a capacidade de reparação tecidual, apesar de resposta inflamatória maior quando comparado ao MTA (6). Em análises com infiltração bacteriana, resultados divergentes colocam o MTA Fillapex como semelhante (7) ou inferior ao AH Plus (8). Em relação a penetração tubular dentinária, testes usando microscopia confocal demonstraram uma maior penetração do MTA Fillapex quando comparado ao AH Plus (9).

O cimento resinoso AH Plus (De trey- Dentsply, Konstanz, Germany) é um cimento insolúvel aos tecidos e com boa capacidade de selamento apical, o que mantém níveis de infiltração menores em relação a outros materiais como o Epiphany, Apexit e Ketac-Endo (10, 11, 12). É o cimento de eleição em estudos comparativos de infiltração (7, 8), e penetração intratubular (9) avaliando cimentos novos, como o MTA Fillapex.

Os métodos mais usados para a avaliação da infiltração das obturações, tem sido o de filtração de fluídos (11, 13) e o de infiltração bacteriana (12, 14, 15). Neste as análises biológicas verificam a penetrabilidade da bactéria na interface dentina/material obturador no sentido coroa ápice.

Pesquisas de infiltração bacteriana geralmente são efetuadas com *Enterococcus faecalis* devido ao fato desta cepa ser uma das mais resistentes nos processos inflamatórios periapicais. Além disso, possui grande capacidade de penetração nos túbulos dentinários, sendo capaz de permanecer viável por meses e até anos, mesmo em condições pouco favoráveis como ambientes

alcalinos, podendo tornar-se patogênica quando o ambiente se torna favorável (12, 16, 17, 18).

As bactérias são mais comumente presentes na região do terço médio do que na região apical dos canais (14), a existência de lesões periapicais estimuladas por estas bactérias se torna menos provável quando há um melhor selamento apical dos canais radiculares (19). As bactérias podem manter-se viáveis por um longo período e a sua completa eliminação dos canais somente com a limpeza é difícil alcançar, mesmo que esta seja realizada de forma adequada (18). Com base no exposto o estudo se propôs a analisar por comparação, os cimentos AH Plus e MTA Fillapex pelos métodos de infiltração bacteriana e microscopia eletrônica de varredura (MEV). A hipótese nula é de que não há diferença entre os materiais seladores AH Plus e MTA Fillapex nos dois testes aplicados.

Materiais e Métodos

Trinta e quatro pré-molares inferiores humanos monorradiculados extraídos foram selecionados junto ao Banco de Dentes da PUCPR para esta pesquisa. O estudo foi previamente aprovado pelo Comitê de Ética em Pesquisa com Seres Humanos, sob protocolo n° 1373/2011. Foram selecionados somente dentes com ápice completo, raízes retas e únicas; dentes que apresentavam cáries extensas, ápices abertos, reabsorções ou fissuras radiculares, dilaceração apical ou bifurcações não foram incluídos.

Para comprovar a presença de canal único foram realizadas radiografias pré-operatórias no sentido vestíbulo-lingual e no sentido mésio-distal. As coroas dentárias de todos os dentes foram removidas com disco diamantado, de modo que cada espécime ficasse padronizado com 16 mm de comprimento radicular a partir do ápice.

Instrumentação e obturação dos canais

Para a remoção do tecido pulpar e determinação do comprimento real do canal, foram utilizados instrumentos 15 K-Flexofile (Dentsply-Maillefer, Ballaigues, Suíça). O instrumento foi introduzindo no canal até que a ponta ficasse visível no forame apical. A partir desta medida, 1 mm foi reduzido para se estabelecer o comprimento de trabalho. A patência apical foi confirmada com a inserção de um instrumento 25 K-Flexofile através do forame apical antes e depois do preparo do canal radicular.

Cada canal radicular foi instrumentado por meio da técnica coroa-ápice, utilizando instrumentos rotatórios de níquel-titânio ProTaper (Dentsply Maillefer, Ballaigues, Suíça), finalizando com a lima F4, sendo que todos os instrumentos foram utilizados até o comprimento de trabalho. A cada troca de instrumento os canais radiculares foram irrigados com 3 mL de hipoclorito de sódio 2,5% (NaOCL)

Após a instrumentação, todos os canais radiculares foram lavados com 5 mL de NaOCL 2,5%. Em seguida foram preenchidos com 3 mL de solução de EDTA 17% por 3 minutos para remoção da *smear layer* (20). A irrigação final foi

realizada com 5 mL de solução salina e os canais foram secos com cones de papel absorvente.

Para a realização da obturação dos canais, das 34 raízes preparadas, 4 foram utilizadas para os controles positivo e negativo e 30 foram divididas aleatoriamente em 2 grupos de 15 espécimes cada. Para cada um destes grupos, um tipo de cimento obturador foi empregado.

- Grupo AH (n=15): cones de guta-percha e cimento AH Plus;
- Grupo FI (n= 15): cones de guta-percha e cimento MTA Fillapex.

Todos os canais foram obturados pela técnica da condensação lateral ativa, onde o cimento foi previamente inserido ao canal radicular com auxílio de uma Lentullo nº 4 (Dentsply Maillefer, Ballaigues, Suíça), seguido da introdução do cone principal F4 (Dentsply Maillefer, Ballaigues, Suíça) no comprimento de trabalho. A execução da técnica de condensação lateral foi realizada com auxílio de um espaçador digital C (Dentsply-Maillefer, Ballaigues, Suíça) permitindo a inserção e compactação lateral de cones acessórios B7, até o total preenchimento do canal radicular, após foi feita a compactação vertical com instrumental de Paiva.

Para os testes de infiltração bacteriana 4 espécimes foram preparados como controle. Para o controle positivo dois espécimes foram obturados somente com guta percha, sem adição de nenhum cimento obturador. Para o controle negativo outros dois espécimes foram obturados seguindo as técnicas dos grupos caso (grupo AH e grupo FI), sendo um espécime para cada cimento.

Concluídas as obturações, todos os dentes foram armazenados em estufa a 100% de umidade e 37 °C por 2 semanas para garantir a presa dos materiais.

Teste de infiltração Bacteriana

Avaliação Microbiológica (20).

- **Impermeabilização dos remanescentes radiculares**

Previamente ao início dos testes a superfície externa dos dentes foi impermeabilizada com duas camadas de adesivo epóxi Araldite de presa rápida –

10 minutos (Brascola, Joinville, SC, Brasil). Nos espécimes dos grupos experimentais e controle positivo, a impermeabilização foi realizada até 1 mm aquém da embocadura do canal radicular e do forame apical. Nos espécimes do grupo controle negativo, a impermeabilização incluiu a região do forame apical, permanecendo sem camada impermeabilizadora apenas a embocadura dos canais radiculares.

- **Montagem dos remanescentes radiculares em tubos plásticos e esterilização dos conjuntos**

Os 34 espécimes foram montados para infiltração bacteriana. Para tanto, foram utilizados micro tubos de polipropileno (tubos para centrifuga do tipo eppendorf) com capacidade de 1,5 mL. As pontas dos tubos foram seccionadas transversalmente com um disco de carborundum, de tal forma que, ao inserir os espécimes, aproximadamente 3 mm da sua porção apical ficou projetada para fora do tubo plástico.

A junção entre o remanescente radicular e o eppendorf foi selada com Araldite de presa rápida. Após a montagem dos remanescentes radiculares, os tubos foram identificados com o número correspondente a cada amostra e grupo. Em seguida, os conjuntos (remanescente radicular e tubos de plástico) foram acondicionados em frascos de vidro de 15ml transparentes, estéreis contendo um corte de papel alumínio (5 x 5 cm). Os frascos de vidro contendo os conjuntos foram tamponados com algodão e embalados individualmente para esterilização em gás de óxido de etileno a 56 °C. Todas as etapas subsequentes à esterilização foram realizadas em câmara de fluxo laminar para evitar a contaminação das amostras.

- **Montagem do dispositivo para o teste de infiltração**

Para o teste de infiltração bacteriana foi utilizado caldo de BHI (Brain Heart Infusion) (Acumedia, EUA) preparado segundo as instruções do fabricante (proporção de 37 g de pó para 1000 mL de água destilada), autoclavado a 120 °C durante 20 minutos e mantido em geladeira por 24 horas. Após este período 7 mL da solução BHI foram dispensadas em cada um dos 34 frascos de vidro esterilizados. Os conjuntos (espécimes-tubos plásticos) foram montados no interior dos frascos de vidro de modo que a porção apical dos espécimes ficasse

imersa no caldo de BHI. A parte superior do dispositivo foi recoberta com papel alumínio estéril e parafilme. Os dispositivos foram identificados numericamente e mantidos em estufa a 37 °C, durante quatro dias, para confirmação da esterilidade do conjunto. Após este período, a amostra que mostrasse turvação do caldo BHI estava contaminada e, portanto, era descartada.

- **Preparo do microrganismo**

Cepas padrão de *Enterococcus faecalis* (cepa ATCC19433/Instituto Adolfo Lutz) foram utilizadas para avaliar a infiltração coronária das obturações. Inicialmente foi realizada coloração Gram e análise da morfologia da cepa para confirmação de sua pureza. O microrganismo foi reativado em 4,0 mL de caldo BHI esterilizado e mantido em estufa a 37 °C, durante 24 horas. Esta alíquota, contendo *E. faecalis* foi transferida para um Elenmeyer contendo 100 mL de caldo BHI esterilizado e incubado em estufa a 37 °C, durante 15 horas. Após este período o número de *E. faecalis* presentes no caldo BHI foi avaliado por leitura em espectrofotômetro. A análise foi realizada por meio da contagem de unidades formadoras de colônia por mililitro de microrganismos, padronizando o inóculo em 10^9 UFC/mL.

- **Teste de infiltração coronária**

Para o teste de infiltração coronária, alíquotas de 500 µL de cultura de *E. faecalis* padronizadas foram transferidas para a parte superior dos tubos plásticos ficando em contato com a porção coronária das obturações. Após sete dias, o caldo de BHI inoculado com *E. faecalis* foi trocado por uma nova alíquota de 500 µL de caldo BHI. Uma porção do caldo foi removida e utilizada para o teste de viabilidade bacteriana. As amostras foram observadas diariamente, por um período de 30 dias. Durante este período, se o meio BHI em contato com a porção apical do remanescente radicular se apresentasse turvo, estava constatada a infiltração coronária. Portanto, nestes casos, houve passagem de *E. faecalis* através da obturação do canal radicular.

- **Teste de viabilidade bacteriana**

Os testes viabilidade bacteriana foram realizados com metade dos espécimes de cada grupo alternados semanalmente. A cada troca do caldo BHI,

100 µL do caldo presente na parte superior do tubo plástico e que seriam descartados, foram transferidos para tubos de rosca de 13x100 mm, contendo 4 mL de caldo BHI estéril, e mantidos em estufa a 37 °C. A viabilidade bacteriana foi constatada através da turvação do meio de cultura.

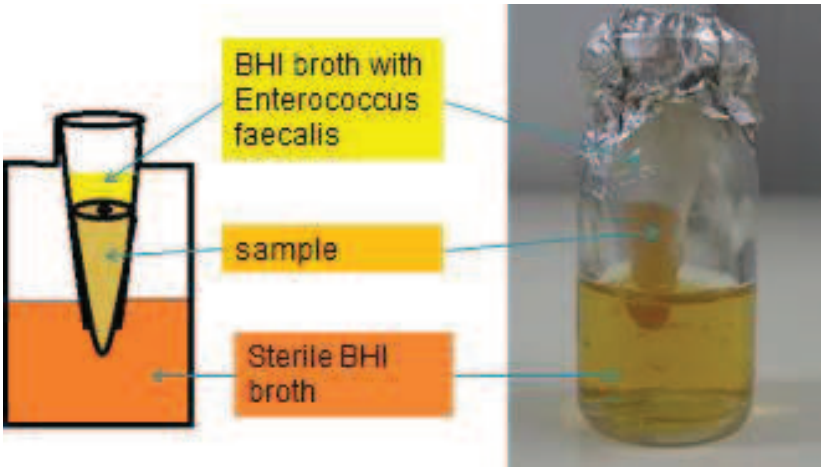

Figura 1 – Montagem dos espécimes

- **Análise dos resultados**

Os dados obtidos com os resultados da infiltração coronária foram tabulados e submetidos à análise estatística, utilizando teste exato de Fisher para a infiltração e Shapiro-Wilk para análise da relação infiltração/dia com nível de 5% de significância.

Avaliações da penetração intratubular por MEV.

Todos os dentes foram seccionados perpendicularmente ao longo eixo da raiz, nas alturas de 5 e 7 mm do ápice da raiz anatômica utilizando disco diamantado de 0,3 mm, em velocidade lenta e com constante refrigeração por água obtendo-se uma secção de 2mm de espessura da amostra de forma circular. A superfície de corte de 5 mm foi selecionada para a análise, sendo, para isso, desmineralizada com EDTA 15% por 10 min. Em seguida, para remoção de resíduos orgânicos, aplicou-se hipoclorito de sódio 5% por 10 min. As amostras foram lavados com água destilada e delicadamente secas com jato de ar, sendo montados em suportes (*stubs*) de alumínio numerados, usando um separador de carbono adesivo, e colocados em estufa a 50 °C por 48 h para completa desidratação. As secções foram levadas ao metalizador ((Denton

Vacuum, mod. Desk II, Moorestown, NJ, EUA) onde foram pulverizadas com ouro antes da observação usando microscópio eletrônico de varredura (MEV). A medida da profundidade máxima de penetração do cimento (Fig.2A) para cada amostra foi obtida por ferramenta de medição calibrada (Fig.2B) incorporada ao sistema de controle do microscópio (JEOL 6390, Tokyo, Japão).

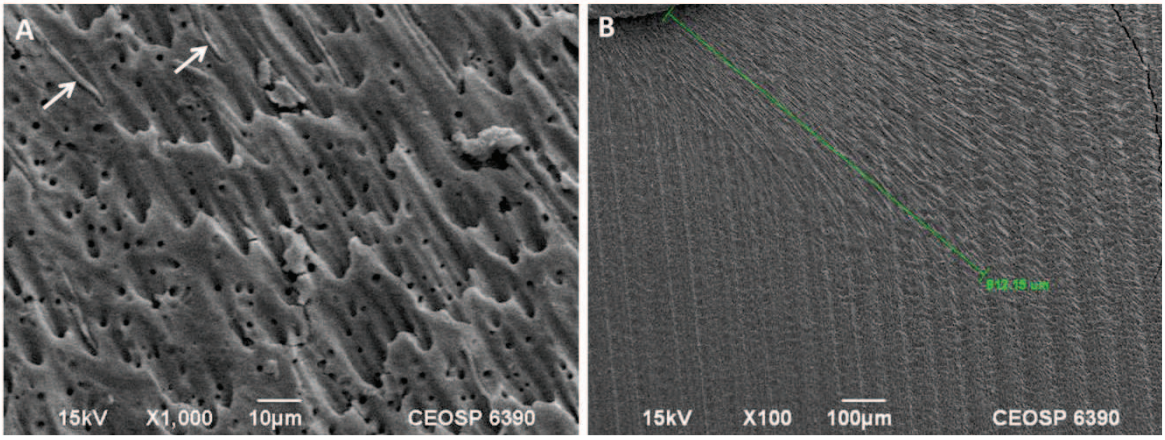

Figura 2 – Imagem de microscopia eletrônica de varredura (MEV) para análise de penetração intratubular. (A) Presença de *tags* de cimento endodôntico. (B) Medição da profundidade de penetração do cimento endodôntico.

Os valores de média e desvio-padrão para a máxima penetração de cimento nos túbulos dentinários de cada um dos grupos experimentais foram calculados. O teste de normalidade aplicado foi o de Shapiro-Wilk. Para análise comparativa da penetração de cada cimento, os resultados foram submetidos ao teste t para amostras independentes. O nível de significância foi estabelecido em 5%.

Resultados

Testes de infiltração bacteriana:

Após 30 dias de análise de infiltração bacteriana, dos 30 espécimes utilizados no estudo, 18 apresentaram infiltração no período de teste. Destes, 12 haviam sido obturados com o cimento endodôntico MTA Fillapex e 6 com o cimento AH Plus (Tabela 1).

	infiltrados	não infiltrados
FI	12	3
AH	6	9

Tabela 1 - Testes de infiltração bacteriana em relação ao tamanho da amostra.

O p valor do teste exato de Fisher para comparação dos grupos (AH e FI) foi igual a 0,0341. Assim, pode-se dizer que no período de 30 dias houve, do ponto de vista estatístico, um maior número de infiltrados no grupo FI (nível de significância de 5%).

Testes de penetração intratubular:

Os testes de penetração intratubular indicaram uma distribuição normal pelos testes de Shapiro-Wilk. Assim, para a variável penetração utilizou-se o teste paramétrico Teste-t para amostras independentes. A análise estatística demonstrou diferença entre os grupos FI e AH ($p < 0,05$).

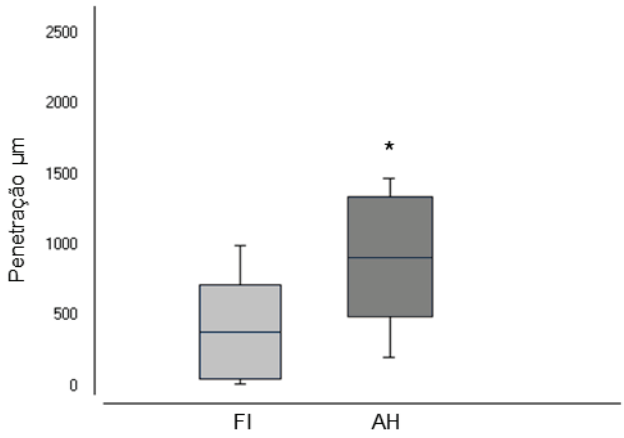

Grafico 1 - Representação gráfica da penetração intratubular (P=0,0006).

Discussão

Em testes de infiltração por fluido, o AH Plus tem se mostrado superior ou semelhante a outros cimentos resinosos (11, 12, 21). Em testes de *push-out* para avaliação da adesão às paredes dentinárias o MTA Fillapex demonstrou ser inferior (22, 23) ou similar (24) ao cimento AH Plus. Em um trabalho com infiltração de corante azul de metileno 2%, a capacidade de selamento apical de AH Plus e MTA foi semelhante, enquanto MTA Fillapex mostrou maior infiltração (25). Os testes de infiltração bacteriana usando o cimento MTA Fillapex tem se mostrado divergentes, sendo então considerado semelhante (7) ou inferior (8) quando comparados ao AH Plus, apesar de nenhum vedar totalmente os canais (7, 8, 26).

O fato de serem metodologias diferentes, tempos diversos e os testes não serem, na sua maioria, com os dois materiais usados neste estudo, dificulta a comparação, mas no presente estudo, as amostras obturadas com AH Plus apresentaram índice de infiltração menor quando comparado com o cimento MTA Fillapex. O cimento AH Plus apresentou, após 30 dias, 6 amostras infiltradas de um total de 15 e o MTA Fillapex 12 de 15. Neste caso a Hipótese nula foi descartada, não concordando com o proposto.

É de conhecimento que o sistema obturador não é capaz de deter a infiltração bacteriana por tempo indeterminado, mas o selamento com materiais obturadores apropriados e restauradores, bem como forradores pode retardar esta contaminação (27).

A imagem produzida usando microscopia eletrônica de varredura (MEV) permite a observação altamente detalhada dos túbulos dentinários, bem como a integridade e a aparência da superfície da interface do cimento e dentina. Permite, ainda, a observação de cimento em locais distantes a partir da parede do canal, possibilitando a medição precisa da profundidade de penetração. A principal desvantagem desta técnica é a incapacidade de obter uma visão geral detalhada em baixa ampliação. Outra desvantagem é o potencial para produção de artefatos durante a preparação das amostras para análise. Situações estas mais fáceis de detectar em trabalhos com corantes e microscopia óptica (26).

A penetração do cimento obturador nos túbulos é potencialmente benéfica para o preenchimento do canal e deve-se às características químicas e físicas

dos cimentos. A penetração pode melhorar a retenção mecânica do material e bloquear mecanicamente os túbulos, o que potencialmente reduz a infiltração (26).

Em estudos usando microscopia confocal, com condensação lateral, o cimento MTA Fillapex apresentou maior penetração nos túbulos do que o AH 26 (28) e AH Plus (9), e, em outro estudo, semelhante ao AH Plus (29). Entretanto, o cimento MTA Fillapex apresenta uma maior solubilidade e quantidade considerável de espaços na interface selante/dentina quando comparado ao cimento AH Plus (29). Esta situação foi comprovada em testes de solubilidade onde o MTA Fillapex obteve maiores alterações morfológicas em todas as superfícies, com perda de matriz evidente, sendo o cimento AH Plus considerado menos solúvel (2, 30, 31). A técnica obturadora tem relevância na penetração de cimentos à base OZE (32), mas não para o AH 26, sugerindo que a penetração dos cimentos à base de resina nos túbulos não é dependente das forças hidráulicas criadas durante o preenchimento e sim arrastado para os túbulos por ação capilar (33).

A penetração de cimento nos túbulos comparada com a infiltração, usando a técnica de infiltração de fluídos, não apresentou correlação tanto na avaliação usando MEV (34) quanto quando utilizada a microscopia optica (35).

No presente trabalho, os dados da penetração intratubular, analisada através de MEV, demonstraram que o MTA Fillapex mostrou resultados estatisticamente inferiores quando comparados ao AH Plus.

Um estudo *in vitro* feito com cimento de Grossman foi efetuado e mesmo sem a limpeza dos túbulos, uma penetração média do cimento foi de 200 μm, e em alguns casos chegou até 900 μm no terço médio (36), o que indica uma média semelhante à que foi encontrada neste trabalho.

No presente trabalho a hipótese nula, onde se acreditava que os materiais se comportariam igualmente foi rejeitada no teste de infiltração bacteriana e também para o teste de penetração intratubular, sendo que o cimento AH Plus (932,36 μm) teve uma maior profundidade de penetração quando comparado ao MTA Fillapex (384,35μm).

Capacidade de escoamento, viscosidade e tamanho das partículas exercem influência significativa na capacidade de penetração dos cimentos no

tecido dentinário. Os testes físico-químicos com o MTA Fillapex indicam que o mesmo está nos padrões ISO 6876/2001(1, 2), o que dificulta o entendimento do motivo pelo qual este cimento teve menor penetração nos túbulos dentinários. Em termos de coibir a penetração da bactéria *Enterococcus Faecalis* seria importante ter mais estudos com a mesma metodologia para analisarmos os dados encontrados, pois os mesmos ainda são conflitantes com pouca literatura existente (7, 8). O método *in vitro* é válido para determinar o selamento e penetração das obturações nos canais radiculares (28), porém, os resultados de pesquisas usando infiltração bacteriana *in vitro* são um indicativo e não podem ser transferidas para as condições *in vivo.* Alguns fatores como o número de bactérias necessário para o início de um processo inflamatório, que ainda permanece desconhecido, e fatores imunológicos, certamente terão efeito sobre o reparo, mas dificilmente poderão ser reproduzidas nos estudos *in vitro*. Fatores como o processamento e limpeza da peça, secagem, variações no dente, tipo de dente, idade do paciente, bem como a utilização de métodos diferentes de visualização podem explicar a diferença de resultados encontrados neste e em outros estudos. Novas pesquisas com metodologias padronizadas seriam importantes para um resultado mais coeso.

Conclusão

Considerando a metodologia empregada e os resultados observados, pode-se concluir que os espécimes obturados com o cimento AH Plus promoveram uma melhor penetração do cimento nos túbulos dentinários em comparação com os obturados com MTA Fillapex.

Em relação à infiltração bacteriana, os resultados mostraram que o MTA Fillapex teve uma maior penetração bacteriana através da coroa do que o AH Plus, sendo esta diferença estatísticamente significante, entre os dois materiais.

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ARTIGO EM INGLÊS

Title page

INFILTRATION ASSESSMENT AND PENETRATION BACTERIAL MTA OF INTRATUBULAR FILLAPEX AND AH PLUS IN HUMAN TEETH FILLED - STUDY IN VITRO.

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Abstract

Objective: To evaluate in vitro penetration depth of two different sealers in dentinal tubules and evaluate bacterial infiltration in fillings with AH Plus and MTA Fillapex cements.

Materials and Methods: Human premolars single rooted were selected. The crowns removed and standardized roots with 16 mm were randomly divided into two groups of 15 specimens instrumented with rotary system Pro Taper, one group obturated with AH cement Plus (AH Group) and the other with MTA Fillapex (FI Group) by lateral condensation technique. 4 specimens were used as negative and positive control. The roots were kept in an oven at 37°C, sterilized in ethylene oxide gas and assembled into eppendorf for assessment of coronary infiltration to bacteria Enterococcus faecalis. The samples were observed daily for 30 days for confirmation or not the turbidity in the culture medium in contact with the apical portion. After the first test, the same specimens were sectioned at 5 and 7 mm from the apex, forming a thickness of 2 mm section, the faces of 5mm sections of the apex were then metallized and analyzed by SEM, to assess the depth penetration in dentinal tubules (magnification 1000x).

Results: For bacterial infiltration, considering the number of infiltrated samples AH and MTA Fillapex, we used the Fisher's exact test ($p = 0.0341$) where there was a statistically significant difference. In the evaluation of intratubular penetration when applied t-test, the difference between the two groups of cement was statistically significant ($P = 0.0006$).

Conclusions: Both cements showed bacterial infiltration and the MTA Fillapex had worse performance, capabilities to enter the dentinal tubules AH Plus was significantly higher than the MTA Fillapex.

Keywords: sealers, intratubular penetration, bacterial infiltration, AH Plus, MTA Fillapex.

Introduction

The MTA Fillapex sealer (Angelus, Londrina, PR, Brazil) has in its formulation a resinous base which makes it suitable for use with shutters cones, has good flow characteristics, working time, and alkaline pH, these important features in filling and maintaining sterility of the root canal system (1, 2), furthermore, one of the first sealers which has in its composition the MTA that is comparable to calcium hydroxide in terms of tissue repair (3) when applied directly to tissues induces the repair, one bioactive materials (4, 5).

The MTA Fillapex shown to have the ability to repair tissue, although greater inflammatory response when compared to MTA (6). In tests with bacterial infiltration, divergent results place the MTA Fillapex as similar (7) or less than AH Plus (8). With respect to dentin tubular penetration tests using confocal microscopy showed a higher penetration when compared to MTA Fillapex AH Plus (9).

The resin cement AH Plus (trey- from Dentsply, Konstanz, Germany) is an insoluble cement to fabrics and with good ability to apical seal, which keeps lower infiltration levels relative to other materials such as Epiphany Apexit and Ketac Endo- (10, 11, 12). It is the preferred cement infiltration in comparative studies (7, 8) and intratubular penetration (9) evaluating new cements such as Fillapex MTA.

The most commonly used methods for evaluating the infiltration of fillings has been the filtration of fluids (11, 13) and the bacterial infiltration (12, 14, 15). This biological analyzes verify the bacteria penetration in the interface dentin / filling material towards apex crown.

Bacterial leakage surveys are usually made with Enterococcus faecalis strain due to the fact this is one of the toughest in periapical inflammatory processes. It also has great penetration capacity in the dentinal tubules, being able to remain viable for months and even years, even in unfavorable conditions such as alkaline environments and can become pathogenic when the environment becomes favorable (12, 16, 17, 18).

The bacteria are most commonly present in the middle third region than in the apical region of the channels (14), the existence of periapical lesions stimulated by these bacteria becomes less likely when there is a better apical seal of the root canal (19). Bacteria can remain viable for a long period and the

complete elimination of the channels only with cleaning is difficult to achieve, even if it is performed adequately (18). Based on the above the study was to analyze by comparison, AH Plus and MTA Fillapex cements the methods of bacterial infiltration and scanning electron microscopy (SEM). The null hypothesis is that there is no difference between the materials sealers AH Plus and MTA Fillapex in both the tests.

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Material and Methods

Thirty-four human premolars extracted monorradiculados were selected by the PUCPR teeth Bank for this research. The study was approved by the Ethics Committee in Research with Human Beings, under n° protocol 1373/2011. Only teeth were selected with complete apex, straight and unique roots; teeth had extensive tooth decay, open apexes, root resorption or cracking apical laceration or forks were not included.

To prove the presence of single channel were performed preoperative radiographs in buccolingually and mesiodistally. Dental crowns of all teeth were removed with diamond disc, so that each specimen stayed standardized 16 mm root length from the apex.

Instrumentation and filling of channels

For the removal of the pulp tissue and determining the real root length were used instruments 15 K-Flexofile (Dentsply-Maillefer, Ballaigues, Switzerland). The instrument was introduced in the channel until the tip was visible at the apical foramen. From this measure 1 mm was reduced to establish the working length. The patency was confirmed by inserting an instrument 25 K-Flexofile through the apical foramen before and after the root canal preparation.

Each root canal was then instrumented by crown-down technique, using rotary instruments of nickel-titanium ProTaper (Dentsply Maillefer, Ballaigues, Switzerland), ending with the F4 file, and all the instruments were used until the length of work. Each exchange tool root canals irrigated with 3 ml of 2.5% sodium hypochlorite (NaOCl - Labarraque solution).

After instrumentation, all root canals were washed with 5 ml of 2.5% NaOCl. Then they were filled with 3 ml 17% EDTA solution for 3 minutes to remove the smear layer (20). A final irrigation was performed with 5 ml of saline solution and the channels were dried with absorbent paper points.

To carry out the filling of canals, 34 roots prepared, 4 were used for the positive and negative controls and 30 were randomly divided into 2 groups of 15 specimens each. For each of these groups, a type of sealer was used.

• AH group (n = 15): gutta-percha and sealer AH Plus;
• FI group (n = 15): gutta-percha and cement MTA Fillapex.

All canals were filled by the technique of lateral condensation, where the cement was previously inserted to the root canal with the help of a Lentullo # 4 (Dentsply Maillefer, Ballaigues, Switzerland), followed by the introduction of the F4 main cone (Dentsply Maillefer, Ballaigues, Switzerland) on the length of work. The implementation of lateral condensation technique was performed with the aid of a digital spacer C (Dentsply-Maillefer, Ballaigues, Switzerland) allowing the insertion and lateral compression B7 accessories cones, until the complete filling of the root canal after the vertical compression is done with instrumental de Paiva.

For bacterial leakage test specimens 4 were prepared as control. For the positive control two specimens were filled only with gutta-percha, without adding any sealer. For the negative control other two specimens were filled following the techniques of case groups (AH group and FI group), one specimen for each cement.

Completed the fillings, all teeth were stored in an oven at 100% humidity and 37 ° C for 2 weeks to ensure the prey of materials.

Bacterial infiltration test

Microbiological Evaluation (20)

- **Waterproofing of root remnants**

Before beginning the tests the outer surface of the teeth was waterproofed with two Araldite epoxy adhesive layer quick setting - 10 minutes (Brascola, Joinville, Brazil). In specimens of the experimental groups and positive control, sealing was carried out by 1 mm below the mouth of the root canal and the apical foramen. Specimens in the negative control group, the waterproofing included the region of the apical foramen, remaining without waterproofing layer only the mouth of the root canals.

- The root remnants Mount plastic pipes and sterilizing sets

The 34 specimens were mounted to bacterial infiltration. To this end, they used micro polypropylene tubes (tubes for centrifuge Eppendorf type) with 1.5 mL capacity. The tips of the tubes were sectioned transversely with a carborundum disk, so that when inserting the specimens, approximately 3 mm from its apical portion was projected out of the plastic tube.

The junction between the root and the remaining eppendorf was sealed with quick-setting Araldite. After assembly of the remaining root, the tubes were labeled with the number corresponding to each sample and group. Then, the sets (root and remainder plastic tubes) were placed in 15ml glass vials transparent, sterile containing a cutting foil (5 x 5 cm). The jars containing the sets were plugged with cotton and individually packaged for sterilization with ethylene oxide gas at 56 ° C. All subsequent steps were performed in the sterilization laminar flow hood to prevent contamination of the samples.

- **Mounting device for leakage testing**

For bacterial penetration test was used BHI broth (Brain Heart Infusion) (Acumedia, USA) prepared according to the manufacturer's instructions (ratio 37 g of powder to 1000 ml of distilled water) and autoclaved at 120 ° C for 20 minutes and kept in the refrigerator for 24 hours. Thereafter 7 mL of the BHI solution was dispensed into each of 34 sterile glass vials. The joint (plastic-tubes specimens) were mounted inside the glass bottles so that the apical portion of the specimens was immersed in BHI broth. The top of the device was covered with aluminum and paper sterile parafilm. The devices were numerically identified and maintained in oven at 37 ° C for four days to confirm the sterility of the assembly. After this period, the sample that showed turbidity of BHI broth was contaminated and therefore was discarded.

- Microorganism preparation

Standard strains of Enterococcus faecalis (ATCC19433 strain / Adolfo Lutz Institute) were used to evaluate the coronal leakage of fillings. Initially Gram staining and analysis of the morphology of the strain to confirm its purity was performed. The microorganism was reactivated in 4.0 ml of sterile BHI broth and maintained in oven at 37 ° C for 24 hours. This aliquot containing E. faecalis was transferred to a Elenmeyer containing 100 ml of sterile BHI broth and incubated in

an incubator at 37 ° C for 15 hours. After this period the number of E. faecalis present in BHI broth was assessed by spectrophotometer reading. The analysis was performed by counting of colony forming units per milliliter of microorganisms, standardizing the inoculum of 109 CFU / mL.

- Coronal leakage test

For coronal leakage test, aliquots of 500 uL standardized culture of E. faecalis were transferred to the top of the plastic tube being in contact with the coronal portion of the filling. After seven days, the BHI broth was inoculated with E. faecalis exchanged for a new aliquot of 500 uL of BHI broth. A portion of the broth was removed and used for the bacterial viability test. Samples were observed daily for a period of 30 days. During this period, the BHI medium in contact with the apical portion of the root remaining cloudy presented itself, the coronal leakage was detected. Therefore, in these cases, there was passage of E. faecalis by root canal filling.

- Bacterial viability test

The bacterial viability tests were performed with half of the specimens of each group alternating weekly. Each exchange BHI broth, 100 uL of this juice on top of the plastic and which would be disposed tube were transferred to a 13x100 mm screw-top tubes containing 4 ml of sterile BHI broth, and maintained in oven at 37 ° C. The bacterial viability was observed by turbidity of the culture medium.

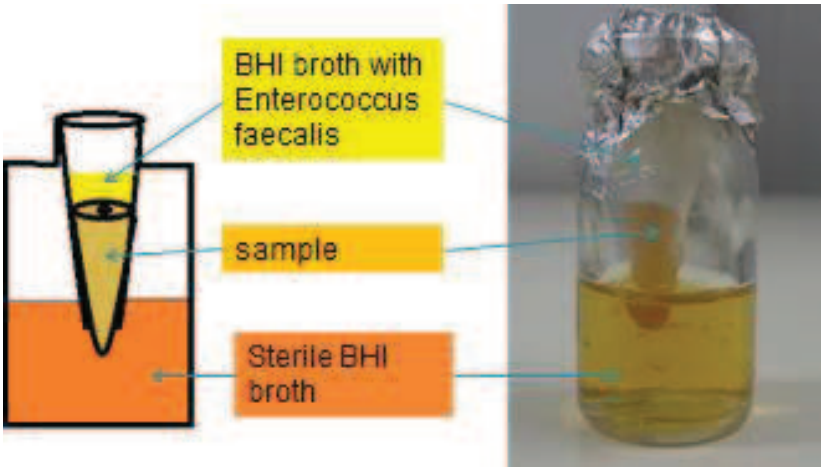


Figure 1 – Installation of the sample

- Analysis of the results

The data obtained from the results of coronal leakage were tabulated and statistically analyzed using Fisher's exact test for infiltration and Shapiro-Wilk for analysis of the relationship infiltration / day with the 5% level of significance.

Reviews penetration intratubular SEM.

All teeth were sectioned perpendicular to the long axis of the root, the heights of 5 to 7 mm of the anatomical root apex using diamond disk of 0.3 mm, with constant speed and slow cooling with water to give a section of 2mm thickness of the sample in a circular fashion. The cut surface of 5 mm was selected for the analysis, and for this, demineralized with 15% EDTA for 10 min, then to remove organic residue was applied to 5% sodium hypochlorite for 10 min. The samples were then washed with distilled water and gently dried with compressed air. The samples were then mounted on supports (stubs) numbered aluminum, using an adhesive carbon tab and placed in an oven at 50 ° C for 48 h to complete dehydration. The sections were then taken to sputter ((Denton Vacuum, mod. Desk II, Moorestown, NJ, USA) where they were sprayed with gold prior to observation using a scanning electron microscope (SEM). The measure of the maximum cement penetration depth (Fig.2A) for each sample was obtained by calibrated measurement tool (Fig.2B) incorporated to the microscope control system (6390 JEOL, Tokyo, Japan).

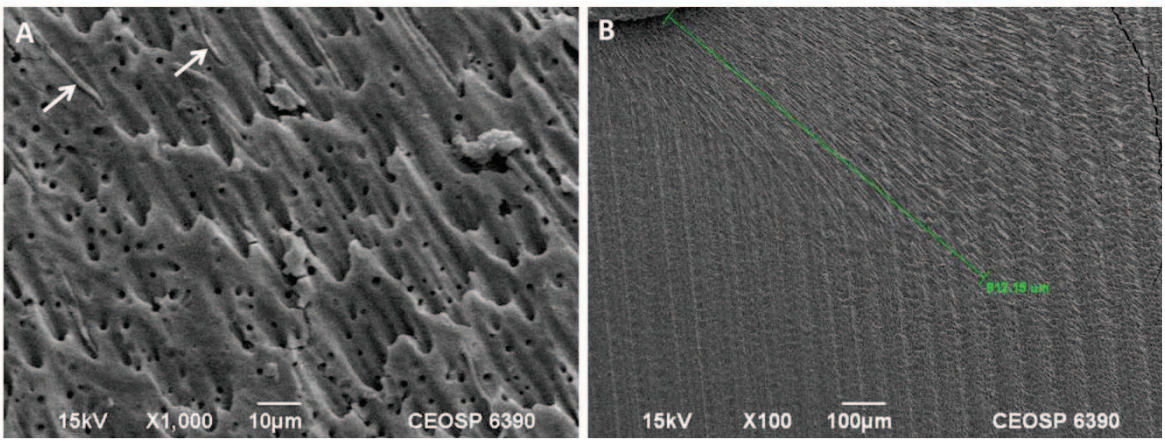


Figure 2 – Image scanning electron microscopy (SEM) to intratubular penetration analysis. (A) Presence of endodontic cement tags. (B) measuring the depth of penetration of the sealer.

The mean and standard deviation for the maximum penetration of the cement dentinal tubules of each of the experimental groups were calculated. The normality test was applied the Shapiro-Wilk. For comparative analysis of each cement penetration was the results were analyzed by t test for independent samples. The level of significance was set at 5%.

Results

Testes de infiltração bacteriana:

After 30 days, analysis of bacterial infiltration of the 30 specimens used in the study, 18 had infiltrating the test period. Of these, 12 had been filled with the sealer and MTA Fillapex 6 with cement AH Plus (Table 1).

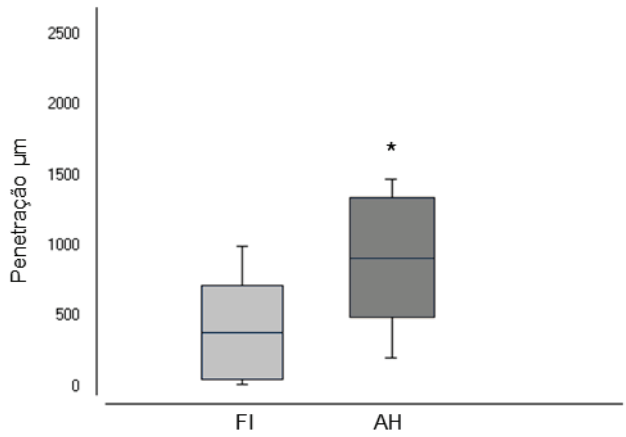
	departed	uninfiltraded
FI	12	3
AH	6	9

Table 1 - bacterial infiltration tests in relation to the size of the sample.

The p value Fisher's exact test to compare groups (AH and FI) was equal to 0.0341. Thus, it can be said that the 30-day period there was a statistical point of view, a greater number of infiltrates in the group FI (5% significance level).

Intratubular penetration tests:

The intratubular penetration tests indicated a normal distribution using the Shapiro-Wilk test. Thus, for variable penetrance used to test the parametric t-test for independent samples. Statistical analysis showed the difference between the FI and AH groups ($p < 0.05$).



Graphical 1 - Graphical representation of intratubular penetration (P = 0.0006).

Discussion

In fluid infiltration tests AH Plus has been shown to be greater than or similar to other resin cements (11, 12, 21). In push-out tests to evaluate the adhesion to the walls dentinal the MTA Fillapex proved to be lower (22, 23) or similar (24) to cement AH Plus. In a paper with blue dye leakage methylene 2%, the apical sealing ability of AH Plus and MTA was similar, while MTA Fillapex showed greater infiltration (25). The bacterial leakage tests using the MTA Fillapex cement has shown conflicting then being considered similar (7) or lower (8) compared to AH Plus, although none completely seal the channels (7, 8, 26).

The fact that they are different methodologies, different times and the tests are not, for the most part, with the two materials used in this study, difficult to compare, but in this study, plugged samples with AH Plus had lower infiltration rate when compared to Fillapex the MTA cement. The AH Plus sealer presented after 30 days, 6 infiltrated samples from a total of 15 and the MTA Fillapex 12 15. In this case the null hypothesis was discarded, not agreeing with the proposal.

It is known that the shutter system is not capable of stopping bacterial infiltration indefinitely, but sealing with appropriate filling materials and restorers as well as liners may slow this contamination (27).

The image produced using scanning electron microscopy (SEM) allows highly detailed observation of dentinal tubules, as well as the integrity and appearance of the surface of the cement and dentin interface. It also allows the cement observation in locations distant from the channel wall, allowing the precise measurement of the depth of penetration. The main disadvantage of this technique is the inability to obtain a comprehensive overview at low magnification. Another drawback is the potential for the production of artifacts during the preparation of samples for analysis. These situations easier to spot when working with dyes and optical microscopy (26).

The penetration of the sealer in the tubules is potentially beneficial to the filling of the canal and is due to chemical and physical characteristics of cement. The penetration can enhance mechanical retention of the material and mechanically blocking the tubules, which potentially reduces the infiltration (26).

In studies using confocal microscopy, with lateral condensation, the MTA Fillapex cement showed the highest penetration in the tubules of the AH 26 (28) and AH Plus (9), and in another study, similar to the AH Plus (29). However, the MTA cement Fillapex presents greater solubility and a considerable amount of space at the interface sealant / dentin compared to AH Plus cement (29). This was confirmed in tests in which the solubility MTA Fillapex more morphological alterations obtained on all surfaces, with obvious loss of matrix and AH Plus considered less soluble cement (2, 30, 31). The technique obturator has relevance in the penetration of cement-based eugenol (32), but not to the HA 26, suggesting that the penetration of resin-based cements in the tubules is not dependent on the hydraulic forces created during filling but drawn to tubules by capillary action (33).

The cement penetration in the tubules compared to infiltration, using the fluid infiltration technique, not correlated both in the evaluation using SEM (34) and when using the optical microscopy (35).

In this study, data from intratubular penetration, analyzed by SEM showed that the MTA Fillapex statistically inferior results when compared to AH Plus.

An in vitro study done with Grossman cement was made and even without cleaning the tubules, an average cement penetration was 200 μm, and in some cases up to 900 μm reached in the middle third (36), which indicates a Average similar to that found in this study.

In this work the null hypothesis, where it was believed that the materials would behave was also rejected in bacterial leakage test and also for intratubular penetration test, and the AH Plus cement (932.36μm) had a greater depth penetration when compared to MTA Fillapex (384,35μm).

Ability to flow, viscosity and particle size exert significant influence on the cement penetration capacity in the dentin tissue. The physical and chemical tests with the MTA Fillapex indicate that it is in the ISO 6876/2001 standards (1, 2), which hinders the understanding of why this cement had lower penetration in the dentinal tubules. In terms of curbing the penetration of Enterococcus faecalis bacteria would be important to have more studies with the same methodology to analyze the data found, as they are still competing with little existing literature (7, 8). The in vitro method is valid to determine the sealing and penetration of fillings

in the root canal (28), however, the results of research using bacterial infiltration in vitro are indicative and may not be transferred to in vivo conditions. Such factors as the number of bacteria required for the initiation of an inflammatory process, which remains unknown, and immunological factors, certainly have effect on the repair, but can hardly be reproduced in vitro studies. Factors such as the processing and cleaning of the workpiece, drying, variations in the tooth, type of tooth, age of the patient as well as the use of different display methods may explain the difference in results of this and other studies. New research with standardized methodologies would be important for a more cohesive result.

Conclusion

Considering the methodology and the results observed, it can be concluded that the specimens filled with AH Plus cement promoted better penetration of cement in dentinal tubules compared to filled with MTA Fillapex.

Regarding the bacterial infiltration, the results showed that the MTA Fillapex had a higher bacterial penetration through the crown of the AH Plus, which is statistically significant difference between the two materials.

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ANEXO

Parecer de comitê de ética

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Pró-Reitoria de Pesquisa e Pós-Graduação
Comitê de Ética em Pesquisa – CEP

Aprovado na
CONEP em 04/08/2000

PARECER 436/2011-CEP

Projeto de Tese de Doutorado, pesquisador responsável: Ediuilson Llo Lisboa do Centro de Ciências Biológicas e da Saúde Campus de Cascavel.

O Comitê de Ética em Pesquisa da Universidade Estadual do Oeste do Paraná analisou em sessão ordinária do dia 24/11/2011, Ata 010/2011 - CEP, o processo CR nº 1373/2011, referente ao projeto, intitulado "Avaliação da Infiltração e penetração intratubular em dentes obturados com os cimentos endodônticos MTA Fillapez e AH Plus".

Período da vigência: Outubro de 2011 a Outubro de 2012.

Assim, em conformidade com os requisitos éticos, somos de parecer favorável à realização do projeto classificando-o como **APROVADO**, pois o mesmo atende aos requisitos fundamentais da Resolução 196/96 e suas complementares do Conselho Nacional de Saúde.
Deverá ser encaminhado ao CEP o relatório final da pesquisa e/ou a publicação de seus resultados, para acompanhamento, bem como comunicada qualquer intercorrência ou a sua interrupção.

Cascavel, 24 de Novembro de 2011.

Aneline M. Ruedell
ANELINE MARIA RUEDELL
Coordenadora do CEP/Unioeste

Metodologia complementar

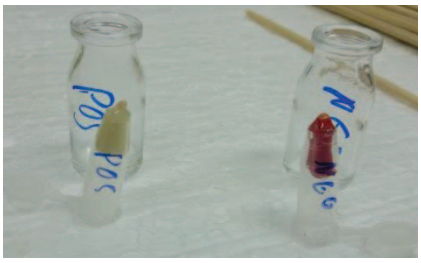

Figura 3 - Eppendorf e recipiente

Figura 4 - Cortadora de precisão

Figura 5 - Disco de Corte

.

Figura 6 - Metalização

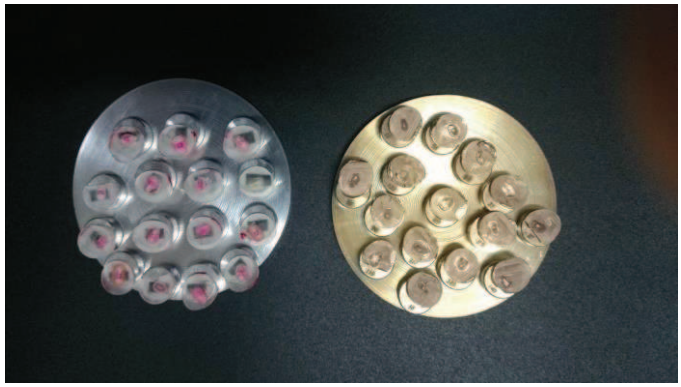

Figura 7 - Espécimes metalizadas (direita) e montadas nos stubs (esquerda)

Figura 8 - Obtenção das imagens

a) **b)** **c)**

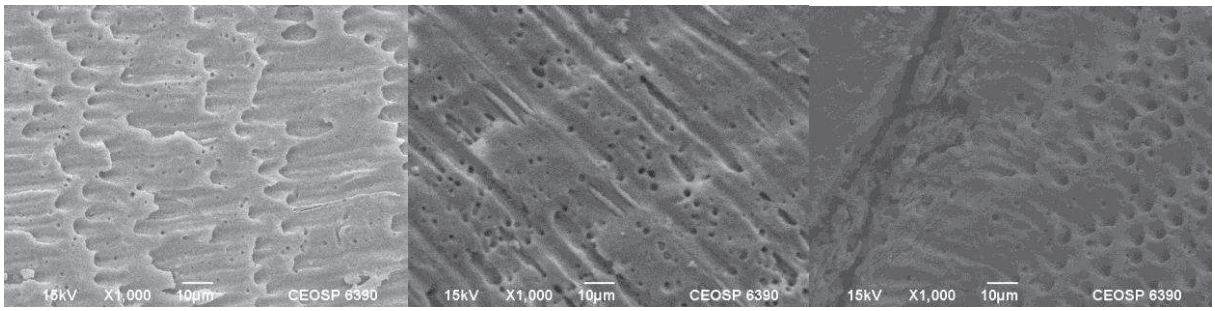

Figura 9 - visualização do cimento no ponto mais distante encontrado do AH Plus (a) do Fillapex (b) e sem penetração do Fillapex (c)

Análise estatística

Teste de normalidade para os dados de penetração intra-tubular (µm)

Teste de Shapiro-Wilk

H0: a variável **penetração** é normalmente distribuída;

H1: a variável **penetração** não apresenta normalidade em sua distribuição;

Resultados	- 1 -	- 2 -
Tamanho da amostra =	15	15
Média =	932.3680	384.0960
Desvio padrão =	426.4659	345.7779
W =	0.9293	0.9006
p =	0.3313	0.0990

Tabela 2 - Representação esquemática dos testes de normalidade (Shapiro-Wilk)

Aceitou-se H0. Os dois grupos apresentam distribuição normal. Assim, para a variável penetração pode-se usar um teste paramétrico.

Teste t para amostras independentes:

H0: não há diferença entre os grupos para a quantidade de penetração;

H1: há diferença entre os grupos para a quantidade de penetração;

	AH Plus	Fillapex
Tamanho =	15	15
Média =	932.3680	384.0960
Variância =	181873.1231	119562.3425
Desvio padrão =	426.4659	345.7779
	Homocedasticidade	---
Variância =	150717.7328	---
t =	3.8676	---
Graus de liberdade =	28	---

p (unilateral) =	0.0003	---
p (bilateral) =	0.0006	---
Poder (0.05)	0.9869	---
Poder (0.01)	0.9379	---
Diferença entre as médias =	548.2720	---
IC 95% (Dif. entre médias) =	257.9490 a 838.5950	
IC 99% (Dif. entre médias) =	156.5911 a 939.9529	---

Tabela 3- Teste t para amostras independentes

Rejeitou-se a H0. Houve diferença estatística significativa entre os grupos para a variável avaliada (p<0,05).

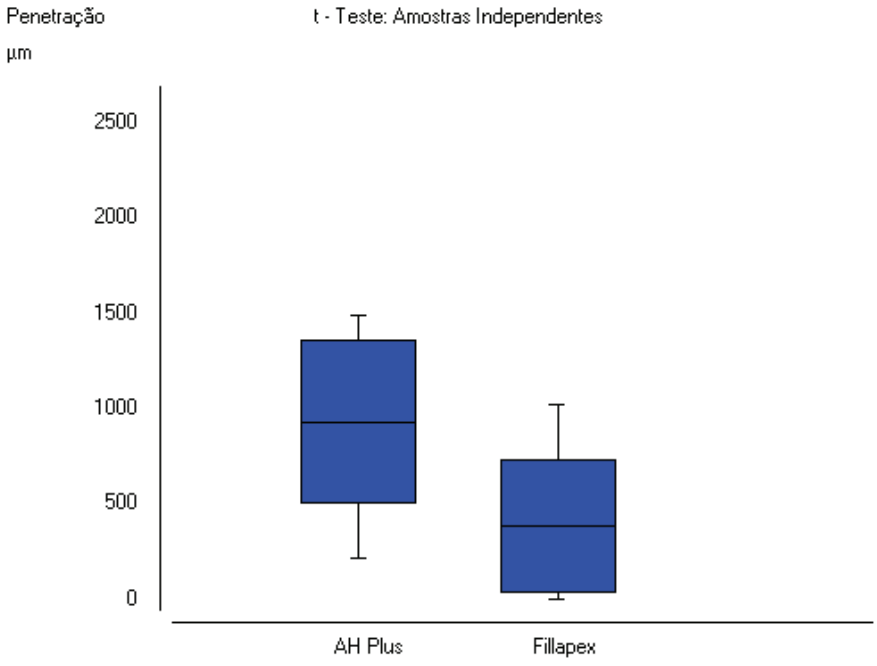

Gráfico 1 - Representação esquemática dos resultados isolados da metodologia de penetração intratubular do AH Plus e Fillapex (em µm).

Resultados Infiltração

Os testes de infiltração bacteriana em relação ao tamanho da amostra:

	infiltrados	não infiltrados
FI	12	3
AH	6	9

Tabela 1 - número de espécimes infiltrados

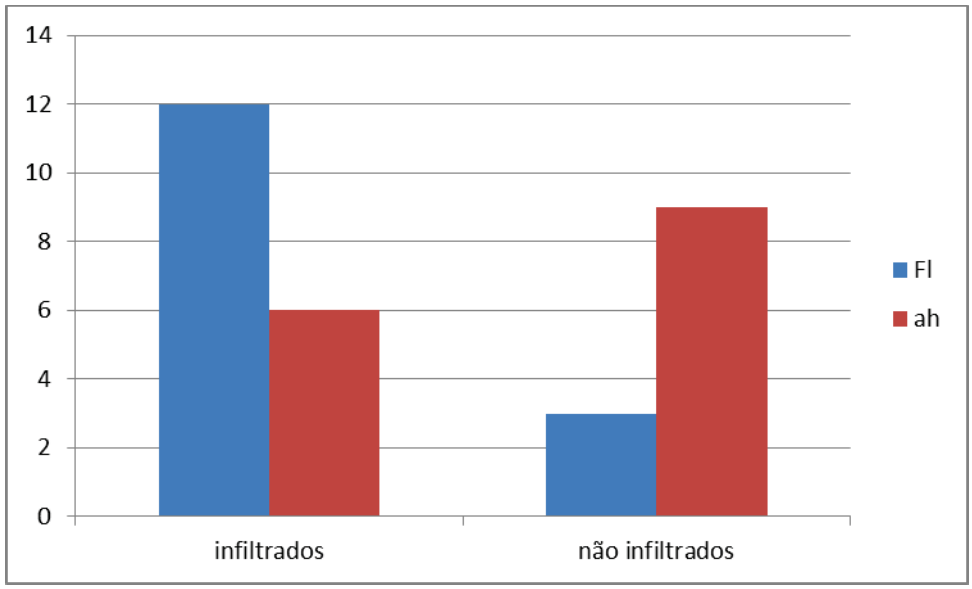

Gráfico 3 - número de espécimes infiltrados

Teste exato de Fisher

O p valor do teste exato de Fisher para comparação dos grupos (ah e Fl) foi igual a 0,0341. Assim, pode-se dizer que no período de 30 dias houve, do ponto de vista estatístico, um maior número de infiltrados no grupo Fl (nível de significância de 5%).

Distribuição da amostra e tabulação:

Dia	FI	Dia	AH	Infiltrações em dias FI	Infiltrações em dias AH
1	0	1	0	0	0
2	1	2	3	2	6
3	2	3	1	6	3
4	1	4	0	4	0
5	1	5	0	5	0
6	0	6	0	0	0
7	2	7	0	14	0
8	0	8	0	0	0
9	0	9	0	0	0
10	0	10	0	0	0
11	0	11	0	0	0
12	0	12	0	0	0
13	0	13	1	0	13
14	1	14	0	14	0
15	1	15	1	15	15
16	0	16	0	0	0
17	0	17	0	0	0
18	0	18	0	0	0
19	0	19	0	0	0
20	0	20	0	0	0
21	1	21	0	21	0
22	0	22	0	0	0
23	0	23	0	0	0
24	0	24	0	0	0
25	0	25	0	0	0
26	0	26	0	0	0
27	1	27	0	27	0
28	0	28	0	0	0
29	1	29	0	29	0
30	0	30	0	0	0
	12		6		

Tabela 6- Resultados da infiltração bacteriana

AH Plus	µm	Fillapex	µm
Amostra 01	1008	Amostra 01	0
Amostra 02	710,5	Amostra 02	308,01
Amostra 03	526,56	Amostra 03	1020
Amostra 04	1350	Amostra 04	670,32
Amostra 05	217,71	Amostra 05	308,76
Amostra 06	1230	Amostra 06	0
Amostra 07	912,15	Amostra 07	0
Amostra 08	1490	Amostra 08	728,38
Amostra 09	233,03	Amostra 09	209,7
Amostra 10	1280	Amostra 10	164,98
Amostra 11	1090	Amostra 11	230,24
Amostra 12	1450	Amostra 12	531,03
Amostra 13	1260	Amostra 13	850,11
Amostra 14	494,51	Amostra 14	0
Amostra 15	733,06	Amostra 15	739,91
Média	932,368		384,096

Tabela 7- Resultados da infiltração penetração intratubular em MEV

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Preparation

General Points on Composition
Authors are strongly encouraged to analyze their final draft with both software (eg, spelling and grammar programs) and colleagues who have expertise in English grammar. References listed at the end of this section provide a more extensive review of rules of English grammar and guidelines for writing a scientific article. Always remember that clarity is the most important feature of scientific writing. Scientific articles must be clear and precise in their content and concise in their delivery because their purpose is to inform the reader. The Editor reserves the right to edit all manuscripts or to reject those manuscripts that lack clarity or precision or that have unacceptable grammar or syntax. The following list represents common errors in manuscripts submitted to the Journal of Endodontics:

a. The paragraph is the ideal unit of organization. Paragraphs typically start with an introductory sentence that is followed by sentences that describe additional detail or examples. The last sentence of the paragraph provides conclusions and forms a transition to the next paragraph. Common problems include one-sentence paragraphs, sentences that do not develop the theme of the paragraph (see also section “c,” below), or sentences with little to no transition within a paragraph.

b. Keep to the point. The subject of the sentence should support the subject of the paragraph For example, the introduction of authors’ names in a sentence changes the subject and lengthens the text. In a paragraph on sodium hypochlorite, the sentence, “In 1983, Langeland et al, reported that sodium hypochlorite acts as a lubricating factor during instrumentation and helps to flush debris from the root canals” can be edited to: “Sodium hypochlorite acts as a lubricant during instrumentation and as a vehicle for flushing the generated debris (Langeland et al, 1983).” In this example, the paragraph’s subject is sodium hypochlorite and sentences should focus on this subject.

c. Sentences are stronger when written in the active voice, that is, the subject performs the action. Passive sentences are identified by the use of passive verbs such as “was,” “were,” “could,” etc. For example: “Dexamethasone was found in this study to be a factor that was associated with reduced inflammation,” can be edited to: “Our results demonstrated that dexamethasone reduced inflammation.” Sentences written in a direct and active voice are generally more powerful and shorter than sentences written in the passive voice.

d. Reduce verbiage. Short sentences are easier to understand. The inclusion of unnecessary words is often associated with the use of a passive voice, a lack of focus, or run-on sentences. This is not to imply that all sentences need be short or even the same length. Indeed, variation in sentence structure and length often helps to maintain reader interest. However, make all words count. A more formal way of stating this point is that the use of subordinate clauses adds variety and information when constructing a paragraph. (This section was written deliberately with sentences of varying length to illustrate this point.)

e. Use parallel construction to express related ideas. For example, the sentence, “Formerly, endodontics was taught by hand instrumentation, while now rotary instrumentation is the common method,” can be edited to “Formerly, endodontics was taught using hand instrumentation; now it is commonly taught using rotary instrumentation.” The use of parallel construction in sentences simply means that similar ideas are expressed in similar ways, and this helps the reader recognize that the ideas are related.

f. Keep modifying phrases close to the word that they modify. This is a common problem in complex sentences that may confuse the reader. For example, the statement, “Accordingly, when conclusions are drawn from the results of this study, caution must be used,” can be edited to “Caution must be used when conclusions are drawn from the results of this study.”

g. To summarize these points, effective sentences are clear and precise, and often are short, simple and focused on one key point that supports the paragraph’s theme.

h. Authors should be aware that the JOE uses iThenticate, plagiarism detection software, to ensure originality and integrity of material published in the journal. The use of copied sentences, even when present within quotation marks, is highly discouraged. Instead, the information of the original research should be expressed by the new manuscript author’s own words, and a proper citation given at the end of the sentence. Plagiarism will not be tolerated and manuscripts will be rejected or papers withdrawn after publication based on unethical actions by the authors. In addition, authors may be sanctioned for future publication.

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Abstract Headings
Introduction, Methods, Results, Conclusions

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Acknowledgements

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Tables are appropriate when it is critical to present exact numeric values; however, not all results need be placed in either a table or figure. Instead of a simple table, the results could state that there was no inhibition of growth from 0.001%-0.03% NaOCl, and a 100% inhibition of growth from 0.03%-3% NaOCl (N=5/group). If the results are not significant, then it is probably not necessary to include the results in either a table or as a figure.

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