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SARA RODRIGUES RENOVATO

Adaptação marginal de cimentos à base de silicato de cálcio à parede dentinária de cavidades retrógadas

Goiânia
2018

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SARA RODRIGUES RENOVATO

Adaptação marginal de cimentos à base de silicato de cálcio à parede dentinária de cavidades retrógadas

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Goiás para obtenção do título de Doutor em Ciências da Saúde

Orientador: Prof. Dr. Carlos Estrela

Co-orientador: Prof. Dr. Julio Almeida Silva

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Ata **da Defesa de Tese de Doutorado** realizada por **Sara Rodrigues Renovato**. **Aos cinco dias do mês de julho do ano de 2018, às 08:30 hs**, reuniu-se no **Auditório da Faculdade de Odontologia/UFG** a Comissão Julgadora infra nomeada para proceder ao julgamento da **Defesa de Tese** intitulada: "**Adaptação marginal de cimentos à base de silicato de cálcio à parede dentinária de cavidades retrógradas"**, como parte de requisitos necessários à obtenção do **título de Doutor**, área de concentração **Patologia Clínica e Tratamento das Doenças Humanas.** O Presidente da Comissão julgadora, **Prof. Dr. Carlos Estrela**, iniciando os trabalhos concedeu a palavra a candidata, para exposição em até **50** minutos do seu trabalho. A seguir, o senhor presidente concedeu a palavra, pela ordem sucessivamente, aos Examinadores, os quais passaram a argüir a candidata durante o prazo máximo de 30 minutos, assegurando-se a mesma igual prazo para responder aos Senhores Examinadores. Ultimada a argüição que se desenvolveu nos termos regimentais, a Comissão, em sessão secreta, expressou seu Julgamento, considerando a candidata aprovada ou reprovada.

Banca Examinadora	**Aprovado(a)/Reprovado(a)**
Prof. Dr. Carlos Estrela - Presidente	Aprovada
Profa. Dra. Ana Helena Gonçalves Alencar – Membro	Aprovada
Prof. Dr. Daniel de Almeida Decúrcio – Membro	Aprovada
Prof. Dr. Jesus Djalma Pécora – Membro	Aprovada
Profa. Dra. Patrícia Correia Siqueira - Membro	Aprovada
Prof. Dr. Júlio Almeida Silva – Suplente	
Prof. Dr. João Batista de Souza – Suplente	

Em face do resultado obtido, a Comissão Julgadora considerou a candidata **Sara Rodrigues Renovato** Habilitada ( ) Não habilitada ( ). Nada mais havendo a tratar, eu **Prof. Dr. Carlos Estrela,** lavrei a presente ata que, após lida e achada conforme foi por todos assinada.

	Assinatura
Prof. Dr. Carlos Estrela - Presidente	[signature]
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A banca examinadora aprovou a seguinte alteração no título da Tese:

Sara Rodrigues Renovato

Sara Rodrigues Renovato

Programa de Pós-Graduação em Ciências da Saúde
da Universidade Federal de Goiás

BANCA EXAMINADORA DA TESE DE DOUTORADO

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Data: 05/07/18

DEDICATÓRIA

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“Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas Graças a Deus, não sou o que era antes”

Marthin Luther King

RESUMO

Objetivo: Analisar a adaptação marginal de cimentos de silicato de cálcio em cavidade retrógada, por meio de MEV. **Materiais e métodos:** Foram utilizadas 50 raízes de incisivos bovinos, que foram instrumentadas com instrumento manual K-File até nº 60 e obturadas com MTA Fillapex® e cones de gutta percha. Após a obturação, as raízes foram apicetomizadas a 3mm do ápice e as cavidades retrógadas foram preparadas com uso de ultrassom. As amostras foram divididas aleatoriamente em cinco grupos experimentais (n=10): 1. Cimento biocerâmico Mk Life; 2. Biodentine®; 3. Bio-C Repair®; 4. Endosequence® BC RRM™; 5. MTA Angelus® (MTA) – controle positivo. Os materiais foram manipulados conforme especificações dos fabricantes e inseridos e adaptados na cavidade com ponta aplicadora, espátula e kit Bernabé. Utilizou-se o EDTA previamente à inserção do material retrobturador em metade das amostras de cada grupo (n=5). As amostras foram preparadas para MEV e foram obtidas fotomicrografias em aumentos de 40x, 150x e 500x. As imagens foram analisadas quanto a presença de fendas e classificada em *scores*: 0 – ausência de fendas; 1 – presença de fenda em 1 área; 2 – presença de fenda em 2 áreas; 3 – presença de fenda em 3 áreas; 4 – presença de fenda em 4 áreas. A análise da dimensão transversal da fenda foi realizada, por meio de mensurações (µm) com o uso do software *Image J*. As variáveis qualitativas foram avaliadas pelo Teste Exato de Fisher, e as variáveis quantitativas pelo Teste de Análise de Variância (ANOVA). O nível de significância foi de 0,05%. **Resultados:** Não observou-se presença de fendas no grupo MTA Angelus® (0%). Presença de fenda foi observada no Cimento biocerâmico MK Life (90%), Biodentine® (80%), Bio-C Repair® (100%) e Endosequence® BC RRM™ (80%). Diferença significativa foi observada quando o MTA foi comparado ao Cimento biocerâmico MK Life, Biodentine® e Bio-C Repair® e Endosequence® BC RRM™ ($p<0,05$). Em relação a dimensão transversal da fenda, não foram observadas diferenças significantes entre os grupos Cimento Biocerâmico MK Life, Biodentine®, Bio-C Repair® e Endosequence® BC RRM™. O MTA não apresentou fendas, mostrando resultado significativamente superior aos grupos do Cimento

Biocerâmico MK Life e Bio-C Repair® ($p<0,05$), porém, sem diferença significante com os grupos Biodentine®, Endosequence® BC RRM™. O uso do EDTA não influenciou na adaptação marginal dos materiais ($p>0,05$). **Conclusão**: O MTA Angelus® apresentou melhor adaptação marginal quando comparado ao Cimento Biocerâmico MK Life, Biodentine®, BioC Repair® e Endosequence® BC RRM™. Não observou-se presença de fendas marginais nas amostra do grupo MTA Angelus®.

Palavras-chave: Adaptação marginal dentária; Cimentos dentários; Microscopia eletrônica de varredura.

ABSTRACT

Objective: To analyse the marginal adaptation of calcium silicate-based cements in root-end cavity by SEM. **Material and Methods:** Fifty bovine roots were prepared with K-File #60 and filled with gutta-percha and MTA Fillapex. Roots were apicetomized and a 3-mm-deep root-end cavity was prepared using ultrasonic tips. Samples were randomly divided into five groups (*n* = 10): 1. Mk Life bioceramic cement; 2. Biodentine®; 3. Bio-C Repair®; 4. Endosequence® BC RRM™; 5. MTA Angelus® (MTA) - positive control. Root-end cavities were filled with the materials prepared according to the manufacturers' instructions. EDTA was used prior the retro-filling material insertion in half of the samples from each group (n = 5). Samples were prepared for SEM and photomicrographs were taked in x40, x150 and x500. The images of root-end fillings were divided into four áreas and distributed into five scores: 0 - absence of gaps; 1 - presence of gap in 1 area; 2 - presence of gap in 2 areas; 3 - presence of gap in 3 areas; 4 - presence of gaps in 4 areas. The analysis of transverse dimension of the gap (μm) was performed using Image J software. Qualitative variables were evaluated by Fisher Exact Test and quantitative variables by Analysis of Variance (ANOVA) . The level of significance was 0.05%. **Results:** No gaps were observed in MTA Angelus® group (0%). Presence of gaps were observed in Mk Life bioceramic cement (90%), Biodentine® (80%), Bio-C Repair® (100%) and Endosequence® BC RRM™ (80%). Significant difference was observed when MTA were compared to Mk Life bioceramic cement, Biodentine®, Bio-C Repair® and Endosequence® BC RRM™ ($p < 0.05$). No significant difference were observed in the transverse dimension of the gap between Mk Life bioceramic cement, Biodentine®, Bio-C Repair® and Endosequence® BC RRM™ groups. MTA show significantly better result than Mk Life bioceramic cement and Bio-C Repair® groups ($p < 0.05$), but without significant difference with Biodentine® and Endosequence® BC RRM™ **Conclusion:** MTA Angelus® showed better marginal adaptation compared to MK Life bioceramic cement, Biodentine® , BioC Repair® and Endosequence® BC RRM™. Marginal gaps were not observed in samples of MTA Angelus® group.

Keywords: Dental marginal adaptation; Dental cements; Scanning electron microscopy.

QUADROS, FIGURAS E TABELAS

SÍMBOLOS, ABREVIATURAS E SIGLAS

$\bar{X}$	Média
#	*Number*
%	Porcentagem
<	Menor que
®	Marca registrada
ANOVA	Análise de Variância
CRTI	Centro Regional para o Desenvolvimento Tecnológico e Inovação
DP	Desvio padrão
EDTA	Ácido etilenodiamino tetra-acético
et al.	e colaboradores
mμ	Micrômetro
MEV	Microscopia eletrônica de varredura
mL	Mililitro
mm	Milímetro
MTA	*Mineral Trioxide Aggregate*
n	Número de amostras
n.	Número
º	Graus
p	Nível de significância
RRM	*Root Repair Material*
™	*Trademark* (marca registrada)

SUMÁRIO

1 INTRODUÇÃO

Materiais à base de silicato de cálcio, conhecidos como materiais biocerâmicos, foram recentemente introduzidos na Odontologia. Estes materiais são resultantes da combinação entre silicatos de cálcio e fosfatos de cálcio, incluindo partículas de alumina, zircônia, vidros bioativos, cerâmicas de vidro e hidroxiapatita (KOCH; BRAVE 2009; SIQUEIRA, 2017).

Os materiais à base de silicato de cálcio apresentam boa capacidade de selamento, tolerância tecidual, ausência de contração após a presa, pH elevado (antibacteriano), facilidade no manuseio e são quimicamente estáveis em ambiente biológico (KOCH; BRAVE 2009; LOUSHINE *et al*., 2011; CANDEIRO *et al*., 2012; LIU *et al*. 2015; LV *et al*., 2017). São considerados bioativos, pois apresentam capacidade de interagir com os tecidos circundantes e promover osteogênese (GANDOLFI *et al.*, 2017). Ainda, a partir da hidratação do material, durante o processo de presa, ocorre formação de cristais de hidroxiapatita entre a superfície do material e a parede de dentina, os quais podem fornecer uma adequada vedação e adaptação marginal nessa interface (LOUSHINE *et al*., 2011; AYATOLLAHI *et al*., 2017; SILVA ALMEIDA *et al.,* 2017).

Os materiais a base de silicato de cálcio foram introduzidos para serem utilizados como cimentos obturadores do canal radicular e em retrobturações (SILVA ALMEIDA *et al.*, 2017). O cimento retrobturador é utilizado em cirurgias parendodônticas, uma opção terapêutica indicada quando se esgotaram as alternativas da terapia endodôntica convencional, e após avaliação do risco/benefício da colocação de um implante dentário (EL-SWIAH; WALKER, 1996; BERNABÉ *et al.*, 2005).

A região apical da raiz possui variações anatômicas que podem ser a fonte do fracasso no tratamento endodôntico, sendo mandatório, portanto, a eliminação dos três últimos milímetros apicais para o máximo de segurança no tratamento cirúrgico (KOKATE; PAWAR, 2012; BERNABÉ *et al.*, 2005). A cirurgia parendodôntica inclui a exposição, remoção do ápice e preparação de uma cavidade retrógada com posterior retrobturação, utilizando-se materiais biocompatíveis. O principal objetivo do uso de um cimento retrobturador é promover um selamento apical para prevenir a infiltração de bactérias e seus subprodutos para os tecidos periapicais (BERNABÉ *et al.*, 2007).

Enfatiza-se que o material retrobturador ideal deve promover o selamento tridimensional do canal radicular, impedir a infiltração bacteriana, ser biologicamente tolerado pelos tecidos periapicais, não reabsorvível, de fácil manipulação, dimensionalmente estável, radiopaco e possibilitar o reparo tecidual (TORABINEJAD; WATSON; PITT FORD, 1993; KOKATE; PAWAR, 2012).

Vários materiais foram propostos para o selamento de cavidades retrógadas, incluindo gutta-percha, amálgama, cimento de óxido de zinco e eugenol, Cavit, resinas compostas, cimentos de ionômero de vidro, Super-EBA, cimentos de hidróxido de cálcio, cimento Portland, agregado de trióxido mineral (MTA), além de alguns cimentos endodônticos (BERNABÉ; HOLLAND 2009).

O amálgama, durante muito tempo, foi utilizado como principal material retrobturador. O MTA, nas duas últimas décadas, tornou-se o material de referência para este fim. O MTA foi desenvolvido em 1993 (LEE *et al*., 1993; (TORABINEJAD; WATSON; PITT FORD, 1993), consiste em um cimento com partículas hidrofílicas à base de silicato tricálcio, silicato dicálcio, aluminato tricálcio e óxido de cálcio (CAMILLERI *et al.*, 2005), e tem sido amplamente aceito devido sua biocompatibilidade e suas propriedades físico-químicas (OLIVEIRA *et al.*, 2013; KÜÇÜKKAYA EREN; PARASHOS, 2018). No entanto, trata-se de um material que apresenta dificuldade de colocação nas cavidades retrógadas e tempo de presa elevado (OROSCO *et al*. 2010; KÜÇÜKKAYA EREN; PARASHOS, 2018).

A busca por um material retrobturador ideal tem direcionado as pesquisas aos novos cimentos a base de silicato de cálcio, que apresentam constituintes semelhantes ao MTA (RAVICHANDRA *et al*., 2014; SHOKOUHINEJAD *et al*., 2014; SOUNDAPPAN *et al*., 2014). Diferentes apresentações comerciais de cimentos de silicato de cálcio estão disponíveis no mercado, tais como: Biodentine®, Endosequence® BC, iRoot SP®, Cimento biocerâmico MK Life, entre outros. A Angelus® recentemente desenvolveu um cimento reparador de silicato de cálcio, o Bio-C Repair®, apresentado de forma experimental (SIQUEIRA, 2017).

A qualidade do selamento apical obtida pelo material retrobturador influencia diretamente no sucesso da cirurgia parendodôntica. A capacidade de selamento dos materiais retrobturadores tem sido avaliada por diversas metodologias, como: penetração de corante, bacteriana e de radioisótopos, método eletroquímico e transporte de fluidos (SHEETAL *et al.*, 2015). A análise da adaptação marginal é uma metodologia que mensura, indiretamente, a capacidade de selamento do material retrobturador (STABHOLZ *et al*., 1985; AYATOLLAHI *et al*., 2017) e a microscopia eletrônica de varredura tem sido o método mais utilizado no estudo da interface do material com a parede dentinária (PETERS; PETERS 2002; XAVIER et al., 2005).

A retrobturação é um procedimento importante que visa impedir a microinfiltração de irritantes aos tecidos periapicais, sendo que, uma retrobturação insuficiente é considerada a principal causa de insucesso no procedimento cirúrgico (BOLHARI *et al.,* 2015). Portanto, a seleção de um material biocompatível e que apresenta boa adaptação marginal torna-se importante, sendo a análise da adaptação marginal capaz de fornecer informações valiosas sobre a capacidade de selamento do material com vistas à aplicação e benefícios na clínica endodôntica (STABHOLZ *et al*., 1985). Considerando a importância dos materiais retrobturadores no contexto do selamento e do sucesso do tratamento endodôntico cirúrgico, aliada à recente introdução dos cimentos de silicato de cálcio no mercado odontolológico, tornou-se oportuno e justificável o presente estudo.

2 OBJETIVOS

2.1 Objetivo geral

Analisar a adaptação marginal de cimentos de silicato de cálcio em cavidades retrógadas.

2.2 Objetivo específico

- Comparar a adaptação marginal do Cimento biocerâmico MK Life, Biodentine®, Bio-C Repair® e Endosequence® BC RRM™ com o MTA Angelus® em cavidade retrógada, por meio de microscopia eletrônica de varredura.

3 MATERIAIS E MÉTODOS

3.1 Seleção e preparo da amostra

Para o presente estudo foram utilizados cinquenta incisivos bovinos com rizogênese completa, ausência de calcificações e de reabsorções internas ou externas, avaliados por radiografias periapicais. Após extração, os dentes foram limpos com curetas periodontais, submetidos à profilaxia com pedra pomes e água e armazenados em timol 0,1%.

Os incisivos foram seccionados transversalmente utilizando-se brocas EndoZ (Dentsply/Maillefer, Ballaigues, Switzerland) em alta rotação com refrigeração, obtendo-se o comprimento radicular de 16 mm. Este comprimento foi determinado por meio de paquímetro digital calibrado de 0,01 mm (Fowler/Sylvac Ultra-Cal Mark IV Electronic Caliper, Crissier, Switzerland), mensurado a partir do ápice radicular.

A odontometria foi realizada pela visualização da lima K-File de n.15 no forame apical seguido de recuo de 1 mm e o preparo do canal radicular foi realizado com instrumentos manuais K-File (Dentsply Maillefer, Ballaigues, Switzerland) até o instrumento nº 60. Os canais radiculares foram irrigados com 3 mL de hipoclorito de sódio a 2,5% (Fitofarma, Goiânia, GO, Brasil) a cada troca de instrumento e, a seguir, secos com cones de papel nº 60 e preenchidos com EDTA a 17% (pH 7,2) (Biodinâmica, Ibiporã, PR, Brazil) por 3 minutos. Foram empregados 3 mL de hipoclorito de sódio a 2,5% na irrigação final e os canais foram secos novamente.

A obturação foi realizada utilizando-se a técnica de condensação lateral com cones de guta-percha (Tanari®, Manacapuru, AM, Brasil) e cimento obturador MTA Fillapex® (Angelus, Londrina, Paraná, Brasil). As amostras foram armazenadas em estufa odontológica (ECB, 1.3 digital, Odontobrás, Ribeirão Preto, SP, Brasil) a 37° por 48 horas.

Após a obturação dos canais radiculares, as amostras foram apicectomizadas de forma perpendicular ao longo eixo da raiz, 3 mm aquém do ápice radicular, com uma broca Zecrya® (28mm - Dentsply/Maillefer) em alta velocidade, sob refrigeração. O preparo das cavidades retrógradas foi realizado com auxílio de lupa de 2,5x de aumento (Bioart, São Carlos, SP, Brasil) a 3 mm de profundidade, empregando-se ponta ultrassônica P1M (Helse, São Paulo, Brasil) acoplada ao aparelho de ultrassom EMS Piezon Master 400 (Electro Medical Systems, Vallée de Joux, Suíça), com constante irrigação de solução fisiológica. Posteriormente, os espécimes foram divididos aleatoriamente em cinco grupos experimentais de acordo com o material retrobturador (Quadro 1). O grupo do MTA Angelus® foi utilizado como controle.

As cavidades retrógadas foram secas com cone de papel absorvente e obturadas com os materiais experimentais (Figura 1), manipulados de acordo com as especificações dos respectivos fabricantes:

- Cimento biocerâmico MK Life, Bio-C Repair® e Endosequence® BC RRM™: apresentados na forma de pasta pré-misturada pronta para uso, comercializados em seringa. Foram inseridos na cavidade com pontas aplicadoras próprias dos fabricantes.

- Cimento Biodentine®: pó apresenta-se dentro de uma cápsula que foi cuidadosamente aberta e aplicadas 5 gotas de água destilada em seu interior. A seguir, a cápsula foi fechada, levada a um amalgamador digital (Astronmix, Dabi Atlante, Ribeirão Preto, SP, Brasil) e agitada por 30 segundos. A cápsula foi removida do aparelho e o material inserido na cavidade com espátula de resina n. ½ (SSWhite Dufles, Juiz de Fora, MG, Brasil).

- MTA Angelus®: proporcionado em uma placa de vidro na medida de 1 dose de pó (pá dosadora) e 1 gota de água destilada, manipulado por 30 segundos e inserido na cavidade com espátula de resina n. ½ (SSWhite Duflex, Juiz de Fora, MG, Brasil).

Quadro 1. Distribuição dos grupos experimentais de acordo com o material retrobturador e composição química dos cimentos de acordo com o fabricante.

Grupos	***n***	**Material Retrobturador**	**Fabricante**	**Composição química**
I	10	Cimento biocerâmico MK-Life	MK Life (Porto Alegre, RS, Brasil)	Òxido de zircônio (35-45%), silicato tricálcio (20-35%), silicato dicálcio (7-15%), hidróxido de cálcio (1-4%), propilenoglicol (2-5%).
II	10	Biodentine®	Septodont (Saint-Maur-des Fosses, França)	Silicato tricálcio, silicato dicálcio, carbonato de cálcio, óxido de cálcio, óxido de ferro e óxido de zircônio.
III	10	Bio-C Repair®	Angelus (Londrina, PR, Brasil)	Silicato de cálcio, óxido de cálcio, óxido de zircônio, óxido de ferro, dióxido de silício e agente de dispersão.
IV	10	Endosequence® BC RRM™	Brasseler (Savannah, GA, USA)	Silicato de cálcio, fosfato de cálcio monobásico, hidróxido de cálcio, óxido de zircônio, óxido de tântalo, agentes espessantes e de preenchimento.
V	10	MTA Angelus®	Angelus (Londrina, PR, Brasil)	Silicato tricálcio, silicato dicálcio, aluminato tricálcio, óxido de cálcio, óxido de bismuto.

Após a inserção dos materiais nas cavidades retrógadas, os cimentos e pasta foram adaptados com kit de calcadores Bernabé (Thimon, São Paulo, SP, Brasil) e cones de papel.

Em todos os grupos, metade das amostras (n=5) tiveram a cavidade inundada com EDTA 17% por 3 minutos, previamente à colocação do material retrobturador seguida de irrigação com solução fisiológica.

Os espécimes foram armazenados em tubos eppendorf por 72 horas e 100% de umidade em estufa odontológica (ECB, 1.3 digital, Odontobrás, Ribeirão Preto, SP, Brasil). Todos os procedimentos foram realizados por um único operador previamente calibrado e com mais de 5 anos de experiência em Endodontia.

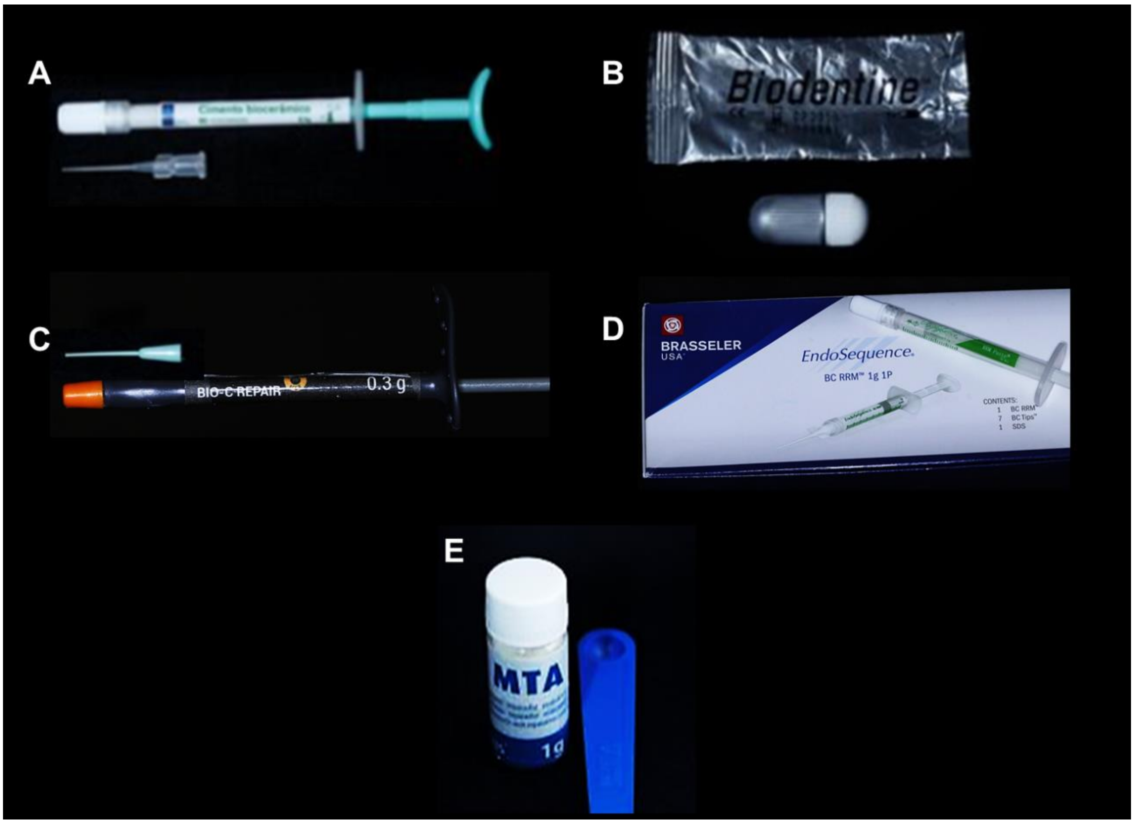

Figura 1. Cimento biocerâmico MK Life (A); Biodentine® (B); Bio-C Repair® (C); Endosequence® BC RRM™ (D); MTA Angelus® (E).

3.2 Preparo dos espécimes para MEV

Para avaliação em MEV, os 3 mm apicais foram seccionados com uma broca Zecrya® (28mm - Dentsply/Maillefer) em alta velocidade, sob refrigeração e, posteriormente, desidratados em álcool 70º, álcool 96º e álcool absoluto, em imersões de 30 minutos em cada, em ordem crescente de concentração, sendo que a solução foi renovada a cada 10 minutos.

As amostras foram analisadas no Centro Regional para o Desenvolvimento Tecnológico e Inovação (CRTI) da Universidade Federal de Goiás, onde permaneceram em estufa e baixo vácuo para completa secagem. As amostras foram codificadas e fixadas em fita adesiva de carbono sobre porta amostras de alumínio e recobertas com ouro. As fotomicrografias foram obtidas no microscópio eletrônico de varredura - MEV (JSM-IT300, JEOL Ltd, Fukuoka, Japão) com aumento de 40x, 150x e 500x para as análises (Figura 2).

3.3 Avaliação da adaptação marginal nas imagens obtidas por MEV

Avaliação da presença de fendas entre o cimento retrobturador e a parede dentinária da cavidade retrógada

Empregando-se a imagem de 40x de aumento, a área total do material retrobturador de cada espécime foi dividida em quatro (Figura 3). As áreas A, B, C e D foram analisadas com aumento de 150x com relação a presença de fendas entre o cimento retrobturador e a parede dentinária da cavidade retrógada.

Cada espécime foi classificado em escores conforme metodologia (Figura 3) empregada por Oliveira *et al*. (2013), constituindo cinco diferentes categorias (Quadro 2).

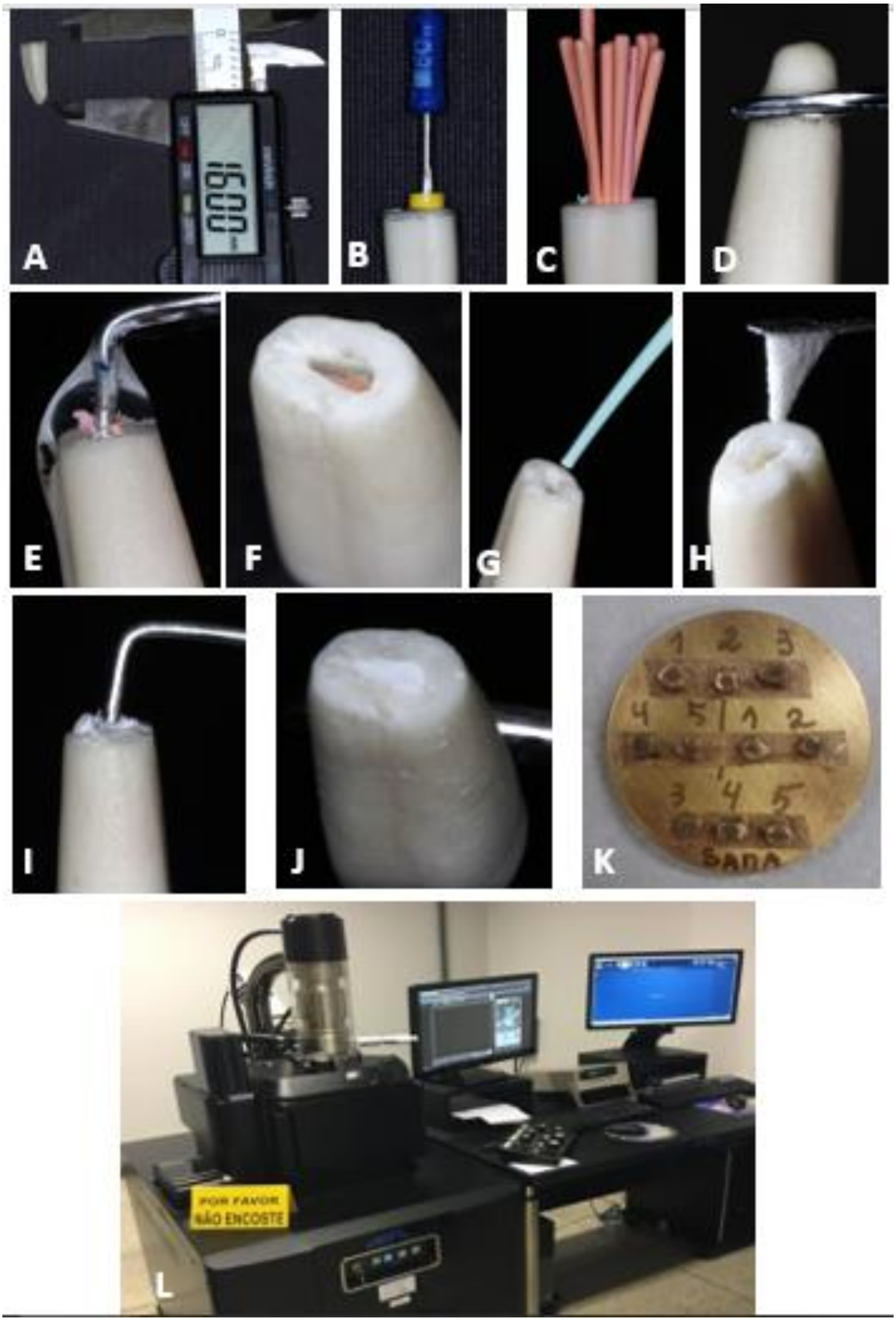

Figura 2. Seccionamento da raiz (A); Instrumentação (B); Obturação (C); Apicectomia (D); Retropreparo (E); Cavidade retrógada (F); Inserção do material retrobturador com seringa (G); Inserção do material retrobturador com espátula (H); Condensação do material retrobturador (I); Retrobturação (J); Amostras preparadas para análise em MEV (K); MEV (JSM - IT300) (L).

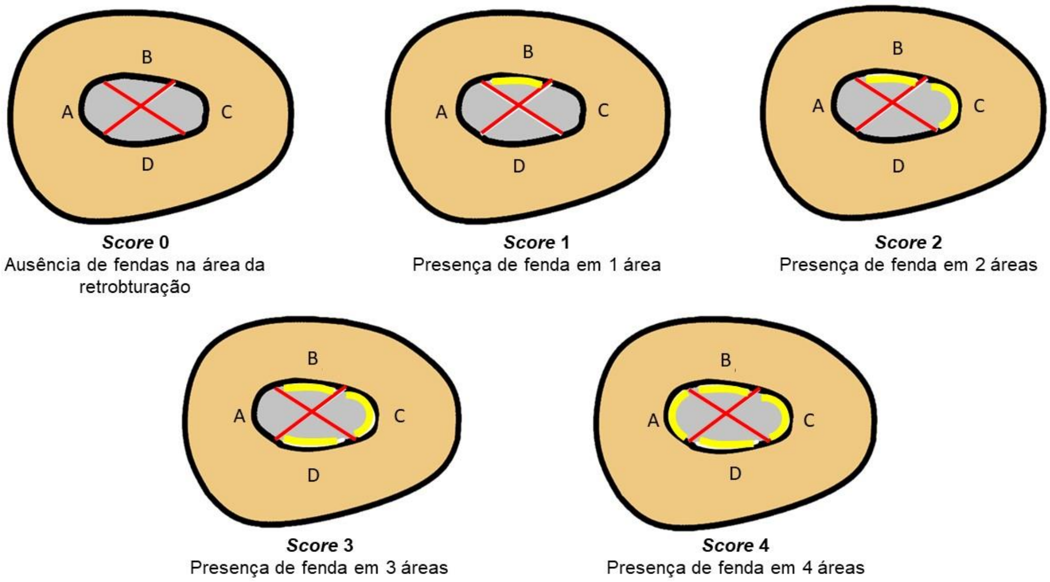

Figura 3. Representação esquemática da divisão da retrobturação em áreas (área A, B, C e D) e da classificação utilizada na avaliação da presença ou ausência de fendas entre o material retrobturador e a parede dentinária da cavidade retrógada.

Quadro 2. Classificação das amostras em *scores* de acordo com a presença de fendas entre o material retrobturador e a parede dentinária da cavidade retrógada.

Categorias	**Descriçâo**
Score 0	Ausência de fendas na área do retro-preparo
Score 1	Presença de fenda em 1 área
Score 2	Presença de fenda em 2 áreas
Score 3	Presença de fenda em 3 áreas
Score 4	Presença de fenda em 4 áreas, ou ausência total de adaptação

Avaliação da dimensão transversal da fenda entre o cimento retrobturador e a parede dentinária da cavidade retrógada

Empregando-se a imagem de 40x de aumento, a área total do material retrobturador de cada espécime foi dividida em quatro (área A, B, C e D), como descrito anteriormente. Nas fotomicrografias com aumento de 150x selecionou-se a área de maior dimensão transversal da fenda entre o cimento retrobturador e a parede dentinária da cavidade retrógada e obteve-se imagens com aumento de 500x para efetuação das mensurações. Com o uso do software *Image J* (NIH, Bethesda, Maryland, EUA), foram realizadas três mensurações lineares (em micrômetros) da maior dimensão transversal da fenda visível. Das três mensurações, utilizou-se a de maior valor de cada área para calcular a média do espécime (Figura 4). As análises foram realizadas por um único avaliador, com experiência em MEV.

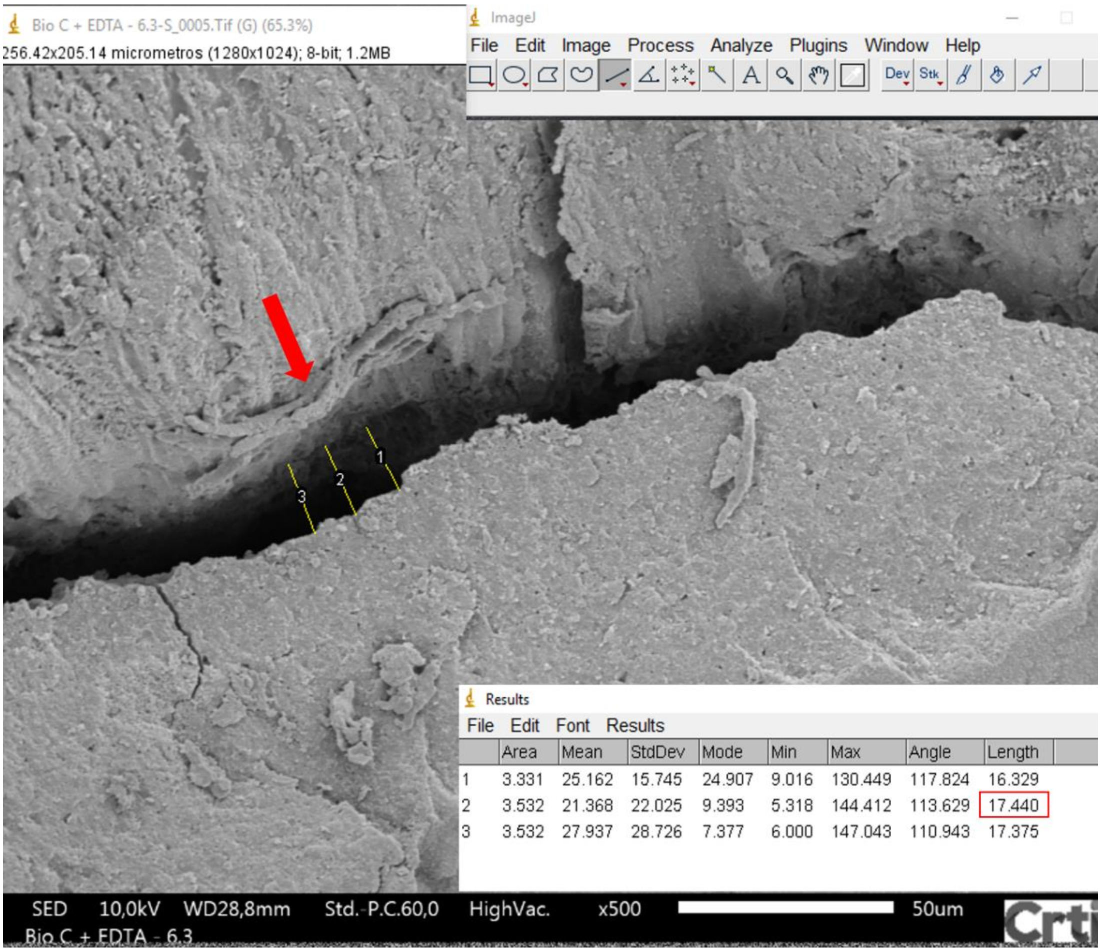

Figura 4. Fotomicrografia de amostra do grupo Bio-C Repair® em aumento de 500x analisada com programa *Image J.* Representação das três mensurações lineares para avaliação da dimensão transversal da fenda.

3.4 Análise estatística

A frequência e porcentagem das variáveis qualitativas e a média e o desvio padrão das variáveis quantitativas foram obtidas. As variáveis qualitativas foram avaliadas pelo Teste Exato de Fisher. A normalidade das variáveis quantitativas foi avaliada pelo teste de Kolmogorov-Smirnov. As variáveis quantitativas foram avaliadas pelo Teste de Análise de Variância (ANOVA) *post hoc* Bonferroni. O nível de significância foi de 0,05%.

4. RESULTADOS

Não observou-se presença de fendas no grupo MTA Angelus® (0%). Presença de fenda foi observada em 90% das amostras do grupo Cimento biocerâmico MK Life, 80% no grupo Biodentine®, 100% no grupo Bio-C Repair® e 80% no grupo Endosequence® BC RRM™.

Quanto a classificação em *scores* (Tabela 1), o grupo do MTA Angelus® apresentou resultados superiores, e estatisticamente significante, quando comparados aos grupos do Cimento biocerâmico MK Life, Biodentine®, Bio-C Repair® e Endosequence® BC RRM™ ($p<0,05$). Ainda, observou-se diferença estatisticamente significante entre os grupos Biodentine® e Bio-C Repair® ($p=0,017$), sendo que o Bio-C Repair® apresentou menor adaptação marginal, com 80% das amostras classificadas em *scores* 3 (40%) e 4 (40%) comparadas a 10% do grupo Biodentine® (10% de *score* 3 e 0% de *score* 4).

Tabela 1. Frequência dos *scores* nos diferentes grupos

Scores	**Cimento biocerâmico MK Life** (*n*=10)	**Biodentine®** (*n*=10)	**Bio-C Repair®** (*n*=10)	**Endosequence BC RRM®** (*n*=10)	**MTA Angelus®** (*n*=10)
0	1	2	0	2	10
1	4	4	0	2	0
2	2	3	2	3	0
3	2	1	4	3	0
4	1	0	4	0	0

Considerando, ainda, a dimensão transversal da fenda, não foram observadas diferenças estatisticamente significantes entre os grupos do Cimento biocerâmico MK Life, Biodentine®, Bio-C Repair® e Endosequence® BC RRM™. O grupo do MTA Angelus® não apresentou fendas, mostrando resultado superior aos grupos do Cimento biocerâmico MK Life e Bio-C Repair® ($p<0,05$), porém, sem diferença estatisticamente significante com os grupos do Biodentine® e Endosequence® BC RRM™ (Tabela 2).

Tabela 2. Comparação da dimensão transversal da fenda (µm) em relação aos cimentos retrobturadores, por meio de MEV (500x).

Grupos	*n*	$\bar{X} \pm DP$	*p**
Cimento biocerâmico MK Life	10	5,58 ± 4,50[a]	0,000
Biodentine®	10	4,13 ± 3,28[a,b]	
Bio-C Repair®	10	8,43 ± 3,90[a]	
Endosequence® BC RRM™	10	4,28 ± 4,14[a,b]	
MTA Angelus®	10	0,00[b]	

*ANOVA *post hoc* Bonferroni. Letras iguais indicam que não há diferença significativa entre os grupos ($p > 0,05$). Letras diferentes indicam diferença significativa entre os grupos ($p < 0,05$).

Em relação à influência do uso do EDTA na dimensão transversal da fenda entre o material retrobturador e a parede da cavidade retrógrada, observou-se que não houve diferença significativa (p>0,05) quando o EDTA foi utilizado previamente à inserção de nenhum dos cimentos retrobturadores utilizados (Tabela 3).

Tabela 3. Comparação da dimensão transversal da fenda (µm) em relação aos cimentos retrobturadores sem e com o uso de EDTA.

Grupos	Sem EDTA		Com EDTA		*p**
	n	$\bar{X} \pm DP$	*n*	$\bar{X} \pm DP$	
Cimento biocerâmico MK Life	5	6,91 ± 4,65	5	4,25 ± 4,41	0,380
Biodentine®	5	4,39 ± 3,60	5	3,87 ± 3,34	0,820
Bio-C Repair®	5	7,12 ± 4,14	5	9,73 ± 3,59	0,319
Endosequence® BC RRM™	5	4,54 ± 3,74	5	4,02 ± 4,94	0,857
MTA Angelus®	5	0,00 ± 0,00	5	0,00 ± 0,00	-

*Teste-t para amostras independentes.

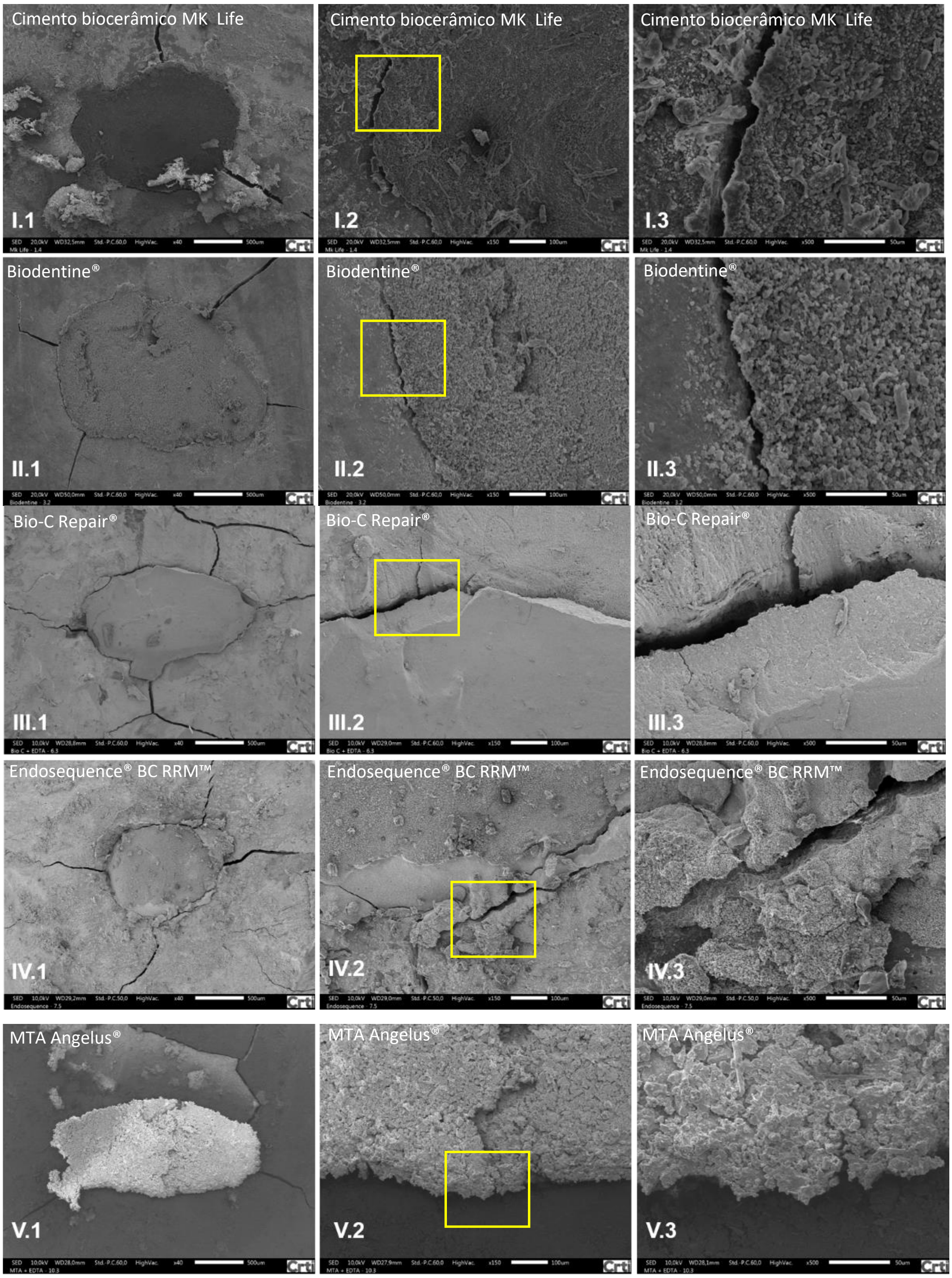

Figura 5. Fotomicrografias de MEV da adaptação marginal com Cimento biocerâmico MK Life (I.1 – I.3), Biodentine® (II.1 – II.3), Bio-C Repair® (III.1 – III.3), Endosequence® BC RRM™ (IV.1 – IV.3) e MTA Angelus® (V.1 – V.3) em magnificações de 40x (1), 150x (2) e 500x (3). Em destaque (amarelo) região selecionada para realizar fotomicrografia em aumento de 500x.

5. DISCUSSÃO

O MTA Angelus® apresentou maior adaptação marginal quando comparado aos demais cimentos de silicato de cálcio avaliados, sendo que não foram observadas fendas em nenhuma amostra deste grupo. Estes resultados são concordantes com os de estudos que demonstraram que o MTA apresentou capacidade de adaptação marginal superior comparado a outros materiais retrobturadores (PETERS; PETERS 2002; GONDIM *et al*., 2003; XAVIER *et al*., 2005; GOMES *et al*., 2009; MUNHOZ *et al*., 2011; ROSALES-LEAL *et al*., 2011; SHAHI *et al*., 2011; ROSA *et al*., 2014; SHOKOUHINEJAD *et al*., 2014; SOUNDAPPAN *et al*., 2014; BOLHARI *et al*., 2015).

Entende-se por selamento, o vedamento da cavidade com finalidade de prevenir microinfiltração de bactérias, fluidos, moléculas ou íons entre a margem da cavidade e o material (KIDD, 1976; MOREIRA *et al.*, 2010). A adaptação marginal pode ser definida como o grau de aproximação ou ajuste do material de preenchimento à superfície dentária (DeCS, 2018). Portanto, a avaliação da adaptação marginal representa uma metodologia que analisa indiretamente a capacidade de selamento do material retrobturador (STABHOLZ *et al*., 1985; AYATOLLAHI *et al*., 2017).

A existência de correlação entre adaptação marginal e capacidade de selamento dos materiais obturadores radiculares tem sido discutida na literatura (BOLHARI *et al*., 2015). Diversos estudos têm mostrado uma relação positiva entre estas duas propriedades (SHANI *et al.*, 1984; STABHOLZ *et al*., 1985; TORABINEJAD *et al*., 1995; TEWARI; TEWARI, 1999; COSTA *et al.*, 2009; AYATOLLAHI *et al*., 2017; KÜÇÜKKAYA EREN; GORGUYSUS; SAHIN, 2017). Mesmo em estudos empregando metodologias diferentes (STABHOLZ *et al*., 1985; AYATOLLAHI *et al*., 2017; KÜÇÜKKAYA EREN; GORGUYSUS; SAHIN, 2017), como microscopia eletrônica de varredura, penetração de radioisótopos e infiltração por transporte de fluidos, esta correlação tem sido evidenciada

O emprego do microscópio eletrônico de varredura tem sido o método mais utilizado para avaliação da adaptação marginal (PETERS; PETERS 2002; XAVIER *et al*., 2005). A principal vantagem da microscopia eletrônica de varredura é a capacidade de fornecer elevada magnificação e resolução. Entretanto, este método também apresenta limitações, visto que, a preparação convencional de amostras biológicas e a evaporação em vácuo pode estar associada à introdução de artefatos, como trincas em tecidos duros (BADR, 2010). Estudos anteriores analisaram a adaptação marginal do material retrobturador utilizando réplicas de resina para evitar artefatos nas amostras (PETERS; PETERS 2002; GONDIM *et al.*, 2003; ROSALES-LEAL *et al*., 2011; SHOKOUHINEJAD *et al.*, 2014; BOLHARI *et al.*, 2015). Orosco *et al.* (2010), relataram que para avaliação da adaptação marginal as amostras podem ser vistas diretamente sob microscopia eletrônica de varredura após recobrimento com ouro, não necessitando de criação de réplicas de resina. Em concordância, no presente estudo, não foram realizadas réplicas das amostras, que foram analisadas diretamente no microscópio eletrônico de varredura sem que houvesse prejuízo ou perdas por formação de artefatos.

Para avaliação da adaptação marginal dos materiais testados, foram realizadas duas análises no estudo: avaliação da presença de fenda com classificação em scores (análise qualitativa) e a mensuração da dimensão transversal da fenda (análise quantitativa), com o objetivo de obter-se resultados que se complementassem, ampliando as possibilidades de discussão sobre o assunto. Em uma revisão sistemática que avaliou trabalhos que compararam a adaptação marginal do MTA com a de outros cimentos retrobturadores, foram apresentados estudos que avaliaram a presença de fendas por análises qualitativas, quantitativas ou utilizando ambas. As análises qualitativas foram realizadas por meio da interpretação das imagens em termos de presença ou ausência de fendas ou utilizando scores para indicar a distribuição das fendas em relação a quadrantes da área da retrobturação. As análises quantitativas foram realizadas com mensurações do comprimento, largura, área ou volume da fenda. As duas análises, por score e por mensuração da dimensão da fenda, também foram realizadas, assim como no presente trabalho (KÜÇÜKKAYA EREN; PARASHOS, 2018).

Apesar das diferenças estruturais entre dentes bovinos e humanos, estudos têm considerados dentes bovinos como possíveis substitutos para dentes humanos em pesquisa odontológica, visto que, a dentina bovina e humana possuem características similares, como, por exemplo, número e diâmetro de túbulos dentinários (Soares *et al*., 2010).

No presente estudo avaliou-se a adaptação marginal dos cimentos de silicato de cálcio (Cimento biocerâmico MK Life, Biodentine®, Bio-C Repair® e Endosequence® BC RRM™), comparados ao MTA Angelus® (controle positivo) e a pasta de hidróxido de cálcio (controle negativo).

O Biodentine® foi introduzido em 2009 pela Septodont® e apresenta indicações endodônticas semelhantes às do MTA, promovendo o reparo dentinário (CAMILLERI; SORRENTINO; DAMIDOT 2013; BOLHARI *et al*., 2015).

No presente estudo, o MTA Angelus® mostrou maior adaptação marginal quando comparado ao Biodentine® . No grupo do MTA Angelus® não foi verificado fenda em nenhuma amostra analisada. O Biodentine® apresentou média de 4,13 ± 3,28 µm de dimensão transversal de fenda, resultados semelhantes com os de Soundappan *et al*. (2014). Este estudo comparou a adaptação marginal do Biodentine e o MTA em MEV. Os resultados, quando o MTA foi utilizado, mostraram-se superiores. Contraditoriamente, Ravichandra *et al*. (2014) observaram melhor adaptação marginal do Biodentine® comparado ao MTA em análise de microscopia confocal de varredura a laser. Outros estudos (BOLHARI *et al.*, 2015; KÜÇÜKKAYA EREN; GORGUYSUS; SAHIN, 2017) demonstraram não haver diferença significativa entre os dois materiais.

Quanto à presença de fenda, o Biodentine® apresentou maior adaptação marginal do que o BioC Repair®, e sem diferença significante quando comparado ao Cimento biocerâmico MK Life ou ao Endosequence® BC RRM™.

O cimento Endosequence e o MTA foram comparados, por microscopia eletrônica de varredura, quanto a adaptação marginal quando utilizados como cimentos retrobturadores (SHOKOUHINEJAD *et al*., 2014; NAGESH *et al.*, 2016). Shokouhinejad *et al*. (2014) observaram que os dois cimentos apresentaram resultados semelhantes. No entanto, Nagesh *et al*. (2016), demonstraram que o Endosequence® apresentou significativamente menor quantidade de fendas marginais quando comparado ao MTA. Os autores relacionaram o resultado às desvantagens do MTA, como a dificuldade de manipulação e o tempo de presa elevado, que podem contribuir para que ocorra infiltração, desintegração da superfície levando à perda de adaptação marginal e de continuidade do material. No presente estudo o MTA apresentou maior adaptação marginal quando comparado ao Endosequence® BC RRM™, que por sua vez, não apresentou diferença significante quando comparado ao Cimento biocerâmico MK Life, Biodentine® ou BioC Repair®.

Há uma variedade muito grande de técnicas e metodologias para avaliar a capacidade de selamento, o que dificulta a sua padronização e comparações entre resultados (SHEETAL *et al.*, 2015). Para Costa *et al.* (2009) e Bolhari *et al*. (2015) os materiais apresentaram uma composição semelhante, tendo o silicato de cálcio como constituinte principal, fato que possivelmente explica os resultados similares de adaptação marginal. Estes cimentos são hidrofílicos, absorvem líquido durante a presa e sofrem uma pequena expansão, além de formarem cristais de hidroxiapatita entre a superfície do material e a parede de dentina, os quais podem fornecer uma apropriada adaptação marginal (AYATOLLAHI *et al*., 2017).

Apesar dos resultados promissores dos trabalhos que avaliaram os novos cimentos de silicato de cálcio, no presente estudo, o MTA Angelus® apresentou maior adaptação marginal quando comparado aos demais materiais testados. O que nos conduz a questionar os motivos que levaram o MTA a apresentar adaptação marginal consideravelmente superior. As justificativas de uma boa adaptação marginal do MTA se enquadram a todos os cimentos a base de silicato de cálcio, o que nos leva a admitir se a presença de compostos que fornecem maior plasticidade aos novos materiais e, consequentemente, facilitam o seu manuseio, poderiam resultar em uma diminuição das propriedades fisico-químicas do material.

Além das características físico-químicas dos materiais, um dos fatores que pode influenciar na qualidade da adaptação marginal em obturações retrógadas é a presença de *smear layer*, porém, esta influência permanece uma questão controversa na literatura (DI LENARDA; CADENARO; SHAIZERO, 2000). Vários agentes como hipoclorito de sódio, EDTA, mistura de detergente ácido de tetraciclina (MTAD) e ácidos orgânicos foram introduzidos para remoção de *smear layer* (DECHICHI; CHRISTIAN, 2006).

Estudos avaliaram a influência do uso do EDTA na adaptação marginal e na capacidade de selamento em retrobturações com MTA. Por meio de microtomografia computadorizada, Al Fouzan *et al.* (2015) observaram uma melhora significativa na adaptação do material à dentina quando o EDTA foi utilizado. No entanto, estudos mostraram que a capacidade de selamento apical, avaliada por transporte de fluidos (YILDIRIM; ORUCOGLU; COBANKARA, 2008) e infiltração microbiana (YILDIRIM *et al*., 2010; ESTRELA *et al.*, 2011) diminuiu significativamente quando a *smear layer* foi removida. No presente estudo, o uso do EDTA previamente a inserção dos materiais retrobturadores testados não influenciou na adaptação marginal dos mesmos.

Os cimentos de silicato de cálcio possuem várias aplicações na Odontologia. O conhecimento atualizado desses novos materiais é essencial para garantir a seleção do mais adequado em diferentes situações clínicas (AL-HADDAD; CHE AB AZIZ, 2016; JITARU *et al*, 2016; RAGHAVENDRA *et al*, 2017). A maioria dos estudos que avaliaram adaptação marginal são laboratoriais, sendo este um aspecto que deve-se ter cuidado. Por se tratar de um modelo *in vitro*, não há presença de umidade e secreções próprias do ambiente cirúrgico, o que interfere na presa e comportamento do material. Considerando condições clínicas complexas, a questão de quanto de dimensão da fenda levaria a uma redução da taxa de sucesso do tratamento precisa ser respondida em futuros estudos clínicos.

6. CONCLUSÃO

O MTA Angelus® apresentou melhor adaptação marginal quando comparado ao Cimento Biocerâmico MK Life, Biodentine®, BioC Repair® e Endosequence® BC RRM™. Não observou-se presença de fendas marginais em nenhuma amostra do grupo MTA Angelus®.

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APÊNDICE

ARTIGO

Title: Marginal adaptation of calcium silicate-based materials to dentine wall in root-end cavities

Renovato SR, Alencar AHG, Estrela C

Sara Rodrigues Renovato, MSc

Department of Stomatologic Sciences, Federal University of Goiás, Goiânia, GO, Brazil

Ana Helena Gonçalves de Alencar, PhD

Department of Stomatologic Sciences, Federal University of Goiás, Goiânia, GO, Brazil

Carlos Estrela, PhD

Department of Stomatologic Sciences, Federal University of Goiás, Goiânia, GO, Brazil

Running title: Calcium silicate-based cements

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Corresponding author:

Sara Rodrigues Renovato

Federal University of Goiás, Department of Stomatologic Sciences, Praça Universitária s/n, Setor Universitário.

CEP 74605-220, Goiânia, GO, Brazil.

Phone number: 55 62 982388504

E-mail address: sara_renovato@hotmail.com

Abstract

Aim: To analyse the marginal adaptation of calcium silicate-based cements in root-end cavity by SEM.

Methodology: Fifty bovine roots were prepared with K-File #60 and filled with gutta-percha and MTA Fillapex. Roots were apicetomized and a 3-mm-deep root-end cavity was prepared using ultrasonic tips. Samples were randomly divided into five groups (*n* = 10): 1. Mk Life bioceramic cement (MKL); 2. Biodentine® (BD); 3. Bio-C Repair® (BCR); 4. Endosequence® BC RRM™ (ERRM); 5. MTA Angelus® (MTA) - positive control. Root-end cavities were filled with materials prepared according to the manufacturers' instructions. EDTA was used prior to the retro-filling material insertion in half of samples from each group (n = 5). Photomicrographs in SEM were taked in x40, x150 and x500. Images of root-end fillings were divided into four áreas and distributed into five scores: 0 - absence of gaps; 1 - presence of gap in 1 area; 2 - presence of gap in 2 areas; 3 - presence of gap in 3 areas; 4 - presence of gaps in 4 areas. Transverse dimension of the gap (μm) was performed using Image J software. Qualitative variables were evaluated by Fisher Exact Test and quantitative variables by Analysis of Variance (ANOVA) . The level of significance was 0.05%.

Results: No gaps were observed in MTA Angelus® group (0%). Presence of gaps were observed in MKL (90%), BD (80%), BCR (100%) and ERRM (80%). Significant difference was observed between MTA to MKL, BD, BCR and ERRM ($p < 0.05$). No significant difference were observed in transverse dimension of gap between MKL, BD, BCR and ERRM groups. MTA show significantly better result than MKL and BCR groups ($p < 0.05$), but without significant difference with BD and ERRM.

Conclusions: MTA Angelus® showed better marginal adaptation compared to MK Life bioceramic cement, Biodentine® , BioC Repair® and Endosequence® BC RRM™. Marginal gaps were not observed in samples of MTA Angelus® group.

Introduction

Calcium silicate-based materials, known as bioceramic materials, were recently introduced in dentistry. These materials are resulting from combination of calcium silicates and calcium phosphates, and include alumina, zirconia, bioactive glass, glass ceramics and hydroxyapatite (Koch & Brave 2009, Siqueira, 2017).

These materials exhibit good sealing ability, biocompatibility, high pH (antibacterial), good handling, do not shrink and are chemically stable in biological environment (Koch & Brave 2009, Loushine *et al*. 2011, Candeiro *et al*. 2012, Liu *et al*. 2015, Lv *et al*. 2017). They are considered bioactive material, as they have ability to interact with the surrounding tissues and promote osteogenesis (Gandolfi *et al*. 2017). From the hydration of the material during setting process occurs hydroxyapatite crystals formation between material surface and dentin wall, which can provide adequate sealing and marginal adaptation in interface (Loushine *et al.* 2011, Ayatollahi *et al.* 2017, Silva Almeida *et al.* 2017).

Calcium silicate-based materials were introduced to be used as root canal sealers and root-end filling material (Silva Almeida *et al*. 2017). Root-end filling materials are used in endodontic surgeries, a therapeutic option indicated when the alternatives of conventional endodontic therapy have been exhausted, and after assessment of the risk / benefit of dental implant placement (El-Swiah & Walker 1996, Bernabe *et al.* 2005).

Apical region of the root has anatomical variations that can be the source of failure in endodontic treatment, so it is mandatory the last three apical millimeters elimination for maximum safety in surgical treatment (Kokate & Pawar 2012, Bernabé *et al.* 2005). Endodontic surgery includes exposure, apex removal, and preparation of a retrograde cavity with subsequent retrofilling, using biocompatible materials. The main objective of a root-end filling material use is promote an apical sealing to prevent bacteria and their by-products infiltration into periapical tissues (Bernabé *et al.* 2007).

An ideal root-end filling material should promote tridimensional filling of root canal, prevent leakage of microrganisms, be biologically tolerated by periapical tissue, non-absorbable, easy to handle, dimensionally stable, radiopaque and allow tissue repair (Torabinejad *et al.* 1993, Kokate & Pawar 2012).

Several materials were proposed for root-end cavity sealing, including gutta-percha, amalgam, zinc oxide and eugenol cement, Cavit, composite resins, glass ionomers, super-EBA, calcium hydroxide cements, Portland cement, mineral trioxide aggregate (MTA), and some endodontic sealers (Bernabé & Holland 2009).

Amalgam, for a long time, was the main root-end filling material used. MTA, in the last two decades, has become the reference material for this purpose. MTA was developed in 1993 (Lee *et al*. 1993, Torabinejad *et al.*, 1993), consisting of a cement with hydrophilic particles composed by tricalcium silicate, dicalcium silicate, tricalcium aluminate and calcium oxide (Camilleri *et al*. 2005), and has been widely accepted because of its biocompatibility and its physico-chemical properties (Oliveira *et al*. 2013, Küçükkaya Eren & Parashos 2018). However, has difficult handling characteristics and long setting time (Orosco *et al*. 2010, Küçükkaya Eren & Parashos 2018).

The search for an ideal root-end filling material has directed researchs to the new calcium silicate-based cements, which have constituents similar to MTA (Ravichandra *et al.* 2014, Shokouhinejad *et al.* 2014, Soundappan *et al.* 2014). Differents commercial presentations of calcium silicate-based cements are available in the market, such as: Biodentine®, Endosequence® BC, iRoot SP®, MK Life Bioceramic Cement, among others. Angelus® recently developed a calcium silicate repair cement, Bio-C Repair®, experimentally presented (Siqueira, 2017).

The apical sealing quality obtained by root-end filling material directly influences at endodontic surgery success. The sealing ability of root-end filling materials has been evaluated by several methodologies, such as: dye, bacterial and radioisotope penetration, electrochemical method and fluid transport (Sheetal *et al.* 2015). The marginal adaptation analysis is a methodology that indirectly measures the sealing ability of the root-end filling material (Stabholz *et al.* 1985, Ayatollahi *et al.* 2017) and scanning electron microscopy has been the most used method in the study of the interface between material and dentin wall (Peters & Peters 2002, Xavier *et al.* 2005).

Retrofilling is an important procedure that aims to prevent microleakage of irritants to the periapical tissues. Insufficient retrofilling is considered the main cause of failure in surgical procedure (Bolhari *et al.* 2015). Therefore, a biocompatible material selection with good marginal adaptation becomes important, and is capable of providing valuable information about material sealing ability for application and benefits in the endodontic clinic (Stabholz *et al*. 1985). Considering the importance of root-end filling materials in sealing ability context and in surgical endodontic treatment succes, and with the recent introduction of calcium silicate-based cements in the dental market, the present study was timely and justifiable.

The aim of the study was too analyze the marginal adaptation of calcium silicate- based cements in root-end cavities, by SEM.

Materials and Methods

Fifty bovine incisors with fully formed apex, absence of calcified root canals, internal or external resorption, evaluated by periapical radiographs, were used. After extraction, teeth were cleaned with periodontal curettes, submitted to prophylaxis with pumice and water and stored on 0.1% thymol solution.

Teeth were cross-sectioned using EndoZ drills (Dentsply / Maillefer, Ballaigues, Switzerland) at high speed to prepare standardized 16-mm tooth length from the root Apex measured by calibrated digital caliper gauge of 0.01 mm (Fowler / Sylvac Electronic caliper Ultra-Cal Mark IV, Crissier, Switzerland).

Odontometry was performed by K-File #15 visualization on apical foramen and canals were prepared using K-Files (Dentsply Maillefer, Ballaigues, Switzerland) up to size #60, 1 mm short of the apical foramen. During instrumentation, the root canals were irrigated with 3 mL of 2,5% sodium hypochlorite (Fitofarma, Goiânia, GO, Brazil) at each change of files. Root canals were dried and filled with 17% EDTA (pH 7.2) (Biodinâmica, Ibiporã, PR, Brazil) for 3 min. After that, 3 mL of 2.5% sodium hypochlorite were used as final irrigation and dried again with paper points (Dentsply Maillefer, Ballaigues, Switzerland).

The root canals were obturated with guttapercha points (Tanari®, Manacapuru, AM, Brazil) and MTA Fillapex® sealer (Angelus, Londrina, Paraná, Brazil) using the conventional lateral compaction technique. After that, samples were placed in an incubator (ECB, 1.3 digital, Odontobrás, Ribeirão Preto, SP, Brazil) at 37ºC for 48 hours.

The apical 3 mm of roots was sectioned perpendicular to the long axis of the tooth with a high-speed Zecrya drill (Dentsply Maillefer, Ballaigues, Switzerland) under continuous air/water spray. Then, a 3-mm-deep root-end cavity was prepared using P1M ultrasonic tips (Helse, São Paulo, Brazil) powered by a EMS Piezon Master 400 (Electro Medical Systems, Vallée de Joux, Switzerland), ultrassonic unit under continuous irrigation with saline solution. Subsequently, samples were randomly assigned to five groups according to root-end filling material tested (Table 1). MTA Angelus® group was used as positive control.

Root-end cavities were dried with paper points and filled with experimental materials that were prepared according to the manufacturers' instruction. MK Life bioceramic cement, Bio-C Repair® and Endosequence® BC RRM™ are marketed in syringes and were inserted into the cavity with applicator tips specific to the manufacturers. Biodentine® and MTA Angelus® were manipulated and inserted into the cavity with resin spatula n. ½ (SSWhite Dufles, Juiz de Fora, MG, Brasil).

Cements and paste were adapted with Bernabé kit (Thimon, São Paulo, SP, Brazil) and paper points. EDTA was used prior to the retro-filling material insertion in half of the samples from each group (n = 5) for 3 minutes, followed by irrigation with saline solution.

Samples were stored in eppendorf tubes for 72 hours and 100% humidity, placed in na incubator (ECB, 1.3 digital, Odontobrás, Ribeirão Preto, SP, Brazil). All procedures were performed by a single operator previously calibrated and with more than 5 years experience in Endodontics.

The apical 3-mm specimens were sectioned with a high-speed Zecrya drill (Dentsply Maillefer, Ballaigues, Switzerland) under continuous air/water spray, then, specimens were dehydrated in increasing concentrations of alcohol (70º, 96º and 99º), in immersions of 30 minutes in each. Samples were analyzed in the Regional Center for Technological Development and Innovation (CRTI) of Federal University of Goiás, where they remained in incubator and under vacuum for complete drying. Photomicrographs were obtained in scanning electron microscope (SEM) (JSM-IT300, JEOL Ltd, Fukuoka, Japan) with an increase of 40x, 150x and 500x for analyzes.

Evaluation of the presence of marginal gaps

Using the 40x magnification image, the root end area of each specimen was divided into four. The areas A, B, C and D were analyzed in a 150x magnification image about the presence of gaps between root-end filling cement and dentin wall of root-end cavity. Each specimen was classified in scores according to methodology (Figure 1) employed by Oliveira *et al.* (2013), constituting five different scores: score 0 - no marginal gap; score 1- marginal gap in one área; score 2 – marginal gap in two áreas; score 3 – marginal gap in three areas and score 4 – marginal gap in four áreas or in entire área.

Evaluation of the transverse dimension of the marginal gap

Using the 40x magnification image, the root end area of each specimen was divided into four (areas A, B, C and D) as previously described. In 150x magnification photomicrographs, greater transverse dimension of the gap was selected and 500x magnification images were obtained for measurements. Using Image J software (NIH, Bethesda, Maryland, USA), three linear measurements (in micrometers) of the largest visible transverse dimension of the gap were performed. The highest value of the three measurements of each area was used to calculate the mean of transverse dimension of the specimen. The analyzes were performed by a single evaluator, with experience in SEM.

Statistical analysis

The frequency and percentage of the qualitative variables and the mean and standard deviation of the quantitative variables were obtained. Qualitative variables were analyzed by Fisher's Exact Test. The normality of the quantitative variables was evaluated by Kolmogorov-Smirnov test. The quantitative variables were compared by Variance Analysis (ANOVA) *post hoc* Bonferroni. The level of significance was 0.05%.

Results

No gaps were observed in MTA Angelus® group (0%). The presence of gaps were observed in 90% of samples in MK Life bioceramic cement group, 80% in Biodentine® group, 100% in Bio-C Repair® group and 80% in Endosequence® BC RRM™ group.

The MTA Angelus® group presented higher and statistically significant results when compared to the MK Life bioceramic cement, Biodentine®, Bio-C Repair® and Endosequence® BC RRM™ groups ($p < 0.05$). There was also a statistically significant difference between Biodentine® and Bio-C Repair® groups ($p = 0.017$). Bio-C Repair® presented the lowest marginal adaptation, with 80% of the samples classified as scores 3 (40%) and 4 (40%) compared to 10% of the Biodentine® group (10% score 3 and 0% score 4).

Considering the transverse dimension of the marginal gap, no statistically significant differences were observed between MK Life bioceramic cement, Biodentine®, Bio-C Repair® and Endosequence® BC RRM™ groups. MTA Angelus® group did not show marginal gaps, showing a superior result to MK Life bioceramic cement and Bio-C Repair® groups ($p < 0.05$), but without significant difference with Biodentine® and Endosequence® BC RRM™ groups (Table 2).

Regarding the influence of the use of EDTA on transverse dimension of the gap between the root-end filling material and the dentine wall of root-end cavity, it was observed that there was no significant difference ($p > 0.05$) when EDTA was used prior the insertion of any material used (Table 3).

Discussion

MTA Angelus® showed better marginal adaptation when compared to the others calcium silicate cements evaluated. In the present study, no gaps were observed in samples of MTA Angelus® group. In agrément, studies have shown that MTA presented greater marginal adaptation compared to other root-end filling materials (Peters & Peters 2002, Gondim *et al*. 2003, Xavier *et al*. 2005, Gomes *et al*. 2009, Munhoz *et al*. 2011, Rosales-Leal *et al*. 2011, Shahi *et al*.

2011, Rosa *et al*. 2014, Shokouhinejad *et al*. 2014, Soundappan *et al*. 2014, Bolhari *et al*. 2015).

The analysis of the marginal adaptation represents a methodology that indirectly analyzes the sealing ability of root-end filling material (Stabholz *et al*. 1985, Ayatollahi *et al.* 2017). It is understood that sealing abilitty is the sealing of the cavity in order to prevent microinfiltration of bacteria, fluids, molecules or ions between the cavity margin and material (KIDD, 1976; MOREIRA et al., 2010). Marginal adaptation can be defined as the degree of approximation or adjustment of the filling material to dental surface (DeCS, 2018).

The correlation between marginal adaptation and sealing ability of root-end filling materials has been discussed in literature (Bolhari *et al*. 2015). Studies have shown a positive relation between these two properties (Shani *et al.* 1984, Stabholz *et al*. 1985, Torabinejad *et al*. 1995, Tewari & tewari 1999, Costa *et al.* 2009, Ayatollahi *et al*. 2017, Küçükkaya Eren *et al.* 2017). Based on diverse methodologies, using scanning electron microscopy, radioisotope penetration and fluid transport filtration, Stabholz *et al.* (1985), Ayatollahi *et al.* (2017) and Küçükkaya Eren *et al.* (2017) verified that exist correlation between marginal adaptation and sealing capacity.

Marginal adaptation can be determined by different techniques. The use of scanning electron microscope has been the most used (Peters & Peters 2002, Xavier *et al*. 2005). The main advantage of scanning electron microscopy is the ability to provide high magnification and resolution. This method also presents limitations, since conventional preparation of biological samples and evaporation in vacuum may be associated with introduction of artifacts, such as cracks in hard tissues (Badr 2010). Previous studies have analyzed marginal adaptation of root-end filling material using resin replicates to avoid artifacts in the samples (Peters & Peters 2002, Gondim *et al.* 2003, Rosales-Leal *et al*. 2011, Shokouhinejad *et al.* 2014, Bolhari *et al.* 2015). Orosco *et al.* (2010), reported that for evaluation of the marginal adaptation the samples can be seen directly under scanning electron microscopy after coating with gold, not requiring the creation of resin replicas. In agreement, in the present study, no replicas of samples were taken, which were analyzed directly in the scanning electron microscope without any damage or losses due to artifact formation.

In order to evaluate marginal adaptation of tested materials, two analyzes were performed in the study: evaluation of the presence of gap with classification in scores (qualitative analysis) and measurement of the transversal dimension of the gap (quantitative analysis), with objective to obtaining results that complement each other, expanding the possibilities of discussion on the subject. In a systematic review that evaluated studies that compared marginal adaptation of MTA with others root-end filling materials, show that they evaluated the presence of gaps by qualitative, quantitative or using both analysis. Two studies of this systematic review, the qualitative analysis was performed by interpretation of the images in terms of presence or absence of gaps, while in four studies scores were used to indicate the distribution of the gaps in relation to quadrants of the root-end cavity area. In 15 studies of this systematic review, quantitative analysis was performed by measurements of the length, width, area or volume of gap, and only two studies performed both the score classification and measurements of the gap analysis as in the present study (Küçükkaya Eren & Parashos 2018).

Despite some structural differences between bovine and human teeth, studies have considered bovine teeth as possible substitutes for human teeth in dental research, since bovine and human dentine have similar characteristics, such as number and diameter of dentinal tubules (Soares *et al.* 2010).

In the present study, marginal adaptation of calcium silicate cements (MK Life bioceramic cement, Biodentine®, Bio-C Repair® and Endosequence® BC RRM™) was evaluated, compared to MTA Angelus® (positive control) and calcium hydroxide paste (negative control).

Biodentine® was introduced by Septodont® in 2009 and presents endodontic indications similar to those of the MTA. It performs an application in dental repair (Camilleri *et al.* 2013, Bolhari *et al.* 2015).

In present study, MTA Angelus® showed better marginal adaptation when compared to Biodentine®. In MTA Angelus® group, no gap was observed in any sample analyzed. Biodentine® presented a mean of 4.13 ± 3.28 μm of transverse gap size, similar results with those of Soundappan *et al.* (2014). This study compared marginal adaptation of Biodentine and MTA by MEV. The results were superior when MTA was used. Ravichandra *et al.* (2014) observed a better marginal adaptation of Biodentine® compared to MTA in confocal laser scanning microscopy. Other studies (Bolhari *et al.* 2015, Küçükkaya Eren *et al.* 2017) showed no significant difference between these two materials.

Regarding the presence of gap, Biodentine® presented better marginal adaptation than BioC Repair®, but without significant difference when compared to MK Life bioceramic cement or Endosequence® BC RRM™.

Endosequence and MTA were compared about marginal adaptation by scanning electron microscopy when used as root-end filling materials (Shokouhinejad *et al.* 2014; Nagesh *et al.* 2016). Shokouhinejad *et al*. (2014) observed that these two cements presented similar results. However, Nagesh et al. (2016), demonstrated that Endosequence® presented significantly less marginal gaps when compared to MTA. The authors related the results to MTA disadvantages, such as the difficulty of handling and the high setting time, which can contribute to infiltration, surface disintegration leading to loss marginal adaptation and loss continuity of material. In the present study, MTA presented greater marginal adaptation when compared to Endosequence® BC RRM™, which did not present significant difference when compared to MK Life bioceramic cement, Biodentine® or BioC Repair®.

There is a very wide variety of techniques and methodologies to evaluate sealing ability, which makes difficult to standardize and compare the results (Sheetal *et al.* 2015). For Costa *et al.* (2009) and Bolhari *et al.* (2015) the materials presented a similar composition, with calcium silicate as main constituent, a fact that possibly explains the similar results of marginal adaptation. These cements are hydrophilic, absorb liquid during setting and suffer a small expansion, in addition they form hydroxyapatite crystals between the surface of material and dentin wall, which can provide an appropriate marginal adaptation (Ayatollahi *et al.* 2017).

In spite of the promising results of the studies that evaluated new calcium silicate cements, in the present study, MTA Angelus® presented better marginal adaptation when compared to other materials tested. This result leads to questioning the reasons that MTA presents considerably higher marginal adaptation. Results (Peters & Peters 2002, Gondim *et al*. 2003, Xavier *et al*. 2005, Gomes *et al*. 2009, Munhoz *et al*. 2011, Rosales-Leal *et al*. 2011, Shahi *et al*. 2011, Rosa *et al*. 2014, Shokouhinejad *et al*. 2014, Soundappan *et al*. 2014, Bolhari *et al*. 2015) that justifie good marginal adaptation of MTA, justifie all calcium silicate-based cements bahavior, which leads us to admit that the presence of compounds that provide greater plasticity to new materials and consequently facilitate their handling could result in a decrease of physicochemical properties of the material.

In addition to materials physicochemical characteristics, one of the factors that may influence the quality of marginal adaptation in root-end fillings is the presence of smear layer, however, this influence remains a controversial issue in literature (Di Lenarda *et al.* 2000) . Several agentes, such as sodium hypochlorite, EDTA, blend of tetracycline acid detergent (MTAD) and organic acids were introduced to remove smear layer (Dechichi & Christian 2006).

Studies have evaluated the influence of EDTA on marginal adaptation and sealing ability on root-end filling with MTA. By computerized microtomography, Al Fouzan *et al.* (2015) observed a significant improvement in the material adaptation to dentin when EDTA was used. However, some studies have shown that apical sealing ability, assessed by fluid transport (Yildirim *et al.* 2008) and bacterial leakage (Yildirim *et al*. 2010; Estrela *et al.* 2011) decreased significantly when the smear layer was removed. The use of EDTA prior the insertion of root-end filling materials tested did not influence their marginal adaptation.

Calcium silicate-based materials have gained several applications in dentistry. The updated knowledge of these new materials is essential to ensure the selection of the most appropriate in different clinical situations (Al-Haddad & Che Ab Aziz, 2016, Jitaru *et al,* 2016, Raghavendra *et al*, 2017). Most of studies that evaluated marginal adaptation are laboratorial, and this should be taken care of. Because it is an in vitro model, there is no presence of moisture and secretions of surgical environment, which interferes with setting and behavior of material. Considering complex clinical conditions, the question of how much dimensiono f the gap would lead to a reduction in treatment success needs to be answered in future clinical studies.

Conclusion

MTA Angelus® presented better marginal adaptation when compared to MK Life bioceramic cement, Biodentine®, BioC Repair® and Endosequence®

BC RRM™. No marginal gaps were observed in all samples tested in MTA Angelus® gropup.

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Table and Figures

Table 1. Experimental groups according to the root-end filling materials

Group	***n***	***Root-end filling materials***
I	10	MK-Life biocerâmic Cement (MK Life, Porto Alegre, RS, Brazil)
II	10	Biodentine® (Septodont, Saint-Maur-des Fosses, France)
III	10	Bio-C Repair® (Angelus, Londrina, PR, Brazil)
IV	10	Endosequence® BC RRM™ (Brasseler, Savannah, GA, USA)
V	10	MTA Angelus® (Angelus, Londrina, PR, Brazil)

Table 2. Transverse dimensiono of gap (µm) according to the root-end filling materials, by SEM (x500).

Groups	***n***	$\bar{X} \pm DP$	***p****
MK Life bioceramic cement	10	5,58 ± 4,50[a,c]	0,000
Biodentine®	10	4,13 ± 3,28[a,b,c]	
Bio-C Repair®	10	8,43 ± 3,90[a]	
Endosequence® BC RRM™	10	4,28 ± 4,14[a,b,c]	
MTA Angelus®	10	0,00[b]	

*ANOVA *post hoc* Bonferroni. Equal letters indicate no significant difference between groups (p> 0.05). Different letters indicate significant difference between groups (p <0.05).

Table 3. Conparison of transverse dimension of gap (μm) in relation to root-end filling cement with and without the use of EDTA.

Groups	Without EDTA		With EDTA		*p**
	n	$\bar{X} \pm DP$	***n***	$\bar{X} \pm DP$	
MK Life bioceramic cement	5	6,91 ± 4,65	5	4,25 ± 4,41	0,380
Biodentine®	5	4,39 ± 3,60	5	3,87 ± 3,34	0,820
Bio-C Repair®	5	7,12 ± 4,14	5	9,73 ± 3,59	0,319
Endosequence® BC RRM™	5	4,54 ± 3,74	5	4,02 ± 4,94	0,857
MTA Angelus®	5	0,00 ± 0,00	5	0,00 ± 0,00	-

*Teste-t for independent samples.

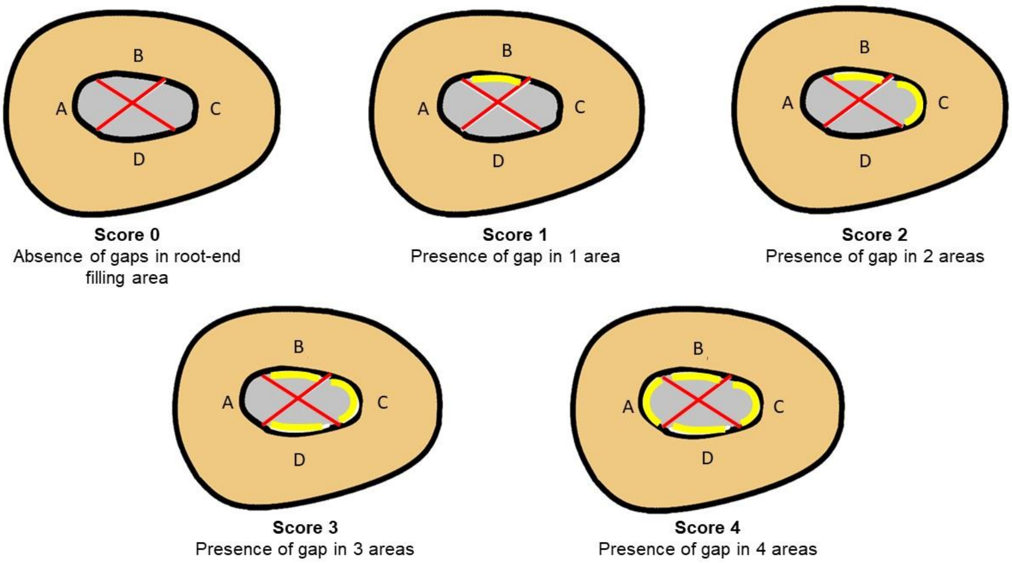

Figure 1. Schematic representation of root-end filling division in areas (A, B, C and D) and classification used to evaluation of the presence or absence of gaps between root-end filling material and root-end cavity dentine.

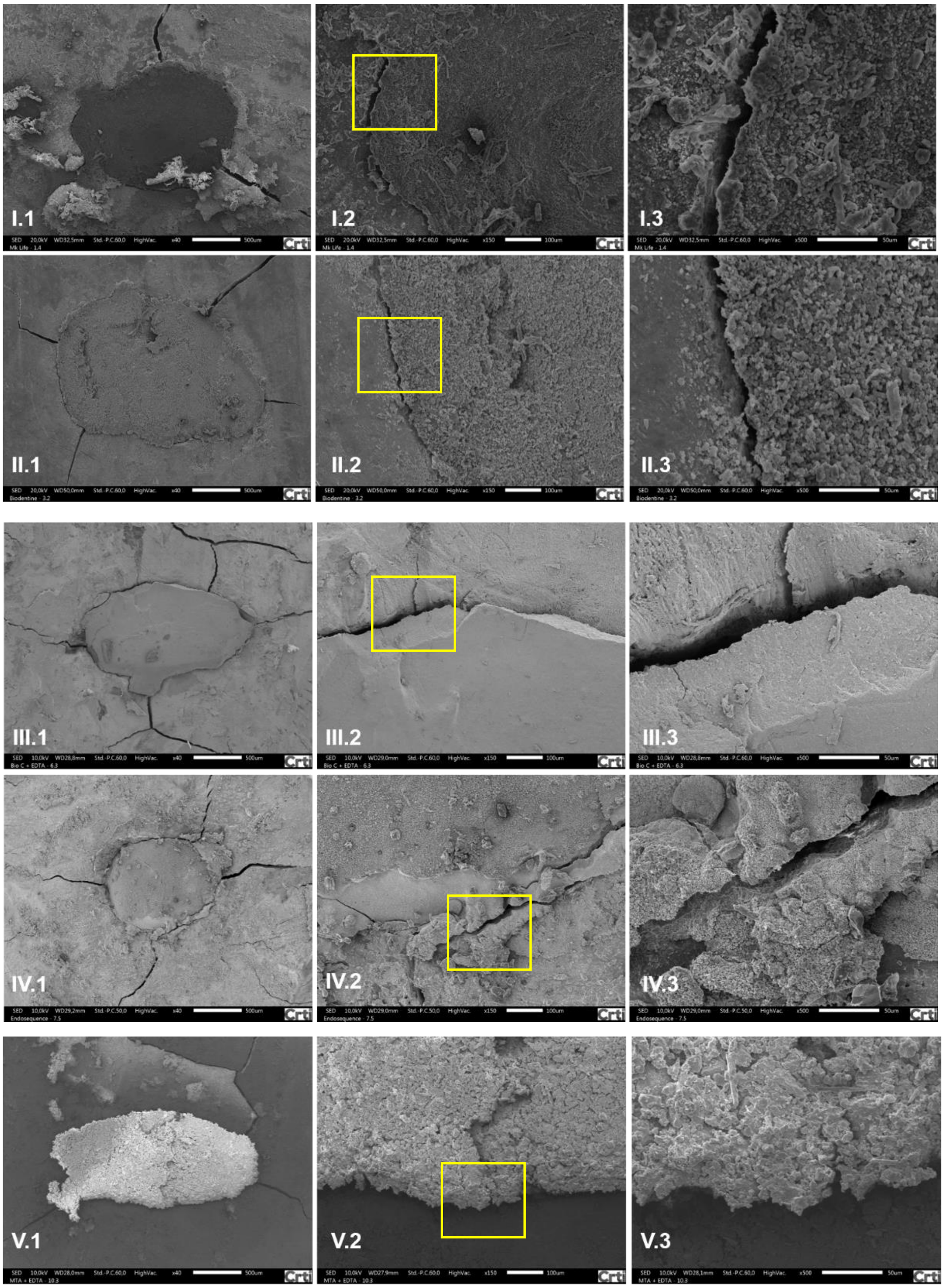

Figure 2. SEM photomicrographs of marginal adaptation of MK Life bioceramic cement (I.1 - I.3), Biodentine® (II.1 - II.3), Bio-C Repair® (III.1 - III.3), Endosequence® BC RRM™ (IV.1 - IV.3), MTA Angelus® (V.1 - V.3) and Calcium hydroxide paste (VI.1 - VI.3) at magnifications of x40 (1), x150 (2) and x500 (3). Spotlight (yellow) region selected to perform photomicrography at 500x magnification.

ANEXO

Normas para publicação

INTERNATIONAL ENDODONTIC JOURNAL
The official journal of the British Endodontic Society and the European Society of Endodontology

Author Guidelines

Content of Author Guidelines:

1. General, 2. Ethical Guidelines, 3. Manuscript Submission Procedure, 4. Manuscript Types Accepted, 5. Manuscript Format and Structure, 6. After Acceptance

Useful Websites:

Submission Site, Articles published in *International Endodontic Journal*, Author Services, Wiley's Ethical Guidelines, Guidelines for Figures

The journal to which you are submitting your manuscript employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

1. GENERAL

International Endodontic Journal publishes original scientific articles, reviews, clinical articles and case reports in the field of Endodontology; the branch of dental sciences dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health. Original scientific articles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in *International Endodontic Journal*. Authors are encouraged to visit Wiley Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

International Endodontic Journal adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal.

International Endodontic Journal adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisiation of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Please find more information on the conflict of interest form in section 2.6.

2.2. Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical AssociationDeclaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study. The authors MUST upload a copy of the ethical approval letter when submitting their manuscript. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

2.3 Clinical Trials

The International Endodontic Journal asks that authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following public clinical trials registries: www.clinicaltrials.gov, https://www.clinicaltrialsregister.eu/, http://isrctn.org/. Other primary registries if named in the WHO network will also be considered acceptable. The clinical trial registration number and name of the trial register should be included in the Acknowledgements at the submission stage.

2.3.1 Randomised control clinical trials

Randomised control clinical trials should be reported using the guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

2.3.2 Epidemiological observational trials

Submitting authors of epidemiological human observations studies are required to review and submit a 'strengthening the reporting of observational studies in Epidemiology' (STROBE) checklist and statement. Compliance with this should be detailed in the materials and methods section. (www.strobe-statement.org)

2.4 Systematic Reviews

Systematic reviews should be reported using the PRISMA guidelines available at http://prisma-statement.org/. A PRISMA checklist and flow diagram (as a Figure) should also be included in the submission material.

2.5 DNA Sequences and Crystallographic Structure Determinations

Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly.

2.6 Conflict of Interest and Source of Funding

International Endodontic Journal requires that all authors (both the corresponding author and co-authors) disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author's objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If authors are unsure whether a past or present affiliation or relationship should be disclosed in the manuscript, please contact the editorial office at iejeditor@cardiff.ac.uk. The existence of a conflict of interest does not preclude publication in this journal.

The above policies are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the International Committee of Medical Journal Editors (http://www.icmje.org/).

It is the responsibility of the corresponding author to have all authors of a manuscript fill out a conflict of interest disclosure form, and to upload all forms individually (do not combine the forms into one file) together with the manuscript on submission. The disclosure statement should be included under Acknowledgements. Please find the form below:

Conflict of Interest Disclosure Form

2.7 Appeal of Decision

The decision on a paper is final and cannot be appealed.

2.8 Permissions

If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

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3. OnlineOpen

OnlineOpen is available to authors of primary research articles who wish to make their article available to non-subscribers on publication, or whose funding agency requires grantees to archive the final version of their article. With OnlineOpen, the author, the author's funding agency, or the author's institution pays a fee to ensure that the article is made available to non-subscribers upon publication via Wiley Online Library, as well as deposited in the funding agency's preferred archive. For the full list of terms and conditions, see http://wileyonlinelibrary.com/onlineopen#OnlineOpen_Terms

Any authors wishing to send their paper OnlineOpen will be required to complete the payment form available from our website at: https://authorservices.wiley.com/bauthor/onlineopen_order.asp

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3.1 MANUSCRIPT SUBMISSION PROCEDURE

Manuscripts should be submitted electronically via the online submission site http://mc.manuscriptcentral.com/iej. The use of an online submission and peer review site enables immediate distribution of manuscripts and consequentially speeds up the review process. It also allows authors to track the status of their own manuscripts. Complete instructions for submitting a paper is available online and below. Further assistance can be obtained from iejeditor@cardiff.ac.uk.

3.2. Getting Started

• Launch your web browser (supported browsers include Internet Explorer 5.5 or higher, Safari 1.2.4, or Firefox 1.0.4 or higher) and go to the journal's online Submission Site: http://mc.manuscriptcentral.com/iej

• Log-in, or if you are a new user, click on 'register here'.

• If you are registering as a new user.

- After clicking on 'register here', enter your name and e-mail information and click 'Next'. Your e-mail information is very important.

- Enter your institution and address information as appropriate, and then click 'Next.'

- Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your areas of expertise. Click 'Finish'.

• If you are registered, but have forgotten your log in details, please enter your e-mail address under 'Password Help'. The system will send you an automatic user ID and a new temporary password.

• Log-in and select 'Author Centre '

3.3. Submitting Your Manuscript

• After you have logged into your 'Author Centre', submit your manuscript by clicking on the submission link under 'Author Resources'.

• Enter data and answer questions as appropriate. You may copy and pastedirectly from your manuscript and you may upload your pre-prepared covering letter.

• Click the 'Next' button on each screen to save your work and advance to the next screen.

• You are required to upload your files.

- Click on the 'Browse' button and locate the file on your computer.

- Select the designation of each file in the drop down next to the Browse button.

- When you have selected all files you wish to upload, click the 'Upload Files' button.

• Review your submission (in HTML and PDF format) before completing your submission by sending it to the Journal. Click the 'Submit' button when you are finished reviewing.

3.4. Manuscript Files Accepted

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected) plus separate figure files. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing. The files will be automatically converted to HTML and PDF on upload and will be used for the review process. The text file must contain the abstract, main text, references, tables, and figure legends, but no embedded figures or Title page. The Title page should be uploaded as a separate file. In the main text, please reference figures as for instance 'Figure 1', 'Figure 2' etc to match the tag name you choose for the individual figure files uploaded. Manuscripts should be formatted as described in the Author Guidelines below.

3.5. Blinded Review

Manuscript that do not conform to the general aims and scope of the journal will be returned immediately without review. All other manuscripts will be reviewed by experts in the field (generally two referees). International Endodontic Journal aims to forward referees´ comments and to inform the corresponding author of the result of the review process. Manuscripts will be considered for fast-track publication under special circumstances after consultation with the Editor.

International Endodontic Journal uses double blinded review. The names of the reviewers will thus not be disclosed to the author submitting a paper and the name(s) of the author(s) will not be disclosed to the reviewers. To allow double blinded review, please submit (upload) your main manuscript and title page as separate files.

Please upload:

• Your manuscript without title page under the file designation 'main document'

• Figure files under the file designation 'figures'

• The title page and Acknowledgements where applicable, should be uploaded under the file designation 'title page'

All documents uploaded under the file designation 'title page' will not be viewable in the html and pdf format you are asked to review in the end of the submission process. The files viewable in the html and pdf format are the files available to the reviewer in the review process.

3.6. Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

3.7. E-mail Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

3.8. Manuscript Status

You can access ScholarOne Manuscripts any time to check your 'Author Centre' for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

3.9. Submission of Revised Manuscripts

To submit a revised manuscript, locate your manuscript under 'Manuscripts with Decisions' and click on 'Submit a Revision'. Please remember to delete any old files uploaded when you upload your revised manuscript.

4. MANUSCRIPT TYPES ACCEPTED

Original Scientific Articles: must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original Scientific Articles must conform to the highest international standards in the field.

Review Articles: are accepted for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should generally include a clearly defined search strategy and take a broad view of the field rather than merely summarizing the authors´ own previous work. Extensive or unbalanced citation of the authors´ own publications is discouraged.

Mini Review Articles: are accepted to address current evidence on well-defined clinical, research or methodological topics. All are refereed by experts in the field who are asked to comment on timeliness, general interest, balanced treatment of controversies, and scientific rigor. A clear research question, search strategy and balanced synthesis of the evidence is expected. Manuscripts are limited in terms of word-length and number of figures.

Clinical Articles: are suited to describe significant improvements in clinical practice such as the report of a novel technique, a breakthrough in technology or practical approaches to recognised clinical challenges. They should conform to the highest scientific and clinical practice standards.

Case Reports: illustrating unusual and clinically relevant observations are acceptable but they must be of sufficiently high quality to be considered worthy of publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered. Illustrative material must be of the highest quality and healing outcomes, if appropriate, should be demonstrated.

Supporting Information: *International Endodontic Journal* encourages submission of adjuncts to printed papers via the supporting information website (see submission of supporting information below). It is encouraged that authors wishing to describe novel procedures or illustrate cases more fully with figures and/or video may wish to utilise this facility.

Letters to the Editor: are also acceptable.

Meeting Reports: are also acceptable.

5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Format

Language: The language of publication is English. It is preferred that manuscript is professionally edited. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication

Presentation: Authors should pay special attention to the presentation of their research findings or clinical reports so that they may be communicated clearly. Technical jargon should be avoided as much as possible and clearly explained where its use is unavoidable. Abbreviations should also be kept to a minimum, particularly those that are not standard. The background and hypotheses underlying the study, as well as its main conclusions, should be clearly explained. Titles and abstracts especially should be written in language that will be readily intelligible to any scientist.

Abbreviations: International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

5.2. Structure

All manuscripts submitted to *International Endodontic Journal* should include Title Page, Abstract, Main Text, References and Acknowledgements, Tables, Figures and Figure Legends as appropriate

Title Page: The title page should bear: (i) Title, which should be concise as well as descriptive; (ii) Initial(s) and last (family) name of each author; (iii) Name and address of department, hospital or institution to which work should be attributed; (iv) Running title (no more than 30 letters and spaces); (v) No more than six keywords (in alphabetical order); (vi) Name, full postal address, telephone, fax number and e-mail address of author responsible for correspondence.

Abstract for Original Scientific Articles should be no more than 250 words giving details of what was done using the following structure:

• **Aim**: Give a clear statement of the main aim of the study and the main hypothesis tested, if any.

• **Methodology**: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.

• **Results**: Give the main results of the study, including the outcome of any statistical analysis.

• **Conclusions**: State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Review Articles should be non-structured of no more than 250 words giving details of what was done including the literature search strategy.

Abstract for Mini Review Articles should be non-structured of no more than 250 words, including a clear research question, details of the literature search strategy and clear conclusions.

Abstract for Case Reports should be no more than 250 words using the following structure:

• **Aim**: Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• **Summary**: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and analysis if any.

• **Key learning points**: Provide up to 5 short, bullet-pointed statements to highlight the key messages of the report. All points must be fully justified by material presented in the report.

Abstract for Clinical Articles should be no more than 250 words using the following structure:

• Aim: Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• Methodology: Describe the methods adopted.

• Results: Give the main results of the study.

• Conclusions: State the primary conclusions of the study.

Main Text of Original Scientific Article should include Introduction, Materials and Methods, Results, Discussion and Conclusion

Introduction: should be focused, outlining the historical or logical origins of the study and gaps in knowledge. Exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation, or hypothesis to be tested.

Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced.

(i) Clinical Trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

(ii) Experimental Subjects: experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National

Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

(iii) Suppliers: Suppliers of materials should be named and their location (Company, town/city, state, country) included.

Results: should present the observations with minimal reference to earlier literature or to possible interpretations. Data should not be duplicated in Tables and Figures.

Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The Discussion section should progress with a review of the methodology before discussing the results in light of previous work in the field. The Discussion should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.

Conclusion: should contain a summary of the findings.

Main Text of Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

Main Text of Mini Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should briefly introduce the subject matter and justify the need and timeliness of the literature review. The Review section should be divided into logical sub-sections to enhance readability and understanding and may be supported by up to 5 tables and figures. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The Conclusions section should present clear statements/recommendations and suggestions for further work. The manuscript, including references and figure legends should not normally exceed 4000 words.

Main Text of Clinical Reports and Clinical Articles should be divided into Introduction, Report, Discussion and Conclusion,. They should be well illustrated with clinical images, radiographs, diagrams and, where appropriate, supporting tables and graphs. However, all illustrations must be of the highest quality

Acknowledgements: *International Endodontic Journal* requires that all sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential conflicts of interest noted. Grant or contribution numbers may be acknowledged, and principal grant holders should be listed. Acknowledgments should be brief and should not include thanks to anonymous referees and editors. See also above under Ethical Guidelines.

5.3. References

It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. The EndNote reference style can be obtained upon request to the editorial office (iejeditor@cardiff.ac.uk). Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

In the text: single or double authors should be acknowledged together with the year of publication, e.g. (Pitt Ford & Roberts 1990). If more than two authors the first author followed by *et al.* is sufficient, e.g. (Tobias *et al.* 1991). If more than 1 paper is cited the references should be in year order and separated by "," e.g. (Pitt Ford & Roberts 1990, Tobias *et al*. 1991).

Reference list: All references should be brought together at the end of the paper in alphabetical order and should be in the following form.

(i) Names and initials of up to six authors. When there are seven or more, list the first three and add *et al.*

(ii)Year of publication in parentheses

(iii) Full title of paper followed by a full stop (.)

(iv) Title of journal in full (in italics)

(v) Volume number (bold) followed by a comma (,)

(vi) First and last pages

Examples of correct forms of reference follow:

Standard journal article

Bergenholtz G, Nagaoka S, Jontell M (1991) Class II antigen-expressing cells in experimentally induced pulpitis. *International Endodontic Journal* **24**, 8-14.

Corporate author

British Endodontic Society (1983) Guidelines for root canal treatment. *International Endodontic Journal* **16**, 192-5.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). *Blood* **54** (Suppl. 1), 26a.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) *Surgical Endodontics*, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. *Endodontics in Clinical Practice*, 3rd edn; pp. 186-223. London, UK: Butterworth.

Published proceedings paper

DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

URLs

Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.
Smith A (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html [accessed on 7 November 2003]

5.4. Tables, Figures and Figure Legends

Tables: Tables should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title.

Figures: All figures should be planned to fit within either 1 column width (8.0 cm), 1.5 column widths (13.0 cm) or 2 column widths (17.0 cm), and must be suitable for photocopy reproduction from the printed version of the manuscript. Lettering on figures should be in a clear, sans serif typeface (e.g. Helvetica); if possible, the same typeface should be used for all figures in a paper. After reduction for publication, upper-case text and numbers should be at least 1.5-2.0 mm high (10 point Helvetica). After reduction, symbols should be at least 2.0-3.0 mm high (10 point). All half-tone photographs should be submitted at final reproduction size. In general, multi-part figures should be arranged as they would appear in the final version. Reduction to the scale that will be used on the page is not necessary, but any special requirements (such as the separation distance of stereo pairs) should be clearly specified.

Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible.

Figures should be on a white background, and should avoid excessive boxing, unnecessary colour, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms should not be truncated to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size.

Figures divided into parts should be labelled with a lower-case, boldface, roman letter, a, b, and so on, in the same typesize as used elsewhere in the figure. Lettering in figures should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature or the nomenclature common to a particular field. Thousands should be separated by a thin space (1 000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general, visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc.)

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