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NARA SOUSA RODRIGUES

# EFEITO DA DESPROTEINIZAÇÃO NO GRAU DE CONVERSÃO E NA RESISTÊNCIA DE UNIÃO DE CIMENTOS RESINOSOS AUTOADESIVOS À DENTINA

FORTALEZA
2016

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará, como requisito parcial para a obtenção do Título de Mestre em Odontologia. Área de Concentração: Clínica Odontológica.

Orientador: Prof. Dr. Vicente de Paulo Aragão Saboia.

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Aprovada em**: __/__/____.**

BANCA EXAMINADORA

___________________________________

Prof. Dr. Vicente de Paulo Aragão Saboia (Orientador)

Universidade Federal do Ceará

___________________________________

Prof[a]. Dr[a]. Vanara Florencio Passos

Universidade de Fortaleza (UNIFOR)

___________________________________

Prof[a]. Dr[a]. Celiane Mary Carneiro Tapety

Universidade Federal do Ceará

A Deus.

Aos meus pais, Erisson e Karla.

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## RESUMO

Os cimentos resinosos autoadesivos não dissolvem completamente a lama dentinária e a consequente interação superficial que ocorre entre o cimento e a dentina pode ser prejudicial. O hipoclorito de sódio (NaOCl) pode causar a remoção de colágeno, sendo uma opção para eliminar essa barreira mecânica, podendo fortalecer a interação química entre cimento e dentina. O presente estudo avaliou o efeito de duas estratégias de desproteinização na resistência de união à dentina (RU), grau de conversão *in situ* (GC) e nanoinfiltração (NI) de dois cimentos resinosos autoadesivos após 24 h e após 200.000 ciclos mecânicos. Cento e catorze terceiros molares humanos foram distribuídos em seis grupos de acordo com o cimento utilizado e a estratégia de desproteinização: RelyX U200 (RU) seguindo as instruções do fabricante (controle), RU após a desproteinização na dentina mineralizada utilizando hipoclorito de sódio (RU - NaOCl), RU após condicionamento ácido (15 s) e desproteinização na dentina (RU - *Etching* + NaOCl), MaxCem Elite (ME) seguindo as instruções do fabricante (controle), ME após a desproteinização na dentina (ME - NaOCl), ME após condicionamento ácido (15 s) e desproteinização na dentina (ME - *Etching* + NaOCl). Os espécimes foram aleatoriamente subdivididos em dois subgrupos para o teste de microtração: testados no período imediato e após 200.000 ciclos mecânicos. Para a NI, duas fatias de cada subgrupo foram infiltradas com solução de nitrato de prata amoniacal, preparadas e analisadas em MEV. O GC *in situ* de três espécimes de cada grupo imediato foi mensurado em espectroscopia micro-Raman. Os dados foram estatisticamente analisados por Análise de Variância a dois critérios e teste de comparações múltiplas de Tukey ($p<0.05$). A desproteinização da dentina sem condicionamento ácido prévio aumentou os valores de RU dos dois cimentos nos grupos imediatos. A desproteinização dentinária diminuiu o GC para os dois cimentos. Após a ciclagem mecânica, essa técnica foi efetiva para o ME, mas não afetou a resistência de união do RU. Os grupos experimentais apresentaram menor NI após o envelhecimento. Os benefícios em longo prazo da desproteinização apresentados nesse trabalho são encorajadores e podem justificar esse passo clínico adicional.

**Palavras-chave:** dentina, hipoclorito de sódio, colágeno, resistência à tração.

**ABSTRACT**

Self-adhesive resin cements do not completely dissolve the smear layer and the consequent superficiality of the interaction between the cement and dentin can be harmful. Sodium hypochlorite (NaOCl) can cause a collagen removal, being an option to eliminate this mechanical barrier which may enhance the chemical interaction between cement and dentin. This study examined the effects of two deproteinization strategies on dentin microtensile bond strength (µTBS), *in situ* degree of conversion (DC) and interfacial nanoleakage (NL) of two self-adhesive resin cements after 24 h or after 200,000 mechanical cycles. One hundred and fourteen human third molars were distributed into six groups according to the type of cement used and the strategy of deproteinization: RelyX U200 (RU) following the manufacturer´s instructions (control); RU after dentin deproteinization using sodium hypochlorite (RU - NaOCl); RU after phosphoric acid etching (15 s) and dentin deproteinization (RU – Etching + NaOCl); MaxCem Elite (ME) following the manufacturer´s instructions (control); ME after dentin deproteinization (ME - NaOCl); ME after phosphoric acid etching (15 s) and dentin deproteinization (ME - Etching + NaOCl). The bonded specimens were randomly subdivided in two subgroups for the microtensile test: tested in the immediate period or after 200,000 load cycles. For NL, two slices from each subgroup were infiltrated with ammoniacal silver nitrate solution, prepared and analyzed in SEM. The *in situ* DC of three bonded-specimens from each immediate group was measured by micro-Raman spectroscopy. Data was statistically analyzed by Two-way ANOVA and Tukey’s test ($p<0.05$). Dentin deproteinization without prior acid etching increased the µTBS of both cements in the immediate groups. Dentin deproteinization decreased the DC of both cements. After load cycling, this technique was effective for ME, but did not affect the bond strength of RU. The experimental groups showed lower NL compared to the control groups after aging. The long term benefits of deproteinization presented in this work were encouraging and might justify this additional clinical step.

**Keywords:** dentin, sodium hypochlorite, collagen, tensile strength.

**SUMÁRIO**

# *Introdução Geral*

# 1 INTRODUÇÃO GERAL

A cimentação adesiva em restaurações indiretas constitui uma parcela expressiva dos tratamentos odontológicos empregados atualmente, visando preservar a estrutura dentária e solucionar problemas estéticos (HIKITA *et al.,* 2007; PRAKKI; CARVALHO, 2001). Os cimentos resinosos foram desenvolvidos para proporcionar elevadas propriedades mecânicas e estéticas para a cimentação de restaurações indiretas. Eles têm sido frequentemente utilizados devido à possibilidade de adesão à estrutura dentária (RODRIGUES *et al.*, 2015) e suas satisfatórias propriedades ópticas. Os cimentos resinosos convencionais requerem o uso de um sistema adesivo com ou sem o condicionamento ácido prévio do substrato (BEVILACQUA; PORTO NETO; MAGNANI, 2000; CARVALHO *et al.*, 2004; VIOTTI *et al.*, 2009), configurando passos clínicos adicionais à cimentação.

Os cimentos autoadesivos foram criados visando simplificar a técnica de cimentação, uma vez que não necessitam do emprego de um sistema adesivo. Portanto, há uma redução no número de passos operatórios, encurtando o tempo de tratamento clínico e diminuindo a sensibilidade da técnica (GORACCI *et al.*, 2006; SOUTO *et al.*, 2010). Os monômeros ácidos funcionais são os responsáveis por desmineralizar e infiltrar o tecido dentinário (FERRACANE; STANSBURY; BURKE, 2011; HAN *et al.*, 2007). O mecanismo de adesão acontece devido à reação química entre os grupos de monômeros ácidos e a hidroxiapatita (GERTH *et al.*, 2006; RADOVIC *et al.*, 2008) pela adesão química aos íons $Ca^{2+}$ do dente (FERRACANE; STANSBURRY; BURKE, 2011), não formando camada híbrida na dentina intertubular, nem *tags* de resina nos túbulos dentinários (AGUIAR *et al*., 2013; MONTICELLI *et al.*, 2008). Portanto, a reação química é apenas superficial sem a liberação de componentes que se difundem através dos túbulos dentinários, gerando significativamente menor sensibilidade pós-operatória (WEISER; BEHR, 2015).

Os cimentos autoadesivos constituem uma boa opção para a cimentação de restaurações indiretas na superfície dentinária, especialmente quando se considera a simplificação da técnica (AGUIAR *et al.*, 2014). Eles podem ser utilizados na cimentação de pinos de fibra de vidro em dentes extensamente destruídos que foram tratados endodonticamente (BITTER *et al.*, 2007; NOVA *et al.*, 2013; SARKIS-ONOFRE *et al*., 2014), entretanto a superficialidade da interação entre o cimento e dentina é o ponto crítico nesta nova abordagem (DE MUNK *et al.,* 2004; GORACCI *et al.,* 2006).

O hipoclorito de sódio (NaOCl) é o irrigante endodôntico mais comumente utilizado devido às suas características antimicrobianas e sua capacidade de dissolver proteínas (CLARKSON *et al*., 2006; SIQUEIRA *et al*., 2000; TAWAKOLI *et al.,* 2015). É considerado um agente desproteinizante não-específico altamente alcalino (pH 11-12,5) que pode causar a dissolução do colágeno por quebrar ligações entre átomos de carbono e oxidar a estrutura primária de proteínas (LAI *et al.*, 2001). O NaOCl pode atuar como agente desproteinizante tanto na dentina desmineralizada como na mineralizada (SAURO *et al.,* 2009; ZHANG *et al.,* 2010), podendo proporcionar a exposição da dentina subsuperficial rica em hidroxiapatita com alta energia de superfície.

A remoção da matriz de colágeno pelo NaOCl, aplicado após condicionamento ácido, tem sido proposta para melhorar o molhamento do substrato dentário (SAURO *et al.,* 2009; WAKABAYASHI *et al.,* 1994), facilitando assim a infiltração de cimentos resinosos. A adesão química dos cimentos resinosos autoadesivos também pode ser melhorada, quando em dentina mineralizada, devido à capacidade do NaOCl alterar o teor mineral da dentina de forma significativa, aumentando a razão de Ca/P da superfície da dentina (DOGAN; SALT, 2001; ZHANG *et al.,* 2010), consequentemente, melhorando a resistência de união da cimentação (DE SOUZA *et al.,* 2011; SOUZA *et al.,* 2016).

Portanto, a remoção do colágeno dentinário associado ao aumento do teor mineral do substrato, provocado pela desproteinização com o NaOCl, pode gerar uma maior interação entre o cimento resinoso autoadesivo e a dentina, resultando em uma maior resistência de união imediata e a longo prazo.

# *Proposição*

# 2 PROPOSIÇÃO

O presente trabalho teve como objetivos:

## 2.1 Objetivo Geral

Avaliar o efeito da remoção de colágeno dentinário com uma solução de NaOCl a 5% por dois minutos no grau de conversão *in situ* e na resistência de união da interface formada por cimentos resinosos autoadesivos e a dentina mineralizada ou parcialmente desmineralizada com ácido fosfórico 37%.

## 2.2 Objetivos Específicos

- Comparar a resistência de união de dois cimentos resinosos autoadesivos aplicados à dentina com e sem remoção do colágeno após 24 horas, avaliando duas estratégias de desproteinização;

- Comparar a resistência de união de dois cimentos resinosos autoadesivos aplicados à dentina com e sem remoção do colágeno após envelhecimento através de ciclagem mecânica, avaliando duas estratégias de desproteinização;

- Avaliar a nanoinfiltração com nitrato de prata amoniacal na interface de união formada pelos cimentos resinosos autoadesivos e a dentina com e sem a remoção de colágeno em microscopia eletrônica de varredura após 24 horas e após envelhecimento;

- Avaliar o grau de conversão *in situ* dos cimentos resinosos autoadesivos aplicados à dentina com e sem remoção do colágeno após 24 horas.

*Capítulo*

## 3 CAPÍTULO

Esta dissertação está baseada no Artigo 46 do Regimento Interno do Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado, e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Por se tratar de estudos envolvendo seres humanos, ou parte deles, o projeto de pesquisa foi submetido à apreciação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, tendo sido aprovado (Anexo A). Assim sendo, esta dissertação é composta de um artigo científico que será submetido ao periódico *Journal of Dentistry*, conforme descrito abaixo:

**EFFECT OF DEPROTEINIZATION ON DEGREE OF CONVERSION AND BOND STRENGTH OF SELF-ADHESIVE RESIN CEMENTS TO DENTIN**

RODRIGUES NS, SOUZA LC, FEITOSA VP, LOGUERCIO AD, SAURO S, D'ARCANGELO C & SABOIA VPA

# EFFECT OF DEPROTEINIZATION ON DEGREE OF CONVERSION AND BOND STRENGTH OF SELF-ADHESIVE RESIN CEMENTS TO DENTIN


## ABSTRACT

Objectives: This study examined the effects of two deproteinization strategies on dentin microtensile bond strength (µTBS), *in situ* degree of conversion (DC) and interfacial nanoleakage (NL) of two self-adhesive resin cements after 24 h or after 200,000 load cycles. Methods: One hundred and fourteen third molars were distributed into six groups according to the type of cement used and the strategy of deproteinization: RelyX U200 (RU) following the manufacturer´s instructions (control), RU after dentin deproteinization using sodium hypochlorite (RU - NaOCl), RU after phosphoric acid etching and dentin deproteinization (RU – Etching + NaOCl), MaxCem Elite (ME) following the manufacturer´s instructions (control), ME after dentin deproteinization (ME - NaOCl), ME after phosphoric acid etching and dentin deproteinization (ME - Etching + NaOCl). The bonded specimens were randomly subdivided in two subgroups for the microtensile test: tested after 24h or after 200,000 load cycles. For NL, two slices from each subgroup were infiltrated with ammoniacal silver nitrate solution, prepared and analyzed in SEM. The *in situ* DC of three bonded-specimens from each immediate group was measured by micro-Raman spectroscopy. Data was statistically analyzed by Two-way ANOVA and Tukey's test ($p<0.05$). Results: Dentin deproteinization without prior acid etching increased the µTBS of both cements in the immediate group and no difference in RU groups were found after load cycling. Dentin deproteinization decreased the DC and NL of both cements. After load cycling, this technique was effective for ME, but did not affect the bond strength of RU. Conclusions: There were long-term benefits on dentin deproteinization for luting indirect restorations with self-adhesive resin cements.

Clinical Significance: The long term benefits of deproteinization presented in this work were encouraging and might justify this additional clinical step.



**KEYWORDS:** dentin, sodium hypochlorite, collagen, tensile strength.

## 1. INTRODUCTION

The clinical success of esthetic indirect restorations depends on the long term bond stability between resin cements and dental tissue [1,2]. Composite cements may be classified as conventional resin cements or self-adhesive resin cements, depending on the bonding strategy [3,4]. Self-adhesive cements were launched advocating to simplify clinically technique-sensitive multi-step procedures for luting indirect restorations. These cements are applied directly to the smear layer-covered dentin without pre-treatment using etchants or bonding solutions [5,6].

The use of adhesive procedures in the restoration of endodontically treated teeth is common. Among the possible restoratives techniques, fiber-reinforced composite posts, which are adhesively luted into the canal, may be used in the treatment of endodontically treated teeth with a considerable loss of tissue providing retention and permitting clinicians to be more conservative [7,8].

During the endodontic treatment, the instrumentation is used to shape and enlarge the root canal and irrigation is indispensable. Sodium hypochlorite (NaOCl) is the most common endodontic irrigant because of its antimicrobial characteristics [9-11]. NaOCl is also a nonspecific oxidizing and proteolytic agent able to oxidize the organic matrix and to remove the organic components of dentin due to its high alkalinity [12-15]. NaOCl pre-treatment associated or not with an etchant has recently demonstrated to improve the bonding of fiber post to radicular dentin by using resin cements [16].

Self-adhesive resin cements do not completely dissolve the smear layer and the superficiality of the interaction between cement and dentin might impair optimal bond strength [17,18]. Acidic functional monomers contained in self-adhesive cements urge low pH and hydrophilic properties in the beginning of the setting reaction. Thereafter, the negatively charged groups of the monomer bind to $Ca^{2+}$ ions and to the dentin. Concomitantly, the alkaline part of the fillers provides further neutralization reaction [19].

NaOCl may cause collagen removal by the deproteinization after etching with phosphoric acid [20], as well as on mineralized dentin [15,21], appearing a feasible alternative to eliminate some mechanical barrier such as the smear-layer. Its use exposes the subsuperficial dentin layer rich in hydroxyapatite and enhances the penetration and the chemical interactions between self-adhesive resin cements and the calcium of the dentin [22,23].

Therefore, the aim of this *in vitro* study was to examine the performance of two self-adhesive resin cements after deproteinization of mineralized and phosphoric acid etched dentin surfaces through microtensile bond strength (µTBS) and interfacial nanoleakage (NL) tests after immediate period or 200,000 load cycles, and *in situ* degree of conversion (DC) assessment.

The following hypotheses of the study are: 1) dentin deproteinization improves the adhesion performance on etched and mineralized dentin for both cements; 2) the DC of the self-adhesive resin cements is not affected by dentin deproteinization and; 3) the mechanical load cycles reduce the adhesion performance of both materials.

## 2. MATERIALS AND METHODS

### *2.1. Tooth preparation*

One hundred and fourteen freshly extracted human non-carious third molars were used in this study after obtaining the patients informed consent for their use, under a protocol approved by the Institution Ethics Committee. The teeth were stored in 0.01% thymol solution at 4°C for no more than 1 month. A flat dentin surface was exposed on each tooth after the occlusal enamel was cut with a water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, USA). Dentin surfaces were exposed and inspected under 80x magnification to ensure that no enamel remnants were left (Leica DM 1000 – Leica Microsystems GmbH - Wetzlar, Germany). The exposed dentin surfaces were further polished with wet 600-grit silicon-carbide papers for 30s to produce a standardized smear layer. Afterwards, each tooth was individually fixed to a sectioning machine (Isomet 1000) and the roots were removed using a diamond disc under cooling to obtain dentin specimens measuring approximately 4 mm thick.

### *2.2. Restorative procedure*

Resin composite (Filtek Z350 - 3M ESPE, Seefeld, Germany) blocks (5.0 x 5.0 x 2.0 mm) of restorative materials were made with the aid of a polyvinylsiloxane matrix. The resin blocks were light activated for 40 s (using incremental technique). The restoration surface in contact with the dentin was roughened by means of wet 400-grit silicon-carbide paper for 10s and then submitted to an ultrasonic bath in distilled water for 10 min. Before the cementation, the internal surface was silanized (Prosil, FGM, Joinville, Brazil) following manufacturer´s instructions.

The self-adhesive resin cements RelyX U200 (3M ESPE, Seefeld, Germany) and MaxCem Elite (Kerr, Orange, USA) were used to luting the resin blocks to the dentin surface. In accordance with the strategy of deproteinization, the specimens were distributed into six groups: RelyX U200 (RU) following the manufacturer´s instructions (control), RU after dentin deproteinization (RU - NaOCl), RU after phosphoric acid etching (15 s) and dentin deproteinization (RU – Etching + NaOCl), MaxCem Elite (ME) following the manufacturer´s instructions (control), ME after dentin deproteinization (ME - NaOCl), ME after phosphoric acid etching (15 s) and dentin deproteinization (ME – Etching + NaOCl) (Table 1). For the deproteinization, the dentin surface was treated with 5% NaOCl for 2 min under rubbing action and rinsed for 30 s. The cementation was done following the manufacturer´s instructions (Table 1). During luting procedure, the pressure onto the restoration was standardized at 20 g/mm$^2$.

*2.3. Specimens preparation and load cycling*

After 24 hours in distilled water at 37°C, the specimens from each group were randomly subdivided in two subgroups for the microtensile bond strength test (n=6): immediately and after load cycling (Figure 1). The cycled specimens were prepared as follows: a PVC cylindrical tube was used to attach the teeth in auto-polymerizing acrylic resin. The teeth were then removed from the acrylic resin and an additional silicone layer was used to support the teeth. The excess silicone was removed with a spatula at the level of the cement-enamel junction. After preparation, the specimens underwent 200,000 mechanical load cycles at 60 N, each load lasting one second with 2Hz frequency, using the chewing simulator CS-4 (SD Mechatronik, Westernham, Germany).

Then, each restored tooth was longitudinally sectioned in both “x” and “y” directions, across the bonded interface, using a diamond blade in an Minitom cutting-machine (Struers A/S, Copenhagen, Denmark) to obtain sticks with cross-sectional areas of approximately 0.8 $mm^{2.}$

*2.4. Microtensile bond strength test (µTBS)*

The sticks were measured individually with digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan) and subjected in a tensile force in a universal testing machine (EMIC DL 2000, São José dos Pinhais, Brazil) at crosshead speed of 1 mm/min.

The dentin side of the fractured sticks was analyzed using stereoscopic light microscopy (Leica DM 1000 – Leica Microsystems GmbH - Wetzlar, Germany) at 80X magnification and classified according to the failure mode as adhesive/mixed (M), cohesive in cement (CC), cohesive in dentin (CD) or cohesive in composite (CC).

*2.5. Interfacial nanoleakage evaluation (NL)*

For interfacial nanoleakage evaluation, two resin-dentin sticks from each subgroup were used. They were placed in ammoniacal silver nitrate solution in darkness for 24 h, rinsed thoroughly in distilled water, and immersed in photodeveloping solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface [24].

Specimens were polished with a 1200-, 2500- and 4000-grit SiC paper and 1 µm diamond paste (Buehler Ltd, Lake Bluff, USA), and ultrasonically cleaned for 3 min between each polish procedure. Thereafter, they were mounted on aluminum stubs, air dried and gold sputter coated for analysis in a field emission gun scanning electron microscope (SEM) (Quanta FEG, FEI, Amsterdam, Netherlands).

In order to standardize image acquisition, three pictures were taken from each specimen. The first micrograph was taken in the center of the bonded slice. The further two were attained to the left and right of the first one. The amount of nanoleakage within the adhesive layer areas was qualitatively evaluated.

*2.6. Degree of Conversion (DC)*

The degree of conversion at the selected bonded interfaces was measured by micro-Raman spectroscopy. Slices of three specimens from each immediate subgroup were obtained as above mentioned. The slices were polished with a 2500- and 4000-grit SiC paper and then ultrasonically cleaned for 3 min between each polishing procedure. Spectra were acquired from each specimen on the cement layer (n=3). Raman spectra were collected using Xplora micro-Raman (Horiba, Paris, France) in the range of 1590–1670 $cm^{-1}$ using the 638 nm laser emission wavelength, with 5 seconds acquisition time and 10 accumulations.

A small amount of uncured resin cement from each material was also obtained and its spectrum was used as unpolymerized reference. The % DC was calculated according to the two-frequency technique using the net peak absorbance areas of the aliphatic C=C stretching vibrations at 1635 $cm^{-1}$ as analytical frequency and the aromatic C···C stretching vibrations at 1610 $cm^{-1}$ as internal reference.

*2.7. Statistical analysis*

The experimental unit in the current study was the tooth. The microtensile bond strength values of all sticks from the same tooth were averaged for statistical purposes. All data were submitted to Kolmogorov-Smirnov normality test. After passing this test, the microtensile bond strength (MPa) data were subjected to Three-way (cement vs. strategy of deproteinization vs. cycling) ANOVA, Two-way (cement vs. strategy of deproteinization) ANOVA and Tukey's *post-hoc* test with $\alpha = 0.05$. For DC, data were statistically analyzed using Two-way ANOVA and Tukey's test.

## 3. RESULTS

*3.1. Microtensile bond strength test (µTBS)*

The three-way ANOVA demonstrated that load cycling did not induce significant effect in any variable. Therefore, two Two-way ANOVA tests were employed separately for the immediate and load cycled data. These tests showed that the interaction was statistically significant for cement and strategy of deproteinization for immediate ($p<0,001$) and cycled ($p=0,001$) subgroups. The µTBS means, standard deviations and number of specimens tested are shown in Tables 2 and 3. The use of the dentin deproteinization significantly increased the bond strength of both cements in the immediate group. After load cycling, ME - NaOCl and ME - Etching + NaOCl presented higher bond strength than ME control, while RU control and deproteinization groups maintained the bond strength values statistically similar ($p>0.05$).

The distribution of failure modes for each group is summarized in Table 4. All experimental groups showed a high incidence of cement cohesive failures. Cohesive fractures within the dentin were not observed for all groups.

### *3.2. Interfacial nanoleakage (NL)*

Representative SEM images of the adhesive interface produced in all conditions tested are depicted in Figure 2 and Figure 3. For both cements observed after 24 h, when dentin was deproteinized (Figure 2B, 2C, 2E and 2F) minimal silver nitrate deposition along the interface was observed. After load cycling, the deproteinized mineralized dentin (NaOCl - Figure 3H and 3K) showed the smallest amount of silver nitrate. The ME control group showed a large gap at the interface for immediate and load cycled control groups (Figure 2D and Figure 3J).

### *3.3. Degree of Conversion (DC)*

The mean and standard deviation values of the %DC are presented in Figure 3. The control groups exhibited the highest %DC, statistically different from other groups ($p < 0.05$). The presence of the NaOCl decreased the %DC for both self-adhesive resin cements. For RelyX U200 resin cement, the use of NaOCl after acid etching resulted in the lowest value of DC. When comparing both materials, RelyX U200 presented higher %DC than Maxcem Elite in all conditions.

## 4. DISCUSSION

According to the present results, both strategies of dentin deproteinization improved the immediate performance of self-adhesive resin cements. For the loaded cycled specimens, the use of NaOCl, regardless the prior etching, improved the bond strength of Maxcem Elite and had no effect with RelyX U200.

Sodium hypochlorite is a nonspecific proteolytic agent that effectively promotes collagen and proteoglycans dissolution. The use of solely NaOCl also alters significantly the mineral content of root dentin, increasing the Ca/P ratio of dentin surface [25], thereby providing more mineralized tissue similar to enamel which could improve the chemical interaction of the self-adhesive resin cements with calcium. NaOCl can penetrate the apatite-encapsulated collagen matrix and remove the organic phase from mineralized dentin [15,21]. Two minutes application of 5% NaOCl treatment was applied once it affects the organization of collagen and glycosaminoglycans in mineralized and partially demineralized dentin [13], improving the bonding results between dentin and self-adhesive resin materials [26,27].

The bonding mechanism of self-adhesive resin cements to dentin relies on mild etching along with shallow formation of an interdiffusion zone as well as on the chemical reaction of acidic functional monomers with calcium and hydroxyapatite [19,28]. The increase in the overall mineral content and the reduction in water content due deproteinization may be advantageous for the hydrophobic cement to chemically interact with hydroxyapatite and enhance the surface wettability, thereby improving the adhesion [29] and reducing the nanoleakage.

Self-adhesive resin cements are acidic initially after mixing in order to demineralize the dentin and approach neutral pH after curing [19,28]. Maxcem Elite maintains its low pH, whilst the pH of RelyX U200 increases after 24 h [30,31]. In the case such low pH is maintained for a long period, for instance with Maxcem Elite, a negative effect might occur on the adhesion between cement and dentin [32,33]. Indeed, the gaps (Figs. 2D and 3J) found in the interface of Maxcem could be explained by this longer low pH. In this case, a continuous etching can affect the optimal interaction of the cement.

Some studies suggest that self-adhesive resin cements have limited capacity to diffuse and decalcify the underlying dentin effectively [17,34]. When NaOCl solution is applied on smear layer-covered dentin, the mineral ratio increases and the smear layer is thinned due to dissolution of its collagen portion [21]. This NaOCl treated smear layer with less organic components may ease the bonding performance of self-adhesive resin cements, especially Maxcem Elite which contains the acidic functional monomer GPDM (glycerol-phosphate di-methacrylate) which has limited interaction with the smear layer-covered dentin.

After load cycling, the μTBS outcomes depicted that deproteinization is more effective for Maxcem Elite cement, but does not affect the bond strength of RelyX U200. Nevertheless, it was observed significant increase of silver infiltration inside the adhesive interface for the RelyX U200 control group and a gap in Maxcem Elite control group (Figures 3G and 3J). However, there was little amount of silver uptake in experimental (NaOCl treated) groups, thereby demonstrating optimal interaction between deproteinized dentin substrate and the self-adhesive resin cements (Figure 2 H, I, K and L). The decrease of nanoleakage for all materials suggests a possible long term benefit of the NaOCl pre-treatment to the indirect restorations luted with self-adhesive resin cements.

Maxcem Elite cement contains an amine-free redox initiator system while RelyX U200 has sodium sulfinate salts that prevent chemical incompatibility between acidic groups and self-curing components [35-37] which may explain the highest *in situ* degree of conversion of RelyX U200 (75,66%). In NaOCl treated groups, the residual hypochlorite and oxygen species may induce incomplete polymerization of resin matrix. The oxygen released by NaOCl molecules may interfere with free radical propagation, inhibiting the polymerization of the cement as described in previous reports [38,39]. One may speculate that deproteinization creates a porous substrate with higher concentrations of $OCl^-$ in such porous, resulting in localized decreased degree of conversion. The negative effect of NaOCl on polymerization may, conversely, be surpassed by the benefits of the improved chemical interaction of self-adhesive resin cements to dentin resulting in overall better performance of these materials.

Even considering the limitations of an in vitro methodology, which only simulates partially the intraoral conditions, the long term benefits of deprotenization presented herein are encouraging and might justify this additional clinical step. However, more investigations should be performed such as reducing the sodium hypochlorite concentration as well as clinical trials.

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Table 1: Resin cements, their application protocols and strategy of cementation

| | Composition | Control | Deproteinized |
|---|---|---|---|
| **RelyX U200 (3M/ESPE, Seefeld, Germany)** | Silane treated glass powder, substituted dimethacrylate, 1-benzyl- 5 -phenyl-barbic-acid, calcium salt, 1,12-dodecane dimethycrylate, sodium p-toluenesulfinate, silane treated silica, calcium hydroxide | 1. Apply the silane on the previously ragged surface of resin block<br>2.Mix cement for 10 s and apply on silanized surface of resin blocks<br>3. Remove excess cement. Wait 3 min and light cure each surface/margin for 20 s | **Etching + NaOCl**<br>1. Apply 37% $H_3PO_4$ Gel (Condac 37%/FGM, Joinville, SC, Brazil) for 15 s<br>2. Rinsing with air spray<br>3. Dry with absorbent paper, keeping dentin moisture<br>**NaOCl**<br>4. Apply 5% NaOCl under rubbing action for 2 min<br>5. Rinsing with air spray for 30 s<br>6. Dry with air spray<br>7. Apply the silane on the previously ragged surface of resin block<br>8.Mix cement for 10 s and apply on silanized surface of resin blocks<br>9. Remove excess cement. Wait 3 min and light cure each surface/margin for 20 s |
| **MaxCem Elite (Kerr, Orange, USA)** | Multifunctional dimethacrylates, Glyceroldimethacrylate Dihydrogen Phosphate (GPDM), proprietary Redox Initiators, photoinitiators, barium, fluoroaluminosilicate, fumed silica (66 wt%) | 1. Apply the silane on the previously ragged surface of resin block<br>2. Place appropriate mixer on dual syringe cartridge.<br>3. Remove excess cement. Excess can be removed in gel state (gel state is achieved in 3 minutes) and light cure each surface/margin for 20 s | **Etching + NaOCl**<br>1. Apply 37% $H_3PO_4$ Gel (Condac 37%/FGM, Joinville, SC, Brazil) for 15 s<br>2. Rinsing with air spray<br>3. Dry with absorbent paper, keeping dentin moisture<br>**NaOCl**<br>4. Apply 5% NaOCl under rubbing action for 2 min<br>5. Rinsing with air spray for 30 s<br>6. Dry with air spray<br>7. Apply the silane on the previously ragged surface of resin block<br>8. Place appropriate mixer on dual syringe cartridge and apply on silanized surface of resin blocks<br>9. Remove excess cement. Wait 3 min and light cure each surface/margin for 20 s |

Table 2: Mean microtensile bond strengths (MPa) and standard derivations (SD) for the immediate groups, as well as, statistical analysis (*)

| *μTBS (MPa)* | **Maxcem** | **U200** |
|---|---|---|
| **Control** | 3.87 (1.02) B,b | 15.3 (2.3) A,c |
| **NaOCl** | 21.2 (3.9) B,a | 26.1 (3.3) A,a |
| **Etching + NaOCl** | 23.3 (2.7) A,a | 20.7 (3.2) A,b |

*Different lower case letters in column and capital letters in row indicate statistical difference ($p<0.05$).

Table 3: Mean microtensile bond strengths (MPa) and standard derivations (SD) for the load cycled groups, as well as, statistical analysis (*)

| *μTBS (MPa)* | **Maxcem** | **U200** |
|---|---|---|
| **Control** | 1.6 (3.82) B,b | 13.7 (6.24) A,a |
| **NaOCl** | 24.9 (6.02) A,a | 14.9 (3.49) B,a |
| **Etching + NaOCl** | 24.7 (5.92) A,a | 7.9 (6.14) B,a |

*Different lower case letters in column and capital letters in row indicate statistical difference ($p<0.05$).

Table 4: Percentage distribution according to the failure modes.

| RelyX U200 | | | | Maxcem Elite | | | |
|---|---|---|---|---|---|---|---|
| Control | Immediate | M | 25% | Control | Immediate | M | 100% |
| | | CC | 73,8% | | | CC | - |
| | | CR | 1,2% | | | CR | - |
| | | CD | - | | | CD | - |
| | Load Cycled | M | 42% | | Load Cycled | M | 100% |
| | | CC | 58% | | | CC | - |
| | | CR | - | | | CR | - |
| | | CD | - | | | CD | - |
| NaOCl | Immediate | M | 28% | NaOCl | Immediate | M | 16,2 % |
| | | CC | 68,2% | | | CC | 78,8 % |
| | | CR | 3,8% | | | CR | 5% |
| | | CD | - | | | CD | - |
| | Load Cycled | M | 30,1% | | Load Cycled | M | 28% |
| | | CC | 65,9% | | | CC | 68,6% |
| | | CR | 4 % | | | CR | 3,4% |
| | | CD | - | | | CD | - |
| Etching + NaOCl | Immediate | M | 29% | Etching + NaOCl | Immediate | M | 22,5% |
| | | CC | 66,3% | | | CC | 73,6% |
| | | CR | 4,7% | | | CR | 3,9% |
| | | CD | - | | | CD | - |
| | Load Cycled | M | 44% | | Load Cycled | M | 30,2% |
| | | CC | 54% | | | CC | 65,8% |
| | | CR | 2% | | | CR | 4% |
| | | CD | - | | | CD | - |

M: mixed/adhesive; CC: cohesive in cement; CR: cohesive in resin; CD: cohesive in dentin

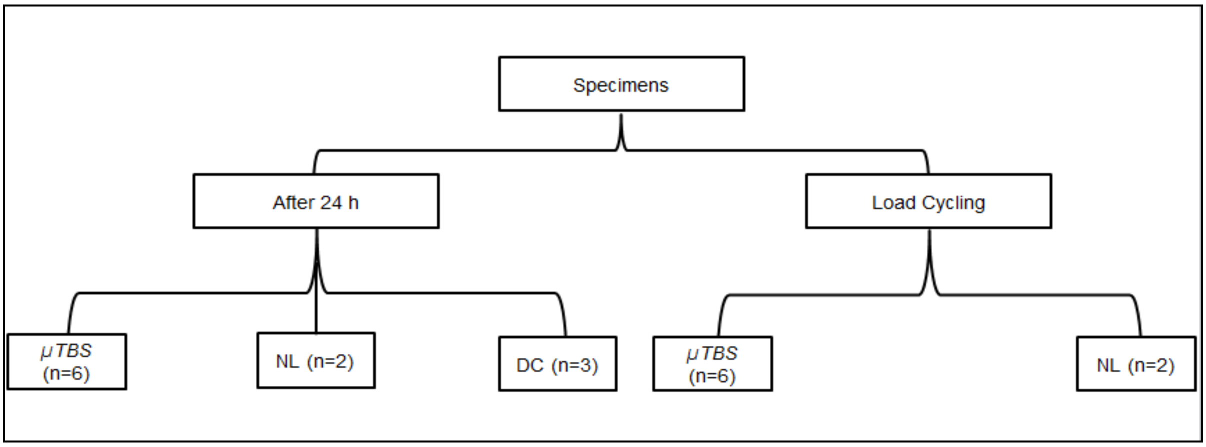


Figure 1 – Flowchart of Experimental Design (µTBS = microtensile bond strength test; NL = interfacial nanoleakage evaluation; DC = degree of conversion)

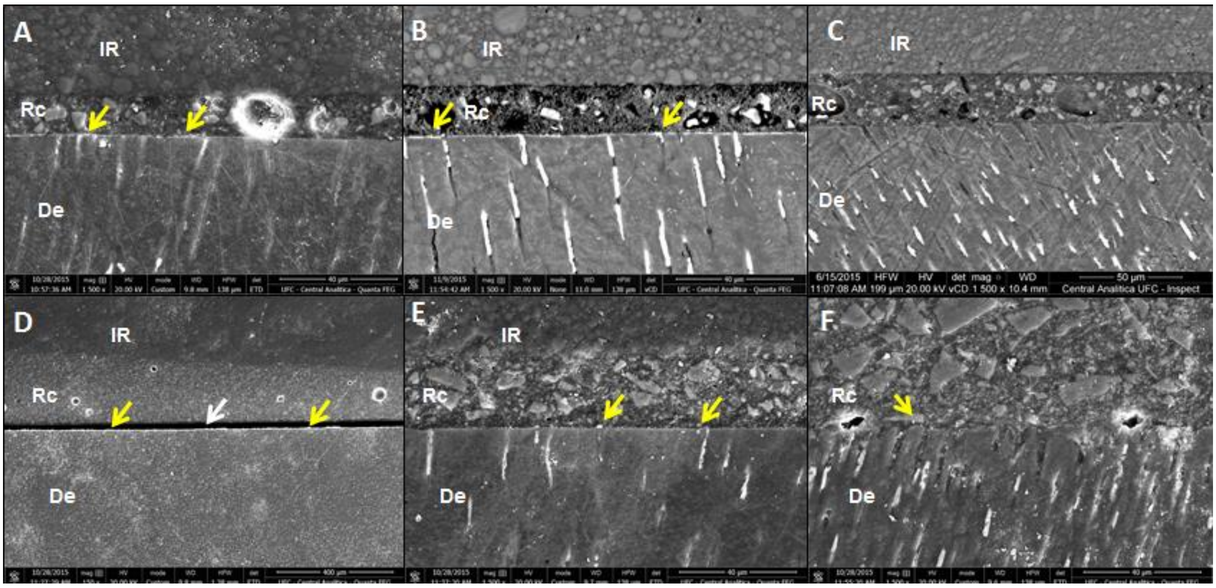


Figure 2 - Representative SEM images of the resin–dentin interfaces bonded with Relyx U200 (RU) and MaxCem Elite (ME) in the immediate groups without (control; A and D) or with dentin deproteinization (NaOCl: B and E; Etching + NaOCl: C and F). Only few areas of silver nitrate uptake were observed within the adhesive interface (yellow arrows). For ME control group (D) a large gap can be seen (white arrow). (IR = indirect restoration; Rc = resin cement; and De = dentin).

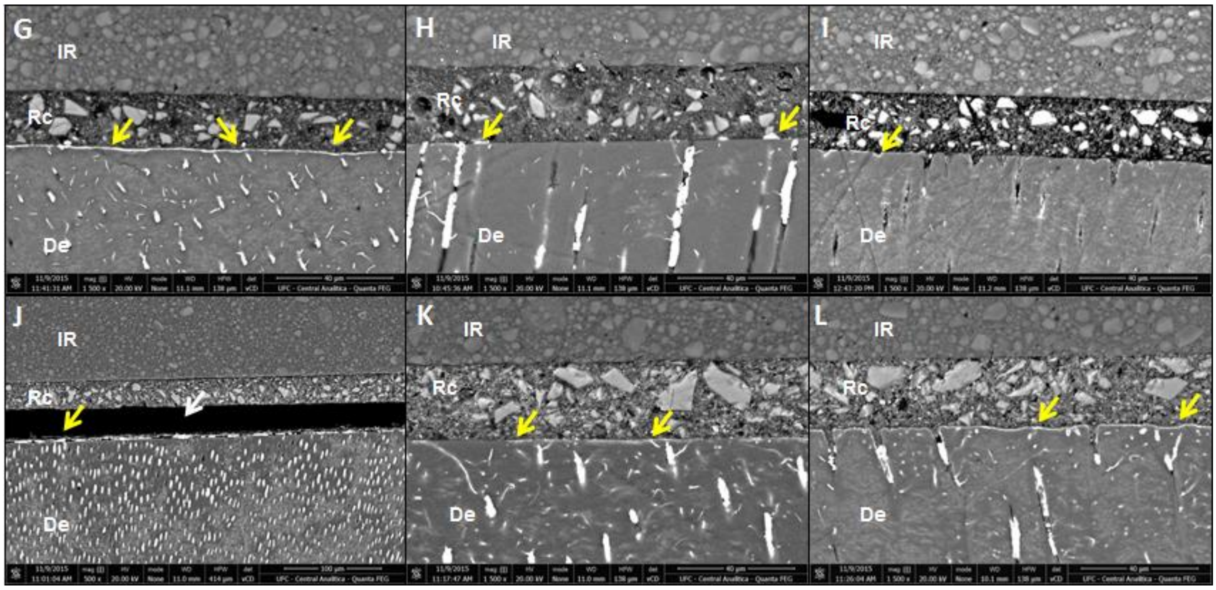


Figure 3 - Representative SEM images of the resin–dentin interfaces bonded with Relyx U200 (RU) and MaxCem Elite (ME) after load cycling without (control; G and J) or with dentin deproteinization (NaOCl: H and K; Etching + NaOCl: I and L). RU control (G) showed a continuous line of silver nitrate uptake. When dentin surface was previously deproteinized, only few areas of silver nitrate uptake were observed within the adhesive interface (yellow arrows). ME control (J) presented a large gap like in immediate group (white arrow). (IR = indirect restoration; Rc = resin cement; and De = dentin).

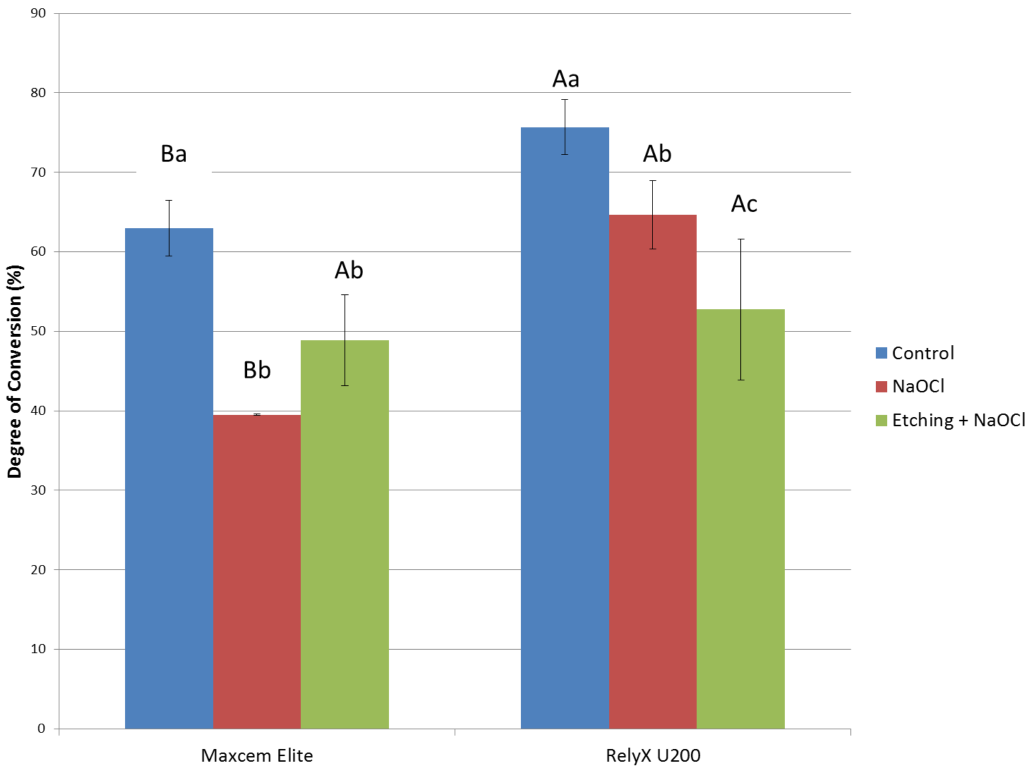


Figure 4. Degree of conversion (%) analysis represented by means and standard deviations for Maxcem Elite and RelyX U200. Different lower case letters intragroup and capital letters intergroup indicate statistical difference ($p<0.05$).

# *Conclusão*

# 4 CONCLUSÃO

De acordo com os resultados obtidos no presente estudo, é possível concluir que houve benefícios em longo prazo quando a desproteinização foi realizada previamente à cimentação, especialmente quando o cimento autoadesivo Maxcem Elite foi utilizado. A desproteinização da dentina sem condicionamento ácido prévio aumentou os valores de resistência de união dos dois cimentos nos grupos imediatos. Após a ciclagem mecânica, essa técnica foi efetiva para o cimento Maxcem Elite, mas não afetou a resistência de união do RelyX U200. Entretanto, presença do NaOCl diminuiu o grau de conversão de ambos os cimentos resinosos estudados. As duas estratégias de remoção de colágeno se mostraram eficazes na diminuição da nanoinfiltração após ciclagem mecânica, sugerindo maior interação entre o cimento autoadesivo e a dentina. Os resultados foram promissores e podem justificar esse passo adicional na conduta clínica, sendo necessários mais estudos.

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# *Anexos*

## ANEXO A – APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

### PARECER CONSUBSTANCIADO DO CEP

**DADOS DO PROJETO DE PESQUISA**

**Título da Pesquisa:** Efeito da desproteinização dentinária na resistência de união de cimentos resinosos à dentina

**Pesquisador:** Lidiane Costa de Souza

**Área Temática:**

**Versão:** 1

**CAAE:** 14049013.3.0000.5054

**Instituição Proponente:** Universidade Federal do Ceará/ PROPESQ

**Patrocinador Principal:** Financiamento Próprio

**DADOS DO PARECER**

**Número do Parecer:** 339.789

**Data da Relatoria:** 25/04/2013

**Apresentação do Projeto:**

Trata-se de um estudo experimental, laboratorial, randomizado, cego, transversal onde serão cimentados em trinta e dois terceiros molares, após a remoção da porção de esmalte coronário e da porção radicular. Os dentes e os blocos de resina serão distribuídos nos seguintes grupos de acordo com a estratégia de cimentação utilizada: 1- RelyX ARC controle (de acordo com as instruções do fabricante); 2- RelyX ARC desproteinizado

(aplicação de ácido fosfórico a 37% + aplicação de NaOCl 5% por 2 min); 3- RelyX U200 controle (de acordo com as instruções do fabricante) 4- RelyX U200 desproteinizado (aplicação de ácido fosfórico a 37% + aplicação de NaOCl 5% por 2 min). Cada dente, após a cimentação, será submetido ao corte em cortadeira metalográfica pela técnica non-trimmed para a obtenção de palitos. Metade dos palitos de cada dente será testada

de forma imediata e metade será testada após 20.000 ciclos térmicos. Os espécimes serão submetidos ao teste de microtração para avaliar a resistência de união. O padrão de fratura será avaliado em lupa estereoscópica. Dois dentes a mais de cada grupo serão preparados para análise do padrão de nanoinfiltração por microscópio eletrônico de varredura (MEV). Os valores de resistência dos espécimes testados serão agrupados por

grupo por meio de uma média aritmética. Será realizada uma análise de variância de medidas

**Endereço:** Rua Cel. Nunes de Melo, 1127
**Bairro:** Rodolfo Teófilo **CEP:** 60.430-270
**UF:** CE **Município:** FORTALEZA
**Telefone:** (85)3366-8344 **Fax:** (85)3223-2903 **E-mail:** comepe@ufc.br

repetidas de 3 fatores (cimento vs. desproteinização vs. termociclagem). O nível de significância será de p0.05

**Objetivo da Pesquisa:**

objetivo primário: avaliar a ação da desproteinização dentinária com NaOCl a 5% na interface de união formada por cimentos resinosos e dentina.

Objetivo secundário: Comparar a resistência de união de um cimento resinoso autoadesivo e de um cimento resinoso convencional aplicado à dentina desproteinizada e à dentina com manutenção de fibras colágenas.

- Comparar a resistência de união resina/dentina de um cimento resinoso autoadesivo e de um cimento resinoso convencional de forma imediata e após 20.000 ciclos térmicos.- Comparar a resistência de união resina/dentina de um cimento

resinoso autoadesivo e de um cimento resinoso convencional aplicados após a desproteinização dentinária de forma imediata e após 20.000 ciclos térmicos.- Avaliar o padrão de fratura dos espécimes após o teste de resistência de microtração.- Analisar a nonoinfiltração da interface resina/dentina com ou sem a manutenção de colágeno, de forma imediata e após 20.000 ciclos térmicos através da microscopia eletrônica de varredura (MEV).- Determinar a quantidade de nitrato de prata presente nas interfaces resina/dentina utilizando um microscópio eletrônico de varredura de emissão de campo operada no modo de elétrons retroespalhados e uso de energia dispersiva de raios-X espectrometria (EDX).

**Avaliação dos Riscos e Benefícios:**

Riscos:

A pesquisa apresenta risco mínimo.

Benefícios:

A desproteinização dentinária pode favorecer a maior longevidade das restaurações indiretas cimentadas com cimentos resinosos

**Comentários e Considerações sobre a Pesquisa:**

Pesquisa relevante para a área odontologia, esta bem escrita e estruturada.

**Considerações sobre os Termos de apresentação obrigatória:**

Todos os termos obrigatórios forma apresentados.

**Recomendações:**

não se aplica

**Conclusões ou Pendências e Lista de Inadequações:**

Projeto aprovado s.m.j.

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**Situação do Parecer:**

Aprovado

**Necessita Apreciação da CONEP:**

Não

**Considerações Finais a critério do CEP:**

FORTALEZA, 24 de Julho de 2013

**Assinador por:**
**FERNANDO ANTONIO FROTA BEZERRA**
**(Coordenador)**

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