UNIVERSIDADE FEDERAL DE GOIÁS
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

FELIPE CAVALCANTI SAMPAIO

Microanálise de superfície e caracterização química de cimentos endodônticos

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduaçãoda Faculdade de Odontologia da Universidade Federal de Goiás para obtenção do título de Mestre em Odontologia.

Orientador: Prof. Dr. Carlos Estrela

Co-orientadora: Prof^{a}. Dr^{a}. Ana Helena Gonçalves de Alencar

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DEDICATÓRIA

Dedico este trabalho a Lindalva Sampaio, que mesmo não estando por perto vem guiando meus passos.

Se você quer ser bem sucedido precisa ter dedicação total, buscar seu último limite e dar o melhor de si.

(Ayrton Senna)

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FIGURAS, QUADROS E TABELAS

SÍMBOLOS E ABREVIATURAS

%at	Porcentagem atômica
%p	Porcentagem de peso atômico
Ag	Prata (elemento químico)
Al	Alumínio (elemento químico)
Ba	Bário (elemento químico)
Bi	Bismuto (elemento químico)
Bo	Boro (elemento químico)
Ca	Cálcio (elemento químico)
Cl	Cloro (elemento químico)
EDX	Espectroscopia de Dispersão de raios-X
Fe	Ferro (elemento químico)
Hf	Háfnio (elemento químico)
I	Iodo (elemento químico)
kV	Quilovolt
mA	Miliampere
MEV	Microscopia Eletrônica de Varredura
Mg	Magnésio (elemento químico)
Mm	Milímetro
MTA	Agregado Trióxido Mineral
Na	Sódio (elemento químico)
Ni	Níquel (elemento químico)
Nm	Nanômetro
S	Enxofre (elemento químico)
Si	Silício (elemento químico)
Ti	Titânio (elemento químico)
W	Tungstênio (elemento químico)
Zn	Zinco (elemento químico)
Zr	Zircônia (elemento químico)

RESUMO

Objetivo:analisar a superfície e avaliar a composição química de materiais obturadores do canal radicular por meio de microscopia eletrônica de varredura (MEV) e espectroscopia de dispersão de raios-X (EDX). **Material e métodos**: dezoito tubos de polietileno padronizados foram preenchidos com os materiais avaliados (n=3):Sealapex®, Sealer 26®, MTA Fillapex®, Pulp Canal Sealer®, Endofill® e AH Plus®. Após 48 horas a 37°C e umidade relativa de 95%, as amostras foram metalizadas com ouro, conduzidas ao MEV e as imagens da superfície analisadas emum aumento de 5.000X. A seguir, a distribuição dos elementos e composição química foram determinadas por meio de EDX. Os resultados foram avaliados qualitativamente (imagens do MEV e mapas de distribuição de elementos) e quantitativamente (porcentagem em peso).**Resultados**: a análise da superfície revelou que os cimentos apresentaram diferentes regularidades em imagens por MEV. As partículas apresentaram-se com distribuição uniforme dos elementos, com tamanhos similares e formas variáveis em microanálises por EDX.Os cimentos à base de óxido ou hidróxido de cálcio(Sealapex® e Sealer 26®) apresentaramquantidades de cálcio de 53,58%p (porcentagem de peso atômico) e 65,00%p, respectivamente, em microanálises por EDX.O cimento MTA Fillapex® apresentou 30,58%p de cálcio e elevadas quantidades de silício (31,02%p) e bismuto (27,38%p). Os cimentos contendo óxido de zinco e eugenol,Pulp Canal Sealer® e Endofill®, apresentaram zinco em quantidades de 67,74%p e 63,16%p, respectivamente.O AH Plus® apresentou maior quantidade de zircônia (64,24%p). Foram encontrados elementos não compatíveis com a composição descrita pelo fabricante. **Conclusões**: as superfícies dos cimentos endodônticos mostraram diferentes regularidades. As partículas apresentaram distribuição uniforme, com tamanhos similares, porém com formas variadas.Foram encontrados nos cimentos endodônticos elementos químicos que não foram descritos pelos fabricantes.

Palavras-chave: Microscopia eletrônica de varredura, espectroscopia de dispersão de raios-X, propriedades químicas, materiais obturadores.

ABSTRACT

Purpose: to assess the surface and evaluate the chemical composition of root canal filling materials by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). **Methods**: eighteen polyethylene standard tubes were filled with the tested materials: Sealapex®, Sealer 26®, MTA Fillapex®, Pulp Canal Sealer®, Endofill® and AH Plus®. After 48 hours at 37°C and 95% relative humidity, the samples were surface-sputtered with gold, led to SEM and the images analyzed at5,000X magnification. Then, the elements distribution and chemical composition were determined by EDX. The results were evaluated qualitatively (SEM images and elements distribution maps) and quantitatively (weight percentage). **Results**: the surface analysis revealed that the sealers presented different regularities, with an uniform distribution of elements, with particles of similar sizes and variable shapes in EDX microanalysis. Calcium oxide and hydroxide based sealers (Sealapex® and Sealer 26®) presented calcium peaks of 53.58wt.% and 65.00wt.%, respectively. MTA Fillapex® presented 30.58wt.% of calcium and high amounts of silicon (31.02 weight%) and bismuth (27.38 weight%). Zinc oxide and eugenol based sealers, Pulp Canal Sealer® and Endofill®, showed zinc quantities of 67.74wt% and 63.16wt.%, respectively. AH Plus® had higher amount of zirconium (64.24wt.%). The materials presented elements incompatible with the composition described by the manufacturer. **Conclusions:** the root canal sealers' surfacesshowed different. The elements presented uniform distribution, with particles of similar sizes and variable shapes. Chemical elements were found in the root canal sealers not described by the manufacturers.

Key Words: Scanning electron microscopy, energy-dispersive X-ray analysis, chemical properties, root canal filling materials.

1.INTRODUÇÃO

O cimento obturador apresenta função de preencher os espaços presentes entre os cones de guta-percha e as paredes dentinináriase possibilitar a perpetuação da sanificação dos canais radiculares. As propriedades biológicas e astécnicas de selamento do canal radicular, em conjunto com a ausência de agressão periapical,favorecem o restabelecimento do estado de normalidade dos tecidos alterados(Orstavik, 2005). A tolerância tecidual ao material obturador, que em algumas situações encontra-se em contato direto com os tecidos periapicais, deve ser cuidadosamente observada durante sua seleção. Outra característica que este material deveria apresentar é a alta resistência de união à dentina(Grossman, 1958; Vujaskovic e Teodorovic, 2010). De um modo geral, a busca por novos materiais obturadores de canais radiculares se deve à necessidade de se obter alternativas com propriedades que superem os materiais existentes(Orstavik, 1983; 2005; Bouillaguet *et al.*, 2008).

A estrutura de superfície e a caracterização química dos componentes encontrados nos cimentos endodônticos permitem estabelecer importantes correlações com a tolerância tecidual, resistência de união à dentina e atividade antimicrobiana(Estrela, 2004).Resende *et al.*(2009) avaliaram as propriedades físico-químicas e a morfologia da superfície dos cimentos endodônticos AH Plus®, Epiphany® e Ephiphany SE®. O AH plus®apresentou-se de acordo com as especificações da ANSI/ADA para todas as propriedades, o que não foi verificado nos demais cimentos. Resultados semelhantes foram encontrados por Versiani *et al.*(2006) e Flores *et al.*(2011).Haragushiku *et al.*(2010)observaram que o AH Plus®apresentoumelhor capacidade de adesão às paredes dentinárias quando comparado ao Apexit®e o Epiphany®.Garrido *et al.*(2010) comparam os cimentosBiosealer®, Sealer 26®, Endofill® e AH Plus® em relação às propriedades físico-químicas e verificaram que o Sealer 26® e o AH Plus® apresentaram tempo de presa mais longo. A espessura e a alteração dimensional do Sealer 26® não foram consideradas satisfatórias, assim como o Endofill® não se mostrou adequado em termos de solubilidade e desintegração.

Na análise de adesão à dentina, oSealapex® e o Apexit® apresentaram pobres resultadosquando comparados ao Sealer 26® e o CRCS®.O EDTA aumentou a adesão dos cimentos endodônticos às paredes dentinárias, à exceção do Sealapex®(Fidel *et al.*, 1994).O Sealapex® mostrou capacidade de penetração em túbulos dentinários em análise por meio de microscopia confocal(Ordinola-Zapata *et al.*, 2009).O hidróxido de cálcio apresentaexcelente potencial de formação de barreira mineralizada eoSealapex®, cimento endodôntico contendo óxido de cálcio, também mostrou capacidade deestimular a deposição de tecido mineralizado após a obturação do canal radicular de dentes de cães e macacos(Holland e de Souza, 1985). Tagger *et al.*(1988)avaliaram a capacidade de liberação de íons cálcio e hidroxila dos cimentos Sealapex®, CRCS® e Hermetic®. OSealapex® apresentou maior capacidade de liberação destes íons e sua solubilidade pode ser a justificativa para a maior liberação em relação aos outros cimentos testados.

Recentemente foi desenvolvido o MTA Fillapex®, com o intuito de adicionar as propriedades biológicas do MTA às propriedades físico-químicas de um cimento endodôntico(Gomes-Filho *et al.*, 2011; Scelza *et al.*, 2012). Gomes-Filho *et al.*(2011) avaliaram a reação de tecido subcutâneo de ratos ao MTA Fillapex® e a capacidade mineralizadora, em comparação ao MTA Angelus® e o Sealapex®. Todos os materiais estudados induziram inflamação moderada e capacidade de indução de mineralização. O MTA Fillapex®mostrou ser um material obturador com boa tolerância tecidual.

A composição química do material obturador, distribuída em nível da estrutura de sua superfície pode caracterizar diferentes propriedades, uma vez que esta superfície possibilita uma interação entre o material e os tecidos com os quais mantêm contato.Entre estas, a biocompatibilidade pode ser diretamente afetada pela composição química do material, uma vez que a presença de compostos irritantes aos tecidos biológicos reduz a tolerância tecidual de um material (Dammaschke *et al.*, 2005).Desta maneira, o conhecimento da composição química da superfície dos cimentos endodônticos pode favorecer o entendimento da interação entre as propriedades biológicas e as físico-químicas.

O objetivo do presente estudo foi analisar a superfície de cimentos endodônticos por meio de imagens de Microscopia Eletrônica de Varredura (MEV) e Espectroscopia de Dispersão de raios-X (EDX), quanto à regularidade da superfície, distribuição dos elementos, forma e tamanho das partículas e caracterizá-los quimicamente.

2. PROPOSIÇÃO

O objetivo do presente estudo foi analisar a superfície dos cimentos endodônticos Sealapex®, Sealer 26®, MTA Fillapex®, Pulp Canal Sealer®,Endofill® e AH Plus® por meio de imagens de Microscopia Eletrônica de Varredura (MEV) e Espectroscopia de Dispersão de raios-X (EDX), quanto à regularidade da superfície, distribuição dos elementos, forma e tamanho das partículas e caracterizá-los quimicamente.

3.MATERIAL E MÉTODOS

Seis cimentos endodônticos comercialmente disponíveis foram utilizados nos experimentos: Sealapex®(SybronEndo, Orange, CA, EUA), Sealer 26®(Dentsply, De Tray, Konstanz, Alemanha), MTA Fillapex®(Angelus Soluções Odontológicas, Londrina, PR, Brasil), Pulp Canal Sealer®(SybronEndo, Orange, CA, EUA), Endofill®(Dentsply, Petrópolis, RJ, Brasil), AH Plus®(Dentsply, De Tray, Konstanz, Alemanha). A composição dos materiais avaliados, como descrito por seus fabricantes, está descrita no Quadro1.

Dezoitotubos de polietileno padronizados (Sonda Levine n° 12, Embramed, Jurubatuba, SP, Brasil), com diâmetro interno de 3 mm e comprimento de 3 mm, foram preparados utilizando um paquímetro digital com resolução de 0,01mm (Mitutoyo MTI Corporation, Tóquio, Japão) e uma lâmina de bisturi nº 11 (SwannMorton, Sheffield, Reino Unido). Três tubos para cada grupo foram colocados em uma placa de vidro polida (75 x 25 x 1 mm) e preenchidoscom pequenas porções dos materiais a serem avaliados com auxílio de uma espátula nº 24 (SS White Duflex, Rio de Janeiro, RJ, Brasil) até que os tubos estivessem completamente preenchidos. Todos os cimentos foram manipulados de acordo com as instruções dos respectivos fabricantes. A seguir, os espécimes foram transferidos para uma estufa com umidade relativa de 95% e temperatura de 37°C, por 48 horas.

Quadro1. Composição dos cimentos endodônticos avaliados

Material	Componentes	
Sealapex®	Catalisador	Base
	Resina Isobutilsalicilato	Resina N-etil tolueno sulfonamida
	Sílica (Dióxido de silicato)	Silica (Dióxido de silicato)
	Trióxido de bismuto	Óxido de zinco
	Pigmento de dióxido de titânio	Óxido de cálcio
Sealer 26®	Pó	Resina
	Hidróxido de cálcio	Éter bisfenol A
	Óxido de bismuto	
	Hexametilenotetramina	
MTA Fillapex®	Componentes	Componentes
	Resina natural	Silicanano particulada
	Resina salicilato	MTA
	Resina diluída	Pigmentos
	Óxido de bismuto	
Pulp Canal Sealer®	Pó	Líquido
	Óxido de zinco	Óleo de cravo
	Prata molecular precipitada	Bálsamo do Canadá
	Oleo resinas	
	Iodeto de timol	
EndoFill®	Pó	Líquido
	Óxido de zinco	Eugenol
	Resina hidrogenada	Óleo de amêndoa
	Subcarbonato de bismuto	
	Sulfato de bário	
	Borato de sódio	
AH Plus®	Pasta A	Pasta B
	Resina epóxi bisfenol-A	Dibenzil diamina
	Resina epóxi bisfenol-F	Aminoadamantano
	Tungstato de cálcio	Triciclodecanodiamina
	Óxido de zircônia	Tungstato de cálcio
	Silica	Óxido de zircônia
	Pigmentos de óxido de ferro	Silica
		Óleo de silicone

Os espécimes foram metalizados com ouro,levados diretamente ao microscópio eletrônico de varredura (MEV Leo Stereoscan 420i, LeicaElectronOptics, Cambridge Instruments, Cambridge, Reino Unido), com tensão 8 a 10 kV e resolução de 2 nm, e examinados sem qualquer preparo ou manipulação. Para a análise foram obtidas imagens de 5.000X de aumento, utilizadas para determinação da regularidade da superfície.

A análise dos elementos químicos dos espécimes foi realizada utilizando espectroscópio de dispersão de raios-X (EDX) com o *software* NSS SpectralAnalysis System 2.3 (Thermo Fisher Scientific Inc., Suwanee, GA, EUA). As mensurações da microanálise por EDX foram conduzidas na região central de cada espécime utilizando um feixe de elétrons de tamanho menor que 50 nm, tensão de aceleração de 25 kV, corrente do feixe de 110mA, determinado de acordo com a composição descrita pelo fabricante, e o espectro foi obtido por 100 segundos.

As avaliações foram realizadas por dois profissionais calibrados a partir de estudo piloto com 10% das amostras e com experiência na área. Uma análise quantitativa foi realizada por meio do *software* NSS SpectralAnalysis System 2.3, em modo de análise não padrão, utilizando método de correção PROZA (Phi-Rho-Z). Os mapas de distribuição de elementos foram obtidos pelo método NetCounts, com alta resolução, por meio do mesmo *software*, e utilizados na análise descritiva da superfície dos cimentos endodônticos.

A análise da superfície por meio de MEV e a microanálise por EDX foram qualitativas, sendo aplicada no estudo uma análise descritiva. A superfície foi descrita de acordo com a regularidade da superfície (regular ou irregular), distribuição dos elementos (uniforme ou não uniforme),forma (globular, forma de agulha, matriz, etc.) e tamanho (similaridade entre o tamanho das partículas, sem mensuração) das partículas presentes.

4.RESULTADOS

As figuras de 1 a 6 mostram as imagens dos cimentos endodônticos pela análise em MEV (A), utilizada para a caracterização da regularidade da superfície, e os mapas de distribuição com os dois elementos principais obtidos pela microanálise por EDX (B), utilizados para caracterizar a superfície quanto à distribuição dos elementos, forma e tamanho das partículas.

OSealapex® apresentou uma superfície irregular (Figura 1A), com distribuição uniforme dos elementos, composta principalmente por cálcio, com partículas de formato globular, e bismuto, que se apresentou em forma de agulhas, e diferentes tamanhos (Figura 1B).O Sealer 26® apresentou uma superfície regular (Figura 2A) composta principalmente por cálcio, com partículas de bismuto de diferentes tamanhos e formas em uma distribuição uniforme (Figura 2B).

O MTA Fillapex® apresentou uma superfície irregular (Figura 3A) com uma distribuição uniforme dos elementos, composta principalmente por uma matriz de silício com partículas de cálcio de aspecto globular e partículas de bismuto de diferentes formas e tamanhos (Figura 3B). O Pulp Canal Sealer®apresentou superfície regular (Figura 4A) com distribuição uniforme dos elementos, composta por partículas globulares, principalmente de zinco e prata (Figura 4B). O Endofill® apresentou superfície regular (Figura 5A), com distribuição uniforme dos elementos, composta por partículas globulares, principalmente zinco e bismuto (Figura 5B).O AH Plus® mostrou uma superfície regular (Figura 6A) com partículas de aspecto globular em uma distribuição uniforme, composto principalmente por zircônia (Figura 6B).

Os resultados quantitativos dos elementos da área dos espécimes avaliados pela microanálise por EDX estão descritos na Tabela 1 em porcentagem atômica (%at) e porcentagem de peso atômico (%p). Os cimentos contendo de óxido ou hidróxido de cálcio (Sealapex® e Sealer 26®) apresentaram picos de cálcio (53,58%p e 65,00%p, respectivamente), em quantidade maior que o material que contém MTA (MTA Fillapex®, 30,58%p), o qual também apresentou elevadas quantidades de silício (31,02%p) e bismuto (27,38%p). Os cimentos que contêm de óxido de zinco e eugenol (Pulp Canal Sealer®e Endofill®) apresentaram picos de zinco (67,74%p e 63,16%p, respectivamente), enquanto que o cimento resinoso (AH Plus®) apresentou maior quantidade de zircônia (64,24%p).

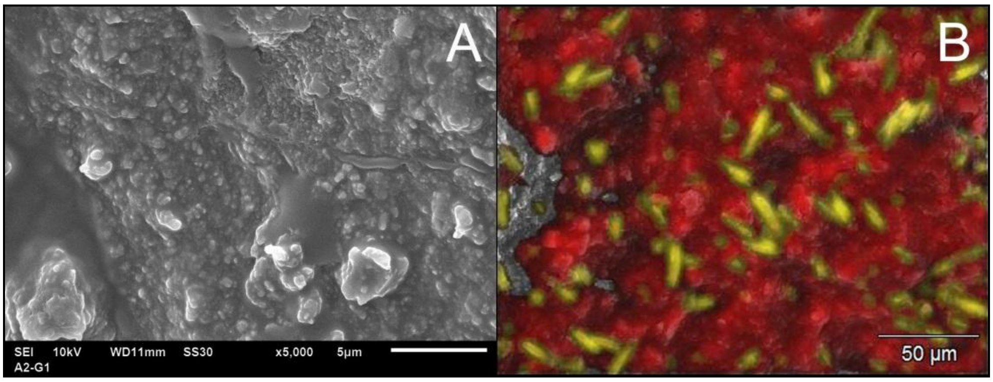

Figura 1. Imagem de microscopia eletrônica de varredura do cimento Sealapex® em aumento de 5.000X (A), evidenciando superfície irregular; mapa de distribuição elementos cálcio (vermelho) e bismuto (amarelo) na superfície do Sealapex® (B) por meio da microanálise por espectroscopia de dispersão de raios-X, em aumento de 50.000X, que mostra a distribuição uniforme dos elementos, com partículas de tamanho e formas variáveis.

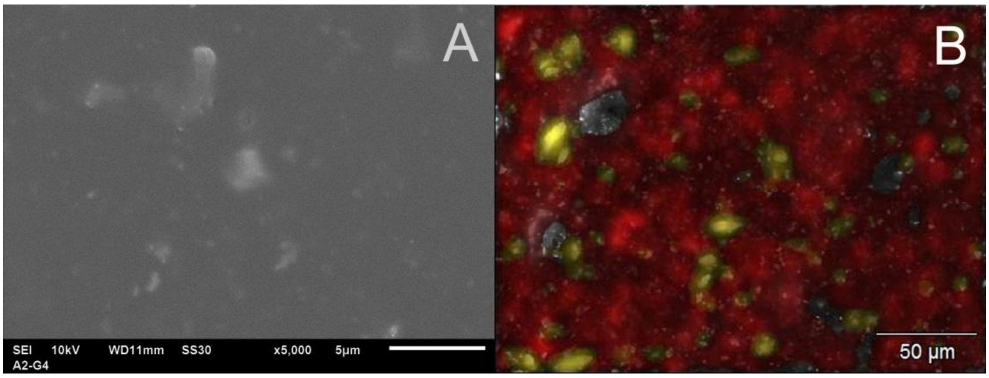

Figura 2. Imagem de microscopia eletrônica de varredura do cimento Sealer 26® em aumento de 5.000X (A), evidenciando superfície regular; mapa de distribuição elementos cálcio (vermelho) e bismuto (amarelo) na superfície do Sealer 26® (B) por meio da microanálise por espectroscopia de dispersão de raios-X,em aumento de 50.000X, que mostra a distribuição uniforme dos elementos, com partículas de tamanho e formas variáveis.

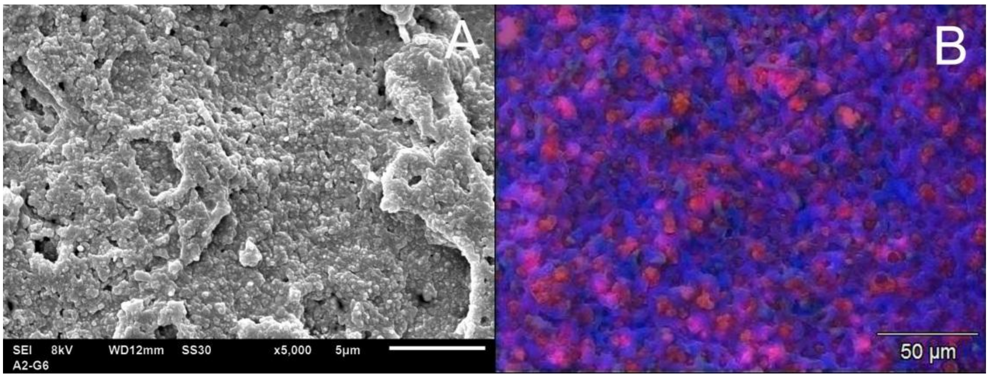

Figura 3. Imagem de microscopia eletrônica de varredura do cimento MTA Fillapex® em aumento de 5.000X (A), evidenciando superfície irregular; mapa de distribuição elementos silício (azul) e cálcio (vermelho) na superfície do MTA Fillapex® (B) por meio da microanálise por espectroscopia de dispersão de raios-X,em aumento de 50.000X, que mostra a distribuição uniforme dos elementos, com partículas de tamanho similar e formas variáveis.

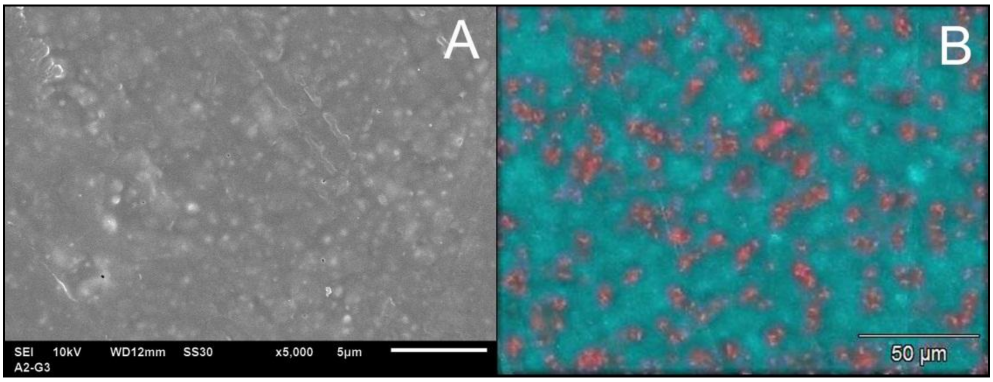

Figura 4. Imagem de microscopia eletrônica de varredura do cimento Pulp Canal Sealer® em aumento de 5.000X (A), evidenciando superfície regular; mapa de distribuição elementos zinco (azul claro) e prata (rosa) na superfície do Pulp Canal Sealer® (B) por meio da microanálise por espectroscopia de dispersão de raios-X,em aumento de 50.000X, que mostra a distribuição uniforme dos elementos, com partículas de tamanho similar e forma globular.

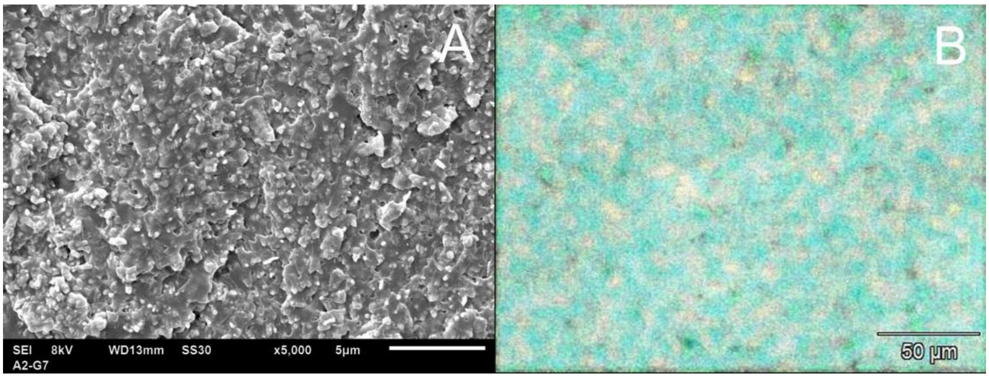

Figura 5. Imagem de microscopia eletrônica de varredura do cimento Endofill® em aumento de 5.000X (A), evidenciando superfície irregular; mapa de distribuição elementos zinco (azul claro) e bismuto (amarelo) na superfície do Endofill® (B) por meio da microanálise por espectroscopia de dispersão de raios-X,em aumento de 50.000X, que mostra a distribuição uniforme dos elementos, com partículas de tamanho similar e forma globular.

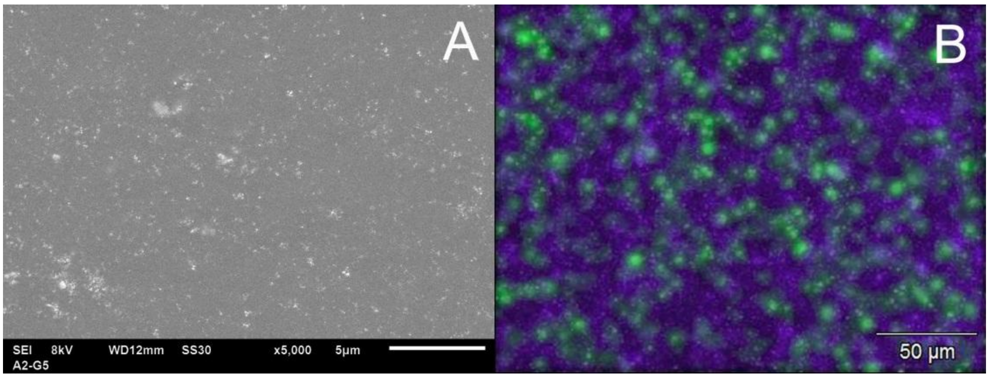

Figura 6. Imagem de microscopia eletrônica de varredura do cimento AH Plus® em aumento de 5.000X (A), evidenciando superfície regular; mapa de distribuição elementos zircônia (roxo) e tungstênio (verde) na superfície do AH Plus® (B) por meio da microanálise por espectroscopia de dispersão de raios-X, em aumento de 50.000X,que mostra a distribuição uniforme dos elementos, com partículas de tamanho similar e forma globular.

Tabela 1 – Elementos encontrados nos cimentos endodônticos analisados por espectroscopia de dispersão de raios-X (EDX).

	Sealapex®		Sealer 26®		MTA Fillapex®		Pulp Canal Sealer®		Endofill®		AH Plus®	
Elemento	Valor atômico (%at)	Peso atômico (%p)	Valor atômico (%at)	Peso atômico (%p)	Valor atômico (%at)	Peso atômico (%p)	Valor atômico (%at)	Peso atômico (%p)	Valor atômico (%at)	Peso atômico (%p)	Valor atômico (%at)	Peso atômico (%p)
Ag	-----	-----	-----	-----	-----	-----	21,69	31,58	-----	-----	-----	-----
Al	0,96	0,51	1,07	0,53	0,97	0,61	1,04	0,38	1,62	0,56	4,16	1,21
Ba	-----	-----	-----	-----	-----	-----	-----	-----	7,91	13,92	-----	-----
Bi	6,15	25,37	8,84	33,80	5,63	27,38	-----	-----	7,10	19,03	-----	-----
Ca	67,74	53,58	88,58	65,00	32,81	30,58	-----	-----	-----	-----	14,56	6,31
Cl	-----	-----	-----	-----	-----	-----	0,36	0,17	-----	-----	1,96	0,75
Fe	0,13	0,14	-----	-----	-----	-----	-----	-----	-----	-----	0,37	0,23
Hf	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0,63	1,22
Mg	0,33	0,16	1,51	0,67	-----	-----	-----	-----	-----	-----	-----	-----
Ni	0,18	0,21	-----	-----	-----	-----	0,16	0,12	0,13	0,10	-----	-----
S	5,90	3,73	-----	-----	11,31	8,43	-----	-----	7,87	3,23	-----	-----
Si	7,97	4,42	-----	-----	47,50	31,02	-----	-----	-----	-----	-----	-----
Ti	5,40	5,11	-----	-----	1,78	1,98	-----	-----	-----	-----	-----	-----
W	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	13,11	26,04
Zn	5,24	6,76	-----	-----	-----	-----	76,75	67,74	75,37	63,16	-----	-----
Zr	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	65,20	64,24

Elementos de baixo peso molecular foram excluídos da microanálise por espectroscopia de dispersão de raios-X, já que a produção de fluorescência diminui à medida que o número atômico do elemento torna-se menor, tornando a análisequantitativa destes elementos imprecisa (Vaughan, 1999).

5 DISCUSSÃO

O conhecimento da superfície e das características químicas de cimentos endodônticos que mantêm íntimo contato com o tecido periapical constitui fator preditivo para a análise das propriedades físico-químicas e biológicas destes materiais(Dammaschke *et al.*, 2005; Estrela *et al.*, 2012).Deste modo, conhecer a composição química dos cimentos endodônticos permite a seleção do melhor material para ser empregado nos variados casos a serem tratados endodonticamente. As superfícies dos cimentos endodônticos mostraram diferentes características de regularidade, de acordo com o cimento avaliado, com elementos distribuídos uniformemente, e partículas de tamanhos e formas similares, na maioria dos cimentos.

A regularidade da superfície é um importante fator que se relaciona à adesão celular ao material, sendo, portanto, fundamental para a avaliação da biocompatibilidade de materiais(Balto e Al-Nazhan, 2003). Os resultados mostraram que os cimentos Sealer 26®, Pulp Canal Sealer® e AH Plus® apresentaram superfícies regulares. Deste modo, pode-se esperar melhores resultados em termos de adesão celular nestes cimentos. Entretanto, cabe ressaltar que outros fatores afetam a adesão celular e a biocompatibilidade de um material, como a composição química deste. Este fato ressalta que os dados de regularidade de superfície não devem ser analisados de modo isolado.

A microanálise por EDX dos cimentos revelou similaridade entre os elementos encontrados e os principais componentes descritos pelos fabricantes. O Sealapex® (53,58%p) e o Sealer 26® (65,00%p) apresentaram elevadas quantidades de cálcio. Estes resultados demonstram que dentre os principais componentes encontram-se oóxido de cálcio presente no Sealapex® e o hidróxido de cálcio presente no Sealer 26®. O MTA Fillapex® apresentou elevadas quantidades de silício (31,02%p), proveniente do dióxido de silício e silicatos do MTA. O Pulp Canal Sealer® e o Endofill® apresentaram elevados valores dezinco (67,74%p e 63,16%p, respectivamente), presente no óxido de zinco, principal componente de suas formulações. O AH Plus®, cimento contendo resina epóxi, apresentou elevadas quantidades de zircônia (64,24%p), procedente do dióxido de zircônia, e também tungstênio (26,04%p), do tungstato de cálcio.

Todos os materiais avaliados apresentaram elementos que não estavam descritos na base de composição dos fabricantes.O Sealapex® apresentou traços de Al, Fe, Mg e Ni, enquantoo Sealer 26®exibiu Al e Mg. Observou-se no MTA Fillapex®a presença de Ti e noPulp Canal Sealer®a de Cl e Ni. Foram ainda verificados Al e Ni no cimento Endofill® e Al, Hf e Mg no cimento AH Plus®. Estes resultados podem ser atribuídos à contaminação durante o processo de fabricação ou mesmo a reserva de mercado.

Alguns elementos químicos descritos por fabricantes não foram encontrados pela microanálise por EDX. O Pulp Canal Sealer®não apresentou iodo, o qual deveria ser proveniente do iodeto de timol, enquanto que o Endofill® não exibiuBo, Cl e Na, que deveriam estar presentes, cuja origem inclui oborato de sódio e a resina hidrogenada.

Estudos demonstraram que o AH Plus® é o cimento endodôntico mais radiopaco dos materiais testados(Tagger e Katz, 2003; Tanomaru *et al.*, 2004;

Carvalho-Junior *et al.*, 2007; Tasdemir *et al.*, 2008; Gorduysus e Avcu, 2009).Há forte evidência de que elementos com elevado peso atômico apresentam maiorradiopacidade. Elementos como o silício, com baixo peso atômico, resultam em materiais radiolucentes, enquanto que elementos com elevado peso atômico, como o bário,identificam materiais radiopacos(Sabbagh *et al.*, 2004). Portanto, a presença de zircônia e tungstênio no AH Plus® pode justificar sua elevadaradiopacidade.

OSealapex®tem sido considerado um cimento que apresenta baixa radiopacidade(Tagger e Katz, 2003; Tanomaru *et al.*, 2004).Estudos recentes demonstraram um aumento na sua radiopacidade em nível similar ao Sealer 26®(Guerreiro-Tanomaru *et al.*, 2009). Essa maior radiopacidade pode ser devido à mudança em sua formulação, a qual foi acrescentada o óxido de bismuto(Tanomaru *et al.*, 2004; Guerreiro-Tanomaru *et al.*, 2009). Os resultados do presente estudo indicam que o Sealapex® teve níveis de bismutosimilares ao do Sealer 26® e do MTA Fillapex®.

A capacidade de adesão de um cimento endodôntico à dentina é fundamental para a obtenção de um bom selamento do canal radicular principal (Schilder, 2006). O AH Plus® é o cimento que apresenta os melhores resultados de adesão à dentina(Nunes *et al.*, 2008; Haragushiku *et al.*, 2010; Sagsen *et al.*, 2011; Haragushiku *et al.*, 2012; Vilanova *et al.*, 2012), o que está relacionado à formação de ligações covalentes entre os anéis da resina epóxi e os grupos amina expostos na rede de colágeno da dentina(Fisher *et al.*, 2007). A presença de cloro no AH Plus® está relacionada à resina epóxi bisfenol-A e bisfenol-F em sua composição.

O Sealer 26® também apresenta bons resultados em termos de adesão(Tagger *et al.*, 2002), enquanto que o Sealapex® apresenta pobre adesão à dentina (Lee *et al.*, 2002). A presença do óxido ou do hidróxido de cálcio pode interferir na adesão dos cimentos endodônticos à dentina, resultando em adesividade com elevada variação(Tagger *et al.*, 2002).

Vários elementos têm sido considerados agressivos às células humanas em determinadas concentrações(Dopp *et al.*, 2011; Field *et al.*, 2011; Kamalov *et al.*, 2011; Thang *et al.*, 2011; Horie *et al.*, 2012; Morris e Levenson, 2012; Ranguelova *et al.*, 2012; Witten *et al.*, 2012; You *et al.*, 2012), como Ag, Al, Ba, Bi, Hf, Ni, S, Zn e Zr. Estes elementos foram encontrados em materiais obturadores, o que justifica osresultados sugestivos de citotoxicidade ou genotoxicidade (Markowitz *et al.*, 1992; Molloy *et al.*, 1992; Figueiredo *et al.*, 2001; Veloso *et al.*, 2006; Ribeiro, 2008; Scelza *et al.*, 2012; Silva *et al.*, 2012).

O Sealapex® apresentou uma elevada quantidade de bismuto e a presença de Al, Ni, S e Zn.Estes elementos químicos têm sido associados acitotoxicidade, somado àinstabilidade deste cimento em meio aquoso, o quepermite o aumento da liberação destes elementos(Geurtsen *et al.*, 1998).Estudos mostraram que o Sealer 26® pode ter efeito citotóxico(Barbosa *et al.*, 1993; Figueiredo *et al.*, 2001; Veloso *et al.*, 2006). Este efeito pode apresentar justificativa devido à grande quantidade de bismuto e à presença de alumínio encontrada. O MTA Fillapex®, apesar de ter sido desenvolvido como tentativa de combinarpropriedades biológicas do MTA às propriedades físico-químicas, tem sido associado à agressão celular(Scelza *et al.*, 2012).Estes efeitos podem ser explicados pela presença de elevadas quantidades de bismuto e a presença de alumínio e enxofre.

O Pulp Canal Sealer® e o Endofill® são cimentos endodônticos que contêm em suas formulações o eugenol, um composto conhecido por seus efeitos citotóxicos às células humanas(Markowitz *et al.*, 1992; Babich *et al.*, 1993; Ribeiro, 2008).No presente estudo verificou-se a presença de elevadas quantidades de prata e zinco no Pulp Canal Sealer®, além do alumínio e níquel.Valores elevados de zinco e baixos de Al, Ba, Bi, Ni e Ssão verificados no Endofill®, os quais têm sido responsáveis pelos efeitos citotóxicos.O AH Plus®é capaz de causar inviabilidade celular, o que se explica pela presença de resina epóxi como principal componente, além da liberação de aminas ou de formaldeído(Cohen *et al.*, 2000; Eldeniz *et al.*, 2007; Karapinar-Kazandag *et al.*, 2011). A elevada quantidade de zircônia pode ser responsável por parte do seu mecanismo de citotoxicidade, assim comoa presença de Al e Hf.

A presença de elementos nos cimentos endodônticos com potencial de causar inviabilidade celular ou efeitos genotóxicos enfatiza que a obturação do canal radicular deve ser mantida dentro do canal dentinário, e não alcançar os tecidos periapicais.

A atividade antibacteriana relaciona-se à composição química dos cimentos. Os elementos Ag, Bi, Cl, S e Ti são conhecidos por sua atividade antibacteriana(Devasconcellos *et al.*, 2012; Ge *et al.*, 2012; Liou e Chang, 2012; Malwal *et al.*, 2012). O óxido de cálcio pode se transformar em hidróxido de cálcio na presença de água, o que faz com que a propriedade antimicrobiana do Sealapex®, Sealer 26® e MTA Fillapex® seja evidenciada, em decorrência do aumento do aumento do pHa partir da liberação de íons hidroxila(Estrela *et al.*, 1995; Estrela *et al.*, 2000; Lai *et al.*, 2001; Duarte *et al.*, 2003; Tanomaru-Filho *et al.*, 2007).OMTA Fillapex® apresentou quantidade consideravelmente menor de cálcio do que o Sealer 26® e o Sealapex®.

O Pulp Canal Sealer® e o Endofill®têm sido associados com atividade antibacteriana devido à presença de eugenol e óxido de zinco(Siqueira *et al.*, 2000; Mickel *et al.*, 2003; Eldeniz *et al.*, 2006; Aal-Saraj *et al.*, 2012).Entretanto, o efeito irritante aos tecidos biológicos do eugenol não justificam o uso desta substância com o intuito de obter ação antibacteriana em um cimento endodôntico.A atividade antibacteriana do Pulp Canal Sealer®parece estar relacionada à presença de pratananoparticulada. OAH Plus® apresenta atividade antibacteriana devido à presença de resina epóxi bisfenol-A e aminas em sua composição(Siqueira *et al.*, 2000; Lai *et al.*, 2001; Yasuda *et al.*, 2008).

Vários estudos indicaram o envolvimento ativo do cálcio no processo de reparo periapical(Holland *et al.*, 1982; Seux *et al.*, 1991; Wakabayashi *et al.*, 1993; Estrela *et al.*, 1995). Estrela *et al.*(1995) descreveram o mecanismo pelo qualos íons cálcio do material participam diretamente na formação da barreira mineralizada pela formação de cristais de calcita.Seux *et al.*(1991) demonstraram a afinidade da fibronectina pelos cristais de calcita, o que permite adesão e diferenciação celular e uma consequente deposição de tecido duro. Deste modo, materiais com elevadas quantidades de cálcio, como o Sealapex® e o Sealer 26®, devem favorecer a obtenção de um selamento biológico. O MTA Fillapex® apresentou quantidades consideravelmente menores de cálcio em comparação aos outros dois, o que pode resultar em capacidade de indução de reparo menor. Entretanto, a maior quantidade de íons cálcio e hidroxila no hidróxido de cálcio, e sua ação mecânica como uma matriz, protegendo contra sobre-obturação, justifica o uso desta medicação previamente à obturação do canal radicular, com a função de indução de reparo periapical e formação de barreira mineralizada no ápice radicular (Estrela *et al.*, 2012).

A microanálise por EDX tem sido utilizada para a caracterização química de materiais endodônticos (Camilleri *et al.*, 2005; Estrela *et al.*, 2012), particularmente capaz de detectar com precisão a presença de elementos químicos em materiais sólidos, principalmente os de alto peso molecular. Entretanto, este método apresenta algumas limitações, como por exemploa detecção de elementos de baixo peso molecular. Além disso, a produção de fluorescência (proporção de eventos ionizantes que resultam em emissão de raios-X) diminui à medida que o número atômico do elemento torna-se menor. Ademais, a pequena energia de raios-X característica dos elementos leves é absorvida pela camada de ouro da metalização, método utilizado neste trabalho(Vaughan, 1999). Por este motivo, a quantificação de compostos orgânicos, que apresentam carbono, oxigênio e hidrogênio, não pôde ser realizada com precisão.

O emprego do NetCounts possibilita a exclusão de possíveis dúvidas quanto ao elemento representativo de cada pico de energia, permitindo uma análise mais precisa quanto à composição química de um material. Entretanto, a análise pode tornar-secomplexa quando a amostra apresenta elementos com picos de energia similares. A análise do AH Plus®revelou a presença de tungstênio, porém o pico de energia deste foi muito semelhante ao de outro elemento descrito pelo fabricante, como o do silício. Deste modo, não se pode concluir se o AH Plus®apresentava silício ou não.

Futuros estudos são necessários com vistas à análise dos efeitos dos principais elementos e compostos químicos formados sobre as células dos tecidos periapicais e sua atividade antibacteriana.

5 CONCLUSÃO

As superfícies dos cimentos endodônticos mostraram diferentes regularidades. As partículas apresentaram distribuição uniforme, com tamanhos similares, porém com formas variadas. Foram encontrados nos cimentos endodônticos elementos químicos que não foram descritos pelos fabricantes.

REFERÊNCIAS

Aal-Saraj AB, Ariffin Z, Masudi SM. 2012. An agar diffusion study comparing the antimicrobial activity of Nanoseal with some other endodontic sealers. Aust Endod J 38:60-63.

Babich H, Stern A, Borenfreund E. 1993. Eugenol cytotoxicity evaluated with continuous cell lines. Toxicol In Vitro 7:105-109.

Balto H, Al-Nazhan S. 2003. Attachment of human periodontal ligament fibroblasts to 3 different root-end filling materials: Scanning electron microscope observation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 95:222-227.

Barbosa SV, Araki K, Spangberg LS. 1993. Cytotoxicity of some modified root canal sealers and their leachable components. Oral Surg Oral Med Oral Pathol 75:357-361.

Bouillaguet S, Shaw L, Barthelemy J, Krejci I, Wataha JC. 2008. Long-term sealing ability of Pulp Canal Sealer, AH-Plus, GuttaFlow and Epiphany. Int Endod J 41:219-226.

Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Ford TR. 2005. The constitution of mineral trioxide aggregate. Dent Mater 21:297-303.

Carvalho-Junior JR, Correr-Sobrinho L, Correr AB, Sinhoreti MA, Consani S, Sousa-Neto MD. 2007. Radiopacity of root filling materials using digital radiography. Int Endod J 40:514-520.

Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS. 2000. An in vitro study of the cytotoxicity of two root canal sealers. J Endod 26:228-229.

Dammaschke T, Gerth HU, Zuchner H, Schafer E. 2005. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. Dent Mater 21:731-738.

Devasconcellos P, Bose S, Beyenal H, Bandyopadhyay A, Zirkle LG. 2012. Antimicrobial Particulate Silver Coatings on Stainless Steel Implants for Fracture Management. Mater Sci Eng C Mater Biol Appl 32:1112-1120.

Dopp E, von Recklinghausen U, Hippler J, Diaz-Bone RA, Richard J, Zimmermann U, Rettenmeier AW, Hirner AV. 2011. Toxicity of volatile methylated species of bismuth, arsenic, tin, and mercury in Mammalian cells in vitro. J Toxicol 2011:503576.

Duarte MA, Demarchi AC, Yamashita JC, Kuga MC, Fraga Sde C. 2003. pH and calcium ion release of 2 root-end filling materials. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 95:345-347.

Eldeniz AU, Erdemir A, Hadimli HH, Belli S, Erganis O. 2006. Assessment of antibacterial activity of EndoREZ. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 102:119-126.

Eldeniz AU, Mustafa K, Orstavik D, Dahl JE. 2007. Cytotoxicity of new resin-, calcium hydroxide- and silicone-based root canal sealers on fibroblasts derived from human gingiva and L929 cell lines. Int Endod J 40:329-337.

Estrela C. 2004. Obturação do canal radicular. In: Ciência Endodôntica. Estrela C, editor. Artes Médicas, São Paulo, pp 457-538.

Estrela C, Bammann LL, Estrela CR, Silva RS, Pecora JD. 2000. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. Braz Dent J 11:3-9.

Estrela C, Estrela CR, Barbin EL, Spano JC, Marchesan MA, Pecora JD. 2002. Mechanism of action of sodium hypochlorite. Braz Dent J 13:113-117.

Estrela C, Sousa Neto MD, Guedes OA, Alencar AHG, Duarte MAH, Pecora JD. 2012. Characterization of calcium oxide in root perforation sealer materials. Braz Dent J 23:539-546.

Estrela C, Sydney GB, Bammann LL, Felippe Junior O. 1995. Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Braz Dent J 6:85-90.

Fidel RA, Sousa Neto MD, Spano JC, Barbin EL, Pecora JD. 1994. Adhesion of calcium hydroxide-containing root canal sealers. Braz Dent J 5:53-57.

Field JA, Luna-Velasco A, Boitano SA, Shadman F, Ratner BD, Barnes C, Sierra-Alvarez R. 2011. Cytotoxicity and physicochemical properties of hafnium oxide nanoparticles. Chemosphere 84:1401-1407.

Figueiredo JA, Pesce HF, Gioso MA, Figueiredo MA. 2001. The histological effects of four endodontic sealers implanted in the oral mucosa: submucous injection versus implant in polyethylene tubes. Int Endod J 34:377-385.

Fisher MA, Berzins DW, Bahcall JK. 2007. An in vitro comparison of bond strength of various obturation materials to root canal dentin using a push-out test design. J Endod 33:856-858.

Flores DS, Rached FJ, Jr., Versiani MA, Guedes DF, Sousa-Neto MD, Pecora JD. 2011. Evaluation of physicochemical properties of four root canal sealers. Int Endod J 44:126-135.

Garrido AD, Lia RC, Franca SC, da Silva JF, Astolfi-Filho S, Sousa-Neto MD. 2010. Laboratory evaluation of the physicochemical properties of a new root canal sealer based on Copaifera multijuga oil-resin. Int Endod J 43:283-291.

Ge R, Chen Z, Zhou Q. 2012. The actions of bismuth in the treatment of Helicobacter pylori infections: an update. Metallomics 4:239-243.

Geurtsen W, Leinenbach F, Krage T, Leyhausen G. 1998. Cytotoxicity of four root canal sealers in permanent 3T3 cells and primary human periodontal ligament fibroblast cultures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 85(5):592-597.

Gomes-Filho JE, Watanabe S, Lodi CS, Cintra LT, Nery MJ, Filho JA, Dezan E, Jr., Bernabe PF. 2011. Rat tissue reaction to MTA FILLAPEX®. Dent Traumatol28:452-456.

Gorduysus M, Avcu N. 2009. Evaluation of the radiopacity of different root canal sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 108:e135-40.

Grossman LI. 1958. An improved root canal cement. J Am Dent Assoc 56:381-385.

Guerreiro-Tanomaru JM, Duarte MA, Goncalves M, Tanomaru-Filho M. 2009. Radiopacity evaluation of root canal sealers containing calcium hydroxide and MTA. Braz Oral Res 23:119-123.

Haragushiku GA, Sousa-Neto MD, Silva-Sousa YT, Alfredo E, Silva SC, Silva RG. 2010. Adhesion of endodontic sealers to human root dentine submitted to different surface treatments. Photomed Laser Surg 28:405-410.

Haragushiku GA, Teixeira CS, Furuse AY, Sousa YT, De Sousa Neto MD, Silva RG. 2012. Analysis of the interface and bond strength of resin-based endodontic cements to root dentin. Microsc Res Tech 75:655-661.

Holland R, de Souza V. 1985. Ability of a new calcium hydroxide root canal filling material to induce hard tissue formation. J Endod 11:535-543.

Holland R, Pinheiro CE, de Mello W, Nery MJ, de Souza V. 1982. Histochemical analysis of the dogs' dental pulp after pulp capping with calcium, barium, and strontium hydroxides. J Endod 8:444-447.

Horie M, Fukui H, Endoh S, Maru J, Miyauchi A, Shichiri M, Fujita K, Niki E, Hagihara Y, Yoshida Y, Morimoto Y, Iwahashi H. 2012. Comparison of acute oxidative stress on rat lung induced by nano and fine-scale, soluble and insoluble metal oxide particles: NiO and TiO_2. Inhal Toxicol 24:391-400.

Kamalov J, Carpenter DO, Birman I. 2011. Cytotoxicity of environmentally relevant concentrations of aluminum in murine thymocytes and lymphocytes. J Toxicol 2011:796719.

Karapinar-Kazandag M, Bayrak OF, Yalvac ME, Ersev H, Tanalp J, Sahin F, Bayirli G. 2011. Cytotoxicity of 5 endodontic sealers on L929 cell line and human dental pulp cells. Int Endod J 44:626-634.

Lai CC, Huang FM, Yang HW, Chan Y, Huang MS, Chou MY, Chang YC. 2001. Antimicrobial activity of four root canal sealers against endodontic pathogens. Clin Oral Investig 5:236-239.

Lee KW, Williams MC, Camps JJ, Pashley DH. 2002. Adhesion of endodontic sealers to dentin and gutta-percha. J Endod 28:684-688.

Liou JW, Chang HH. 2012. Bactericidal effects and mechanisms of visible light-responsive titanium dioxide photocatalysts on pathogenic bacteria. Arch Immunol Ther Exp (Warsz) 60:267-275.

Malwal SR, Sriram D, Yogeeswari P, Konkimalla VB, Chakrapani H. 2012. Design, synthesis, and evaluation of thiol-activated sources of sulfur dioxide (SO_2) as antimycobacterial agents. J Med Chem 55:553-557.

Markowitz K, Moynihan M, Liu M, Kim S. 1992. Biologic properties of eugenol and zinc oxide-eugenol. A clinically oriented review. Oral Surg Oral Med Oral Pathol 73:729-737.

Mickel AK, Nguyen TH, Chogle S. 2003. Antimicrobial activity of endodontic sealers on Enterococcus faecalis. J Endod 29:257-258.

Molloy D, Goldman M, White RR, Kabani S. 1992. Comparative tissue tolerance of a new endodontic sealer. Oral Surg Oral Med Oral Pathol 73:490-493.

Morris DR, Levenson CW. 2012. Ion channels and zinc: mechanisms of neurotoxicity and neurodegeneration. J Toxicol 2012:785647.

Nunes VH, Silva RG, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. 2008. Adhesion of Epiphany and AH Plus sealers to human root dentin treated with different solutions. Braz Dent J 19:46-50.

Ordinola-Zapata R, Bramante CM, Graeff MS, del Carpio Perochena A, Vivan RR, Camargo EJ, Garcia RB, Bernardineli N, Gutmann JL, de Moraes IG. 2009. Depth and percentage of penetration of endodontic sealers into dentinal tubules after root canal obturation using a lateral compaction technique: a confocal laser scanning microscopy study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 108:450-457.

Orstavik D. 1983. Physical properties of root canal sealers: measurement of flow, working time, and compressive strength. Int Endod J 16:99-107.

Orstavik D. 2005. Materials used for root canal obturation: technical, biological and clinical testing. Endodontic Topics 12:25-38.

Ranguelova K, Rice AB, Khajo A, Triquigneaux M, Garantziotis S, Magliozzo RS, Mason RP. 2012. Formation of reactive sulfite-derived free radicals by the activation of human neutrophils: an ESR study. Free Radic Biol Med 52:1264-1271.

Resende LM, Rached-Junior FJ, Versiani MA, Souza-Gabriel AE, Miranda CE, Silva-Sousa YT, Sousa Neto MD. 2009. A comparative study of physicochemical properties of AH Plus, Epiphany, and Epiphany SE root canal sealers. Int Endod J 42:785-793.

Ribeiro DA. 2008. Do endodontic compounds induce genetic damage? A comprehensive review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 105:251-256.

Sabbagh J, Vreven J, Leloup G. 2004. Radiopacity of resin-based materials measured in film radiographs and storage phosphor plate (Digora). Oper Dent 29:677-684.

Sagsen B, Ustun Y, Demirbuga S, Pala K. 2011. Push-out bond strength of two new calcium silicate-based endodontic sealers to root canal dentine. Int Endod J 44:1088-1091.

Scelza MZ, Linhares AB, da Silva LE, Granjeiro JM, Alves GG. 2012. A multiparametric assay to compare the cytotoxicity of endodontic sealers with primary human osteoblasts. Int Endod J 45:12-18.

Schilder H. 2006. Filling root canals in three dimensions. 1967. J Endod 32:281-290.

Seux D, Couble ML, Hartmann DJ, Gauthier JP, Magloire H. 1991. Odontoblast-like cytodifferentiation of human dental pulp cells in vitro in the presence of a calcium hydroxide-containing cement. Arch Oral Biol 36:117-128.

Silva EJ, Accorsi-Mendonca T, Almeida JF, Ferraz CC, Gomes BP, Zaia AA. 2012. Evaluation of cytotoxicity and up-regulation of gelatinases in human fibroblast cells by four root canal sealers. Int Endod J 45:49-56.

Siqueira JF, Jr., Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP. 2000. Antimicrobial activity and flow rate of newer and established root canal sealers. J Endod 26:274-277.

Tagger M, Katz A. 2003. Radiopacity of endodontic sealers: development of a new method for direct measurement. J Endod 29:751-755.

Tagger M, Tagger E, Kfir A. 1988. Release of calcium and hydroxyl ions from set endodontic sealers containing calcium hydroxide. J Endod 14:588-591.

Tagger M, Tagger E, Tjan AH, Bakland LK. 2002. Measurement of adhesion of endodontic sealers to dentin. J Endod 28:351-354.

Tanomaru-Filho M, Tanomaru JM, Barros DB, Watanabe E, Ito IY. 2007. In vitro antimicrobial activity of endodontic sealers, MTA-based cements and Portland cement. J Oral Sci 49:41-45.

Tanomaru JM, Cezare L, Goncalves M, Tanomaru Filho M. 2004. Evaluation of the radiopacity of root canal sealers by digitization of radiographic images. J Appl Oral Sci 12:355-357.

Tasdemir T, Yesilyurt C, Yildirim T, Er K. 2008. Evaluation of the radiopacity of new root canal paste/sealers by digital radiography. J Endod 34:1388-1390.

Thang ND, Yajima I, Kumasaka MY, Ohnuma S, Yanagishita T, Hayashi R, Shekhar HU, Watanabe D, Kato M. 2011. Barium promotes anchorage-independent growth and invasion of human HaCaT keratinocytes via activation of c-SRC kinase. PLoS One 6:e25636.

Vaughan D. 1999. Energy-Dispersive X-ray Microanalysis: An Introduction. Middleton: NORAN Instruments. 59 p.

Veloso HH, do Santos RA, de Araujo TP, Leonardi DP, Baratto Filho F. 2006. Histological analysis of the biocompatibility of three different calcium hydroxide-based root canal sealers. J Appl Oral Sci 14:376-381.

Versiani MA, Carvalho-Junior JR, Padilha MI, Lacey S, Pascon EA, Sousa-Neto MD. 2006. A comparative study of physicochemical properties of AH Plus and Epiphany root canal sealants. Int Endod J 39:464-471.

Vilanova WV, Carvalho-Junior JR, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. 2012. Effect of intracanal irrigants on the bond strength of epoxy resin-based and methacrylate resin-based sealers to root canal walls. Int Endod J 45:42-48.

Vujaskovic M, Teodorovic N. 2010. Analysis of sealing ability of root canal sealers using scanning electronic microscopy technique. Srp Arh Celok Lek 138:694-698.

Wakabayashi H, Horikawa M, Funato A, Onodera A, Matsumoto K. 1993. Bio-microscopical observation of dystrophic calcification induced by calcium hydroxide. Endod Dent Traumatol 9:165-170.

Witten ML, Sheppard PR, Witten BL. 2012. Tungsten toxicity. Chem Biol Interact 196:87-88.

Yasuda Y, Kamaguchi A, Saito T. 2008. In vitro evaluation of the antimicrobial activity of a new resin-based endodontic sealer against endodontic pathogens. J Oral Sci 50:309-313.

You C, Han C, Wang X, Zheng Y, Li Q, Hu X, Sun H. 2012. The progress of silver nanoparticles in the antibacterial mechanism, clinical application and cytotoxicity. Mol Biol Rep 39:9193-9201.

ANEXOS

Original Research Article:

SURFACE MICROANALYSIS AND CHEMICAL CHARACTERIZATION OF ENDODONTIC SEALERS

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SURFACE MICROANALYSIS AND CHEMICAL CHARACTERIZATION OF ENDODONTIC SEALERS

Abstract

Purpose: to assess the surface and evaluate the chemical composition of root canal filling materials by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). *Methods*: eighteen polyethylene standard tubes were filled with the tested materials: Sealapex®, Sealer 26®, MTA Fillapex®, Pulp Canal Sealer®, Endofill® and AH Plus®. After 48 hours at 37°C and 95% relative humidity, the samples were surface-sputtered with gold, led to SEM and the images analyzed at 5,000X magnification. Then, the elements distribution and chemical composition were determined by EDX. The results were evaluated qualitatively (SEM images and elements distribution maps) and quantitatively (weight percentage). *Results*: the surface analysis revealed that the sealers presented different regularities, with an uniform distribution of elements, with particles of similar sizes and variable shapes in EDX microanalysis. Calcium oxide and hydroxide based sealers (Sealapex® and Sealer 26®) presented calcium peaks of 53.58wt.% and 65.00wt.%, respectively. MTA Fillapex® presented 30.58wt.% of calcium and high amounts of silicon (31.02 weight%) and bismuth (27.38 weight%). Zinc oxide and eugenol based sealers, Pulp Canal Sealer® and Endofill®, showed zinc quantities of 67.74wt% and 63.16wt.%, respectively. AH Plus® had higher amount of zirconium (64.24wt.%). The materials presented elements incompatible with the composition described by the manufacturer. *Conclusions:*the root canal sealers' surfaces showed different. The elements presented uniform distribution, with particles of similar sizes and variable shapes. Chemical elements were found in the root canal sealers not described by the manufacturers.

*Key Words:*Scanning electron microscopy, energy-dispersive X-ray analysis, chemical properties, root canal filling materials.

INTRODUCTION

The root canal sealer has the role of filling the present spaces between gutta-percha points and dentinal walls and allowing to perpetuate root canal cleaning. The biological and technical properties of the root canal sealing along with the absence of periapical aggression favor the reestablishment of the normality status of the injured tissues (Orstavik, 2005). The tissue tolerance to the endodontic sealers, which are in direct contact with the periapical tissues in some situations, must be carefully taken into account for the material selection. Other characteristic that the material should present is the bond strength to dentinal walls (Grossman, 1958; Vujaskovic and Teodorovic, 2010). In general, the search for new endodontic sealers is due to the need to obtain alternatives with better properties than the existing materials (Bouillaguet et al., 2008; Orstavik, 1983; 2005).

The surface structure and chemical characterization of the compounds found in the endodontic sealers may provide important correlations with the tissue tolerance, dentinal bond strength and antimicrobial activity (Estrela, 2004). Resende et al.(2009) evaluated the physicochemical properties and the surface morphology of the sealers AH Plus®, Epiphany® and Ephiphany SE®. AH plus® was in accordance with the ANSI/ADA specifications for all properties, results not achieved by the other sealers tested. Similar results were found by Versiani et al.(2006) and Flores et al.(2011). Haragushiku et al.(2010) observed that AH Plus® showed the best ability to adhere to dentinal walls when compared to Apexit® and Epiphany. Garrido et al.(2010) compared the physicochemical properties of the sealers Biosealer®, Sealer 26®, Endofill® and AH Plus®. Sealer 26® and AH Plus® presented longer setting time. Thickness and dimensional alteration of Sealer 26® were not satisfactory, as Endofill® was not adequate for solubility and disintegration.

Sealapex® and Apexit® presented poor results for dentinal bonding when compared to Sealer 26® and CRCS®. EDTA increased the adhesion of endodontic sealers to dentinal walls, in exception of Sealapex®(Fidel et al., 1994). Sealapex® showed ability to penetrate the dentinal tubules assessed by confocal microscopy (Ordinola-Zapata et al., 2009). Calcium hydroxide presents excellent mineralization potential to form a mineralized barrier and

Sealapex®, an endodontic sealer that contains calcium oxide, also showed ability to stimulate the deposition of mineralized tissue after the root canal fillings of dogs' and monkeys' teeth (Holland and de Souza, 1985). Tagger et al.(1988)assessedthecapabilityofSealapex®, CRCS®andHermetic®toliberatecalciumandhydroxylions. Sealapex® presented the most capability to liberate these ions and the solubitity of this sealer could be the reason for increased liberation of ions than other sealers tested.

MTA Fillapex® was recently developed in order to add biological properties from MTA to the physicochemical properties of an endodontic sealer (Gomes-Filho et al., 2011; Scelza et al., 2012). Gomes-Filho et al.(2011) evaluated the reaction of rats' subcutaneous tissue to MTA Fillapex® and the mineralizing ability of this sealer in comparison to MTA Angelus® and Sealapex®. All the studied materials showed presence of mild inflammation and capability to induce mineralization. The MTA Fillpapex® was considered a root canal filling material with good tissue tolerance.

The chemical composition of a root canal filling material, distributed at surface structural level may characterize different properties, once this surface allows the interaction between the material and the tissues in contact. Among these, the biocompatibility might be directly affected by the chemical composition of the material, once the presence of irritant compounds adjacent to biological tissues decrease the tissue tolerance to the material (Dammaschke et al., 2005). Therefore, the knowledge of the endodontic sealers surface chemical composition might facilitate to understand the interaction between the biological and physicochemical properties.

The aim of this study was to assess the root canal sealer's surface by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX), for surface regularity, elements distribution, shape and size of particles, and chemical characterization.

MATERIALS AND METHODS

Specimens Preparation

Six commercially available endodontic sealers were used for the experiments: Sealapex® (SybronEndo, Orange, CA, USA), Sealer 26® (Dentsply, De Tray, Konstanz, Germany), MTA Fillapex® (Angelus

SoluçõesOdontológicas, Londrina, PR, Brazil), Pulp Canal Sealer® (SybronEndo, Orange, CA, USA), Endofill® (Dentsply, Petropolis, RJ, Brazil), AH Plus® (Dentsply, De Tray, Konstanz, Germany). The composition of the evaluated materials, as described by their manufacturers, is shown in Table 1.

Eighteen polyethylene standard tubes (Number 12 Levine Probe, Embramed, Jurubatuba, SP, Brazil) with an internal diameter of 3 mm and a length of 3 mm were prepared using a digital caliper with a resolution of 0.01 mm (Mitutoyo MTI Corporation, Tokyo, Japan) and a scalpel blade number 11 (Swann Morton, Sheffield, United Kingdom). Three tubes for each group were placed on a polished glass slab (75 x 25 x 1 mm) and filled with the tested materials aided by a spatula #24 (SS White Duflex, Rio de Janeiro, RJ, Brazil). All sealers were mixed according to the manufacturers´ instructions. Then, the specimens were transferred to a chamber with 95% relative humidity and a temperature of 37^{o}C for 48 hours.

Microscopy and elemental analysis

The specimens were surface-sputtered with gold and conducted to Scanning Electron Microscope (SEM) Leo Stereoscan 420i (Leica Electron Optics, Cambridge instruments, Cambridge, UK), using 8-10 kV and resolution of 2 nm, and examined without any preparation and manipulation. Images of 5,000X magnification were taken for determining the surface regularity.

The analysis of chemical elements was performed by Energy Dispersive X-ray analysis (EDX) with the software NSS Spectral Analysis System 2.3 (Thermo Fisher Scientific Inc., Suwanee, GA, EUA). to examine the elemental composition of the tested sealers and the elements distribution. EDX measurements were conducted from the central region of each specimen using electron beam spot sizes lower than 50 nm, with an accelerating voltage of 25 kV, 110 mA beam current, determined in accordance to the composition described by the manufacturers, and the spectra were obtained for 100 seconds live time.

The evaluations were performed by two professionals calibrated at a pilot study with 10% of the sample and who had experience in the area. The quantitative analysis was performed using software NSS Spectral Analysis System 2.3, in non-standard analysis mode, using PROZA (Phi-Rho-Z) correction method. The elemental maps were achieved by NetCounts method, with high resolution, using the same software. The surface analysis by SEM and EDX microanalysis were qualitative, and a descriptive analysis was applied in the study.

The surface was described for the surface regularity (regular and irregular), distribution of the elements (uniform or non-uniform), shape (globular-like, needle-like, matrix) and sizes (similarities between particles without measurements) of the present particles.

RESULTS

Figures 1 to 6 show the sealers SEM images (A), used to characterize the surface regularity, and the distribution maps with the 2 main elements achieved by EDX microanalysis (B), used to characterize the surfaces' elements distribution, size and shape of the particles.

Sealapex® presented an irregular surface (Figure 1A) with a uniform distribution of elements, composed mainly by calcium globular-like particles and bismuth needle-like particles, and different sizes (Figure 1B). Sealer 26® had a regular surface (Figure 2A) composed mostly by calcium with bismuth particles of different sizes and shapes in a uniform distribution (Figure 2B).

MTA Fillapex® presented an irregular surface (Figure 3A) with uniform distribution of elements, mainly composed by a silicon matrix with calcium globular-like particles and bismuth particles of different sizes and shapes (Figure 3B). Pulp Canal Sealer® presented aregular surface (Figure 4A) with a uniform distribution of elements, composed mainly by globular-like particles of zinc and silver (Figure 4B).Endofill® presented an irregular surface (Figure 5A) with a uniform distribution of elements, composed mainly by globular-like particles of zinc and bismuth (Figure 5B). AH Plus® showed a regular surface (Figure 6A) with globular-like particles in a uniform distribution, mostly composed by zirconium (Figure 6B).

The elements quantitative results of the specimens' area assessed by EDX microanalysis are described in Table 2. The calcium oxide or hydroxide based sealers (Sealapex® and Sealer 26®) showed peaks of calcium (53.58wt.% and 65.00wt.%, respectively), in a higher amount than MTA based material (MTA Fillapex, 30.58wt.%), which also showed high amounts of silicon (31.02wt.%) and bismuth (27.38wt.%). Zinc oxide and eugenol based sealers (Pulp Canal Sealer® and Endofill®) presented peaks of zinc (67.74wt.%and 63.16wt.%, respectively), while the resin based material (AH Plus®) had greater amount of zirconium (64.24wt.%).

DISCUSSION

The knowledge of the material surface and the chemical composition of endodontic sealers that keep intimal contact with the periapical tissue is a predictive factor for the physicochemical and biological properties analysis (Dammaschke et al., 2005; Estrela et al., 2012). Thus, the knowledge of the chemical composition allow the selection of the best material to be used in different patients and conditions that must be endodontically treated. ofThe endodontic sealers’ surface showed different regularities, varying from each cement evaluated, uniform distribution of the elements and particles of similar sizes and shapes for most root canal sealers.

The surface regularity is an important factor that is related to cellular adhesion to the material. Thus, this characteristic is essential for evaluating materials’ biocompatibility (Balto and Al-Nazhan, 2003). The results showed that Sealer 26®, Pulp Canal Sealer® and AH Plus®, presented regular surfaces. Therefore, better results could be expected for cellular adhesion to these root canal sealers. However, other factors influence cellular adhesion and biocompatibility of a material, like the chemical composition. This fact highlights that the surface regularity data should not be analyzed isolated.

The EDX microanalysis of the root canal sealers revealed similarity between the elements found and the main compounds described by their manufacturers. Sealapex® (53.58wt.%) and Sealer 26® (65.00wt.%) had high amounts of calcium. These results demonstrate that among the main components are calcium oxide in Sealapex® and calcium hydroxide in Sealer 26®. MTA Fillapex® presented high quantities of silicon (31.02wt.%), from silicon dioxide and MTA silicates. Pulp Canal Sealer® and Endofill® presented high values of zinc (67.74wt.% and 63.16wt.%, respectively), present in zinc oxide, main component of their formulas. AH Plus®, an epoxy resin based sealer, presented higher amounts of zirconium (64.24wt.%), from zirconium dioxide and also tungsten (26.04wt.%), from calcium tungstate.

All the evaluated materials presented elements that were not described by their manufacturers. Sealapex® showed traces of Al, Fe, Mg and Ni, while Sealer 26® exhibited Al and Mg. MTA Fillapex® presented Ti and Pulp Canal Sealer® had Cl and Ni. Still, Al and Ni were found in Endofill® and Al, Hf and Mg in AH Plus®. These results could be attributed to contamination during the manufacturing process or industrial secret.

Some elements described by the manufacturers were not found by the EDX microanalysis. Pulp Canal Sealer® had not iodine, which should be derived from thymol iodine, while Endofill® did not exhibited boron, sodium and chlorine, which should be present in sodium borate and hydrogenated resin.

Studies demonstrated that AH Plus® is the most radiopaque endodontic sealer from the materials tested (Carvalho-Junior et al., 2007; Gorduysus and Avcu, 2009; Tagger and Katz, 2003; Tanomaru et al., 2004; Tasdemir et al., 2008). There is strong evidence that elements with high molecular weight have higher radiopacity. Elements like silicon, with low atomic number, should result in radiolucent materials, while elements with high atomic number, as barium, identify radiopaque materials (Sabbagh et al., 2004). Therefore, the presence of zirconium and tungsten in AH Plus® could justify its high radiopacity.

Sealapex® has been considered a sealer with the low radiopacity (Tagger and Katz, 2003; Tanomaru et al., 2004). However, recent studies demonstrated increased radiopacity of this sealer, at similar level to Sealer 26®(Guerreiro-Tanomaru et al., 2009). This enhanced radiopacity must be due to the change in Sealapex® formulation, with increased percentage of bismuth oxide (Guerreiro-Tanomaru et al., 2009; Tanomaru et al., 2004). The results of this study indicate that Sealapex® had similar levels of bismuth than Sealer 26® and MTA Fillapex®.

The root canal sealers ability to adhere to dentin is essential for obtaining a good root canal sealing (Schilder, 2006). AH Plus® have the best bond strength results (Haragushiku et al., 2010; Haragushiku et al., 2012; Nunes et al., 2008; Sagsen et al., 2011; Vilanova et al., 2012), which is related to the formation of covalent bonds between epoxide rings and amine groups exposed in the collagen net (Fisher et al., 2007). The presence of chlorine in AH Plus® is related to the presence of Bisphenol-A and Bisphenol-F epoxy resins in its composition.

Sealer 26® also presents good results of bond strength (Tagger et al., 2002), while Sealapex® have poor results in terms of adhesion to dentin (Lee et al., 2002). The presence of calcium oxide or hydroxide could interfere in the bond strength of root canal sealers to dentine, resulting in high variation to bond strength (Tagger et al., 2002).

Several elements are considered aggressive to human cells at determined concentrations (Dopp et al., 2011; Field et al., 2011; Horie et al., 2012; Kamalov et al., 2011; Morris and Levenson, 2012; Ranguelova et al., 2012; Thang et al., 2011; Witten et al., 2012; You et al., 2012), like Ag, Al, Ba, Bi, Hf, Ni, S, Zn, and Zr. These elements were found in the root canal filling materials, justifying their results suggestive of cytotoxicity or genotoxicity (Figueiredo et al., 2001; Markowitz et al., 1992; Molloy et al., 1992; Ribeiro, 2008; Scelza et al., 2012; Silva et al., 2012; Veloso et al., 2006)].

Sealapex® had high amount of bismuth and presence of Al, Ni, S and Zn. These chemical elements have been associated to cytotoxicity, plus the instability of this sealer at aqueous environment, that allows the enhanced liberation of these elements. (Geurtsen et al., 1998). Studies show that Sealer 26® might have cytotoxic effects (Barbosa et al., 1993; Figueiredo et al., 2001; Veloso et al., 2006). This effect must be due to the high amount of Bi and presence of Al found. MTA Fillapex®, despite being developed in an attempt to combine the biological properties of MTA to the physicochemical properties, also has been associated to cell agression(Scelza et al., 2012). These effects may be explained by the presence of high amounts of bismuth and presence of Al and S.

Pulp Canal Sealer® and Endofill® are endodontic sealers which present eugenol in their composition, a compound known for its cytotoxic effects to human cells (Babich et al., 1993; Markowitz et al., 1992; Ribeiro, 2008). The present study found the presence of high amounts of silver and zinc in Pulp Canal Sealer®, along with Al and Ni. High values of zinc and presence of Al, Ba, Bi, Ni and S were verified in Endofill®, which have been responsible for cytotoxic effects. AH Plus® is able to cause cytotoxic effects, which may be explained by the presence of epoxy resin as its main component and the release of amines, or formaldehyde (Cohen et al., 2000; Eldeniz et al., 2007; Karapinar-Kazandag et al., 2011). The high amount of zirconium might be responsible for part of this cytotoxic mechanism, along with Al and Hf.

The presence of elements in the endodontic sealers with potential to cause cell inviability or genotoxic effects emphasize that the root canal filling should be kept inside the dentinal root canal, and not reach to the periapical region.

The antimicrobial activity is directly related to the chemical composition of the sealers. Elements like Ag, Bi, Cl, S and Ti are known for their activity against bacteria (Devasconcellos et al., 2012; Estrela et al., 2002; Ge et al., 2012; Liou and Chang, 2012; Malwal et al., 2012). Calcium oxide could turn into calcium hydroxide in the presence of water, what highlights the antimicrobial activity mechanism of Sealapex®, Sealer 26® and MTA Fillapex®, due to the induction of increased pH by the release of hydroxyl ions (Duarte et al., 2003; Estrela et al., 2000; Estrela et al., 1995; Lai et al., 2001; Tanomaru-Filho et al., 2007). MTA Fillapex® had considerably lower amount of calcium than Sealer 26® and Sealapex®.

Pulp Canal Sealer® and Endofill® have been associated to antimicrobial activity due to the presence of eugenol and zinc oxide (Aal-Saraj et al., 2012; Eldeniz et al., 2006; Mickel et al., 2003; Siqueira et al., 2000). However, the irritant effect to biological tissues of eugenol does not justify the use of this substance in order to obtain antimicrobial action from an endodontic sealer.The antimicrobial activity of Pulp Canal Sealer® could be associated to the presence of nanoparticulated silver. AH Plus® presents antimicrobial activity due to the bysphenol-A epoxy resin and the amines in its composition (Lai et al., 2001; Siqueira et al., 2000; Yasuda et al., 2008).

Several studies indicated the active involvement of calcium in the periapical repair process (Estrela et al., 1995; Holland et al., 1982; Seux et al., 1991; Wakabayashi et al., 1993). Estrela et al.(1995) described the mechanism by which calcium ions from the material directly participate in the mineralized barrier formation, by the formation of calcite crystals. Seux et al.(1991) showed the affinity of fibronectin for calcite crystals, which allows cellular adhesion and differentiation and consequently hard tissue deposition. Therefore, materials with high levels of calcium, like Sealapex® and Sealer 26®, may favor a biological obturation. MTA Fillapex® had considerably lower levels of calcium in comparison to the other two materials. Thus, a lower capacity of inducing periapical repair is expected from this sealer. However, the higher amount of calcium and hydroxyl ions from calcium hydroxide and its mechanical action as a matrix, protecting overfilling, justifies the use of this medication previously to root canal filling, intended to induce periapical repair and mineralized barrier formation in root apex (Estrela et al., 2012).

The EDX microanalysis has been used for chemical characterization of endodontic sealers (Camilleri et al., 2005; Estrela et al., 2012), particularly capable to precisely detect chemical elements of solid materials, mainly high molecular weight elements. However, this method has some limitations, for example the detection of elements of light molecular weight. Besides, the fluorescent yield (the proportion of ionization events which result in the emission of an X-ray) decreases with decreasing atomic number of the element. Moreover, the low energy X-rays characteristic of light elements are absorbed in the in the Au contact layer, method used in this study (Vaughan, 1999). For this reason, the quantification of organic compounds, composed by carbon, oxygen and hydrogen, could not be performed with precision.

The NetCounts use allowed the exclusion of possible doubts for the representative chemical element of each energy peak, enabling a more accurate analysis for the material chemical composition. However, the analysis might be difficult when the sample has elements of similar energy peaks. The AH Plus® samples analysis revealed the presence of W, but its energy peak was very similar to other element described by the manufacturer, Si. Thus, it could not be concluded if AH Plus® presented silicon or not.

Future studies are necessary to assess the effects of the main chemical elements and compounds formed over the periapical tissue cells and their antimicrobial activity.

CONCLUSIONS

The root canal sealers' surfaces showed different regularities. The particles presented an uniform distribution, with similar sizes and variable shapes. There were chemical elements found in the root canal sealers not described by their manufacturers.

REFERENCES

Aal-Saraj AB, Ariffin Z, Masudi SM. 2012. An agar diffusion study comparing the antimicrobial activity of Nanoseal with some other endodontic sealers. Aust Endod J 38:60-63.

Babich H, Stern A, Borenfreund E. 1993. Eugenol cytotoxicity evaluated with continuous cell lines. Toxicol In Vitro 7:105-109.

Balto H, Al-Nazhan S. 2003. Attachment of human periodontal ligament fibroblasts to 3 different root-end filling materials: Scanning electron microscope observation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 95:222-227.

Barbosa SV, Araki K, Spangberg LS. 1993. Cytotoxicity of some modified root canal sealers and their leachable components. Oral Surg Oral Med Oral Pathol 75:357-361.

Bouillaguet S, Shaw L, Barthelemy J, Krejci I, Wataha JC. 2008. Long-term sealing ability of Pulp Canal Sealer, AH-Plus, GuttaFlow and Epiphany. Int Endod J 41:219-226.

Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Ford TR. 2005. The constitution of mineral trioxide aggregate. Dent Mater 21:297-303.

Carvalho-Junior JR, Correr-Sobrinho L, Correr AB, Sinhoreti MA, Consani S, Sousa-Neto MD. 2007. Radiopacity of root filling materials using digital radiography. Int Endod J 40:514-520.

Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS. 2000. An in vitro study of the cytotoxicity of two root canal sealers. J Endod 26:228-229.

Dammaschke T, Gerth HU, Zuchner H, Schafer E. 2005. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. Dent Mater 21:731-738.

Devasconcellos P, Bose S, Beyenal H, Bandyopadhyay A, Zirkle LG. 2012. Antimicrobial Particulate Silver Coatings on Stainless Steel Implants for Fracture Management. Mater Sci Eng C Mater Biol Appl 32:1112-1120.

Dopp E, von Recklinghausen U, Hippler J, Diaz-Bone RA, Richard J, Zimmermann U, Rettenmeier AW, Hirner AV. 2011. Toxicity of volatile methylated species of bismuth, arsenic, tin, and mercury in Mammalian cells in vitro. J Toxicol 2011:503576.

Duarte MA, Demarchi AC, Yamashita JC, Kuga MC, Fraga Sde C. 2003. pH and calcium ion release of 2 root-end filling materials. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 95:345-347.

Eldeniz AU, Erdemir A, Hadimli HH, Belli S, Erganis O. 2006. Assessment of antibacterial activity of EndoREZ. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 102:119-126.

Eldeniz AU, Mustafa K, Orstavik D, Dahl JE. 2007. Cytotoxicity of new resin-, calcium hydroxide- and silicone-based root canal sealers on fibroblasts derived from human gingiva and L929 cell lines. Int Endod J 40:329-337.

Estrela C. 2004. Obturação do canal radicular. In: Ciência Endodôntica. Estrela C, editor. Artes Médicas,São Paulo, pp 457-538.

Estrela C, Bammann LL, Estrela CR, Silva RS, Pecora JD. 2000. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. Braz Dent J 11:3-9.

Estrela C, Estrela CR, Barbin EL, Spano JC, Marchesan MA, Pecora JD. 2002. Mechanism of action of sodium hypochlorite. Braz Dent J 13:113-117.

Estrela C, Sousa Neto MD, Guedes OA, Alencar AHG, Duarte MAH, Pecora JD. 2012. Characterization of calcium oxide in root perforation sealer materials. Braz Dent J 23:539-546.

Estrela C, Sydney GB, Bammann LL, Felippe Junior O. 1995. Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Braz Dent J 6:85-90.

Fidel RA, Sousa Neto MD, Spano JC, Barbin EL, Pecora JD. 1994. Adhesion of calcium hydroxide-containing root canal sealers. Braz Dent J 5:53-57.

Field JA, Luna-Velasco A, Boitano SA, Shadman F, Ratner BD, Barnes C, Sierra-Alvarez R. 2011. Cytotoxicity and physicochemical properties of hafnium oxide nanoparticles. Chemosphere 84:1401-1407.

Figueiredo JA, Pesce HF, Gioso MA, Figueiredo MA. 2001. The histological effects of four endodontic sealers implanted in the oral mucosa: submucous injection versus implant in polyethylene tubes. Int Endod J 34:377-385.

Fisher MA, Berzins DW, Bahcall JK. 2007. An in vitro comparison of bond strength of various obturation materials to root canal dentin using a push-out test design. J Endod 33:856-858.

Flores DS, Rached FJ, Jr., Versiani MA, Guedes DF, Sousa-Neto MD, Pecora JD. 2011. Evaluation of physicochemical properties of four root canal sealers. Int Endod J 44:126-135.

Garrido AD, Lia RC, Franca SC, da Silva JF, Astolfi-Filho S, Sousa-Neto MD. 2010. Laboratory evaluation of the physicochemical properties of a new root canal sealer based on Copaifera multijuga oil-resin. Int Endod J 43:283-291.

Ge R, Chen Z, Zhou Q. 2012. The actions of bismuth in the treatment of Helicobacter pylori infections: an update. Metallomics 4:239-243.

Geurtsen W, Leinenbach F, Krage T, Leyhausen G. 1998. Cytotoxicity of four root canal sealers in permanent 3T3 cells and primary human periodontal ligament fibroblast cultures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 85(5):592-597.

Gomes-Filho JE, Watanabe S, Lodi CS, Cintra LT, Nery MJ, Filho JA, Dezan E, Jr., Bernabe PF. 2011. Rat tissue reaction to MTA FILLAPEX®. Dent Traumatol28:452-456.

Gorduysus M, Avcu N. 2009. Evaluation of the radiopacity of different root canal sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 108:e135-40.

Grossman LI. 1958. An improved root canal cement. J Am Dent Assoc 56:381-385.

Guerreiro-Tanomaru JM, Duarte MA, Goncalves M, Tanomaru-Filho M. 2009. Radiopacity evaluation of root canal sealers containing calcium hydroxide and MTA. Braz Oral Res 23:119-123.

Haragushiku GA, Sousa-Neto MD, Silva-Sousa YT, Alfredo E, Silva SC, Silva RG. 2010. Adhesion of endodontic sealers to human root dentine submitted to different surface treatments. Photomed Laser Surg 28:405-410.

Haragushiku GA, Teixeira CS, Furuse AY, Sousa YT, De Sousa Neto MD, Silva RG. 2012. Analysis of the interface and bond strength of resin-based endodontic cements to root dentin. Microsc Res Tech 75:655-661.

Holland R, de Souza V. 1985. Ability of a new calcium hydroxide root canal filling material to induce hard tissue formation. J Endod 11:535-543.

Holland R, Pinheiro CE, de Mello W, Nery MJ, de Souza V. 1982. Histochemical analysis of the dogs' dental pulp after pulp capping with calcium, barium, and strontium hydroxides. J Endod 8:444-447.

Horie M, Fukui H, Endoh S, Maru J, Miyauchi A, Shichiri M, Fujita K, Niki E, Hagihara Y, Yoshida Y, Morimoto Y, Iwahashi H. 2012. Comparison of acute oxidative stress on rat lung induced by nano and fine-scale, soluble and insoluble metal oxide particles: NiO and TiO_2. Inhal Toxicol 24:391-400.

Kamalov J, Carpenter DO, Birman I. 2011. Cytotoxicity of environmentally relevant concentrations of aluminum in murine thymocytes and lymphocytes. J Toxicol 2011:796719.

Karapinar-Kazandag M, Bayrak OF, Yalvac ME, Ersev H, Tanalp J, Sahin F, Bayirli G. 2011. Cytotoxicity of 5 endodontic sealers on L929 cell line and human dental pulp cells. Int Endod J 44:626-634.

Lai CC, Huang FM, Yang HW, Chan Y, Huang MS, Chou MY, Chang YC. 2001. Antimicrobial activity of four root canal sealers against endodontic pathogens. Clin Oral Investig 5:236-239.

Lee KW, Williams MC, Camps JJ, Pashley DH. 2002. Adhesion of endodontic sealers to dentin and gutta-percha. J Endod 28:684-688.

Liou JW, Chang HH. 2012. Bactericidal effects and mechanisms of visible light-responsive titanium dioxide photocatalysts on pathogenic bacteria. Arch Immunol Ther Exp (Warsz) 60:267-275.

Malwal SR, Sriram D, Yogeeswari P, Konkimalla VB, Chakrapani H. 2012. Design, synthesis, and evaluation of thiol-activated sources of sulfur dioxide (SO_2) as antimycobacterial agents. J Med Chem 55:553-557.

Markowitz K, Moynihan M, Liu M, Kim S. 1992. Biologic properties of eugenol and zinc oxide-eugenol. A clinically oriented review. Oral Surg Oral Med Oral Pathol 73:729-737.

Mickel AK, Nguyen TH, Chogle S. 2003. Antimicrobial activity of endodontic sealers on Enterococcus faecalis. J Endod 29:257-258.

Molloy D, Goldman M, White RR, Kabani S. 1992. Comparative tissue tolerance of a new endodontic sealer. Oral Surg Oral Med Oral Pathol 73:490-493.

Morris DR, Levenson CW. 2012. Ion channels and zinc: mechanisms of neurotoxicity and neurodegeneration. J Toxicol 2012:785647.

Nunes VH, Silva RG, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. 2008. Adhesion of Epiphany and AH Plus sealers to human root dentin treated with different solutions. Braz Dent J 19:46-50.

Ordinola-Zapata R, Bramante CM, Graeff MS, del Carpio Perochena A, Vivan RR, Camargo EJ, Garcia RB, Bernardineli N, Gutmann JL, de Moraes IG. 2009. Depth and percentage of penetration of endodontic sealers into dentinal tubules after root canal obturation using a lateral compaction technique: a confocal laser scanning microscopy study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 108:450-457.

Orstavik D. 1983. Physical properties of root canal sealers: measurement of flow, working time, and compressive strength. Int Endod J 16:99-107.

Orstavik D. 2005. Materials used for root canal obturation: technical, biological and clinical testing. Endodontic Topics 12:25-38.

Ranguelova K, Rice AB, Khajo A, Triquigneaux M, Garantziotis S, Magliozzo RS, Mason RP. 2012. Formation of reactive sulfite-derived free radicals by the activation of human neutrophils: an ESR study. Free Radic Biol Med 52:1264-1271.

Resende LM, Rached-Junior FJ, Versiani MA, Souza-Gabriel AE, Miranda CE, Silva-Sousa YT, Sousa Neto MD. 2009. A comparative study of physicochemical properties of AH Plus, Epiphany, and Epiphany SE root canal sealers. Int Endod J 42:785-793.

Ribeiro DA. 2008. Do endodontic compounds induce genetic damage? A comprehensive review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 105:251-256.

Sabbagh J, Vreven J, Leloup G. 2004. Radiopacity of resin-based materials measured in film radiographs and storage phosphor plate (Digora). Oper Dent 29:677-684.

Sagsen B, Ustun Y, Demirbuga S, Pala K. 2011. Push-out bond strength of two new calcium silicate-based endodontic sealers to root canal dentine. Int Endod J 44:1088-1091.

Scelza MZ, Linhares AB, da Silva LE, Granjeiro JM, Alves GG. 2012. A multiparametric assay to compare the cytotoxicity of endodontic sealers with primary human osteoblasts. Int Endod J 45:12-18.

Schilder H. 2006. Filling root canals in three dimensions. 1967. J Endod 32:281-290.

Seux D, Couble ML, Hartmann DJ, Gauthier JP, Magloire H. 1991. Odontoblast-like cytodifferentiation of human dental pulp cells in vitro in the presence of a calcium hydroxide-containing cement. Arch Oral Biol 36:117-128.

Silva EJ, Accorsi-Mendonca T, Almeida JF, Ferraz CC, Gomes BP, Zaia AA. 2012. Evaluation of cytotoxicity and up-regulation of gelatinases in human fibroblast cells by four root canal sealers. Int Endod J 45:49-56.

Siqueira JF, Jr., Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP. 2000. Antimicrobial activity and flow rate of newer and established root canal sealers. J Endod 26:274-277.

Tagger M, Katz A. 2003. Radiopacity of endodontic sealers: development of a new method for direct measurement. J Endod 29:751-755.

Tagger M, Tagger E, Kfir A. 1988. Release of calcium and hydroxyl ions from set endodontic sealers containing calcium hydroxide. J Endod 14:588-591.

Tagger M, Tagger E, Tjan AH, Bakland LK. 2002. Measurement of adhesion of endodontic sealers to dentin. J Endod 28:351-354.

Tanomaru-Filho M, Tanomaru JM, Barros DB, Watanabe E, Ito IY. 2007. In vitro antimicrobial activity of endodontic sealers, MTA-based cements and Portland cement. J Oral Sci 49:41-45.

Tanomaru JM, Cezare L, Goncalves M, Tanomaru Filho M. 2004. Evaluation of the radiopacity of root canal sealers by digitization of radiographic images. J Appl Oral Sci 12:355-357.

Tasdemir T, Yesilyurt C, Yildirim T, Er K. 2008. Evaluation of the radiopacity of new root canal paste/sealers by digital radiography. J Endod 34:1388-1390.

Thang ND, Yajima I, Kumasaka MY, Ohnuma S, Yanagishita T, Hayashi R, Shekhar HU, Watanabe D, Kato M. 2011. Barium promotes anchorage-independent growth and invasion of human HaCaT keratinocytes via activation of c-SRC kinase. PLoS One 6:e25636.

Vaughan D. 1999. Energy-Dispersive X-ray Microanalysis: An Introduction. Middleton: NORAN Instruments. 59 p.

Veloso HH, do Santos RA, de Araujo TP, Leonardi DP, Baratto Filho F. 2006. Histological analysis of the biocompatibility of three different calcium hydroxide-based root canal sealers. J Appl Oral Sci 14:376-381.

Versiani MA, Carvalho-Junior JR, Padilha MI, Lacey S, Pascon EA, Sousa-Neto MD. 2006. A comparative study of physicochemical properties of AH Plus and Epiphany root canal sealants. Int Endod J 39:464-471.

Vilanova WV, Carvalho-Junior JR, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. 2012. Effect of intracanal irrigants on the bond strength of epoxy resin-based and methacrylate resin-based sealers to root canal walls. Int Endod J 45:42-48.

Vujaskovic M, Teodorovic N. 2010. Analysis of sealing ability of root canal sealers using scanning electronic microscopy technique. Srp Arh Celok Lek 138:694-698.

Wakabayashi H, Horikawa M, Funato A, Onodera A, Matsumoto K. 1993. Bio-microscopical observation of dystrophic calcification induced by calcium hydroxide. Endod Dent Traumatol 9:165-170.

Witten ML, Sheppard PR, Witten BL. 2012. Tungsten toxicity. Chem Biol Interact 196:87-88.

Yasuda Y, Kamaguchi A, Saito T. 2008. In vitro evaluation of the antimicrobial activity of a new resin-based endodontic sealer against endodontic pathogens. J Oral Sci 50:309-313.

You C, Han C, Wang X, Zheng Y, Li Q, Hu X, Sun H. 2012. The progress of silver nanoparticles in the antibacterial mechanism, clinical application and cytotoxicity. Mol Biol Rep 39:9193-9201.

Table 1. Chemical composition of evaluated sealers

Material tested	Components	
Sealapex®	Catalyst	Base
	Isobutyl salicylate resin	N-ethyltoluenesulfonamideresin
	Fumed silica (silicon dioxide)	Fumed silica (silicon dioxide)
	Bismuth trioxide	Zinc oxide
	Titanium dioxide pigment	Calcium oxide
Pulp Canal Sealer®	Powder	Liquid
	Zinc oxide	Oil of cloves
	Precipitated molecular silver	Canada Balsam
	Oleo resins (white resin)	
	Thymoliodide	
Sealer 26®	Powder	Resin
	Calcium hydroxide	Bisphenol A ether
	Bismuth oxide	
	Hexamethylenetetramine	
AH Plus®	Paste A	Paste B
	Bisphenol-A epoxy resin	Dibenzyldiamine
	Bisphenol-F epoxy resin	Aminoadamantane
	Calcium tungstate	Tricyclodecanediamine
	Zirconium oxide	Calcium tungstate
	Silica	Zirconium oxide
	Iron oxide pigments	Silica
		Silicone oil
MTA Fillapex®	Components	Components
	Natural resin	Nanoparticulated silica
	Salicylate resin	MTA
	Diluting resin	Pigments
	Bismuth oxide	
EndoFill®	Powder	Liquid
	Zinc oxide	Eugenol
	Hydrogenated resin	Sweet almond oil
	Bismuth subcarbonate	
	Barium sulfate	
	Sodium borate	

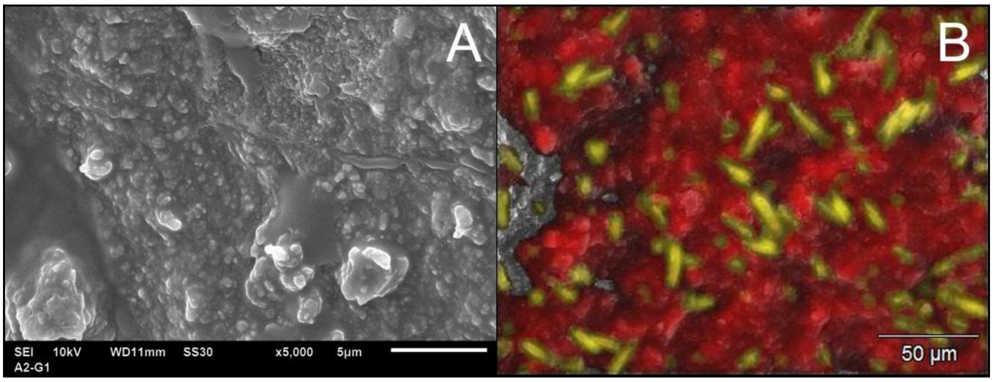

Figure 1. Sealapex® SEM image with 5,000X magnification (A), showing irregular surface. Elements distribution maps of calcium (red) and bismuth (yellow) at the surface of Sealapex® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of variable sizes and shapes.

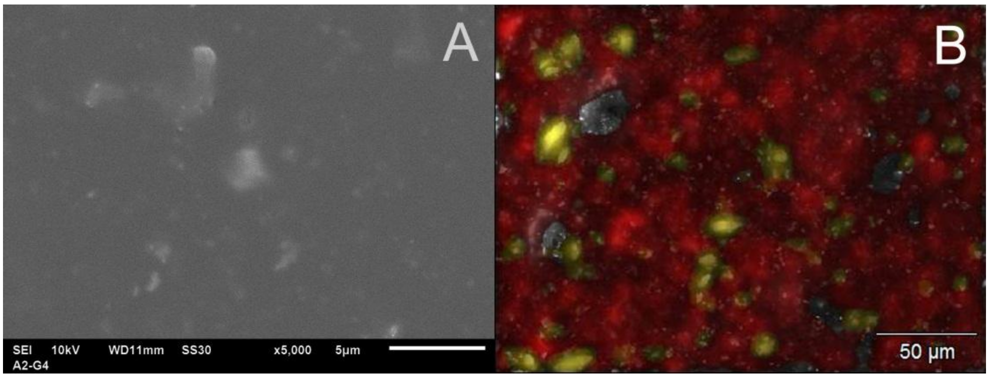

Figure 2. Sealer 26® SEM image with 5,000X magnification (A), showing regular surface. Elements distribution maps of calcium (red) and bismuth (yellow) at the surface of Sealer 26® (B) assessed by EDX microanalysis,with 50,000X magnification, which showed an uniform distribution of elements, with particles of variable sizes and shapes.

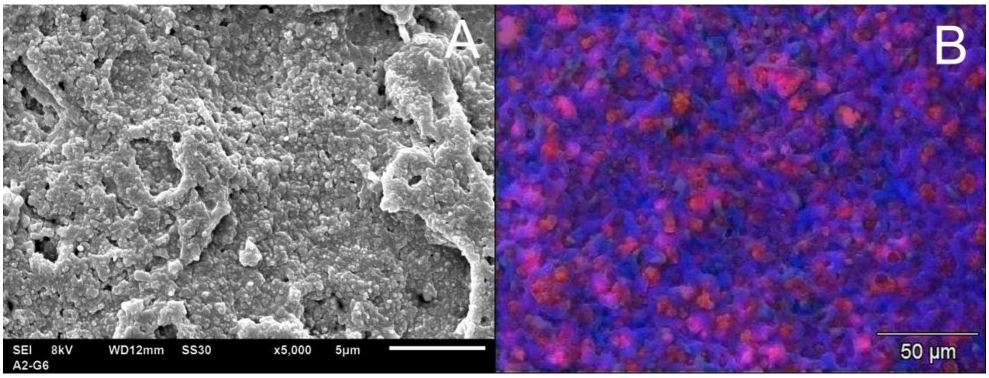

Figure 3. MTA Fillapex® SEM image with 5,000X magnification (A), showing irregular surface. Elements distribution maps of silicon (blue) and calcium (red) at the surface of MTA Fillapex® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of similar sizes and variable shapes.

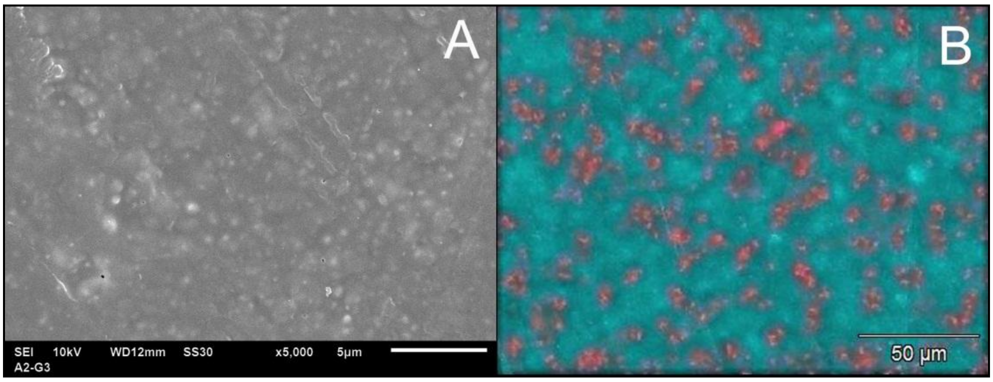

Figure 4. Pulp Canal Sealer® SEM image with 5,000X magnification (A), showing regular surface. Elements distribution maps of zinc (light blue) and silver (pink) at the surface of Pulp Canal Sealer® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of similar sizes and globular-like shapes.

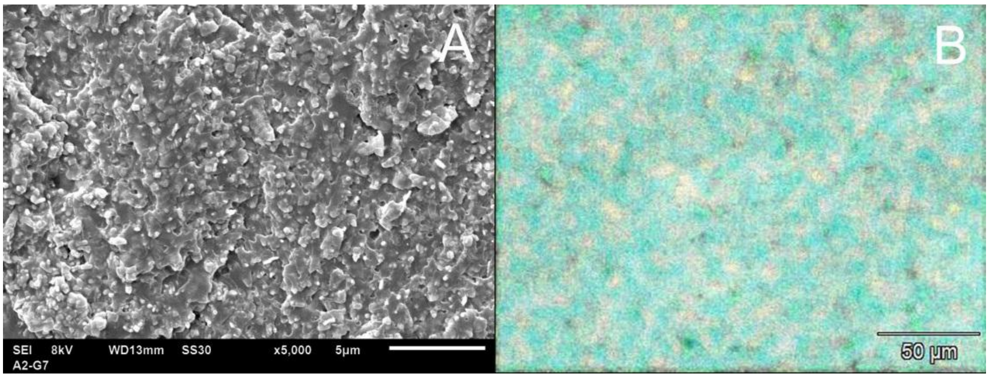

Figure 5. Endofill® SEM image with 5,000X magnification (A), showing irregular surface. Elements distribution maps of zinc (light blue) and bismuth (yellow) at the surface of Endofill® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of similar sizes and globular-like shapes.

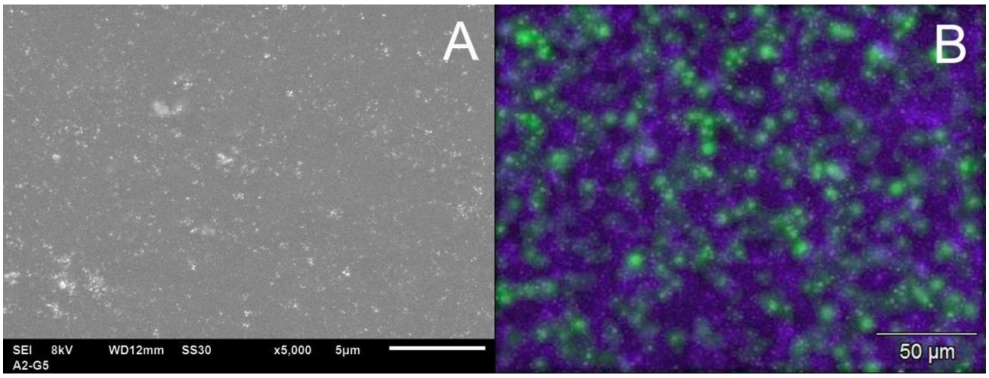

Figure 6. AH Plus® SEM image with 5,000X magnification (A), showing irregular surface. Elements distribution maps of zirconium (purple) and tungsten (green) at the surface of AH Plus® (B) assessed by EDX microanalysis, with 50,000X magnification, which showed an uniform distribution of elements, with particles of similar sizes and globular-like shapes.

Table 2 –Elements found on the endodontic sealers analyzed by energy dispersive X-ray analysis (EDX).

	Sealapex®		Sealer 26®		MTA Fillapex®		Pulp Canal Sealer®		Endofill®		AH Plus®	
Element	at.%	wt.%	at.%	wt.%	at.%	wt.%	at.%	wt.%	at.%	wt.%	at.%	wt.%
Ag	-----	-----	-----	-----	-----	-----	21.69	31.58	-----	-----	-----	-----
Al	0.96	0.51	1.07	0.53	0.97	0.61	1.04	0.38	1.62	0.56	4.16	1.21
Ba	-----	-----	-----	-----	-----	-----	-----	-----	7.91	13.92	-----	-----
Bi	6.15	25.37	8.84	33.80	5.63	27.38	-----	-----	7.10	19.03	-----	-----
Ca	67.74	53.58	88.58	65.00	32.81	30.58	-----	-----	-----	-----	14.56	6.31
Cl	-----	-----	-----	-----	-----	-----	0.36	0.17	-----	-----	1.96	0.75
Fe	0.13	0.14	-----	-----	-----	-----	-----	-----	-----	-----	0.37	0.23
Hf	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.63	1.22
Mg	0.33	0.16	1.51	0.67	-----	-----	-----	-----	-----	-----	-----	-----
Ni	0.18	0.21	-----	-----	-----	-----	0.16	0.12	0.13	0.10	-----	-----
S	5.90	3.73	-----	-----	11.31	8.43	-----	-----	7.87	3.23	-----	-----
Si	7.97	4.42	-----	-----	47.50	31.02	-----	-----	-----	-----	-----	-----
Ti	5.40	5.11	-----	-----	1.78	1.98	-----	-----	-----	-----	-----	-----
W	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	13.11	26.04
Zn	5.24	6.76	-----	-----	-----	-----	76.75	67.74	75.37	63.16	-----	-----
Zr	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	65.20	64.24

Light weight elements were excluded from the EDX microanalysis, since the fluorescence production decrease as the atomic number is lower, which makes the quantitative analysis of these elements imprecise (Vaughan, 1999).

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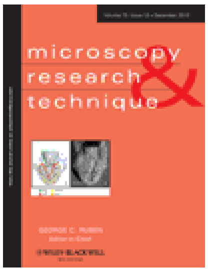

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Scaling, cropping, and rotating should be performed in the originating application. We recommend Adobe Photoshop for generating pixel-based graphics and either Adobe Illustrator or Macromedia's Freehand for generating vector-based graphics. Black and white (B/W) line art is either pixel based (typically generated by scanning the image) or vector based (generated using a drawing program such as Freehand or Illustrator). Pixel-based artwork should be supplied in TIFF format and at a resolution of not less than 1,000 dots per square inch (dpi). Vector-based artwork should be supplied as EPS files. B/W halftones should be supplied in TIFF format. The halftone should be provided at the proper resolution. The finished resolution should be at least 300 dpi. Color artwork should be provided as an EPS or TIFF file. Files should be converted from RGB to CMYK. The finished resolution should be at least 300 dpi.

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