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CENTRO DE CIÊNCIAS DA SAÚDE
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ODONTOLÓGICAS**

**EFEITO DE SOLUÇÕES ATIVADAS POR
ULTRASSOM NA RESISTÊNCIA ADESIVA DE PINOS
DE FIBRA DE VIDRO CIMENTADOS COM CIMENTO
RESINOSO AUTOADESIVO**

DISSERTAÇÃO DE MESTRADO

Mirela Sangoi Barreto

Santa Maria, RS, Brasil

2015

**EFEITO DE SOLUÇÕES ATIVADAS POR ULTRASSOM NA
RESISTÊNCIA ADESIVA DE PINOS DE FIBRA DE VIDRO
CIMENTADOS COM CIMENTO RESINOSO AUTOADESIVO**

Mirela Sangoi Barreto

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, ênfase em Endodontia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Ciências Odontológicas**

Orientador: Prof. Dr. Carlos Alexandre Souza Bier

Santa Maria, RS, Brasil

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**Universidade Federal de Santa Maria
Centro de Ciências da Saúde
Programa de Pós-Graduação em Ciências Odontológicas**

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elaborada por

Mirela Sangoi Barreto

como requisito parcial para obtenção do grau de
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“Se você quer transformar o mundo, experimente primeiro promover o seu aperfeiçoamento pessoal e realizar inovações no seu próprio interior”.

(Dalai Lama)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Odontológicas
Universidade Federal de Santa Maria

EFEITO DE SOLUÇÕES ATIVADAS POR ULTRASSOM NA RESISTÊNCIA ADESIVA DE PINOS DE FIBRA DE VIDRO CIMENTADOS COM CIMENTO RESINOSO AUTOADESIVO

AUTOR: MIRELA SANGOI BARRETO

ORIENTADOR: CARLOS ALEXANDRE SOUZA BIER

Data e Local da Defesa: Santa Maria, 07 de Julho de 2015

O presente estudo tem por objetivo avaliar o efeito de diferentes soluções irrigantes e quelantes na resistência adesiva de pinos de fibra de vidro cimentados com cimento autoadesivo RelyX U200. 60 pré-molares inferiores extraídos de humanos, padronizados em 14 mm, foram preparados com o sistema ProTaper Universal até o instrumento F3, obturados com cimento AH Plus e cones F3 do mesmo sistema. Os espécimes foram desobturados parcialmente, restando 4 mm apicais de material obturador e realocados em quatro grupos experimentais e um controle (n=12), de acordo com a solução de tratamento de superfície dentinária prévia à cimentação dos pinos: EDTA 17% (EDTA), QMix (QM), SmearClear (SC), Soro Fisiológico (SS) e NaOCl 2,5% (NaOCl). As soluções foram ativadas por ultrassom em três etapas, durante 20 segundos cada e os canais foram secos com pontas de papel absorventes. Os pinos de fibra de vidro do Sistema Exacto Translúcido N2 foram cimentados com RelyX U200. Em um espécime por grupo foi incorporada Rodamina B ao cimento autoadesivo como marcador fluorescente para análise em Microscopia Confocal à laser (MECL). Foram seccionados 3slices por espécime, sendo 1 slice por terço radicular, e submetidos ao teste de *push-out* na máquina de ensaio universal EMIC. Estereomicroscópio e MECL foram utilizados para analisar o padrão de falha dos espécimes e o padrão de penetração do cimento autoadesivo nos túbulos dentinários, respectivamente. A média de resistência adesiva de cada espécime foi calculada. Testes ANOVA e Bonferroni foram utilizados para analisar as diferenças entre os grupos. SS demonstrou a maior média de resistência adesiva, sendo superior aos grupos QM, SC ($P < .05$). NaOCl apresentou valores intermediários de resistência adesiva, similares ao EDTA e SS ($P > .05$). QM e SC apresentaram as menores médias de resistência adesiva ($P < .05$). Pode-se concluir que o uso de SS e NaOCl parece ser adequado para lavagem do canal prévia à cimentação de pinos de fibra de vidro, com uso de cimento resinoso autoadesivo RelyX U200. O uso de soluções quelantes como EDTA, QM e SC parece interferir negativamente nos valores de resistência adesiva.

Palavras-chave: Endodontia. Resistência adesiva. Pinos de fibra de vidro. Cimento autoadesivo. Soluções irrigadoras.

ABSTRACT

Master Course Degree
Post Graduate Program in Dental Science
Federal University of Santa Maria

EFFECT OF INTRACANAL IRRIGANTS ON BOND STRENGTH OF FIBER POSTS CEMENTED WITH A SELF-ADHESIVE RESIN CEMENT

AUTHOR: MIRELA SANGOI BARRETO
ADVISER: CARLOS ALEXANDRE SOUZA BIER
Defense Place and Date: Santa Maria, July 7th, 2015

The current study aimed to evaluate the effect of irrigating and chelating solutions on bond strength of fiber glass posts cemented with RelyX U200. 60 single-rooted premolars were sectioned at 14 mm from the apex, prepared with ProTaper Universal system until F3 instrument and filled with F3 master cone and AH Plus sealer. Root canal filling was partially removed, remaining 4 mm of gutta-percha at the apical third. Specimens were randomly divided into 5 experimental groups (n=12), according to the solution used before fiber post cementation (dentin surface treatment), as described: EDTA 17% (EDTA); QMix (QM); SmearClear (SC); NaOCl 2.5% (NaOCl) and saline solution as control group (SS). Ultrasonic activation was performed three times, during 20 seconds each and root canals were dried with paper points. Exacto N2 glass fiber posts were cemented with RelyX U200. Rhodamine B dye was mixed to RelyX U200 in one specimen per group, in order to provide fluorescence for confocal laser scanning microscopy (CLSM) assessment. A cutting machine was used for root's sectioning, providing 3 slices, one for each root third. Next, push-out test was performed. Stereomicroscope and CLSM were used to analyze the failure mode and to illustrate the pattern of distribution of RelyX U200 inside dentinal tubules, respectively. Bond strength mean were calculated and ANOVA and Bonferroni tests were used for statistical analysis. SS showed the higher mean of bond strength values, superior to QM, SC ($P < .05$). NaOCl presented intermediary bond strength values, similar to EDTA and SS ($P > .05$). QM and SC showed the lowest mean of bond strength ($P < .05$). In conclusion, SS and NaOCl associated to ultrasonic activation seems to be adequate solutions for root canal cleaning before fiber post cementation with RelyX U200, while chelating solutions, such as EDTA, QMix and SmearClear, should not be used for dentin pre-treatment when cementing with RelyX U200.

Key Words: Endodontics. Bond strength. Glass fiber posts. Self-adhesive resin-cement.

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INTRODUÇÃO GERAL

A restauração de dentes tratados endodonticamente constitui um desafio ao clínico, em função da redução na quantidade de remanescente coronário. Dentre as principais causas de perda de estrutura dentária destacam-se a cárie dentária e os traumatismos (Torbjoner et al. 1995). Devido a esse fato, é comum que seja necessário a cimentação de retentores intrarradiculares nos condutos, quando metade ou mais do remanescente coronário está comprometido (Morgano & Brackett 1999; Boone et al. 2001).

Os pinos de fibra de vidro são amplamente utilizados em função de sua simples técnica de inserção, podendo ser preparado em uma única sessão, por seu baixo custo e por suas vantagens estéticas quando comparados aos pinos metálicos (Malferrari et al. 2003). Além disso, apresentam características biomecânicas similares às da dentina, como seu módulo de elasticidade e rigidez (Ferrari et al. 2000; Malferrari et al. 2003), o que favorece a distribuição mais homogênea das cargas mastigatórias através da estrutura radicular como indicam estudos que utilizaram a análise de elementos finitos (Sorrentino et al. 2007; Spazzin et al. 2007).

A sensibilidade na técnica de cimentação dos pinos de fibra de vidro levou ao desenvolvimento de cimentos resinosos autoadesivos. O principal objetivo da utilização destes cimentos é simplificar a técnica, diminuindo os passos clínicos - como condicionamento ácido, aplicação de primer e adesivo - e consequentemente a chance de erros inerentes à técnica de cimentação (Behr et al. 2004). Alguns estudos demonstraram que a resistência adesiva de pinos de fibra de vidro cimentados com cimentos autoadesivos mostrou-se similar ou até mesmo superior àqueles cimentados com sistema convencional de três passos (Amaral et al. 2011; Munck et al. 2004). Essa resistência adesiva ocorre através de retenção micromecânica na interface cimento/dentina e da interação química entre o monômero e a hidroxiapatita (Gerth et al. 2006).

Recentemente introduzido no mercado, o cimento RelyX U200 (3M ESPE, Saint Paul, MN, EUA) consiste em um cimento resinoso autoadesivo, composto por uma pasta base e uma pasta catalisadora. Segundo o próprio fabricante, este cimento traz um monômero adicional em relação ao RelyX U100 (3M ESPE) e propriedades reológicas modificadas, o que melhoraria sua resistência mecânica, resistência adesiva, escoamento e fluidez. No entanto, são poucos os estudos que avaliaram as propriedades mecânicas deste cimento

isoladamente e em comparação com os demais cimentos da categoria, como RelyX U100 (Rodrigues et al. 2015).

Deve-se considerar também que para que se obtenha adesão satisfatória dos pinos ao canal radicular, é requisito fundamental a remoção da *smear layer* ou lama dentinária, bem como a completa remoção de todos os traços de cimento endodôntico e de guta-percha sobre as paredes do canal (Monaco et al. 2003). A *smear layer* é resultado do processo de instrumentação dos canais radiculares e consiste em uma mistura de substâncias orgânicas e inorgânicas, incluindo raspas de dentina, processos odontoblásticos, proteínas coaguladas e elementos microbianos (Mccomb & Smith 1977). É capaz de prejudicar a penetração e a difusão da medicação intracanal nos túbulos dentinários a adaptação e adesão do material obturador às paredes do canal (Violich & Chandler 2010) e a penetração do sistema adesivo e cimento resinoso nos túbulos dentinários (Ørstavik & Haapasalo 1990).

Nesse sentido, as soluções quelantes são utilizadas com o objetivo de remover a *smear layer*, especialmente antes da colocação da medicação intracanal e da obturação dos canais radiculares (Prado et al. 2013). A solução quelante mais utilizada atualmente é o ácido etilenodiaminotetracético (EDTA) na concentração de 17%, que é capaz de atuar sobre a porção inorgânica da dentina (Kuah et al. 2009), expondo os túbulos dentinários (Bystrom & Sundqvist 1985). Por outro lado, essa solução não apresenta potencial antimicrobiano e seu uso pode causar erosão na dentina intrarradicular (Ordinola-Zapata et al. 2012).

Com o objetivo de aumentar o potencial antimicrobiano, sem afetar a estrutura dentinária radicular, novas soluções quelantes vem sendo estudadas. SmearClear (SybronEndo, West Collins, Orange, CA) é uma solução composta por EDTA e cetrimida. Foi especificamente desenvolvida para a remoção da *smear layer* e para limpeza dos canais radiculares (Silva et al. 2008; De-Deus et al. 2008; Nelson-Filho et al. 2009; Wu et al. 2012). Estudos demonstraram que a agitação desta substância utilizando limas manuais é capaz de remover completamente a *smear layer* de regiões críticas dos canais radiculares como o terço apical (Syedmukhtar-Um-Nisarandrabi et al. 2013).

O QMix (DentsplyMaillefer, Ballaigues, Suíça), por sua vez, apresenta em sua composição EDTA, digluconato de clorexidina e agente surfactante. Este detergente atua diminuindo a tensão superficial, melhorando sua molhabilidade (Wang et al. 2012). O QMix possui potencial antimicrobiano frente ao *Enterococcus faecalis* e possibilita a remoção do *smear layer* (Stojicic et al. 2012). Segundo o fabricante, foi desenvolvido para ser utilizado como irrigação final por 60 à 90 segundos, em substituição ao EDTA, causando menor desmineralização e mantendo a matriz colágena da dentina intacta.

A fim de otimizar a ação das soluções irrigadoras, a irrigação ultrassônica passiva (PUI) pode ser utilizada. A PUI consiste na ativação de uma solução irrigadora, sem que haja contato com as paredes do canal, através de um aparelho ultrassônico que transforma energia elétrica em ondas ultrassônicas. Esse processo altera a pressão hidrostática, formando ondas que eclodem contra as paredes do canal, melhorando a remoção de debris e *smear layer* (Van der Sluis et al. 2007). Estudos que compararam a PUI e irrigação convencional (seringa) demonstraram que a PUI foi mais efetiva na remoção de tecido pulpar, debris dentinários e bactérias planctônicas (Passarinho-Neto et al. 2006).

No que diz respeito aos aspectos metodológicos, o teste de *push-out* é conduzido a fim de testar novos produtos e investigar variáveis experimentais. Este teste é capaz de distribuir o stress de forma mais homogênea, produzindo menor variabilidade nos resultados. Por isso, o teste de *push-out* é recomendado para determinar a resistência adesiva dos pinos de fibra de vidro à dentina radicular (Goracci et al. 2007).

O uso da microscopia vem possibilitando grandes avanços na prática odontológica. A microscopia eletrônica confocal à laser (MECL) produz informações detalhadas à respeito da presença e distribuição de cimentos endodônticos e sistemas adesivos dentro dos túbulos dentinários e em toda a circunferência do canal (D'Alpino et al. 2006; Ordinola-Zapata et al. 2009; Kok et al. 2014), através da incorporação de marcadores fluorescentes - como a Rodamina B - ao cimento endodôntico, cimentos resinosos e sistemas adesivos. De acordo com D'Alpino et al. 2006, a Rodamina B incorporada ao cimento na proporção de 0,1% não interfere nas propriedades físico-químicas dos cimentos endodônticos.

Apesar dos estudos citados, ainda são poucos os trabalhos que avaliaram o efeito do uso de substâncias irrigadoras e quelantes na dentina intrarradicular, no que tange a questão de resistência adesiva entre pinos de fibra de vidro e cimentos resinosos (Gu et al. 2009). Além disso, as informações relacionadas à resistência adesiva de pinos de fibra cimentados com o cimento RelyX U200 ainda são escassas.

1. OBJETIVOS:

OBJETIVO GERAL:

(1) - Avaliar o efeito de diferentes soluções ativadas por ultrassom sobre a resistência adesiva de pinos de fibra de vidro cimentados com cimento resinoso autoadesivo RelyX U200;

Para efeitos de apresentação esta Dissertação intitulada **“Efeito de soluções ativadas por ultrassom na resistência adesiva de pinos de fibra de vidro cimentados com cimento resinoso autoadesivo”** está no formato de artigo científico, submetido à publicação.

ARTIGO:

**EFFECT OF INTRACANAL IRRIGANTS ON BOND STRENGTH OF FIBER POSTS
CEMENTED WITH A SELF-ADHESIVE RESIN CEMENT**

Enviado para publicação no “Operative Dentistry”

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2. ARTIGO

EFFECT OF INTRACANAL IRRIGANTS ON BOND STRENGTH OF FIBER POSTS CEMENTED WITH A SELF-ADHESIVE RESIN CEMENT

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Effect of intracanal irrigants on bond strength of fiber posts cemented with a self-adhesive resin cement

Short title: *Bond strength of fiber posts cemented with RelyX U200*

Clinical significance:

When cementing fiber posts with self-adhesive cement *RelyX U200*, NaOCl 2.5% and 0.9% saline solution ultrasonically activated as dentin pre-treatment should be used for bond improvements.

Abstract

Objectives: to evaluate the effect of five intracanal irrigants on bond strength of fiber posts cemented with newer self-adhesive resin cement. **Methods:** 60 extracted human premolar single-rooted, sectioned at 14 mm, were prepared with ProTaper Universal system until F3 instrument and filled with F3 master cone and AH Plus. Root canal filling was partially removed, remaining 4 mm of apical gutta-percha. Specimens were randomly assigned into 5 groups (n=12), according to the solution used for dentin surface treatment before fiber post cementation, as described: EDTA 17% (EDTA); QMix (QM); SmearClear (SC); NaOCl 2.5% (NaOCl) and 0.9% saline solution (SS). Ultrasonic activation was performed (three times, 20 seconds each) and root canals were dried with paper points. Fiber posts were cemented with RelyX U200. In one specimen per group, Rhodamine B dye was mixed to RelyX U200 to provide adequate fluorescence for confocal laser scanning microscopy (CLSM) assessment. Specimens were transversally sectioned and three slices were obtained, one for each root third. Next, push-out test was performed. Stereomicroscope and CLSM were used to analyze the failure modes and to illustrate the pattern of infiltration of RelyX U200 into dentinal tubules, respectively. Bond strength means were calculated and ANOVA and Bonferroni tests were used for statistical analysis. **Results:** SS showed the higher mean of bond strength values (11.5 ± 5.3), superior to QM (5.1 ± 3.1), SC (5.1 ± 3.3). NaOCl presented intermediary bond strength values (9.7 ± 5.0), similar to EDTA (7.7 ± 2.9) and SS. QM and SC showed the lowest mean of bond strength ($P < .05$). Adhesive failures between cement/dentin were predominant (53.9%). **Conclusion:** SS and NaOCl associated to ultrasonic activation seems to be adequate solutions for root canal cleaning before fiber post cementation with RelyX U200, while chelating solutions, such as EDTA, QMix and SmearClear, should not be used for dentin pre-treatment when cementing with RelyX U200.

Effect of intracanal irrigants on bond strength of fiber posts cemented with a self-adhesive resin cement

INTRODUCTION

Endodontically treated teeth may exhibit pronounced coronal destruction and the amount of residual coronal dentine can influence the clinical survival of posts and restorations¹. Recent studies indicate a direct relationship between fracture resistance and the amount of remaining tooth structure². Due to this fact, fiber post cementation consists in a viable alternative to restore weakened teeth.

Glass fiber posts were introduced as an alternative to cast metal posts, because it presents mechanical properties similar to dentin^{3,4}, such as elastic moduli, which improves the distribution of functional loads to the root canal when compared with cast posts⁵. Several factors lead to the use of glass fiber posts such as aesthetic advantages, low costs⁴, simplify and less time consuming technique⁶.

Self-adhesive resin cements, such as RelyX U200 and RelyX U100 (3M ESPE, Saint Paul, MN, USA), have been recently introduced in order to reduce the sensibility of pre-treatment steps and to prevent application errors of cementation procedures⁷. This fact may increase bond strength when compared to conventional three steps system⁸. Nevertheless, some studies indicate that self-adhesive cements produce lower bond strength in enamel and in dentin than conventional resin cement⁹.

Ideally, a post cement system will provide a tight seal impermeable to oral bacteria¹⁰, however debonding failures have been reported³. Several variables may be associated with these failures, such as: the action of irrigant solutions on dentine collagen (sodium hypochlorite, hydrogen peroxide, chlorhexidine); the peculiar conditions of root canal dentine and the type of agent used to condition the substrate; the polymerization stress of resin cement; and the chemical and physical properties of the posts¹¹.

To enable satisfactory adhesion of posts to root dentin, smear layer has to be removed¹². It consists in an agglomeration of dentin, irrigant solutions, and organic tissues poorly adhered to the root canal walls¹³. Smear layer is able to damage penetration and adaptation of self-adhesive resin sealers inside dentinal tubules¹², which may decrease bond strength.

In this sense, chelating solutions play an important role in removing debris and smear layer^{14,15}. Ethylenediaminetetraacetic acid 17% (EDTA) is the most widely used chelating solution, because it enables dissolution of the inorganic portion of dentine and smear layer¹⁶. On the other hand, EDTA can cause erosion of root canal dentin¹⁷ and presents lower antimicrobial action¹⁸.

With the aim of increase antimicrobial activity, without producing dentin erosion, new irrigants has been proposed¹⁹. EDTA-based formulations have been developed as final rinse solutions, such as SmearClear and QMix²⁰. SmearClear (SybronEndo, West Collins, Orange, CA) presents EDTA and cetrimide in its formulation, while QMix (Dentsply Tulsa Dental, Tulsa, OK, USA) contains EDTA, chlorhexidine (CHX) and a surfactant agent. This one-step final rinse is supposed to combine the antimicrobial and substantivity properties of CHX with smear layer removing properties of EDTA²¹.

Passive ultrasonic irrigation (PUI) activates the irrigant without contacting root canal walls, increasing smear layer and debris removal²². PUI associated to EDTA or sodium hypochlorite (NaOCl) is more effective than conventional irrigation at removing debris from root canal²³.

Despite of the reported literature, few studies have evaluated the effect of irrigating and chelating solutions on bond strength to root dentin. The use of PUI in Endodontics is well supported, but no study evaluated the use of PUI previously to fiber post cementation. Moreover, information related to fiber posts cemented with RelyX U200 is still poor.

Therefore, the main goal of this *ex vivo* study was to evaluate the effect of different intracanal irrigants ultrasonically activated on bond strength between root dentine and fiber posts cemented with RelyX U200.

The null hypothesis tested was that different irrigant solutions have no influence on fiber glass post bond strength.

METHODS AND MATERIALS

Experimental design

Roots (N = 60) were randomly allocated (www.random.org) into 5 groups (n = 12), considering 1 factor (irrigant solutions) at five levels. The main outcome was the ‘push-out bond strength’ and the experimental unit was the ‘root’. The operators were blinded for intracanal solutions’ applications, post cementations, push-out tests, and failure analyses.

Tooth selection

This study was submitted and approved by the Ethical Committee of the Federal University of Santa Maria (number #855.457). Sixty single-rooted human mandibular premolars, with similar dimensions, were selected and stored in a 0.9% saline solution at 4°C until use. Periapical radiographs were performed to confirm the presence of one root canal. All roots were observed at x8 magnification with a stereomicroscope (Zeiss Stemi SV6; Carl Zeiss, Jena, Germany) in order to exclude external cracks, incomplete root formation, root resorption, coronal root canal diameter greater than 2 mm, as measured with a digital caliper (Starrett 727; Starrett, Itu, SP, Brazil).

Specimens were decoronated at the cervical root third, to standardize a remaining root length of 14 mm, using a diamond blade (Komet, Santo André, SP, Brazil) under cool water.

Root canal preparation

Canal patency was established with a size 10 K-file (Dentsply Maillefer, Chemin du Verger, Ballaigues, Switzerland), followed by PathFile 1, 2, 3 (Dentsply Maillefer) instruments. The working length was set a 1mm from the apex. Root canals were prepared by using the ProTaper Universal System (Dentsply Maillefer). Initially, the cervical and middle portions of the roots were prepared by using S1, SX, and S2 instruments. Later, S1, S2, F1, F2, and F3 files were sequentially used for all of the working lengths. Each canal was irrigated with 2 mL of a freshly prepared 2.5% sodium hypochlorite (NaOCl) (Asfer, São Paulo, SP, Brazil) between each instrument change. Specimens were irrigated with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) (Biodinâmica, Iporã, PR, Brazil) during 3 minutes and subsequently rinsed with 2 mL of NaOCl. Next, they were dried by using size 30 paper points (Dentsply Maillefer).

Root canal filling

AH Plus (Dentsply Maillefer) was mixed according to the manufacturer's instructions and placed in working length by using a 400-rpm lentulo spiral (Dentsply Maillefer) for 5 seconds²⁴. Single cone technique was performed by using F3 (Dentsply Maillefer) main gutta-percha cones, coated with sealer and placed into root canals to the working length. The excess gutta-percha in the coronal portion was removed with a flame-heated plugger, and the access cavity was sealed with Filtek Z350 (3M ESPE) composite resin. Roots were stored for 1 week at 37°C and 100% humidity to allow the sealers to set.

Post space preparation

Root canal filling was partially removed using sizes 1, 2, 3, 4 Largo drills (Dentsply Maillefer), alternately with 0.9% saline solution irrigation, in 10 mm length, remaining 4 mm

of apical guta-percha. Post space preparation was completed using the Exacto Translúcido Angelus N2 (Angelus, Londrina, PR, Brazil) bur at 10 mm. Periapical radiographs were performed to confirm root filling removal.

Apical root portions were included in a chemically cured acrylic resin (Dencrilay Dencril, Pirassununga, SP, Brazil) block. The specimens were fixed on a parallelometer, with the long axes of the teeth and the resin block parallel to each other and perpendicular to the ground.

Irrigation protocols

As aforementioned, specimens were randomly divided into five groups, according to final flushing after post space preparation, as described: **EDTA** (EDTA 17%); **QM** (QMix); **SC** (SmearClear); **NaOCl** (NaOCl 2.5%) and **SS** (0.9% saline solution).

EDTA, QM, SC, NaOCl and SS were delivered into root canals using Ultradent syringes (Ultradent Products Inc., South Jordan, UT, USA) and 30G EndoEzeTip needles (Ultradent Products Inc).

The irrigation protocols for all experimental and control group was: (1) root canals were rinsed with 1 mL of the corresponding irrigant; (2) ultrasonic activation with a size 20/0.1 ultrasonic tip (Capelli e Fabris Ind., Santa Rosa do Viterbo, SP, Brazil), attached to an NAC Plus ultrasonic device (Adiel LTDA, São Paulo, SP, Brazil) was performed during 20 seconds, without touching root canal walls. This procedure was repeated twice again and the irrigant was renewed²²; (3) a final continuous irrigation with 2 mL of 0.9% saline solution was performed in all groups.

Specimens were dried with paper points. Exacto Translúcido N2 (Angelus, Londrina, PR, Brazil) glass fiber posts were cleaned with ethyl alcohol 70%, coated with silane (Angelus) and put out to dry for five minutes, in order to solvent evaporation.

Rhodamine B dye in a ratio of 0.1%²⁵ was mixed to RelyX U200 (3M ESPE) in one specimen per group, in order to provide the fluorescence which enabled CLSM assessment. This procedure allowed the illustration of the patterns of self-adhesive resin cement distribution into dentinal tubules.

RelyX U200 was mixed according to manufacturer's specification inserted into root canal using Automix tips (3M ESPE) and immediately, fiber post was inserted by manual pressure. The cement was light-cured for 40 s using an LED light-curing unit (Radii Cal; SDI, Melbourne, Australia) previously calibrated, maintaining the light guide tip of the light-curing unit placed perpendicular to the post²⁶. A single operator performed all procedures. The coronal access was sealed with composite resin (Filtek Z350; 3M ESPE). Roots were stored for 1 week at 37°C.

Sample preparation for CLSM analysis

In order to illustrate the pattern of distribution of RelyX U200 inside dentinal tubules of each group, one specimen per group was prepared for CLSM.

A cutting machine (Extec Labcut 1010, Enfield, CT, USA) was used for sectioning transversally the roots, providing 3 slices, one for each root third. For CLSM assessment, surfaces were polished with Arotec paste (Arotec, Cotia, SP, Brazil) in order to eliminate dentin debris generated during the cutting procedures. The coronal surface of the samples was examined with the Olympus FluoView Confocal Laser 1000 Microscope (Olympus Corporation, Tokyo, Japan). The absorption and emission wavelengths for rhodamine B was 540 nm and 494 nm, respectively. Dentin samples were analyzed using the ×10 oil lens.

Push-out test

The first cervical slice (approximately 1 mm thick) was discarded due the excess of cement, which could negatively influence the adhesive resistance. Three other slices per specimen (thickness: 2 ± 0.3 mm) were obtained. Each slice was positioned on a metallic device with a central opening ($\varnothing=3$ mm) larger than the canal diameter. The most coronal portion of the specimen was placed downward. For push-out test, a metallic cylinder (\varnothing extremity = 0.8 mm) induced a load on the post in an apical to coronal direction, without applying any pressure to the cement and/or dentin.

Push-out test was performed in a universal testing machine (Emic DL-2000; Emic, Sao Jose dos Pinhais, PR, Brazil) at a speed of 1 mm/min. Bond strength values (σ) in MPa were obtained as followed: $\sigma=F/A$, where **F** = load for specimen rupture (N) and **A** = bonded area (mm^2). To determine the bonded interface area, a formula was used: $A=2\pi g(\mathbf{R1} + \mathbf{R2})$, where $\pi=3.14$, **g**= slant height, **R1**= smaller base radius, **R2**= larger base radius. To determine the slant height, the following calculation was used: $g^2=(h^2 + [\mathbf{R2}- \mathbf{R1}]^2)$, where **h**= section height. **R1** and **R2** were obtained by measuring the internal diameters of the smaller and larger base, respectively, which corresponded to the internal diameter between the root canal walls²⁷. The diameters and **h** were measured using a digital caliper (Starrett 727, Starrett, Itu, SP, Brazil).

Failure mode analysis

Dentin slices were analyzed first in stereomicroscope (Zeiss Stemi SV6) and some samples were selected for scanning electron microscopy (SEM) in order to categorize and illustrate the failure modes, respectively. The failure modes were categorized as follow: Ac/d = Predominant Adhesive at cement/dentin interface failure; Ac/p = Predominant Adhesive at cement/post interface failure; CC = Cement cohesive failure; DC = Dentin cohesive failure;

PC = Post cohesive failure. Specimens presenting cohesive fracture of the fiber post or dentin were excluded from the study once these types of failures do not represent real push-out bond strength.

Data Analysis

The Kappa test was used to analyze the agreement between the intra-examiner readings about failure mode, at different times.

The mean of bond strength distribution was checked with Shapiro Wilk test. One-way ANOVA and Bonferroni tests (SPSS 12.0; SPSS Inc., Chicago, IL, USA) were used for statistical analysis. The significance level was set at 5%.

RESULTS

The Kappa value was 0.84. After push-out test, some dentine cohesive failures were observed and those specimens were excluded from the bond strength calculations.

One-way ANOVA revealed a significant difference among the groups ($P = 0.0009$). SS showed the higher mean of bond strength values, superior to QM, SC ($P < .05$). NaOCl presented intermediary bond strength values, similar to EDTA and SS ($P > .05$). QM and SC showed the lowest mean of bond strength ($P < .05$) (**Table 1**).

Table 1 also presents failure modes distribution. Adhesive failures between cement/dentin (Ac/d) were predominant (53.9%), followed by adhesive failures between cement/post (Ac/p) (19.5%), while dentin cohesive (DC) failures represent 26.6% of the specimens. Post cohesive failures (PC) and cement cohesive failures (CC) were not observed.

Figure 1 represents the failure modes.

Figure 2 shows the infiltration patterns of RelyX U200 into dentinal tubules. Images A (EDTA), C (NaOCl) and E (SS) showed the penetration of RelyX U200 into dentinal

tubules. SS presented the most homogenous penetration into dentinal tubules, along entire perimeter of root canal. Images B (QM) and D (SC) showed RelyX U200 limited to root canal perimeter, but not into dentinal tubules.

DISCUSSION

This current investigation depicted the tested intracanal irrigants promoted different push-out bond strength, thus the null hypothesis was rejected. The higher mean of bond strength was found in SS and NaOCl groups, while SC and QMix presented the lowest means and EDTA presented intermediary mean values.

This study tested the RelyX U200 resin cement, and the respective manufacturer recommends the use of NaOCl flushing previously to fiber post cementation; however the criteria used for choosing this irrigant is not clear. At the moment, none study support this indication, as well the NaOCl concentration is not clear.

It is known that NaOCl acts upon organic components of the dentine and improves the penetration of monomers into dentine structure²⁸. However, NaOCl presents potential of collagen degradation, which could affect bond strength to root dentin¹¹. Several studies have been demonstrating that the dentin exposure for a long period to a high concentration of NaOCl is able to reduce the bond strength and could result in root fracture²⁹, contrasting with the results of the current study, which NaOCl 2.5% was chosen to standardize as the same concentration used in root canal preparation. This fact may be explained because NaOCl was ultrasonically activated just for 60 seconds, contributing to root canal cleaning and not to collagen degradation.

Saline Solution presents no antimicrobial and chelating properties. In Endodontics, it is frequently associated to CHX 2% gel, for root canal preparation³⁰. Due to the fact that saline is not able to dissolve organic tissue, the ultrasonic activation of this solution

previously to fiber post cementation seems not contribute to dentin collagen degradation, resulting in higher bond strength values in this study.

According to the manufacturer's brochure, QMix is a proprietary blend of 2% CHX, EDTA, and a surfactant. The lower bond strength values presented in this group may be explained by the interaction between CHX and NaOCl used in root canal preparation. This association produces an orange-brown precipitate¹⁵ which contains para-chloroaniline, that been shown to be toxic³¹. This precipitate can lead to tooth discoloration and might interfere with the sealing of root fillings³². Moreover, this precipitate seems to interfere negatively on bond strength values presented in the current study.

SmearClear formula contains EDTA, cetrimide and a special surfactant. Cetrimide presents low detergent capacity and high antimicrobial activity. Previous studies showed that the presence of surfactants in SC did not improve its efficiency in removing the smear layer³³. However, information related to the influence of SC as irrigant on bond strength of fiber post to root dentin is still poor.

As a mild chelating agent, EDTA could remove the hydroxyapatite and noncollagenous protein selectively, avoiding major alterations of the native collagen fibrillar structure³⁴. The results of this current study are in contrast to the findings of Gu et al. 2009³⁵ which found that EDTA removed the smear layer effectively and, as a result, increased the bond strength, comparing to NaOCl and saline solution. However, in the study EDTA was applied manually for 60 seconds without PUI activation, which may lead to the fact that PUI on chelating solutions may contribute for collagen degradation and bond strength reduction, as the results of this study showed.

It is important to highlight that irrigating solutions that present EDTA-based formulation promoted the lower bond strength values, in this study (EDTA, QM, SC). SS and NaOCl were capable to promote root canal cleaning without cause dentin degradation. Thus,

root canal cleaning before fiber post cementation has to consider chemical composition of intracanal irrigants.

The cleaning efficacy of PUI implies the effective removal of dentine debris, microorganisms (planktonic or in biofilm) and organic tissue from root canal²². In this sense, PUI was used to enhance irrigating and chelating potential of the solutions, exposing dentinal tubules for cement penetration, which could contribute to higher bond strength values. Smear layer removal plays an important role especially when self-adhesive resin cements are used³⁵, due to the fact that no previous acid etching is indicated. Rodrigues et al. 2015 evaluated the effect of 37% phosphoric acid etching on bond strength of fiber post cemented with conventional and self-adhesive resin cement. Phosphoric acid etching previously to self-adhesive resin cement did not improve the bond strength values, which were similar to those obtained using conventional resin cements and acid etching.

Especially in Endodontics, CLSM is ideal to determine the degree of adaptation and penetration of the root canal filling to dentine walls and into dentinal tubules, respectively³⁸, through the use of fluorescent rhodamine dye. According to D'Alpino et al. 2006²⁵, rhodamine dye must be incorporated to cement in a ratio of 0.1%, and it did not interfere on physical-chemical proprieties of endodontic sealers. Up to date, it is not clear if this dye is able to affect resin cements behavior used to fiber post cementation.

Figure 2 shows that SS, NaOCl and EDTA presented self-adhesive resin penetration into dentinal tubules. In QM and SC groups, resin cement remained restricted to root canal perimeter. Taking into considerations bond strength and CLSM findings, it can be stated that groups presenting self-adhesive resin penetration into dentinal tubules resulted in higher bond strength values. For instance, SS showed more evident and homogeneous penetration of resin cement into dentinal tubules, and also resulted in the greatest bond values. However, as limitation, just one specimen per group was prepared for CLSM. More studies have to be

conducted in order to establish the relation between depth-continuity of self-adhesive resin cement penetration and bond strength outcomes.

Bond strength studies should be conducted to screen new products and investigate experimental variables³⁸. This way, push-out tests distribute stress more homogenously and produce less variability in mechanical testing results³⁹. Therefore, they are recommended to determine bond strength of fiber posts to root dentine⁴⁰.

Bonding to root canal dentin might be a challenge because of the anatomy of the root, handling characteristics of the adhesive systems, and adhesive procedures³. Into root canal, the factor cavity configuration (C-factor) is critical, increasing the stress polymerization of resin cements³⁹. The force of polymerization shrinkage into root canal may be greater than the adhesion of the cement to dentine, resulting in gaps that affect adhesive interface and may compromise the restoration longevity⁴¹.

Several *in vitro* studies have demonstrated that predominant failures of bonding occur at the cement–dentin interface⁴². In the current study, adhesive failure between cement/dentin (Ac/d) was predominant (53.9%), as expected, followed by adhesive failure between cement/post (Ac/p) (19.5%). Dentin cohesive (DC) failures represent 26.6% of the specimens.

One of the limitations of this study is that samples were not submitted to thermal and mechanical cycling, to simulate intra-oral conditions more precisely. Moreover, detailed information about the composition and adhesive properties of self-adhesive cements is still limited. Future studies have to be conducted in order to establish the relation between chemical irrigating solutions and self-adhesive resin cements, and also the relation between endodontic sealers and self-adhesive resin cements.

CONCLUSIONS

Saline solution and NaOCl 2.5%, followed by ultrasonic activation, promoted the highest bond strength and seems to be adequate irrigants for root canal cleaning before fiber post cementation with RelyX U200 cement; Chelating solutions, such as EDTA, QMix and SmearClear, presented lower bond strength values and should not be indicated for intracanal irrigation, using PUI, when cementing with RelyX U200.

Conflict of Interest

The authors claim none conflict of interest.

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Figures

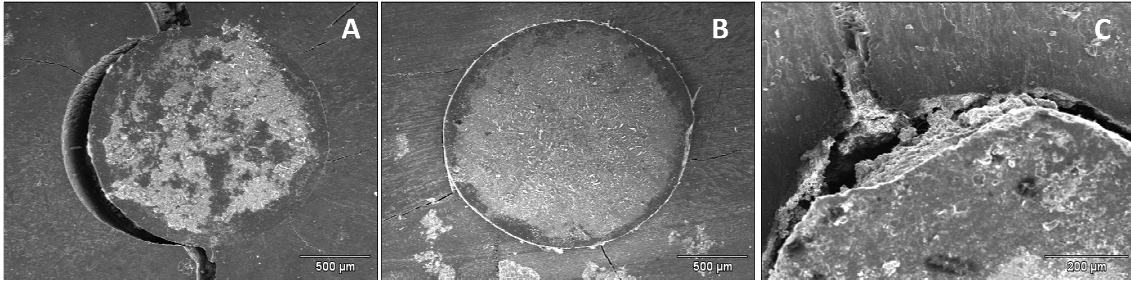


Figure 1- Scanning electron microscopy photomicrographs of fiber post/self-adhesive resin cement and self-adhesive resin cement/root dentin interfaces. Failure modes are illustrated in A, B and C images. (A) DC - Dentin cohesive failure (50x); (B) Ac/d = Predominant Adhesive at cement/dentin failure (50x); (C) Ac/p = Predominant Adhesive at cement/post interface failure (150x);

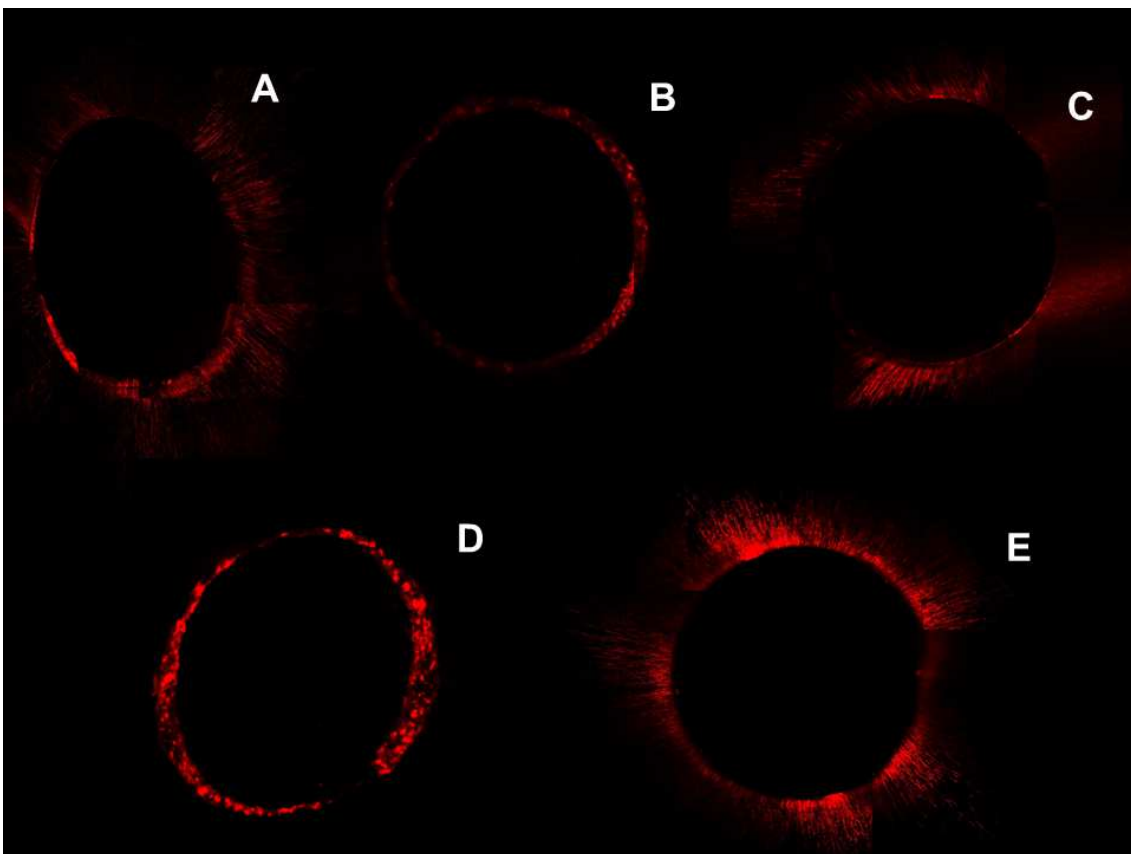


Figure 2 - CLSM ($\times 10$) of self-adhesive resin cement penetration into dentin tubules: EDTA (A); QM (B); NaOCl (C); SC (D); SS (E). Red: RelyX U200 stained with Rhodamine 0.1%. Note that in B and D the self-adhesive resin cement is confined into root canal perimeter with absence of tubule penetration.

Table**Table 1** - Mean of bond strength values and failure modes distribution after push-out test for the irrigants used previously to the post cementation

Groups	Bond strength	Failure					TOTAL
		Ac/d	Ac/p	DC	PC	CC	
SS	11.5 ± 5.3 ^a	19	4	13	-	-	36
NaOCl	9.7 ± 5.0 ^{ab}	18	8	10	-	-	36
QM	5.1 ± 3.1 ^c	16	15	5	-	-	36
SC	5.1 ± 3.3 ^c	18	6	12	-	-	36
EDTA	7.7 ± 2.9 ^{bc}	26	2	8	-	-	36
TOTAL		97	35	48	0	0	180
		(53.9%)	(19.5%)	(26.6%)	(0%)	(0%)	(100%)

Different superscript letters identify statistically significant differences at the $P < .05$ level. Failure modes: Ac/d = Predominant Adhesive at cement/dentin interface failure; Ac/p = Predominant Adhesive at cement/post interface failure; CC = Cement cohesive failure; DC = Dentin cohesive failure; PC = Post cohesive failure.

3. CONSIDERAÇÕES FINAIS

Durante o preparo dos canais radiculares são formadas raspas de dentina pela ação dos instrumentos endodônticos que, associadas à matéria orgânica proveniente de remanescentes de tecido pulpar e microrganismos, se aderem à superfície dentinária formando a *smear layer* (Prado et al. 2013). Muitos aspectos relacionados a essa camada de lama dentinária vem sendo estudados ao longo dos anos. Aspectos que envolvem o poder de desmineralização e erosão dentinária, atividade quelante, associação entre soluções quelantes e irrigantes, influência da ativação por ultrassom (De-Deus et al. 2011). No entanto, não foram encontradas, até o momento, evidências científicas que comprovem a influência de diferentes substâncias irrigantes e quelantes ativadas por ultrassom na resistência adesiva de pinos de fibra de vidro, cimentados com cimento resinoso autoadesivo RelyX U200.

Outro aspecto a ser considerado quando da escolha da solução irrigadora é a adesão à dentina que pode ser afetada por inúmeros fatores. Algumas pesquisas demonstram que a utilização de soluções irrigadoras e medicações durante o tratamento endodôntico podem ter um efeito deletério sobre a união de agentes adesivos às estruturas dentinárias da cavidade pulpar (Zhang et al. 2010; Renovato et al. 2013).

Sabe-se que o NaOCl apresenta excelente potencial antimicrobiano e atua frente aos componentes orgânicos da dentina (Dietschi et al. 2007), mas sua ação na remoção de *smear layer* é limitada. O efeito deletério envolvendo as propriedades mecânicas da dentina, atribuído ao uso do hipoclorito de sódio, depende da concentração e do tempo de exposição (Zhang et al. 2010). Em altas concentrações essa solução pode degradar a rede de fibras colágenas e, com isso reduzir a resistência adesiva (Morris et al. 2001), podendo resultar em fratura radicular (Zhang et al. 2010). No presente estudo, a concentração de 2,5%, mesma concentração utilizada no preparo dos canais, parece não ter sido capaz de modificar a estrutura da dentina radicular, a ponto de interferir nos valores de resistência adesiva.

De acordo com o fabricante, QMix é uma solução que combina CHX, EDTA e agente surfactante. Os baixos valores de resistência adesiva encontrados neste grupo podem ter ocorrido pela interação entre a CHX e o NaOCl utilizado durante o preparo. Essa associação entre CHX e NaOCl forma um precipitado castanho-alaranjado, que contém paracloroanilina (Magro et al. 2015), que parece ser tóxico. Além disso, esse precipitado promove a descoloração dentária e interfere negativamente no selamento da obturação endodôntica.

Dessa maneira, a interação entre os diferentes compostos desta solução interferiu negativamente nos valores de resistência adesiva encontrados (Magro et al. 2014).

A fórmula do SmearClear contém EDTA, cetrimida e um surfactante especial. Cetrimida apresenta baixa capacidade detergente e alta atividade antimicrobiana. Estudos prévios demonstraram que a presença do surfactante não aumentou a eficácia de remoção de *smear layer* (Ulusoy et al. 2013). No presente estudo, o grupo SC apresentou os menores valores de resistência adesiva, demonstrando que a associação de diversas substâncias em uma mesma solução ativada ultrassonicamente pode ter colaborado para colabar a rede de fibras colágenas, interferindo negativamente nos valores de resistência de união.

O EDTA, solução quelante mais utilizada atualmente, é capaz de dissolver material inorgânico, inclusive hidroxiapatita, auxiliando na remoção do *smear layer*, preparando as paredes dos canais radiculares para receber medicação intracanal e materiais obturadores (Carvalho et al. 2014). Como a união dos cimentos resinosos autoadesivos à dentina é totalmente dependente da hidroxiapatita (Rodrigues et al. 2014), a remoção desse mineral pela ação quelante do EDTA sob a ação da PUI pode ter sido responsável pelos valores baixos de resistência adesiva encontrados.

Já a solução de soro fisiológico não apresenta potencial antimicrobiano e quelante. Em função disso, a ativação ultrassônica dessa solução parece não ter contribuído para degradação das fibras colágenas, resultando nos maiores valores de resistência adesiva encontrados no estudo. Em endodontia, seu uso é frequentemente associado à Clorexidina 2% gel, para instrumentação de canais radiculares (Ferraz et al. 2001).

A fim de potencializar o efeito das soluções previamente citadas, a Irrigação Ultrassônica Passiva (PUI) foi utilizada neste estudo. A PUI contribui para a remoção de *debris* e *smear layer* (Van der Sluis et al. 2007), expondo maior número de túbulos dentinários para a penetração do cimento RelyX U200. Quando cimentos resinosos convencionais são utilizados – a exemplo do RelyX ARC, o condicionamento ácido prévio da estrutura dentinária é o responsável pela remoção da *smear layer*. Já quando utilizamos um sistema resinoso autoadesivo, que dispensa o condicionamento ácido, a remoção prévia da *smear layer* desempenha um papel fundamental para que haja retenção micromecânica e interação do monômero do cimento com a hidroxiapatita dentinária (Rodrigues et al. 2015).

Sob as condições apresentadas nesse estudo, podemos concluir que o uso de solução salina (SS) e hipoclorito de sódio (NaOCl) 2,5%, ativados pela PUI, parece ser adequado para lavagem do canal prévia à cimentação de pinos de fibra de vidro cimentados com

cimento resinoso autoadesivo RelyX U200. O uso de soluções quelantes parece interferir negativamente nos valores de resistência adesiva.

No entanto, informações detalhadas a respeito da composição e propriedades adesivas dos cimentos autoadesivos ainda são limitadas. Mais estudos precisam ser conduzidos a fim de estabelecer a relação entre soluções irrigadoras e quelantes com o uso de cimentos resinosos autoadesivos e entre cimentos endodônticos e cimentos resinosos autoadesivos.

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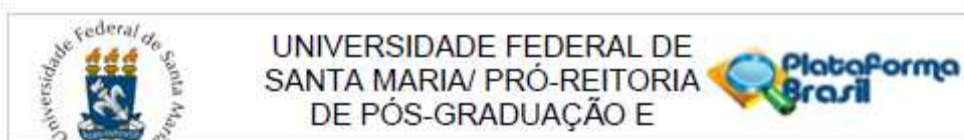
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ANEXOS

Anexo A - Parecer de aprovação do Comitê de Ética em Pesquisa (CEP)



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DE SOLUÇÕES QUELANTES ATIVADAS POR ULTRASSOM NA RESISTÊNCIA ADESIVA DE PINOS DE FIBRA DE VIDRO CIMENTADOS COM CIMENTO AUTOADESIVO RelyX U200

Pesquisador: Carlos Alexandre Souza Bier

Área Temática:

Versão: 2

CAAE: 38307514.4.0000.5348

Instituição Proponente: Universidade Federal de Santa Maria/ Pró-Reitoria de Pós-Graduação e

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 855.457

Data da Relatoria: 02/11/2014

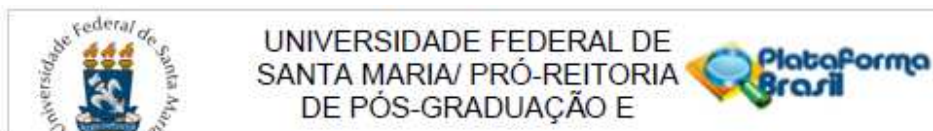
Apresentação do Projeto:

O projeto tem o título: "EFEITO DE SOLUÇÕES QUELANTES ATIVADAS POR ULTRASSOM NA RESISTÊNCIA ADESIVA DE PINOS DE FIBRA DE VIDRO CIMENTADOS COM CIMENTO AUTOADESIVO RelyX U200" e é vinculado ao curso de mestrado em Ciências Odontológicas da UFSM.

Objetivo da Pesquisa:

Objetivo geral: Avaliar o efeito de diferentes soluções quelantes na resistência adesiva de pinos de fibra de vidro cimentados com cimento autoadesivo RelyX U200.

Objetivos específicos: Comparar os valores de resistência adesiva nos diferentes níveis do pino de fibra (cervical, médio, apical), dentro de um mesmo grupo e entre os diferentes grupos testados; Descrever, através de microscopia eletrônica de varredura, os padrões de falha apresentados por cada grupo, após o teste de resistência adesiva; Analisar, através de microscopia eletrônica confocal à laser, a área de penetração e profundidade máxima de penetração do cimento autoadesivo nos túbulos dentinários após a utilização das soluções quelantes.



Continuação do Parecer: 855.457

Avaliação dos Riscos e Benefícios:

Riscos: Não há riscos biológicos associados ao estudo uma vez que os dentes que serão utilizados serão obtidos a partir de um banco de dentes (Banco de dentes permanentes da universidade federal de santa maria) que segue as normas de biossegurança para coleta e armazenagem dos mesmos.

Benefícios: Os benefícios envolvem a possibilidade de conhecermos melhor as propriedades de um novo cimento resinoso autoadesivo para cimentação de pinos intraradiculares (RelyX U200), bem como avaliar a influência de diferentes substâncias quelantes na resistência adesiva de pinos de fibra, contribuindo, assim, para a evolução do tratamento endodôntico e protético, com retorno para toda a comunidade científica, além de oferecer tratamento adequado aos pacientes.

Os riscos e benefícios estão apresentados.

Comentários e Considerações sobre a Pesquisa:

.

Considerações sobre os Termos de apresentação obrigatória:

Foi apresentado o Termo de Confidencialidade, uma declaração do Banco de Dentes, uma autorização da chefia do departamento e uma declaração da coordenação do mestrado (Autorização Institucional). O TCLE foi dispensado.

Recomendações:

.

Conclusões ou Pendências e Lista de Inadequações:

As pendências foram resolvidas. A pesquisa atende os quesitos básicos e pode ser realizada.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Anexo B - Authors Guide for publication on Operative Dentistry:

Manuscript submission General Requirements

Operative Dentistry requires electronic submission of all manuscripts. All submissions must be sent to Operative Dentistry using the Allen Track upload site. A mandatory and nonrefundable \$25.00 fee is required at submission. Your manuscript will only be considered officially submitted after it has been approved through our initial quality control check, and any quality problems have been resolved. You will have 6 days from when you start the process to submit and approve the manuscript. After the 6 day limit, if you have not finished the submission, your submission may be removed from the server. You are still able to submit the manuscript, but you must start from the beginning. Be prepared to submit the following manuscript files in your upload:

- A Laboratory or Clinical Research Manuscript file must include: a title; a running (short) title; a clinical relevance statement; a concise summary (abstract); Current as of: introduction, methods & materials, results, discussion and conclusion; references (see Below).
- The manuscript body MUST NOT include any:
 - o Author identifying information such as: ♣ Authors names or titles ♣ Acknowledgements ♣ Correspondence information ♣ Response to reviewer files should also NOT include any author identifying information, such as a signature at the end, etc. Figures, Graphs, Tables.
- An acknowledgement, disclaimer and/or recognition of support (if applicable) must in a separate file and uploaded as supplemental material.
- All figures, illustrations, graphs and tables must also be provided as individual files. These should be high-resolution images, which are used by the editor in the actual typesetting of your manuscript. Please refer to the instructions below for acceptable formats and sizes.
- All other manuscript types use this template, with the appropriate changes as listed below.

Complete the online form (which includes complete author information, copyright release and conflict of interest), and select the files you would like to send to Operative Dentistry. Manuscripts that do not meet our formatting and data requirements listed below will be sent back to the corresponding author for correction.

Important Information

- All materials submitted for publication must be submitted exclusively to Operative Dentistry.

- The editor reserves the right to make literary corrections.
- Currently, color will be provided at no cost to the author if the editor deems it essential to the manuscript. However, we reserve the right to convert to gray scale if color does not contribute significantly to the quality and/or information content of the paper.
- The author(s) retain(s) the right to formally withdraw the paper from consideration and/or publication if they disagree with editorial decisions.
- International authors whose native language is not English must have their work reviewed by a native English speaker prior to submission. Manuscripts that are rejected before peer-review for English correction should be entered as a new manuscript upon resubmission. In the manuscript comments box the comment, “this is a resubmission of manuscript number XX-XXX” should be noted. Manuscripts that are rejected after peer-review are not eligible for resubmission. Manuscripts that have major revisions requested (i.e. For English correction) are entered as a resubmission of the original article.
- Spelling must conform to the American Heritage Dictionary of the English Language, and SI units for scientific measurement are preferred.
- While we do not currently have limitations on the length of manuscripts, we expect papers to be concise; authors are also encouraged to be selective in their use of figures and tables, using only those that contribute significantly to the understanding of the research.
- Acknowledgement of receipt is sent automatically upon acceptance through quality control. This may take up to 7 days. If you do not receive such an acknowledgement, please check your author homepage at <http://jopdent.allentrack.net> if the paper does not appear there please resend your paper.

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Manuscript Type Requirements

All Manuscripts

CORRESPONDING AUTHOR must provide a WORKING / VALID e-mail address which will be used for all communication with the journal.

NOTE: Corresponding authors MUST update their profile if their e-mail or postal address changes. If we cannot contact authors within seven days, their manuscript will be removed from our publication queue.

AUTHOR INFORMATION must include:

- full name of all authors
- complete mailing address for each author
- valid email address for each author
- degrees (e.g. DDS, DMD, PhD)
- affiliation (e.g. Department of Dental Materials, School of Dentistry, University of Michigan)

MENTION OF COMMERCIAL PRODUCTS/EQUIPMENT must include:

- full name of product
- full name of manufacturer
- city, state and country of manufacturer

MANUSCRIPTS must be provided as Word for Windows files. Files with the .doc and .docx extensions are accepted.

TABLES may be submitted as either Word (.doc and .docx) or Excel (.xls and .xlsx) files. All tables must be legible, with fonts being no smaller than 7 points. Tables have the following size limitations: In profile view a table must be no larger than 7 x 9 inches; landscape tables should be no wider than 7 inches. It is the Editor's preference that tables not need to be rotated in order to be printed, as it interrupts the reader's flow.

ILLUSTRATIONS, GRAPHS AND FIGURES must be provided as TIFF or high resolution JPEG files with the following parameters:

- line art (and tables that are submitted as a graphic) must be sized with the short edge being no shorter than 5 inches. It should have a minimum resolution of 600 dpi and a maximum resolution of 17 Current as of: 1200 dpi. This means the shortest side should be no smaller than 3000 pixels.

- gray scale/black & white figures must be sized with the short edge being no shorter than 5 inches. It should have a minimum resolution of 300 dpi and a maximum of 400 dpi. This means the shortest side should be no smaller than 1500 pixels.
- color figures and photographs must be sized with the short edge being no shorter than 3.5 inches. It should have a minimum resolution of 300 dpi and a maximum of 400 dpi. This means that the shortest side should be no smaller than 1050 pixels.

Other Manuscript Type – Additional Requirements

CLINICAL TECHNIQUE/CASE STUDY MANUSCRIPTS must include as part of the narrative:

- a running (short) title
- purpose
- description of technique
- list of materials used
- potential problems
- summary of advantages and disadvantages
- references (see below)

LITERATURE AND BOOK REVIEW MANUSCRIPTS must include as part of the narrative:

- a running (short) title
- a clinical relevance statement based on the conclusions of the review
- conclusions based on the literature review...without this, the review is just an exercise and will not be published
- references (see below). References must be numbered (superscripted numbers) consecutively as they appear in the text and, where applicable, they should appear after punctuation. The reference list should be arranged in numeric sequence at the end of the manuscript and should include:

1. Author(s) last name(s) and initial (ALL AUTHORS must be listed) followed by the date of publication in parentheses.

2. Full article title.

3. Full journal name in italics (no abbreviations), volume and issue numbers and first and last page numbers complete (i.e. 163-168 NOT attenuated 163-68).

4. Abstracts should be avoided when possible but, if used, must include the above plus the abstract number and page number.

5. Book chapters must include chapter title, book title in italics, editors' names (if appropriate), name of publisher and publishing address.

6. Websites may be used as references, but must include the date (day, month and year) accessed for the information.

7. Papers in the course of publication should only be entered in the references if they have been accepted for publication by a journal and then given in the standard manner with "In press" following the journal name.

8. DO NOT include unpublished data or personal communications in the reference list. Cite such references parenthetically in the text and include a date.

9. References that contain Crossref.org's DOIs (Digital Object Identifiers) should always be displayed at the end of the reference as permanent URLs. The prefix <http://dx.doi.org/> can be appended to the listed DOI to create this URL. i.e. <http://dx.doi.org/10.1006/jmbi.1995.0238>

Reference Style Guide

- Journal article-two authors: Evans DB & Neme AM (1999) Shear bond strength of composite resin and amalgam adhesive systems to dentin American Journal of Dentistry 12(1) 19-25.
- Journal article-multiple authors: Eick JD, Gwinnett AJ, Pashley DH & 19 Current as of: 3-Sep-14 Robinson SJ (1997) Current concepts on adhesion to dentin Critical Review of Oral and Biological Medicine 8(3) 306-335.
- Journal article: special issue/supplement: Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P & Vanherle G (2001) Adhesives and cements to promote preservation dentistry Operative Dentistry (Supplement 6) 119-144.
- Abstract: Yoshida Y, Van Meerbeek B, Okazaki M, Shintani H & Suzuki K (2003) Comparative study on adhesive performance of functional monomers Journal of Dental Research 82(Special Issue B) Abstract #0051 p B-19.
- Corporate publication: ISO-Standards (1997) ISO 4287 Geometrical Product Specifications Surface texture: Profile method – Terms, definitions and surface texture parameters Geneva: International Organization for Standardization 1st edition 1-25.
- Book-single author: Mount GJ (1990) An Atlas of Glass-ionomer Cements Martin Duntz Ltd, London.

- Book-two authors: Nakabayashi N & Pashley DH (1998) Hybridization of Dental Hard Tissues Quintessence Publishing, Tokyo.
- Book-chapter: Hilton TJ (1996) Direct posterior composite restorations In: Schwarts RS, Summitt JB, Robbins JW (eds) Fundamentals of Operative Dentistry Quintessence, Chicago 207-228.
- Website-single author: Carlson L (2003) Web site evolution; Retrieved online July 23, 2003 from: <http://www.d.umn.edu/~lcarlson/cms/evolution.html>
- Website-corporate publication: National Association of Social Workers (2000) NASW Practice research survey 2000. NASW Practice Research Network, 1. 3. Retrieved online September 8, 2003 from: <http://www.socialworkers.org/naswprn/default>
- Journal Article with DOI: SA Feierabend, J Matt & B Klaiber (2011) A Comparison of Conventional and New Rubber Dam Systems in Dental Practice. Operative Dentistry 36(3) 243-250, <http://dx.doi.org/10.2341/09-283-C> 20.

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