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FERNANDA RIBEIRO SANTANA

**Influência da terapia endodôntica e do envelhecimento
artificial acelerado na resistência de união de pinos de
fibra de vidro à dentina intrarradicular**

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envelhecimento artificial acelerado na resistência de
união de pinos de fibra de vidro à dentina
intrarradicular**

Tese de Doutorado apresentada ao
Programa de Pós-Graduação em Ciências da
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Orientador: Prof. Dr. Carlos Estrela

Coorientador: Prof. Dr. Carlos José Soares

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Dedicatória

Dedico este trabalho às pessoas mais importantes da minha vida: meus pais Eduardo e Margareth, meu esposo Elvis, e minhas irmãs Fabiana e Flávia. Agradeço eternamente a vocês pelo amor, incentivo, confiança, apoio e companheirismo para a realização deste trabalho. Amo vocês.

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"Só pelo amor o homem se realiza plenamente." (Platão)

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"Grandes obras são feitas por pessoas que não têm medo de serem grandes." (Fernando Flores)

*"O professor se liga à eternidade. Ele nunca sabe quando cessa a sua influência."
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*"Fale, e eu esquecerei, ensina-me, e eu poderei lembrar, envolva-me, e eu aprenderei."
(Benjamin Franklin)*

Só os sonhadores podem nos ensinar a voar." (Anne Marie Pierce)

"Só são verdadeiramente grandes aqueles que são verdadeiramente bons." (George Chapman)

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"Não basta ensinar ao homem uma especialidade, porque se tornará assim uma máquina utilizável e não uma personalidade. É necessário que adquira um sentimento, senso prático daquilo que vale a pena ser empreendido, daquilo que é belo, do que é moralmente correto." (Albert Einstein)

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construímos, das lições que aprendemos e das amizades que
fizemos.”(Barbosa Filho)*

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***“Só sabemos com exatidão quando
sabemos pouco; à medida que vamos
adquirindo conhecimentos, instala-se a dúvida.”
(Johann Wolfgang von Goethe)***

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Símbolos, siglas e abreviaturas

n	Número
=	Igual
%	Porcentagem
EDTA	Ácido etilenodiamino tetra-acético
°C	grau Celsius
MPa	Megapascal
ANOVA	Análise de variância
α	Nível de confiabilidade
Mm	Milímetro
<i>et al.</i>	e outros
&	e (comercial)
RJ	Rio de Janeiro
SP	São Paulo
mL	Mililitro
GO	Goiás
Ltda.	Limitada
L	Litro
g/h	Gramas por hora
min	Minuto
PR	Paraná
MN	Minnesota
EUA	Estados Unidos da América
mW/cm ²	Miliwatts por centímetro quadrado
s	Segundo
"	Polegada
CT	Connecticut
IL	Illinois
cm	Centímetro
kgf	Quilograma-força

mm/min	Milímetro por minuto
N	Newton
SAS	Statistical analysis system
NC	North Carolina
ANOVA Two-way	Análise de variância dois fatores
ANOVA One-way	Análise de variância um fator
<i>P</i>	Probabilidade
<	Menor
X	Magnitude de aumento de tomada de imagem
UFU	Universidade Federal de Uberlândia
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
#	Número

Resumo

Objetivo: Analisar *in vitro* a influência das técnicas de instrumentação do canal radicular, dos irrigantes e cimentos endodônticos e do envelhecimento artificial acelerado na resistência de união do pino de fibra de vidro à dentina intrarradicular em dentes bovinos. **Metodologia: Parte 1.** Cento e vinte incisivos bovinos foram divididos em doze grupos experimentais (n=10) resultantes da interação entre três fatores em estudo: técnica de instrumentação do canal radicular (PAI – preparo do canal radicular com instrumentos de aço inoxidável - K-File; PNiTi – preparo do canal radicular com instrumentos de Níquel-Titânio - K3); irrigante endodôntico (NaOCl- hipoclorito de sódio 1%; CHX- clorexidina 2%; O₃- água ozonificada 1,2%) (em todas as amostras o EDTA 17% foi utilizado para a remoção de smear layer) e envelhecimento artificial acelerado dos espécimes (Imediato, teste sem envelhecimento; Mediato, teste após 2 meses de envelhecimento em água a 37°C). Após o preparo dos canais radiculares não foi realizada a obturação endodôntica. Pinos de fibra de vidro foram cimentados com cimento resinoso auto-adesivo (RelyX U100, 3M-ESPE) e as raízes foram seccionadas para obtenção de duas fatias de cada terço. As amostras foram submetidas ao teste de *micropush-out* e os dados de resistência de união (MPa) foram analisados por ANOVA com parcela subdividida e teste de Tukey ($\alpha = 0,05$). Os padrões de falha foram avaliados por meio de microscopia confocal. **Parte 2.** Sessenta incisivos bovinos foram divididos em seis grupos experimentais (n=10) resultantes da interação entre dois fatores em estudo: cimento endodôntico (SX- Sealapex; S26- Sealer 26; AH- AH Plus) e envelhecimento artificial acelerado dos espécimes (Imediato, teste sem envelhecimento; Mediato, teste após 2 meses de envelhecimento em água a 37°C). Foram empregados dois grupos controles (sem obturação do canal radicular) representados pelos grupos PNiTiNaOCl imediato e PNiTiNaOCl mediato da parte 1 do presente estudo. Nos seis grupos experimentais os canais radiculares foram preparados 1mm aquém do ápice com instrumentos de Níquel-Titânio - K3 associado a

irrigação com NaOCl 1% e EDTA 17%. Em seguida, foram obturados com gutapercha e o cimento endodôntico específico de cada grupo, usando a técnica de condensação lateral. Pinos de fibra de vidro foram cimentados com cimento resinoso auto-adesivo (RelyX U100, 3M-ESPE) e as raízes foram seccionadas para obtenção de duas fatias de cada terço. As amostras foram submetidas ao teste de *micropush-out* e os dados de resistência de união (MPa) foram analisados por ANOVA com parcela subdividida e teste de Tukey ($\alpha = 0,05$). Comparações com os grupos controles foram feitas pelo teste de Dunnett ($\alpha = 0,05$). Os padrões de falha foram avaliados por meio de microscopia confocal.

Resultados: Parte 1. Nos espécimes submetidos ao envelhecimento artificial em água, PNiTi apresentou maiores valores de resistência de união que PAI no terço apical irrigado com NaOCL ou CHX. A irrigação com NaOCL resultou em maior resistência de união comparada a O₃. O envelhecimento artificial resultou em aumento significativo da resistência de união, exceto para os terços médio e apical de PAIO₃ e apical de PNiTiO₃. A resistência de união reduziu significativamente no terço apical. A prevalência de falha adesiva cimento-dentina foi verificada em todos os grupos.

Parte 2. Os cimentos endodônticos não mostraram diferenças significantes entre si, entretanto apresentaram valores de resistência de união significativamente menores que os grupos controles (sem obturação), exceto no terço cervical dos grupos testados imediatamente. O envelhecimento artificial não interferiu na resistência de união à dentina intrarradicular. Houve uma diminuição significativa na resistência de união do terço cervical para o apical. A prevalência de falha adesiva cimento-dentina foi verificada em todos os grupos.

Conclusões: Parte 1. O preparo do canal radicular com instrumentos de NiTi associado a irrigação com NaOCl e uso do EDTA aumentou a resistência de união de pinos de fibra de vidro cimentados com cimento auto-adesivo à dentina intrarradicular.

Parte 2. Os cimentos endodônticos interferiram negativamente na união de pinos de fibra de vidro cimentados com cimento auto-adesivo à dentina intrarradicular.

Abstract

Aim: To evaluate *in vitro* the influence of root canal instrumentation techniques, endodontic irrigants, endodontic sealers and artificial accelerated aging on fibreglass post bond strength to bovine intraradicular dentine. **Methodology:**

Part 1. 120 bovine incisors were divided into 12 experimental groups (n=10) resulting from the interaction among 3 study factors: root canal instrumentation technique (RCPSS- root canal preparation with stainless steel instruments - K-File; RCPNiTi- root canal preparation with K3 Nickel-Titanium instruments), endodontic irrigant (NaOCl- 1% sodium hypochlorite; CHX- 2% chlorhexidine; O₃- 1.2% ozonated water) (in all samples 17% EDTA was used to remove the smear layer) and specimens artificial accelerated aging (Immediate, test with no aging; Mediate, test performed after 2 months of water storage at 37°C). After root canal preparation, endodontic filling was not performed. Fibreglass posts were cemented with self-adhesive resin cement (RelyX U100, 3M-ESPE) and roots were cross-sectioned to obtain two slices of each third. Samples were submitted to micropush-out test and bond strength values (MPa) were analyzed by ANOVA in a split-plot arrangement and Tukey's test ($\alpha = 0.05$). Failure modes were evaluated under a confocal microscope. **Part 2.** 60 bovine incisors were divided into 6 experimental groups (n=10) resulting from the interaction between 2 study factors: endodontic sealer (Sx- Sealapex; S26- Sealer 26; AH-AH Plus) and specimens artificial accelerated aging (Immediate, test with no aging; Mediate, test performed after 2 months of water storage at 37°C). Two control groups were employed (without root canal filling), represented by groups RCPNiTiNaOCl immediate and RCPNiTiNaOCl mediate of part 1 of the present study. In the six experimental groups, root canals were prepared 1mm from the apex with K3 Nickel-Titanium instruments associated with 1% sodium hypochlorite irrigation and 17% EDTA. They were then filled with gutta-percha and the specific sealer of each group, using the lateral compaction technique. Fibreglass posts were cemented with self-adhesive resin cement (RelyX U100, 3M-ESPE) and roots were cross-sectioned to obtain two slices of each third.

Samples were submitted to micropush-out test and bond strength values (MPa) were submitted to ANOVA in a split-plot arrangement and Tukey's test ($\alpha = 0,05$). Comparisons with control groups were made using Dunnet test ($\alpha = 0.05$). Failure modes were evaluated under a confocal microscope. **Results: Part 1.** In specimens submitted to water artificial aging, RCPNiTi presented higher bond strength values than RCPSS in apical third irrigated with NaOCl or CHX. Irrigation with NaOCl resulted in higher bond strength than O₃. Artificial aging resulted in significant bond strength increase, except for middle and apical thirds of RCPSSO₃ and apical of RCPNiTiO₃. Bond strength significantly reduced in apical third. The prevalence of adhesive cement-dentine failure was verified in all groups. **Part 2.** Endodontic sealers showed no significant differences among them, however they presented significantly lower bond strength values than control groups (without filling), except in cervical third of groups tested immediately. Artificial aging did not interfere on bond strength to intraradicular dentine. There was a significant decrease on bond strength from cervical to apical third. The prevalence of adhesive cement-dentine failure was verified in all groups. **Conclusions: Part 1.** Root canal preparation with NiTi instruments associated with NaOCl irrigation and EDTA increased the bond strength of fiberglass posts cemented with self-adhesive resin cement to intraradicular dentine. **Part 2.** Endodontic sealers interfered negatively on bonding of fibreglass posts cemented with self-adhesive resin cement to intraradicular dentine.

1. Introdução

A restauração de dentes tratados endodonticamente representa um desafio, uma vez que geralmente apresentam estrutura coronária insuficiente para reter o material restaurador (Menezes *et al.*, 2008; Santana *et al.*, 2011). Assim, tem sido sugerido o uso de pino intrarradicular para melhorar a retenção do núcleo de preenchimento (Morgano *et al.*, 2004; Cheung, 2005; Tang *et al.*, 2010; Santana *et al.*, 2011).

Pinos de fibra de vidro em associação ao núcleo de preenchimento em compósito são amplamente aceitos como alternativa a núcleos metálicos moldados e fundidos na restauração de dentes tratados endodonticamente (Schwartz & Robbins, 2004; Bitter & Kielbassa, 2007; Naves *et al.*, 2011). Os pinos de fibra de vidro apresentam elevada resistência flexural e módulo de elasticidade similar ao da dentina, o que minimiza a transmissão de tensões para as paredes do canal radicular e reduz a possibilidade de fratura (Lassila *et al.*, 2004; Schwartz & Robbins, 2004; Cecchin *et al.*, 2011). Estes pinos têm sido cimentados com materiais adesivos (Kececi *et al.*, 2008; Bitter *et al.*, 2009; Cecchin *et al.*, 2011).

A união entre pino-cimento e cimento-dentina é necessária para o sucesso do procedimento restaurador em dentes tratados endodonticamente (Soares *et al.*, 2008a). A falta de união é o modo de falha mais comum em restaurações com pino de fibra e compósito (Ferrari *et al.*, 2007; Cagidiaco *et al.*, 2008; Albashaireh *et al.*, 2010; Jongsma *et al.*, 2010). Este fato provavelmente ocorre devido a ausência de visibilidade, características anatômicas (Mjör *et al.*, 2001; Bitter *et al.*, 2008; Pelegrine *et al.*, 2010) e limitada capacidade de dissipar as tensões geradas pela contração de polimerização em um espaço estreito e longo preparado para o pino, exibindo um fator de configuração cavitária altamente desfavorável (Tay *et al.*, 2005; Pelegrine *et al.*, 2010).

Procedimentos endodônticos realizados antes da cimentação dos pinos podem interferir na união à dentina intrarradicular (Ari *et al.*, 2003; Erdemir *et al.*, 2004; Hayashi *et al.*, 2005; Muniz & Mathias, 2005; Bitter *et al.*, 2008; Pelegrine *et al.*, 2010). Na terapia endodôntica, o processo de sanificação de canais radiculares infectados tem início com a ação mecânica dos instrumentos e ação química dos irrigantes endodônticos (Estrela *et al.*, 2003, 2004). Estes fatores podem induzir alterações químicas e estruturais na superfície dentinária e comprometer a interação com os materiais restauradores (Cecchin *et al.*, 2010; Shokouhinejad *et al.*, 2010).

No preparo do canal radicular, uma efetiva solução irrigante é essencial para o processo de sanificação porque ela favorece a limpeza, modelagem e neutraliza o conteúdo necrótico, o que favorece o alargamento do canal radicular para subsequente obturação. Diferentes agentes químicos auxiliares ao preparo do canal radicular têm sido propostos e a seleção de um irrigante ideal depende de sua ação nos micro-organismos e tecidos periapicais. O hipoclorito de sódio e a clorexidina são agentes antimicrobianos frequentemente usados no tratamento de infecções endodônticas (Zehnder, 2006; Estrela *et al.*, 2003, 2004, 2007).

O hipoclorito de sódio (NaOCl) é a solução irrigante mais utilizada na endodontia, porque seu mecanismo de ação causa alterações biossintéticas no metabolismo celular e destruição de fosfolípidios, formação de cloraminas que interferem no metabolismo celular, ação oxidativa com inativação enzimática irreversível na bactéria e degradação de lipídeos e ácidos graxos (Estrela *et al.*, 2002). A clorexidina (CHX) é um potente antisséptico com uma ação antimicrobiana de amplo espectro, substantividade (Okino *et al.*, 2004; Pelegrine *et al.*, 2010) e habilidade de decomposição (Barbin *et al.*, 2008), entretanto, é incapaz de dissolver tecidos pulparem (Okino *et al.*, 2004; Pelegrine *et al.*, 2010). A água ozonificada (O₃) no tratamento de infecções endodônticas tem sido estudada (Nagayoshi *et al.*, 2004; Hems *et al.*, 2005; Estrela *et al.*, 2007; Bitter *et al.*, 2008). O ozônio (O₃) é um agente oxidante

poderoso cujo efeito antimicrobiano resulta da oxidação de componentes celulares microbianos (Estrela *et al.*, 2007).

Todavia, verifica-se ausência de consenso sobre os possíveis efeitos dos agentes químicos utilizados durante o preparo de canais radiculares em relação à união do pino de fibra à dentina intrarradicular (Morris *et al.*, 2001; Ari *et al.*, 2003; Varela *et al.*, 2003; Erdemir *et al.*, 2004; Muniz & Mathias, 2005; Hayashi *et al.*, 2005; Bitter *et al.*, 2008; Demiryürek *et al.*, 2009; Pelegrine *et al.*, 2010), uma vez que estes efeitos dependem do sistema adesivo empregado (Hayashi *et al.*, 2005; Bitter *et al.*, 2008).

A obturação do canal radicular, importante fase do tratamento endodôntico, apresenta como objetivo o selamento biológico endodôntico (Estrela *et al.*, 2007). Para tanto, utiliza-se a associação de materiais sólidos de preenchimento (como cones de guta-percha) e cimento endodôntico. Este material deveria aderir às paredes do canal radicular e fornecer uma configuração em bloco único permitindo o selamento do espaço do canal (Schwartz, 2006; Teixeira *et al.*, 2009). Este processo de adesão envolve forças mecânicas que permitem o entrelaçamento do material com a estrutura dentinária e pode resultar em uma capacidade maior de selamento, reduzindo assim o risco de microinfiltração no canal radicular e manutenção de uma massa de preenchimento coesiva (Saleh *et al.*, 2002; Teixeira *et al.*, 2009).

Houve uma busca permanente ao longo dos últimos anos de um cimento endodôntico que atendesse as propriedades físico-químicas ideais de selamento, radiopacidade, tempo de endurecimento e escoamento, bem como a tolerância tecidual (Duarte *et al.*, 2010; Holland & Souza, 1985). O Sealapex constitui um cimento endodôntico contendo óxido de cálcio, que apresenta capacidade de estimular a formação de tecido mineralizado (Holland & Souza, 1985). O Sealer 26 representa outro cimento endodôntico que incorpora hidróxido de cálcio, porém associado à resina epóxica (Gomes *et al.*, 2004; Nunes *et al.*, 2008; Demiryürek *et al.*, 2010). O AH Plus é um cimento à base de resina epóxica com boas características físico-químicas (Kopper *et al.*, 2003; Solano *et al.*, 2005; Grecca *et al.*, 2009; Demiryürek *et al.*, 2010) e uma

adequada adesão (Sousa-Neto *et al.*, 2005; Nunes *et al.*, 2008). A composição dos cimentos endodônticos pode interferir na adesão do pino à dentina intrarradicular (Hagge *et al.*, 2002; Muniz & Mathias, 2005; Baldissara *et al.*, 2006; Menezes *et al.*, 2008; Teixeira *et al.*, 2008; Demiryürek *et al.*, 2010; Cecchin *et al.*, 2011; Dimitrouli *et al.*, 2011).

Diante do exposto, verifica-se que a compatibilidade entre os diferentes materiais empregados na terapia endodôntica e na cimentação de pinos de fibra constitui aspecto importante a ser considerado para uma restauração satisfatória. Além disso, a qualidade de união à dentina radicular pode ser afetada pela densidade e orientação dos túbulos dentinários nos diferentes níveis das paredes do canal radicular (Ferrari *et al.*, 2000; Goracci *et al.*, 2004) e pela facilidade de acesso aos terços do canal radicular (Ferrari *et al.*, 2000; Ferrari *et al.*, 2001; Goracci *et al.*, 2004; Wang *et al.*, 2008; da Cunha *et al.*, 2010).

Vários métodos mecânicos têm sido empregados para mensurar *in vitro* a resistência de união do pino de fibra de vidro à dentina intrarradicular, como os testes de microtração e *micropush-out* (Soares *et al.*, 2008b). O método de *micropush-out* resulta em menor índice de falhas prematuras dos espécimes, menor variabilidade na distribuição dos dados (Goracci *et al.*, 2004; Soares *et al.*, 2008b) e distribuição de tensões mais homogênea (Soares *et al.*, 2008b) comparado ao método de microtração, constituindo assim uma metodologia mais segura (Goracci *et al.*, 2004) e apropriada para a avaliação de pinos de fibra de vidro unidos à dentina intrarradicular (Soares *et al.*, 2008b).

Entretanto, estes métodos mecânicos fornecem uma avaliação estática da resistência de união interfacial (Goracci *et al.*, 2004). Alguns estudos têm incluído procedimentos de envelhecimento artificiais, tais como a ciclagem térmica, simulação mastigatória e/ou armazenamento em água para avaliar a durabilidade da união (Balbosh *et al.*, 2005; De Munck *et al.*, 2005; Radovic *et al.*, 2007; Albashaireh *et al.*, 2010). O armazenamento em água é considerado como um teste *in vitro* de envelhecimento acelerado para as interfaces de união (Radovic *et al.*, 2007).

Portanto, torna-se oportuno avaliar a influência das técnicas de instrumentação do canal radicular, da composição química dos irrigantes e cimentos endodônticos e do envelhecimento artificial acelerado dos espécimes na resistência de união do pino de fibra de vidro à dentina intrarradicular, em função da profundidade do canal radicular.

2. Objetivo

O objetivo deste estudo foi avaliar os efeitos da terapia endodôntica (técnica de instrumentação, irrigantes e cimentos endodônticos) e do envelhecimento artificial acelerado dos espécimes na resistência de união de pinos de fibra de vidro cimentados adesivamente (RelyX U100, 3M-ESPE) ao canal radicular em incisivos bovinos, em função da profundidade do canal radicular.

O estudo foi desenvolvido em duas partes. Na parte 1, as hipóteses testadas foram que (1) as técnicas de instrumentação do canal radicular, (2) a composição química dos irrigantes endodônticos e (3) o envelhecimento artificial acelerado dos espécimes influenciam na resistência de união do pino de fibra de vidro à dentina intrarradicular, dependendo da profundidade do canal radicular.

Na parte 2, as hipóteses testadas foram que (4) os cimentos endodônticos e (5) o envelhecimento artificial acelerado dos espécimes influenciam na resistência de união do pino de fibra de vidro à dentina intrarradicular, dependendo da profundidade do canal radicular.

3. Material e métodos

Seleção e preparo dos dentes

A partir de 1000 incisivos inferiores bovinos recém-extraídos, limpos com curetas periodontais (SS White Duflex, Rio de Janeiro, RJ, Brasil) e submetidos a profilaxia com pedra pomes (Vigodent, Rio de Janeiro, RJ, Brasil) e água, foram selecionados 180 dentes (aprovação pelo Comitê de Ética da Universidade Federal de Goiás, n. 256/10), sendo 120 dentes para a parte 1 e 60 para a parte 2 do presente estudo. O critério de seleção usado foi a similaridade na morfologia anatômica externa e interna de dentes de animais adultos, no qual o diâmetro do canal radicular deveria ser menor que 1mm. Os dentes foram seccionados perpendicularmente ao longo eixo com disco diamantado de dupla face (KG Sorensen, São Paulo, SP, Brasil), sob refrigeração em água, permanecendo remanescente radicular de 15 mm a partir da porção apical da raiz (Figura 1).

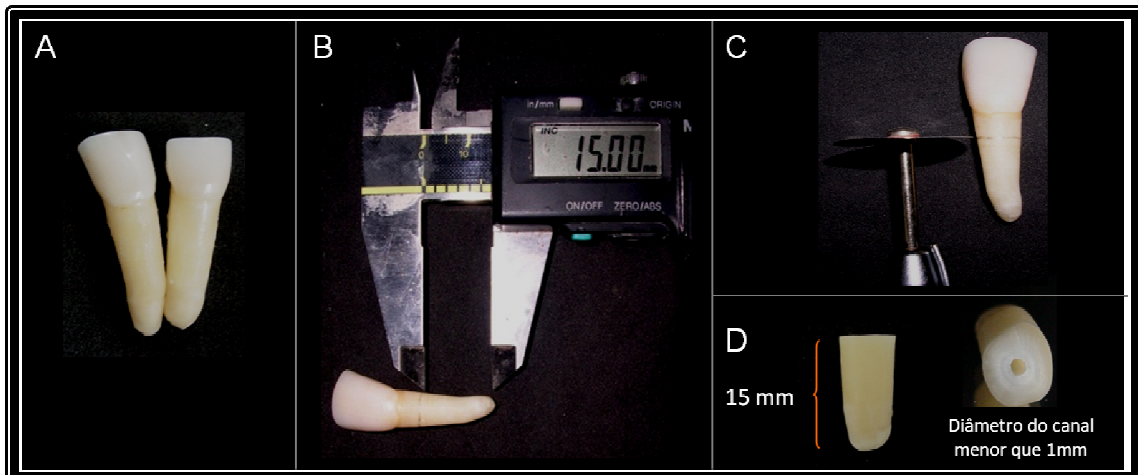


Figura 1. Seleção e preparo dos dentes: Critério da similaridade externa (A), Demarcação de 15 mm a partir da porção apical da raiz (B), Seccionamento do dente (C), Remanescente radicular de 15 mm e critério da similaridade interna (D).

Parte 1

Grupos experimentais

Cento e vinte raízes foram divididas aleatoriamente em 12 grupos experimentais (n=10) resultantes da interação entre três fatores em estudo: técnica de instrumentação do canal radicular (**PAI**- preparo do canal radicular com instrumentos de aço inoxidável - K-File; **PNiTi**- preparo do canal radicular com instrumentos rotatórios de Níquel-Titânio - sistema K3), irrigante endodôntico (**NaOCl**- hipoclorito de sódio 1%; **CHX**- clorexidina 2%; **O₃**- água ozonificada 1,2%) e envelhecimento artificial acelerado dos espécimes (**Imediato**, teste sem envelhecimento; **Mediato**, teste após 2 meses de envelhecimento em água a 37°C) (Figura 2).

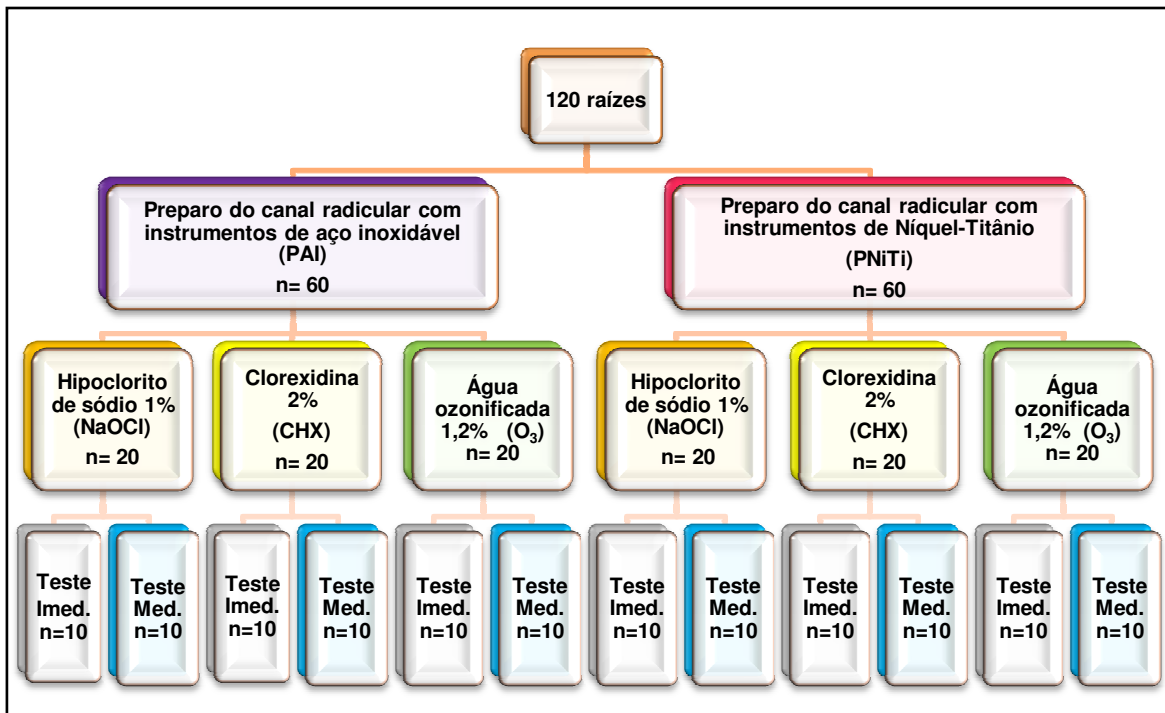


Figura 2. Organograma dos grupos experimentais da parte 1.

Preparo do canal radicular

Os canais radiculares foram preparados 1 mm aquém do ápice por meio da técnica coroa-ápice. Nos grupos PAI, o preparo do canal radicular foi realizado utilizando-se brocas Gates Glidden n. 1,2 (Dentsply Maillefer, Ballaigues, Suíça) (Comprimento de Trabalho - CT 10 mm), brocas Largo n. 2 (Dentsply Maillefer) (CT 10 mm) e instrumentos de aço inoxidável (K-File; Dentsply Maillefer) até a lima n. 45 K-File (CT 14 mm). Nos grupos PNiTi, os canais radiculares foram preparados com brocas Gates Glidden n. 1,2 (Dentsply Maillefer) (CT 10 mm) e instrumentos rotatórios de Níquel-Titânio do sistema K3 (SybronEndo, Optimum, São Paulo, SP, Brasil) na seguinte sequência: lima 25 taper 10 (CT 10 mm); limas 15-25 taper 2 (CT 14 mm); lima 25 taper 4 (CT 14mm); lima 25 taper 6 (CT 14 mm); limas 30-45 taper 2 (CT 14 mm).

Durante a instrumentação, a cada troca de lima, os canais foram irrigados com 2 mL de uma das soluções irrigantes testadas no presente estudo: NaOCl- hipoclorito de sódio 1% (Fitofarma, Goiânia, GO, Brasil); CHX- clorexidina 2% (Fitofarma); O₃- água ozonificada 1,2%. O ozônio foi produzido por uma descarga elétrica através de uma corrente de oxigênio (PXZ3507, Eaglesat Tecnologia em Sistemas Ltda., São José dos Campos, SP, Brasil) e inserido em 1L de água destilada estéril com uma vazão de ozônio de 7 g/h (1,2%) (Estrela *et al.*, 2007). Em todos os grupos, 3 mL de EDTA 17% (Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brasil) foram utilizados por 5 min para a remoção de smear layer. A irrigação final foi realizada com 5 mL da mesma solução irrigante empregada no preparo do canal radicular. Após o preparo dos canais radiculares não foi realizada a obturação endodôntica (Figura 3).

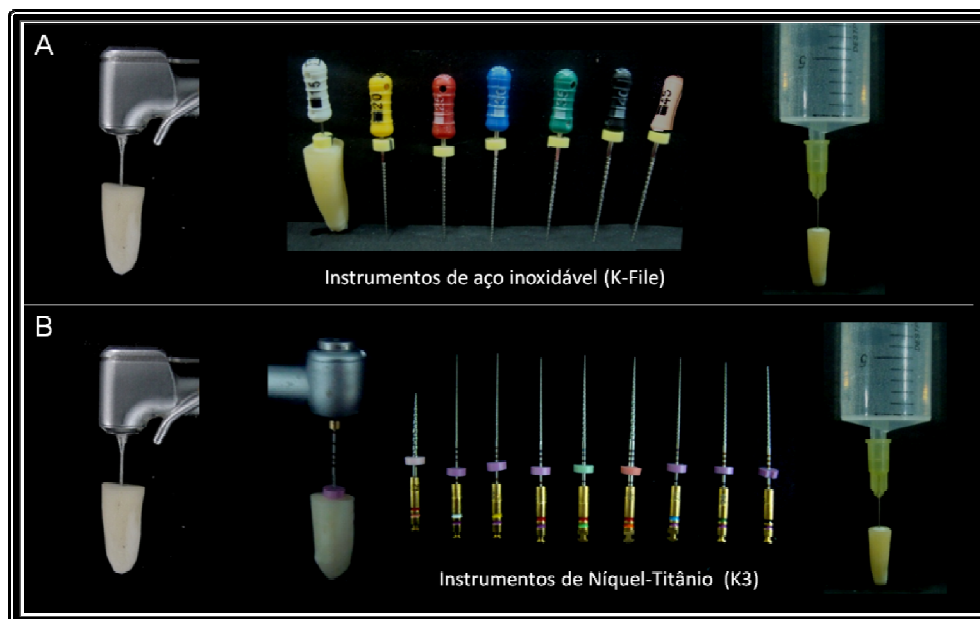


Figura 3. Preparo do canal radicular para os grupos PAI (A) e PNiTi (B).

Preparo para o pino intrarradicular

O preparo para o pino foi realizado usando brocas Largo n. 3-5 (Dentsply Maillefer) (CT 10 mm) correspondente ao pino de fibra de vidro paralelo e serrilhado de 1,5 mm de diâmetro (Reforpost n. 3; Angelus, Londrina, PR, Brasil). Os canais radiculares foram irrigados, a cada troca de brocas e após o preparo, com 2 mL da mesma solução irrigante usada previamente, e foram secos com cones de papel absorventes. Todas as raízes foram cobertas externamente com cera utilidade para evitar polimerização lateral pelo fotoativador (Soares *et al.*, 2012) (Figura 4).

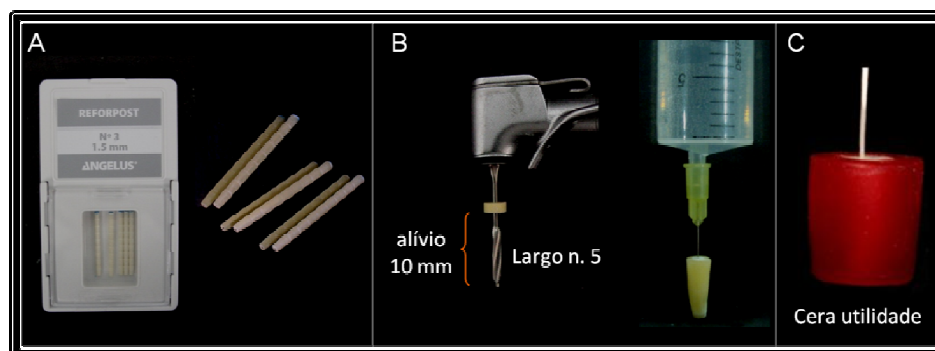


Figura 4. Pinos de fibra de vidro (Reforpost n. 3; Angelus) (A), Preparo para o pino intrarradicular (B), Recobrimento da raiz externamente com cera utilidade (C).

Cimentação do pino intrarradicular

Os pinos de fibra foram limpos com álcool 70% e, em seguida, em uma única aplicação usando um microbrush, e após a secagem, o agente silano foi aplicado por um minuto (Silano; Angelus). O cimento resinoso auto-adesivo (RelyX U100; 3M-ESPE, St. Paul, MN, EUA) foi manipulado de acordo com as instruções do fabricante, introduzido no canal radicular com limas K-File e aplicado na superfície do pino. O pino foi inserido no canal com pressão digital. O excesso de cimento foi removido após 1 min. Após 5 min, o cimento resinoso foi fotopolimerizado com 1200 mW cm⁻² (Radii-Cal; SDI, Bayswater, Austrália) por 40 s na face cervical da raiz, em direção ao longo eixo da raiz, e obliquamente nas superfícies vestibular e lingual, totalizando 120 s. A interface pino-cimento-dentina na face cervical foi selada com resina composta e as raízes foram armazenadas em água destilada a 37°C por 24 horas (Figura 5).

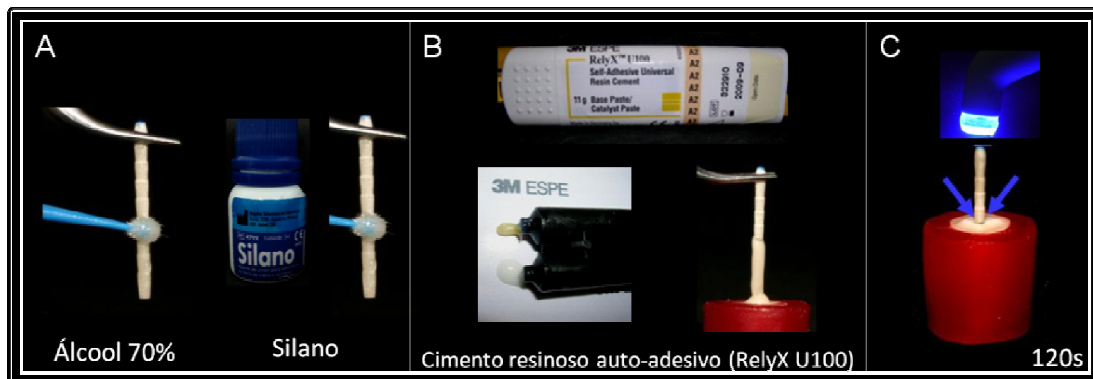


Figura 5. Tratamento de superfície do pino de fibra de vidro (A), Cimentação do pino com o cimento RelyX U100 (3M-ESPE) (B), Fotoativação do cimento (C).

Preparo dos espécimes para o ensaio de *micropush-out*

As raízes foram coladas em placa acrílica com auxílio de adesivo a base de cianoacrilato (Loctite Super Bonder, Henkel Loctite Corporation, EUA) e godiva, e então seccionadas perpendicularmente ao seu longo eixo com disco diamantado dupla face (4" Diameter x 0,012" Thickness x 1/2" Arbor, Extec, Enfield, CT, EUA) em baixa velocidade e sobre refrigeração com água (Isomet 1000, Buehler, Lake Bluff, IL, EUA) para obtenção de duas fatias, com

aproximadamente 1 mm de espessura, de cada terço radicular (cervical, médio e apical), totalizando seis fatias por raiz (Figura 6).

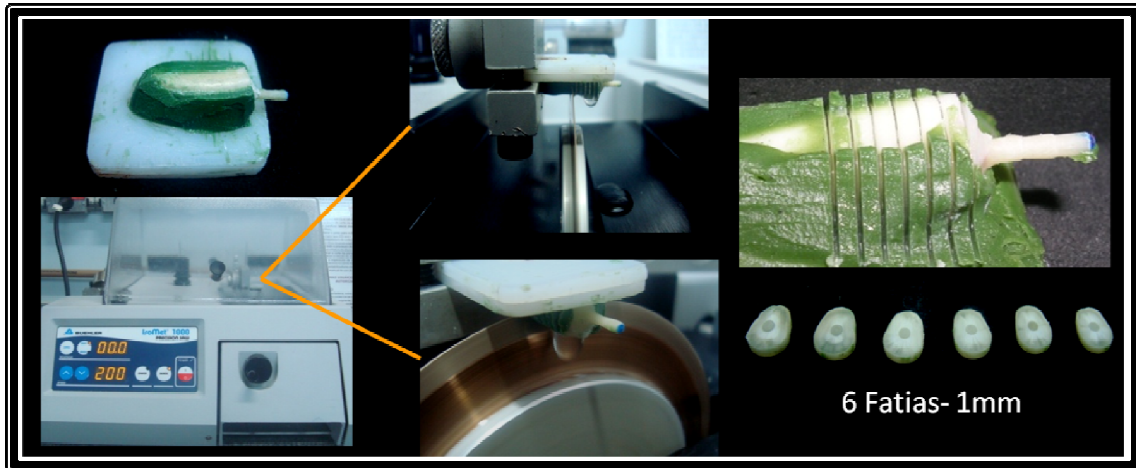


Figura 6. Seccionamento da raiz com disco diamantado de dupla face montado em micrótomo de tecido duro.

Envelhecimento artificial acelerado dos espécimes

Nos grupos mediatos, as fatias foram individualmente armazenadas em *ependorfs* com água destilada e mantidas em estufa a 37°C por dois meses antes do teste de *micropush-out* (Figura 7).

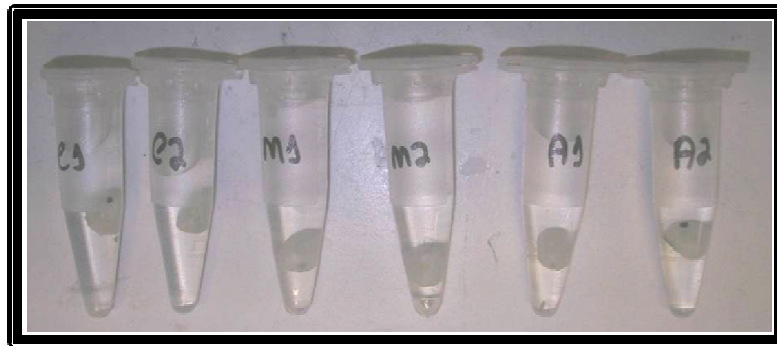


Figura 7. Espécimes armazenados individualmente em *ependorfs* com água destilada para envelhecimento em estufa a 37°C por dois meses.

Ensaio de *micropush-out*

Para a realização do ensaio de *micropush-out* foi utilizado dispositivo desenvolvido especificamente para este teste (Menezes *et al.*, 2008), constituído por base metálica em aço inoxidável com 3 cm de diâmetro, contendo orifício de 2 mm na região central e ponta aplicadora de carga com 1

mm de diâmetro e 3 mm de comprimento. Após o posicionamento do conjunto na base da máquina de ensaio mecânico (EMIC DL 2000, São José dos Pinhais, PR, Brasil) contendo célula de carga de 20 Kgf, as fatias foram posicionadas de forma que a ponta aplicadora de carga coincidisse com o orifício da base metálica. Em seguida, foi aplicado carregamento de compressão no sentido ápice/coroa sob velocidade de 0,5 mm/min, até ocorrer a extrusão do pino. A resistência de união foi calculada em MPa dividindo-se a carga que levou a falha do sistema (N) pela área da interface de união. A área da interface de união foi calculada pela fórmula: $A = 2\pi rh$, sendo **A** a área da interface de união; π a constante 3,14; **r** o raio do segmento do pino (mm) e **h** a espessura do segmento do pino (mm) (Goracci *et al.*, 2004; Soares *et al.*, 2008b; Soares *et al.*, 2012) (Figura 8).

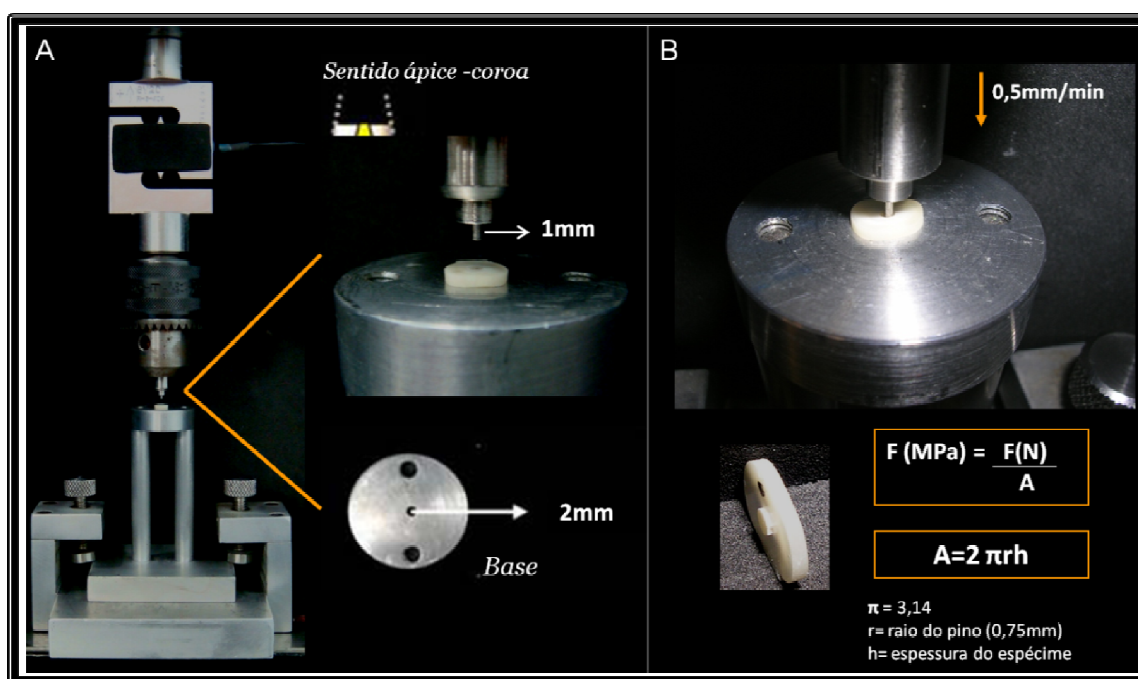


Figura 8. Posicionamento do espécime no dispositivo para o ensaio de *micropush-out* (A), Aplicação de carregamento de compressão em ensaio de *micropush-out* e fórmula para cálculo da resistência de união (B).

Análise estatística

A análise estatística foi realizada utilizando o programa estatístico SAS (Institute Inc., Cary, NC, EUA, Release 9,2). Os dados foram submetidos ao teste de normalidade Shapiro-Wilk. Os efeitos, na resistência de união, da

técnica de instrumentação, do irrigante endodôntico e da profundidade do canal radicular foram analisados usando ANOVA Two-way em esquema de parcela subdividida, com as parcelas representadas pelos fatores técnica de instrumentação, irrigante endodôntico e suas interações, e a sub-parcela representada pelos terços do canal radicular (cervical, médio e apical). Comparações relacionadas ao envelhecimento artificial foram realizadas por ANOVA One-way com a parcela representada por este fator e a sub-parcela representada pelo terços do canal radicular. Comparações múltiplas foram feitas pelo teste de Tukey ($\alpha=0,05$).

Definição dos padrões de falha por Microscopia Confocal a laser de varredura

Para determinar os padrões de falha, todos os espécimes fraturados (n=720) foram secos com jato de ar e analisados em microscópio de varredura confocal a laser (Carl Zeiss Laser Scanning Systems, LSM510, META, Oberkochen, Alemanha). As imagens foram analisadas usando o Zeiss LSM Image Browser (META, Alemanha). Os padrões de falha foram classificados em seis tipos: (I) falha adesiva entre o pino e o cimento resinoso; (II) falha adesiva entre o cimento resinoso e a dentina intrarradicular; (III) falha coesiva no cimento; (IV) falha coesiva na dentina; (V) falha coesiva no pino (Castellan *et al.*, 2010) e (VI) falha mista entre pino, cimento resinoso e dentina intrarradicular (Bitter *et al.*, 2009).

Parte 2

Grupos experimentais

Sessenta raízes foram divididas aleatoriamente em seis grupos experimentais (n=10) resultantes da interação entre dois fatores em estudo: cimento endodôntico (**SX**- Sealapex, cimento à base de óxido de cálcio; Kerr Corporation, Orange, EUA; **S26**- Sealer 26; cimento à base de hidróxido de cálcio; Dentsply Maillefer, Petrópolis, RJ, Brasil; **AH**- AH Plus; cimento à base de resina epóxica; Dentsply DeTrey GmbH, Konstanz, Alemanha) e envelhecimento artificial acelerado dos espécimes (**Imediato**, teste sem envelhecimento; **Mediato**, teste após 2 meses de envelhecimento em água a 37°C) (Figura 9).

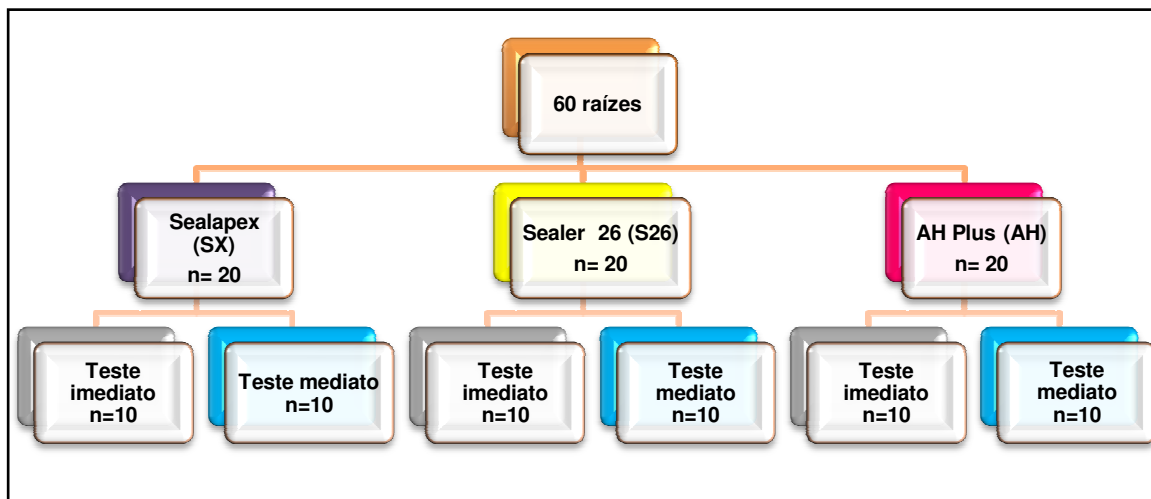


Figura 9. Organograma dos grupos experimentais da parte 2.

Nesta parte do presente estudo, foram empregados dois grupos controles (sem obturação do canal radicular), sendo um controle imediato e um controle mediato. Os valores de resistência de união e padrões de falha dos grupos controle imediato e mediato foram obtidos dos grupos PNiTiNaOCl imediato e PNiTiNaOCl mediato da parte 1, respectivamente.

Preparo do canal radicular

Nos grupos experimentais, os canais radiculares foram preparados 1 mm aquém do ápice por meio da técnica coroa-ápice com instrumentos rotatórios de Níquel-Titânio do sistema K3 (SybronEndo, Optimum, São Paulo, SP, Brasil) associados aos agentes químicos NaOCl 1% e EDTA 17%. Assim, os canais radiculares foram preparados com brocas Gates Glidden n. 1,2 (Dentsply Maillefer, Ballaigues, Suíça) (CT 10 mm) e instrumentos rotatórios NiTi na seguinte sequência: lima 25 taper 10 (CT 10 mm); limas 15-25 taper 2 (CT 14 mm); lima 25 taper 4 (CT 14mm); lima 25 taper 6 (CT 14 mm); limas 30-45 taper 2 (CT 14 mm).

Durante a instrumentação, a cada troca de lima, os canais foram irrigados com 2 mL de NaOCl 1% (Fitofarma, Goiânia, GO, Brasil). Em todos os grupos, 3 mL de EDTA 17% (Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brasil) foram utilizados por 5 min para a remoção de smear layer. A irrigação final foi realizada com 5 mL de NaOCl 1% (Fitofarma).

Os canais radiculares foram secos com cones de papel absorventes e obturados com cones de guta-percha (Dentsply Maillefer) e o cimento endodôntico específico de cada grupo, utilizando a técnica de condensação lateral. Os cimentos endodônticos foram preparados e utilizados de acordo com as instruções dos fabricantes. Após a obturação, as aberturas dos canais radiculares foram seladas com cimento de ionômero de vidro (Vidrion R; SSWhite, Rio de Janeiro, RJ, Brasil) e as amostras foram armazenadas em água destilada a 37°C por 24 horas (Figura 10).



Figura 10. Cimentos endodônticos empregados no presente estudo: SX-Sealapex (Kerr), S26-Sealer 26 (Dentsply), AH- AH Plus (Dentsply) (A), Obturação do canal radicular (B) e Selamento da abertura do canal radicular com ionômero de vidro (C).

Preparo para o pino intrarradicular

Após 24 horas, o material obturador foi removido com brocas Gates Glidden na profundidade de 10 mm mantendo-se 5 mm de selamento apical.

O preparo para o pino foi realizado usando brocas Largo n. 3-5 (Dentsply Maillefer) (CT 10 mm) correspondente ao pino de fibra de vidro paralelo e serrilhado de 1,5 mm de diâmetro (Reforpost n. 3; Angelus, Londrina, PR, Brasil). Os canais radiculares foram irrigados, a cada troca de brocas e após o preparo, com 2 mL de NaOCl 1% e foram secos com cones de papel absorventes. Todas as raízes foram cobertas externamente com cera utilidade para evitar polimerização lateral pelo fotoativador (Soares *et al.*, 2012).

Cimentação do pino intrarradicular

Os pinos de fibra foram limpos com álcool 70% e, em seguida, em uma única aplicação usando um microbrush, e após a secagem, o agente silano foi aplicado por um minuto (Silano; Angelus). O cimento resinoso auto-adesivo (RelyX U100; 3M-ESPE, St. Paul, MN, EUA) foi manipulado de acordo com as instruções do fabricante, introduzido no canal radicular com limas K-File e aplicado na superfície do pino. O pino foi inserido no canal com pressão digital.

O excesso de cimento foi removido após 1 min. Após 5 min, o cimento resinoso foi fotopolimerizado com 1200 mW cm^{-2} (Radii-Cal; SDI, Bayswater, Austrália) por 40 s na face cervical da raiz, em direção ao longo eixo da raiz, e obliquamente nas superfícies vestibular e lingual, totalizando 120 s. A interface pino-cimento-dentina na face cervical foi selada com resina composta e as raízes foram armazenadas em água destilada a 37°C por 24 horas.

Preparo dos espécimes para o ensaio de *micropush-out*

As raízes foram coladas em placa acrílica com auxílio de adesivo a base de cianoacrilato (Loctite Super Bonder, Henkel Loctite Corporation, EUA) e godiva, e então seccionadas perpendicularmente ao seu longo eixo com disco diamantado dupla face (4" Diameter x 0,012" Thickness x 1/2" Arbor, Extec, Enfield, CT, EUA) em baixa velocidade e sobre refrigeração com água (Isomet 1000, Buehler, Lake Bluff, IL, EUA) para obtenção de duas fatias, com aproximadamente 1 mm de espessura, de cada terço radicular (cervical, médio e apical), totalizando seis fatias por raiz.

Envelhecimento artificial acelerado dos espécimes

Nos grupos mediatos, as fatias foram individualmente armazenadas em *ependorfs* com água destilada e mantidas em estufa a 37°C por dois meses antes do teste de *micropush-out*.

Ensaio de *micropush-out*

Para a realização do ensaio de *micropush-out* foi utilizado dispositivo desenvolvido especificamente para este teste (Menezes *et al.*, 2008), constituído por base metálica em aço inoxidável com 3 cm de diâmetro, contendo orifício de 2 mm na região central e ponta aplicadora de carga com 1 mm de diâmetro e 3 mm de comprimento. Após o posicionamento do conjunto na base da máquina de ensaio mecânico (EMIC DL 2000, São José dos Pinhais, PR, Brasil) contendo célula de carga de 20 Kgf, as fatias foram

posicionadas de forma que a ponta aplicadora de carga coincidisse com o orifício da base metálica. Em seguida, foi aplicado carregamento de compressão no sentido ápice/coroa sob velocidade de 0,5 mm/min, até ocorrer a extrusão do pino. A resistência de união foi calculada em MPa dividindo-se a carga que levou a falha do sistema (N) pela área da interface de união. A área da interface de união foi calculada pela fórmula: $A = 2\pi rh$, sendo **A** a área da interface de união; π a constante 3,14; **r** o raio do segmento do pino (mm) e **h** a espessura do segmento do pino (mm) (Goracci *et al.*, 2004; Soares *et al.*, 2008b; Soares *et al.*, 2012).

Análise estatística

A análise estatística foi realizada utilizando o programa estatístico SAS (Institute Inc., Cary, NC, EUA, Release 9,2). Os dados foram submetidos ao teste de normalidade Shapiro-Wilk. Os efeitos, na resistência de união, dos cimentos endodônticos e do envelhecimento artificial foram analisados usando ANOVA One-way em esquema de parcela subdividida, com a parcela representada pelo cimento endodôntico ou pelo envelhecimento, e a sub-parcela representada pelos terços do canal radicular (cervical, médio e apical). Comparações múltiplas foram feitas pelo teste de Tukey ($\alpha=0,05$). Comparações com os grupos controles imediato e mediato foram feitas pelo teste de Dunnet ($\alpha=0,05$).

Definição dos padrões de falha por Microscopia Confocal a laser de varredura

Para determinar os padrões de falha, todos os espécimes fraturados (n=360) foram secos com jato de ar e analisados em microscópio de varredura confocal a laser (Carl Zeiss Laser Scanning Systems, LSM510, META, Oberkochen, Alemanha). As imagens foram analisadas usando o Zeiss LSM Image Browser (META, Alemanha). Os padrões de falha foram classificados em seis tipos: (I) falha adesiva entre o pino e o cimento resinoso; (II) falha adesiva

entre o cimento resinoso e a dentina intrarradicular; (III) falha coesiva no cimento; (IV) falha coesiva na dentina; (V) falha coesiva no pino (Castellan *et al.*, 2010) e (VI) falha mista entre pino, cimento resinoso e dentina intrarradicular (Bitter *et al.*, 2009).

4. Resultados

Parte 1

A análise de variância two-way (técnica de instrumentação X irrigante endodôntico) com sub-parcela (terço do canal radicular) dos grupos testados imediatamente mostrou diferença significativa para o irrigante endodôntico ($p=0,0023$) e terço do canal radicular ($p=<0,0001$). Os valores médios de resistência de união e os desvios padrões estão na Tabela 1. O teste de Tukey indicou que a irrigação com NaOCl resultou em resistência de união maior comparada a O_3 , exceto no terço apical de PNiTi no qual foi similar. A irrigação com CHX resultou em valores de resistência de união intermediários que foram estatisticamente semelhantes aos valores obtidos nos grupos irrigados com O_3 nos terços cervical/apical de PAI e cervical/médio de PNiTi, e em todos os grupos irrigados com NaOCl. Independente da técnica de preparo do canal radicular e do irrigante endodôntico, o terço cervical apresentou maiores valores de resistência de união que o terço apical.

Tabela 1. Médias de resistência de união em MPa (desvio padrão) dos grupos testados imediatamente (sem envelhecimento) e categorias estatísticas definidas pelo teste de Tukey (n = 10).

Irrigante endodôntico	Técnica de instrumentação						Total
	Instrumentos de aço inoxidável (PAI)			Instrumentos de Níquel-Titânio (PNiTi)			
	Terço radicular			Terço radicular			
	Cervical	Médio	Apical	Cervical	Médio	Apical	
NaOCl	12,90(2,41) ^{Aa}	10,30(4,03) ^{Ba}	7,10(3,78) ^{Ca}	13,52(2,12) ^{Aa}	11,39(3,26) ^{ABa}	9,33(4,80) ^{BCa}	10,75(4,03) ^a
CHX	11,39(3,27) ^{Aab}	9,81(3,89) ^{ABa}	6,74(3,88) ^{Cab}	11,46(3,84) ^{Aab}	9,99(4,91) ^{ABab}	7,08(5,55) ^{BCa}	9,41(4,52) ^{ab}
O ₃	8,65(6,83) ^{Ab}	6,15(5,04) ^{Bb}	3,53(2,92) ^{Cb}	9,49(3,39) ^{Ab}	7,40(1,55) ^{ABb}	6,77(1,42) ^{BCa}	7,00(4,30) ^b
Total	8,51(4,86) ^B			9,60(4,15) ^A			

Letras maiúsculas foram utilizadas para comparar os grupos nas linhas horizontais e letras minúsculas para comparar os grupos nas linhas verticais. Categorias estatísticas com letras iguais, definidas pelo teste Tukey, não são estatisticamente significantes entre si ($p < 0,05$).

PAI, preparo do canal radicular com instrumentos de aço inoxidável (K-File); PNiTi, preparo do canal radicular com instrumentos de Níquel-Titânio K3); NaOCl, hipoclorito de sódio 1%; CHX, clorexidina 2%; O₃, água ozonificada 1,2%.

A análise de variância two-way (técnica de instrumentação X irrigante endodôntico) com sub-parcela (terço do canal radicular) dos grupos mediatos, testados após envelhecimento acelerado dos espécimes, mostrou diferença significativa para a técnica de instrumentação ($p=0,0356$), irrigante endodôntico ($p<0,0001$), terço do canal radicular ($p<0,0001$) e para a interação entre irrigante e terço do canal radicular ($p=0,0047$). Os valores médios de resistência de união e os desvios padrões estão na Tabela 2. No terço apical de espécimes irrigados com NaOCl ou CHX, PNiTi resultou em maior resistência de união que PAI. Em relação ao irrigante endodôntico, o NaOCl resultou em maiores valores de resistência de união que O₃ em todos os grupos. CHX apresentou valor estatisticamente menor que NaOCl apenas no terço cervical de PAI e similaridade a NaOCl nos outros grupos. O terço cervical apresentou valores maiores que o apical, exceto nos grupos PNiTiNaOCl e PNiTiCHX nos quais foi similar.

Tabela 2. Médias de resistência de união em MPa (desvio padrão) dos grupos mediatos, testados após envelhecimento artificial em água, e categorias estatísticas definidas pelo teste de Tukey (n = 10).

Irrigante Endodôntico	Técnica de instrumentação						Total
	Instrumentos de aço inoxidável (PAI)			Instrumentos de Níquel-Titânio (PNiTi)			
	Terço radicular			Terço radicular			
	Cervical	Médio	Apical	Cervical	Médio	Apical	
NaOCl	24,08(5,77) ^{Aa}	20,31(7,70) ^{Ba}	15,46(8,63) ^{Ca}	25,10(6,44) ^{Aa}	22,55(6,08) ^{ABa}	21,64(6,33) ^{ABa}	21,52(7,31) ^a
CHX	18,85(4,26) ^{ABb}	16,16(3,99) ^{Ba}	13,28(7,20) ^{Ca}	21,41(3,44) ^{Aab}	20,83(6,27) ^{ABa}	18,35(7,67) ^{ABa}	18,15(6,14) ^a
O ₃	17,36(4,84) ^{Ab}	9,94(3,98) ^{Bb}	6,42(3,35) ^{Cb}	17,44(4,85) ^{Ab}	10,99(5,34) ^{Bb}	7,06(3,78) ^{BCb}	11,54(6,15) ^b
Total	15,76(7,52) ^B			18,37(7,77) ^A			

Letras maiúsculas foram utilizadas para comparar os grupos nas linhas horizontais e letras minúsculas para comparar os grupos nas linhas verticais. Categorias estatísticas com letras iguais, definidas pelo teste Tukey, não são estatisticamente significantes entre si ($p < 0,05$).

PAI, preparo do canal radicular com instrumentos de aço inoxidável (K-File); PNiTi, preparo do canal radicular com instrumentos de Níquel-Titânio K3); NaOCl, hipoclorito de sódio 1%; CHX, clorexidina 2%; O₃, água ozonificada 1,2%.

A análise de variância one-way (envelhecimento artificial) com sub-parcela (terço do canal radicular) no grupo PAlNaOCl mostrou significância para o envelhecimento artificial ($p < 0,001$) e terço do canal radicular ($p < 0,001$). No grupo PAICHX houve significância para o envelhecimento artificial ($p < 0,001$) e terço radicular ($p < 0,001$). No grupo PAIO₃ houve significância para o envelhecimento artificial ($p < 0,001$), terço do canal radicular ($p < 0,001$) e para a interação entre estes fatores ($p = 0,032$). No grupo PNiTlNaOCl houve significância para o envelhecimento artificial ($p < 0,001$) e terço do canal radicular ($p = 0,005$). No grupo PNiTlCHX houve significância para o envelhecimento artificial ($p < 0,001$) e terço do canal radicular ($p = 0,008$). No grupo PNiTlO₃ houve significância para o envelhecimento artificial ($p = 0,008$), terço do canal radicular ($p < 0,001$) e para a interação entre estes fatores ($p < 0,001$). Os valores médios de resistência de união e os desvios padrões dos grupos estão na Tabela 3. O teste de Tukey demonstrou um aumento significativo na resistência de união após o envelhecimento artificial acelerado, exceto para os terços médio e apical de PAIO₃ e apical de PNiTlO₃ nos quais foram similares.

Tabela 3. Médias de resistência de união em MPa (desvio padrão), como resultado do envelhecimento artificial e terço radicular, e categorias estatísticas definidas pelo teste de Tukey para cada grupo (n = 10).

Grupos	Teste	Terço radicular		
		Cervical	Médio	Apical
PAINaOCl	Imediato	12,90±2,41 ^{Ab}	10,30±4,03 ^{ABb}	7,10±3,78 ^{Bb}
	Mediato	24,08±5,77 ^{Aa}	20,31±7,70 ^{Ba}	15,46±8,63 ^{Ca}
PAICHX	Imediato	11,39±3,27 ^{Ab}	9,81±3,89 ^{ABb}	6,74±3,88 ^{Bb}
	Mediato	18,85±4,26 ^{Aa}	16,16±3,99 ^{ABa}	13,28±7,20 ^{Ba}
PAIO ₃	Imediato	8,65±6,83 ^{Ab}	6,15±5,04 ^{ABa}	3,53±2,92 ^{Ba}
	Mediato	17,36±4,84 ^{Aa}	9,94±3,98 ^{Ba}	6,42±3,35 ^{Ba}
PNiTiNaOCl	Imediato	13,52±2,12 ^{Ab}	11,39±3,26 ^{ABb}	9,33±4,80 ^{Bb}
	Mediato	25,10±6,44 ^{Aa}	22,55±6,08 ^{Aa}	21,64±6,33 ^{Aa}
PNiTiCHX	Imediato	11,46±3,84 ^{Ab}	9,99±4,91 ^{ABb}	7,08±5,55 ^{Bb}
	Mediato	21,41±3,44 ^{Aa}	20,83±6,27 ^{Aa}	18,35±7,67 ^{Aa}
PNiTiO ₃	Imediato	9,49±3,39 ^{Ab}	7,40±1,55 ^{ABb}	6,77±1,42 ^{Ba}
	Mediato	17,44±4,85 ^{Aa}	10,99±5,34 ^{Ba}	7,06±3,78 ^{Ca}

Em cada grupo, letras maiúsculas foram utilizadas para comparar os grupos nas linhas horizontais e letras minúsculas para comparar os grupos nas linhas verticais. Categorias estatísticas com letras iguais, definidas pelo teste Tukey, não são estatisticamente significantes entre si ($p < 0,05$).

PAI, preparo do canal radicular com instrumentos de aço inoxidável (K-File); PNiTi, preparo do canal radicular com instrumentos de Níquel-Titânio K3); NaOCl, hipoclorito de sódio 1%; CHX, clorexidina 2%; O₃, água ozonificada 1,2%; Imediato, teste sem envelhecimento; Mediato, teste após 2 meses de envelhecimento em água a 37°C.

A distribuição e representação dos padrões de falha estão apresentadas na Tabela 4 e Figura 11, respectivamente. A prevalência de (II) falha adesiva entre o cimento resinoso e a dentina intrarradicular foi verificada em todos os grupos.

Tabela 4. Padrões de falha (%) para os grupos experimentais da parte 1.

Grupos	Adesiva: Pino e cimento (I)	Adesiva: Cimento e dentina (II)	Coesiva: Cimento (III)	Coesiva: Dentina (IV)	Coesiva: Pino (V)	Mista (VI)
PAINaOCl mediato	-	32 (53,3)	-	4 (6,7)	1 (1,7)	23 (38,3)
PAICHX mediato	1 (1,7)	33 (55,0)	-	3 (5,0)	1 (1,7)	22 (36,7)
PAIO ₃ mediato	3 (5,0)	33 (55,0)	-	-	1 (1,7)	23 (38,3)
PNiTiNaOCl mediato	2 (3,3)	33 (55,0)	-	6 (10,0)	2 (3,3)	17 (28,3)
PNiTiCHX mediato	2 (3,3)	34 (56,7)	-	2 (3,3)	2 (3,3)	20 (33,3)
PNiTiO ₃ mediato	2 (3,3)	47 (78,3)	-	1 (1,7)	-	10 (16,7)
PAINaOCl mediato	2 (3,3)	27 (45,0)	-	-	11 (18,3)	20 (33,3)
PAICHX mediato	3 (5,0)	31 (51,7)	-	1 (1,7)	7 (11,7)	18 (30,0)
PAIO ₃ mediato	3 (5,0)	40 (66,7)	-	-	2 (3,3)	15 (25,0)
PNiTiNaOCl mediato	1 (1,7)	20 (33,3)	-	9 (15,0)	15 (25,0)	15 (25,0)
PNiTiCHX mediato	-	42 (70,0)	-	-	10 (16,7)	8 (13,3)
PNiTiO ₃ mediato	1 (1,7)	35 (58,3)	1 (1,7)	-	3 (5,0)	20 (33,3)

PAI, preparo do canal radicular com instrumentos de aço inoxidável (K-File); PNiTi, preparo do canal radicular com instrumentos de Níquel-Titânio K3); NaOCl, hipoclorito de sódio 1%; CHX, clorexidina 2%; O₃, água ozonificada 1,2%; Imediato, teste sem envelhecimento; Mediato, teste após 2 meses de envelhecimento em água a 37°C.

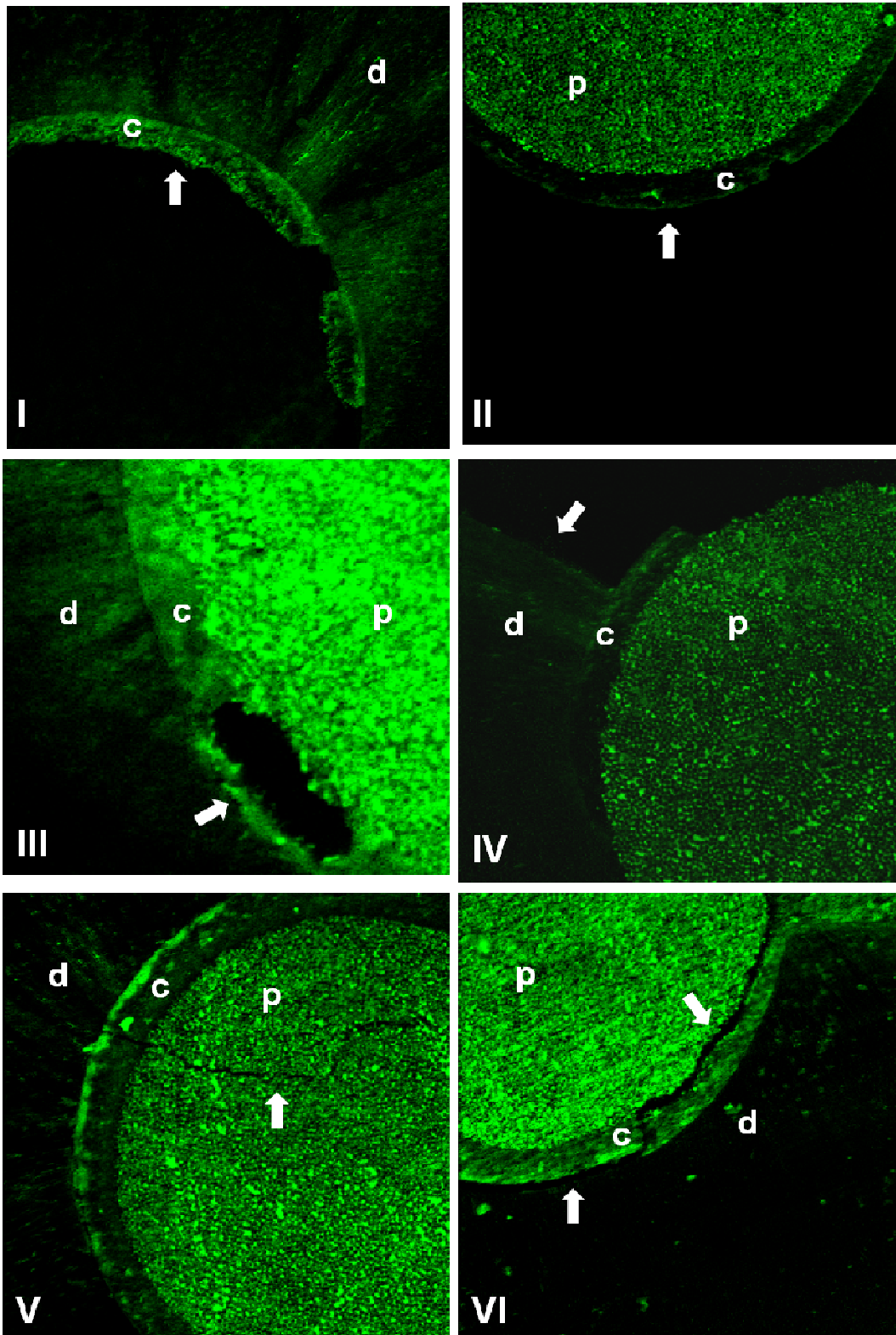


Figura 11. *d – dentina; c – cimento resinoso; p – pino de fibra de vidro.* Parte 1. Microscopias dos padrões de falha após o teste de micropush-out (aumento 10X): (I) falha adesiva entre o pino e o cimento resinoso; (II) falha adesiva entre o cimento resinoso e a dentina; (III) falha coesiva no cimento; (IV) falha coesiva na dentina; (V) falha coesiva no pino; (VI) falha mista entre pino, cimento resinoso e dentina.

Parte 2

A análise de variância one-way (cimento endodôntico) com sub-parcela (terço do canal radicular) dos grupos testados imediatamente e dos grupos mediatos (envelhecimento acelerado antes do teste) mostrou diferença significativa apenas para o terço do canal radicular ($p < 0,001$). Os valores médios de resistência de união e os desvios padrões dos grupos imediatos e mediatos estão nas Tabelas 5 e 6, respectivamente. O teste de Tukey indicou uma redução significativa nos valores de resistência de união do terço cervical para o apical, independente do cimento endodôntico.

Comparações com os grupos controles imediato e mediato estão apresentadas nas Tabelas 5 e 6, respectivamente. O teste de Dunnet mostrou que o uso do cimento endodôntico resultou em valores de resistência de união menores comparado aos grupos controles (sem obturação), com diferença estatisticamente significativa nos terços médio ($p = 0,0294$) e apical ($p = 0,0207$) dos grupos testados imediatamente (Tabela 5), e nos terços cervical ($p < 0,0001$), médio ($p < 0,0001$) e apical ($p < 0,0001$) dos grupos testados após o envelhecimento (Tabela 6).

Tabela 5. Médias de resistência de união em MPa (desvio padrão) dos grupos testados imediatamente (sem envelhecimento) e categorias estatísticas definidas pelo teste de Tukey e teste de Dunnet ($n = 10$).

Cimento endodôntico	Terço radicular		
	Cervical	Médio	Apical
Controle imediato	13,52±2,12	11,39±3,26	9,33±4,80
Sealapex	10,29±1,96 ^{Aa}	7,41±1,95 ^{*Ba}	5,36±1,34 ^{*Ba}
Sealer 26	13,83±6,64 ^{Aa}	8,16±4,16 ^{*Ba}	3,63±4,72 ^{*Ca}
AH Plus	12,91±2,54 ^{Aa}	8,08± 2,59 ^{*Ba}	4,77±2,96 ^{*Ca}

Letras maiúsculas foram utilizadas para comparar os grupos nas linhas horizontais e letras minúsculas para comparar os grupos nas linhas verticais. Categorias estatísticas com letras iguais, definidas pelo teste Tukey, não são estatisticamente significantes entre si ($p < 0,05$).

O (*) indica diferença significativa com o grupo controle pelo teste de Dunnet ($p < 0,05$).

Tabela 6. Médias de resistência de união em MPa (desvio padrão) dos grupos mediatos, testados após envelhecimento artificial em água, e categorias estatísticas definidas pelo teste de Tukey e teste de Dunnet (n = 10).

Cimento endodôntico	Terço radicular		
	Cervical	Médio	Apical
Controle mediato	25,10±6,44	22,55±6,08	21,64±6,33
Sealapex	10,94±5,12 ^{*Aa}	7,50±1,54 ^{*ABa}	5,47±3,96 ^{*Ba}
Sealer 26	14,02±1,94 ^{*Aa}	8,40±4,97 ^{*Ba}	3,47±3,91 ^{*Ca}
AH Plus	13,34±3,43 ^{*Aa}	9,12± 3,83 ^{*Ba}	4,87±4,02 ^{*Ca}

Letras maiúsculas foram utilizadas para comparar os grupos nas linhas horizontais e letras minúsculas para comparar os grupos nas linhas verticais. Categorias estatísticas com letras iguais, definidas pelo teste Tukey, não são estatisticamente significantes entre si ($p < 0,05$).

O (*) indica diferença significativa com o grupo controle pelo teste de Dunnet ($p < 0,05$).

A análise de variância one-way (envelhecimento artificial) com sub-parcela (terço do canal radicular) para cada cimento endodôntico (SX, S26, AH) mostrou diferença significativa apenas para o terço do canal radicular ($p < 0,001$). Os valores médios de resistência de união e os desvios padrões para cada cimento endodôntico estão na Tabela 7. O teste de Tukey revelou uma redução significativa nos valores de resistência de união do terço cervical para o apical, independente do envelhecimento artificial.

Tabela 7. Médias de resistência de união em MPa (desvio padrão), como resultado do envelhecimento artificial e terço radicular, e categorias estatísticas definidas pelo teste de Tukey para cada cimento endodôntico (n = 10).

Cimento endodôntico	Teste	Terço radicular		
		Cervical	Médio	Apical
Sealapex	Imediato	10,29±1,96 ^{Aa}	7,41±1,95 ^{ABa}	5,36±1,34 ^{Ba}
	Mediato	10,94±5,12 ^{Aa}	7,50±1,54 ^{ABa}	5,47±3,96 ^{Ba}
Sealer 26	Imediato	13,83±6,64 ^{Aa}	8,16±4,16 ^{Ba}	3,63±4,72 ^{Ca}
	Mediato	14,02±1,94 ^{Aa}	8,40±4,97 ^{Ba}	3,47±3,91 ^{Ca}
AH Plus	Imediato	12,91±2,54 ^{Aa}	8,08± 2,59 ^{Ba}	4,77±2,96 ^{Ba}
	Mediato	13,34±3,43 ^{Aa}	9,12± 3,83 ^{Ba}	4,87±4,02 ^{Ca}

Em cada cimento endodôntico, letras maiúsculas foram utilizadas para comparar os grupos nas linhas horizontais e letras minúsculas para comparar os grupos nas linhas verticais. Categorias estatísticas com letras iguais, definidas pelo teste Tukey, não são estatisticamente significantes entre si ($p < 0,05$).

A distribuição e representação dos padrões de falha estão apresentadas na Tabela 8 e Figura 12, respectivamente. A prevalência de (II) falha adesiva entre o cimento resinoso e a dentina intrarradicular foi verificada em todos os grupos.

Tabela 8. Padrões de falha (%) para os grupos experimentais da parte 2.

Grupos (%)	Adesiva: Pino e cimento (I)	Adesiva: Cimento e dentina (II)	Coesiva: Cimento (III)	Coesiva: Dentina (IV)	Coesiva: Pino (V)	Mista (VI)
SX imediato	2 (3,3)	35 (58,3)	3 (5,0)	1 (1,7)	1 (1,7)	18 (30,0)
S26 imediato	3 (5,0)	31 (51,7)	6 (10,0)	-	3 (5,0)	17 (28,3)
AH imediato	3 (5,0)	26 (43,3)	9 (15,0)	1 (1,7)	1 (1,7)	20 (33,3)
SX mediato	2 (3,3)	42 (70,0)	7 (11,7)	-	1 (1,7)	8 (13,3)
S26 mediato	-	33 (55,0)	2 (3,3)	-	4 (6,7)	21 (35,0)
AH mediato	1 (1,7)	44 (73,3)	3 (5,0)	1 (1,7)	-	11 (18,3)

SX, Sealapex; S26, Sealer 26; AH, AH Plus; Imediato, teste sem envelhecimento; Mediato, teste após 2 meses de envelhecimento em água a 37°C).

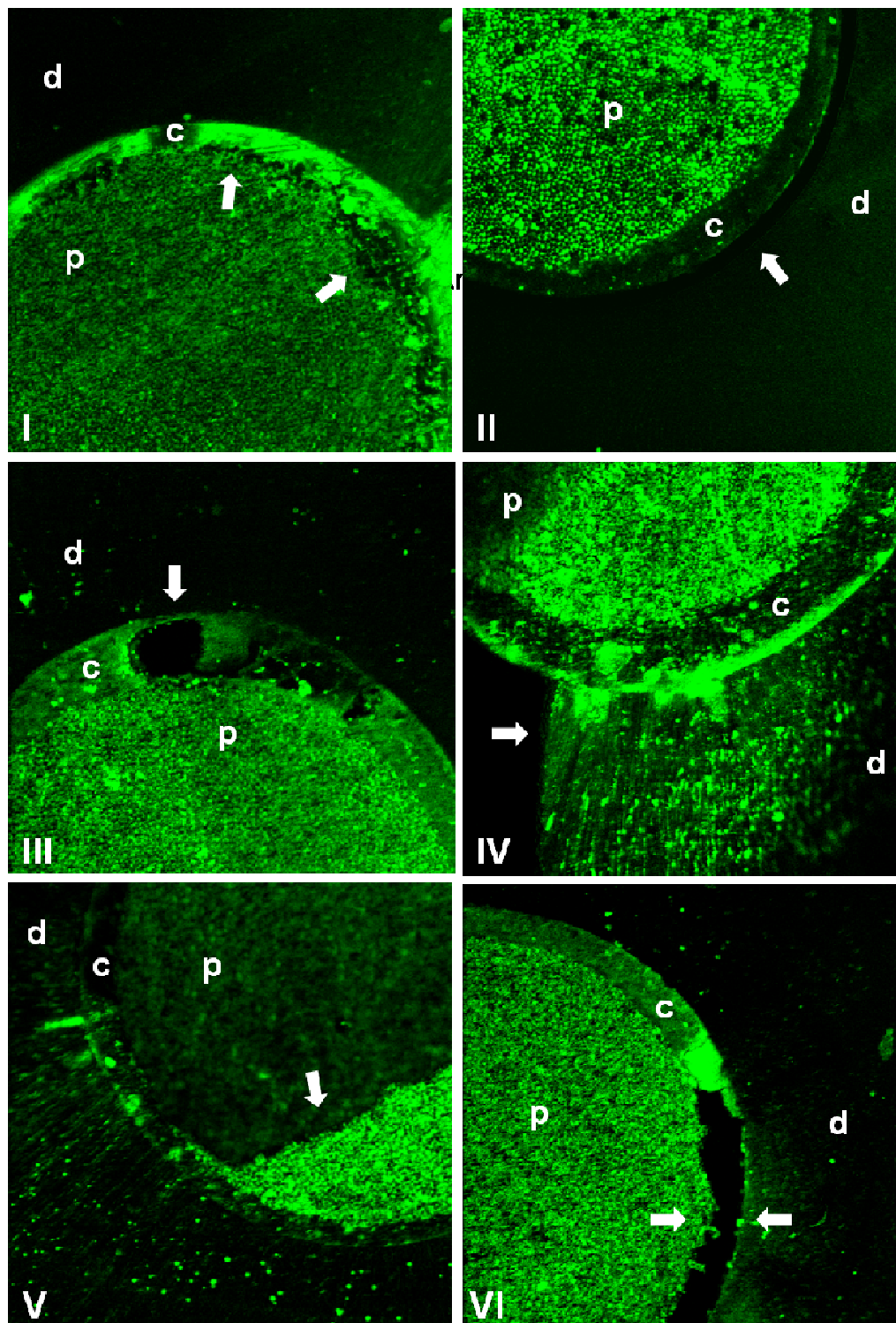


Figura 12. *d – dentina; c – cimento resinoso; p – pino de fibra de vidro.* Parte 2. Microscopias dos padrões de falha após o teste de micropush-out (aumento 10X): (I) falha adesiva entre o pino e o cimento resinoso; (II) falha adesiva entre o cimento resinoso e a dentina; (III) falha coesiva no cimento; (IV) falha coesiva na dentina; (V) falha coesiva no pino; (VI) falha mista entre pino, cimento resinoso e dentina.

5. Discussão

Parte 1

As técnicas de instrumentação do canal radicular, a composição química dos irrigantes endodônticos e o envelhecimento artificial das amostras influenciaram na resistência de união de pinos de fibra de vidro. Além disto, a união à dentina radicular foi afetada pelos diferentes terços do canal radicular. As hipóteses testadas foram aceitas.

O preparo do canal radicular apresenta como finalidade o esvaziamento, alargamento e sanificação endodôntica (Estrela *et al.*, 2007). Os instrumentos de aço-inoxidável têm sido geralmente utilizados para tal finalidade. Os instrumentos de Níquel-Titânio (NiTi) acionados a motor têm sido sugeridos com vistas a favorecer o preparo de canais curvos, devido à flexibilidade e superelasticidade (Thompson, 2000). A qualidade da modelagem, a eficiência, o menor tempo de trabalho e a diminuição do stress profissional constituem outras qualidades inerentes ao preparo com instrumentos de NiTi (Thompson, 2000; Peters *et al.*, 2001a; Peters & Paqué, 2010). O preparo com os instrumentos em aço inoxidável tem permitido que paredes não sejam tocadas pelos instrumentos (Peters *et al.*, 2001b). Considerando que estes instrumentos apresentam ação de raspar, e não de excisar dentina, como ocorre com os instrumentos de NiTi, sugere-se maior possibilidade de compactação de smear layer dentro dos túbulos dentinários, caracterizando maior presença de smear plug. Este pode ter atuado como barreira à penetração e interação do cimento com a dentina intrarradicular, o que pode estar relacionado à diminuição da resistência de união verificada em grupos preparados com instrumentos de aço inoxidável.

O NaOCl é um composto halogenado utilizado rotineiramente na endodontia (Zehnder, 2006), que possui efeito antimicrobiano (Byström &

Sundqvist, 1981; Estrela *et al.*, 2002), capacidade de dissolução tecidual (Byström & Sundqvist, 1981; Spanó *et al.*, 2009; Estrela *et al.*, 2002) e compatibilidade biológica aceitável em soluções menos concentradas (Estrela *et al.*, 2002, 2003, 2004). Entretanto, ele não atua na porção inorgânica da dentina, que constitui grande parte da *smear layer* (Garberoglio & Becce, 1994; Cecchin *et al.*, 2010). O EDTA produz desmineralização da dentina e proporciona uma excelente limpeza das paredes do canal radicular (Spanó *et al.*, 2009), melhorando a penetração de substâncias químicas e promovendo um contato mais íntimo do material obturador com a dentina radicular (Cecchin *et al.*, 2010). Ele atua sobre os componentes inorgânicos da *smear layer*, levando à descalcificação da dentina peri e intertubular (Cecchin *et al.*, 2010).

A associação dessas substâncias é largamente empregada na terapia endodôntica porque elas atuam nas porções orgânica e inorgânica da dentina (Estrela *et al.*, 2007; Zhang *et al.*, 2010; Cecchin *et al.*, 2010), com dissolução quase completa da *smear layer*, desobstruindo os orifícios dos túbulos dentinários (Wu *et al.*, 2009). Em contrapartida, a clorexidina e a água ozonificada não possuem capacidade de dissolver tecidos orgânicos, e assim, debris podem permanecer aderidos às paredes do canal radicular obstruindo os túbulos dentinários (Menezes *et al.*, 2003; Bitter *et al.*, 2008; Bodrumlu *et al.*, 2010), o que pode influenciar negativamente na união à dentina intrarradicular, como visto no presente estudo.

Bitter *et al.* (2009) relataram que a hibridização da dentina foi detectada apenas esporadicamente com o emprego do cimento resinoso auto-adesivo RelyX Unicem (3M-ESPE). O cimento auto-adesivo usado no presente estudo, RelyX U100 (UC), e o RelyX Unicem (UN) foram desenvolvidos pelo mesmo fabricante e são comercializados sob o mesmo nome em alguns países (Viotti *et al.*, 2009). Segundo o fabricante, a única diferença entre estes produtos é o sistema de dispensar o material. Enquanto o UN requer um ativador, triturador e aplicador, o UC pode ser misturado manualmente (Viotti *et al.*, 2009). As propriedades adesivas deste cimento são baseadas em monômeros ácidos que desmineralizam e infiltram o substrato dentário, e criam retenção

micromecânica e união química à hidroxiapatita (De Munck *et al.*, 2004; Gerth *et al.*, 2006; Zicari *et al.*, 2008; Bitter *et al.*, 2009; Cecchin *et al.*, 2011).

Bitter *et al.* (2009) descreveram que a penetração deste cimento nos túbulos dentinários foi encontrada apenas em alguns espécimes e concluíram que a *smear layer* não é dissolvida consistentemente na interface dentina-cimento. Estes dados confirmam os resultados de estudos conduzidos previamente que também descreveram uma interação morfológica apenas superficial (De Munck *et al.*, 2004; Al-Assaf *et al.*, 2007; Monticelli *et al.*, 2008). Assim, frente ao baixo efeito desmineralizante do cimento RelyX U100 empregado no presente estudo, a capacidade do NaOCl em remover os componentes orgânicos da dentina, principalmente colágeno, resultando em uma superfície rugosa (Hayashi *et al.*, 2005), poderia aumentar a penetração do cimento (Ari *et al.*, 2003) na estrutura de dentina parcialmente desmineralizada (Monticelli *et al.*, 2008) favorecendo a retenção micromecânica e, conseqüentemente, o aumento da resistência de união.

Adicionalmente, Gerth *et al.* (2006) documentaram uma intensa interação química do cimento RelyX Unicem com a hidroxiapatita. Em concordância, Bitter *et al.* (2009) relataram que interações químicas entre o cimento resinoso auto-adesivo e a hidroxiapatita podem ser efetivas no interior do canal radicular, e indicaram que esta interação pode ser mais crucial para a união à dentina radicular do que a capacidade do mesmo material em hibridizar a dentina. Então, como o NaOCl atua na dissolução da porção orgânica expondo a inorgânica, isto pode favorecer essas interações químicas entre o cimento e a hidroxiapatita, o que pode justificar os valores mais elevados de resistência de união encontrados nos grupos em que esse irrigante foi usado.

O aumento ou a ausência de efeito adverso do NaOCl na resistência de união de cimentos resinosos à dentina intrarradicular foram relatados anteriormente (Varela *et al.*, 2003; Muniz & Mathias, 2005; Hayashi *et al.*, 2005; Pelegrine *et al.*, 2010), enquanto outros estudos descreveram um efeito adverso deste irrigante na resistência de união (Morris *et al.*, 2001; Ari *et al.*, 2003; Erdemir *et al.*, 2004; Demiryürek *et al.*, 2009). O aumento foi explicado pela

remoção do colágeno da *smear layer*, o que resultou em uma superfície dentinária rugosa, e a diminuição da resistência de união foi explicada pela mudança do potencial redox do substrato de união devido ao NaOCl residual. No entanto, estes estudos apresentam diferentes metodologias da utilizada no presente estudo, no que diz respeito a concentração e tempo de exposição do NaOCl, dificultando a comparação adequada com os resultados obtidos.

Em oposição aos efeitos positivos do NaOCl no presente estudo, a água ozonificada reduziu significativamente a resistência de união ao canal radicular. O ozônio (O₃) é uma forma altamente reativa de oxigênio que é gerado pela passagem de oxigênio através de alta tensão (Estrela *et al.*, 2007). Ele é um gás azul, contendo três átomos de oxigênio, e é um poderoso agente antimicrobiano, irritante, tóxico e instável (Stübinger *et al.*, 2006; Estrela *et al.*, 2007; Azarpazhooh & Limeback, 2008; Rodrigues *et al.*, 2011). No entanto, devido à sua elevada instabilidade, o ozônio rapidamente se transforma em oxigênio (Stübinger *et al.*, 2006; Estrela *et al.*, 2007; Rodrigues *et al.*, 2011), o qual pode inibir a polimerização do cimento resinoso e, assim, reduzir a resistência de união (Bitter *et al.*, 2008). Portanto, uma possível interação com os componentes iniciadores do cimento resinoso, assim como certa influência das propriedades do material (Bitter *et al.*, 2008), como a incapacidade de dissolução tecidual, poderiam explicar a redução significativa na resistência de união, encontrada no presente estudo, quando a sanificação do canal foi realizada com água ozonificada.

Apesar dos pinos de fibra cimentados em canais radiculares não estarem diretamente expostos aos fluidos bucais, o armazenamento em água é considerado um teste *in vitro* de envelhecimento acelerado para as interfaces de união (Radovic *et al.*, 2007). No presente estudo, o envelhecimento artificial em água por dois meses aumentou significativamente a retenção do pino. Este fato pode estar relacionado à habilidade de união ou polimerização aumentada durante o armazenamento em água, ao relaxamento das tensões pela expansão higroscópica como consequência da sorção de água durante o armazenamento ou expansão higroscópica dos materiais de cimentação (Sadek

et al., 2006; Albashaireh *et al.*, 2010). A expansão higroscópica do cimento resinoso, em particular, pode ter contribuído para uma maior adaptação do cimento ao substrato dentinário. Uma importante contribuição para a resistência de união no teste de *push-out* está prevista para ocorrer como conseqüência do atrito interfacial deslizante (Sadek *et al.*, 2006) decorrente da aplicação da força compressiva. Assim, as resistências interfaciais mais elevadas encontradas após dois meses de envelhecimento podem ter sido causadas pelo aumento no atrito interfacial, em função da maior adaptação do cimento à dentina conseqüente à expansão higroscópica (Sadek *et al.*, 2006; Albashaireh *et al.*, 2010). É importante ressaltar que o método aplicado é um modelo simplificado de envelhecimento acelerado, que tem sido comumente realizado para avaliação da união entre resina-dentina (De Munck *et al.*, 2005; Radovic *et al.*, 2007).

Em relação ao efeito dos diferentes níveis do canal radicular no presente estudo, a resistência de união foi predominantemente superior no terço cervical e inferior no terço apical. Isto pode ser devido a uma melhor polimerização do cimento no terço cervical pela maior proximidade da fonte de luz em relação ao terço apical; a uma melhor interação do cimento com a dentina cervical e ao acesso mais difícil ao terço apical, associado a possíveis limitações no escoamento do cimento (de Durão Mauricio *et al.*, 2007; da Cunha *et al.*, 2010), já que o cimento empregado no presente estudo possui uma elevada viscosidade (De Munck *et al.*, 2004). Além disso, a adesão à dentina radicular também é influenciada pela concentração e direção dos túbulos dentinários nos diferentes níveis das paredes do canal radicular (Ferrari *et al.*, 2000; Ferrari *et al.*, 2001; Wang *et al.*, 2008; da Cunha *et al.*, 2010).

A análise fractográfica fornece informações importantes que ajudam a prever o desempenho do material adesivo. No presente estudo, a microscopia confocal foi empregada e ela parece ser uma alternativa viável para a análise do padrão de falha, uma vez que consome menos tempo e não requer qualquer preparo dos espécimes (Castellan *et al.*, 2010).

Como visto, o cimento auto-adesivo RelyX U100 possui uma reduzida capacidade de desmineralização e hibridização dentinária, apresentando uma interação morfológica apenas superficial (De Munck *et al.*, 2004; Gerth *et al.*, 2006; Bitter *et al.*, 2009; Al-Assaf *et al.*, 2007; Monticelli *et al.*, 2008). Esta observação pode explicar a predominância de (II) falhas adesivas entre cimento-dentina em todos os grupos do presente estudo (Tabela 4). Em geral, resistências de união mais elevadas resultaram em um percentual superior de falhas coesivas no pino. Nestes casos, a resistência de união à dentina, bem como ao pino, foi mais elevada em comparação à estabilidade do próprio pino.

Limitações do presente estudo incluem o fato de que os espécimes não foram submetidos à ciclagem térmica e mecânica, a fim de simular as condições intrabucais mais precisamente. Adicionalmente, dentes bovinos foram usados porque as preocupações bioéticas tornam difícil a coleta e utilização de dentes humanos para fins de pesquisa (Menezes *et al.*, 2008; Soares *et al.*, 2012). A coleta de dentes bovinos é mais fácil e a idade do dente pode ser melhor padronizada (Menezes *et al.*, 2008; Soares *et al.*, 2012). Além disso, diversos estudos têm demonstrado propriedades semelhantes entre os dentes humanos e bovinos (Nakamichi *et al.*, 1983; Dong *et al.*, 2003). Estudos adicionais precisam ser conduzidos para analisar os efeitos de diferentes concentrações de NaOCl; da associação de um agente antioxidante, tal como o ascorbato de sódio, com a água ozonificada; e do aumento do tempo de envelhecimento artificial na resistência de união à dentina intrarradicular.

Considerando os resultados obtidos no presente estudo, sugere-se que na prática clínica a irrigação do canal com NaOCl 1% e uso do EDTA 17% seja o protocolo mais indicado no preparo e sanificação do canal radicular, quando a cimentação de um pino de fibra de vidro com cimento resinoso auto-adesivo estiver indicada.

Parte 2

A retenção de pinos de fibra de vidro cimentados com cimento resinoso auto-adesivo à dentina intrarradicular foi influenciada pelo cimento endodôntico e profundidade do canal radicular, mas não pelo envelhecimento artificial. As hipóteses testadas foram parcialmente aceitas.

Quando cimentos endodônticos são introduzidos no canal radicular e forças são aplicadas durante a obturação, é provável que os constituintes do cimento endodôntico penetrem nos túbulos dentinários (Boone *et al.*, 2001; Shokouhinejad *et al.*, 2011). A penetração do cimento endodôntico aumenta a interface entre o material e as paredes dentinárias, o que pode melhorar a retenção mecânica do material pelo embricamento do cimento endodôntico no interior dos túbulos, e potencialmente reduzir a infiltração (Mamootil & Messer, 2007; Shokouhinejad *et al.*, 2011). Além disso, a atividade antibacteriana do cimento endodôntico pode ser eficaz para matar e sepultar as bactérias que permanecem no interior dos túbulos dentinários (Heling & Chandler, 1996). A capacidade do cimento endodôntico em penetrar nos túbulos dentinários de forma consistente e eficaz é um dos muitos fatores que influenciam a escolha do material para a obturação do canal radicular (Mamootil & Messer, 2007; Shokouhinejad *et al.*, 2011).

O uso do EDTA, que é capaz de agir sobre a matriz mineral do dente e promover a remoção da *smear layer* formada durante o preparo do canal radicular, permite uma melhor penetração do cimento endodôntico nos túbulos dentinários, aumentando a superfície de contato do material de preenchimento com a dentina (Nunes *et al.*, 2008).

Assim, durante o preparo para o pino, a remoção mecânica do cimento endodôntico impregnado na dentina intrarradicular é um passo crítico na obtenção de retenção satisfatória para o pino quando um cimento resinoso é usado (Boone *et al.*, 2001). No presente estudo, houve uma diminuição significativa na retenção do pino quando os cimentos endodônticos foram empregados, em comparação aos grupos controles (sem obturação), o que pode sugerir que o preparo para o pino pode não ter removido todo o cimento

endodôntico impregnado na dentina, não garantindo uma superfície "limpa" na qual o cimento resinoso pudesse aderir (Boone *et al.*, 2001).

Em concordância com este achado, Dimitrouli *et al.* (2011) revelaram que a resistência de união do pino de fibra no grupo sem obturação do canal radicular (WRF) foi maior em comparação aos outros grupos (guta-percha/AH Plus (GP); gutta-percha/Guttaflow (GF); obturação do canal radicular pré-existente (PRF). Demiryürek *et al.* (2010) também mostraram que os cimentos endodônticos (AH Plus, Endofill e Sealapex) têm efeitos significativos sobre a resistência de união de pinos de fibra de vidro. Neste estudo, o grupo controle (apenas gutta-percha, sem cimento endodôntico) apresentou a maior média de resistência de união pelo teste de *micropush-out*. Em contraste, Cecchin *et al.* (2011) documentaram que o AH Plus e Sealer 26 não interferiram na resistência de união de pinos de fibra de vidro comparado ao grupo controle (cones gutta-percha e nenhum cimento endodôntico). Menezes *et al.* (2008) também não encontraram diferença significativa entre o grupo controle (sem obturação) e os grupos obturados com Sealer 26.

A ação de brocas utilizadas na remoção do material obturador do canal radicular, com o intuito de criar espaço para o pino, produz uma nova *smear layer* rica em cimento endodôntico e remanescentes de gutta-percha plastificados pelo calor de atrito da broca (Serafino *et al.*, 2004; Demiryürek *et al.*, 2010). Independente do tipo de cimento endodôntico, esta *smear layer* pode atuar como isolante contra qualquer tipo de material adesivo destinado a promover a união à dentina intrarradicular (Dimitrouli *et al.*, 2011). Além disso, no presente estudo, os canais radiculares foram irrigados após o preparo para o pino apenas com NaOCl 1% que age seletivamente sobre a remoção de partículas orgânicas (Byström & Sundqvist, 1981; Spanó *et al.*, 2009; Estrela *et al.*, 2002) e não pode dissolver partículas inorgânicas (Garberolio & Becce, 1994, Cecchin *et al.*, 2010). Portanto, ele não remove efetivamente a *smear layer* formada sobre as paredes do canal radicular após o preparo para o pino (Nunes *et al.*, 2008), o que pode reduzir a penetração e ação química do cimento resinoso auto-adesivo (RelyX U100, 3M-ESPE).

A influência da profundidade do canal foi observada pela diminuição da resistência de união do terço cervical para apical em todos os grupos. Este fato pode ser atribuído à dificuldade de visualização e acesso à parte apical do sistema de canais radiculares (de Durão Mauricio *et al.*, 2007; Onay *et al.*, 2010), à dificuldade em eliminar a *smear layer* ou os remanescentes de cimento endodôntico/guta-percha sobre as paredes dentinárias neste terço (Serafino *et al.*, 2004), à configuração de túbulos dentinários menos densa na porção apical do sistema de canais radiculares (Ferrari *et al.*, 2000; Ferrari *et al.*, 2001; Wang *et al.*, 2008; da Cunha *et al.*, 2010), ao fator de configuração cavitária (Goracci *et al.*, 2004; Tay *et al.*, 2005), a uma melhor polimerização do cimento no terço cervical pela maior proximidade da fonte de luz em relação ao terço apical e à restrições no escoamento do cimento resinoso à parte apical do canal radicular (de Durão Mauricio *et al.*, 2007; da Cunha *et al.*, 2010). Todos estes fatores comprometem a continuidade interfacial (Silva *et al.*, 2011) e, conseqüentemente, a resistência de união.

Em relação à análise fractográfica, a predominância de (II) falhas adesivas cimento-dentina em todos os grupos do presente estudo (Tabela 8) pode ser atribuída à dificuldade em eliminar a *smear layer* ou os remanescentes de cimento endodôntico/guta-percha sobre as paredes dentinárias (Serafino *et al.*, 2004; Silva *et al.*, 2011; Demiryürek *et al.*, 2010) e à reduzida capacidade de desmineralização e hibridização dentinária do cimento auto-adesivo RelyX U100 (De Munck *et al.*, 2004; Al-Assaf *et al.*, 2007; Monticelli *et al.*, 2008; Bitter *et al.*, 2009).

Limitações do presente estudo incluem o fato de que os espécimes não foram submetidos à ciclagem térmica e mecânica, a fim de simular as condições intrabucais mais precisamente. Estudos adicionais precisam ser conduzidos para analisar os efeitos do uso do EDTA, após o preparo para o pino, na resistência de união à dentina intrarradicular em cada terço do canal radicular (cervical, médio e apical).

Considerando os resultados obtidos no presente estudo, observa-se que na prática clínica a remoção total da *smear layer* e dos remanescentes de

cimento endodôntico/guta-percha das paredes dentinárias do canal radicular, previamente a cimentação de um pino de fibra de vidro, é um fator crítico para a união satisfatória do cimento resinoso auto-adesivo à dentina intrarradicular.

6. Conclusões

Parte 1

O preparo do canal radicular com instrumentos de NiTi associado a irrigação com NaOCl 1 % e uso do EDTA aumentou a resistência de união de pinos de fibra de vidro cimentados com cimento auto-adesivo à dentina intrarradicular.

Parte 2

Os cimentos endodônticos interferiram negativamente na união de pinos de fibra de vidro cimentados com cimento resinoso auto-adesivo à dentina intrarradicular.

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8. Publicações

Artigo 1:

Influence of endodontic therapy and artificial accelerated aging on fibreglass post bond strength to intraradicular dentine. Part 1. Instrumentation techniques and endodontic irrigants

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Influence of endodontic therapy and artificial accelerated aging on fibreglass post bond strength to intraradicular dentine. Part 1. Instrumentation techniques and endodontic irrigants

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Running title (no more than 30 letters and spaces)

Post bond strength to dentine

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Abstract

Aim To evaluate *in vitro* the influence of instrumentation techniques, endodontic irrigants and specimens aging on fibreglass post bond strength to bovine intraradicular dentine. **Methodology** 120 teeth were divided into 12 groups (n=10) resulting from the interaction among 3 study factors: instrumentation technique (SS- root canal preparation with stainless steel instruments - K-File; and NiTi- root canal preparation with K3 Nickel-Titanium instruments); endodontic irrigant (NaOCl- 1% sodium hypochlorite; CHX- 2% chlorhexidine; and O₃- 1.2% ozonated water) and specimens aging (Immediate, test with no aging; and Mediate, test performed after 2 months of water storage at 37°C). After root canal preparation, endodontic filling was not performed. Posts were cemented with self-adhesive resin cement (RelyX U100) and roots were cross-sectioned to obtain two slices of each third. Samples were submitted to push-out test and bond strength values were analysed by ANOVA in a split-plot arrangement and Tukey's test ($\alpha = 0.05$). Failure modes were evaluated under a confocal microscope. **Results** In specimens submitted to water artificial aging, NiTi presented higher bond strength values than SS in apical third irrigated with NaOCl or CHX. Irrigation with NaOCl resulted in higher bond strength than O₃. Artificial aging resulted in significant bond strength increase, except for middle and apical thirds of SSO₃ and apical of NiTiO₃. Was verified the prevalence of adhesive cement-dentine failure for all groups. **Conclusions** Root canal preparation with NiTi instruments associated with NaOCl irrigation and EDTA increased the bond strength of fibreglass posts cemented with self-adhesive resin cement to intraradicular dentine.

Introduction

The restoration of endodontically treated teeth represent a challenge in function of they often have insufficient coronal structure to retain the restorative material (Menezes *et al.* 2008, Santana *et al.* 2011). On occasions, they require the use of a post to provide sufficient retention for the core (Morgano *et al.* 2004, Cheung 2005, Tang *et al.* 2010, Santana *et al.* 2011).

Fibreglass posts in combination with composite core foundation materials are widely accepted as alternative to cast posts in the restoration of endodontically treated teeth (Schwartz & Robbins 2004, Bitter & Kielbassa 2007, Naves *et al.* 2011). Fibreglass posts have a high flexural strength and elasticity modulus similar to that of dentine, which minimize the transmission of stresses to root canal walls and decrease the possibility of fractures (Lassila *et al.* 2004, Schwartz & Robbins 2004, Cecchin *et al.* 2011). Furthermore, they can also be cemented in the root canal using adhesive techniques (Kececi *et al.* 2008, Bitter *et al.* 2009, Cecchin *et al.* 2011).

An optimal bond between post-cement and cement-dentine is necessary for the success of the restorative procedure in endodontically treated teeth (Soares *et al.* 2008a), however, debonding is the most common mode of failure of the fibre-reinforced composite post restoration (Ferrari *et al.* 2007, Cagidiaco *et al.* 2008, Albashaireh *et al.* 2010, Jongsma *et al.* 2010). This occurs probably to poor visibility, anatomical features (Mjör *et al.* 2001, Bitter *et al.* 2008, Pelegrine *et al.* 2010) and limited capacity to dissipate polymerization shrinkage stresses in long narrow post spaces, exhibiting a highly unfavourable configuration factor (Tay *et al.* 2005, Pelegrine *et al.* 2010), and was found to be less effective than bonding to coronal dentine (Bitter *et al.* 2009).

Endodontic procedures performed before the luting of posts also may interfere on bonding to intraradicular dentine (Ari *et al.* 2003, Erdemir *et al.* 2004, Hayashi *et al.* 2005, Muniz & Mathias 2005, Bitter *et al.* 2008, Pelegrine *et al.* 2010). In endodontic therapy, the process of sanitizing infected root canals begins with the mechanical action of instruments and chemical action of endodontic irrigants (Estrela *et al.* 2003, Estrela *et al.* 2004), what can induce chemical and structural changes of dentine surface and affect the interaction with restorative materials (Cecchin *et al.* 2010, Shokouhinejad *et al.* 2010).

An effective irrigating solution in root canal preparation is essential for the sanitization process, because it favours cleaning, shaping and neutralizes necrotic content, which favours root canal enlargement for subsequent filling. Different auxiliary chemical agents for root canal preparation have been proposed and the selection of an ideal irrigant depends on its action on microorganisms and periapical tissues. Sodium hypochlorite and chlorhexidine are antimicrobial agents frequently used in the treatment of endodontic infections (Zehnder 2006, Estrela *et al.* 2003, 2004, 2007).

Sodium hypochlorite (NaOCl) is the most used irrigating solution for endodontic treatment, because its mechanism of action causes biosynthetic alterations in cellular metabolism and phospholipid destruction, formation of chloramines that interfere in cellular metabolism, oxidative action with irreversible enzymatic inactivation in bacteria, and lipid and fatty acid degradation (Estrela *et al.* 2002). Chlorhexidine (CHX) is a potent antiseptic with a broad-spectrum antimicrobial action, substantivity (Okino *et al.* 2004, Pelegrine *et al.* 2010) and ability of decomposition (Barbin *et al.* 2008), however, is unable to dissolve pulp tissue (Okino *et al.* 2004, Pelegrine *et al.* 2010). Ozonated water (O₃) in endodontic infection treatment has been studied (Nagayoshi *et al.* 2004, Hems *et al.* 2005, Estrela *et al.* 2007, Bitter *et al.* 2008). Ozone (O₃) is a powerful oxidizing agent and has been used in the water industry for many years to kill bacteria. The antimicrobial effect of ozone results from oxidation of microbial cellular components (Estrela *et al.* 2007).

Nonetheless, there is no consensus in the literature about the possible effects of the chemical agents commonly used during the biomechanical preparation of root canals regarding bonding to intraradicular dentine (Morris *et al.* 2001, Ari *et al.* 2003, Varela *et al.* 2003, Erdemir *et al.* 2004, Muniz & Mathias 2005, Hayashi *et al.* 2005, Bitter *et al.* 2008, Demiryürek *et al.* 2009, Pelegrine *et al.* 2010), since these depend on the dentine bonding system used (Hayashi *et al.* 2005, Bitter *et al.* 2008). Thus, the compatibility among different materials used in endodontic therapy with the luting of fibre-reinforced posts to root canal is an important aspect to be considered for a successful restoration. Moreover, the quality of bonding to root dentine may be affected by the density and orientation of dentine tubules at different levels of the root canal walls (Ferrari *et al.* 2000, Goracci *et al.* 2004) and the ease of access to the root

canal thirds (Ferrari *et al.* 2000, Ferrari *et al.* 2001, Goracci *et al.* 2004, Wang *et al.* 2008, da Cunha *et al.* 2010).

The aim of the present study was to evaluate the effects of endodontic therapy (instrumentation technique and endodontic irrigant), artificial accelerated aging of specimens and depth of root canal on bond strength of adhesively cemented (RelyX U100; 3M-ESPE, St. Paul, MN, USA) fibreglass posts in root canal of bovine incisor teeth. The hypothesis tested was that (i) the instrumentation techniques of root canal, (ii) the chemical composition of the endodontic irrigants and (iii) the artificial accelerated aging of specimens influence on fibreglass post bond strength to intraradicular dentine, depending on the depth of the root canal.

Materials and methods

From among 700 freshly extracted bovine incisor teeth, 120 were selected (approved by the Federal University of Goiás Ethics Committee, # 256/10). The selection criterion used was the similarity of the external and internal anatomical morphology and a root canal diameter smaller than 1mm. Roots 15 mm in length were produced after removing the coronal portion. Roots were randomly divided into 12 groups (n=10) resulting from the interaction among three study factors: instrumentation technique of root canal (SS- root canal preparation with stainless steel instruments - K-File; NiTi- root canal preparation with K3 Nickel-Titanium instruments), endodontic irrigant (NaOCl - 1% sodium hypochlorite; CHX- 2% chlorhexidine; O₃- 1.2% ozonated water) and specimens aging (Immediate, test with no aging; Mediate, test performed after artificial accelerated aging - 2 months of water storage at 37 °C).

Root canals were prepared 1 mm from the apex by the crown-down technique. In groups SS, root canal preparation was carried out using size # 1,2 Gates Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland) (Working Length - WL 10mm), size # 2 Largo burs (Dentsply Maillefer) (WL 10mm) and stainless steel instruments (K-File; Dentsply Maillefer) up to # 45 K-File (WL 14mm). In groups NiTi, roots canals were prepared with size # 1,2 Gates Glidden burs (Dentsply Maillefer) (WL 10mm) and K3 Nickel-Titanium instruments (SybronEndo, Optimum, São Paulo, SP, Brazil) in the following

sequence: # 25 .10 taper (WL 10mm); # 15-25 .02 taper (WL 14mm); # 25 .04 taper (WL 14mm); # 25 .06 taper (WL 14mm); # 30-45 .02 taper (WL 14mm).

During instrumentation, at each change of file, root canals were irrigated with 2 mL of one of the irrigant solutions tested in the present study: NaOCl- 1% sodium hypochlorite (Fitofarma, Goiânia, GO, Brazil); CHX- 2% chlorhexidine (Fitofarma); O₃- 1.2% ozonated water. Ozone was produced by electric discharge through oxygen current (PXZ3507, Eaglesat Tecnologia em Sistemas Ltda., São José dos Campos, SP, Brazil) and bubbled into 1 L sterile distilled water at 7g/h ozone flow rate (1.2%) (Estrela *et al.* 2007). In all groups, 3 mL of 17% EDTA (Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brazil) was used for 5 min to remove the smear layer. The final irrigation was performed with 5 mL of the same solution used in root canal preparation. After root canal preparation, endodontic filling was not performed.

Post space was prepared using size # 3-5 Largo burs (Dentsply Maillefer) (WL 10mm) corresponding to the fibreglass parallel-sided and serrated post 1.5-mm-diameter (Reforpost # 3; Angelus, Londrina, PR, Brazil). Root canals were irrigated, at each change of burs and after the preparation, with 2 mL of the same irrigant solution used previously and dried with absorbent paper points. All roots were covered externally with utility wax to avoid lateral polymerization (Soares *et al.* 2012).

Fibreglass posts were cleaned with 70% alcohol, then in a single application using a microbrush, and after drying, a silane agent was applied for 1 min (Silano; Angelus). The self-adhesive resin cement (RelyX U100; 3M-ESPE, St. Paul, MN, USA) was manipulated in accordance with the manufacturer's instructions, introduced into the canal with K-File and placed on the post. The post was seated to full depth by finger pressure. Excess cement was removed after 1 min. After 5 min, the resin cement was light-cured with 1200 mW cm⁻² (Radii-Cal; SDI, Bayswater, Australia) for 40 s each on the cervical face of the root, in the direction of the long axis of the root, and obliquely to the buccal and lingual surfaces, totalling 120 s. The interface post-cement-dentine on the cervical face was then sealed with composite resin and the roots were stored in distilled water at 37°C for 24h. After 24 h, each root was serial sectioned perpendicular to its long axis with a double face diamond disc (4" Diameter x 0.012" Thickness x 1/2" Arbor, Extec, Enfield, CT, USA) at

low speed under water cooling (Isomet 1000, Buehler, Lake Bluff, IL, USA) to obtain two slices, approximately 1 mm thick, of each root third (cervical, middle and apical), totalling six slices per root.

In immediate groups, slices were submitted immediately (with no aging) to push-out test, while in mediate groups, slices were stored in distilled water at 37°C for 2 months (artificial accelerated aging) prior to testing. The push-out test was performed in a testing machine (EMIC DL 2000, São José dos Pinhais, PR, Brazil) by applying a compressive load at 0.5 mm min⁻¹ in the apical-coronal direction until post segment extrusion. The bond strength was calculated in MPa by dividing the load at failure (in N) by the area of the bonded interface. The area of the bonded interface was calculated as follows: $A = 2\pi rh$, where A is the area of the bonded interface, $\pi = 3.14$, r is the radius of post segment (mm) and h is the thickness of the post segment (mm) (Goracci *et al.* 2004, Soares *et al.* 2008b, Soares *et al.* 2012).

Statistical analysis was performed using SAS statistical program (Institute Inc., Cary, NC, USA). Data were submitted to the Shapiro-Wilk test of normality. The effects, on bond strength, of instrumentation technique, endodontic irrigant and depth of root canal were analysed using two-way analysis of variance in a split-plot arrangement, with the plot represented by the factors instrumentation technique, endodontic irrigant and their interaction, and the sub-plot represented by the root canal thirds (cervical, middle and apical). Comparisons with respect the artificial aging was performed by one-way analysis of variance with the plot represented by this factor and the sub-plot represented by the root canal thirds. Multiple comparisons were made using Tukey's test ($\alpha = 0.05$).

To determine the failure modes, all fractured specimens were air dried and analysed under a confocal laser scanning microscope (Carl Zeiss Laser Scanning Systems, LSM510, META, Oberkochen, Germany). Images were analysed using Zeiss LSM Image Browser (META, Germany). The failure modes were classified into six types: (I) adhesive between the post and resin cement; (II) adhesive between resin cement and intraradicular dentine; (III) cohesive in cement; (IV) cohesive in dentine; (V) cohesive in post (Castellan *et al.* 2010) and (VI) mixed, among post, resin cement and intraradicular dentine (Bitter *et al.* 2009).

Results

Two-way analysis of variance (instrumentation technique X endodontic irrigant) with sub-plot (root canal third) of immediate groups showed significant difference for endodontic irrigant ($P=.0023$) and root canal third ($P<.0001$). The mean push-out bond strengths and standard deviations are in Table 1. Tukey's test indicated that irrigation with NaOCl resulted in higher bond strength than with O_3 , except in the apical third of NiTi that was similar. The irrigation with CHX resulted on intermediate values that were statistically similar to values obtained in groups irrigated with O_3 in thirds cervical/apical of SS and cervical/middle of NiTi, and in all groups irrigated with NaOCl. Regardless of root canal preparation and endodontic irrigant, cervical third had higher bond strength values than apical third.

Two-way analysis of variance (instrumentation technique X endodontic irrigant) with sub-plot (root canal third) of mediate groups, artificial accelerated aging prior to testing, showed significant difference for instrumentation technique ($P=.0356$), endodontic irrigant ($P<.0001$), root canal third ($P<.0001$) and the interaction between irrigant and root canal third ($P=.0047$). The mean push-out bond strengths and standard deviations are in Table 2. In apical third of specimens irrigated with NaOCl or CHX, NiTi resulted on higher bond strength than SS. Regarding the endodontic irrigant, NaOCl resulted on higher bond strength values than O_3 in all groups. CHX presented statistically lower value than NaOCl only in cervical third of SS and similarity to NaOCl in the other groups. Cervical third presented higher values than apical third, except in groups NiTiNaOCl and NiTiCHX that were similar.

One-way analysis of variance (artificial aging) with sub-plot (root canal third) in group SSNaOCl showed significance for artificial aging ($P<.001$) and root canal third ($P<.001$). In group SSCHX there was significance for artificial aging ($P<.001$) and root canal third ($P<.001$). In group SSO₃ there was significance for artificial aging ($P=.006$), root canal third ($P<.001$) and interaction between the factors ($P=.032$). In group NiTiNaOCl there was significance for artificial aging ($P<.001$) and root canal third ($P=.005$). In group NiTiCHX there was significance for artificial aging ($P<.001$) and root canal third ($P=.008$). In group NiTiO₃ there was significance for artificial aging ($P=.008$), root canal third

($P < .001$) and interaction between the factors ($P < .001$). The mean push-out bond strengths and standard deviations of all groups are in Table 3. Tukey's test demonstrated a significant bond strength increase after artificial accelerated aging, except for middle and apical thirds of SSO₃ and apical of NiTiO₃ that was similar.

Failure modes of tested specimens and its distribution are presented in Figure 1 and Table 4, respectively. The prevalence of (II) adhesive cement-dentine failure was verified in all groups.

Discussion

The instrumentation techniques of root canal, the chemical composition of endodontic irrigants and the artificial accelerated aging of samples influenced on fibreglass post bond strength. Moreover, bond to root dentine was affected by the different thirds of root canal. The tested hypothesis was accepted.

Various mechanical methods have been used to measure *in vitro* the bond strength of fibreglass post to intraradicular dentine, as microtensile bond strength and push-out tests (Soares *et al.* 2008b). The push-out method was shown to have fewer premature specimen failures, a lower data distribution variability (Goracci *et al.* 2004, Soares *et al.* 2008b) and a more homogenous stress distribution (Soares *et al.* 2008b) compared to microtensile method during the bond strength evaluation of fibreglass posts to intraradicular dentin. The push-out laboratory test would seem to be safer than the microtensile test (Goracci *et al.* 2004) and a more appropriate methodology for the evaluation of fibreglass posts bonded to intraradicular dentine (Soares *et al.* 2008b).

Root canal preparation aims the emptying, enlargement and endodontic sanitizing (Estrela *et al.* 2007). The stainless-steel instruments have generally been used for this purpose. The motor driven nickel-titanium instruments have been suggested in order to facilitate the preparation of curved canals, due to the flexibility and super-elasticity (Thompson 2000). The quality of modelling, efficiency, less time of work and decrease of stress professional are other qualities inherent in the preparation with NiTi instruments (Thompson 2000, Peters *et al.* 2001a, Peters & Paqué 2010). The preparation with stainless steel instruments has allowed that walls have not been touched by the instruments (Peters *et al.* 2001b). Since these instruments act as scrapers, and not by

excision of dentine, as occur with NiTi instruments, it can be suggested a greater possibility of compaction of smear layer into dentinal tubules, featuring greater presence of smear plug. This may have acted as a barrier to penetration and interaction of cement with intraradicular dentine, which may be related to the decrease on bond strength observed in groups prepared with stainless steel instruments in the present study.

NaOCl is a halogenated compound routinely used in endodontics (Zehnder 2006), which has antimicrobial effect (Byström & Sundqvist 1981, Estrela *et al.* 2002), tissue dissolution capacity (Byström & Sundqvist 1981, Spanó *et al.* 2009, Estrela *et al.* 2002) and acceptable biologic compatibility in less concentrated solutions (Estrela *et al.* 2002, Estrela *et al.* 2002,2003,2004). However, it does not act on the inorganic portion of dentine, which constitutes great part of the smear layer (Garberolio & Becce 1994, Cecchin *et al.* 2010). EDTA produces dentine demineralization and provides an excellent cleaning of the root canal walls (Spanó *et al.* 2009), improving the penetration of chemical substances and promoting a more intimate contact of the filling material with the root dentine (Cecchin *et al.* 2010). It acts on the inorganic components of the smear layer, leading to decalcification of the peri- and intertubular dentine (Cecchin *et al.* 2010).

The association of both substances is largely used in endodontic therapy because they act in organic and inorganic portion of dentine (Estrela *et al.* 2007, Zhang *et al.* 2010, Cecchin *et al.* 2010), with almost complete dissolution of the smear layer and exposition of the dentinal tubules (Wu *et al.* 2009). Chlorhexidine and ozonated water, however, do not have the ability to dissolve organic tissues, and debris can remain adhered to root canal walls obstructing the dentinal tubules (Menezes *et al.* 2003, Bitter *et al.* 2008, Bodrumlu *et al.* 2010), which can negatively influence the bond to root dentine, as seen in the present study.

Bitter *et al.* (2009) related that hybridization of dentine was only detected sporadically for the self-adhesive cement RelyX Unicem (3M-ESPE). The self-adhesive cement RelyX U100 (UC) used in the present study and the RelyX Unicem (UN) were developed by the same manufacturer and are marketed under the same name in some countries (Viotti *et al.* 2009). According to the manufacturer, the only difference between these products is the delivery

system. While UN requires an activator, triturator and applicator, UC can be hand mixed (Viotti *et al.* 2009). The adhesive properties of this cement is claimed to be based upon acidic monomers that demineralize and infiltrate the tooth substrate, and create micromechanical retention and chemical adhesion to hydroxyapatite (De Munck *et al.* 2004, Gerth *et al.* 2006, Zicari *et al.* 2008, Bitter *et al.* 2009, Cecchin *et al.* 2011).

Bitter *et al.* (2009) reported that the penetration of this cement into the dentinal tubules was found in only a few specimens and concluded that the smear layer did not dissolve consistently at the dentine-cement interface. This finding corroborates with the results of investigations conducted previously that also described a superficial morphological interaction (De Munck *et al.* 2004, Al-Assaf *et al.* 2007, Monticelli *et al.* 2008). Since the RelyX U100 has low desmineralization capacity, the ability of NaOCl to remove the organic components of dentine may result in a rough surface (Hayashi *et al.* 2005). Probably, this fact may increase the penetration of the cement (Ari *et al.* 2003) into the partially demineralized dentine structure (Monticelli *et al.* 2008) favouring the micromechanical retention and increasing the bond strength.

Moreover, an intense chemical interaction of RelyX Unicem with hydroxyapatite has been documented (Gerth *et al.* 2006). Chemical interactions between the self-adhesive resin cement and hydroxyapatite may be effective inside the root canal, and indicate that this interaction might be more crucial for root dentine bonding than the ability of the same material to hybridize dentine (Bitter *et al.* 2009). As NaOCl acts on organic portion dissolution exposing the inorganic, this action may favour the chemical interactions between hydroxyapatite and cement, justifying the highest bond strength values found when this irrigant was used.

The effect of NaOCl on the bond strength of resin cements to intraradicular dentine is controversial, several studies has demonstrated benefit when this irrigant is used (Varela *et al.* 2003, Muniz & Mathias 2005, Hayashi *et al.* 2005, Pelegrine *et al.* 2010), whereas other studies described an adverse effect of this irrigant on bonding (Morris *et al.* 2001, Ari *et al.* 2003, Erdemir *et al.* 2004, Demiryürek *et al.* 2009). The increase has been explained by the removal of the collagen of the smear layer, which resulted in the rough surface of the dentine. On the other hand, the decrease of bond strength has

been explained by the change of the redox potential of bonding substrate due to the residual NaOCl. However, these studies present different methodologies from that used on the present study, as regarding concentration and exposure time of NaOCl, hindering appropriate comparison with the obtained results.

Ozonated water reduced significantly the bond strength to root canal. Ozone (O₃) is a highly reactive form of oxygen that is generated by passing oxygen through high-voltage (Estrela *et al.* 2007). It is a blue gas, containing three oxygen atoms, and it is a power antimicrobial agent, irritant, toxic and unstable (Stübinger *et al.* 2006, Estrela *et al.* 2007, Azarpazhooh & Limeback 2008, Rodrigues *et al.* 2011). However, because of its high instability, ozone rapidly turns into oxygen (Stübinger *et al.* 2006, Estrela *et al.* 2007, Rodrigues *et al.* 2011), which may inhibit the polymerization of the resin cement and thus reduce bond strength (Bitter *et al.* 2008). Therefore, a possible interaction with initiator components of resin cements as well as a certain influence of the material properties (Bitter *et al.* 2008), such as the inability to dissolve organic tissue, may explain the significant reduction in bond strength.

Although fiber posts luted in root canals are not directly exposed to oral fluids, water storage is considered as *in vitro* accelerated aging test for bonded interfaces (Radovic *et al.* 2007). In the present study, water artificial aging for two months significantly increased post retention. Increases in interfacial strength may be related to enhanced bonding ability or setting during water storage, stress relaxation by hygroscopic expansion as a consequence of water sorption during storage or hygroscopic expansion of luting materials (Sadek *et al.* 2006, Albashaireh *et al.* 2010). The hygroscopic expansion of the resin cement, in particular, could have contributed to a greater adaptation of the cement to dentine substrate. A major contribution to retentive strength in push-out test is expected to occur as a consequence of the interfacial sliding friction (Sadek *et al.* 2006) resulting from application of a compressive force. Thus, the higher 2-months interfacial strengths achieved may have been caused by the increase in interfacial friction, due to the greater adaptation of the cement to dentine consequent to hygroscopic expansion (Sadek *et al.* 2006, Albashaireh *et al.* 2010). It is important to emphasize that the applied method is a simplified model of accelerated aging which has been commonly performed for challenging resin-dentine adhesion (De Munck *et al.* 2005, Radovic *et al.* 2007).

In relation to the effect of the different levels of root canal in the present study, bond strength was predominantly higher in cervical third and lower in apical third. This may be due to a better interaction of the cement with cervical dentine and to the more difficult access to the apical third, associated with possible limitations of cement flow (de Durão Mauricio *et al.* 2007, da Cunha *et al.* 2010), as the cement used in the present study has a high viscosity (De Munck *et al.* 2004). In addition, bond to root dentine is also influenced by the concentration and direction of dentine tubules at different levels of the root canal walls (Ferrari *et al.* 2000, Ferrari *et al.* 2001, Wang *et al.* 2008, da Cunha *et al.* 2010), being the cervical third with higher density and diameter of dentinal tubules (Ferrari *et al.* 2000).

Fractographic analysis provides important information that helps to predict the adhesive material performance. In the present study, confocal microscopy was used and appears to be a noteworthy alternative for failure modes analysis, since it is less time consuming and does not require any preparation of the specimens (Castellan *et al.* 2010). The RelyX U100 has a superficial morphological interaction (De Munck *et al.* 2004, Gerth *et al.* 2006, Bitter *et al.* 2009, Al-Assaf *et al.* 2007, Monticelli *et al.* 2008). This observation may explain the predominance of adhesive cement-dentine failures in all groups of the present study (Table 4).

Limitations from the present study include the fact that the specimens were not submitted to thermal and mechanical cycling, in order to simulate intra-oral situations more precisely. Further, bovine teeth were used because bioethical concerns make it difficult to collect and utilize human teeth for research purposes (Menezes *et al.* 2008, Soares *et al.* 2012). Bovine teeth collecting is easier and tooth age can be standardized (Menezes *et al.* 2008, Soares *et al.* 2012). Further, several studies have demonstrated similar properties between human and bovine teeth (Nakamichi *et al.* 1983, Dong *et al.* 2003). Further studies need to be conducted to analyse the effects of different concentrations of NaOCl; the association of a reversal agent such as ascorbic or sodium ascorbate with ozonated water in order to reverse the oxidized dentin; and the increase of the artificial aging time on bond strength to intraradicular dentine.

Conclusions

Root canal preparation with NiTi instruments associated with NaOCl irrigation and EDTA increased the bond strength of fibreglass posts cemented with self-adhesive resin cement to intraradicular dentine.

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Table 1 Bond strength means in MPa (standard deviation) of groups tested immediately (no aging) and statistical categories defined by Tukey's test (n = 10).

Endodontic irrigant	Instrumentation technique						Total
	SS			NiTi			
	Root canal third			Root canal third			
	Cervical	Middle	Apical	Cervical	Middle	Apical	
NaOCl	12.9 (2.4) ^{Aa}	10.3 (4.0) ^{Ba}	7.10 (3.8) ^{Ca}	13.5 (2.1) ^{Aa}	11.4 (3.3) ^{ABa}	9.3 (4.8) ^{BCa}	10.8 (4.0) ^a
CHX	11.4 (3.3) ^{Aab}	9.8 (3.9) ^{ABa}	6.7 (3.9) ^{Cab}	11.5 (3.8) ^{Aab}	10.0 (4.9) ^{ABab}	7.1 (5.6) ^{BCa}	9.4 (4.5) ^{ab}
O ₃	8.7 (6.8) ^{Ab}	6.2 (5.0) ^{Bb}	3.5 (2.9) ^{Cb}	9.5 (3.4) ^{Ab}	7.4 (1.6) ^{ABb}	6.8 (1.4) ^{BCa}	7.0 (4.3) ^b
Total	8.5 (4.9) ^B			9.6 (4.2) ^A			

Capital letters were used to compare groups in the horizontal lines and lower-case letters were used to compare groups in the vertical lines. Tukey categories with same letters are not statistically significant from each other ($P < 0.05$).

SS, root canal preparation with stainless steel instruments - K-File; NiTi, root canal preparation with K3 Nickel-Titanium instruments; NaOCl, 1% sodium hypochlorite; CHX, 2% chlorhexidine; O₃- 1.2% ozonated water.

Table 2 Bond strength means in MPa (standard deviation) of groups tested after water artificial accelerated aging and statistical categories defined by Tukey's test (n = 10).

Endodontic Irrigant	Instrumentation technique						Total
	SS			NiTi			
	Root canal third			Root canal third			
	Cervical	Middle	Apical	Cervical	Middle	Apical	
NaOCl	24.1 (5.8) ^{Aa}	20.3 (7.7) ^{Ba}	15.5 (8.6) ^{Ca}	25.1 (6.4) ^{Aa}	22.6 (6.1) ^{ABa}	21.6 (6.3) ^{ABa}	21.5(7.3) ^a
CHX	18.9 (4.3) ^{ABb}	16.2 (4.0) ^{Ba}	13.3 (7.2) ^{Ca}	21.4 (3.4) ^{Ab}	20.8 (6.3) ^{ABa}	18.4 (7.7) ^{ABa}	18.2(6.1) ^a
O ₃	17.4 (4.9) ^{Ab}	9.9 (4.0) ^{Bb}	6.4 (3.4) ^{Cb}	17.4 (4.9) ^{Ab}	11.0 (5.3) ^{Bb}	7.1 (3.8) ^{BCb}	11.5(6.2) ^b
Total	15.8 (7.5) ^B			18.4 (7.8) ^A			

Capital letters were used to compare groups in the horizontal lines and lower-case letters were used to compare groups in the vertical lines.

Tukey categories with same letters are not statistically significant from each other ($P < 0.05$).

SS, root canal preparation with stainless steel instruments - K-File; NiTi, root canal preparation with K3 Nickel-Titanium instruments; NaOCl, 1% sodium hypochlorite; CHX, 2% chlorhexidine; O₃- 1.2% ozonated water.

Table 3 Bond strength means in MPa (standard deviation), as a result of artificial accelerated aging and root canal third, and statistical categories defined by Tukey's test for each group (n = 10).

Groups	Test	Root canal third		
		Cervical	Middle	Apical
SSNaOCl	Immediate	12.9 (2.4) ^{Ab}	10.3 (4.0) ^{ABb}	7.1 (3.8) ^{Bb}
	Mediate	24.1 (5.8) ^{Aa}	20.3 (7.7) ^{Ba}	15.5 (8.6) ^{Ca}
SSCHX	Immediate	11.4 (3.3) ^{Ab}	9.8 (3.9) ^{ABb}	6.7 (3.9) ^{Bb}
	Mediate	18.8 (4.3) ^{Aa}	16.2 (4.0) ^{ABa}	13.3 (7.2) ^{Ba}
SSO ₃	Immediate	8.7 (6.8) ^{Ab}	6.2 (5.0) ^{ABa}	3.5 (2.9) ^{Ba}
	Mediate	17.4 (4.9) ^{Aa}	9.9 (4.0) ^{Ba}	6.4 (3.4) ^{Ba}
NiTiNaOCl	Immediate	13.5 (2.1) ^{Ab}	11.4 (3.3) ^{ABb}	9.3 (4.8) ^{Bb}
	Mediate	25.1 (6.4) ^{Aa}	22.6 (6.1) ^{Aa}	21.6 (6.3) ^{Aa}
NiTiCHX	Immediate	11.5 (3.8) ^{Ab}	10.0 (4.9) ^{ABb}	7.1 (5.6) ^{Bb}
	Mediate	21.4 (3.4) ^{Aa}	20.8 (6.3) ^{Aa}	18.4 (7.7) ^{Aa}
NiTiO ₃	Immediate	9.5 (3.4) ^{Ab}	7.4 (1.6) ^{ABb}	6.8 (1.4) ^{Ba}
	Mediate	17.4 (4.9) ^{Aa}	11.0 (5.3) ^{Ba}	7.1 (3.8) ^{Ca}

In each group, capital letters were used to compare values in the horizontal lines and lower-case letters were used to compare values in the vertical lines. Tukey categories with same letters are not statistically significant from each other ($P < 0.05$).

SS, root canal preparation with stainless steel instruments - K-File; NiTi, root canal preparation with K3 Nickel-Titanium instruments; NaOCl, 1% sodium hypochlorite; CHX, 2% chlorhexidine; O₃- 1.2% ozonated water.

Table 4 Failure modes (%) for experimental groups.

Groups	Adhesive: post and cement (I)	Adhesive: cement and dentine (II)	Cohesive: Cement (III)	Cohesive: Dentine (IV)	Cohesive: Post (V)	Mixed (VI)
SSNaOCl Immediate	-	32 (53.3)	-	4 (6.7)	1 (1.7)	23 (38.3)
SSCHX Immediate	1 (1.7)	33 (55.0)	-	3 (5.0)	1 (1.7)	22 (36.7)
SSO ₃ Immediate	3 (5.0)	33 (55.0)	-	-	1 (1.7)	23 (38.3)
NiTiNaOCl Immediate	2 (3.3)	33 (55.0)	-	6 (10.0)	2 (3.3)	17 (28.3)
NiTiCHX Immediate	2 (3.3)	34 (56.7)	-	2 (3.3)	2 (3.3)	20 (33.3)
NiTiO ₃ Immediate	2 (3.3)	47 (78.3)	-	1 (1.7)	-	10 (16.7)
SSNaOCl Mediate	2 (3.3)	27 (45.0)	-	-	11 (18.3)	20 (33.3)
SSCHX Mediate	3 (5.0)	31 (51.7)	-	1 (1.7)	7 (11.7)	18 (30.0)
SSO ₃ Mediate	3 (5.0)	40 (66.7)	-	-	2 (3.3)	15 (25.0)
NiTiNaOCl Mediate	1 (1.7)	20 (33.3)	-	9 (15.0)	15 (25.0)	15 (25.0)
NiTiCHX Mediate	-	42 (70.0)	-	-	10 (16.7)	8 (13.3)
NiTiO ₃ Mediate	1 (1.7)	35 (58.3)	1 (1.7)	-	3 (5.0)	20 (33.3)

SS, root canal preparation with stainless steel instruments - K-File; NiTi, root canal preparation with K3 Nickel-Titanium instruments; NaOCl, 1% sodium hypochlorite; CHX, 2% chlorhexidine; O₃- 1.2% ozonated water; Immediate, test with no aging; Mediate, test performed after 2 months of water storage at 37°C.

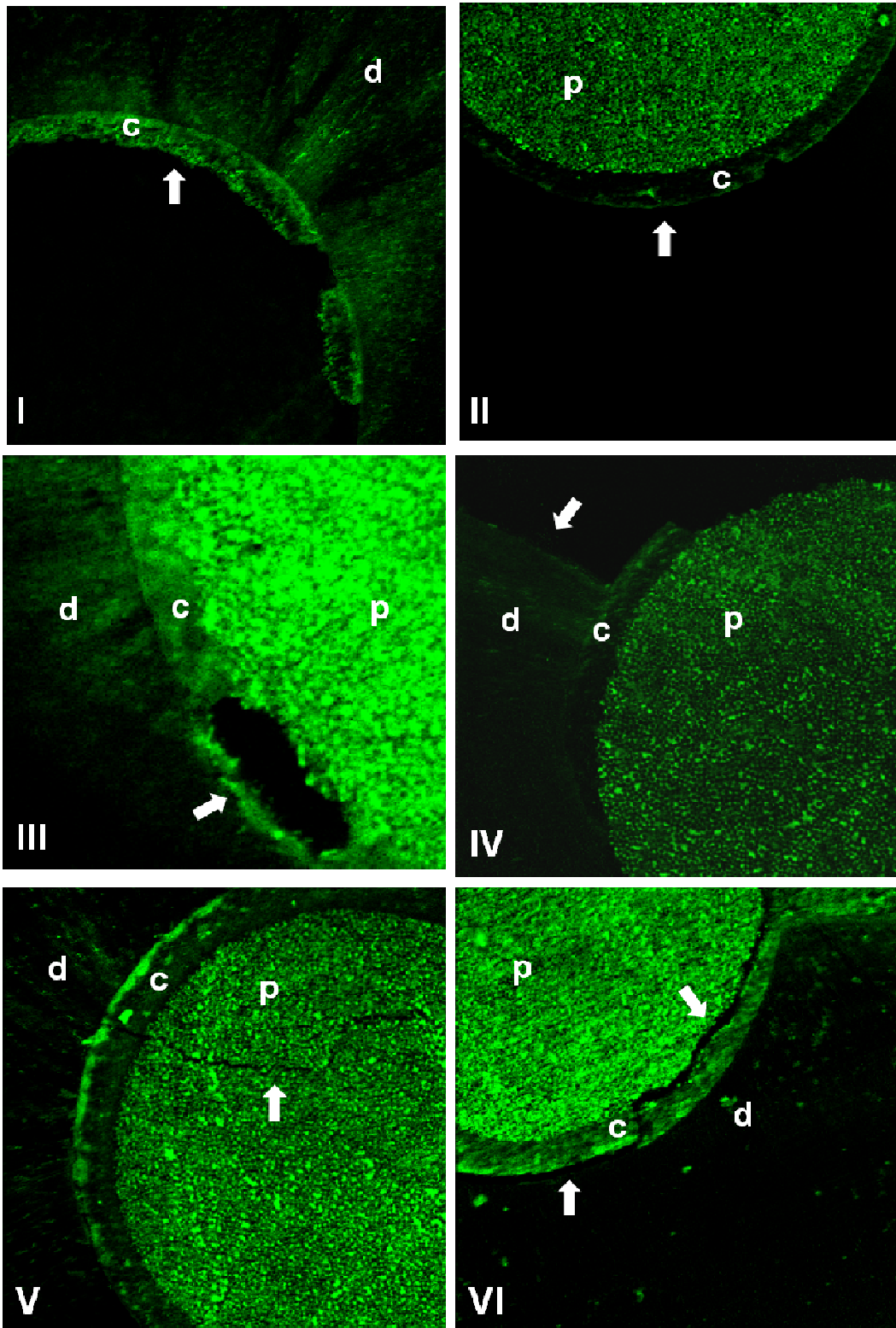


Figure 1. *d* – dentine; *c* – resin cement; *p* – fibreglass post. Microscopic images of failure modes after micropush-out test (magnification 10X): (I) adhesive between the post and resin cement; (II) adhesive between resin cement and intraradicular dentine; (III) cohesive in cement; (IV) cohesive in dentine; (V) cohesive in post; (VI) mixed among post, resin cement and intraradicular dentine.

Artigo 2:

Influence of endodontic therapy and artificial accelerated aging on fibreglass post bond strength to intraradicular dentine. Part 2. Endodontic sealers

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Influence of endodontic therapy and artificial accelerated aging on fibreglass post bond strength to intraradicular dentine. Part 2. Endodontic sealers

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Running title (no more than 30 letters and spaces)

Post bond strength to dentine

Key words: fibreglass post, bond strength, root dentine, endodontic sealer, aging.

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Abstract

Aim To evaluate *in vitro* the influence of endodontic sealers and specimens aging on fibreglass post bond strength to bovine intraradicular dentine.

Methodology 80 teeth were divided into 8 groups (n=10), 2 controls and 6 experimental groups resulting from the interaction between 2 study factors: endodontic sealer (SX- Sealapex; S26- Sealer 26; AH- AH Plus) and specimens aging (Immediate, test with no aging; Mediate, test performed after 2 months of water storage at 37°C). Root canals were prepared with K3 Nickel-Titanium-instruments associated with 1% NaOCl and 17% EDTA. In control groups (immediate and mediate), after root canal preparation, endodontic filling was not performed. In experimental groups, root canals were filled with gutta-percha and the specific sealer for each group. In all groups, posts were cemented with self-adhesive resin cement (RelyX U100). Roots were cross-sectioned to obtain two slices of each third. Samples were submitted to push-out test and data were analysed by ANOVA in a split-plot arrangement and Tukey's test ($\alpha = 0.05$). Comparisons with controls were made using Dunnet test ($\alpha = 0.05$). Failure modes were evaluated under a confocal microscope.

Results No significant difference was detected among endodontic sealers, however they presented significantly lower bond strength values than controls, except in cervical third of groups tested immediately. Artificial aging did not interfere on bond strength to intraradicular dentine. The prevalence of adhesive cement-dentine failure was verified in all groups.

Conclusions Endodontic sealers interfered negatively on bonding of fibreglass posts cemented with self-adhesive resin cement to intraradicular dentine.

Introduction

Root canal filling is an important step in the last phase of endodontic treatment, which is completed with coronal restoration. The correct sealing aims to prevent reinfection of the root canal and promote the process of biological sealing (Estrela *et al.* 2007). This procedure is achieved with the association of a solid filling material, such as gutta-percha, and endodontic sealer. One of the key roles of the endodontic sealer is to aggregate the root filling material and maintain it as compact mass with no gaps, which adheres to the canal walls and provides a single block configuration that seals hermetically the canal space (Schwartz 2006, Teixeira *et al.* 2009). This adhesion process involves mechanical forces that yield the intertwining of the material with dentine structures and may result in a greater sealing ability, thus reducing the risk of root canal microleakage and maintaining a cohesive filling mass (Saleh *et al.* 2002, Teixeira *et al.* 2009).

There has been an ongoing search for an endodontic sealer that meets the ideal physicochemical properties of sealing, radiopacity, setting time and flow as well as tissue tolerance over the last few years (Duarte *et al.* 2010, Holland & Souza 1985). Sealapex is an endodontic sealer containing calcium oxide which has the capacity to stimulate the formation of mineralized tissue (Holland & Souza 1985). Sealer 26 is an endodontic sealer that incorporates calcium hydroxide into a epoxy resin-based sealer (Gomes *et al.* 2004, Nunes *et al.* 2008, Demiryürek *et al.* 2010). AH Plus is an epoxy resin-based sealer with good physicochemical properties (Kopper *et al.* 2003, Solano *et al.* 2005, Grecca *et al.* 2009, Demiryürek *et al.* 2010) and good adhesion (Sousa-Neto *et al.* 2005, Nunes *et al.* 2008).

The compatibility among different materials used in endodontic therapy with luting material to cement fibre posts is an important aspect to be considered for a successful restoration of root treated teeth. Depending on its composition, the endodontic sealer might interfere with the bonding of a post to root dentine (Hagge *et al.* 2002, Muniz & Mathias 2005, Baldissara *et al.* 2006, Menezes *et al.* 2008, Teixeira *et al.* 2008, Demiryürek *et al.* 2010, Cecchin *et al.* 2011,

Dimitrouli *et al.* 2011). Moreover, the quality of bonding to root dentine may be affected by the density and orientation of dentine tubules at different levels of the root canal walls (Ferrari *et al.* 2000, Goracci *et al.* 2004) and the real access to the root canal thirds (Ferrari *et al.* 2000, Ferrari *et al.* 2001, Goracci *et al.* 2004, Wang *et al.* 2008, da Cunha *et al.* 2010).

The aim of this study was to evaluate the effects of the endodontic sealer and artificial accelerated aging of specimens on bond strength of adhesively cemented fibreglass posts in root canal of bovine teeth, depending on the depth of the root canal. The hypothesis tested was that (i) the chemical composition of endodontic sealers and (ii) the artificial accelerated aging of specimens influence on fibreglass post bond strength to root dentine, depending on the depth of the root canal.

Materials and methods

From among 300 freshly extracted bovine incisor teeth, 80 were selected (approved by the Federal University of Goiás Ethics Committee, # 256/10). The selection criterion used was the similarity of the external and internal anatomical morphology and a root canal diameter smaller than 1mm. Roots 15 mm in length were produced after removing the coronal portion. Roots were randomly divided into eight groups (n=10), two controls and six experimental groups resulting from the interaction between two study factors: endodontic sealer (SX- Sealapex, Kerr Corporation, Orange, EUA; S26- Sealer 26, Dentsply Maillefer, Petrópolis, RJ, Brazil; AH- AH Plus, Dentsply DeTrey GmbH, Konstanz, Germany) and specimens aging (Immediate, test with no aging; Mediate, test performed after artificial accelerated aging - 2 months of water storage at 37°C). Root canals were prepared 1 mm from the apex by the crown-down technique with size # 1,2 Gates Glidden burs (Dentsply Maillefer) (WL 10mm) and K3 Nickel-Titanium instruments (SybronEndo, Optimum, São Paulo, SP, Brazil) in the following sequence: # 25 .10 taper (WL 10mm); # 15-25 .02 taper (WL 14mm); # 25 .04 taper (WL 14mm); # 25 .06 taper (WL 14mm); # 30-45 .02 taper (WL 14mm). During instrumentation, at each change of file, root canals were irrigated with 2

mL of 1% sodium hypochlorite (NaOCl) (Fitofarma, Goiânia, GO, Brazil). In all groups, 3 mL of 17% EDTA (Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brazil) was used for 5 min to remove the smear layer. The final irrigation was performed with 5 mL of 1% NaOCl (Fitofarma).

In control groups (immediate and mediate control group), after root canal preparation, endodontic filling was not performed. In the six experimental groups, roots canals were dried with absorbent paper points and filled with gutta-percha (Dentsply Maillefer) and the specific endodontic sealer prepared and used according to manufacturers' instructions, using the lateral compaction technique. After root filling, root canal openings were filled with glass ionomer cement (Vidrion R; SSWhite, Rio de Janeiro, RJ, Brazil) and samples were stored in distilled water at 37°C for 24 h. After 24 h, the filling material was removed with Gates Glidden burs to a depth of 10 mm with 5 mm of apical seal maintained.

In all groups post space was prepared using size # 3-5 Largo burs (Dentsply Maillefer) (WL 10mm) corresponding to the fibreglass parallel-sided and serrated post 1.5-mm-diameter (Reforpost # 3; Angelus, Londrina, PR, Brazil). Root canals were irrigated, at each change of burs and after the preparation, with 2 mL of 1% NaOCl and dried with absorbent paper points. All roots were covered externally with utility wax to avoid lateral polymerization (Soares *et al.* 2012).

Fibreglass posts were cleaned with 70% alcohol, then in a single application using a microbrush, and after drying, a silane agent was applied for 1 min (Silano; Angelus). The self-adhesive resin cement (RelyX U100; 3M-ESPE, St. Paul, MN, USA) was manipulated in accordance with the manufacturer's instructions, introduced into the canal with K-File and placed on the post. The post was seated to full depth by finger pressure. Excess cement was removed after 1 min. After 5 min, the resin cement was light-cured with 1200 mW cm⁻² (Radii-Cal; SDI, Bayswater, Australia) for 40 s each on the cervical face of the root, in the direction of the long axis of the root, and obliquely to the buccal and lingual surfaces, totalling 120 s. The interface post-cement-dentine on the

cervical face was then sealed with composite resin and the roots were stored in distilled water at 37°C for 24h. After 24 h, each root was serial sectioned perpendicular to its long axis with a double face diamond disc (4" Diameter x 0.012" Thickness x 1/2" Arbor, Extec, Enfield, CT, USA) at low speed under water cooling (Isomet 1000, Buehler, Lake Bluff, IL, USA) to obtain two slices, approximately 1 mm thick, of each root third (cervical, middle and apical), totalling six slices per root.

In immediate groups, slices were submitted immediately (with no aging) to push-out test, while in mediate groups, slices were stored in distilled water at 37°C for 2 months (artificial accelerated aging) prior to testing. The push-out test was performed in a testing machine (EMIC DL 2000, São José dos Pinhais, PR, Brazil) by applying a compressive load at 0.5 mm min⁻¹ in the apical-coronal direction until post segment extrusion. The bond strength was calculated in MPa by dividing the load at failure (in N) by the area of the bonded interface. The area of the bonded interface was calculated as follows: $A = 2\pi rh$, where A is the area of the bonded interface, $\pi = 3.14$, r is the radius of post segment (mm) and h is the thickness of the post segment (mm) (Goracci *et al.* 2004, Soares *et al.* 2008, Soares *et al.* 2012).

Statistical analysis was performed using SAS statistical program (Institute Inc., Cary, NC, USA, Release 9.2). Data was submitted to the Shapiro-Wilk test of normality. The effects, on bond strength, of endodontic sealers and artificial accelerated aging of specimens were analysed using One-way analysis of variance in a split-plot arrangement, with the plot represented by the endodontic sealer or the aging, and the sub-plot represented by the root canal thirds (cervical, middle and apical). Multiple comparisons were made using Tukey's test ($\alpha = 0.05$). Comparisons with control groups (immediate control and mediate control) were made using Dunnet test ($\alpha = 0.05$).

To determine the failure modes, all fractured specimens were air dried and analysed under a confocal laser scanning microscope (Carl Zeiss Laser Scanning Systems, LSM510, META, Oberkochen, Germany). Images were analyzed using Zeiss LSM Image Browser (META, Germany). The failure modes

were classified into six types: (I) adhesive between the post and resin cement; (II) adhesive between resin cement and intraradicular dentine; (III) cohesive in cement; (IV) cohesive in dentine; (V) cohesive in post (Castellan *et al.* 2010) and (VI) mixed, among post, resin cement and intraradicular dentine (Bitter *et al.* 2009).

Results

One-way analysis of variance (endodontic sealer) with sub-plot (root canal third) of immediate groups and mediate groups (aging prior to testing) showed significant difference only for root canal third ($P < .001$). The mean push-out bond strengths and standard deviations are shown in Tables 1,2. Significant reduction on bond strength values from the cervical to apical third, regardless of the endodontic sealer was found.

Comparisons with immediate and mediate control groups are presented in Tables 1 and 2, respectively. Dunnet test showed that the use of an endodontic sealer resulted on lower bond strength values than control groups (without filling), with statistically significant difference in middle ($P = .0294$) and apical thirds ($P = .0207$) of groups tested immediately (Table 1), and in cervical ($P = <.0001$), middle ($P = <.0001$) and apical ($P = <.0001$) thirds of groups tested after aging (Table 2).

One-way analysis of variance (artificial accelerated aging) with sub-plot (root canal region) for each endodontic sealer (SX, S26, AH) showed significance only for root canal third ($P = <.001$). The mean micropush-out bond strengths and standard deviations for each endodontic sealer are in Table 3. Tukey's test revealed a significant reduction on bond strength values from cervical to apical third, regardless of the artificial aging.

Failure modes of tested specimens and its distribution are presented in Figure 1 and Table 4, respectively. The prevalence of (II) adhesive cement-dentine failure was verified in all groups.

Discussion

The retention of fibreglass post luted with self-adhesive resin cement to root dentine was influenced by the endodontic sealer and the depth of root canal, but not by the artificial accelerated aging. The tested hypothesis was partially accepted.

Various mechanical methods have been used to measure the bond strength of a fibreglass post to intraradicular dentine, as microtensile bond strength and push-out tests (Soares *et al.* 2008), however the push-out laboratory test would seem to be safer than the microtensile test (Goracci *et al.* 2004) and a more appropriate methodology for the evaluation of fibreglass posts bonded to intraradicular dentine (Soares *et al.* 2008).

When endodontic sealers are introduced in root canal and obturation forces are applied, it is likely that endodontic sealer constituents penetrate into the dentinal tubules (Boone *et al.* 2001, Shokouhinejad *et al.* 2011). Endodontic sealer penetration increases the interface between material and dentinal walls, what may improve the mechanical retention of the material by interlocking of the endodontic sealer plug inside the tubules and potentially reduces leakage (Mamootil & Messer 2007, Shokouhinejad *et al.* 2011). Furthermore, the endodontic sealer's antibacterial activity can be effective for killing and entombing bacteria that remain inside the dentinal tubules (Heling & Chandler 1996). The ability of the endodontic sealer to penetrate dentinal tubules consistently and effectively is one of many factors influencing the choice of material for root canal filling (Mamootil & Messer 2007, Shokouhinejad *et al.* 2011).

The use of EDTA, that is able to act on tooth mineral matrix and promote removal of the smear layer formed during root canal preparation, allows a better penetration of endodontic sealers into the dentinal tubules, increasing the contact surface of the filling material with dentine (Nunes *et al.* 2008). The mechanical removal of the endodontic sealer, impregnated in the root dentine, during post-space preparation is a critical step in achieving optimum post retention when resin cement is used (Boone *et al.* 2001). In the present study

there was a significant decrease on post retention for all endodontic sealers compared to control groups, in which no endodontic sealer was used, what can suggest that post-space preparation may not have removed all endodontic sealer-impregnated in dentine; not ensuring a “freshened” surface in which resin cement could bond (Boone *et al.* 2001).

In agreement with this finding, previous study revealed that fibre post bond strength in the group without root canal filling was higher compared to the other groups that use gutta-percha/AH Plus, gutta-percha/Guttaflow and pre-existing root canal filling (Dimitrouli *et al.* 2011). Other study showed that endodontic sealers (AH Plus, Endofill and Sealapex) have significant negative effects on fibreglass post bond strength (Demiryürek *et al.* 2010). In contrast, other study reported that AH Plus and Sealer 26 did not interfere on the adhesive bond of fibreglass posts compared to control group that used only gutta-percha points (Cecchin *et al.* 2011). Menezes *et al.* (2008) also did not find significant difference between control group (unfilled) and groups filled with Sealer 26.

The action of drills used to remove the root filling material to create post space produces a new smear layer rich in endodontic sealer and gutta-percha remnants plasticized by the friction heat of the drill (Serafino *et al.* 2004, Demiryürek *et al.* 2010). Independently from the type of endodontic sealer, this smear layer can act as insulation against any kind of adhesive material intended to bond to intraradicular dentine (Dimitrouli *et al.* 2011). Moreover, in the present study, root canals were irrigated after post preparation only with 1% NaOCl that acts selectively on the removal of organic particles (Byström & Sundqvist 1981, Spanó *et al.* 2009, Estrela *et al.* 2002) and cannot dissolve inorganic particles (Garberolio & Becce 1994, Cecchin *et al.* 2010). Therefore, it does not effectively remove the smear layer formed on root canal walls after post mechanical preparation (Nunes *et al.* 2008), what may reduce the penetration and chemical action of the self-adhesive resin cement (RelyX U100, 3M-ESPE).

The influence of canal depth was observed as the bond strength decreased from the cervical to apical third in all groups. The explanation for this

result can be attributed to various factors such as the difficulty of visualization and access to the apical part of the root canal system (de Durão Mauricio *et al.* 2007, Onay *et al.* 2010), the difficulty to eliminate the smear layer or remaining endodontic sealer/gutta-percha covering dentine walls in this third (Serafino *et al.* 2004), the less dense dentinal tubule configuration in the apical portion of the root canal system (Ferrari *et al.* 2000, Ferrari *et al.* 2001, Wang *et al.* 2008, da Cunha *et al.* 2010), the cavity configuration factor (Goracci *et al.* 2004, Tay *et al.* 2005) and restrictions in the flow of the resin cement to this part of the root canal (de Durão Mauricio *et al.* 2007, da Cunha *et al.* 2010). All these factors compromise the interfacial continuity (Silva *et al.* 2011) and, consequently, the bond strength.

The predominance of adhesive cement-dentine failures in all groups observed in the present study (Table 4) may be attributed to the difficulty to eliminate the smear layer or remaining endodontic sealer/gutta-percha covering dentine walls (Serafino *et al.* 2004, Silva *et al.* 2011, Demiryürek *et al.* 2010) and to the reduced ability of dentine demineralization and hybridization of the self-adhesive cement RelyX U100 (De Munck *et al.* 2004, Al-Assaf *et al.* 2007, Monticelli *et al.* 2008, Bitter *et al.* 2009).

Limitations of the present study include the fact that the specimens were not submitted to thermal and mechanical cycling, in order to simulate intra-oral situations more precisely. Further studies need to be conducted to analyse the effects of the use of EDTA, after post space preparation, on the bond strength to intraradicular dentin at each third of the root canal.

Conclusions

Endodontic sealers, Sealapex, Sealer 26 and AH Plus, interfered negatively on bonding of fibreglass posts luted with self-adhesive resin cement to intraradicular dentine irrespective of root depth.

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Table 1 Bond strength means in MPa (standard deviation) of groups tested immediately (no aging) and statistical categories defined by Tukey's test and Dunnet test (n = 10).

Endodontic sealer	Root canal third		
	Cervical	Middle	Apical
Immediate control	13.5 (2.1)	11.4 (3.3)	9.3 (4.8)
Sealapex	10.3 (2.0) ^{Aa}	7.4 (2.0) ^{*Ba}	5.4 (1.3) ^{*Ba}
Sealer 26	13.8 (6.6) ^{Aa}	8.2 (4.2) ^{*Ba}	3.6 (4.7) ^{*Ca}
AH Plus	12.9 (2.5) ^{Aa}	8.1 (2.6) ^{*Ba}	4.8 (3.0) ^{Ca}

Capital letters were used to compare groups in the horizontal lines and lower-case letters were used to compare groups in the vertical lines. Tukey categories with same letters are not statistically significant from each other ($P < 0.05$).

The (*) indicate significant difference compared with control group by Dunnet test ($P < 0.05$).

Table 2 Bond strength means in MPa (standard deviation) of groups tested after water artificial accelerated aging and statistical categories defined by Tukey's test and Dunnet test (n = 10).

Endodontic sealer	Root canal third		
	Cervical	Middle	Apical
Mediate control	25.1 (6.4)	22.6 (6.1)	21.6 (6.3)
Sealapex	10.9 (5.1) ^{*Aa}	7.5 (1.5) ^{*ABa}	5.5 (4.0) ^{*Ba}
Sealer 26	14.0 (1.9) ^{*Aa}	8.4 (5.0) ^{*Ba}	3.5 (3.9) ^{*Ca}
AH Plus	13.3 (3.4) ^{*Aa}	9.2 (3.8) ^{*Ba}	4.9 (4.0) ^{*Ca}

Capital letters were used to compare groups in the horizontal lines and lower-case letters were used to compare groups in the vertical lines. Tukey categories with same letters are not statistically significant from each other ($P < 0.05$).

The (*) indicate significant difference with control group by Dunnet test ($P < 0.05$).

Table 3 Bond strength means in MPa (standard deviation) as a result of artificial accelerated aging and root canal third, and statistical categories defined by Tukey's test for each endodontic sealer (n = 10).

Endodontic sealer	Test moment	Root canal third		
		Cervical	Middle	Apical
Sealapex	Immediate	10.3 (2.0) ^{Aa}	7.4 (2.0) ^{ABa}	5.4 (1.3) ^{Ba}
	Mediate	10.9 (5.1) ^{Aa}	7.5 (1.5) ^{ABa}	5.5 (4.0) ^{Ba}
Sealer 26	Immediate	13.8 (6.6) ^{Aa}	8.2 (4.2) ^{Ba}	3.6 (4.7) ^{Ca}
	Mediate	14.2 (1.9) ^{Aa}	8.4 (5.0) ^{Ba}	3.5 (3.9) ^{Ca}
AH Plus	Immediate	12.9 (2.5) ^{Aa}	8.08 (2.6) ^{Ba}	4.8 (3.0) ^{Ba}
	Mediate	13.3 (3.4) ^{Aa}	9.12 (3.8) ^{Ba}	4.9 (4.0) ^{Ca}

In each sealer, capital letters were used to compare values in the horizontal lines and lower-case letters were used to compare values in the vertical lines. Tukey categories with same letters are not statistically significant from each other ($P < 0.05$).

Table 4 Failure modes (%) for experimental groups.

Groups	Adhesive: post and cement (I)	Adhesive: cement and dentine (II)	Cohesive: Cement (III)	Cohesive: Dentine (IV)	Cohesive: Post (V)	Mixed (VI)
SX immediate	2 (3.3)	35 (58.3)	3 (5.0)	1 (1.7)	1 (1.7)	18 (30.0)
S26 immediate	3 (5.0)	31 (51.7)	6 (10.0)	-	3 (5.0)	17 (28.3)
AH immediate	3 (5.0)	26 (43.3)	9 (15.0)	1 (1.7)	1 (1.7)	20 (33.3)
SX mediate	2 (3.3)	42 (70.0)	7 (11.7)	-	1 (1.7)	8 (13.3)
S26 mediate	-	33 (55.0)	2 (3.3)	-	4 (6.7)	21 (35.0)
AH mediate	1 (1.7)	44 (73.3)	3 (5.0)	1 (1.7)	-	11 (18.3)

SX, Sealapex; S26, Sealer 26; AH, AH Plus; Immediate, test with no aging; Mediate, test performed after 2 months of water storage at 37°C.

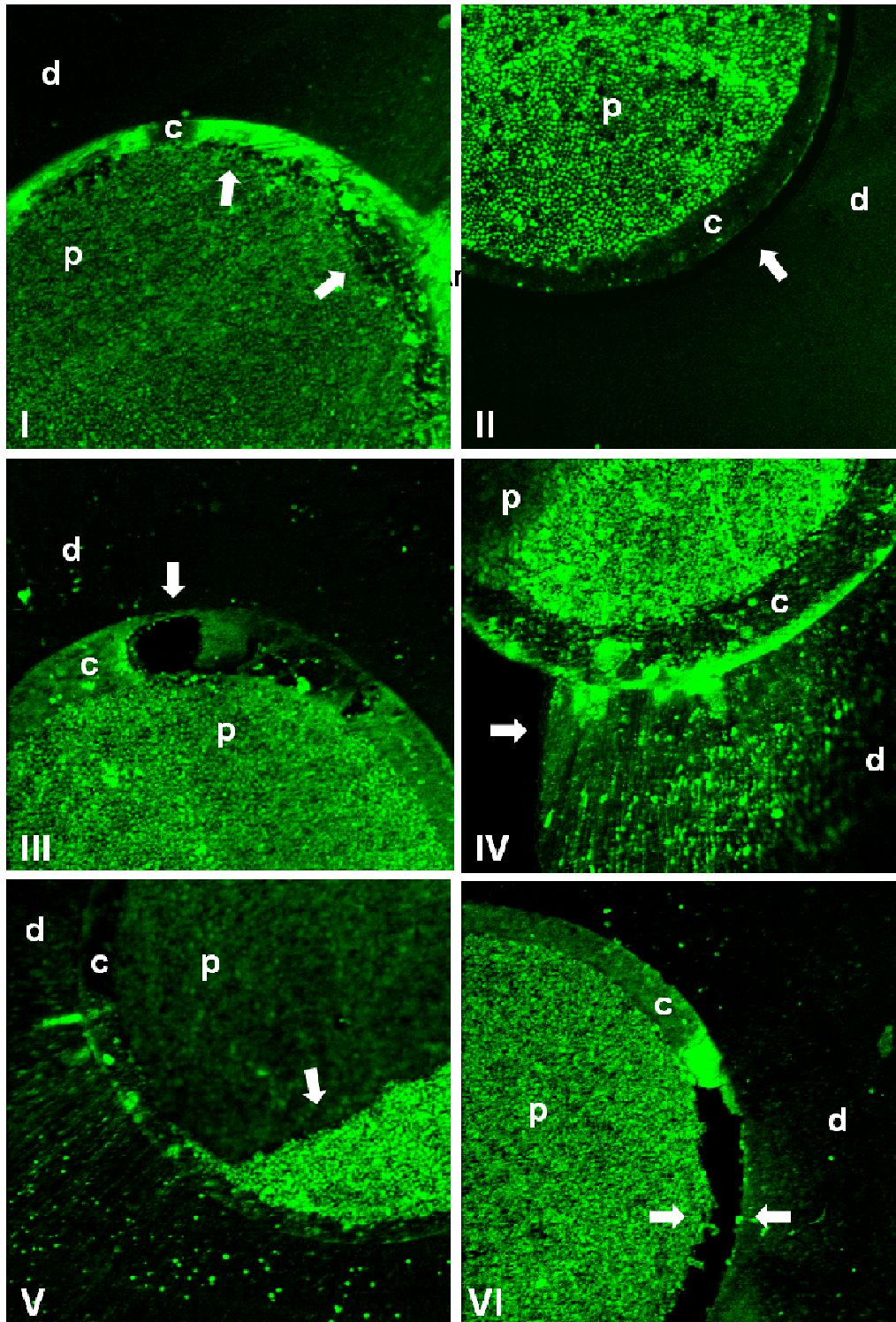


Figure 1. *d* – dentine; *c* – resin cement; *p* – fibreglass post. Microscopic images of failure modes after micropush-out test (magnification 10X): (I) adhesive between the post and resin cement; (II) adhesive between resin cement and intraradicular dentine; (III) cohesive in cement; (IV) cohesive in dentine; (V) cohesive in post; (VI) mixed among post, resin cement and intraradicular dentine.

9. Anexos

Anexo 1. Publicações no triênio 2010/2011/2012.

Artigos científicos publicados em periódicos internacionais, com corpo editorial:

- 1) Castro CG, Santana FR, Roscoe MG, Simamoto PC Jr, Santos-Filho PC, Soares CJ. Fracture resistance and mode of failure of various types of root filled teeth. *Int Endod J.* 2012 Mar 8.
- 2) Santana FR, Castro CG, Simamoto-Júnior PC, Soares PV, Quagliatto PS, Estrela C, Soares CJ. Influence of post system and remaining coronal tooth tissue on biomechanical behaviour of root filled molar teeth. *Int Endod J.* 2011 May;44(5):386-94.
- 3) Naves LZ, Santana FR, Castro CG, Valdivia AD, Da Mota AS, Estrela C, Correr-Sobrinho L, Soares CJ. Surface treatment of glass fiber and carbon fiber posts: SEM characterization. *Microsc Res Tech.* 2011 Dec;74(12):1088-92.
- 4) Soares CJ, Roscoe MG, Castro CG, Santana FR, Raposo LH, Quagliatto PS, Novais VR. Effect of gamma irradiation and restorative material on the biomechanical behaviour of root filled premolars. *Int Endod J.* 2011 Nov; 44(11):1047-54.
- 5) Soares CJ, Barbosa LM, Santana FR, Soares PB, Mota AS, Silva GR. Fracture strength of composite fixed partial denture using bovine teeth as a substitute for human teeth with or without fiber-reinforcement. *Braz Dent J.* 2010;21(3):235-40.

Artigos científicos enviados para publicação em periódicos nacionais e internacionais, com corpo editorial:

- 1) Renovato SR, Santana FR, Ferreira JM, Souza JB, Soares CJ, Estrela C. Influence of Calcium Hydroxide and Endodontic Irrigants on Fibreglass Post Bond Strength to Root Canal Dentine. *International Endodontic Journal.*
- 2) Ferreira JM, Santana FR, Renovato SR, Soares CJ, Estrela C. Effect of internal bleaching agents on fibreglass post bond strength to root dentin. *International Endodontic Journal.*
- 3) Soares CJ, Valdivia ADCM, Silva GR, Santana FR, Menezes MS. Longitudinal clinical evaluation of post systems: A literature review. *Brazilian Dental Journal.*
- 4) Fabiana Ribeiro Santana, Fernanda Ribeiro Santana, Giselle Vieira dos Anjos, Thiago Vieira Campos, Patrícia Carla Teixeira Lima, Mayara Maia Lopes, Rafaela Pereira de Lima, Normalene Sena de Oliveira, Claci Fátima Weirich, Cinira Magali Fortuna. Ações de saúde na estratégia saúde da família à luz da integralidade: município goiano. *Revista Eletrônica de Enfermagem.*

Trabalhos concluídos em fase de elaboração de artigo científico para publicação em periódico internacional, com corpo editorial:

- 1) Santana FR, Castro CG, Naves LZ, Valdivia ADCM, Mota AS, Estrela C, Soares CJ. Push-out Bond strength between glass fibre and carbon fibre posts with composite resin core: effect of post surface treatments. *Operative Dentistry.*
- 2) Guimarães RM, Santana FR, Silveira RE, Moreira FCL, Estrela C, Torres EM, Fonseca RB, Lopes LG, Souza JB. Influence of surface treatment on bond strength of repair resin-based silorane: analysis after aging. *Operative Dentistry.*
- 3) Silveira RE, Santana FR, Guimarães RM, Moreira FCL, Estrela C, Torres EM, Fonseca RB, Lopes LG, Souza JB. Influence of surface treatment on bond strength of repair resin-based silorane: immediate analysis. *Operative Dentistry.*

Anexo 2. Normas de publicação do periódico

INTERNATIONAL ENDODONTIC JOURNAL

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

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

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

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

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

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Clinical trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A [CONSORT checklist](#) should also be included in the submission material.

The International Endodontic Journal encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries: www.clinicaltrials.gov, <http://clinicaltrials.ifpma.org/clinicaltrials/>, <http://isrctn.org/>. The clinical trial registration number and name of the trial register will then be published with the paper.

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

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5. MANUSCRIPT FORMAT AND STRUCTURE

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

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

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- **Methodology:** Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.
- **Results:** Give the main results of the study, including the outcome of any statistical analysis.
- **Conclusions:** State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

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

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

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

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- **Summary:** Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and analysis if any.
- **Key learning points:** Provide up to 5 short, bullet-pointed statements to highlight the key messages of the report. All points must be fully justified by material presented in the report.

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- **Results:** Give the main results of the study.
- **Conclusions:** State the primary conclusions of the study.

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

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Conclusion: should contain a summary of the findings.

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

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

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

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

- (i) Names and initials of up to six authors. When there are seven or more, list the first three and add *et al.*
- (ii) Year of publication in parentheses
- (iii) Full title of paper followed by a full stop (.)
- (iv) Title of journal in full (in italics)
- (v) Volume number (bold) followed by a comma (,)
- (vi) First and last pages

Examples of correct forms of reference follow:

Standard journal article

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Corporate author

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

Journal supplement

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

Books and other monographs

Personal author(s)

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

Chapter in a book

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

Published proceedings paper

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

Agency publication

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

Dissertation or thesis

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

URLs

Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.

Smith A (1999) Select committee report into social care in the community [WWW document]. URL <http://www.dhss.gov.uk/reports/report015285.html> [accessed on 7 November 2003]

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

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7 Guidelines for reporting of DNA microarray data

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Prospective authors are also encouraged to search for previously published microarray data with relevance to their own data, and to report whether such data exists. Furthermore, they are encouraged to use the previously published data for qualitative and/or quantitative comparison with their own data, whenever suitable. To fully acknowledge the original work, an appropriate reference should be given not only to the database in question, but also to the original article in which the data was first published. This open approach will increase the availability and use of these large-scale data sets and improve the reporting and interpretation of the findings, and in increasing the comprehensive understanding of the physiology and pathology of endodontically related tissues and diseases, result eventually in better patient care.

Anexo 3. Parecer do Comitê de Ética em Pesquisa - UFG



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Goiânia, 27/ 09/10

**PARECER CONSUBSTANCIADO REFERENTE AO PROJETO DE PESQUISA,
PROTOCOLADO NESTE COMITÊ SOB O N.: 256/10**

I – Identificação

- Título do projeto: Influência da terapia endodôntica na resistência adesiva de pinos de fibra à dentina radicular
- Pesquisador Responsável: Carlos Estrela
- Pesquisadores participantes: Fernanda Ribeiro Santana; Lawrence Gonzaga Lopes;
- Instituição onde será realizado o estudo: Faculdade de Odontologia - UFG
- Data de apresentação ao CEP/UFG: 02/09/10
- Área Temática: Grupo III, Ciência da Saúde - Odontologia

Comentários do relator frente à Resolução CNS 196/96 e complementares em particular sobre:

II – Estrutura do Protocolo

CD; Ficha de protocolo do projeto de pesquisa com assinatura do responsável pela pesquisa e do responsável pela instituição; Declaração de disponibilidade e viabilidade de infra-estrutura e equipamento; Termo de responsabilidade assinado por todos os componentes da pesquisa; TCLE da doação dos dentes bovinos do frigorífico JBS- Friboi; Certidão de ATA do Conselho Diretor da FO-UFG de aprovação da pesquisa. Projeto de Pesquisa.

III – Projeto de pesquisa

OBJETIVO GERAL: Analisar diferentes parâmetros da terapia endodôntica que podem exercer influência na resistência adesiva de retentores intra-radulares de fibra à dentina radicular. Em Específico: - Avaliar a influência dos materiais e técnicas utilizados na terapia endodôntica na resistência adesiva de pinos de fibra à dentina intra-radicular, em função dos diferentes terços do canal radicular, por meio de ensaio mecânico de *micropush-out* e análise em MEV, variando: 1) - Técnica de preparo do canal radicular: Preparo com instrumentos de aço inoxidável e com níquel-titânio. 2)- Sanificação do canal radicular com Hipoclorito de sódio 2,5%; Clorexidina 2%; Gás ozônio, EDTA 17%; 3) Obturação do canal radicular utilizando: Cimento contendo óxido de cálcio (Sealapex); contendo óxido de zinco e eugenol (EndoFill) e à base de resina epóxica (AH Plus).

Análise das questões éticas. Nesta pesquisa não será realizada nenhuma intervenção no animal. **Serão utilizados apenas dentes bovinos que serão extraídos de mandíbulas provenientes de animais que já foram abatidos em frigorífico.** Assim, o abate destes animais se dá por razões que não as envolvidas nesta pesquisa e, para a extração dos dentes, os pesquisadores terão contato apenas com as mandíbulas provenientes dos animais já abatidos no frigorífico. Utilizar-se-á 220 dentes incisivos inferiores bovinos. O projeto será desenvolvido de forma seqüencial e as raízes distribuídas aleatoriamente nos grupos.

- Quanto a Descrição do alojamento/alimentação e hidratação/temperatura/umidade, não se aplica, pois não será realizada nenhuma intervenção no animal, uma vez que os dentes bovinos a serem utilizados serão provenientes de mandíbulas de animais já abatidos em frigorífico, cuja carne é destinada ao consumo humano.

- Os dentes bovinos serão armazenados em solução aquosa tamponada de timol a 0,2% após a extração. Os dentes serão limpos e a parte coronária será removida sob refrigeração em água, a fim de permanecer remanescente radicular de 15mm a partir da porção apical de cada raiz

- Considerações sobre o sofrimento imposto aos animais: Não se aplica

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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO



COMITÊ DE ÉTICA EM PESQUISA

- Descrição do método de eutanásia e destino dos animais após a experimentação: Não se aplica a esta pesquisa, uma vez que para a extração dos dentes bovinos os pesquisadores terão contato apenas com as mandíbulas provenientes de animais já abatidos no frigorífico. Assim, o próprio frigorífico possui os seus protocolos de abate e destino de resíduos.

- Riscos aos pesquisadores/alunos (físicos, biológicos, psicológicos, sociais): Para a obtenção dos dentes existirão riscos físicos e biológicos, uma vez que terão contato com material biológico e utilizarão instrumentos perfuro-cortantes. Assim, para a extração, atenderão aos princípios de biossegurança por meio da utilização de barreiras ou equipamentos de proteção individual (luvas, avental impermeável, gorro, máscara e óculos de proteção), com o objetivo de prevenir a exposição ao sangue, e tomarão os devidos cuidados para prevenir acidentes com os instrumentos perfuro-cortantes. Os instrumentos necessários serão previamente esterilizados. Ressalta-se que os pesquisadores envolvidos são cirurgiões-dentistas, sendo assim capacitados para a realização das extrações dentárias nas mandíbulas reservadas pelo frigorífico. As extrações serão realizadas em ambiente reservado no próprio frigorífico com supervisão do veterinário, e, após a coleta dos dentes bovinos, as mandíbulas permanecerão no frigorífico.

- Análise do Termo de consentimento dos responsáveis por animais: Segue assinado por Osvaldo Caetano de Abreu, Fiscal Federal Agropecuário, Médico Veterinário CRMV-GO 0328 a autorização para extração dos dentes bovinos junto ao frigorífico acima mencionado.

- Cronograma: Adequado

- Análise da metodologia e sua adequação aos objetivos da pesquisa: A metodologia encontra-se adequada e relatam que estes dentes serão utilizados exclusivamente neste experimento

- Verificação das condições para realização da pesquisa. Condições adequadas e os currículos dos pesquisadores são compatíveis com a pesquisa proposta.


V- Parecer do CEP

Protocolo "APROVADO":

VI - Data da reunião: 27/09/2010

Assinatura do(a) relator(a):

Assinatura do(a) Coordenador(a)/ CEP/UFG:


Prof. João Carlos de Rocha Medrado
Coordenador do Comitê de Ética em Pesquisa
Pró-Reitoria de Pesquisa e Pós-graduação/UFG