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ORIGINAL ARTICLE

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The effects of TiO_2 nanotubes on bond strength and radiopacity of a self-adhesive resin cement in self-curing mode

Os efeitos de nanotubos de TiO_2 na resistência de união e radiopacidade de um cimento resinoso auto-adesivo no modo auto-polimerizável

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ABSTRACT

Objective: The aim of this in vitro study was to analyze the influence of the titanium dioxide nanotubes in a self-cure mode polymerization of a dual resin luting agent through push out bond strength and radiopacity tests. **Material and Methods:** After mixed with a commercial dual self-adhesive resin cement, three concentrations of titanium dioxide nanotubes (0.3, 0.6, and 0.9% by weight) were analyzed in self-curing mode. The bond strength to bovine root dentin and fiberglass posts was assessed with the push out bond strength test and was evaluated in three thirds (cervical, middle and apical) (n=10), followed by failure mode analysis (SEM), and the ISO standard 9917-2 was followed for radiopacity test (n=10). Data were statistically analyzed by one-way ANOVA test, followed by Tukey's test (α=0.05). **Results:** Reinforced self-adhesive resin cement with 0.6% titanium dioxide nanotubes showed significant difference compared to the control group for push out test (p=0.00158). The modified groups did not show significant difference among thirds (p=0.782). Radiopacity showed higher value for group with 0.9% titanium dioxide nanotubes in comparison with control group (p<0.001). **Conclusion:** The addition of titanium dioxide nanotubes to a self-adhesive resin cement increased the bond strength to dentin and radiopacity values in the self-cure polymerization mode.

KEYWORDS

Bond strength; Dental cements; Fiber post; Nanotubes; Radiopacity; Titanium.

RESUMO

Objetivo: O objetivo deste estudo in vitro foi analisar a influência de nanotubos de dióxido de titânio na polimerização química de um agente cimentante resinoso dual através de testes de resistência à união e radiopacidade. **Material e Métodos:** Após misturado com um cimento resinoso auto-adesivo comercial, três concentrações de nanotubos de dióxido de titânio (0,3, 0,6 e 0,9% em peso) foram analisadas. A resistência da união para a dentina da raiz bovina e os pinos de fibra de vidro foi avaliada pelo teste de push-out e avaliada em três terços (cervical, médio e apical) (n = 10), seguido pelo análise de modo de falha (MEV) e a norma ISO 9917-2 foi seguido para teste de radiopacidade (n = 10). Os dados foram analisados estatisticamente pelo teste ANOVA um fator seguido do teste de Tukey (α = 0,05). **Resultados:** O cimento resinoso auto-adesivo reforçado com nanotubos de dióxido de titânio a 0,6% mostrou diferença significativa em comparação com o grupo controle para teste de push-out (p=0,00158). Os grupos modificados não mostraram diferença significativa entre os terços (p=0,782). A radiopacidade mostrou maior valor para o grupo com nanotubos de dióxido de titânio 0,9% em comparação com o grupo controle (p<0,001). Conclusão: A adição de nanotubos de dióxido de titânio a um cimento resinoso auto-adesivo aumentou a os valores de resistência de união à dentina e radiopacidade no modo de polimerização química do agente cimentante.

PALAVRAS-CHAVE

Resistência adesiva; Cimentos odontológicos; Pino de fibra; Nanotubos; Radiopacidade; Titânio.

INTRODUCTION

Self-adhesive resin cements are capable of bonding to tooth without an adhesive or etchant phase due to the presence of fillers able to neutralize the early low pH, functional acid monomers, and a dual polymerization system [1,2]. The resin cement interaction with apatite present at tooth substrate [2-4] and with the metal oxides from the basic acid-soluble inorganic fillers results in the gradual neutralization of the resin cement acidity as the reaction progress [1,2,5,6]. This is quite significant, once self-adhesive resin cements polymerization can be considerably delayed by low pH via the deactivation of free radicals, what ultimately can compromise the curing reaction [2].

There is scarce literature on the evaluation of self-cure mode of dual self-adhesive resin cements when used in the absence of light-curing. This knowledge is of crucial importance, once decreased mechanical properties have been demonstrated in the cement line areas where light penetration is not sufficient [7]. In addition, in specific cases where light penetration still results in low intensity being delivered to the material, studies have shown that the redox portion of the polymerization may be jeopardized by a partially gelled/vitrified structure. That is known to lead to lower hardness values when compared to a material that undergoes redox by itself, where bond strength is indirectly compromised [7].

Bond strength of self-cure mode polymerization to dentin is still a complex issue, especially when related to root canal areas. This chemical interaction has but a series of factors that can influence and harm it of proceeding in a satisfactory way. For fiber posts cementation cases, the smear layer and debris created during the instrumentation, particular characteristics of the root canal as density and orientation of the tubules, accessibility and variations in different depths of the same root canal, can compromise this interaction and be a challenge for the cementation procedure [8-11].

In order to overcome those limitations and improve mechanical and adhesive properties, several studies in literature reported a variability of nanostructures being added to dental composites, such as TiO_2-nt [12-15]. Compared to other forms and sizes, the addition of nanotubes may lead to significantly improved physical and mechanical properties, in behalf of a high ratio of surface area to volume achieved [14,16]. Positive results for a self-adhesive resin cement enhanced by TiO_2-nt were shown in literature, including improvement in specific mechanical, biological and physical-chemical properties [15]. These findings, mainly about the auto-cure mode, raise questions if other properties might also be affected by the nanotubes inclusion.

Factors as satisfactory visualization of cementation line, excess cement and marginal adaptation are indispensable for a correct diagnosis and an adequate follow-up of restorative treatments. Accordingly, radiopacity of these materials is not only required [17-19], but a very effective tool to achieve suitable results, especially considering areas where other forms of examinations besides radiographic are not achievable. The addition of metal oxides might strongly interfere in this property, producing an increase of the photoelectric cross section interaction [20]. However, there is still a lack in literature of radiopacity data for self-adhesive resin cements enhanced by TiO_2-nt.

Therefore, the aim of this investigation was to determine the bond strength to bovine root dentin and the radiopacity values of a self-adhesive resin cement modified by TiO_2-nt at three concentrations only in self-cured polymerization mode. The null hypothesis of the present study was that the addition of different concentrations of TiO_2-nt would not affect bond strength and radiopacity values of self-adhesive dual resin cement.

MATERIALS AND METHODS

Experimental design

In this in-vitro study, different concentrations of TiO_2-nt (0.3, 0.6 and, 0.9 wt%) [14] were added to a self-adhesive resin cement (RelyX U200,3M ESPE, St. Paul, MN). The dual cement was evaluated only in self-cured mode, and specimens (sps) were tested through push-out bond strength test (PBS) and radiopacity test (RO). In accordance with Ramos-Tonello et al. [15], the sps were randomly divided in four groups: (**SCG**) self-adhesive resin cement, without TiO_2-nt (control group); (**S03**) self-adhesive resin cement with 0.3 wt% of TiO_2-nt; (**S06**) self-adhesive resin cement with 0.6 wt% of TiO_2-nt; (**S09)** self-adhesive resin cement with 0.9 wt% of TiO_2-nt.

Specimen preparation

The TiO_2-nt were manufactured and characterized according to the method described by Arruda et al. [21]. Equal portions of base and catalyst pastes of RelyX U200 were dispensed by clicker packing on a paper pad and weighed using a scale (Pinnacle P-214, Denver Instrument, NY). After, pre-stablished percentages of TiO_2-nt were weighed using the same scale. TiO_2-nt were mixed by hand to the base paste for 10 s and handled with the catalyst paste for more 10 s in a room with ambient light, controlled temperature (23ºC) and humidity (50%) [15].

Push-out bond strength

Forty bovine anterior teeth, with internal root canal diameter less than 3 mm and a minimum length of 30 mm, with not curves, were selected according to the Animals Use Ethics Committee. The teeth were cleaned and stored under refrigeration in a 0.1% Thymol solution. The roots were separated from the crows to create a standard access to the root canal and to obtain 17mm length. Next, the root canal shaping and cleaning were performed with the working length established at 16 mm with nickel-titanium rotary instruments (ProTaper Universal, Dentsply Maillefer, Ballaigues, Switzerland) to the final F5. After each instrument, 2.5% NaOCl solution (Rioquímica, São José do Rio Preto, Brazil) irrigation was carried out. The protocol to remove smear layer was followed with 1 ml 2.5% NaOCl to rinse the canals, 1 ml of EDTA (Biodinâmica, Ibiporã, Brazil) for 30 s, in sequence by final irrigation with 1 ml 2.5% NaOCl and 1ml distilled water. The root canals were dried with paper points (Tanari, Manaus, Brazil).

The root canals were filled by lateral condensation technique with gutta-percha points size F5 (ProTaper Universal, Dentsply Maillefer, Ballaigues, Switzerland) and epoxy calcium hydroxide-based sealer (Sealer 26, Dentsply, Petrópolis, Brazil). Roots were coronally sealed with glass ionomer cement (Maxxion R, FGM, Joinville, Brazil) and stored at 37 ±1ºC in 100% humidity for 14 days to complete setting the sealer.

After that, the root canals were unsealed up to 13 mm length using low-speed drills (Gates-Glidden and Largo Peeso Reamer, Dentsply Maillefer, Ballaigues, Switzerland). A low-speed drill, provided by the posts-system manufacturer (Whitepost DC #2, FGM, Joinville, Brazil) was used to prepare the posts-space into the root canals (13 mm length). After rinsed the root canals with distilled water and dried with paper points (Tanari, Manaus, Brazil), the glass fiber posts (Whitepost DC #2, FGM, Joinville, Brazil) were tested in the root canals to check the position and fitting, cleaned with 70% ethanol, dried, silanized (Silane, Angelus, Londrina, Brazil) with microbush (Cavibrush, FGM, Joinville, Brazil) for 1 min and dried again. For the luting procedure, one click of the clicker packing of the resin cement was used for each post and distributed randomly in four groups according to experimental design (n=10). The fiber glass posts were covered with the resin cement modified or not with TiO_2-nt and inserted into the root canals as seen in Figure 1.

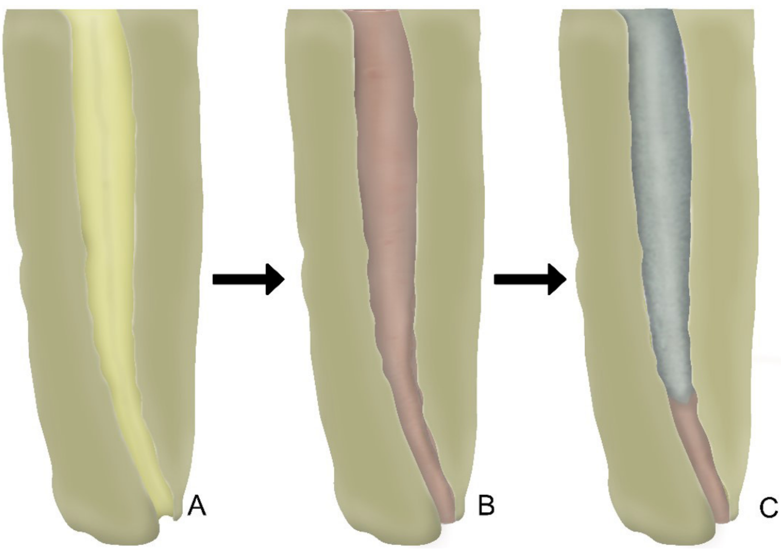

Figure 1 - Methods for PBS: root canal shaped (A), root canal filled with gutta-percha cones and epoxy calcium hydroxide-based sealer (B), and root canal unsealed until 13 mm length and fiberglass post luted (C).

This stage was carried out in a room with ambient light, controlled temperature (23ºC) and humidity (50%) to ensure the self-cure mode, and after 30 min, the sps were stored in an oven at 37 ± 1ºC in 100% humidity (artificial saliva solution).

After 24 h, the roots were fixed on a low-speed cutting-machine (Isomet, Buehler, Lake Bluff, IL) and sectioned with a diamond disc, under water-cooling, perpendicularly to the long axis. Nine sps were obtained out of each root: three cervical, three medial, and three apical as seen in Figure 2. Each slice (1.0 ±0.2 mm thick) was measured with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan), marked with a permanent pen on their apical side and stored in 3 ml of artificial saliva solution at 37 ±1ºC in a container with coded identifier, not disclosed to the operator (blind trial). After 7 days, the push-out bond strength test was performed in a universal testing machine Instron 3342 (Instron Co., Canton, MA) with a 500 Kg (50 N) load-cell at a cross-head speed of 0.5 mm/min in the apical-coronal direction. Each slice was placed on the test base with its coronal side directed to the device, and aligned with the corresponding perforation. A plunger compatible with the post's diameter (0.9 – 1.1 mm) pushed the post portion, making no contact with the dentin.

The load failure recorded in Kgf was divided by the area (mm^2) to converted the values in MPa. The formula $A = \varpi (R2+R1) [h^2 + (R2-R1)^2]^{0.5}$ was used to find the bonding area where ϖ value it is 3.14, R2 represents the fragment coronal radius [9].

After testing, the failure modes were analyzed with a 200 × magnification optical microscope (Dino - Lite Plus Digital Microscope, AnMo Eletronics Co., Taipei, Taiwan) and categorized as: 1) A - C/D (adhesive between the cement and the dentin); 2) A - C/P (adhesive between the cement and the post); 3) CP (cohesive in the post); 4) CC (cohesive in the cement); 5) Mixed (adhesive and cohesive simultaneously). The three more representative failures of each group were processed for analysis in Scanning Electron Microscopy (SEM) by variable pressure, APEX Express (APEX Corporation, Delmont, PA) with 400 and 1000 × magnification.

Radiopacity

Forty resin cement sps were manufactured with a split polytetrafluoroethylene mold, as seen in Figure 3A, according to ISO 9912-2:2010 [22], by the same operator and divided in the groups determined: SCG, S03, S06 and S09 (n=10). Three clicks of the clicker packing resin cement were used and the TiO_2–nt were added according to the group being prepared. The resin cement was handled in a room with ambient light and inserted in a single portion in the mold to slightly overfill it. After 30 min, the resin cement sps were removed from the mold and checked with a digital caliper to guarantee 1.0 ±0.1 mm of final thickness. Then, sps were stored in grade 3 water (ISO 9912-2:2010), during 7 days.

The RO test was carried out up to 30 min after removing the sps from deionized water. The images were obtained by an occlusal film size X-ray sensor (Intraoral image plate #4, VistaScan, Dürr Dental, Bietigheim-Bissingen, Germany), previously calibrated for use with single-phase dental X-ray unit with appropriate software (VistaScan Perio Plus, Dürr Dental, Bietigheim-Bissingen, Germany). One specimen of each group and an aluminum step wedge were placed on the top of the film, as seen in Figure 3B. This aluminum device was used to convert the radiopacity in equivalent mm of aluminum.

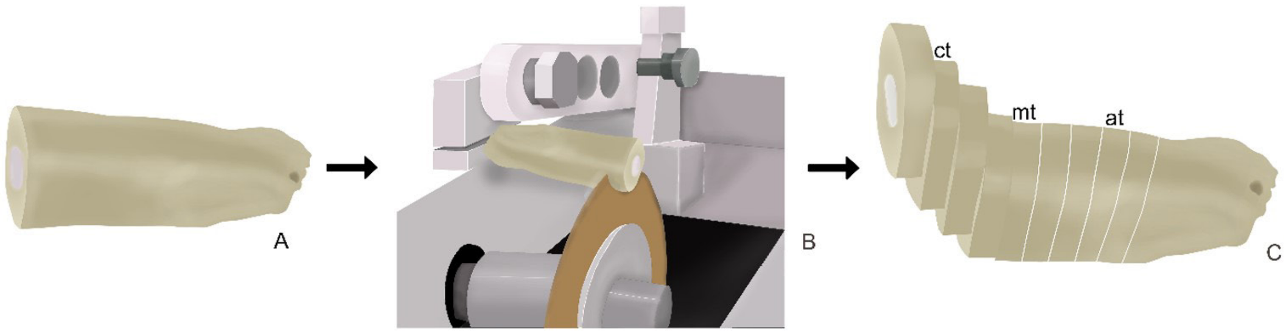

Figure 2 - Methods for PBS: root with fiberglass post luted (A), root at the cutting machine (B), and slices of the root (specimens): tree of each third (*ct* – cervical third; *mt* – medium third; *at* – apical third) (C).

Radiographic images were obtained with a conventional dental X-ray equipment (Yoshida Kaycor, X-707, Tokyo, Japan), at 70 kVp and 7 mA. The exposure time 30 s at a distance of 400 mm as seen in Figure 3C. Three images were obtained of each set X-rayed, which was filed in 1070 dpi resolution, in JPG format as seen in Figure 3D. Digital images were evaluated for optical density by grey scale analysis software (Adobe Photoshop CC 2017, Adobe Systems Incorporated, CA), by the same operator. The grey scale values for the aluminum stepwedge (3 points in each step) and for all specimens (5 points in each specimen) were measured and the correspondent means were calculated.

The RO value was converted in aluminum millimeters by the formula A × 1/B + mm/Al immediately below RDM where A represents the aluminum stepwedge increment immediately below to the RDM, 1 the mm increment between each aluminum stepwedge, B the aluminum stepwedge immediately above to the RDM and RDM is the radiographic density of the material [23].

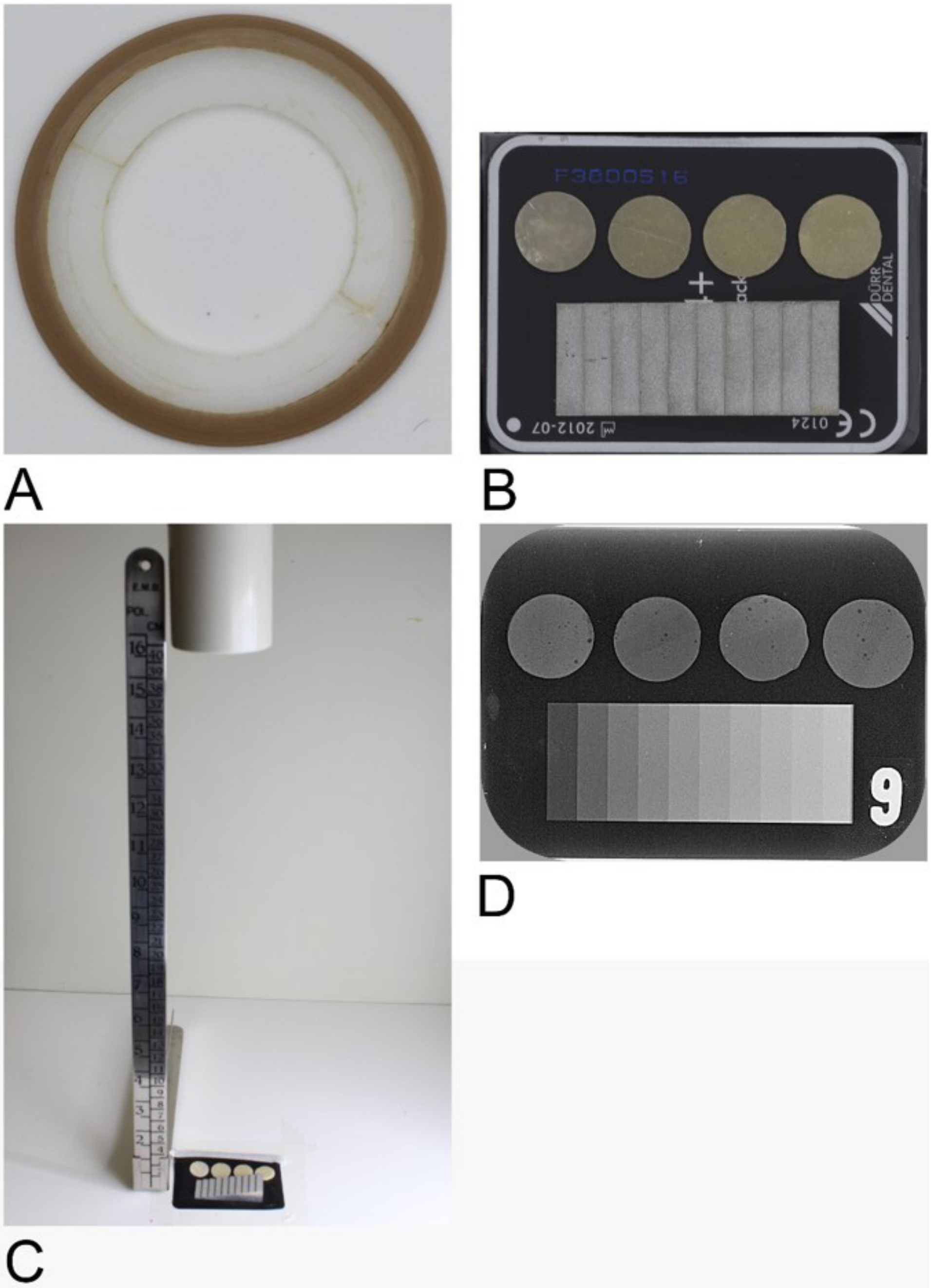

Figure 3 - Methods for radiopacity: Split polytetrafluoroethylene mold (15.0 mm ø x 1.0 mm) (A). X-ray sensor, specimens and aluminum step wedge (B). X-ray sensor, specimens and aluminum step wedge positioned at 400mm from the X-Ray device (C). Digital image in JPG format (D).

Statistical analysis

Statistical analysis was carried out with the software Stat Soft (Statistica v10.0 Entrerprise, TBICO Software Inc., CA). PBS and RO values were subjected to the Kolmogorov-Smirnov and Shapiro-Wilk normality tests and data were normally distributed. Then, data were analyzed by one-way ANOVA test, followed by post hoc multiple comparisons Tukey's test. ANOVA with repeated measures was performed to compare PBS per thirds at the $\alpha = 0.05$ significance level.

RESULTS

The results for PBS per root are presented in Table I. S06 (7.12 ± 1.89 MPa) group showed significant difference for the SCG (4.30 ± 1.03 MPa) (p=0.001580). For PBS per thirds, the results are presented in Figure 4, which the modified groups did not show significant difference among thirds (p=0,782). Figure 5 shows the failure distribution (in %) for PO. The PO failure analysis - SEM images obtained for each failure type in representative samples from the evaluated groups are presented in Figure 6A–E.

The RO analysis is present in Table II. The variance of the RO values showed correlation with the addition of TiO_2-nt. All groups complied with the minimal value established. S09 (2.27 ± 0.11) group showed significant difference for the SCG (2.00 ± 0.16) (p<0.001).

Table I - Means and standard deviations for PBS (per root)

Groups	PBS (MPa)
SCG	4.30 (1.03)[b]
S03	4.12 (0.99) [b]
S06	7.12 (1.89)[a]
S09	5.16 (2.04)[b]

Lowercase letters show significant statistical differences among groups (p=0.00040).

Table II - Means and standard deviations for radiopacity (RO) presented as follows

Groups	RO
SCG	2.00 (0.16)[b,c]
S03	1.96 (0.15)[c]
S06	2.19 (0.20)[a,b]
S09	2.27 (0.11)[a]

Lowercase letters show significant statistical differences between lines (p=0.000254).

DISCUSSION

The null hypothesis of the present study was rejected since it showed that adding variable concentrations of titanium dioxide nanotubes affects bond strength and radiopacity of self-adhesive resin cement in self-cure mode. RelyX U200 is indicated for the luting of fiberglass posts, and in fact, self-adhesive resin cement has shown higher push-out bond strength values than conventional resin cement in all thirds of the root canal dentin [24,25]. However, the dual resin cements rely on the light-curing process to reach the highest conversion degree. As a result, it is considered that a low conversion degree can cause poor bond strength values, mainly in areas where light-curing is weaker, such as root canal apical portions [25].

According to the literature, the push-out test chosen for this study is considered the best simulation of clinical conditions and provides a better assessment of the adhesion mechanism once it evaluates the structural variations of the dentin substrate in the root canal [8,26]. In this study, the results for PBS showed an increase in the S06 group compared to SCG.

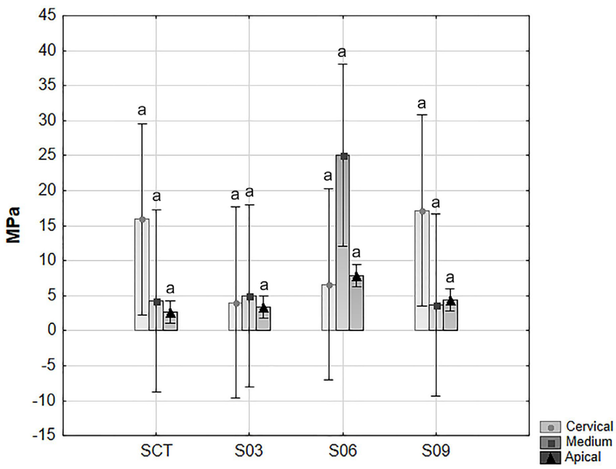

Figure 4 - PBS values (mean ± standard deviation) of all groups tested (per thirds).

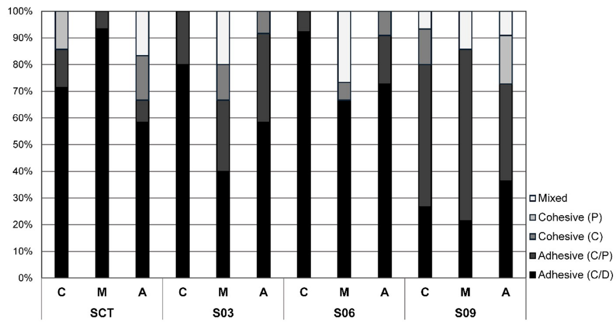

Figure 5 - Values (%) failure analysis of each group tested for PBS.

An increased conversion can be suggested to be the reason for that result, as previous reports showed an enhanced degree of conversion of an auto-adhesive resin cement with the addition of TiO_2-nt at 0.3% to 0.9 wt% [15]. Adding these percentages may play a role in initial viscosity, which influences the mobility of polymerizing species [27]. The increase in viscosity was sufficient to decrease the rate of termination. This fact allowed propagation to proceed to a greater extent in conversion, but not as dramatic to decrease the propagation rate – the net result is likely an increase in conversion [27]. Even though polymerization kinetics was not evaluated here, the increase in push-out bond strength in tandem with previously reported results [15] adds evidence for using TiO_2-nt modified materials in clinical conditions relying predominantly on the self-cure mode, as glass fibers posts.

The decrease in bond strength at deeper portions of the root canal is a concern that remains in literature. Many studies exhibited lower values of push-out strength in the apical third when compared to the cervical and middle thirds; this has been attributed to the difficulty to access deep and tight areas with the instruments available, the deficient elimination of the smear layer before the luting procedure, and the low penetration of resin cement into the dentin root canal [8,24,28]. In addition, these regions are far from photoactivation unit access, possibly affecting the conversion degree of the resin cement. It is well stablished that the use of light activation during polymerization of dual-cured materials leads to higher degree of conversion values [26,29]. This study has shown promising results in comparison per thirds, once the result of apical thirds presented the same value compared to the medium and cervical thirds. These results showed no statistical difference among the thirds for all groups evaluated, however, an analysis of the photopolymerization factor could respond other influence of the modification of these cements. Besides, the failure analysis exhibited results in accordance with literature [24,30,31] with predominance of adhesive failures in all thirds and all groups. These results revealed that the cement/dentin interface was more prevalent in the SCG, S03 and S06, while in the S09 group the cement/post interface was the most observed, as observed in SEM images on Figure 6.

Another crucial property for limited light-curing access areas in cementation procedures is radiopacity, which plays a critical role in secondary caries diagnosis, detection of cement excess, examination of open gingival margins or marginal excesses [20,32-34]. The International Organization for Standardization (ISO) for resin-modified cements requires radiopacity values equal to or greater than that of the same thickness of aluminum (ISO 9912-2:2010) [22].

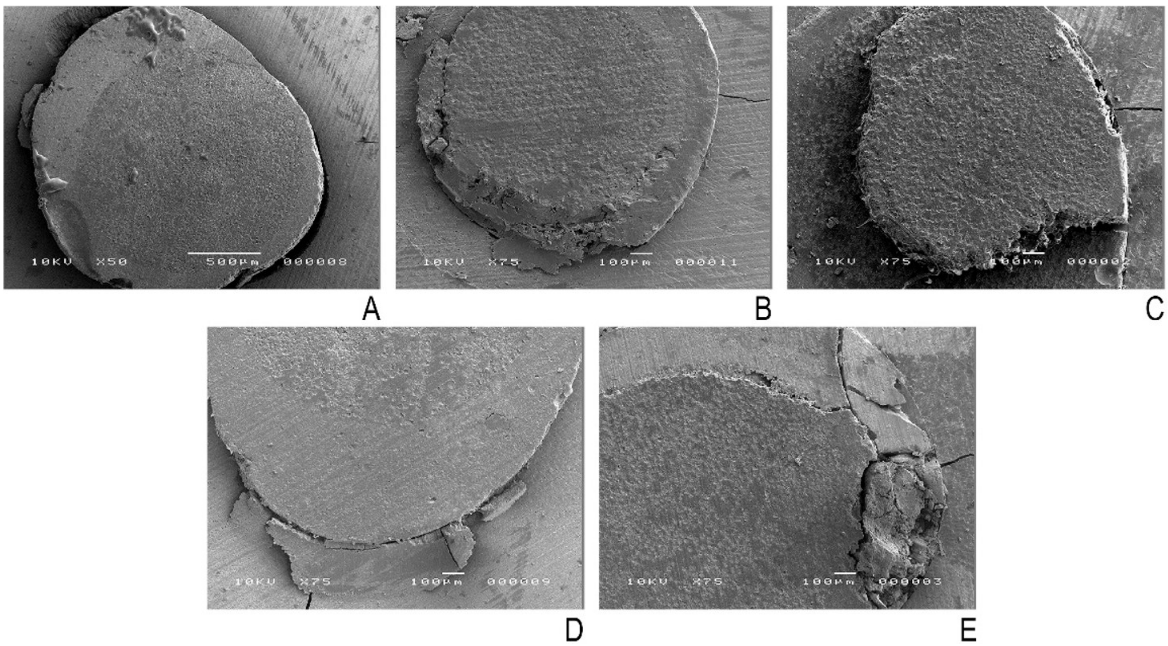

Figure 6 - PBS failure analysis - SEM image of each group showing failure type: Failure 1 (A-C/D) of a slice #3 of the cervical third of S03 group (A); Failure 2 (A-C/P) of a slice #2 of the cervical third of S03 group (B); Failure 3 (CP) of a slice #2 of the cervical third of S09 group (C); Failure 4 (CC) of the slice #1 of the apical third of S03 group (D); Failure 5 (M) of a slice #2 of the cervical third of S09 group (E).

The radiopacity values of dentin and enamel vary between 0.9 - 1.0 and 1.8 - 2.0 mm Al [35]. Furthermore, several studies have been carried out using radiopacifiers to modify the radiopacity of dental cements [36,37]. Overall, groups SCG, S03, S06, S09 complied for material radiopacity and exceeded the radiopacity of enamel and dentin, with values ranging between 1.90 - 2.37 mm Al. Furthermore, S06 and S09 groups exhibited higher values in comparison with SCG which both demonstrated significant difference in the statistical analysis. These results are in accordance with the literature, which reports TiO_2-nt as a suitable radiologic contrast agent [20], and are interesting from a clinical point of view, enabling more accurate follow-up of the respective cases.

It is important to understand the self-cured reaction of the dual self-adhesive resin cements as an element to better predict the cement behavior in this condition. This cure mode should be considered especially in areas with restricted access to light as fiberglass posts cementation [38]. The data performed in the present study along with the good results already presented in literature for degree of conversion, mechanical strength, cell viability [15]. Thus, the feasibility of use of this modified material in the future depends on further studies and clinical trials, in order to be grounded in solid evidence to predict better clinical longevity for the respective indications. Both characteristics evaluated can benefit clinical situation increasing longevity of indirect restorative procedures, with better adhesion of fiberglass posts to dentin in regions with lower light access. Similarly, a major radiopacity may help the diagnosis of the proper sealing of the resin cement.

CONCLUSIONS

The addition of 0.6% and 0.9% TiO2-nt increased the bond strength and radiopacity values in a self-cure mode of a dual self-adhesive resin cement, respectively. Based on the nanostructure's influence on the materials' behavior, adding TiO2 nanotubes proved to be a promising additive for these materials, aiming to improve the adhesive longevity of indirect restorations.

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Author's Contributions

LEP: Conceptualization, Methodology, Formal Analysis, Writing - Original Draft Preparation. APRM, CMRT: Conceptualization Methodology, Investigation, Formal Analysis. LSB, OPG: Methodology, Investigation, Formal Analysis. LJAS: Methodology, Investigation, Formal Analysis. AYF: Formal Analysis, Writing - Review & Editing, Visualization. PNLF and CSCP: Formal Analysis, Writing - Review & Editing, Visualization. AFSB: Conceptualization, Writing - Review & Editing, Visualization, Project Administration. PASF: Conceptualization, Writing - Review & Editing, Visualization, Supervision, Project Administration, Funding Acquisition.

Conflict of interest

There are no conflicts of interest to declare.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of: CEUA/FOB/USP.

The approval code for this study is: 003/2019.

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