UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

LETÍCIA DURANTE

Avaliação da ação antibacteriana e resistência de união de um sistema adesivo com adição de nanotubos funcionalizados com Cálcio e Zinco

Antibacterial properties and bond strength evaluation of Ca and Zn-functionalized nanotubes added to an adhesive system

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Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, como parte dos requisitos exigidos para a obtenção do título de Mestra em Materiais Dentários.

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Orientadora: Profª Drª Regina Maria Puppin Rontani

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PROFª. DRª. REGINA MARIA PUPPIN RONTANI

PROFª. DRª. GIOVANA SPAGNOLO ALBAMONTE DE ARAÚJO

PROF. DR. AMERICO BORTOLAZZO CORRER

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno.

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RESUMO

O objetivo deste estudo foi avaliar as propriedades antibacteriana e mecânica de um sistema adesivo experimental contendo nanotubos de TiO_2 funcionalizados com Zinco (Zn) e Cálcio (Ca). Materiais e Métodos: A avaliação antibacteriana foi realizada pelo teste halo de inibição. Foram confeccionados 9 discos de papel filtro (n=3) de 5 mm de diâmetro, sendo impregnados com o primer experimental (EX) ou com o primer comercial CLEARFIL™ SE BOND (controle-CT) e os outros 3 discos com clorexidina a 0.12% (controle de método). Os discos foram dispostos em placas de petri contendo *Streptococus mutans* (UA159). Em seguida, foi realizado teste de microtração e análise do padrão de fratura em MEV. Para este teste, foram utilizados 20 terceiros molares humanos recém-extraídos, divididos em 2 grupos (n=10). A face oclusal e as raízes foram seccionadas no sentido perpendicular ao longo eixo do dente; as superfícies cortadas foram desgastadas com lixas de carbeto de silício até a completa eliminação do esmalte e exposição da superfície planificada de dentina. Os adesivos (CT e EX) foram aplicados seguindo-se instruções do fabricante, e, sobre este fora construídos blocos de resina composta Filtek Z250 (Cor A1 – 3M ESPE) de 4 mm de altura. Os conjuntos resina/dentina foram armazenados por 24h em água a 37^oC e posterioremente, seccionados paralelamente ao longo eixo do dente, obtendo-se palitos com 1,0 mm^2 de secção transversal. O ensaio de microtração foi realizado na máquina de ensaios universal EZ test (Shimadzu Co, Kyoto, Japão) e submetidos à carga de tração, com velocidade de 1 mm/minuto, utilizando célula de carga de 500 N, até que ocorresse a fratura do palito. Os palitos foram analisados e classificados de acordo com o padrão de fratura. Os dados foram submetidos aos testes estatísticos ANOVA 1 critério, com α=5%. Resultados: Para o teste de halo de inibição o grupo controle positivo obteve os maiores valores, seguido do grupo EX e grupo CT, com diferenças estatisticamente significativas ($p<0,01$). Para resistência de união não houve diferenças entre os grupos CT e EX, sendo apenas dois tipos de padrões de fraturas observados: tipo 1- adesiva, 13,43% no experimental e 23,18% no grupo CT; e fratura tipo 3 – mista, 86,57% no grupo EX e 76,82% no CT. A adição de nanotubos de TiO_2 funcionalizados com Ca e Zn ao sistema adesivo, resultou em um aumento na zona antimicrobiana formada, sem prejudicar a resistência de união do material.

Palavras chave: Nanotubos,Titânio, Propriedades Físicas.

ABSTRACT

The aim of this study was to evaluate the antimicrobial and mechanical properties of an experimental adhesive system containing Zn and Ca functionalized TiO2 nanotubes. Materials and Methods: The antimicrobial evaluation was performed by the halo inhibition test. Nine filter paper discs (n = 3) of 5 mm in diameter were impregnated with the experimental primer-EX or the commercial primer CLEARFIL™ SE BOND (CT-control) and the other 3 discs with 0.12% chlorhexidine (CHX-method control). The disks were arranged in petri dishes containing Streptococus *mutans* (UA159). After that, a microtensile bond strength test was performed and analysis of the fracture pattern in SEM. For this test, 20 recently extracted human third-molar were used, which were divided into 2 groups (n = 10). The occlusal face and roots were sectioned perpendicularly to the long axis of the tooth; the cut surfaces were abraded with silicon carbide sanding strips until complete removal of the enamel and exposure of a plain dentin surface. The adhesive systems (CT and EX) were applied following manufacturer's instructions, and a composite resin (Filtek Z250 - Color A1 - 3M ESPE) block with 4 mm height was incrementally built on top of the bonded dentin surface. The resin / dentin sets were stored for 24 h in 37 ° C water and posteriorly, sectioned parallely to the long axis of the tooth, obtaining sticks with 1.0 mm^2. The microtensile bond strength test was performed on the universal test machine EZ test (Shimadzu Co, Kyoto, Japan) and subjected to tensile force at a speed of 1 mm / min using a 500 N load cell until fracture occurred. The sticks were analyzed and classified according to the fracture pattern. The Data were submitted to statistical tests one way ANOVA, with α = 5%. Results: For the halo inhibition test, the positive control group obtained the highest values, followed by the EX group and the CT group, with statistically significant differences ($p < 0.01$). Bond strength results showed no differences between CT and EX groups, with only two failure modes observed: type 1- adhesive, 13.43% in the EX group and 23.18% in the CT group; and type 3 - mixed, 86.57% in EX group and 76.82% in CT. Conclusion: adding Ca and Zn-funcionalized nanotubes to the adhesive system resulted in an increase in the antimicrobial effect, with no decrease to the bond strength of the material.

Key-words: Nanotubes, Titanium, Physical properties.

SUMÁRIO

1. INTRODUÇÃO

O surgimento da odontologia adesiva mudou os conceitos de intervenção à cárie dental para uma Odontologia Minimamente Invasiva, evitando a remoção de dentina afetada. Mesmo após a remoção da dentina infectada, ainda podem existir microorganismos cariogênicos na cavidade, sendo interessante o desenvolvimento de primers e adesivos com agentes antimicrobianos e remineralizantes que eliminem ou inibam o acúmulo destas bactérias na interface da restauração, pois apesar do desenvolvimento de novos adesivos dentinários, a interface de união substrato/material restaurador continua sendo o elo mais fraco em uma restauração adesiva.

A geração de sistemas adesivos autocondicionantes, como o Clearfil SE Bond (Kuraray, Japão), elimina a necessidade de condicionamento prévio do substrato com ácido fosfórico, uma vez que os primers destes sistemas apresentam em sua composição monômeros como o 10-MDP (10-metacriloloxidecildihidrogênio fosfato), com objetivo de aumentar a molhabilidade das superfícies dentais (Ceballos et al., 2003; Manuja et al., 2012). Além disto, estes materiais possuem propriedades de desmineralização menos agressivas, que o ácido fosfórico, podendo criar irregularidades de aproximadamente 1 µm de profundidade, bem menores que a encontrada quando do uso do ácido fosfórico (Breschi et al., 2010).

Já é sabido que o zinco (Zn) possui propriedades antimicrobianas (Fang et al., 2006) e que partículas em escala nanométricas apresentam eficácia ainda maior devido à sua maior área de contato (Adams et al., 2006). Kasraei et al., 2014 demonstraram que compósitos resinosos contendo nanopartículas de ZnO apresentaram atividade antibacteriana frente aos microorganismos *Streptococcus mutans* e *Lactobacillus.*

O Zn tem demonstrado, além da capacidade antimicrobiana, ser importante nos processos de mineralização e remineralização (Osorio et al., 2014), e na inibição enzimas metaloproteínases (MMPs) presentes em estado latente na dentina e ativadas após a exposição das fibrilas colágeno durante a desmineralização da dentina no procedimento restaurador. As MMPs atuam degradando o colágeno desprotegido, enfraquecendo a união adesiva. O Zn age unindo-se ao sítio de ligação da proteína, impedindo que as enzimas (MMPs) se

unam e degradem sua estrutura (Rosenberg et al. 1998).

Toledano, et al., 2012 demonstraram que nanopartículas carregadas com Zn e Cálcio (Ca) infiltradas na dentina desmineralizada facilitaram a manutenção das fibrilas colágenas e a remineralização da camada híbrida. Segundo os autores, as nanopartículas de Zn auxiliaram a deposição do Ca, induzindo a melhoria das propriedades mecânicas da região após avaliação de 3 meses de armazenamento (Toledano, et al., 2012).

Com a vasta aplicação dos nanomateriais tem havido um grande interesse na utilização destes materiais, principalmente por suas propriedades antibacterianas, físico/mecânicas e biocompatibilidade (Cheng et al. 2013), é o caso dos nanotubos de TiO_2, medindo aproximadamente 10 nm de diâmetro e 200 nm de comprimento (Arruda et al. 2015). Bottino et al. 2013 sugeriram que a estrutura oca dos nanotubos poderiam potencialmente ser usadas como um método de entrega de medicamentos para encapsulamento e liberação de agentes terapêuticos, tais como inibidores de MMPs, aumentando a longevidade das restaurações adesivas.

O emprego da nanotecnologia nos materiais dentários, como um novo conceito no desenvolvimento de materiais com melhores propriedades mecânicas e antimicrobianas, capazes de manter a estabilidade da camada hibrída com diminuição da colonização bacteriana devem ser considerados.

Com base no grande número de aplicações, este estudo avaliou a incorporação de nanotubos de TiO_2 funcionalizados por Ca e Zn ao primer do sistema adesivo autocondicionante de dois passos Clearfil SE Bond (Kuraray, Japão), quanto a ação antibacteriana e a resistência de união imediata.

Este trabalho foi apresentado no formato alternativo de tese de acordo com as normas estabelecidas pela deliberação 002/06 da Comissão Central de Pós-Graduação da Universidade Estadual de Campinas.

2. Artigo

"Antibacterial and mechanical properties of an adhesive system with Ca and Zn-functionalized nanotubes"

ABSTRACT

Objectives: evaluate the antimicrobial and mechanical properties of an experimental adhesive system containing Ca and Zn-functionalized TiO_2 nanotubes.

Materials and Methods: The antimicrobial evaluation was performed by the pour plate test. Nine filter paper discs (n = 3) of 5 mm in diameter were impregnated with the experimental primer-EX or the commercial primer (CT-control) and the other 3 discs with 0.12% chlorhexidine (CHX-method control). The disks were arranged in Petri dishes containing S. *mutans* (UA159). After that, a microtensile bond strength-μTBS test and analysis of the fracture pattern in SEM were performed. For μTBS, 20 recently extracted human third-molar were used, which were divided into 2 groups (n = 10). The occlusal surface and roots were sectioned perpendicularly to the long axis of the tooth; the cut surfaces were abraded with silicon carbide sanding strips until complete removal of the enamel and exposure of a plain dentin surface. The adhesive systems (CT and EX) were applied following manufacturer's instructions, and a composite resin (Filtek Z250 - Color A1 - 3M ESPE) block with 4 mm height was incrementally built on top of the bonded dentin surface. The resin / dentin sets were stored for 24 h in 37 ° C water and posteriorly, sectioned parallel to the long axis of the tooth, obtaining 1.0 mm^2 sectioned area sticks. The μTBS test was performed on the universal test machine EZ test and subjected to tensile force at a speed of 1 mm / min using a 500 N load cell until fracture occurred. The sticks were analyzed and classified according to the fracture pattern. The data were submitted to one way ANOVA and Tukey´ statistical tests, with α=5.

Results: For the inhibition zones test the CHX showed the highest significant values, followed by the EX and the CT groups ($p < 0.01$). For bond strength there was no significant difference between CT and EX groups, with only two types of fracture patterns observed: type 1-adhesive, 13.43% (EX) and 23.18% (CT); and failure type 4 - mixed, 86.57% (EX) and 76.82% (CT).

Conclusion: the addition of Ca and Zn-funcionalized nanotubes to the adhesive system resulted in an increase in the antimicrobial zone formed, without harming the bond strength of the material.

Key-words: Nanotubes, Titanium, Physical properties.

INTRODUCTION

Adhering restorative materials to tooth structure is a complex process. The achievement of an efficient and stable bonding requires the formation of a specific structure called hybrid layer, which consists in a layer of interpenetrated luting agent and tooth structure[1]. This interface can be achieved through the application of adhesive systems, which are responsible for demineralizing the tooth surface, promoting irregularities required for the luting agent penetration, as well as bonding properties to restorative materials. The adhesive systems can be classified basically as etch-and-rinse and self-etch systems. The etch-and-rinse system requires the application of phosphoric acid in order to create irregularities up to 5µm in depth on the tooth structure previously to the application of the luting agent[2]. The self-etch adhesive systems does not require the application of phosphoric acid; instead, its active component consists in a phosphate monomer, which possesses demineralization properties milder than phosphoric acid, being able to create irregularities of approximately 1µm in depth[2]. However, it has been shown in the literature that the depth of these irregularities promoted by both types of adhesive systems results in voids which are not completely sealed by the penetration of the luting agents, resulting in sites susceptive of accumulation of fluids and bacteria, which can lead to adverse outcomes such as secondary caries[3,4,5].

Although the prevalence of caries has decreased in the latest years, the replacement of tooth restorations caused by secondary caries is very frequent in the clinical daily practice, being the most common cause for the failure of bonded restorations[6,7], as a result of the accumulation of cariogenic microorganisms on the restoration's margin[8].

The removal of failed restorations always implies in some level of wear on sound dentin and enamel, resulting in loss of the remaining tooth structure. In order to minimize this outcome, incorporations of anti-bacterial agents to the adhesive

systems may be useful to prevent or decrease bacterial accumulation, especially gram-positive bacteria like *Streptococus mutans*, on the tooth/restoration interface[9].

In this regard, one of the most noticeable anti-bacterial agents is Zinc (Zn). It is known for its lasting anti-bacterial effect, biocompatibility and nanometric scale activity, as well as re-mineralization properties through the formation of zinc-phosphate crystals, which provide ions of calcium and phosphorus to demineralized dentin[10]. Therefore, the addition of Zn to adhesive systems seems a reasonable attempt, although the influence of this type of addition on the adhesive material's properties such as bond strength and biocompatibility is unknown.

Alongside with the application of anti-bacterial agents, the incorporation of some specific nanomaterials are known for resulting in improvements of other material's mechanical and biological properties[11]. Following this direction, a nanomaterial consisting of titanium dioxide (TiO_2) nanotubes with 200 nm length and 10nm diameter was developed by Lisboa-Filho et al. 2014[12]. These nanotubes are prone to being functionalized with agents such as calcium (Ca) and Zn, in order to deliver these agents to the adjacent substrate, while no decrease on the mechanical properties of the material is expected.

Thus, the purpose of this study was to evaluate the properties of an experimental self-etch adhesive system containing Ca and Zn-functionalized TiO_2 nanotubes. The null hypotheses tested are that: 1) there are no significant differences on the anti-bacterial properties between the experimental and control group; 2) there are no significant differences on bond strength between the experimental and control group.

MATERIAL AND METHODS

TECHNICAL INFORMATION

This study was approved by Research Ethics Committee, under the CAAE: 71750417.4.0000.5418. Adhesive sytems and composite resin are described on Table 1. For experimental group, the adhesive system's primer used was modified by the addition of funcionalized nanutubes with Ca and Zn. For 1ml of primer, 20µg / ml of nanotubes of TiO_2 anatase (Aldrich, 99%) in powder functionalized with Zn and Ca (Aldrich, 99%) was added. The TiO_2 nanotubes were obtained from alkaline syntheses followed by successive heat treatments and washes in acidic medium,

resulting in nanotubes with diameter around a few nanometers, being a relatively simple and inexpensive process. The incorporation of the powder into the primer was done manually[12,13].

Table 1. Composition, manufacturer and application technique according to manufacturer of adhesive and composite used in the study.

Materials	Composition	Manufacturer and batch number	Method of application
FILTEK™ Z250	BisEMA, UDMA, silane treated ceramic, Filled to 60% by volume with zircon silica filler, average particle size = 0.6 µm and water.	3M DIVISION:3M ESPE Dental Products; LOT: 644674	Incremental insertion of 2mm; light curing (20s).
CLEARFIL™ SE BOND (CT)	**Primer:** MDP, HEMA, CQ, N,N-Diethanol p-toluidine, hydrophilic dimethacrylate and water (pH = 2) **Bond:** MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, CQ, N,N-Diethanol p-toluidine and silanated colloidal silica.	Kuraray Medical Inc, Kurashiki, Tokyo, Japan Primer: 9N0168 Bond: 9U0269	Application of Primer (20s); Dry gently; Application of the adhesive and light curing (10s).
CLEARFIL SE BOND (EX)	**Primer:** MDP, HEMA, CQ, N,N-Diethanol p-toluidine, hydrophilic dimethacrylate and water (pH = 2) Ca, Zn nanotubes TiO_2 **Bond:** MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, CQ, N,N-Diethanol p-toluidine and silanated colloidal silica	Kuraray Medical Inc, Kurashiki, Tokyo, Japan Primer: 9N0169 Bond: 9U0270	Application of Primer (20s); Dry gently; Application of the adhesive and curing light (10s).

POUR PLATE

The antimicrobial evaluation was performed by the inhibition zone test. Nine filter paper discs with diameter of 5 mm were impregnated with the experimental primer (EX) (n=3), the commercial primer (CT-control)(n=3) or 0.12% chlorhexidine digluconate (positive control)(n=3). The disks were placed on petri dishes filled with

agar and containing *S. mutans* (strain UA159), and stored in a bacteriological oven at 37° for 24 hours. After this period, a measurement of the inhibition zones with a digital caliper (Mitutoya, Suzhou, China) around each sample was carried out.

BOND STRENGTH TEST - PREPARATION OF THE SPECIMENS

Twenty extracted human third molars were selected and cleaned in order to remove residues of periodontal tissue. The teeth were randomly distributed in to two groups: control (CT) and experimental (EX) (n=10). Each tooth was then taken to a cutting machine (Isomet 1000 - Buehler Ltd., Lake Bluff, IL, USA), and sectioned perpendicularly to the long axis of the tooth, with a diamond impregnated disc (15LC Diamond Series - Isomet Buehler- Microstructural Analysis Division, Lake Bluff, Illinois, 60044-USA), in order to remove the occlusal surface and roots. After that, parallel cuts were executed in order to remove the enamel layer. The specimens were then grinded on all surfaces with 320 grit silicon carbide sanding (Carborundum Abrasivos, Recife, PE, Brazil) under water irrigation in order to completely remove the remaining enamel areas. Dentin disks were obtained for each tooth, which were polished using 600 grit silicon carbide sanding (Carborundum Abrasivos, Recife, PE, Brazil) under distilled water irrigation for one minute in order to promote a standardized surface. The adhesive primers were applied to each group following the manufacturer instructions and light cured using a LED curing light unit (700mW/cm2, BluePhase, Ivoclair Vivadent - Schaan, Liechtenstein) for 20 seconds. Then, a composite resin was placed on the adhesive coated surface with horizontal increments of 2 mm and light cured for 20 seconds building a block of approximately 4 mm height was obtained. The resin-dentin blocks were stored in deionized water at 37° C for 24 hours. Next, the block was fixed with wax on acrylic supports. Cuts parallel to the long axis of the tooth, starting in the lingual-buccal direction and then in the mesio-distal direction were performed, maintaining a distance between the cuts of 1.3 mm, throughout the block. Stick-shaped specimens measuring approximately 1 ± 0.3 mm^2 section area were obtained from each resin-dentin blocks and they were stored in deionized water at 37° C for 24 hours.

MICROTENSILE BOND STRENGTH (μTBS) TEST

Specimens were individually placed and fixed with a cyanoacrylate gel glue (Super Bonder Gel - Locite Brasil LTDA - São Paulo, Brazil) in a custom jig which was coupled to a universal test machine (EZ-S - Shimadzu - Kyoto, Japan). The specimens were stressed under tensile force until failure at a crosshead speed of 1 mm/min. The μTBS was expressed in MPa. The tooth was considered as an experimental unit in this study (n = 10), using the arithmetic mean of the μTBS per tooth measurements for statistical analysis.

FAILURE MODE

The mode of failure for each specimen was firstly determined using light microscope (M35 - Zeiss - Oberkochen, Germany). Failure modes were classified according to the following categories: Type 1- adhesive failure; Type 2- cohesive failure in the dentin; Type 3 - cohesive failure in the resin; Type 4- mixed failure (cohesive failure in the luting agent and adhesive). Then, a representative specimen of each failure mode from each group, was selected and analyzed using scanning electron microscope. In order to confirm the failure mode, the specimens were placed on an aluminum stub, sputter-coated with gold-palladium alloy (SCD 050 - Balzers - Schaan, Liechtenstein) and analyzed using a scanning electron microscope (JSM 5600 LV - JEOL - Tokyo, Japan) operating at 15 kV. Each debonded surface was analyzed at several magnifications, and a representative micrograph was taken at magnifications 85x in order to illustrate the failure pattern.

STATISTICAL ANALISYS

Data from μTBS and inhibition zone tests were submitted to Shapiro-Wilk's Levene's test, verifying a normal distribution of data and homogeneity of variances, respectively (α=0.05). Then data were submitted to one-way ANOVA and Tukey´s test with significant level set on α=0.05, using BioEstat 5.3 (Mamirauá - AM - Brasil).

RESULTS

POUR PLATE

The results of the measurements of the inhibition zone are shown in Table 2. Adding Ca and Zn-funcionalized TiO_2 nanotubes to the primer of Clearfil SE Bond adhesive system provided a higher antimicrobial effect showed by the higher inhibition zone observed for EX than CT ($p<0.01$)

Table 2: Averages and standard deviations (mm) of the inhibition zones for each group.

	CHX	CT	EX
S. mutans	19.485 ^{A} - 0.199	15.59 ^{C} – 0.303	16.68 ^{B} - 0.310

Different uppercase letters mean statistical differences between groups ($p<0.01$). CHX – Chlorhexidine – control of method; CT – Clearfil SE Bond adhesive system - control group; EX- Clearfil SE Bond+Ca/ Zn funcionalized nanotubes.

MICROTENSILE BOND STRENGTH (µTBS)

Adding Ca and Zn-functionalized nanotubes to the adhesive system did not affect the µTBS, there was no significant statistical differences between groups concerning µTBS ($p=0.97$). µTBS averages from both groups are displayed on Table 3.

Table 3- Microtensile bond strength averages (MPa) and standard deviations from both groups, CT and EX.

CT	EX
5.75^{a}-7,12	25.66^{a}-7,02

Similar lower case letters mean no statistical significant differences between groups ($p= 0.97$). ; CT – Clearfil SE Bond adhesive system - control group; EX- Clearfil SE Bond+ Zn funcionalized nanotubes.

FAILURE MODE PREVALENCE

The most prevalent failure was type 4 (mixed failure), being 86.57% on EX and 76.82% on CT; followed by type 1 - adhesive failure, 13.43% on EX compared to the 23.18% on CT. Other types of failure mode were not found. The failure mode prevalence is illustrated on Figure 2.

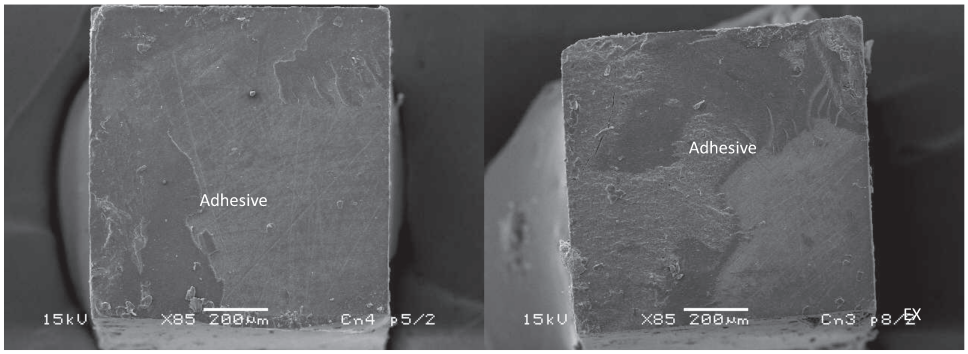

Figure 2- Representative SEM images from the two types of failure mode found; adhesive and mixed.

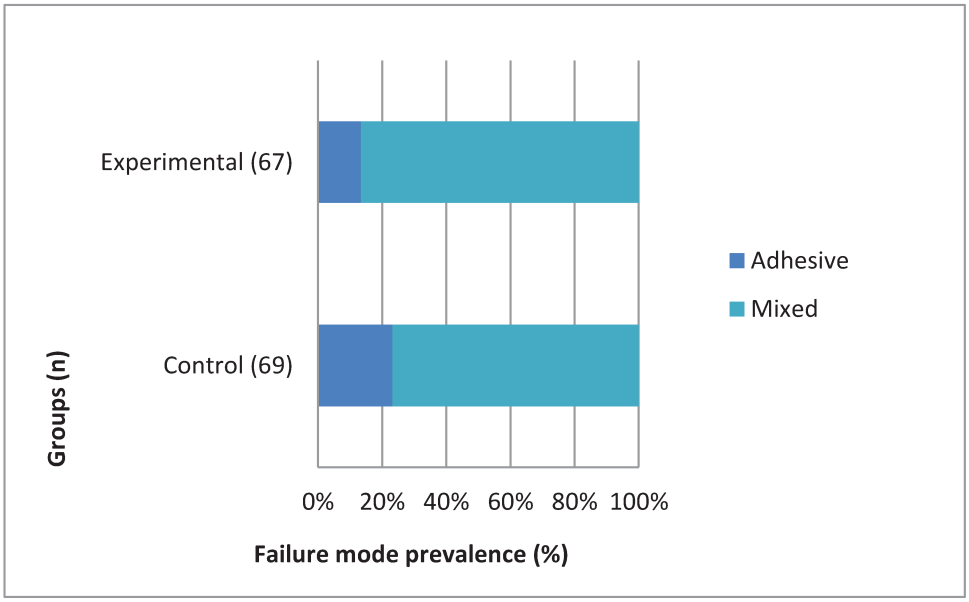

Figure 3 – Failure mode prevalence from both groups.

DISCUSSION

The development of synthetic materials capable of bioactive activity as participation mineralization, inhibiting MMPs, antibacterial activity with absence of undesirable effects, which can be used safely in medical and dental procedures, are of great interest[14]. In this matter, the use of nanomaterials, due to their different applications, physical/mechanical properties as well as biocompatibility has been highly demanded[11].

Therefore, this study was conducted in order to prove the hypotheses that additions of nanotubes to the primer of Clearfil SE adhesive system influence the microtensile bond strength (μTBS) between the adhesive and human dentin, as well as on its antibacterial activity. The first hypothesis was rejeited since there was statistically significant difference between groups on the antibacterial properties; while the second null hypothesis was accepted, the results confirmed the first null hypothesis, in which there was no statistically significant difference in bond strength between the Control and Experimental groups;

The absence of statistical difference between the experimental and control groups is an important result, since the inclusion of TiO_2 nanotubes did not interfere in the bond strength values nor in the failure mode of the adhesive system. Moreover, considering the results over time, the release of Zn and Ca in the dentin demineralized by the acid primers could at the same time disconnect one of the main degradation pathways of the interface by the MMPs and strengthen the demineralized area by the presence of Ca. Therefore, micro-TBS long-term studies have to be accomplished in order to observe that.

A recent study showed that the addition of TiO_2 nanotubes to a composite light cured resin resulted in an increase in the degree of conversion, at times shorter than that recommended by the manufacturer, and better mechanical properties, such as hardness and modulus of elasticity in a proportion of 0.3% [13].

The addition of Zn to the adhesive has been shown to be important in the remineralization of tooth structure, Zn induces the deposition of Ca and P in the adhesive / tooth surface. Although it is not possible to guarantee that this deposition results in an effective remineralization of collagen[11]. Addition of Zn was found not to affect bonding strength immediately, but increased its union stability in 3 months[17].

Despite the concern of the addition of bioactive materials such as Zn to resinous composites in relation to mechanical properties, the results found in this study and in the literature have many advantages when administered in an adequate proportion.

The potential action of the using of Zn-funcionalized nanotubes on primer is to consider the possibility of releasing Zinc ions in the hybrid layer. The specific adhesive system used on this study contains an unpolimeryzed primer, which can easly disseminate on dentin surface.

In addition, to the bonding strength results, this study showed a higher significant antimicrobial property by the Zn-funcionalized nanotubes associated with Clearfil SE bond against *S. mutans*. These results corroborate the results found by other studies in the literature that showed satisfactory action of Zn against cariogenic bacteria present in the oral cavity, more frequently *S. mutans*[15,16]. It has to be considered that Zn ions can be easily released from the primer, since the absence of photoinitiator system it cannot be polymerized. Then, it can be freely released from the primer and act as an antibacterial agent preventing the potential bacteria colonization at the resin/dentin interface. Besides the antibacterial property, Zinc ion when is in intimate contact with dentin, it has the ability to inhibit the MMPs, responsible for the degradation of collagen without protection by the inorganic phase of the dentin that was removed by the demineralization produced by the first acid[17,18]. Thus, greater resistance to chemical degradation is expected when observed in the long run. Many authors have tested the efficacy of chlorhexidine as an antibacterial agent, used as a control of the method in this study, with the highest values of inhibition[19,20,21].

Even with the highly positive results found in this study, one should consider the limitations of applying these results directly, as it is an *in vitro* study and have most of its external variables highly controlled. In addition, other events occurring *in vivo* are not possible to be simulated *in vitro*, such as other sources of MMPs other than dentin, the active participation of cells and biochemical events in the healing of the affected area that may contribute to other outcomes in alive organisms.

Therefore, future research evaluating inhibition of MMPs, long-term bond strength, and also the biocompatibility of the adding Ca/Zn-funcionalized TiO_2 nanotubes in adhesive primers are required before *in vivo* tests are be conducted.

CONCLUSIONS

Based on the results obtained and within the limitations of this study, the following conclusions can be drawn:

1. The incorporation of Zinc and Calcium functionalized nanotubes to the adhesive primer improved the antibacterial properties of the adhesive primer Clearfil SE Bond;

2. The incorporation of Zinc and Calcium functionalized nanotubes to the adhesive primer did not affect the bond strength of this material.

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3. CONCLUSÃO

Com base nos resultados obtidos e nas limitações deste estudo, é possível concluir :

1. A incorporação de nanotubos funcionalizados com zinco e cálcio no primer adesivo melhorou as propriedades antibacterianas do primer adesivo Clearfil SE Bond;
2. A incorporação de nanotubos funcionalizados com zinco e cálcio no primer adesivo não afetou a resistência de união deste material.

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COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Avaliação da adição de nanotubos funcionalizados a um sistema adesivo sobre a ação das metaloproteinases**", CAAE **71750417.4.0000.5418**, dos pesquisadores **Letícia Durante** e **Regina Maria Puppin Rontani**, satisfaz as exigências das resoluções específicas sobre ética em pesquisa com seres humanos do Conselho Nacional de Saúde – Ministério da Saúde e foi aprovado por este comitê em 01/11/2017.

The Research Ethics Committee of the School of Dentistry of Piracicaba of the University of Campinas (FOP-UNICAMP) certifies that research project "**Evaluation of the addition of functionalized nanotubes to an adhesive system on the action of metalloproteinases**", CAAE **71750417.4.0000.5418**, of the researcher's **Letícia Durante** and **Regina Maria Puppin Rontani**, meets the requirements of the specific resolutions on ethics in research with human beings of the National Health Council - Ministry of Health, and was approved by this committee on First of November of 2017.

Profa. Fernanda Miori Pascon

Vice Coordenador
CEP/FOP/UNICAMP

Prof. Jacks Jorge Junior

Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo e a lista de autores aparecem como fornecidos pelos pesquisadores, sem qualquer edição.
Notice: The title and the list of researchers of the project appears as provided by the authors, without editing.