Unesp – Universidade Estadual Paulista

“Júlio de Mesquita Filho”

Faculdade de Odontologia de Araraquara

ANNA THEREZA PEROBA REZENDE RAMOS

EFEITO DA TERAPIA FOTODINÂMICA SOBRE A RESISTÊNCIA DE UNIÃO E A PENETRABILIDADE DENTINÁRIA DE DIFERENTES PROTOCOLOS DE CIMENTAÇÃO DE PINOS DE FIBRA DE VIDRO

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Orientador: Prof. Dr. Marcelo Ferrarezi de Andrade

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Dissertação para obtenção do grau de Mestre em Ciências Odontológica.

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Araraquara, 16 de março de 2017.

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2008 – 2013:

Graduação em Odontologia.

Universidade Federal de Alagoas, UFAL, Brasil.

Título: ANÁLISE, IN VITRO E IN SITU, DO POTENCIAL EROSIVO DE BEBIDAS ISOTÔNICAS SOB A REALIZAÇÃO DE EXERCÍCIO FÍSICO.

Orientador: Natanael Barbosa dos Santos.

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2012 – 2013:

Aperfeiçoamento em Odontologia estética integrada. (Carga Horária: 192h).

NEO Odontologia, NEO, Brasil.

2013 – 2015:

Especialização em Odontologia Estética Restauradora.

Associação Brasileira de Odontologia - Seção de Alagoas, ABO/AL, Brasil.

Título: Can endodontic sealer residues affect the bond strength of self-etching adhesive systems to dentine?.

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2015 – atual:

Mestrado em andamento em Ciências Odontológicas (Conceito CAPES 5).

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2016 – 2017:

Aperfeiçoamento em andamento em Prótese sobre implantes e dentes naturais.

Associação Paulista de Cirurgiões Dentista, APCD, Brasil.

À minha mãe, razão da minha felicidade!

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"Quer saber quanto custa uma saudade:

Tenha amor, queira bem e viva ausente!"

Ramos ATPR. Efeito da terapia fotodinâmica sobre a resistência de união e a penetrabilidade dentinária de diferentes protocolos de cimentação de pinos de fibra de vidro [Dissertação de Mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2017.

RESUMO

Essa pesquisa avaliou o efeito da Terapia Fotodinâmica (PDT) sobre diferentes protocolos de cimentação de pinos de fibra de vidro com cimentos resinosos convencionais ou autoadesivos, assim como os cimentos ionoméricos, através da resistência de união e penetrabilidade dentinária por meio de ensaio mecânico “push-out”, análise do padrão de fratura e microscopia confocal à laser. Foram utilizados dentes humanos, com raízes padronizadas em 15mm e tratados endodonticamente com o sistema ProTaper (Dentsply, Ballaigues, SW), irrigados com hipoclorito de sódio a 2,5% e obturados pela técnica do cone único com cimento endodôntico contendo resina epóxi (AH Plus; Dentsply DeTrey GmbH, Konstanz, Germany). Após armazenamento em estufa com umidade a 37ºC por 7 dias, foi preparado o espaço e instalado o pino de fibra de vidro com o sistema White Post DC 2 (FGM, Joinville, SC, BR). De forma aleatória, as raízes foram divididas em 6 grupos (n=10) de acordo com o sistema de cimentação e a utilização da PDT. Os grupos que fazem parte da utilização da PDT tiveram seu canal radicular irrigado com soro fisiológico, seco, preenchido com fotossensibilizador (azul de metileno), permaneceu em repouso por 5 minutos e irradiado por uma fibra ótica adaptada em fonte de emissão, então foi removido o corante e seguido o protocolo de cimentação do pino conforme o fabricante recomenda tanto para o tratamento do pino, quanto para o tratamento da dentina radicular e manipulação do cimento. Nessa etapa foi adicionado rodamina B à cimentação para permitir avaliação em microscopia confocal à laser. Após inserção das raízes verticalmente em matrizes plásticas e completa polimerização dos cimentos, o conjunto foi seccionado em 3 fatias de acordo com os terços radiculares. Os pinos de fibra de vidro foram submetidos ao teste de push-out, determinado o padrão de fratura e avaliação da penetrabilidade dentinária do cimento. A análise estatística foi feita através do teste ANOVA e pós-teste Tukey, com nível de significância de 5%. A utilização da PDT exerce efeito negativo sobre a resistência de união do sistema de cimentação RelyX ARC na dentina cervical, e sobre a penetração dentinária do sistema RelyX ARC, no terço apical e cervical radicular. A PDT exerce efeito negativo sobre a resistência de união quando utilizado CIV no terço cervical, contudo não influenciou sobre a penetração dentinária.

Palavras-chave: Fotoquimioterapia. Cimentos de resina. Espécies de oxigênio reativas. Pinos dentários.

Ramos ATPR. Effect of photodynamic therapy on the bond strength and dentinal penetrability using different glass-fiber post cementation systems [Dissertação de Mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2017.

ABSTRACT

The aim of this study was to evaluate the effects of photodynamic therapy (PDT) on the glass-fiber post cementation protocols using conventional or self-adhesive resin cements and a glass ionomer cement (GIC) on the bond strength and dentinal penetrability. The specimens were submitted to the push-out test, failure mode and laser confocal microscopy evaluation. Human canine roots standardized in 15mm were endodontically-treated using ProTaper (Dentsply, Ballaigues, SW) system, irrigated with 2.5% sodium hypochlorite and obturated with single cone technique and epoxi-based sealer (AH Plus; Dentsply DeTrey GmbH, Konstanz, Germany). The specimens were stored under 100% relative humidity, at 37^{o}C for 7 days. Afterwards, the intracanal prosthetic space was prepared and the glass-fiber post (White Post DC 2, FGM, Joinville, SC, BR) was placed. The roots were randomly divided into 6 groups (n = 10) according to the cementation system and PDT use. The groups that used PDT were irrigated with saline, dried, filled with photosensitizer (methylene blue), left untouched for 5min and irradiated using a laser emission source. The pigment was removed, the post cementation protocol was handled according to the manufacturer´s recommendations. Rhodamine B was added in the cementation system to facilitate the evaluation of confocal laser microscopy. The roots were vertically placed inside plastic matrices and filled with polyester resin. After 24h, the set was sectioned in 3 slices according to the root thirds. The glass-fiber posts were subjected to the push-out test, the failure mode and dentinal penetrability analysis. The data were submitted to ANOVA and Tukey post-tests, at 5% significance level. PDT presented a negative effect on the bond strength of RelyX ARC cementation system to the cervical dentin and on the dentinal penetrability of RelyX ARC system in the apical and cervical thirds. Moreover, PDT showed a negative effect on the bond strength of GIC to the cervical third; however, it did not influence the dentinal penetrability.

Keywords: Photochemotherapy. Resin Cements. Reactive oxygen species. Dental pins.

SUMÁRIO

1 INTRODUÇÃO

O preparo do espaço protético para a colocação de um pino de fibra de vidro implica na remoção parcial da obturação do canal radicular, nos terços cervical e médio, expondo a superfície dentinária ao meio bucal. Em situações em que o preparo estrategicamente não tenha sido realizado com isolamento absoluto do campo operatório poderá ocorrer a contaminação microbiana do local durante os procedimentos, comprometendo o sucesso do tratamento (Bitter, Kielbassa[5], 2007; Cheung[6], 2005).

Visando controlar essa contaminação microbiana, alguns recursos têm sido preconizados para a antissepsia do espaço protético, previamente à cimentação dos pinos de fibra de vidro, tais como a irrigação com solução de hipoclorito de sódio e digluconato de clorexidina (Lima et al.[13], 2015). Entretanto, os compostos halógenos tendem a interferir negativamente sobre a resistência de união dos cimentos resinosos à dentina radicular (Martinho et al.[14], 2015; Saraiva et al.[16], 2013).

A solução de hipoclorito de sódio origina ácido hipocloroso e hidróxido de sódio (Kuga et al.[12], 2011). Por sua vez, a degradação química do ácido hipocloroso ocasiona a liberação de cloro livre e oxigênio *singlet* (Estrela et al.[8], 2002; Guida[10], 2006). A presença destes radicais livres tende a interferir sobre as cadeias aminas dos cimentos resinosos, comprometendo o processo de polimerização e a integridade do procedimento de cimentação dos pinos de fibra (Bitter et al.[4], 2013). Similar situação também pode ocorrer com as soluções de clorexidina, uma vez que também produzem produtos residuais contendo radicais livres oxidativos (Bitter et al.[3], 2014).

Uma vez que estas substâncias podem comprometer a integridade da adesão dos cimentos resinosos à dentina, outras alternativas têm sido sugeridas (Arslan et al.[2], 2015; Bitter et al.[3], 2014). A utilização de métodos mecânicos, tais como o ultrassom, proporcionam satisfatória limpeza da parede dentinária do canal radicular, porém necessitam estar associada a alguma solução antisséptica para exercerem uma adequada atividade antimicrobiana (Aranda-Garcia et al.[1], 2013; Jiang et al.[11], 2011).

Por sua vez, a utilização da terapia fotodinâmica (PDT) com fotossensibilizantes específicos não tóxicos, tais como o azul de metileno, pode ser uma alternativa interessante para suplementar as deficiências demonstradas pelos demais recursos (Gergova et al.[9], 2016). Tal afirmação é fundamentada no fato de que a irrigação dos canais radiculares com hipoclorito de sódio a 5,25% associado à terapia fotodinâmica demonstra ser o método mais efetivo para reduzir a contaminação microbiana em canais radiculares instrumentados pela técnica do instrumento único (de Oliveira et al.[7], 2015).

O mecanismo de ação do PDT consiste em utilizar um comprimento de onda específico para ativar um corante não tóxico (fotossensibilizante), levando à formação de radicais livres oxidativos, que danificam as proteínas, membrana celular e ácidos nucleicos microbianos, promovendo a morte bacteriana (Singh et al.[18], 2015). Uma vez que a dinâmica do uso do PDT também envolve a liberação de radicais livres, tais como oxigênio *singlet*, similar ao que ocorre nas soluções halogenadas, são desconhecidos os efeitos que este método poderia exercer sobre a resistência de união dos sistemas de cimentação de pino de fibra na dentina radicular.

Adicionalmente, o cimento de ionômero de vidro é um promissor agente de cimentação de pino de fibra, apresentando satisfatórios valores de resistência de união à dentina radicular (Pereira et al.[15], 2014). Como o seu mecanismo de ação e adesão são diferentes dos cimentos resinosos, há o questionamento se os radicais livres liberados tanto pelas substâncias halogenadas como pelo PDT poderiam interferir na cimentação dos pinos de vidro (Sidhu, Watson[17], 1995).

Sendo assim, torna-se pertinente avaliar a solução de hipoclorito de sódio e a PDT sobre os sistemas de cimentação de pinos de fibra com cimentos resinosos convencionais ou autoadesivos, bem como como os cimentos ionoméricos.

2 PROPOSIÇÃO

2.1 Proposição Geral

O presente estudo tem por objetivo geral avaliar o efeito da terapia fotodinâmica sobre a resistência de união e a penetrabilidade dentinária de diferentes protocolos de cimentação de pinos de fibra de vidro.

2.2 Proposição Específica

O objetivo do presente estudo será avaliar:

1. A resistência de união do sistema de adesão de pinos de fibra de vidro na dentina radicular, utilizando o sistema adesivo “condiciona e lava” (Adper Scotchbond Multiuso Plus) com o cimento resinoso (RelyX ARC) comparado a um cimento resinoso autocondicionante (RelyX U200) na dentina radicular ou um cimento ionomérico (GC Gold Label 1 C), após a utilização da terapia fotodinâmica associada ao fotossensibilizante azul de metileno (0,005%);
2. A penetrabilidade intradentinária dos sistemas de adesão após a utilização dos métodos de cimentação citados e a utilização da terapia fotodinâmica.
3. Avaliar o padrão de fratura encontrado após a cimentação com os métodos supracitados e a utilização da terapia fotodinâmica.

3 PUBLICAÇÕES

3.1 Publicação 1

Effects of photodynamic therapy on the adhesive interface of fiber posts cementation protocols*

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Abstract

Introduction: The aim of this study was to evaluate the effects of photodynamic therapy (PDT) on the bond strength and dentinal penetrability of cementation protocols using conventional resin cement (Relyx ARC) or self -adhesive (Relyx U200), after the glass-fiber post cementation . **Methods**: Forty human canine roots were endodontically-treated and prepared for fiber post. The roots were divided into four groups, according to the cementation protocol and PDT use: G1, Relyx ARC; G2, Relyx U200 cement; G3, PDT + Relyx ARC; and G4, PDT + Relyx U200. After the fiber posts cementation, the roots were cross-sectioned and then, specimens from the cervical, middle and apical thirds of the prosthetic space were obtained. The specimens were submitted to the push-out test and dentinal penetration evaluation of the cementation protocol using laser confocal microscopy. **Results**: G3 presented the lowest bond strength to root dentin in the cervical third ($P < 0.05$). In the middle and apical thirds, all groups presented similar bond strength ($P > 0.05$). G3 presented the lowest dentinal penetration of the adhesive system in the cervical and apical thirds ($P < 0.05$). **Conclusions**: PDT presented negative effects on the bond strength to dentin in cervical third after cementation using Relyx ARC, and on the dentinal penetrability of the etch-and-rinse adhesive system in the cervical and apical thirds of the prosthetic space.

Key Words: bond strength, fiber posts, photodynamic therapy, push-out bond strength, self-adhesive cement.

Introduction

The intracanal preparation of prosthetic space for fiber post requires a partial removal of the root canal obturation. During this procedure, a local contamination may occur, which compromises the success of endodontic and / or restorative treatments (1, 2). Sodium hypochlorite or chlorhexidine digluconate have been recommended for the prosthetic space irrigation, but they have shown negative effects on the bond strength of the resin cements to root dentin (3-5).

Free radicals participate in the polymerization process of resinous compounds inducing chemical reactions in the methacrylates structure (6). Thus, the degree of conversion and the adhesion of resinous materials to the dentin are compromised by the substances that interact with these free radicals, such as sodium hypochlorite, which degrades in sodium hydroxide and hypochlorous acid, and consequently leads to the singlet oxygen formation (7-9). Moreover, the oxygen presence can also work as a barrier in the adhesive interface, which hampers the hybrid layer formation in dentin (10).

Since the sodium hypochlorite may cause those undesirable effects, other alternatives have been sought to perform the prosthetic space antisepsis. Henceforth, the photodynamic therapy (PDT) with specific photosensitizers, such as 0.005% or 0.01% methylene blue, has been an interesting option (11) owing to its satisfactory antimicrobial activity in contaminated root canals (12).

The mechanism of action of PDT occurs when a photosensitizing substance absorbs the photons from the irradiation source and their electrons enter an excitation state. Then, the energy is transferred to a specific substrate, forming the reactive oxygen species (ROS), mainly singlet oxygen, which irreversibly oxidize the cellular components causing bacterial death (11, 13, 14). However, it is still unknown whether those free radicals, from the ROS release, affect the adhesive interface between the dentin and fiber post cement, after different cementation protocols, similarly to those decontamination protocols using sodium hypochlorite.

Therefore, the aim of this study was to evaluate the effects of photodynamic therapy using 0.005% methylene blue in the intracanal prosthetic space on the bond strength and intra-dentinal penetrability using conventional (Relyx ARC) or self-adhesive (Relyx U200) resin cements, in different root thirds, after the fiber post cementation protocols. The null hypothesis was that photodynamic therapy does not affect the adhesion and the dentinal penetration of fiber posts cements.

Materials and Methods

The study was approved by the Research Ethics Committee of FOAr-UNESP (1.603.859) (attachment). Forty human canines with similar root anatomy and absence of structural alterations were selected and kept in 0.1% thymol solution at 4^{o}C.

The dental crowns were removed about 15mm from the root apex. Then, the chemical-mechanical preparation and root canal obturation were carried out according to Aranda-Garcia et al (15). After the vertical condensation obturation, the cervical opening was sealed using temporary cement (Coltosol, Coltene, Rio de Janeiro, Brazil) and the roots were stored under 100% relative humidity, at 37^{o}C for 7 days.

The preparation of the intracanal prosthetic space was performed using #2 bur (White Post DC System; FGM, Joinville, SC, Brazil), 11mm length. Then, it was irrigated using 10mL of distilled water and dried with absorbent paper points. The specimens were randomly divided into four groups (n=10), according to the cementation protocol and PDT application in the prosthetic space: G1, conventional cement (Relyx ARC); G2, self-adhesive cement (Relyx U200); G3, photodynamic therapy and conventional cement (PDT + Relyx ARC) and G4, photodynamic therapy and self-adhesive cement (PDT + Relyx U200).

The fiber post surface was cleansed using 95% ethanol, etched with 37% phosphoric acid (Power Etching, BM4, Palhoça, SC, Brazil) for 1 minute, then the silane (Prosil; FGM, Joinville, BR) and dentin adhesive (Adper Scotchbond Multiuso Plus; 3M ESPE, St. Paul, MN, USA) were applied throughout its length. Afterwards, the whole set was light-cured for 60s (Bluephase; Ivoclar Vivadent, Barueri, SP, Brazil) (appendage).

PDT was carried out in G3 and G4. Initially, the prosthetic space was filled with 1000µL of 0.005% methylene blue (Chimiolux; DMC, São Carlos, SP, Brazil), and the root cervical face was covered with laminated paper and left untouched for 5 minutes. After that, an optical fiber (Twin Flex Evolution, MMO Opto-Electronic Equipment, São Carlos, SP, Brazil) was inserted into the entire prosthetic space, in at static position and the prosthetic space irradiated for 30s using a laser emission source (Twin Flex Evolution, MMO Opto-Electronic Equipment, São Carlos, SP, Brazil), output power 30 J/cm^{2}.

Afterwards, the methylene blue was aspirated and the prosthetic space was irrigated with 3mL of saline solution and dried with absorbent paper points. Prior to the fiber posts cementation, 0.01% (by mass) of Rhodamine B isothiocyanate was added to the primer of the adhesive system (Adper Scotchbond Multiuso Plus; 3M ESPE, St. Paul, MN, USA) and used in G1 and G3. Rhodamine B isothiocyanate was also added to the cements and used in G2 and G4. All specimens were subjected to laser confocal microscopy evaluation.

G1 and G3 were acid etched (Power Etching; BM4, Palhoça, SC, Brazil) for 15s, irrigated with distilled water for 30s and dried with absorbent paper points. The adhesive system (Adper Scotchbond Multiuso Plus, 3M ESPE, St. Paul, MN, USA) was applied throughout the prosthetic space and light-cured for 20s (Bluephase; Ivoclar Vivadent, Barueri, SP, Brazil).

The cements were handled according to the manufacturers' recommendations and are described in Table 1. Immediately after the cementation of #2 (FGM, Joinville, SC, BR) fiber posts, the roots were vertically centralized inside a PVC matrix (16.5 diameter x 15.0mm length) and checked using parallelogram (BioArt B2, São Carlos, SP, BR). The matrices were filled with polyester resin (Maxi Rubber, Diadema, SP, Brazil), leaving 1.0mm of the root cervical outside the inclusion. The whole set was left undisturbed for 24 hours. Then, the specimens were removed from the matrices and were sectioned perpendicular to their long axis with a diamond disk using hard tissues cutting machine (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under running water-cooling. Three sections were performed with 2.0mm ± 0.1mm thickness from the apical, middle and cervical thirds of the prosthetic space. The cervical, medial and apical sections were carried out respectively from 1.0mm, 5.0mm and 8.0mm from the root cervical face. The sections irregularities were removed using #1200 (Norton, São Paulo, SP, Brazil) sandpaper.

The specimens of each root third were submitted to a push-out test using an electromechanical test machine (EMIC, São José dos Pinhais, PR, Brazil), at 0.5mm/min speed using 5kN load cell, until the complete displacement of all root canal walls. The force required to the displacement occurs was obtained in N (Newton) and it was transformed into bond strength (MPa), as according to Magro et al (16). Subsequently, each specimen was assessed using a stereomicroscope, at 20X magnification to determine the failure mode. The failure mode was classified as: type 1 (Adhesive): when it ocurred between the fiber post and the cement; Type 2 (Adhesive): between dentin and cement; Type 3 (Cohesive) within the cement and type 4 (Mixed) when both types of failure were combined, as according to Elnaghy (17).

The sections were analyzed using a laser confocal microscope at 100X magnification to determine the root canal perimeter with the materials penetration within the dentinal tubules. The images were evaluated using Image J software. The perimeter of the root canal and the cement penetration in dentin were measured, and the material penetration in the dentinal tubules percentage was obtained.

Statistical Analysis

The data were submitted to ANOVA and Tukey tests at 5% significance level.

Results

Regarding the bond strength of the fiber post cements to root dentin, all groups presented similar results in the middle and apical thirds of the prosthetic space ($P > 0.05$). G3 was the only group that presented the lowest value in the cervical third ($P < 0.05$). Table 2 shows the mean and standard deviation of bond strength (in MPa) of each third according to the PDT use in the prosthetic space.

In relation to the dentinal penetration of the fiber post cementation protocols, all groups presented similar results in the middle third ($P > 0.05$). In the cervical and apical thirds, G3 presented the lowest dentinal penetration ($P < 0.05$). Table 3 shows the mean and standard deviation of the dentinal penetration (%) of the fiber posts cementation protocols in all thirds of the prosthetic space, according to the cementation protocol and PDT use. Figure 1 shows the dentinal penetration pattern of the groups.

Regarding the failure mode, G3 and G4 presented higher incidence of type 2 failure. G1 presented higher incidence of type 1 failure. G2 presented higher incidence of type 4. Figure 2 displays the failure mode distribution.

Discussion

PDT negatively affected the bond strength of the Relyx ARC system to cervical third of the prosthetic space. In addition, the dentinal penetration of this cementation system was compromised in the cervical and apical thirds of the intracanal prosthetic space. Thus, the null hypothesis was rejected.

The presence of oxygen affects the adhesive interface of resinous compounds in dentin (18) owing to the competition the free radicals responsible for the polymerization reactions of methacrylates. Moreover, the accumulation of ROS in dentin hampers the hybrid layer formation (19). Since PDT releases ROS, it is assumed that it also affects the adhesion of fiber-posts cementation protocols (13).

However, the bond strength reduction was only observed in the cervical third of the prosthetic space after the conventional cement (Relyx ARC) use. Prior to this resin cement use, the etch-and-rinse (Adper Scotchbond Multi-Purpose Plus) adhesive system was applied, therefore, it is possible that products from the photoactivation of 1% methylene blue, mainly the singlet oxygen, competed with the free radicals, which reacts in the chemical reaction of camphorquinone and aliphatic amines. It negatively affected the polymerization and adhesion of the adhesive system in dentin (20, 21).

Futhermore, Garcez et al. (11) have reported that the light distribution and oxygen formation was uniform when the optical fiber was used in spiral movements for approximately 10 minutes. These steps may have influenced the results, since this study used an optical fiber in at static position for 30 seconds, consequently, the irradiation may have been concentrated mainly in the radicular cervical third.

PDT did not present effect on the interface when Relyx U200 cement was used. The mechanism of adhesion of this cement is mainly based in the chemical interaction between the acidic monomers and the hydroxyapatite and it does not only depend of mechanical micro-retention in root dentin (6), thus the ROS interference on the adhesion of this cement to root dentin was null, in accordance to Barreto et al (22).

Laser confocal microscopy has been used to evaluate the materials´s penetrability and adaptation in root dentin (23). G3 presented lower dentinal penetrability in the cervical and apical thirds. It can be associated to the high concentration of oxygen, which worked as mechanical barrier and hampered the adhesive systems penetration in the dentinal tubules (10). Moreover, Grandini et al. (24) have observed that higher incidence of gaps and cementation failures occurred in the apical third of the prosthetic space, similarly to G3, due to the presence of oxygen.

G3 and G4 presented the highest incidence of the failure mode between the root dentin and the cementation protocol, owing to the ROS release in the adhesive interface. On the other hand, the protocol using etch-and-rinse adhesive system is considered as reference standard in fiber post cementation, which favored type 2 failure mode occurrence (6, 25). G2 showed mixed failure mode, which agreed with previous studies that used this cement (6, 26, 27).

Despite of PDT performs the prosthetic space antisepsis, it releases ROS, mainly the singlet oxygen. The present study has shown that PDT affects the fiber post cementation protocols, however, further studies should be carried out to evaluate the interaction of PDT with other photosensitizing substances and new protocols for fiber posts cementation, such as ionomeric cements (28).

In conclusion, PDT with 0.005% methylene blue presented negative effects- on the bond strength of the cementation protocol using conventional cement (Relyx ARC) in cervical third, and - on the dentinal penetrability of the etch-and-rinse adhesive system in the cervical and apical thirds of the prosthetic space.

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Table 1. Materials, manufacturers, origin and chemical composition of materials and groups

Material	Composition	Groups
Relyx ARC (3M ESPE, St. Paul, MN, USA)	Paste A: BisGMA, TEGDMA, zirconia silica, pigments, amine and photoinitiator system. Paste B: BisGMA, TEGDMA, zirconia silica, benzoyl peroxide	G1 and G3
Relyx U200 (3M ESPE, St. Paul, MN, USA)	Base paste: glass powder treated silane, 2-propenoic acid, 2-metil 1,1'-[1-(hydroxymetil)-1,2- ethanodlyl] ester, triethylene dimethylacrylate with silane, glass fiber, sodium persulfate and t-butyl per-3,5,5–trimethyl-hexanoate. Catalyst paste: silane-treated glass powder, substituted dimethacrylate, silanated silica, sodium p-toluene sulfonate, 1-benzyl-5-phenyl-baric acid, calcium salts, 1,12-dodecane dimethacrylate, calcium hydroxide and titanium dioxide	G2 and G4
Adper Scotchbond Multi Purpose (3M ESPE, St. Paul, MN, USA)	Primer: 2-hydroxyethyl methacrylate in aqueous solution (HEMA) and polyalkanoic acid copolymer. Adhesive: Bisphenol diglycidyl dimethacrylate solution (Bis-GMA), 2-hydroxyethyl methacrylate (HEMA) and camphorquinone	G1 and G3

Table 2. Mean and standard deviation of bond strength (in MPa), in the root thirds of the prosthetic space according to the PDT use in the prosthetic space

Groups	Cervical	Middle	Apical
G1 – Relyx ARC	4.21 ± 1.06 [a]	3.56 ± 1.13 [a]	3.96 ± 1.96 [a]
G2 – Relyx U200	6.09 ± 1.66 [a]	4.38 ± 2.22 [a]	3.51 ± 1.54 [a]
G3 – PDT + Relyx ARC	2.45 ± 0.78 [b]	3.27 ± 1.56 [a]	3.65 ± 1.52 [a]
G4 – PDT + Relyx U200	4.55 ± 1.57 [a]	4.71 ± 1.06 [a]	4.66 ± 1.37 [a]

[ab] Different letters in the same column indicate significant differences ($P < 0.05$).

Table 3. Mean and standard deviation of the penetration (%) of the fiber post cement in root dentin in all thirds of the prosthetic space, according to the cementation protocol and PDT use.

Groups	Cervical	Middle	Apical
G1 - Relyx ARC	34.85 ± 6.60 [a]	39.14 ± 19.80 [a]	16.01 ± 1.46 [a]
G2 - Relyx U200	42.89 ± 12.44 [a]	49.29 ± 19.33 [a]	19.56 ± 5.18 [a]
G3 – PDT + Relyx ARC	11.82 ± 3.02 [b]	26.52 ± 16.08 [a]	6.36 ± 3.18 [b]
G4 – PDT + Relyx U200	41.28 ± 16.10 [a]	41.76 ± 23.78 [a]	18.19 ± 3.21 [a]

[ab] Different letters in the same column indicate significant differences ($P < 0.05$).

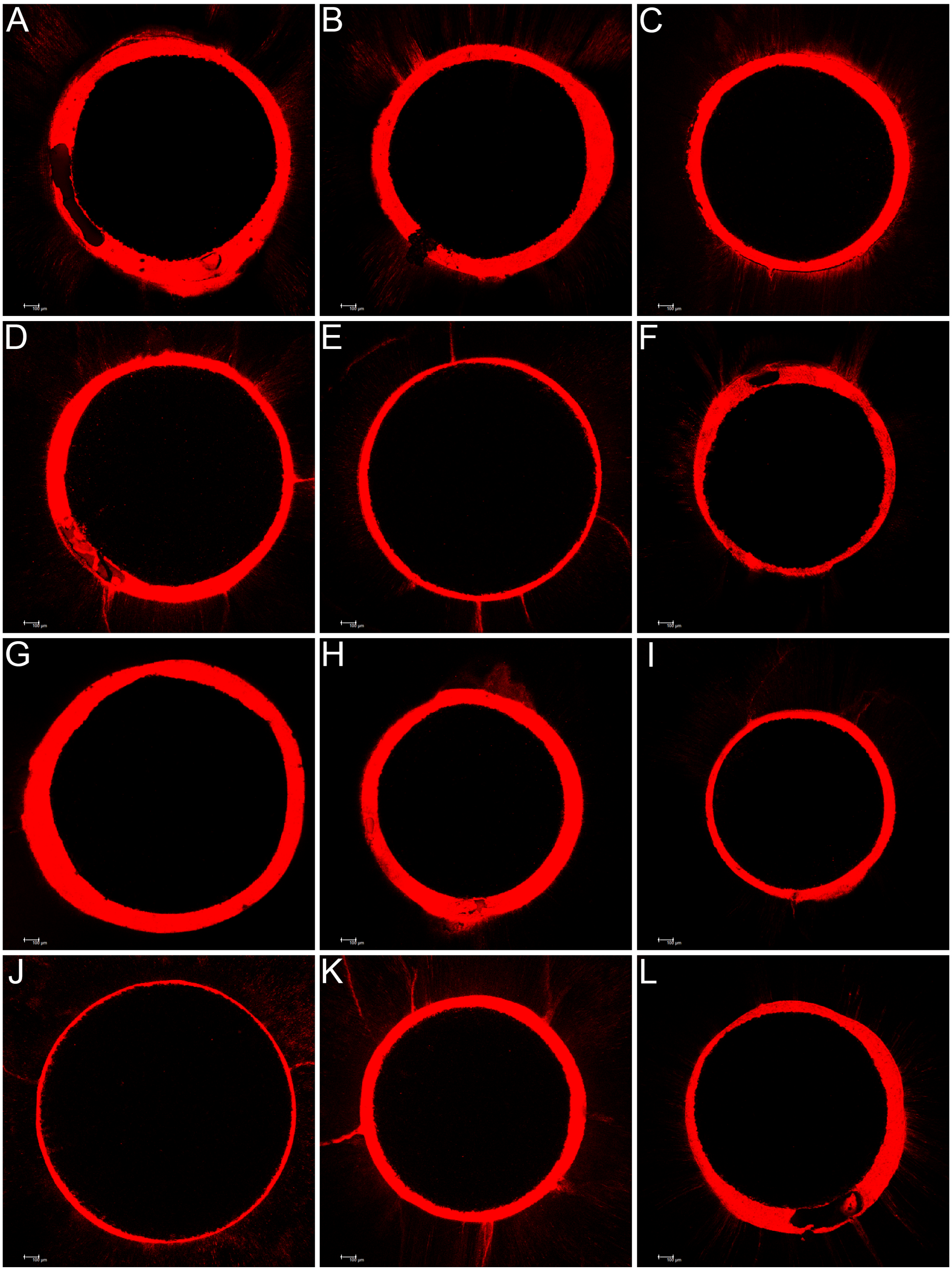

Figure 1. Representative image of the dentinal penetrability in the groups evaluated. (A, B and C) G1, cervical, middle and apical third, respectively; (D, E and F) G2, cervical, middle and apical third, respectively; (G, H and I) G3, cervical, middle and apical third, respectively; and (J, K and L) G4, cervical, middle and apical third, respectively. G1, Relyx ARC; G2, Relyx U200; G3, PDT + Relyx ARC and G4, PDT + Relyx U200.

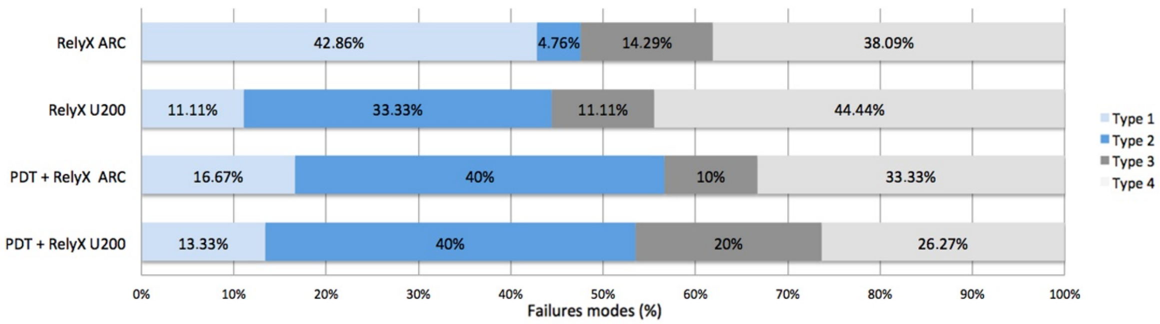

Figure 2. Distribution of the failure mode in each group.

3.2 Publicação 2

Effects of photodynamic therapy on the adhesive interface using two fiber posts cementation systems*

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Abstract

Introduction: The aim of this study was to evaluate the effects of photodynamic therapy (PDT) on the adhesive interface between the dentin and different glass-fiber post cementation systems using a self-adhesive resin cement (Relyx U200) and a glass ionomer cement (Gold Label 1). **Methods**: Forty human canine roots were endodontically-treated and prepared for fiber post. The roots were divided into four groups (n = 10), according to the PDT use and cementation protocol: G1, Relyx U200; G2, Gold Label 1; G3, PDT + Relyx U200 e G4, PDT + Gold Label 1. Afterwards, the roots were cross-sectioned and specimens from the cervical, middle and apical thirds of the prosthetic space were obtained and submitted to the push-out test. After that, the dentinal penetrability of the cementation protocol was assessed using laser confocal microscopy **Results**: No significant differences were found in the bond strength values among the groups in all thirds ($P > 0.05$). G2 and G4 presented lower dentinal penetrability than the other groups, only in the cervical third ($P < 0.05$) **Conclusions:** Photodynamic therapy did present effects on the bonding interface between root dentin and fiber post cementation systems in both Relyx U200 and Gold Label 1, which were similar to each other.

Key Words: photodynamic therapy, fiber posts, glass-ionomer cement, self-adhesive cement.

Introduction

The coronary restoration after the root canal obturation is a determinant fator associated with success of the endodontic treatment (1, 2). After the root canal obturation is completed, its recontamination must be avoided since it compromises the periradicular repair process (3, 4). However, the contamination can occur during the preparation of the intraradicular prosthetic space and / or in the manipulation of restorative materials (5, 6).

Several substances have been recommended for the prosthetic space irrigation with both purposes of dentin cleaning and also local antisepsis (7- 9). Sodium hypochlorite is the solution most widely used for the root canal irrigation, which degrades and releases hypochlorous acid and singlet oxygen (10, 11). However, these byproducts from the sodium hypochlorite decomposition can negatively affect in the adhesive interface between conventional glass fiber posts cementation systems and root dentin (12-14).

In order to solve those problems, other alternatives have been evaluated for the prosthetic space antisepsis without negative effects on the bonding interface of fiber post cementation systems to root dentin. Photodynamic therapy (PDT) is an interesting alternative to decontaminate the root canal, and, consequently could be carried out in the prosthetic space antisepsis (15,16). PDT action occurs when a photosensitizing substance absorbs the photons from the irradiation source and their electrons enter an excitation state. Then, the energy is transferred to a specific substrate, forming the reactive oxygen species (ROS), mainly singlet oxygen, which irreversibly oxidize the cellular components causing bacterial death (15, 17, 18). PDT is an innovation in prosthetic space antisepsis; however, the effects of reactive oxygen specimens (ROS) are still unknown.

Glass ionomer cement has been recently used for the fiber posts cementation (19). Its bonding to dentin substrate is different from the conventional resin cements. It is unknown whether the photodynamic therapy would present any effect on the adhesive interface when the glass ionomer cement was used (20).

Therefore, the aim of the present study was to evaluate the bond strength and intra-dentinal penetrability using self-adhesive (Relyx U200) resin cements and glass ionomer cement (Gold Label 1) after PDT using 0.005% methylene blue in the intracanal prosthetic space. The null hypothesis was that photodynamic therapy does not affect the bonding interface of fiber post cements.

Materials and Methods

The study was approved by the Research Ethics Committee of FOAr-UNESP (1.603.859) (attachment). Forty human canines with similar root anatomy and absence of structural alterations were selected and kept in 0.1% thymol solution at 4^{o}C.

Prosthetic space preparation

The roots were standardized with 15mm from the root apex. Then, chemical-mechanical preparation and root canal obturation were carried out according to Aranda-Garcia et al (21). After the vertical condensation obturation, the cervical opening was sealed using temporary cement (Coltosol, Coltene, Rio de Janeiro, Brazil) and the roots were stored under 100% relative humidity, at 37^{o}C for 7 days. The preparation of the intracanal prosthetic space was performed using #2 bur (White Post DC System; FGM, Joinville, SC, Brazil), 11mm length. Then, it was irrigated using 10mL of distilled water and dried with absorbent paper points.

PDT use

The specimens were randomly divided into four groups (n=10), according to the glass-fiber cementation protocol and PDT application in the prosthetic space: G1, self-adhesive (Relyx U200); G2, glass ionomer cement(Gold Label 1) ; G3, PDT and self-adhesive (PDT + Relyx U200) and G4, PDT and glass ionomer cement (PDT + Gold Label 1).

The photodynamic therapy was carried out in G3 and G4. Initially, the prosthetic space was filled with 1000μL of 0.005% methylene blue (Chimiolux; DMC, São Carlos, SP, Brazil) and left untouched for 5 minutes. After that, an optical fiber (Twin Flex Evolution, MMO Opto-Electronic Equipment, São Carlos, SP, Brazil) was inserted into the entire prosthetic space, in at static position and the prosthetic space irradiated for 30s using a laser emission source (Twin Flex Evolution, MMO Opto-Electronic Equipment, São Carlos, SP, Brazil), output power 30 J/cm^2. Afterwards, the methylene blue was aspirated and the prosthetic space was irrigated with 3mL of saline solution and dried with absorbent paper points.

Fiber posts cementation

The fiber post surface was cleansed with 95% ethanol, etched using 37% phosphoric acid (Power Etching, BM4, Palhoça, SC, Brazil) for 1 minute, then the silane (Prosil; FGM, Joinville, BR) and dentin adhesive (Adper Scotchbond Multiuso Plus; 3M ESPE, St. Paul, MN, USA) were applied throughout its length. Then, the whole set was light-cured for 60s (Bluephase; Ivoclar Vivadent, Barueri, SP, Brazil).

Prior to the fiber posts cementation, 0.01% (by mass) of Rhodamine B isothiocyanate pigment was added to the primer of the adhesive system (Adper Scotchbond Multiuso Plus; 3M ESPE, St. Paul, MN, USA) and used in G1 and G3. Rhodamine B isothiocyanate was also added to the cements and used in G2 and G4. All specimens were subjected to laser confocal microscopy evaluation.

The cements were handled according to the manufacturers' recommendations. Immediately after the cementation of #2 (FGM, Joinville, SC, BR) fiber posts, the roots were vertically centralized inside a PVC matrix (16.5 diameter x 15.0mm length) and checked using parallelogram (BioArt B2, São Carlos, SP, BR). The matrices were filled with polyester resin (Maxi Rubber, Diadema, SP, Brazil), leaving 1.0mm of the root cervical outside the inclusion. The whole set was left undisturbed for 24 hours.

Push-out test

After that, the specimens were removed from the matrixes and sectioned perpendicular to their long axis using a diamond disk in a hard tissues cutting machine (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under running water-cooling. Three sections were performed with 2.0mm ± 0.1mm thickness from the apical, middle and cervical thirds of the prosthetic space. The cervical, medial, and apical sections were carried out respectively from 1.0mm, 5.0mm and 8.0mm from the root cervical face. The sections irregularities were removed using #1200 (Norton, São Paulo, SP, Brazil) sandpaper under running water-cooling .

The specimens of each root third were submitted to a push-out test using an electromechanical test machine (EMIC, São José dos Pinhais, PR, Brazil), at 0.5mm/min speed using 5kN load cell, until the complete displacement of all root canal walls. The force required for the displacement was obtained in N (Newton) and it was transformed into bond strength (MPa), as according to Magro et al (16).

Subsequently, each specimen was assessed using a stereomicroscope, at 20X magnification to determine the failure mode. The failure mode was classified as: type 1 (Adhesive): when it occurred between the fiber post and the cement; Type 2 (Adhesive): between dentin and cement; Type 3 (Cohesive) within the cement and type 4 (Mixed) when both types of failure were combined, according to Elnaghy (17).

Dentinal penetrability test

The sections were analyzed using a laser confocal microscope at 100X magnification to determine the root canal perimeter with the materials penetration within the dentinal tubules. The images were evaluated using Image J software. The perimeter of the root canal and the cement penetration in dentin were measured, and the material penetration in the dentinal tubules percentage was obtained.

Statistical Analysis

The data were submitted to ANOVA and Tukey tests at 5% significance level ($P = 0.05$).

Results

Regarding the bond strength of the fiber post cements to the root dentin, all groups presented similar results regardless of the PDT use in the prosthetic space ($P > 0.05$). Table 2 shows the mean and standard deviation of bond strength (in MPa) of cements to root dentin and PDT application in the prosthetic space.

In relation to the dentinal penetration of the fiber post cementation protocols, the glass ionomer cement (Gold Label 1) presented penetrability lower than the resinous cement (Relyx U200) regardless of the PDT in the cervical third of prosthetic space ($P < 0.05$). Table 3 shows the mean and standard deviation of the dentinal penetrability (%) of the fiber posts cementation protocols. Figure 1 shows the dentinal penetrability pattern of the cementation protocols in all thirds of the prosthetic space.

Regarding the failure mode, type 1 failure was more frequent when ionomer cement was used. However, types 2 and 4 were more frequent for resinous cement (RElyx U200) with and without previous use of photodynamic therapy. Figure 2 shows the frequency of incidence of failure mode in all groups.

Discussion

PDT did not affect the bond strength and the dentinal penetrability of fiber posts cementation systems regardless the evaluated root third. This study has showed that only the cervical third of the prosthetic space presented less dentinal penetrability of glass ionomer cement (Gold Label 1) than resin cement (Relyx U200), regardless of the PDT use. Therefore, the null hypothesis was accepted.

Oxygen from the degradation of chemicals is routinely used in Dentistry, such as hydrogen peroxide and sodium hypochlorite, which negatively affect the bond strength of conventional adhesive systems to dentin substrate (12, 24). PDT causes the methylene blue photoactivation, which releases reactive oxygen species (ROS), mainly singlet oxygen (15), it is presumed it has a negative effect on the bonding interface between the fiber post cementation system and the root dentin (12, 24).

The present study has observed that the bond strength of the self-etching resin cement (Relyx U200) to dentin of the prosthetic space was not affected after PDT. The adhesion mechanism of the self-etching resin cements is more related to chemical interactions between their chemical components and the hydroxyapatite from the dentin substrate than only through the hybrid layer formation in dentin surface (25). According to that property, the presence of ROS did not affect the fiber posts cementation protocol, in accordance to Barreto et al (14).

A resistência de união do cimento de ionômero de vidro na dentina radicular também não foi afetada pela terapia fotodinâmica. O mecanismo de adesão do cimento de ionômero de vidro à estrutura dentária ainda não está totalmente esclarecido, porém há evidências que ocorre trocas iônicas entre o material e o substrato mineralizado (26). Consequentemente, os efeitos dos ROS sobre a interface de adesão foram praticamente nulos, semelhantemente ao ocorrido com o U200. Moreover, the bond strength of the glass ionomer cement to root dentin was not affected by PDT. Despite of the adhesion mechanism of the glass ionomer cement to the dental structure has *not* been *fully understood*, there is evidence that ionic exchange occurs between the material and the mineralized substrate (26). Consequently, the effects of ROS on the adhesion interface were practically null, similar to the U200 cement.

Although the bond strength values of glass ionomer cements to dentin were relatively low (27), they were similar to resin cement (Relyx U200). Even though, the adhesive failure mode between cement and fiber post was the most frequent when the glass ionomer cement was used, regardless of the previous use of PDT. On the other hand, the mixed and adhesive failure modes between the cement and the dentin were more frequent when Rylex U200 was used, regardless of the previous use of PDT. Thus, although the bond strength values to the root dentin were similar, the type of failure mode showed that the use of glass ionomer cement favored its use, especially when PDT was used.

The adhesion of fiber posts cementation system to root dentin is related to several factors (28, 29), such as the dentinal tubules penetration and the hybrid layer formation in the dentin substrate (14, 29). This study has observed that PDT did not present any effect on the cements penetrability in root dentin. Only the third cervical root has showed that the glass ionomer cement presented less dentinal penetration than the resin cement. It may have occurred since the glass ionomer presented lower flow rate, in accordance to Sidhu (26).

In contrast, the other thirds have not showed any differences, probably because to the fiber post had higher adaptation and compression in the prosthetic space, since the apical and middle thirds are the places that fiber post fits better inside the root canal (9). Since the adhesion of these cements is more related to a chemical interaction between the dentin substrate and the cement, the dentinal penetrability values are not correlated to the adhesion efficiency, as it was observed in this study.

PDT may contribute to antissepsis of the intracanal prosthetic space after a local contamination has occurred. The oxidative radical specimens (ROS) did not affect the bond strength and dentinal penetrability of the cementation systems evaluated in this study, and it can be a good alternative to avoid the deleterious effects caused by sodium hypochlorite and chlorhexidine (14, 29).

In conclusion, this study has showed that PDT with methylene blue presented no effect on the adhesion interface between the root dentin and the fiber posts cementation systems, neither for the resin cement (Relyx U200) nor for the glass ionomer cement (Gold Label 1) that were similar to each other.

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Table 1. Materials, manufacturers, origin and chemical composition of materials and groups according to the fiber post cementation

Cement	Composition	Groups
RelyX U200 (3M ESPE, St. Paul, MN, USA)	Base paste: glass powder treated silane, 2-propenoic acid, 2-metil 1,1'-[1-(hydroxymetil)-1,2-ethanodlyl] ester, triethylene dimethylacrylate with silane, glass fiber, sodium persulfate and t-butyl per-3,5,5–trimethyl-hexanoate. Catalyst paste: silane-treated glass powder, substituted dimethacrylate, silanated silica, sodium p-toluene sulfonate, 1-benzyl-5-phenyl-baric acid, calcium salts, 1,12-dodecane dimethacrylate, calcium hydroxide and titanium dioxide	G1 and G3
GOLD LABEL 1 (GC America Inc., (Alsip, IL, USA)	Powder: fluoralumino-silicate glass (95%), polyacrylic acid (5%) Liquid: polyacrylic acid (30-40%), distilled water (50-55%)	G2 and G4

Table 2. Mean and standard deviation of bond strength (in MPa), in the root thirds of the prosthetic space according to the PDT use in the prosthetic space

Groups	Cervical	Middle	Apical
G1 - RelyX U200	4.21 ± 1.06 [a]	3.56 ± 1.13 [a]	3.61 ± 1.46 [a]
G2 - Gold Label 1	3.47 ± 1.12 [a]	3.82 ± 1.24 [a]	3.60 ± 0.66 [a]
G3 - RelyX U200 + PDT	4.55 ± 1.19 [a]	4.71 ± 1.06 [a]	4.66 ± 1.37 [a]
G4 - Gold Label 1 + PDT	3.45 ± 0.65 [a]	4.60 ± 1.78 [a]	3.58 ± 0.45 [a]

* No statistically significant difference was found among the groups, regardless of the root third ($P < 0.05$).

Table 3. Mean and standard deviation of the dentinal penetrability (%) of the fiber post cement according to the cementation protocol and PDT use in the prosthetic space

Groups	Cervical	Middle	Apical
G1 - RelyX U200	42.89 ± 12.44 [a]	48.27 ± 18.66 [a]	19.56 ± 5.18 [a]
G2 - Gold Label 1	13.35 ± 2.06 [b]	33.10 ± 25.21 [a]	15.33 ± 4.02 [a]
G3 - RelyX U200 + PDT	41.28 ± 16.10 [a]	41.76 ± 23.78 [a]	18.19 ± 3.21 [a]
G4 - Gold Label 1 + PDT	8.96 ± 3.48 [b]	33.40 ± 21.63 [a]	17.90 ± 7.94 [a]

[ab] Different letters in the same column indicate significant differences ($P < 0.05$).

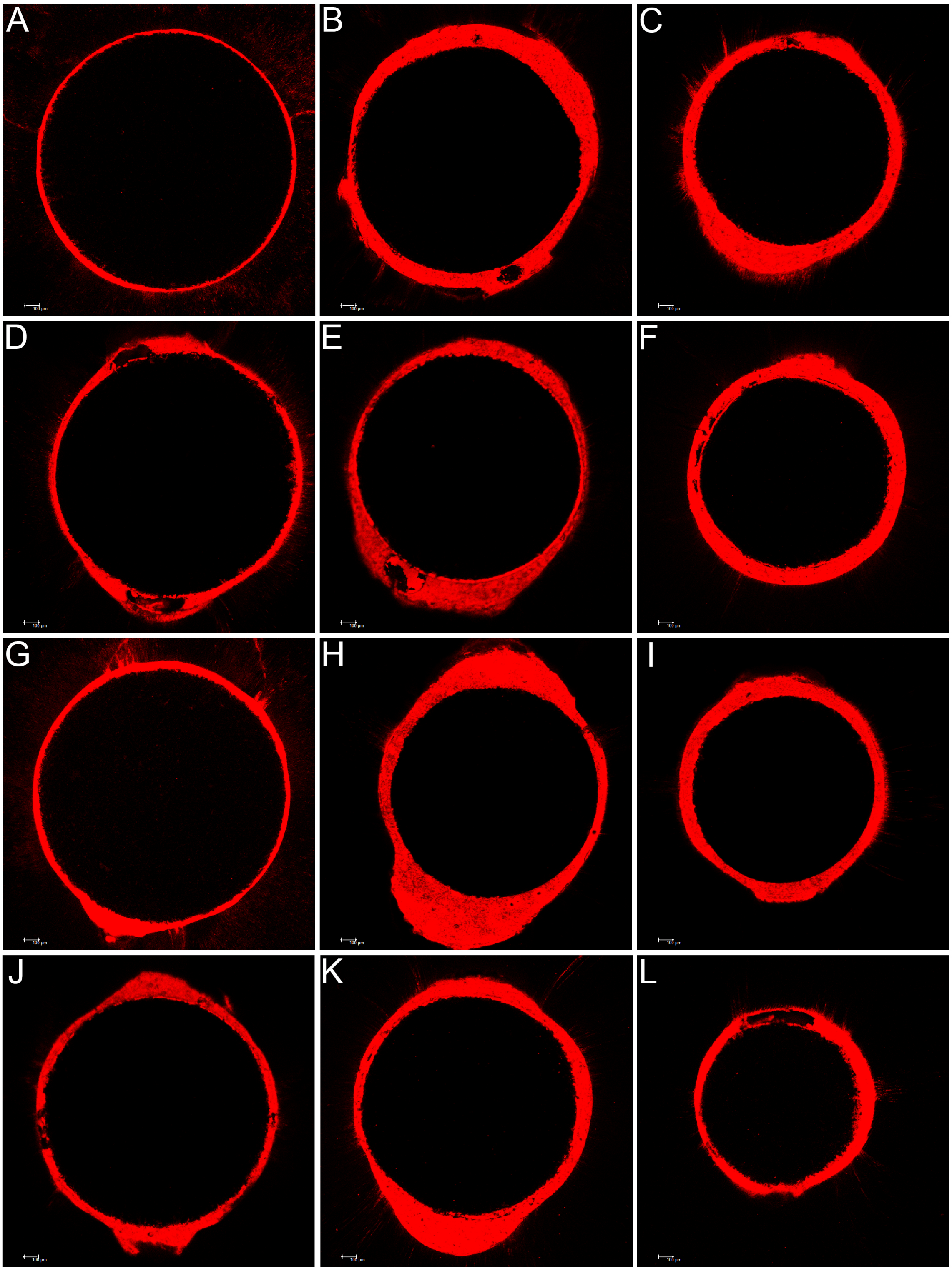

Figure 1. Representative image of the dentinal penetrability in the groups. (A, B and C) G1, cervical, middle and apical third, respectively; (D, E and F) G2, cervical, middle and apical third, respectively; (G, H and I) G3, cervical, middle and apical third, respectively; and (J, K and L) G4, cervical, middle and apical third, respectively. G1, Relyx U200; G2, Gold Label 1; G3, PDT + Relyx U200 and G4, PDT + Gold Label 1.

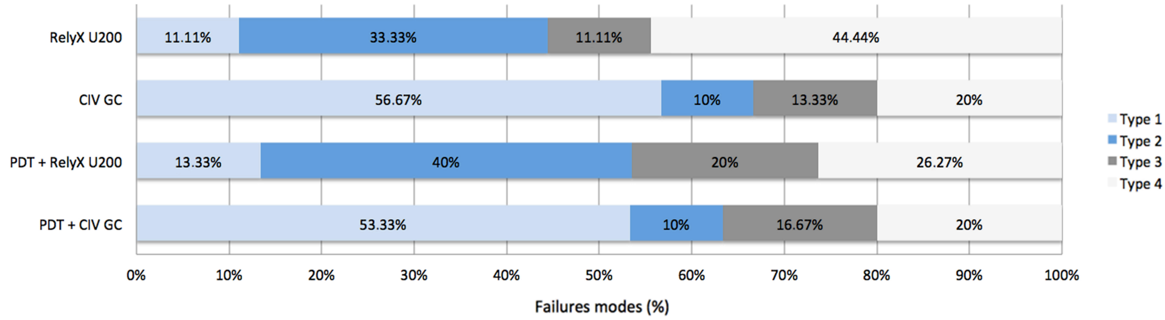

Figure 2. Distribution of the failure mode in each group.

4 CONCLUSÃO

A utilização do PDT no espaço protético:

- Exerce efeito negativo significante sobre a resistência de união do sistema de adesão RelyX ARC na dentina cervical, ao contrário do sistema RelyX U200 que não é afetado por este protocolo de tratamento do espaço protético.
- Exerce efeito negativo significante sobre a resistência de união do sistema de adesão utilizando o cimento de ionômero de vidro (GC) na dentina radicular cervical.
- Interfere negativamente sobre a penetração dentinária apenas do sistema RelyX ARC, no terço apical e cervical radicular.
- No terço cervical radicular, o cimento de ionômero de vidro demonstrou menor penetração dentinária que o RelyX U200, independentemente do uso prévio do PDT.
- Aumentou o padrão de fratura do tipo adesiva nos cimentos resinosos, tanto convencional, quanto autoadesivo.

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APÊNDICE

MATERIAIS E METODOS

Após aprovação do Comitê de Ética em Pesquisa da Faculdade de Odontologia de Araraquara, da Universidade Estadual Paulista – parecer 1.603.859. Setenta e dois caninos humanos recém extraídos, obtidos do Banco de Dentes da Faculdade de Odontologia de Araraquara, da Universidade Estadual Paulista, e mantidos em solução de timol a 0,1%, na temperatura de 4^{o}C. Após a lavagem em água corrente por 12 horas, os dentes foram analisados em estereomicroscópio, com aumento de 10x, com o objetivo de descartar e substituir aqueles que apresentarem fissuras e/ou trincas radiculares e radiografados, no sentido vestíbulo-lingual e mésio-distal, a fim de averiguar a presença de um único canal radicular principal. As raízes foram mensuradas e segmentados transversalmente na extensão de 15mm a partir do ápice radicular.

Inicialmente foi feito um estudo piloto com 12 dentes, sendo 2 representando cada grupo. Foi feito o desenho experimental de forma rigorosamente igual aos grupos experimentos.

Os canais radiculares inicialmente explorados com a lima K#10 (Maillefer, Petrópolis, RJ, BR), obtido o *glyde-path* e realizado da patência apical com a lima K#15, instrumentados e ampliados até o instrumento F5 (ProTaper; Dentsply, Ballaigues, SW). Durante todo o preparo mecânico, os canais radiculares foram irrigados com 5mL de solução de hipoclorito de sódio a 2,5% (Asfer, São Caetano do Sul, SP, BR) a cada troca de instrumento e ao final irrigados com 5mL de EDTA a 17% (Biodinâmica, Ibiporã, PR, BR), mantidos em seu interior por 3 minutos e, ao final irrigados com 5mL de solução de hipoclorito de sódio a 2,5% para neutralização.

Após a secagem com ponta de papel absorvente F5 (Dentsply, Petrópolis, RJ, BR), os canais radiculares foram obturados pela técnica do cone único utilizando ponta de guta percha F5 (Dentsply, Petrópolis, RJ, BR) e cimento endodôntico contendo resina epóxi (AH Plus; Dentsply DeTrey GmbH, Konstanz, Germany). Após o corte cervical, realização da condensação vertical da obturação endodôntica e vedamento da abertura cervical dos canais radiculares com cimento provisório (Coltosol; Coltene, Rio de Janeiro, RJ, BR), as raízes foram mantidas em estufa, com umidade relativa a 100%, a 37^{o}C, por 7 dias, para aguardar a presa total do material obturador.

O preparo do espaço para a colocação do pino de fibra foi confeccionado com a broca #2 do sistema White Post DC (FGM, Joinville, SC, BR), na extensão de cervical para apical de 11mm. Após a realização do preparo, os canais radiculares foram irrigados com 10mL de água destilada e novamente secos com pontas de papel absorvente. A partir deste momento, os espécimes foram aleatoriamente divididos em seis grupos (n = 10), de acordo com a utilização da terapia fotodinâmica no espaço protético e o tipo de cimento empregado para a cimentação dos pinos de fibra de vidro, conforme demonstrado na Tabela 1.

Tabela 1 - Grupos experimentais, de acordo com o tipo de pré-tratamento e cimento utilizado.

	Pré-tratamento	Cimento
G1	-	Relyx ARC
G2	-	Relyx U200
G3	-	CIV GC
G4	PDT	Relyx ARC
G5	PDT	Relyx U200
G6	PDT	CIV GC

FONTE: elaboração dos autores.

Em todos os grupos, inicialmente os pinos de fibra foram limpos com etanol 95% e ácido fosfórico 37% (Power Etching; BM4 , Palhoça, SC, BR) por 1 minuto, aplicado o silano (Prosil; FGM, Joinville, SC, BR) em toda a sua extensão, aguardado 1 min e então seco com jato de ar para total evaporação do solvente, então aplicado o bond, do sistema adesivo (Adper Scotchbond Multiuso Plus; 3M ESPE, St. Paul, MN, USA), e fotopolimerizado por 60s (Bluephase; Ivoclar Vivadent, Barueri, SP, BR).

Em G1, G2 e G3, ou seja, os grupos que não utilizaram uso da PDT o espaço radicular não foi submetido a processo de antissepsia. Enquanto os demais grupos, os quais utilizaram a PDT, o espaço foi preparado, irrigado com 3mL de soro fisiológico, seco com pontas de papel absorvente e preenchido com 1000µL de azul de metileno (MMO Equipamentos Opto-Eletrônicos, São Carlos, SP, BR). Após repouso por período de 5 minutos, coberto com papel laminado para que a luz ambiente não altere o corante, o canal radicular foi irradiado com a fibra ótica por 30 segundos (Fibras Óticas Twin Flex Evolution; MMO Equipamentos Opto-Eletrônicos, São Carlos, SP, BR), na potência de $30J/cm^2$, adaptada na fonte de emissão (Twin Flex Evolution – Arcada; MMO Equipamentos Opto-Eletrônicos, São Carlos, SP, BR). Após este período, o corante foi removido através de sucção e lavagem com 3mL de soro fisiológico e seco com pontas de papel absorvente.

Os cimentos descritos na Tabela 1 foram manipulados de acordo com as recomendações dos respectivos fabricantes. Nos G1 e G4, previamente à inserção do cimento resinoso, a dentina do canal radicular foi submetida ao condicionamento ácido (Power Etching; BM4 , Palhoça, SC, BR), por 15s, lavado com água destilada por 30s e seca com pontas de absorvente. Em seguida aplicado o primer do sistema adesivo (Adper Scotchbond Multiuso Plus; 3M ESPE, St. Paul, MN, USA) aguardou-se a evaporação do solvente por 20seg e jato de ar, aplicação do bond do mesmo sistema, remoção do excesso e polimerização por 20s (Bluephase; Ivoclar Vivadent, Barueri, SP, BR). Nos demais grupos não houve preparo do conduto radicular por não haver necessidade, de acordo com os fabricantes. Previamente à cimentação dos pinos de fibra, no primer do sistema adesivo de G1 e G4 e nos cimentos dos demais grupos foi incorporado 0,01% (em massa) de isotiocianato de Rodamina B com o objetivo de permitir a futura avaliação em microscopia confocal à laser.

Imediatamente após o processo de cimentação, as raízes foram verticalmente posicionadas e centralizadas em uma matriz de PVC (16,5 de diâmetro X 15,0 mm de comprimento), a fim de manter a correta verticalização no interior das matrizes, as mesmas tiveram seus ápices radiculares fixados com cera em uma placa de vidro e o posicionamento conferido com um paralelômetro (BioArt B2, São Carlos, SP, BR). Os anéis plásticos foram preenchidos com resina poliéster (Maxi Rubber, Diadema, SP, Brasil), mantendo 1,0 mm do segmento cervical da raiz fora da inclusão. Todo o conjunto permaneceu intacto por 24 horas, para a que ocorresse a completa polimerização da resina. Após a desinclusão do conjunto das matrizes plásticas, foram seccionadas perpendicularmente ao seu longo eixo, utilizando um disco diamantado adaptado em máquina de corte para tecidos duros (Isomet; Buehler Ltd, Lake Bluff, IL, USA), sob intensa refrigeração.

Três secções foram obtidas, com espessura de 2.0 mm + 0.1, dos terços apical, médio e cervical do espaço protético de cada raiz. A secção cervical, média e apical será obtida respectivamente a partir de 1,0 mm, 5,0 mm e 8,0 mm da face cervical da raiz. As irregularidades das secções foram removidas com lixa d'água de granulação 1200 (Norton, São Paulo, SP, BR), limpas com pincel e jatos de ar.

Os pinos de fibra de vidro cimentados no espaço protético de cada um dos terços das raízes foram submetidos ao teste de *push-out* em máquina eletromecânica de ensaios (EMIC, São José dos Pinhais, PR, Brasil), calibrada em uma velocidade de 0,5 mm/min, até que ocorresse o completo deslocamento do pino de fibra das paredes do canal radicular. Os valores da força necessária para que ocorresse o deslocamento foi obtido em N (Newton) e, posteriormente, transformado em resistência de união (MPa) através da fórmula: MPa = F/AD. A área de adesão foi calculada utilizando a seguinte fórmula: AD = $\pi \cdot (R + r) \cdot g$, onde R = raio cervical do canal radicular, em mm; r = raio apical do canal radicular, em mm; *g* = altura relativa do cone invertido, em mm.

O diâmetro cervical e apical dos canais radiculares foi obtido individualmente através de mensuração com auxílio de estereomicroscópio, com magnificação de 20X (Leica Microsystems, Wetzlar, Germany). O valor de *g* foi obtido a partir da equação: $g = \sqrt{(R - r)2 + (2.0)2}$.

Cada espécime foi analisado em estereomicroscopia, com aumento de 20X para determinar o padrão de fratura ocorrido, sendo classificado em quatro categorias, conforme descrito por Elnaghy (2014):

Tipo 1 – Adesiva: entre o pino de fibra e o cimento;

Tipo 2 – Adesiva: entre a dentina e o cimento;

Tipo 3 – Coesiva: dentro do cimento;

Tipo 4 – Mista.

As secções foram analisadas sob microscópio confocal à laser com aumento de 100X para determinação do perímetro do canal com penetração do cimento nos túbulos dentinários. As imagens obtidas foram analisadas pelo software Image J, o qual foi devidamente calibrado tomando-se por base a régua adaptada abaixo dos espécimes. Foi mensurado o perímetro total do canal e o perímetro de dentina com penetração de cimento nos túbulos dentinários, calculando-se assim a porcentagem do perímetro do canal com penetração do cimento nos túbulos dentinários em porcentagem.

Os dados obtidos nos estudos citados foram submetidos ao teste de Shapiro-Wilk, confirmando a distribuição normal da amostra. Então avaliados pelo teste de ANOVA e pós-teste de Tukey, com nível de significância de 5%.

ANEXO

Comitê de Ética

UNESP - FACULDADE DE ODONTOLOGIA DE ARARAQUARA

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito da terapia fotodinâmica sobre a resistência de união e a penetrabilidade dentinária de diferentes protocolos de cimentação de pinos de fibra de vidro

Pesquisador: Milton Carlos Kuga

Área Temática:

Versão: 2

CAAE: 55134815.7.0000.5416

Instituição Proponente: Faculdade de Odontologia de Araraquara - UNESP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.603.859

Apresentação do Projeto:

O projeto "Efeito da terapia fotodinâmica sobre a resistência de união e a penetrabilidade detinária de diferentes protocolos de cimentação de pinos de fibra de vidro" foi devidamente reapresentado. Todas as questões levantadas no primeiro parecer foram devidamente esclarecidas. O projeto foi revisado e a versão final apresenta-se de forma clara e bem informativa. O número de amostra foi adequado e apresenta a autorização do banco de dentes. Todos os participantes da pesquisa foram incluídos no projeto de pesquisa da Plataforma Brasil. O resumo foi adequado corretamente sendo o mesmo apresentado no projeto e na Plataforma Brasil. A aluna de mestrado Anna Thereza Peroba Rezende possui autorizações de outros laboratórios da faculdade para utilização tanto das dependências destes laboratórios, como também dos materiais necessários para realização da pesquisa. O orçamento financeiro encontra-se adequado, uma vez que serão empregados recursos próprios do pesquisador responsável.

Objetivo da Pesquisa:

1. A resistência de união do sistema adesivo de pinos de fibra de vidro na dentina radicular, utilizando o sistema "condiciona e lava" (Adper Scotchbond Multiuso Plus) com o cimento resinoso convencional (RelyX ARC) ou um autocondicionante (RelyX U200), comparado a um cimento ionomérico (GC Gold Label 1 C), após a utilização da terapia fotodinâmica associada ao

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fotossensibilizante azul de metileno (0,005%);

2. A penetrabilidade intradentinária dos sistemas de adesão após a utilização dos métodos de cimentação citados e a utilização da terapia fotodinâmica.

Avaliação dos Riscos e Benefícios:

Riscos: A cimentação pode ocorrer de forma insatisfatória em grupos que hajam interferências de um procedimento junto a outro (conforme descrito na metodologia) e o pino solte da raiz, caso isso seja detectado, por se tratar de um estudo in vitro serão aconselhados novas pesquisas para confirmação do fato in vivo e assim tenha um objetivo final otimizar o tratamento para que o paciente seja beneficiado desta informação.

Benefícios: Aumentar qualidade e longevidade de cimentação, o que proporcionará uma melhor qualidade de vida ao paciente, posto que terão tratamentos mais duradouros, e necessidade de retratamentos em menor quantidade visto a otimização do procedimento inicial.

Comentários e Considerações sobre a Pesquisa:

O projeto trará contribuição para a área ao qual se aplica.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos foram apresentados.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

O projeto encontra-se bem estruturado com uma proposta clara, bem definida e de execução imediata. A dispensa do TCLE se justifica neste estudo. O projeto encontra-se dentro do período de execução para início no mês de julho de 2016.

Considerações Finais a critério do CEP:

Protocolo APROVADO em reunião de 23 de junho de 2016.

O pesquisador deverá encaminhar relatórios parciais a cada 01 (um) ano até o prazo final da pesquisa, quando deverá encaminhar o relatório final.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_638413.pdf	24/05/2016 23:55:20		Aceito

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Continuação do Parecer: 1.603.859

Projeto Detalhado / Brochura Investigador	Projeto.pdf	24/05/2016 23:54:49	Milton Carlos Kuga	Aceito
Folha de Rosto	folhaderosto.pdf	22/03/2016 16:04:12	Milton Carlos Kuga	Aceito
Outros	LabSupAcademico.pdf	03/12/2015 12:33:46	Milton Carlos Kuga	Aceito
Outros	Labensaiosmecanicos.pdf	03/12/2015 12:33:23	Milton Carlos Kuga	Aceito
Outros	LabDentistica.pdf	03/12/2015 12:32:57	Milton Carlos Kuga	Aceito
Outros	LabBiomateriais.pdf	03/12/2015 12:31:32	Milton Carlos Kuga	Aceito
Outros	BDentes.pdf	03/12/2015 12:30:00	Milton Carlos Kuga	Aceito
Outros	Termodecompromisso.pdf	03/12/2015 12:29:36	Milton Carlos Kuga	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	03/12/2015 12:28:36	Milton Carlos Kuga	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

ARARAQUARA, 24 de Junho de 2016

Assinado por:
Lígia Antunes Pereira Pinelli
(Coordenador)

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