UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE ODONTOLOGIA

Programa de Pós-Graduação em Odontologia

Área de Concentração Clínica Odontológica – Materiais Dentários

NANOTUBOS DE NITRETO DE BORO COMO CARGA PARA RESINAS
ADESIVAS

Felipe Weidenbach Degrazia

Orientador: Prof. Dr. Fabrício Mezzomo Collares

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LINHA DE PESQUISA: BIOMATERIAIS E TÉCNICAS TERAPÊUTICAS EM ODONTOLOGIA

NANOTUBOS DE NITRETO DE BORO COMO CARGA PARA RESINAS ADESIVAS

Defesa de Tese de Doutorado, requisito obrigatório para a obtenção do título de Doutor em Clínica Odontológica - Materiais Dentários.

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DEDICATÓRIA

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RESUMO

O objetivo do presente estudo foi desenvolver e avaliar a incorporação de nanotubos de Nitreto de Boro (BNNT) como carga em uma resina adesiva. Foi formulada uma resina adesiva utilizando-se os seguintes reagentes: 2-hidróxietil metacrilato (HEMA), Bisfenol A glicol dimetacrilato (BisGMA), canforoquinona (CQ) e Etil 4-dimetilaminobenzoato (EDAB). Os BNNTs foram caracterizados por Microscopia Eletrônica de Varredura (MEV) e Microscopia Eletrônica de Transmissão (MET). O adesivo experimental com diferentes concentrações de BNNT (0, 0,05, 0,075, 0,1 e 0,15%) foi caracterizado quanto ao ângulo de contato e energia livre de superfície (ELS), dureza e amolecimento em solvente (solução de etanol) em microdurômetro, deposição mineral até 14 dias e grau de conversão por espectroscopia Raman e resistência coesiva. O adesivo experimental com diferentes concentrações de BNNT após imersão por 3 h em 65% HNO_3 e 48 h em H_2O_2 foi caracterizado por grau de conversão e cinética de polimerização por Espectroscopia de infravermelho por transformada de Fourier (FTIR), ângulo de contato em dentina por tensiômetro, citotoxicidade por sulforodamina B (SRB) e resistência de união imediata e longitudinal. Os resultados da análise de microscopia eletrônica de varredura e de transmissão demonstraram nanotubos de nitreto de boro com diâmetros de 5 a 10 nanometros com término fechado. Nenhuma modificação no grau de conversão ocorreu após a incorporação de BNNTs até 0,15%. Os ângulos de contato de água e α-bromonaftaleno aumentaram ($p < 0,05$) e consequentemente a ELS diminuiu após a incorporação de BNNTs ao adesivo. Houve aumento da microdureza e da resistência ao amolecimento em solvente após incorporação de 0,075, 0,1 e 0,15% de BNNTs. Não houve diferença significativa na resistência coesiva entre os diferentes grupos. Foi encontrada deposição mineral nos espécimes com BNNT após sete dias de imersão em SBF (fluido corporal simulado). Após o tratamento dos BNNTs com HNO_3 e H_2O_2 e incorporação ao adesivo, houve aumento do grau de conversão e da taxa de polimerização máxima após incorporação de 0,075 e 0,1% de BNNT. O ângulo de contato à dentina aumentou após a incorporação de 0,15% BNNT. Não houve diferença significativa na resistência de união entre os

grupos após 24 h (p >0,05). Após 6 meses a resistência de união diminuiu significativamente para o grupo 0% e 0,15% BNNT. Entretanto, os grupos que tiveram incorporação de BNNT obtiveram maiores valores que 0% (p <0,05). Não houve diferença no crescimento celular (p >0,05) entre os grupos em comparação ao grupo controle positivo (100% de crescimento). A incorporação de BNNT até 0,15% melhorou as propriedades físico-químicas do adesivo dentário, promoveu deposição mineral e aumentou a estabilidade longitudinal sem apresentar citotoxicidade a fibroblastos, fornecendo benefícios consideráveis frente aos materiais disponíveis no mercado.

ABSTRACT

The aim of this study was to develop and evaluate the incorporation of boron nitride nanotubes (BNNTs) into an adhesive resin. An adhesive resin was developed using the following reagents: 2-hydroxyethyl methacrylate (HEMA), bisphenol-A glycidyl dimethacrylate (BisGMA), camphorquinone (CQ) e ethyl 4-dimethylaminobenzoate (EDAB). BNNTs were characterized by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The experimental adhesive with different concentrations of BNNT (0, 0.05, 0.075, 0.1 e 0.15%) was characterized by contact angle and surface free energy (SFE), hardness and softening in solvent (ethanol solution) by microdurometer, mineral deposition up to 14 days and degree of conversion by Raman spectroscopy and ultimate tensile strength test. The experimental adhesive with different concentrations of BNNT after immersion for 3 h in 65% HNO_3 and 48 h in H_2O_2 was characterized by degree of conversion and polymerization kinetics by Fourier Transform Infrared Spectroscopy (FTIR), contact angle in dentin by tensiometer, sulforhodamine B (SRB) cytotoxicity and immediate and longitudinal bond strength. The results of scanning and transmission electron microscopy showed boron nitride nanotubes with diameters of 5 to 10 nanometers with closed end tips. No change in degree of conversion occurred after the incorporation of BNNTs up to 0.15%. The contact angles of water and α-bromonaphthalene increased (p <0.05) and consequently SFE decreased after the incorporation of BNNTs to adhesive. After incorporation of 0.075, 0.1 and 0.15% BNNTs an increase was shown in microhardness and resistance to softening in solvent. No significant difference in ultimate tensile strength was found among groups. Mineral deposition was found on BNNT specimens after seven days of immersion in SBF (simulated body fluid). After BNNTs´ treatment with HNO_3 and H_2O_2 and incorporation into the adhesive, there was an increase in the degree of conversion and the maximum polymerization rate after incorporation of 0.075 and 0.1% of BNNT. Contact angle in dentin increased after incorporation of 0.15% BNNT. There was no significant difference in microtensile bond strength among groups after 24 h (p> 0.05). After 6 months the bond strength decreased significantly for 0% group and 0.15% BNNT. However, the groups that had BNNT incorporation obtained higher values than

0% (p <0.05). There was no difference in cell growth (p> 0.05) among groups compared to the positive control group (100% growth). The incorporation of BNNT up to 0.15% improved the physicochemical properties of dental adhesive, promoted mineral deposition and increased longitudinal stability with no cytotoxicity to fibroblasts, providing considerable benefits against dental materials available in the market.

Key words: resin cements; dental cements; remineralization

SUMÁRIO

1. INTRODUÇÃO

1.1 ODONTOLOGIA ADESIVA

O condicionamento dentinário, descrito por FUSAYAMA *et al*. (1979) e a demonstração da infiltração da resina em uma dentina desmineralizada formando uma matriz resinosa reforçada por fibras colágenas, denominada de camada híbrida (NAKABAYASHI; KOJIMA; MASUHARA, 1982) aumentaram a previsibilidade e consequente avanço da odontologia adesiva. Mesmo assim, a degradação da interface adesiva ao longo do tempo vem sendo uma das principais causas de mudanças de cor e perdas prematuras da resistência adesiva e que, consequentemente, interferem na longevidade do tratamento restaurador (MJÖR *et al*., 2000; DE MUNCK *et al*., 2003; DE MUNCK *et al*., 2005). Uma revisão sistemática de adesivos odontológicos de 1, 2 e 3 passos, demonstrou taxas de falhas anuais entre 3,1 e 5,8% de restaurações de resina composta (PEUMANS *et al*., 2014). Recentemente, uma revisão sobre sobrevivência longitudinal de restaurações compostas anteriores demonstrou taxa de falha anual que pode chegar a 4,1% e encontraram como principais razões de falha a fratura do dente ou restauração e perda da restauração (DEMARCO *et al*., 2015).

Por esse motivo, o desenvolvimento de materiais odontológicos de base polimérica que apresentam melhores propriedades físicas, mecânicas e biológicas em função do tempo vem sendo o objetivo de diversos estudos (PASHLEY *et al*., 2004; TAY; PASHLEY, 2009; LEITUNE *et al*., 2011). As principais características requeridas destes materiais são: maior resistência à fratura (VAN MEERBEEK *et al*., 1993; KIM *et al*., 2005), à degradação

hidrolítica (BRESCHI *et al*., 2008; PASHLEY *et al*., 2011) e enzimática (PASHLEY *et al*., 2004) e a capacidade de interação com estruturas biológicas, prevenindo lesões de cárie (FERREIRA-ZANDONÁ *et al*., 2012; ZHANG *et al*., 2013) e estimulando a remineralização dentária (GU *et al*., 2010; VASCONCELLOS *et al*., 2013).

As propriedades mecânicas e características dos materiais adesivos dependem da qualidade das ligações formadas e da presença de partículas inorgânicas (VAN LANDUYT *et al*., 2007; FERRACANE *et al*., 2011). Os agentes de carga podem interferir na disponibilidade de energia luminosa no interior do polímero, dificultando a mobilidade das cadeias durante a propagação de polimerização (ANDRZEJEWSKA, 2001). No entanto, se incorporados na devida concentração, podem ser empregados em adesivos odontológicos para diminuir a proporção de matriz orgânica, acarretando em redução da contração e do estresse de polimerização (GONÇALVES; KAWANO; BRAGA, 2010), aumentar a resistência mecânica (LI, J. *et al*., 2012) e à degradação (SIDERIDOU *et al*., 2004) e contribuir com propriedades antimicrobianas (STOPIGLIA *et al*., 2012) e de deposição mineral (COLLARES *et al*., 2014).

Diferentes agentes para remineralização dentinária têm sido investigados com a finalidade de estabilizar a camada híbrida, que, por sua vez, sofre degradação pela permanência de fibras colágenas expostas devido à incompleta infiltração da resina adesiva após o condicionamento ácido (TJÄDERHANE, 2015). O emprego de análogos biomiméticos ou nanomateriais bioativos consiste em utilizar compostos que desempenham duas funções: (1) produção de nanoprecursores de fosfato de cálcio amorfo

que são pequenos o suficiente e estão estabilizados para que penetrem na matriz colágena desmineralizada antes de serem transformados em nanocristais de apatita, (2) simulação dos sítios de ligação dos cristais ao colágeno, guiando a ancoragem dos nanoprecursores à matriz colágena, tanto ao longo das microfibrilas (remineralização intrafibrilar), quanto na superfície das fibras colágenas (remineralização interfibrilar) (TAY E PASHLEY, 2008). Assim, o estímulo da deposição mineral ocorre também para auxiliar no processo de restauração da rede estrutural de hidroxiapatita em tecidos que foram afetados por ácidos de origem bacteriana decorrentes da doença cárie ou provenientes da dieta ou origem gástrica (BESINIS; VAN NOORT; MARTIN, 2014). Com a utilização desses análogos biomiméticos ou agentes bioativos incorporados a um material restaurador que estimule a deposição mineral, é sugerido que uma remineralização intrafibrilar da matriz colágena da dentina possa ser alcançada (ABUNA *et al*., 2016).

1.2 NANOTUBOS DE NITRETO DE BORO

A nanotecnologia vem contribuindo para o aperfeiçoamento de materiais, especialmente na resolução de falhas de escala nanométrica. Uma ampla variedade de nanocargas na forma de cápsulas (OUYANG *et al*., 2011), varetas (SADAT-SHOJAI *et al*., 2010), fibras (KIM *et al*., 2014a) e tubos (YU *et al*., 2014) vem sendo utilizada. O tamanho da escala, concomitantemente ao aumento da área de superfície dessas cargas, rende uma combinação que aumenta as propriedades mecânicas por meio do reforço da matriz polimérica (LIN *et al*., 2010). Além disso, as nanopartículas podem ser utilizadas como

carreadoras de fármacos (FEITOSA *et al.*, 2014; GENARI *et al.*, 2016), apresentando vantagens como melhorar a estabilidade da droga (OURIQUE *et al.*, 2008) e possuir liberação controlada do fármaco (AZGHANDI; FARAHANI; DEHGHANI, 2017; SCIORTINO *et al.*, 2017). Assim, diversas composições de nanopartículas já foram testadas em Odontologia com estes propósitos (OSORIO *et al.*, 2014; GARNER; BARBOUR, 2015; SHRESTHA; DIOGENES; KISHEN, 2015) e, recentemente, a literatura odontológica vem abordando a utilização de estruturas nanométricas tubulares como forma de incrementar as propriedades físico-químicas e biológicas dos sistemas adesivos (CHEN *et al.*, 2012; ALKATHEERI *et al.*, 2014; DEGRAZIA *et al.*, 2016).

Nos últimos anos, tem surgido um aumento das evidências de que o elemento químico Boro, classificado como um semi-metal de número atômico 5, é um componente importante para seres humanos (BENDERDOUR *et al.*, 1998; HUNT, 2012). Fármacos vêm sendo elaborados utilizando Boro como agente terapêutico com atividades anticâncer, antiviral, antibacteriana e antifúngica (BAKER *et al.*, 2009; CIANI; RISTORI, 2012). Há estudos também que relatam o aumento da resistência e da composição mineral de ossos, demonstrando ações bioativas do Boro em relação ao crescimento e manutenção óssea (HAKKI *et al.*, 2013). Recentemente, nanotubos de nitreto de boro vêm sendo explorados na área biomédica. Os nanotubos de nitreto de boro (BNNT) possuem uma estrutura similar a nanotubos de carbono, no entanto eles têm atraído um interesse ainda maior da comunidade científica devido as suas melhores propriedades mecânicas (SURYAVANSHI *et al.*, 2004) e estabilidade química (CHEN *et al.*, 2004) e térmica (TERRONES *et al.*, 2007). Uma adequada biocompatibilidade pelos BNNTs foi relatada,

comprovando uma boa interação com moléculas orgânicas como proteínas (ZHI *et al.*, 2005) e osteoblastos (LAHIRI *et al.*, 2011a). Essas características tornam os BNNTs nanovetores promissores de diversas aplicações biomédicas (CIOFANI *et al.*, 2010; WENG *et al.*, 2014), incluindo a deposição mineral (LAHIRI *et al.*, 2011b) e estímulo da diferenciação osteogênica de células mesenquimais (LI *et al.*, 2017). Não obstante, uma modificação da ligação covalente na sua superfície poderia abrir interessantes perspectivas quanto à liberação de drogas, marcação celular e, de modo geral, em todos os campos da nanomedicina (ZHI *et al.*, 2011).

Recentemente, foi demonstrado que zonas do elemento Boro (B) podem ser ativadas por oxidação com peróxido de hidrogênio sob altas temperaturas (ZHI *et al.*, 2009). Além disso, conseguiu-se, após tratamento com ácido nítrico concentrado, sua funcionalização com agentes silano, promovendo sua compatibilidade com outros polímeros para futura aplicação na matriz polimérica de compósitos (CIOFANI *et al.*, 2012). A ligação entre o Boro e Nitrogênio (B-N) é altamente estável, pois, apesar de covalente, possui um caráter iônico devido às diferenças de eletronegatividade entre os dois elementos (COHEN; ZETTL, 2010). Por esse motivo, os BNNTs possuem alta estabilidade térmica (CHEN *et al.*, 2004), um módulo de elasticidade (Young) de aproximadamente 1 TPa (CHOPRA; ZETTL, 1998), resistência à tração de 61 GPa (ARENAL *et al.*, 2011) e, por sua natureza fibrilar, de aspecto emaranhado, são hidrófobos, podendo atingir ângulos de contato de até 170° (BOINOVICH *et al.*, 2012), servindo assim como base de produtos autolimpantes (LEE *et al.*, 2009; PAKDEL *et al.*, 2011). A diminuição da energia livre de superfície promovida por esse material, segundo AHN *et al.* (2010)

poderia inibir a deposição bacteriana pela diminuição da colonização dos micro-organismos.

A produção de materiais que promovam bioatividade, diminuam a retenção do biofilme e melhorem as propriedades físico-químicas de resinas adesivas é uma demanda atual da Odontologia. Assim, apesar do incremento no estudo das estruturas nanotubulares em Odontologia e dos nanotubos de nitreto de boro na engenharia e química, até o momento, há escassez na utilização dos nanotubos de nitreto de boro em sistemas adesivos odontológicos.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar as propriedades de uma resina adesiva com a incorporação de nanotubos de Nitreto de Boro.

2.2 Objetivos específicos

- Analisar os nanotubos de Nitreto de Boro;

- Avaliar o comportamento mecânico, químico e biológico de uma resina adesiva experimental com a incorporação de nanotubos de Nitreto de Boro.

3 MANUSCRITOS

A presente tese é composta por dois manuscritos. O manuscrito I foi submetido para o periódico *Journal of Dentistry* e encontra-se publicado (doi: http://dx.doi.org/10.1016/j.jdent.2017.05.013) (DEGRAZIA *et al*., 2017). O manuscrito II foi submetido para o periódico *Dental Materials* e encontra-se publicado (doi: http://dx.doi.org/10.1016/j.dental.2017.11.024).

Os manuscritos foram formatados de acordo com as normas dos periódicos aos quais foram submetidos e encontram-se a seguir.

3.1 MANUSCRITO I

Boron nitride nanotubes as novel fillers for improving the properties of dental adhesives

Boron nitride nanotubes as novel fillers for dental adhesives

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Keywords: resin cement; dental cement; remineralization

Boron nitride nanotubes as novel fillers for improving the properties of dental adhesives

Objective. This study aimed to evaluate the physical-chemical properties of experimental dental adhesives containing boron nitride nanotubes (BNNTs) as inorganic fillers.

Methods. An experimental adhesive resin was prepared using HEMA-BisGMA, 66/33 wt% (control). Inorganic BNNT fillers were first analyzed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) and then incorporated into the adhesive at different concentration (0.05, 0.075, 0.1, 0.15 wt%). Degree of conversion (DC), ultimate strength, contact angle, surface free energy (SFE) microhardness, softening in solvent and bioactivity were assessed.

Results. Scanning and transmission electron microscopy (SEM and TEM) showed BNNTs with diameter ranging from 5 to 10 nm with close end tips. No changes in DC were observed after incorporating BNNTs up to 0.15 wt%. The contact angles of water and α-bromonaphthalene increased ($p < .05$) and consequently the SFE decreased after incorporating BNNTs to the polymer matrix. Microhardness and solvent degradation strength increased after incorporation of 0.075, 0.1 and 0.15 wt% BNNTs. Mineral deposition was found after 7 days of immersion on adhesive specimens after incorporation of BNNT.

Conclusions. The incorporation of BNNTs up to 0.15 wt% improved the chemical and mechanical properties of dental adhesives and promoted mineral deposition.

Significance: Incorporation of boron nitride nanotubes into adhesive resin materials improved physical-chemical properties and increased mineral deposition on its surface allowing enhanced properties of the resin-dentin interface. Thus, the novel adhesive material is promising as a dental adhesive and may contribute to the stability of the dentin-resin bonding.

Key-words: resin cement; dental cement; remineralization

1. INTRODUCTION

Degradation of the bonded interface is a long-standing concern in the dental adhesive field because it influences bonding effectiveness and consequently the long-term success rate of restorations[1]. After acid-etching and primer/adhesive procedures, exposed collagen fibrils may remain incompletely infiltrated, and therefore prone to degradation[2,3]. For this reason, several attempts to regenerate dentinal tissue have been recently reported[4,5 and 6]. The ability of bioactive ion-releasing adhesives promoting mineral deposition is a suitable strategy to backfill the mineral-depleted dentin collagen and improve mechanical strength within the hybrid layer[7,8].

Notwithstanding, hydrolytic resin degradation within the hybrid layer due to a breaking process of covalent bonds between polymers is considered one of the main reasons for adhesives' mass loss and reduction in bond strengths to dentin[9,10]. To overcome these issues, the development of dental adhesives with improved physical and mechanical properties as a function of time has been proposed[11]. Hydrolytic stability[12,13], enzymatic degradation resistance[14], and the ability to interact with biological structures[15,16] are the main required characteristics for bonding materials. The increase of mechanical and chemical strength of dental adhesives is directly involved in the clinical longevity of the hybrid layer and related to the presence of inorganic fillers that reduce the proportion of organic matrix[17,18].

Nanotechnology has contributed to the improvement of adhesive fillers, especially the resolving of nanoscale failures. A wide variety of nanofillers have been used in the form of capsules, rods, tubes, and fibers. Notably, the size of the scale concomitant to surface area increase of these fillers renders a unique combination of mechanical properties by reinforcing the polymeric matrix[19]. Among these nanofillers, boron nitride nanotubes (BNNT) have drawn attention due to their structures being similar to

carbon and graphene nanotubes, and hence to their superior mechanical properties[20] and chemical[21] and thermal stability[22]. The covalent bond between boron and nitrogen (B-N) is highly stable; therefore, BNNTs achieve high modulus of elasticity reaching up to 1.2 TPa[23]. BNNTs' fibrillar nature and tangled aspect make them superhydrophobic, reaching contact angles (CAs) with water up to 170°[24], thus serving as the basis of self-cleaning products[25]. A suitable biocompatibility of BNNTs was reported, demonstrating a good interaction with organic molecules such as proteins[26] and osteoblasts[27]. These characteristics make them promising nanovectors in biomedical applications[28]. Although most of the studies carried out have explored BNNTs as reinforcing agents to improve their mechanical properties, some of them have demonstrated bioactive properties[27,29].

BNNTs have been also proposed as fillers for polymeric grafts and scaffolds[30,31]. The addition of BNNTs into dental adhesives could improve the mechanical properties of the material and stimulate mineral deposition. Thus, the purpose of this study was to evaluate the influence of BNNTs' incorporation in experimental adhesive resin. The null hypothesis is that the addition of BNNTs will not influence the properties of adhesive resin.

2. MATERIAL AND METHODS

2.1. Scanning and Transmission Electron Microscopy

BNNTs (BNNT, LCC, Newport News, VA, USA) synthesized via a pressurized vapor/condenser method[32] were examined using scanning electron microscopy (EVO MA10, Zeiss, Oberkochen, Germany) with a voltage of 0.2 to 30 kV and maximum resolution of 3.0 nm, and transmission electron microscopy (JEM2010, Jeol, Tokyo,

Japan) operating in a voltage of 200 kV with a point resolution of 0.25 nm and line resolution of 0.14 nm (Figure 1).

2.2. Formulation of Experimental Adhesive Resin

The polymeric matrix was composed by bisphenol A-glycol dimethacrylate (BisGMA) and hydroxyethyl methacrylate (HEMA) purchased from Sigma-Aldrich (St. Louis, MO, USA) and mixed in mass ratio of 66.6 and 33.3 wt.%, respectively. The photo-initiators camphorquinone (CQ), ethyl 4-dimethylamino benzoate (EDAB) and diphenyliodonium hexafluorophosphate (DPIHFP) (Aldrich Chemical, Milwaukee, MI, USA) were added to 1 mol%, as previously reported[6].

BNNT were incorporated into the polymeric matrix at concentrations of 0.05, 0.075, 0.1 and 0.15 wt%. A control group was maintained without nanotubes. All components were weighed in an analytical balance (AUW220D, Shimadzu Corp., Kyoto, Japan), mixed, and ultrasonicated for 2 h. For all evaluations performing photo-activation, a light-emission diode curing unit (Radii Cal, SDI, Bayswater, VIC, Australia) with an irradiation value of 1.200 mW/cm^2, determined with a digital radiometer (RD-7, Ecel Ind., Ribeirão Preto, Brazil) was used.

2.3. Degree of Conversion

The specimens of each group (n = 3), were analyzed by micro-Raman spectroscopy using Senterra equipment (Bruker Optik GmbH, Ettlingen, Baden-Württemberg, Germany) with 3 coadditions for 5 s of irradiation using a 100 mW diode laser with 785-nm wavelength and 50 x 1000 μm aperture size. Spectra were obtained with a spectral resolution of ~3.5 cm^{-1} at 440 to 1800 cm^{-1} range, the same parameters used in mineral deposition evaluation. Spectra were collected in the adhesive at three individual points both previous and after polymerization for 20 s. The average value of

the measurements was used for calculation of the ratio of double bond content of monomer to polymer in the adhesive. The aliphatic and aromatic peaks were 1635 cm^{-1} and 1608 cm^{-1}.

2.4. Microhardness and Softening in Solvent

Cylindrical resin specimen with standardized dimensions (thickness: 2 mm; diameter: 5 mm) were created using a polyvinylsiloxane mold. In brief, each tested adhesive was inserted into a mold and the outer surfaces were covered with a polyester strip and pressed with a glass slide to squeeze out any excess of adhesive before the light-curing procedures. The specimens were light cured for 20 s on both sides and polished using #600, #1200, and #2000 grits SiC abrasive papers under water irrigation (30 s each).

Five specimens were used for each experimental adhesive. The specimens were stored for 24 h and submitted to initial Knoop microhardness (KHN1) assessment, as previously described[33]. Then, specimens were immersed in a solution of 50% ethanol and 50% water mixture for 2 h at 37°C and were again submitted to microhardness (KHN2) assessment. KHN measurements were taken using an indenter HMV-2 (Shimadzu Corp., Kyoto, Japan) under a load of 10 g for 5 s. Three indentations were performed on the upper face of each specimen. The Knoop microhardness values were recorded as the average of the three indentations per specimen. The KHN1 and the KHN2 values were processed as KHN% by calculation of the Δ between both.

2.5. Ultimate Tensile Strength

Eleven hourglass-shaped samples of each group measuring 8 mm long, 2 mm wide, 1 mm thick, and cross-sectional area of 1 mm^2 were subjected to ultimate tensile strength test. The adhesive resins were inserted into a metallic matrix and a polyester

strip was placed on them before light curing at the bottom and at the top of the specimens. The samples were stored for 24 h at 37°C and submitted to strength test using a mechanical testing machine (Shimadzu EZ-SX, Shimadzu Corp., Kyoto, Japan) at a crosshead speed of 1 mm/min until rupture of the specimen.

2.6. Contact Angle and Surface Free Energy Measurement

Five specimens were created with same manufacturing process and dimensions, as described in section 2.7. These were embedded in acrylic resin and analyzed using an optical tensiometer Theta (Biolin Scientific, Stockholm, Sweden) to measure the static CA (θ) to distilled water and to α-bromonaphthalene droplets and the surface free energy. The drop out size, drop rate, displacement rate, and speed dispersion of water or α-bromonaphthalene were: 3.0 µL, 2.0 µL/s, 20.0 µL/s, and 50 mm/min, respectively. The test period was performed over 20 s and the mean contact angle (θ) between the droplet and the solid surface was registered after 10 s. The surface free energy calculation (mN/m) was performed following the OWRK/Fowkes* equation with OneAttension software (Biolin Scientific, Stockholm, Sweden) as previously described[34].

$$*\gamma_{ls} = \sigma_l + \sigma_s - 2(\sqrt{\sigma_l^D . \sigma_s^D} + \sqrt{\sigma_l^P . \sigma_s^P})$$

2.7. Mineral Deposition

Three specimens for each experimental group tested in this study were generated using cylindrical silicon molds (3.0 mm diameter and 2.0 mm height). The same specimens used to assess degree of conversion were used to evaluate mineral deposition, as previously described[6]. Once each adhesive resin was inserted into the molds, a polyester strip was placed on the top and then light cured for 20 s on both sides

of the specimen. Specimens were then polished using a #1200 grit silicon-carbide paper under constant irrigation and immediately immersed in 15 mL of simulated body fluid (SBF) solution prepared according to Kokubo et al.(2006)[35] for 14 days at 37°C.

Raman spectroscopy (Senterra, Bruker Optics, Ettlingen, Germany) was employed to analyze the mineral phase precipitated on the specimens after different periods of storage in SBF. Three standard areas (100 x 100 μm) were analyzed (200 equidistant points) for each specimen. The integral of the 962 cm^{-1} peak absorbance (symmetric stretching υ1 mode of phosphate) was calculated using the spectroscopy software (Opus 7.5, Bruker Optics, Ettlingen, Germany). Absorbance increase of the peaks at 962 cm^{-1} indicated the deposition of calcium phosphate on the sample surfaces, and the results were processed to obtain digitalized images of the phosphate deposition using Sigma Plot version 12.0 for Windows (Systat Software Inc, San Jose, CA, USA).

2.8. Statistical analysis

Statistical analysis was done on Sigma Plot. The normality of data was evaluated using the Kolmogorov-Smirnov test ($p > 0.05$ for all tests). Homogeneity of variance was calculated using the Levene´s test. For all tests the variances were homoscedastic ($p > 0.05$) except for Degree of Conversion ($p < 0.05$). The statistical analyses were performed using ANOVA on ranks for Degree of Conversion, one-way ANOVA and Tukey's post hoc at the 0.05 level of significance for KHN1, KHN2, softening in solvent, ultimate tensile strength, contact angles for water and α-bromo, and surface free energy. Paired Student's t-tests were performed between KHN1 and KHN¬2. Sample size for each assay was based on previous studies[5,6,36 and 37].

3. RESULTS

SEM and TEM images showed BNNTs' widths ranging from 5 to 10 nm, hollow structures and closed end tips (Fig. 1). The results for the degree of conversion, microhardness, and softening in solvent are shown in Table 1. No changes in degree of conversion were observed after 0.15 wt% incorporation of BNNT. Initial microhardness values increased ($p < 0.05$) after incorporation of BNNT from 19.64 ± 1.57 (0 wt%) to 24.62 ± 1.44 (0.075 wt%), 25.84 ± 1.10 (0.1 wt%) and 28.20 ± 1.32 (0.15 wt%). The incorporation of BNNT up to 0.15 wt% decreased softening in solvent from 66.90 ± 4.86 % (0 wt%) to 28.27 ± 4.76 % (0.15 wt%).

The results of ultimate strength, contact angle, and surface free energy are shown in Table 2. The mean ultimate strength values varied from 52.28 ± 15.14 MPa (0 wt%) to 65.47 ± 13.00 MPa (0.15 wt%), however no statistical difference was shown ($p > 0.05$). The contact angle of water and α-bromo increased significantly ($p < 0.05$) after incorporation of 0.075, 0.1 and 0.15 wt%, consequently the surface free energy decreased significantly ($p < 0.05$) from 50.63 ± 2.87 mN/m (0 wt%) to 41.55 ± 1.41 mN/m (0.15 wt%) of BNNT. Mineral deposition was found on specimens with incorporation of BNNT after 7 days of immersion into SBF solution. An increase of mineral amount was shown for groups 0.075 and 0.1 wt% after 14 days of immersion (Fig. 2). SEM images (Fig. 3) showed precipitation of particles with the typical morphology of hydroxyapatite after 14 days of immersion in SBF solution.

4. DISCUSSION

Nanofillers have been widely studied in dental adhesives because they present some important issues as degradation decrease[5], bioactivity[5,16], and improvement of chemical and mechanical properties[38]. In this study, BNNTs were successfully incorporated as fillers into a methacrylate-based adhesive resin and as far as we know, this is the first report of boron nitride particles being incorporated into an adhesive

resin. As shown in the literature, BNNTs' sidewalls favorably interact with compounds presenting aromatic groups as Bis-GMA via π-π stacking or hydrophobic interactions[39]. The addition of these nanotubes increased solvent resistance and contact angles of water and bromonaphthalene. Bioactivity was demonstrated by mineral deposition observed on specimens with BNNTs after 7 days of immersion in SBF. Thus, the incorporation of BNNTs has influenced the properties of the methacrylate-based adhesive and the null hypothesis of this study must be rejected.

As a proof-of-concept strategy, biomimetic mineralization replaces water from voids and bare areas of the hybrid layer with apatite crystallites in the extra- and intrafibrillar compartments of the collagen matrix[40]. Remineralization of demineralized dentin has important consequences for the control of dentinal caries as well as improvement of dentin bonding stability[16,38 and 41]. Besides that, mineral formation on the resin-dentin interface could act as a shield protecting from acids produced by oral bacteria. A previous investigation conducted by Lahiri et al.(2011)[29] has shown the ability of BNNT soaked for 7 days in SBF to form both amorphous and crystal hydroxyapatite precipitation. Amorphous apatite formation could be related to crystallinity difference between hydroxyapatite and BNNT and kinetics of nucleation. One of the mechanisms of homogenous nucleation that occurs in nature (no seed crystallites formation) involves sequestration of the amorphous mineral phase by polyanionic extracellular matrix protein molecules[4]. Our study showed the induction of mineral precipitation on the surface of an adhesive incorporated with BNNT. This possible bottom-up biomineralization may mineralize apatite-depleted collagen fibrils, thereby being a useful mechanism in extending the longevity of resin–dentin bonds via restoring the dynamic mechanical properties of the denuded collagen within the hybrid layer[42].

Monomers themselves or their degradation products have been demonstrated to induce cytotoxic and mutagenic, effects[43,44]. Thus, effective polymerization is thought to be of paramount importance to avoid monomer leaching[45] and to keep the dentin-restoration interface sealed against the entrance of bond-degradation substances. Hexagonal boron nitride has already been used due to its wide bandgap energy (ranging from 3.6 to 7.1 eV) to produce a photocatalytic effect. A previous report[46] of boron nitride showed single peak of ultraviolet luminescence at 5.765 eV (215.0 nm), and its intrinsic fundamental absorption spectra also had a structure in a range from 5.822 to 5.968 eV that could be readily assigned as an s-like direct exciton structure, and it explained the interaction between the N-group of BNNT and the amine group of EDAB donating H* radicals presented on the BNNT surface to the reaction. This findings classified crystal samples of boron nitride as direct-gap semiconductors. Degree of conversion was measured in this study using micro-Raman spectroscopy instead of the more common ATR-FTIR. Although the degree of conversion was not significantly increased, higher mean values of degree of conversion with increasing BNNT concentration were found.

BNNTs have been cited in efforts to find interesting uses in polymeric composites[47] and potentially to surpass carbon nanotubes due to their superior thermal and chemical stabilities[21,48], rigidity[49], straight shapes and elasticity[50]. Although a previous study[51] has demonstrated an increase between 30 - 50% of |E| modulus of polymers reinforced by only 1 - 3 wt% addition of BNNT, no significant increase was found comparing ultimate strength values of the different tested groups. Despite this, higher values of ultimate strength with increasing weight fractions of BNNT were found. This may be due to a high Young modulus of BNNT previously measured by Chopra and Zettl (1998)[52]. The importance of the |E| modulus in

dentistry was evidenced in a study of Benetti et al. (2014)[53] in which resin materials with high |E| modulus are related with lower amounts of marginal gaps. Furthermore, the addition of BNNT has improved mechanical and chemical performances of adhesives based on the results of microhardness and solvent degradation. As known, solvents may plasticize the polymer network producing a relaxation process that leads to a separation of polymer chains by reduction of the frictional forces[1]. It is worth mentioning that organic solvents have the ability to penetrate the polymer network, promoting swelling and thus allowing the release of unreacted and leachable monomers[54]. The results of this study might be explained by the BNNTs' chemically stable nature due to the partially ionic character between B and N bonding and even more due to the small interlayer distance of ~0.333 nm[32]. The ionic character of B-N bonds may induce a polarized surface suitable for interaction with polar polymers as Bis-GMA. Besides that, the nanoscale surface roughness may enhance mechanical interlocking with polymer chains[55].

In the same pathway, the incorporation of BNNT into adhesives has led to an increase of water and bromo contact angles. This may be due the superhydrophobic feature of BNNTs[25]. This hydrophobic behavior may prevent biofilm formation on the material surface while acting as a protective barrier against two issues: (1) water uptake and ability to undergo hydrolysis, and (2) bacterial degradation of adhesive resin compounds[56]. Although surface free energy decreased after incorporation of BNNTs, a previous study[57] has shown no influence of surface wettability in a long-term evaluation of adhesive bond strength.

BNNTs incorporation into polymers has been recently studied and is of great interest in the development of chemically and physically reinforced polymeric materials. Based on our results, the incorporation of BNNTs in order to fulfil dental

restorative needs is in direction of the key targets of on-demand novel nanomaterials with improved physicochemical properties. Also, one of their key features that have attracted much effort is the ability of their nanotube surfaces to be chemically functionalized, which opens pathways to future researches. Thus, dentinal adhesives reinforced by BNNTs may be useful for prolonging longevity of dental restorations.

5. CONCLUSION

The incorporation of BNNTs up to 0.15 wt% improved the chemical and mechanical properties of dental adhesives and promoted mineral deposition, providing considerable benefits over the available materials.

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Table 1. Mean values ± SD of degree of conversion (DC, %), microhardness and softening in solvent (Δ%) after 2h immersion in a mixture of water/ethanol.

Groups	Degree of Conversion	Microhardness Initial	Microhardness Final	Δ%
0%	87.97 ± 1.65^A	$19.64 \pm 1.57^{C,a}$	$6.46 \pm 0.83^{C,b}$	66.90 ± 4.86^C
0.05%	88.46 ± 3.73^A	$20.98 \pm 1.08^{C,a}$	$11.70 \pm 1.28^{B,b}$	44.09 ± 7.05^B
0.075%	89.54 ± 2.66^A	$24.62 \pm 1.44^{B,a}$	$11.62 \pm 1.58^{B,b}$	52.73 ± 6.63^B
0.1%	91.74 ± 0.43^A	$25.84 \pm 1.10^{B,a}$	$12.28 \pm 1.59^{B,b}$	52.58 ± 4.80^B
0.15%	89.54 ± 1.29^A	$28.20 \pm 1.32^{A,a}$	$20.26 \pm 2.01^{A,b}$	28.27 ± 4.76^A

Different upper case means significant difference in the same column ($p < 0.05$).
Different lower case means significant difference in the same row ($p < 0.05$).

Table 2. Mean values ± SD of ultimate strength (MPa), contact angle (θ) and surface free energy (mN/m) of adhesive resin after BNNT incorporation.

Groups	Ultimate Strength	Contact Angle Water	Contact Angle α-bromo	Surface Free Energy
0%	52.28 ± 15.14^A	63.64 ± 4.27^B	28.07 ± 6.71^{AB}	50.63 ± 2.87^A
0.05%	62.46 ± 13.13^A	70.32 ± 2.75^{AB}	26.40 ± 6.73^B	47.26 ± 1.93^A
0.075%	58.38 ± 13.21^A	72.58 ± 3.81^A	37.76 ± 7.62^A	42.53 ± 3.39^B

0.1%	61.29±16.25^A	70.33±4.92^{AB}	38.48±3.94^A	42.87±1.71^B
0.15%	65.47±13.00^A	77.23±4.02^A	36.12±0.90^{AB}	41.55±1.41^B

Different letters mean significant difference in the same column ($p < 0.05$).
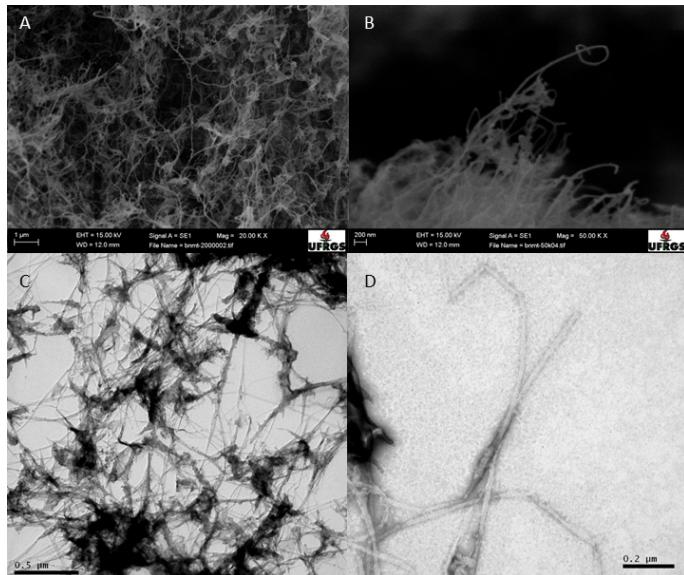
Fig. 1 – SEM and TEM images (A and C) showing BNNTs as a cobweb-like form. Images B and D show in higher magnification the folding structure of BNNT with closed end tips.
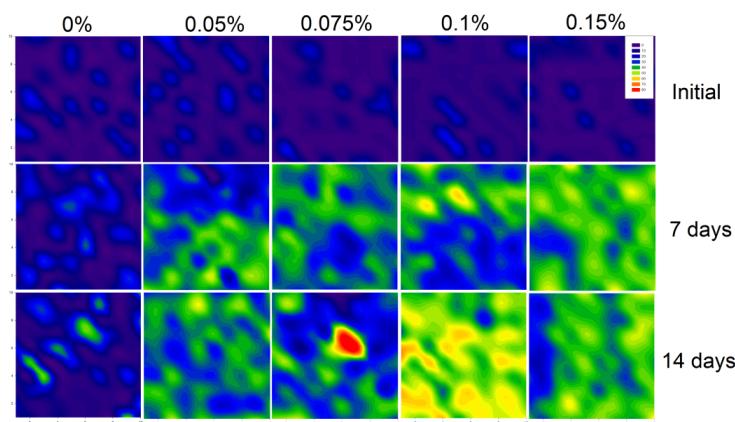
Fig. 2 – Images obtained after calculation the integral of the 962 cm^{-1} Raman peak absorbance. Mineral deposition observed after 7 and 14 days for groups with BNNT incorporation. Increase of mineral deposition was found on the surface of groups with higher amount of BNNT concentration.
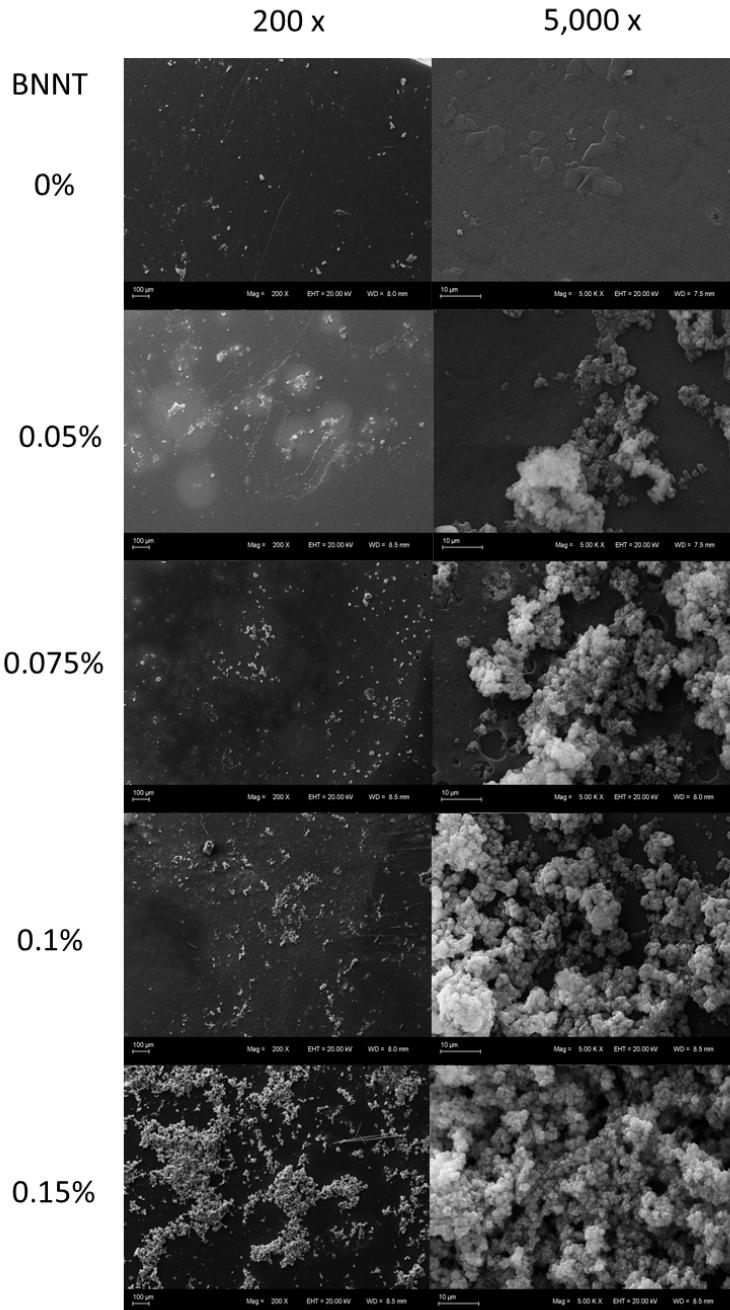
Fig. 3 – SEM images after immersion into SBF medium during 14 days. Mineral deposition of hydroxyapatite aggregates is shown on the surface of each specimen after incorporation of BNNT.

3.2 MANUCRITO II

Long-term stability of dental adhesive incorporated by boron nitride nanotubes

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Long-term stability of dental adhesive incorporated by boron nitride nanotubes

ABSTRACT

Objective. The aim of this study was to evaluate physicochemical properties, long-term microtensile bond strength and cytotoxicity of methacrylate-based adhesive containing boron nitride nanotubes (BNNTs) as fillers.

Methods. A dental adhesive was formulated using BisGMA/HEMA, 66/33 wt% (control). Inorganic BNNT fillers were incorporated into the adhesive at different concentrations (0.05, 0.075, 0.1 and 0.15 wt%). Degree of conversion (DC), polymerization rate [$Rp.(s^{-1})$], contact angle (CA) on dentin, after 24 h and 6 months microtensile bond strength (µTBS- 24h and 6 mo) and cytotoxicity sulforhodamine B (SRB) of fibroblast cells were assessed.

Results. DC and max. polymerization rate increased ($p < 0.05$) after incorporating 0.075 and 0.1 wt% BNNT. The contact angle on dentin increased ($p < 0.05$) after incorporating 0.15 wt% BNNT. The µTBS-24h showed no changes ($p > 0.05$) after incorporating up to 0.15 wt% BNNT comparing to control. After 6 months, µTBS decreased ($p < 0.05$) for control and 0.15% BNNT, however all groups showed higher µTBS values than control ($p < 0.05$). No change of cell growth was found ($p > 0.05$) comparing all groups to positive control group (100%).

Significance. Incorporating boron nitride nanotubes up to 0.1 wt% into dental adhesive increased the long-term stability to dentin without decreasing viability of fibroblast cell growth. Thus, the use of BNNTs as filler may decrease failure rate of current dentinal adhesives.

Keywords: dental materials; adhesion; nanotubes; biocompatibility; boron nitride.

1. Introduction

The resin–dentin bonding is a major reason for dentin demineralization apart from caries[1]. This occurs due to the presence of non-entangled exposed collagen fibrils in the partially or completely demineralized dentin, after the etching step from acids or acidic resin monomers[2]. The incomplete monomer infiltration into the collagen matrix leads to the presence of mineral-depleted, resin-sparse, water-rich fibrils along the bonded interface challenging the interfacial integrity between resin and dentin[3]. Since the discovery of the microhybrid layer, efforts have been focused on displace water within the extra- and intrafibrillar compartments of demineralized collagen matrix[4,5]. Notwithstanding, hydrophilic monomers used to promote adhesive diffusion into demineralized dentin leads to higher water uptake and thus decrease its mechanical properties over time[6,7].

A recent review of innovative applications in dentistry[8] claimed that fortifying adhesives with nanomaterials that possess biological merits and enhancing their mechanical and physical properties help to attain and maintain a durable adhesive joint with enhanced longevity. Therefore, novel functionalizing nanofillers to improve current dental adhesives have been evaluated by several studies[9,10,11 and 12]. One-dimensional nanotubes have been investigated[13,14] and offer an excellent choice to reinforce dental composites with bioactive properties. Among such, boron nitride nanotubes (BNNTs) have been recently used as nanofillers of a dental adhesive[15] showing bioactive and improved physicochemical properties. The mechanical properties of their nanocomposites is enhanced by various functional groups such as amines and nitriles[16,17] due to improvement in their dispersion in polymer matrices that facilitate better polymer–nanomaterial interaction and allow efficient load transfer from the polymer to the nanomaterial[18]. Besides, their hydrophobic[15] character could act as

a shield against water remained on demineralized collagen matrix avoiding degradation of dentin fibrils and hydrolytic softening of the polymer matrix thus improving the longevity of the adhesive bonding to dentin.

Therefore, the purpose of this paper was to evaluate the long-term stability on dentin of a methacrylate-based adhesive incorporated by BNNTs and their cytotoxicity to dental fibroblast cells. The null hypothesis is that the incorporation of BNNT into a dental adhesive will not influence its chemical and mechanical properties, and cytotoxicity among groups.

2. Material and methods

2.1 Adhesive formulation and incorporation of BNNT

The experimental adhesives were formulated by mixing 66.6 wt% bisphenol A glycerolate dimethacrylate (BisGMA) and 33.3 wt% of 2-hydroxyethyl methacrylate (HEMA) from Sigma-Aldrich (St. Louis, MO, USA) as described in a previous study[12]. BNNTs (BNNT, LCC, Newport News, VA, USA) synthesized via a pressurized vapor/condenser method[19] were first immersed in a 65% HNO_3 bath for 3 h and into H_2O_2 for 48 h[20]. BNNTs were then used in four concentrations (0.05, 0.075, 0.1 and 0.15 wt%) and firstly incorporated into BisGMA, dispersed with ultrasonification for 1 h, and then HEMA was mixed. A control group without BNNT was used. As a photoinitiator system, camphorquinone and ethyl 4-dimethylaminobenzoate at 1 mol% each were added to all groups.

2.2 Polymerization kinetic

The degree of conversion (DC) and polymerization rate [Rp.(s^{-1})] of each experimental adhesives were evaluated using Fourier transform infrared (FTIR) spectrometer Vertex

70 (Bruker Optics, Ettlingen, Germany) equipped with Attenuated Total Reflectance (ATR) device. A support was coupled to fix the light source and standardize the distance between the tip and the top of the 2 mm-height sample. Analysis was performed at a controlled room temperature of 23±1 °C and 60±1 % relative humidity during sample (3 μL) polymerization, which was directly dispensed onto the diamond crystal and light activated for 50 s (n=3) by a light-emitting diode (RadiiCal, SDI, Bayswater, VIC, Australia) .

DC and Rp.(s^{-1}) were calculated for the experimental adhesives as described in a previous study[21] based on the intensity of the C=C stretching vibrations (peak height) as a function of time at 1635 cm^{-1} and using the symmetric ring stretching at 1608 cm^{-1} from the polymerized and non-polymerized samples as an internal standard. The IR-Solution software was used to set the standard parameters and to check the scanning in Happ-Genzel apodization function mode in a range of 4000–800 cm^{-1}, resolution of 4 cm^{-1}, and scanning time of 50 s. This setup allowed the acquisition of each single scan, every 0.49 s during adhesive photo-activation. The data was plotted in SigmaPlot 12.0 (Systat Software Inc, San Jose, CA, USA) and a sigmoidal curve fitting method was applied using linear regression. DC (%) × time (s) and [Rp.(s^{-1})] x time (s) were plotted for each amount of BNNT .

2.3 Contact angle

Twenty-five bovine teeth were embedded in acrylic resin and their superficial dentin was exposed and ground flat (600-grit silicon-carbide paper) under running water for 1 min. A dentin area of 5 mm diameter was etched with 37% phosphoric acid for 15 s. Analysis was performed using an optical tensiometer (Theta, Biolin Scientific, Stockholm, Sweden) to measure the static CA (θ) among dentin and adhesive. The drop

out size, drop rate, displacement rate, and speed dispersion of adhesive were: 3.0 μL, 2.0 μL/s, 20.0 μL/s, and 50 mm/min, respectively. The test period was performed over 20 s and the mean contact angle (θ) between the droplet and the solid surface was registered after 10 s.

2.4 Microtensile bond strength (μTBS) and Failure Pattern analysis

One hundred and twenty bovine permanent incisors (n=12) were horizontally sectioned in order to expose a 5 mm² area of superficial dentin. A 600-grit silicon-carbide paper was used under running water for 30 s. The dentin was etched with 37 % phosphoric acid for 15 s, rinsed with distilled water and dried for 30 s. A commercial primer (Primer Scotch Bond MultiPurpose; 3M ESPE) was actively applied for 20 s, and the solvent evaporated for 5 s. The experimental adhesives were applied and light cured for 20 s using previously described light-emitting diode. A composite build-up was performed (Z350; 3M ESPE) by two increments of 2 mm and light cured for 20 s each.

The samples were stored in distilled water at 37º for 24 h and sectioned into beams of 0.5 mm² of cross-sectional area. Half of the sample from each group (n=60) have their beams evaluated for 24 h, and all beams from the other half were stored for 6 mo before evaluating. The beams were fixed with cyanoacrylate adhesive in a metallic device and submitted to universal testing machine (EZ-SX series, Shimadzu Corp., Kyoto, Japan) at a crosshead speed of 1 mm/min. The fracture pattern was evaluated with a stereomicroscope (HMV-2, Shimadzu Corp., Kyoto, Japan) at x40 magnification and classified as adhesive, mixed or cohesive in dentin or composite.

2.5 Cytotoxicity Sulforhodamine B (SRB) colorimetric assay

Fibroblasts were derived from two intact human third molars with incomplete root formation (having extraction indication) of one patient without systematic health problems. Patients were asked to donate tooth for the study and after agreement they signed the Informed Consent (IC). After the extractions, the teeth were immersed in 1,0 mL of Dulbecco′s Modified Eagle Medium (DMEM), supplemented with Hepes, fetal bovine serum 10 % and 100 U/mL penicillin, 100 µg/mL streptomycin (Thermo Fischer Scientific, Waltham, MA, USA), and then incubated at 37 °C humidified atmosphere with 5 % CO_2. Culture medium was changed 24 h after the initial plating and from this moment on, every 2 days. After expansion to reach sufficient numbers of cells, experiments were performed with cells in fifth passage.

Fibroblasts obtained from human dental pulp were used for the cytotoxicity assay. Eluate was prepared immersing the specimen in 1 mL of medium during 72 h. Cells were seeded in triplicate at a concentration of 5 x 10^3 in 96 well plates, and then treated with 100 µL of eluate. After 72 h cells were fixed with a concentration of 10 % trichloroacetic acid (TCA), they were incubated at 4°C for 1 h and subsequently were washed 6 times under running water and dried at room temperature. Sulforhodamine B (SRB, Sigma-Aldrich, St. Louis, USA) at 4 % was added to stain the cells and the plate was incubated for 30 min at room temperature. Posteriorly, the plate was washed 4 times with 1 % acetic acid to remove the unbound excess dye and allowed to dry completely at room temperature. Trizma solution was added and the plate incubated for 1 h to allow complete solubilization of the dye. At the end of this process the microplates were read at 560 nm. Cell viability was normalized against initial control free of drugs and expressed in percentage.

2.6 Statistical Analysis

Statistical analysis was done on Sigma Plot. The normality of data was evaluated using the Kolmogorov-Smirnov test ($p \geq 0.05$ for all tests). All data were homoscedastic calculated by Levene´s test ($p \geq 0.05$). The statistical analyses were performed using one-way ANOVA and Tukey´s post hoc at the 0.05 level of significance for degree of conversion, maximum polymerization rate, contact angle and cytotoxicity. Two-way ANOVA was performed for microtensile bond strength.

3. Results

The results of degree of conversion, maximum polymerization rate, microtensile bond strength and contact angle are shown in Table 1. The 0.075 and 0.1% BNNT adhesives achieved higher degree of conversion (68.9±0.6 and 70.2±0.8, respectively) and maximum polymerization rates (14.5±2.4 and 14.1±1.8, respectively) as compared to 0% BNNT ($p < 0.05$). The incorporation of 0.15% BNNT has shown increased contact angle compared to control group. No significant differences ($p > 0.05$) were shown among contact angles after incorporating up to 0.1% BNNT and 0% BNNT. The immediate microtensile bond strength of adhesives incorporated up to 0.15% BNNT has showed no alterations to control group. After 6 months, adhesives with 0 and 0.15% BNNT have showed significant decrease ($p < 0.05$), while adhesives with 0.05, 0.075 and 0.1% BNNT have showed no difference compared to 24 h ($p > 0.05$). After 6 months, BNNTs' groups incorporated up to 0.15% presented higher values of microtensile bond strength compared to 0% BNNT. The summary of the percentage failure modes of debonded specimens according to groups is shown in Table 1. Mixed fractures were frequently identified in all groups.

The behavior of polymerization kinetics is presented in Figure 1. Figure 1a presented a faster development of degree of conversion for adhesives with 0.075 and

0.1% BNNT achieving almost final degree of conversion within 10 s of photo-activation. Figure 1b showed maximum polymerization rate of all groups occurring within similar period of time. Adhesives with 0.075 and 0.1% have a faster decrease of polymerization rate then 0, 0.05 and 0.15% BNNT. Adhesives with 0, 0.05 and 0.075% BNNT have shown an increased cell viability of 13, 19 and 14%, respectively compared to their positive control expressed by the 100% line. No significant difference in cell viability was found ($p > 0.05$) comparing adhesives up to 0.15% BNNT (Fig. 2).

4. Discussion

Over the past years, BNNTs have been used as mechanical reinforcement, thermal insulating[22] and bioactive promoter agent[23]. Thus BNNTs have been recently evaluated showing improvements of the chemical and mechanical properties of dental adhesive. Following the long-term goal of several studies[24,25] to avoid the degradation of collagen matrix within hybrid layer, we incorporated BNNTs as agent fillers to dental methacrylate-based adhesive. Their enhanced properties associated to promotion of mineral deposition could support the theory that remineralization of demineralized dentin has important consequences for the control of dentin bonding stability. In this study, the addition of BNNTs increased degree of conversion and maximum polymerization rate, contact angle and microtensile bond strength. Moreover, since no cytotoxicity was found among groups, we have partially rejected the null hypothesis of this study.

The current challenge of adhesive dentistry is to increase the resistance of adhesive-dentin interface against aging, thus enhancing longevity of restorative treatments. In this study, aging of samples was performed in distilled water, already related as a well-validated method to perform bond strength durability[26]. Although

previous *in vitro* studies have demonstrated degradation of the hybrid layer after aging specimens for 12 months, the threat of stability bonding of specimens immersed into water has been shown with similar results by several studies at early stages of storage, as 6 months[27,28]. Notwithstanding, higher values of microtensile bond strength are always desired in adhesive dentistry. The goal of predicting performance of dental adhesives was previously confirmed[29]. In Van Meerbeek's study they did find a correlation between bond strength data gathered after *in vitro* aging appearing more clinically relevant and predicting the clinical failure rate of adhesives on the long term of 5 years to some extent. Therefore, the results of μTBS of this study are promising to increase clinical performance of current dental adhesives. The BNNT addition up to 0.1% into BisGMA/HEMA adhesive has stabilized μTBS. This outcome is far of a coincidence since two issues may have occurred: (1) higher degree of conversion and polymerization reactivity and (2) lower degradation of micro hybrid layer.

High percentages of degree of conversion lead to less unreacted monomers within the polymer matrix[7,30], and thus we may expect enhancing the physicochemical properties of adhesive. Indeed, the results of DC and maximum polymerization rate means achieved in this study have demonstrated higher photocatalytic performance of groups incorporated by BNNT up to 0.1 wt%. This is in line with measurements conducted by Watanabe et al., 2004[31] that found hexagonal-BN is a direct-gap semi-conductor with bandgap energy of 5.971 eV. This photocatalysis phenomenon may have occurred due to this wide bandgap energy given to its fundamental absorption spectra a direct exciton structure[31] that probably explains the interaction between the N-group of BNNT and the amine group of EDAB. Moreover, immersion into HNO_3 was shown to open the end tip of nanotubes and link –OH to its surface, whereas H_2O_2 links H* radicals to BNNTs' outer surface, thus forming excitons[32,33] and increasing photo

induced oxidation as previously demonstrated[34]. The presence of hydroxyl groups within B-N bonds strongly improves the hydrogen bonding ability, resulting in a significant increase of the photopolymerization reactivity[35,36], as also shown in this study.

Previous reports[37,38 and 39] have demonstrated BNNTs´ chemically stable nature due to ionic character and small interlayer distance of B-N bonds[19]. BNNTs showed strong interactions to polymers[40]. The ionic character may induce a polarized surface with hydroxyls on its backbone, turning them suitable for interactions with polar polymers as BisGMA. This high stability in addition to previous reports of lower surface free energy and softening in solvent[15,41] after incorporating BNNTs into methacrylate-based adhesive have probably led to lower water sorption of the polymer, thus decreasing degradation of hybrid layer. Despite incorporating concentrations above 0.1 wt% of BNNT have decreased μTBS after 6 months, the mean values of 0.15% BNNT were higher than 0% BNNT. This decrease may have occurred due to a lower degree of conversion and mostly, an increase of adhesive contact angle measured on dentin. Higher contact angles reduce surface free energy and thus, hinder long-term bonding of adhesive to dentin[42]. Although lower wettability was found on dentin surface, BNNT incorporation into adhesive may have avoided water-filled spots within intrafibrillar spaces of 5 nm due to higher hydrophobicity[15]. The thin width (5-10 nm) of hydrophobic BNNTs as previously shown[15] could promote the displacement of the free and loosely-bound intrafibrillar water by adhesive[43] avoiding collagen degradation.

Cytotoxicity of nanomaterials is regarded as a crucial point to nanotechnology development of health products, especially in oral environment[44]. Nonetheless, regarding polymeric materials used in restorative dentistry; inflammatory reactions may

be induced due to their degradation that lowers microenvironment pH, hampering cell survival[45]. Although BNNTs have been used as vectors for drug delivery[46] their application in biomedicine remain controversial. Recent studies[47,48] demonstrated that BNNTs could inhibit cell viability due to their morphology resembling highly toxic asbestos. However, regarding SRB assay of this study, fibroblast viability were not affected by BNNT incorporation. Much like the cases of other nanomaterials, its cytotoxicity could depend on their purity, concentration, functionalization, size and structure, as well as the investigated cell type[49]. The cytotoxicity results of this study are in line with a recent study[50] that found lower cytotoxicity of fibroblasts when monomers with at least 1.0 wt% of photoinitiators based on their total weight were used in curable resins. Moreover, an enhance growth up to 19% was achieved when 0.05 wt% BNNT were incorporated into methacrylate-based adhesive. This high cell viability confirms that BNNTs incorporated to methacrylate-based materials may have other dental applications as cements, sealants and composites.

5. Conclusions

Based on the results of this study, it is possible to conclude that boron nitride nanotubes were successfully incorporated into an adhesive resin, promoting improved physicochemical properties and long-term stability without decreasing viability of fibroblast cell growth.

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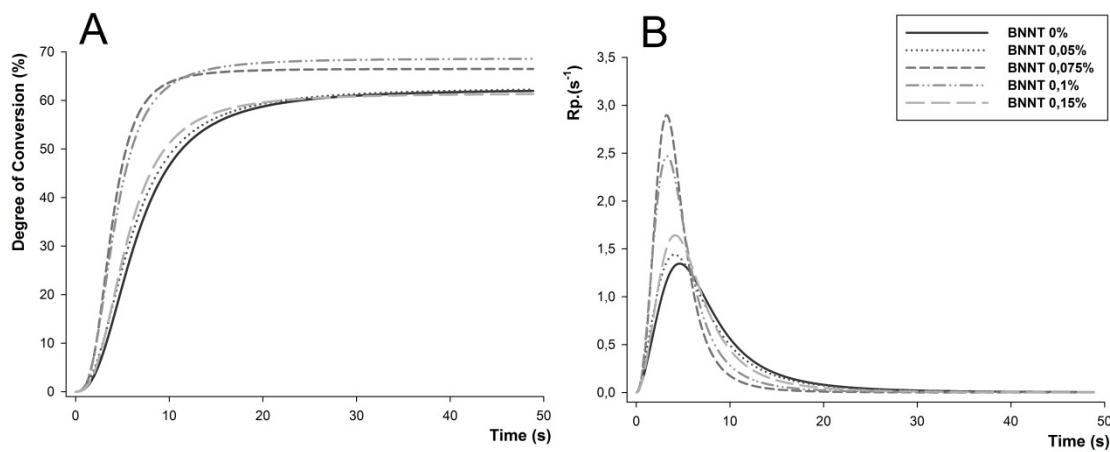
Figure 1 - Polymerization kinetic profiles after 50 s of photo-activation. (A) Degree of conversion (%) as a function of time showing higher DC after incorporating BNNT 0.075 and 0.1%. (B) Polymerization rate as a function of time showing higher $Rp.(s^{-1})$ after incorporating BNNT 0.075 and 0.1% at similar time.
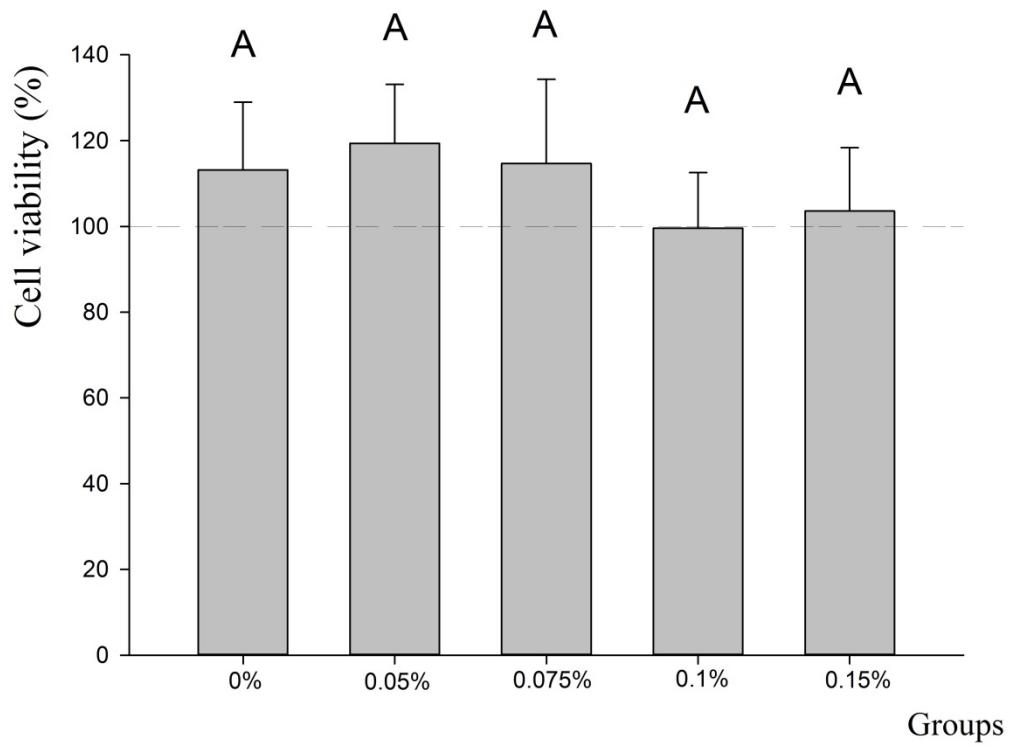
Figure 2 – Cell viability of fibroblasts after 72h. Dashed line shows the control free of drug as 100% cell viability. Same letters means no statistical difference ($p > 0.05$).

Table 1. Degree of conversion (%) after 50 s of photo-activation, maximum polymerization rate [Max. Rp.(s^{-1})],

contact angle (θ) on dentin after 10 s and microtensile bond strength (μTBS, in MPa) after 24 h and 6 months.

Groups	Degree of Conversion	Max. Rp.(s^{-1})	Contact Angle	μTBS 24 h	μTBS 6 months	A_0	M_0	C_0	A_6	M_6	C_6
0% BNNT	62.9±0.5^A	5.3±0.3^C	25.2±3.3^A	55.1±4.7^{AB,a}	35.9±6.5^{B,b}	36	50	14	20	57	23
0.05% BNNT	63.0±0.3^A	5.1±0.1^C	32.7±1.6^A	50.2±5.8^{AB,a}	51.6±13.7^{A,a}	40	46	14	35	53	12
0.075% BNNT	68.9±0.6^B	14.5±2.4^A	32.2±4.4^A	48.1±7.4^{B,a}	51.4±9.5^{A,a}	22	71	7	31	62	7
0.1% BNNT	70.2±0.8^B	14.1±1.8^A	32.8±6.8^A	57.8±8.3^{A,a}	53.5±10.5^{A,a}	25	64	11	41	48	11
0.15% BNNT	63.2±0.7^A	9.8±2.1^B	35.8±3.4^B	55.9±6.2^{AB,a}	48.2±7.6^{A,b}	24	64	12	37	50	13

Different capital letters mean statistical significance within columns ($p < 0.05$).
Different lowercase letters mean statistical significance among columns ($p < 0.05$).
A_0 adhesive, M_0 mixed, C_o cohesive – immediate.
A_6 adhesive, M_6 mixed, C_6 cohesive – after 6 months.

CONSIDERAÇÕES FINAIS

A procura por materiais que contribuam para o controle de falhas restauradoras e, assim, aumentem a longevidade clínica das restaurações dentárias tem sido o objeto de diversos estudos (TAY; PASHLEY, 2008; LEITUNE *et al.*, 2013a; ABUNA *et al.*, 2016; DEGRAZIA *et al.*, 2016). A degradação hidrolítica que ocorre na interface adesiva atingindo o polímero e o colágeno tem sido apontada como a principal causa das falhas restauradoras (DE MUNCK *et al.*, 2003; BRESCHI, 2008). Sendo assim, nanotubos de Nitreto de Boro foram utilizados como carga e incorporados a uma resina adesiva a base de polímeros metacrilatos resultando em um compósito com melhores propriedades físico-químicas e biológicas.

Os resultados deste estudo demonstraram favoravelmente a interação dos BNNTs com os monômeros do adesivo. Essa interação ocorre por meio de ligações π-π com os grupamentos aromáticos do BisGMA e interações hidrófobas do tipo van der Waals (KIM *et al.*, 2014b), resultando em um compósito homogêneo e por isso, diminuindo a chance de formação de pontos de estresse específicos, conforme demonstrado nos resultados de resistência coesiva. Além disso, o grau de conversão quando o adesivo foi avaliado na forma pura dos BNNTs não resultou em diferença do adesivo sem BNNT, demonstrando que o efeito da luz na fotoativação não foi alterado pela presença das nanopartículas. Após o tratamento dos BNNTs com ácido nítrico e peróxido de hidrogênio foi encontrada uma alteração na cinética de polimerização com aumento da taxa e do grau de conversão do adesivo.

Apesar de sua natureza iônica (SURYAVANSHI *et al.*, 2004), isto é, de forte ligação e estabilidade entre os átomos de Boro e Nitrogênio, a superfície

dos nanotubos possui reatividade devido a presença de elétrons livres, principalmente grupamentos –OH e radicais H (Golberg *et al*., 2010). A capacidade de reagir com o ácido nitrico, causando sua hidroxilação, e com o peróxido de hidrogênio aumentando a concentração de radicais livres na sua superfície conferiram ao adesivo um aumento da oxidação foto induzida e consequentemente aumentando o grau de conversão do polímero.

A estabilidade demonstrada na resistência de união após 6 meses em água foi consequência das melhores propriedades físico-químicas conferidas ao adesivo após a incorporação de BNNT. Os principais fatores que determinaram esta melhora foram: a diminuição do percentual da diferença de amolecimento em solvente após imersão na solução de degradação, a diminuição da energia livre de superfície e o maior grau de conversão.

A diminuição do percentual da diferença de amolecimento pode ser explicada pela resistência ao solvente orgânico devido à estabilidade química da carga, pequena distância interplanar, de aproximadamente 0,333 nm, e o engrenamento mecânico com as cadeias poliméricas. A diminuição da energia livre de superfície do adesivo é resultante da hidrofobicidade dos BNNTs conferida pela morfologia da superfície e adsorção de hidrocarbonetos (BOINOVICH *et al*., 2012). A diminuição da energia livre de superfície diminui a absorção de água e a degradação polimérica de origem bacteriana. Além disso, o molhamento do adesivo à dentina demonstrou diminuir apenas quando a maior concentração de BNNT foi incorporada, o que explicaria a diminuição da resistência de união após 6 meses neste grupo. Já o alto grau de conversão tem sido atribuído a materiais com melhores propriedades mecânicas (RODRIGUES *et al*., 2015).

A deposição mineral encontrada após 14 dias faz com que a adição de BNNTs ao adesivo resulte na substituição de lacunas com água, formadas na base da camada híbrida, por minerais. Esta mineralização pode ocorrer nos compartimentos inter e intrafibrilares da matriz de colágeno da dentina (TAY; PASHLEY, 2009). Tal modificação resulta em menor degradação do colágeno exposto após a etapa do condicionamento ácido da dentina e, consequentemente, aumento da longevidade do material restaurador. Além disso, não foi constatada neste estudo citotoxicidade para fibroblastos pelos adesivos com BNNT, podendo ser utilizados com este fim. Além disso, este resultado permite que outros estudos utilizando BNNTs sejam realizados ampliando sua indicação, como em: forramentos, infiltrantes, resinas ortodônticas e resinas compostas para restaurações.

O uso de adesivo com nanotubos de nitreto de boro pode ser uma alternativa que providencia propriedades biológicas e físico-químicas adequadas para um desempenho clínico de maior longevidade. O material utilizado alia-se à nanotecnologia, sendo uma fronteira do conhecimento em crescimento nos últimos anos. Diferentes campos da engenharia e química vem trazendo os BNNTs como substitutos do seu análogo nanotubos de carbono como reforço de estruturas poliméricas e por não modificarem sua cor. Assim, este estudo demonstra a viabilidade de uso dos BNNTs em adesivos odontológicos.

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