UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE ODONTOLOGIA

Programa de Pós-Graduação em Odontologia

Área de Concentração Clínica Odontológica – Materiais Dentários

INFLUÊNCIA DA WOLASTONITA COMO CARGA EM RESINAS ADESIVAS

Islam Mahmoud Bendary

Orientador: Prof. Dr. Vicente Castelo Branco Leitune

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LINHA DE PESQUISA: BIOMATERIAIS E TÉCNICAS TERAPÊUTICAS EM ODONTOLOGIA

INFLUÊNCIA DA WOLASTONITA COMO CARGA EM RESINAS ADESIVAS

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

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"Conhecimento na alienação é uma pátria
e Ignorância na pátria é Alienação.."

Ibne Ruxide (1126 – 1198)

DEDICATÓRIA

Pelos meus pais Nadia e Mahmoud, não há palavras para descrever o quanto o amor, a gratidão e o orgulho da minha família me deram todo o amor generoso, carinho e apoio em cada etapa da minha vida.

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RESUMO

O objetivo deste estudo foi formular adesivos dentários experimentais com wollastonita e avaliar as propriedades físicas, químicas e de bioatividade das resinas. A wollastonita foi caracterizada por espectroscopia de infravermelho com transformada de Fourier, análises de difração de raios-X e laser e microscopia eletrônica de varredura. Uma resina adesiva experimental foi formulada e a wollastonita foi usada como carga inorgânica a 0 (grupo controle), 0,5, 1 ou 2%, em peso. Os adesivos experimentais foram avaliados quanto à sua radiopacidade, grau de conversão (DC%), microdureza Knoop, amolecimento em solvente, resistência à tração (UTS), resistência da união adesiva à microtração após 24 horas e um ano (µTBS), deposição mineral e cor dos adesivos. Partículas de wollastonita mostraram uma forma semelhante a agulha, um diâmetro médio de 70 (± 30) µm, picos químicos característicos e fase β-CaSiO3 cristalina pura. Não houve diferenças significativas (p>0,05) para radiopacidade, amolecimento em solvente e mudança de cor. O grupo com 2%, em peso, de wollastonita apresentou maior microdureza Knoop e UTS em comparação ao grupo controle (p <0,05). Após um ano, o grupo controle apresentou menor µTBS em comparação ao valor imediato (p <0,05). Os grupos com Wollastonita apresentaram µTBS estável após um ano em comparação ao µTBS imediato (p> 0,05). A Wollastonita induziu a deposição mineral na superfície adesiva ao longo do tempo de armazenamento em fluido corporal simulado. A adição de wollastonita melhorou o comportamento mecânico do adesivo sem alterar as propriedades químicas analisadas.

Palavras-chave:

Agentes de união de dentina, silicatos de cálcio, polímeros, polimerização

ABSTRACT

The aim of this study was to formulate experimental dental adhesives with wollastonite and evaluate the physical, chemical, and bioactivity properties of the resins. Wollastonite was characterized by Fourier transform infrared spectroscopy, X-ray and laser diffraction analyses, and scanning electronic microscopy. An experimental adhesive resin was formulated, and wollastonite was used as an inorganic fillers at 0 (control group), 0.5, 1, or 2% wt. Radiopacity, degree of conversion (DC%), Knoop micro-hardness, softening in solvent, ultimate tensile strength (UTS), 24 hours and one year- micro tensile bond strength (μTBS), mineral deposition, and color of the experimental adhesives were evaluated. Wollastonite particles showed a needle-like shape, a mean diameter of 70 ($\pm$ 30) μm, characteristic chemical peaks, and pure crystalline β-CaSiO3 phase. There were no significant differences ($p > 0.05$) for radiopacity, softening in solvent, and color change. The group with 2 wt. % of wollastonite showed higher Knoop micro-hardness and UTS in comparison to the control group ($p < 0.05$). After one year, the control group showed reduced μTBS compared to the immediate value ($p < 0.05$). The groups with wollastonite presented stable μTBS after one year in comparison to the immediate μTBS ($p > 0.05$). Wollastonite induced mineral deposition on the adhesive surface over the time of storage in simulated body fluid. The addition of wollastonite improved the mechanical behavior of the adhesive without changing the analyzed chemical properties.

Keywords:

Dentin-bonding agents, Calcium silicates, Polymers, Polymerization

SUMÁRIO

1. INTRODUÇÃO

1.1 ODONTOLOGIA ADESIVA

As técnicas de restauração contemporâneas baseadas nas propriedades adesivas foram introduzidas pela primeira vez pelo Dr. Hagger em (1949), que foi o primeiro a introduzir um material de vedação de cavidade para ser usado em combinação com a resina de cura química. O primeiro experimento de adesão ao esmalte por ataque ácido foi realizado por Buonocore em (1955). Enquanto, o processo de condicionamento foi descrito por Fusayama et al. (1979) como a infiltração da resina no tecido desmineralizado permitindo a formação de uma matriz de resina reforçada por fibras de colágeno. Nakabayashi, em (1982) foi o primeiro a demonstrar uma verdadeira formação de camada híbrida que é considerada o principal mecanismo de ligação dos agentes de ligação (Edward et al., 1995). O objetivo principal dos adesivos dentais é fornecer retenção para restaurações com resinas compostas ou cimentos resinosos. Vários estudos revelaram que de acordo com a exposição o ambiente oral, a falha clínica das restaurações ocorre com mais frequência devido à inadequação selamento levando à perda de retenção (Pashley et al., 2004, Ferreira et al., 2007 e Breschi et al., 2008).

Os sistemas adesivos são incapazes de se infiltrar completamente na rede de colágeno com monômeros de resina, resultando em espaços ricos em água ao longo das fibrilas de colágeno desprotegidas e responsáveis pela nanoinfiltração e micropermeabilidade dentro das camadas híbridas (Sauro et al., 2009). Camadas híbridas com presença de água e solvevnte residual são mais propensas à degradação ao longo do tempo. Além disso a rede de fibrilas de colágeno parcialmente exposta está sujeita à degradação enzimática de MMPs endógenos e cistepsinas de cisteína, ativadas por componentes ácidos de sistemas adesivos (Mazzoni et al., 2013). A combinação de hidrólise do componente de resina, atividade colagenolítica, temperatura, ácidos bacterianos e tensões mecânicas comprometem a integridade interfacial de longo prazo de restaurações adesivas (Frassetto et al., 2016).

Em relação a esses problemas, vários métodos têm sido preconizados para reduzir a degradação da camada híbrida dentro das interfaces resina-dentina, incluindo a inibição das metaloproteinases da matriz dentinária (MMPs), que causam degradação enzimática da matriz de colágeno, pela adição de agentes reticulantes (Seseogullari-Dirihan et al., 2016). Além disso, a melhoria das propriedades químicas e físicas do sistema adesivo pode contribuir para o aumento

da resistência à degradação hidrolítica e aumentando a estabilidade ao longo do tempo (Dos Santos et al., 2011, Dodiuk-Kenig et al., 2012, Leitune et al., 2013 , Burujeny et al., 2015).

Diversos autores sugeriram que a adição de cargas inorgânicas em pequenas concentrações às resinas adesivas pode fortalecer a camada adesiva (Schulz et., 2008, Leitune et al., 2013, Degrazia et al., 2016, Almeida et al., 2017, Garcia et al., 2018 e Balbinot et al., 2020). Dependendo de sua composição química, as cargas também podem fornecer radiopacidade, o que pode ser importante no diagnóstico diferencial de cáries adjacentes à restauração (Furtos et al., 2012), ação antimicrobiana (Cruzetta et al., 2020) e ação remineralizante (Garcia et al., 2017). Em relação à composição carga, podem ser compostos por íons silicato (Si-O) acredita-se que eles possam desempenhar um papel na melhoria das propriedades mecânicas dos adesivos resinosos (Belli et al., 2014). Além disso, os íons de sílica hidratada liberados com carga negativa fornecem um local favorável para a formação de apatita na interface da dentina aderida (Gandolfi et al., 2011).

A remineralização das interfaces resina-dentina visa substituir o espaço desmineralização durante do condicionamento ácido e não preenchido por polímeros, bem como regiões que sofreram algum tipo de desmineralização (Profeta et al 2012). Com isso, seria possível aumentar as propriedades mecânicas da interfase dentina-resina e proteger o colágeno exposto de desafios externos (He et al., 20019).

1.2 WOLLASTONITA

A wollastonita ($CaSiO_3$) é um mineral natural de silicato de cálcio de cor branca composto por 51,7% de SiO_2 e 48,3% de CaO (Asar et al., 2010). Apresenta excelente estabilidade térmica, estabilidade química e propriedades mecânicas adequadas, além de sua capacidade de liberar cálcio (De Aza et al., 1994). Possui grande importância em aplicações clínicas, seja na forma de pó como enchimento ósseo ou como material a bulk para confecção de próteses da crista ilíaca, vértebras artificiais e discos intervertebrais (Park and Lakes 2007). Acredita-se que o $CaSiO_3$ induza o crescimento do osso como uma camada de apatita ligada ao osso vivo em um curto período de tempo, mantendo altas propriedades mecânicas no ambiente corporal (Cannillo et al., 2009).

O $CaSiO_3$ é usado em aplicações médicas (Dziadek et al., 2017) e dentais como um potencial material de revestimento de superfície de implante dentário para regeneração de tecido

ósseo (Saadaldin et al., 2014). Além disso, tem sido utilizado como carga em polímeros e cimentos para melhoria de suas propriedades mecânicas e bioativas (Chen et al., 2016, Flores-Ledesma et al., 2017 e Herrera et al., 2018). A bioatividade das partículas à base de $CaSiO_3$ está relacionada à alcalinização promovida pelos íons hidroxila liberados da fase cristalina do hidróxido de cálcio. O aumento do pH local inibe a atividade de MMP e favorece a precipitação mineral, além de exercer atividade antimicrobiana (Profeta et al., 2013). A deposição mineral do material depende da capacidade da sílica com carga negativa liberada encontrada na superfície do tecido para formar um local favorável para a formação de apatita por atração de íons de cálcio liberados pelas partículas e íons de fosfato presentes em fluidos fisiológicos (De Aza et al., 1994). Esse mecanismo supostamente fornece uma condição favorável para a remineralização dos tecidos duros dentais por meio de efeitos terapêuticos / protetores que podem aumentar a longevidade da interface resina-dentina (Profeta et al., 2013).

A degradação da camada híbrida dentro das interfaces resina-dentina pode ser atribuída ao aumento das concentrações de monômeros hidrofílicos e ácidos presentes na composição dos adesivos de resina (Van Landuyt et al., 2007). Além disso, a degradação hidrolítica da ligação éster em tais monômeros pode apresentar um efeito adverso nas propriedades mecânicas da rede polimérica (Van Landuyt et al., 2008). A aplicação de preenchimentos inorgânicos na composição de sistemas adesivos dentais tentou reduzir a degradação do colágeno dentinário mediada por MMP e aumentar a durabilidade das ligações resina-dentina (Toledano et al., 2012). No entanto, sua propriedade de liberação de íons assumida para demonstrar uma eficácia na remineralização de regiões esparsas de resina empobrecida de minerais da matriz de colágeno desmineralizado que supostamente aumentam a qualidade e a longevidade das interfaces resina-dentina (Tay et al., 2008). Em contraste, a incorporação de materiais à base de silicato nos monômeros específicos da resina, foi proposta para melhorar as propriedades mecânicas e a resistência de união aos tecidos dentais (Sauro et al., 2012).

2.Objetivo

2.1 Objetivos gerais:

O objetivo deste estudo foi avaliar o efeito da adição de wollastonita como carga em adesivos dentais experimentais em diferentes concentrações.

3 MANUSCRITO

A presente tese é composta por um manuscrito. O manuscrito foi submetido para o periódico *Journal of Dentistry* e encontra-se publicado (https://doi.org/10.1016/j.jdent.2020.103472) (Bendary et al., 2020).

Wollastonite as filler of an experimental dental adhesive

INFLUENCE OF WOLASTONITE AS FILLERS APPLIED IN RESIN ADHESIVE

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ABSTRACT

Objective: The aim of this study was to formulate experimental dental adhesives with wollastonite and evaluate the physical, chemical, and bioactivity properties of the resins.

Methods: Wollastonite was characterized by Fourier transform infrared spectroscopy, X-ray and laser diffraction analyses, and scanning electronic microscopy. An experimental adhesive resin was formulated, and wollastonite was used as filler at 0 (control group), 0.5, 1, or 2 wt. %. Radiopacity, degree of conversion (DC%), micro-hardness, softening in solvent, ultimate tensile strength (UTS), 24 h- and 1 year- microtensile bond strength (µTBS), mineral deposition, and color of the adhesives were evaluated.

Results: Wollastonite particles showed a needle-like shape, a mean diameter of 70 ($\pm$ 30) µm, characteristic chemical peaks, and pure crystalline β-CaSiO$_3$ phase. There were no significant differences ($p > 0.05$) for radiopacity, softening in solvent, and color change. The group with 2 wt. % of wollastonite showed higher microhardness and UTS in comparison to the control group ($p < 0.05$). After one year, the control group showed reduced µTBS compared to the immediate value ($p < 0.05$). The groups with wollastonite presented stable µTBS after one year in comparison to the immediate µTBS ($p > 0.05$). Wollastonite induced mineral deposition on the adhesive surface over the time of storage in simulated body fluid.

Conclusion: The addition of wollastonite improved the mechanical behavior of the adhesive without changing the analyzed chemical properties. The adhesives with this filler presented mineral deposition and acceptable clinical color. Moreover, dentin treated with wollastonite-doped adhesives showed higher bonding stability after one year of aging.

Clinical significance: Wollastonite, a silicate-based material, provided bioactivity for the adhesives, which assists in producing therapeutic tooth-restoration interfaces. Moreover, incorporation of this mineral improOfiller improves the biological properties of adhesives and assists in dentin-restoration stability.

1. Introduction

The maintenance of a long-lasting bonding of restorative materials to dentin is challenging due to degradation processes [1–3], which affect the adhesive or collagen network [1–3]. The adhesive degradation occurs via water sorption and catalysis of ester groups because of water presence or esterases from microorganisms and saliva [1,2]. The collagen degradation occurs via hydrolytic degradation, mainly in areas of exposed collagen, or due to the activity of endogenous dentin matrix metalloproteinases (MMPs) [4]. These events may lead to marginal discolorations, leakage, and loss of restoration over time [1].

The incorporation of inorganic fillers in adhesives has been proposed to address these concerns. Tantalum oxide [5,6], niobium pentoxide [7], calcium phosphates [8–10], zinc chloride [11], titanium dioxide [12], and zirconium dioxide [13] were used to improve the organic matrix properties. The particles increase the mechanical properties of polymers via toughening mechanisms, and they can act as obstacles in front of crack propagation [14]. Moreover, some particles present ion-releasing ability that induces mineral precipitation and collagen remineralization, improving the bonding longevity [15,16]. The mineral deposition on collagen has also been reported as a feasible approach to fossilize MMPs and protect dentin against biodegradation [17].

Calcium silicates ($CaSiO_3$) are widely used to coat implants [18], promote pulp and bone regeneration, and induce remineralization of dental tissues [19]. Mineral trioxide aggregate and Portland cement are two classic examples of $CaSiO_3$-based materials used in operative dentistry and endodontics [19]. These cements release ions of calcium (Ca^{2+}) and hydroxyl groups (OH^-) that favor the remineralization process [20]. However, they present drawbacks such as the longer setting time in comparison to light-curable materials and increased dissolution rate [19]. The development of light-curable restorative materials with bio-interactivity via ion-releasing property, such as that presented by $CaSiO_3$, could favor the adhesion to dentin. Therefore, $CaSiO_3$-derived fillers were incorporated into adhesives to provide them bioactivity [16,21,22]. In those studies, improved dental bonding was achieved when $CaSiO_3$-derived fillers were incorporated into the adhesives [16,21,22]. In these previous *in vitro* studies [16,22], the adhesives doped with $CaSiO_3$-derived fillers were tested for bonding

effectiveness and bioactivity. However, the adhesives were tested after aging for only six months [16,22], while an extended period of aging assists in increasing the correlation between the microtensile bond strength test and *in vivo* outcomes [23]. Adhesives doped with $CaSiO_3$ could be further explored regarding other physical and chemical properties, such as radiopacity, degree of conversion, ultimate tensile strength, softening in solvent, and color evaluation. This set of analyzed properties, together with an extended period of aging of teeth restored with adhesives doped with $CaSiO_3$-derived fillers, could assist in understanding materials' behavior deeply.

In this context, wollastonite is a $CaSiO_3$ that occurs in nature, and that was not tested in dental adhesives so far. The availability of wollastonite in the environment reduces the steps of syntheses of this $CaSiO_3$, which may increase the chances of transferring the knowledge about wollastonite-doped adhesives for clinical scenarios. This mineral can be arranged as masses or tabular crystals of metamorphosed limestone [24]. It presents bioactivity via the release of Ca^{2+}, inducing apatite formation [25], which could provide therapeutic properties to adhesives and protective effects to dental tissues [15,26]. The aim of this study was to formulate experimental dental adhesives with wollastonite and evaluate the physical, chemical, and bioactivity properties of the resins. For this purpose, after the characterization of the wollastonite used as filler, we also analyzed the adhesives for possible effects on the radiopacity, degree of conversion, micro-hardness, softening in solvent, ultimate tensile strength, microtensile bond strength (μTBS) immediately and after one year of aging, mineral deposition, and color. The null hypothesis to be tested is that the addition of wollastonite up to 2 wt. % does not affect the evaluated properties of the dental adhesives.

2. Materials and methods

The methods applied in this study to evaluate the wollastonite particles and the experimental adhesive resins are indicated in Fig. 1.

2.1. Chemical characterization of wollastonite

Wollastonite (NYCO Minerals, Inc., NY, USA) was analyzed by Fourier Transform infrared spectroscopy (FTIR) using a Vertex 70 spectrophotometer (Bruker Optics, Ettlingen, Germany) with wave-length ranging from 4000 to 400 cm^{-1} and resolution of 4 cm^{-1}. The

powder of wollastonite was placed on the diamond crystal of an attenuated total reflectance (ATR) accessory of FTIR to be chemically characterized.

2.2. Determination of the crystalline phase of wollastonite by X-ray diffraction analysis

The determination of the crystalline phase of wollastonite was car- ried out by X-ray diffraction analysis (XRD). A diffractometer (X'pert- PRO, PANalytical MPD, Eindhoven, Netherlands) was used with copper Kα radiations at 40 kV and 40 mA at an angular range between 5° and 100° with step size 0.02° over 2 s.

2.3. Evaluation of wollastonite morphology by scanning electron microscopy

The powder of wollastonite was placed on a metallic stub and was coated with a gold-sputter (15 25 nm) (SCD 050, Baltec, Vaduz, Liechtenstein). Particle morphology was analyzed with scanning electron microscopy (SEM, JSM-56060, Jeol, Akishima, Tokyo, Japan) set at 3 kV. Images were magnified at 100 and 330 X.

2.4. Evaluation of particle size of wollastonite by laser diffraction

The powder was dispersed in ultrasound for 10 s with isopropyl alcohol. Then, the particle size of wollastonite was measured by laser diffraction (CILAS 1180, Orleans, France).

2.5. Adhesive resins formulation

The adhesive resin was formulated by mixing bisphenol A-glycol dimethacrylate and hydroxyethyl methacrylate (Sigma-Aldrich, St. Louis, MO, USA) in a mass ratio of 66.66 wt% and 33.33 wt. %, respectively. The photo-initiation system composed of camphorquinone and ethyl 4-dimethylamino benzoate was added at 1 mol% to the mixture. The adhesive was divided into four groups according to wollastonite concentration: unfilled adhesive, which was used as a control group ($G_{0\%}$); 0.5 wt.% (G0.5 %); 1 wt.% ($G_{1\%}$); 2 wt.% ($G_{2\%}$). The three filled experimental adhesives were homogenized by hand- mixing for 5 min, sonicated for 180 s, and hand-mixed for more 5 min [6].

2.6. Radiopacity

The determination of the radiopacity of the experimental adhesives was performed according to the International Standards Organization (ISO) 4049/2009 [27]. Five samples per group with a diameter of 5.0 mm (±0.5 mm) and a thickness of 1.0 mm (±0.2 mm) were photo-activated for 20 s on both sides by a light-emitting diode (LED) unit (Radii Cal 1200 mW/cm^2, SDI LTD., Bayswater, VIC, Australia). X-ray images were obtained using a digital system with phosphorous plates (VistaScan, Dürr Dental GmbH & Co. KG, Bietigheim-Bissingen, Germany) at 70 kV and 8 mA with 0.4 s of exposure and a focus-film distance of 40 cm. One sample from each group was positioned on the film, while an aluminum step-wedge was exposed with the samples for the acquisition of each image. The aluminum step-wedge thickness ranged from 0.5 to 5.0 mm in increments of 0.5 mm. A total of five images were acquired. The images were saved in the tagged image file format (TIFF) for less compressed files. The digital images were processed using Photoshop software (Adobe Systems Incorporated, CA, USA) to obtain the pixel density for each group.

2.7. Degree of conversion

The degree of conversion (DC%) was obtained using FTIR-ATR (Vertex 70, Bruker Optics, Ettlingen, Germany). Three samples from each group were evaluated. The analysis was performed at a controlled room temperature of 23 (±2) °C and 60 (±5) % humidity. Samples were directly dispensed in a polyvinyl silicone mold on the diamond crystal of ATR, and they were analyzed before and after photoactivation for 20 s. The DC% was calculated from the ratio between the integrated area of absorption bands of the aliphatic C—C bond (1638 cm^{-1}) to that of the aromatic C=C bond (1609 cm^{-1}) obtained from the polymerized and unpolymerized sample according to Eq. (1).

$$DC\% = 100 \times \left[1 - \left(\frac{Rpolymerized}{Runpolymerized} \right) \right] \tag{1}$$

Where *R* is the ratio between the integrated area of 1638 cm^{-1} and 1609 cm^{-1}.

2.8. Microhardness and softening in solvent

Five samples per group were prepared with 5 mm diameter and 1 mm thickness after the photoactivation for 20 s on each side. The samples were polished (Model 3v, Arotec, Cotia, SP, Brazil) using #600, #1200, #2000 grits abrasive papers (30 s each with water irrigation), and a felt disc saturated with an alumina suspension. The samples were subjected to a microhardness test in which five indentations (10 g/ 5 s) with magnification at 40X were performed per sample (HMV 2, Shi- madzu, Tokyo, Japan). These values represented the initial Knoop hardness (KHN1). The samples were immersed in a solvent solution (30% water mixed with 70 % ethanol) for 2 h, and then they were tested again to obtain the final Knoop hardness (KHN2). The Knoop hardness values were recorded as the average of the five indentations per sample. Softening in solvent (ΔKHN%) was calculated by the percentage difference between KHN1 and KHN2 [28].

2.9. Ultimate tensile strength

Five samples per group were prepared in a metallic mold with an hourglass design with dimensions of 8 mm long, 2 mm wide, 1 mm thick, and with a cross-sectional area of 1 mm^2. The samples were photo-activated for 20 s on each side and then fixed with a cyanoacrylate adhesive to a metallic device. The samples were tested using a universal testing machine (EZ-SX Series; Shimadzu) at a crosshead speed of 1 mm/ min until the samples fracture. The values were expressed in MPa [29].

2.10. Microtensile bond strength and failure pattern analysis

One-hundred sixty extracted sound bovine teeth were used in the microtensile bond strength (μTBS) test, totalizing eighty teeth for the immediate μTBS and another eighty for the μTBS after one year of aging. Each tooth was prepared to expose the superficial dentin surface. In order to create a standardized smear layer, each surface was polished with 600-grit silicon carbide paper under running water for 30 s. The prepared dentin surface was etched using 37 % phosphoric acid for 15 s and rinsed for 15 s. Excess water was removed with an absorbent paper. A thin layer of a commercial primer (3 M ESPE, Adper Scotchbond, St. Paul, MN, USA) was actively applied for 20 s, and the solvent was evaporated with air-spray for 5 s. A layer of the experimental adhesives was applied and photoactivated for 20 s. Finally, two layers with 2 mm thickness each one

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(Z350, 3 M ESPE, St. Paul, MN, USA) were applied and photoactivated for 40 s with the light-tip 1 mm from the top of resin surface. The samples were sectioned into a series of beams under constant water cooling using a precision cutting device and a diamond disc (Law Speed Saw, Buehler Ltd, Lake Bluff, IL, USA). Four beams from the center of each resin composite restoration were measured using a digital caliper to check their thickness in the bonding area and tested for µTBS. The sample unit was the tooth. Specimens were subject to the µTBS test immediately or after one year of storage in simulated body fluid (SBF), which was changed every month [30]. Each beam was attached to a metallic jig with cyanoacrylate resin. The jig was placed in the lower fixed compartment of the universal testing machine, and the upper part was pulled at a constant speed of 1 mm/min. The required load for debonding the substrate-adhesive interface of each beam was recorded in MPa. The fracture pattern was evaluated with a stereomicroscope (HMV-2, Shimadzu Corp., Kyoto, Japan) at 10x magnification and classified as adhesive, mixed, cohesive within the dentin, or cohesive within the resin composite.

2.11. Mineral deposition

Three samples from each experimental adhesive group with a diameter of 6 mm and 2 mm in height were used for mineral deposition analysis. They were then polished using a #1200-grit abrasive paper under constant irrigation. Samples were immersed in SBF for 1, 7, and 28 days at 37 °C. After each storage period, samples were washed with distilled water and analyzed using micro-Raman spectroscopy (Senterra, Bruker Optics, Ettlingen, Germany). Chemical changes on the sample surfaces were evaluated by irradiating an area of 500 500 µm that was analyzed by a 785 nm-laser of 100 mW on 100 equidistant points for 3 s per each point. Spectra were obtained at wavelengths ranging from 1800 to 440 cm^{-1}. Integration of the 960 cm^{-1} peak (symmetric stretching $\upsilon 1$ mode of phosphate) was calculated using the spectroscopy software (Opus 7.5, Bruker Optics, Ettlingen, Germany). Sigma Plot version 12.0 (Systat Software Inc, San Jose, CA, USA) was used to convert data obtained from the spectroscopy software into digitalized images [31].

2.12. Color evaluation

Five samples for each group with a diameter of 5 mm and a thickness of 1 mm were evaluated. All samples were polished using #600-grit abrasive paper to standardize the surface. Samples

were measured with reflectance spectroscopy (Cary5000 UV–vis, Agilent, Santa Clara, USA). This instrument utilizes a double beam ratio integrated with a sphere reflectometer that collects specular and diffuse reflectance. All samples were scanned from 400 to 700 nm, with a data interval of 1 nm at a speed of 480 nm/min. CIE L*a*b* was used to quantitatively show the color difference, movements on the white-black (value scale L), red- green (a), and yellow-blue (b) scales. Colour planes (chroma scales) were compiled by the Perkin Elmer color program (Perkin Elmer, Norwalk, CT, USA) converted into L*a*b* values, which was recorded and analyzed. Changes in overall color (ΔE) were calculated for each group comparing their parameters with those obtained for $G_{0\%}$ (Eq. (2)).

$$\textbf{\textit{Change in color } } \Delta E = \sqrt{(\Delta L2) + (\Delta a2) + (\Delta b2)} \qquad\qquad (2)$$

2.13. Statistical analysis

The characterization of wollastonite via FTIR, X-ray analysis, laser diffraction analysis, and SEM, as well as the mineral deposition on adhesives surfaces, were descriptively analyzed. The physicochemical properties of the adhesives were statistically analyzed using SigmaPlot software (version 12.0, Systat Software, San Jose, CA, USA). Shapiro- Wilk test was used to analyze the data distribution. The data of radio- pacity, microhardness, softening in solvent, DC%, ultimate tensile strength (UTS), and color change were analyzed via one-way ANOVA and Tukey post-hoc test. The differences between KHN1 and KHN2 within each group were assessed via paired t-tests. Two-way ANOVA and Tukey post-hoc test were used to analyze the data of μTBS, considering the two factors: concentration of $CaSiO_3$ in the adhesive (0.5, 1, or 2 wt.%) and time of storage of the beams (24 h or 1 year). All tests were analyzed at a significance level of 0.05.

3. Results

Results for wollastonite characterization by SEM and laser diffraction showed particles distributed in a needle-like form (Fig. 2) with a mean diameter of 70 ($\pm$30) μm. FTIR spectra (Fig. 3) showed broadband stretching of hydroxyl groups (υO–H) due to moisture content of the sample in the region of 1,150 cm^{-1} Wollastonite showed characteristic peaks of the amorphous SiO$_2$ (υSi–O–Si) and Si–O–Ca at 1,025 and 900 cm^{-1}, respectively. The band near 450 cm^{-1} resulted from the vibration of the Si–O bond Fig. 3. The XRD pattern indicated the pure crystalline β-CaSiO$_3$ phase via the intensity peak shown in Fig. 4.

The results of radiopacity, DC%, microhardness, and softening in solvent are described in Table 1. The radiopacity evaluation indicated that the pixel density ranged from 31.9 ($\pm$ 4.6) for G$_{0\%}$ to 35.9 ($\pm$ 6.0) for G$_{1\%}$, with no statistically significant differences among the unfilled and filled adhesives ($p > 0.05$). DC% results of the experimental adhesive showed values ~60 % after 20 s of photoactivation for all groups, without statistically significant differences among them ($p > 0.05$). The KHN1 was significantly higher for the group containing 2 wt. % of wollastonite in comparison to all other groups ($p < 0.05$). The KHN2 was lower than KHN1 within each group ($p < 0.05$). There was no statistically significant difference among groups for ΔKHN% ($p > 0.05$).

The results of UTS and μTBS are displayed in Table 2. UTS ranged from 34.9 ($\pm$7.1) MPa for G$_{0\%}$ to 46.0 ($\pm$4.0) MPa for G$_{2\%}$, with a statistically significant difference between these groups ($p < 0.05$). The two-way ANOVA revealed that the outcomes of μTBS were concentration-dependent for the immediate test, and that the time affected the outcomes depending on the concentration of wollastonite added. G$_{0\%}$ had the highest value (61.0 $\pm$ 8.0 MPa) after 24 h of storage, with a statistically significant difference in comparison to G$_{2\%}$ (40.6 $\pm$ 9.3 MPa) ($p < 0.05$) and no differences compared to G0.5 % or G$_{1\%}$ ($p > 0.05$). There were no statistically significant differences among groups after one year of aging in SBF ($p > 0.05$). When the values of 24 h- and 1 year- μTBS are compared within each group, G$_{0\%}$ decreased from 61.0 ($\pm$8.0) MPa in the 24 h-μTBS to 43.4 ($\pm$9.7) MPa in the 1 year- μTBS ($p < 0.05$). Groups containing wollastonite showed no statistically significant differences between 24 and 1

year- µTBS (p > 0.05) within each adhesive. The failure pattern differed among groups, with high percentages of mixed and adhesive failures for all groups Fig. 5.

The mineral deposition on the adhesives' surface increased for all samples containing wollastonite compared to $G_{0\%}$. The images obtained from micro-Raman spectroscopy demonstrated an increase in the mineral deposition after 7 and 28 days of immersion in SBF, as illustrated in Fig. 6.

Mean and standard deviation values of the color analysis showed that the experimental adhesives with wollastonite had ΔE values ($G_{0\%}$ used as a pattern) of 2.6 (± 1.7), 4.3 (± 0.3), and 4.3 (± 2.6) for G0.5 %, $G_{1\%}$, and $G_{2\%}$, respectively. There was no statistically significant difference among groups (p > 0.05).

4. Discussion

The maintenance of adhesive properties and collagen stability are considered critical factors in restoration success [1,3]. The formulation of adhesives with improved mechanical properties and bioactivity may extend the survival of restorations [3,32]. In this study, an experimental dental adhesive was doped with wollastonite, which reinforced the methacrylate-based resin, besides inducing mineral deposition on adhesives surfaces and supporting the µTBS over time. Thus, the addition of wollastonite up to 2 wt. % changed some of the evaluated properties. Therefore, the null hypothesis must be partially rejected.

Wollastonite, a $CaSiO_3$ that can be found in nature, was characterized and incorporated in an organic matrix of dental adhesive. The addition of inorganic particles into adhesives can increase the radiopacity of these restorative materials due to the higher atomic weight of fillers in comparison to the organic elements of polymers [6,33,34]. The enhanced radiopacity of adhesive resins may assist clinicians in differentiating radiolucent areas of caries to restorative materials [35]. The results of this study showed a slight increase in the mean values of radiopacity for wollastonite groups in comparison to the unfilled adhesive. However, there was no statistical difference among groups from 0 to 2 wt. % of wollastonite. This finding may have occurred due to the low concentrations of wollastonite tested. The resins were doped with wollastonite up to 2 wt. % to maintain the manipulation features and adequate DC% of the adhesives. The modification of experimental $CaSiO_3$ to incorporate elements with a higher

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atomic number could be a strategy to keep the suitable properties of $CaSiO_3$ and supply radiopacity for the adhesives.

As aforementioned, the incorporation of inorganic fillers in dental adhesives should not compromise the DC%. The proper conversion of carbon-carbon double bonds into single bonds of monomers is an essential chemical property that these materials must present with the photoactivation. An efficient DC% is related to the achievement of suitable mechanical properties, such as high UTS, and better hydrolytic stability, such as lower sorption and solubility [29]. However, it is not unlikely to decrease the DC% by increasing the filler content in the polymer [6,7,34]. This effect is attributed to the mismatch of refractive indexes between fillers and organic matrix [36] or the lower light transmittance with the increase of filler content [37]. In the present study, all groups showed DC% higher than 50 %, which is commonly observed for commercial adhesives [38], and no significant differences from 0 to 2 wt. % of wollastonite. The slight variation (~1.5- 1.6) between the refractive indexes of wollastonite [39] and organic matrix [7] may have assisted in the availability of light in the adhesive during the photoactivation, maintaining high DC% for all groups.

The mechanical properties of the experimental adhesives were tested via UTS and microhardness. Wollastonite incorporated at 2 wt. % in the organic matrix increased the values of KHN1 and UTS in comparison to the control group. The rationale for that is the higher hardness of the inorganic filler compared to the methacrylate resin [8] and increased elastic modulus of the composite (wollastonite adhesive) in comparison to the unfilled adhesive [14]. Another reason for the higher UTS of $G_{2\%}$ could be the behavior of the inorganic particles within the organic matrix when the samples were tensioned in the universal testing machine. Toughening mechanisms such as crack deflection and plastic deformation around the particles can be involved and support the higher mechanical properties of filled adhesives [14,40].

The softening in solvent assessment is commonly used to measure the crosslink density of the polymers and to analyze the hydrophilic behavior of the adhesives with different fillers addition [28,41]. KHN2 values were lower than KHN1 for all groups, which is in accordance with previous analyses of adhesives [6,8,28]. Methacrylate networks, as used in this study, swell during the storage in solvents, leading to carbon chain relaxation or degradation [41]. One could

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think that the incorporation of the intrinsically hydrophilic $CaSiO_3$ into the organic matrix would facilitate the hydrolysis under organic solvents storage. However, the addition of wollastonite did not affect the $\Delta KHN\%$, showing that the crosslink density and the hydrophilic characteristics of the polymers probably did not change among groups. The high DC% that all groups presented probably assisted in the achievement of proper physico- chemical properties.

The bonding to dentin is a complex process that relies on the permeation and polymerization *in situ* of the adhesive in the heterogeneous dentin structure [1]. The failure pattern showed high percentages of adhesive and mixed modes for all groups, indicating that the failure probably started in similar areas regardless of the filler concentration [42]. The addition of 2 wt. % of wollastonite may have changed the adhesive viscosity, which could explain its lower 24 h-µTBS [43]. However, $G_{0\%}$ was the only group that showed lower µTBS after 1 year in comparison to 24 h-µTBS. During the aging, the collagen fibrils and adhesive are prone to degradation after the swelling of the polymer and hydrolysis [32]. The activity of MMPs on collagen cleavage contributes to the instability of bond strength over time [4]. Consequently, it is essential to analyze the new adhesives formulations for their bonding effectiveness to dentin over time.

$G_{0\%}$ showed lower µTBS after 1 year. On the other hand, groups containing wollastonite presented no difference within each adhesive when 24 h-µTBS was compared to 1 year-µTBS. The degradation may have occurred more accented for the unfilled adhesive in comparison to those with wollastonite. These results were in agreement with Profeta et al. [22], who demonstrated the effect of $CaSiO_3$-based materials as reinforcing fillers, maintaining the bond strength after six months of aging. Despite there was no difference among groups in the µTBS after one year, the results could be jeopardized for $G_{0\%}$ in comparison to wollastonite groups after more extended periods of storage. Therefore, the use of wollastonite as filler in the adhesives could provide a promising long-term result for dentin-resin bonds.

The bioactivity of wollastonite could assist in explaining the protective effect observed in 1 year-µTBS. The ability to induce mineral deposition on adhesives surfaces was analyzed via micro-Raman after SBF storage. This analysis indicated more phosphate groups on the surfaces of filled adhesives. The mineral deposition on polymers with wollastonite occurs via the following steps [44]: Ca^{2+} is released from $CaSiO_3$ to the SBF. The Ca^{2+} is exchanged with

hydroxyl groups, and silanol groups are formed on the polymer's surface. The increased silanol content on the surface makes the polymer more negatively charged and prone to receive Ca^{2+} from the SBF. Over time, wollastonite has promising sites for apatite nucleation [44]. This process could induce mineral deposition on dentin-resin interfaces of groups with wollastonite during the storage of beams for µTBS in the SBF.

The micro-Raman analysis shows that wollastonite has the potential to provide bioactivity for the adhesive-dentin interface. As a limitation of this study, we did not evaluate the possible biomimetic remineralization of collagen. This approach would be tested with bio-mimetic agents to induce the nucleation of calcium phosphates in specific sites of collagen. For instance, it could be applied biomimetic solutions based on poly-L-aspartic acid to treat the dentin before employing the adhesive systems on dentin and storing the teeth in artificial saliva or simulated body fluid [45,46]. Interestingly, previous studies without biomimetic analogous showed lower nanoleakage, improvements on mineral-depleted sites of dentin, and higher µTBS when adhesives doped with bioactive fillers were used [16,22,47]. The authors attributed these results to a possible biomimetic activity of silanol species [47]. In addition to this effect, the formation of Ca/P-MMPs species with high molecular weight was reported as a feasible explanation to decrease MMPs activity via fossilization, increasing µTBS when bioactive fillers are used [22,47]. Further studies about the importance of biomimetic analogous in this scenario could be addressed. However, the evaluation of mineral deposition on the polymerized samples, as performed in this study, supports that the adhesives with wollastonite have the potential to be used for remineralization purposes.

Finally, the adhesives were evaluated regarding their color change in comparison to the unfilled resin. The addition of inorganic fillers into adhesives can change the color of the material [48]. The specification of ΔE thresholds depends on the material, observer, and incident light [49]. Diamantopoulou et al. [50] analyzed the effects of water aging of composites regarding their color. The authors used ΔE = 2.6 as a perceptible threshold and ΔE 5.5 as an acceptable threshold [50]. These values were applied according to previous research that analyzed how much color difference mismatch is considered perceivable and acceptable to observers of an intraoral dental prosthesis [51]. In the present study, the ΔE for adhesives with wollastonite showed values between 2.6 (±1.7) and 4.3 (± 2.6), which are nearly those

clinically perceptible threshold and below the acceptable threshold [50,51]. The rationale for that is the similar refractive index between the wollastonite particles and the adhesive. The optical property observed for the formulated materials may encourage the use of wollastonite as novel bioactive filler for dental adhesives.

5. Conclusion

The addition of wollastonite improved the mechanical behavior of the adhesive without changing the analyzed chemical properties. The adhesives with this filler presented mineral deposition and acceptable clinical color. Moreover, dentin treated with wollastonite-doped adhesives showed higher bonding stability after one year of aging. Wollastonite is a promising filler to improve the biological properties of adhesives and assist in dentin-restoration stability.

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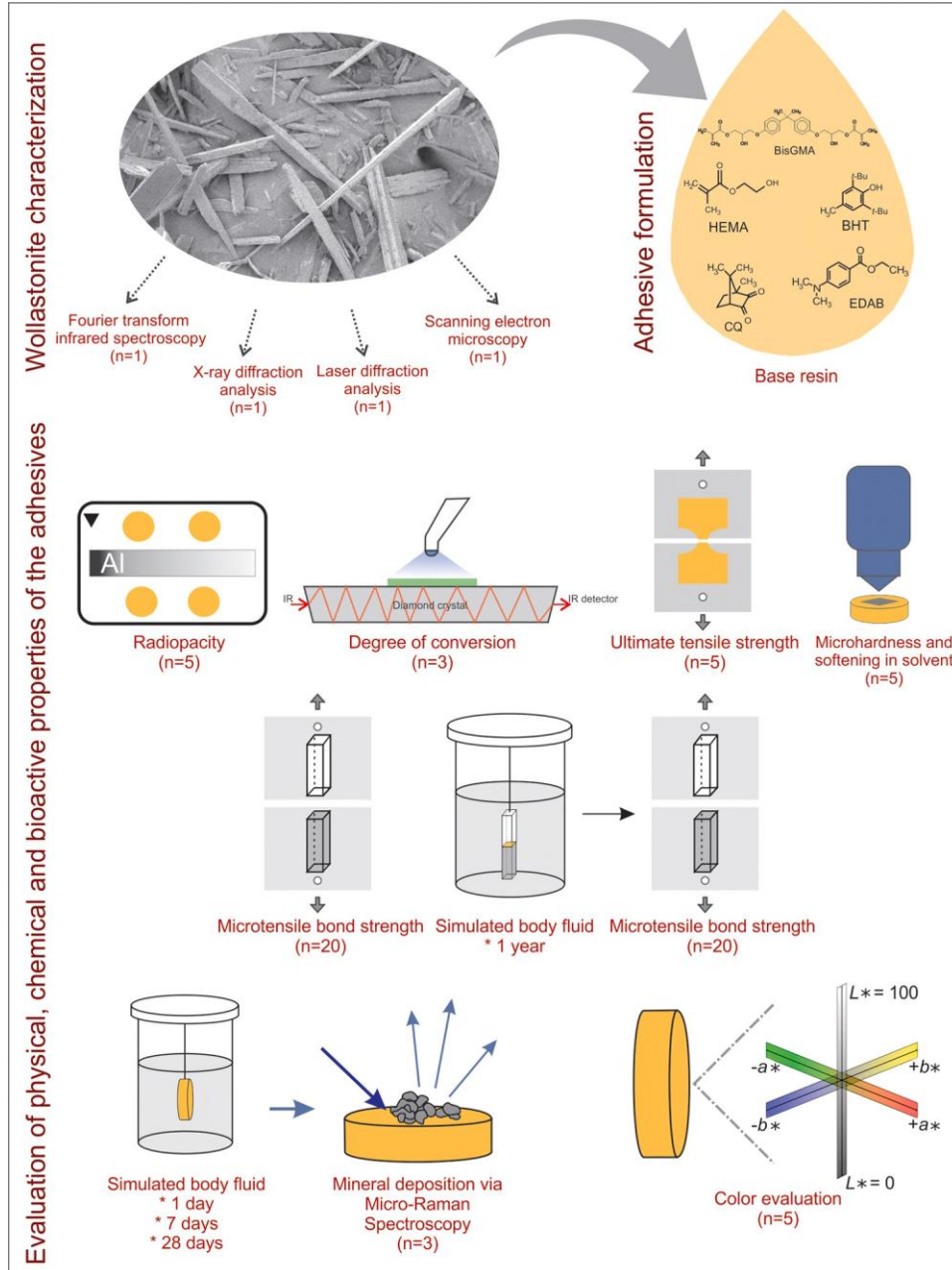
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Fig. 1. Schematic representation of the experimental procedures applied in the present study. The particles of wollastonite were characterized and used as filler for an experimental adhesive resin. The adhesive resins without wollastonite and 0.5 wt. %, 1 wt. %, or 2 wt. % of wollastonite were evaluated regarding their physical, chemical, and bioactive properties.
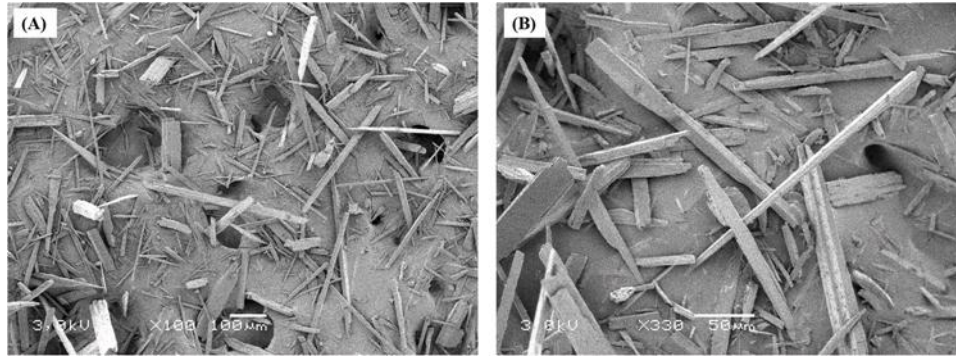
Fig. 2. SEM images of the CaSiO$_3$ used in the adhesives' formulation, evidencing the needle-like morphology of the particles. The images were acquired with 100 **(A)** and 330 **(B)** magnification.
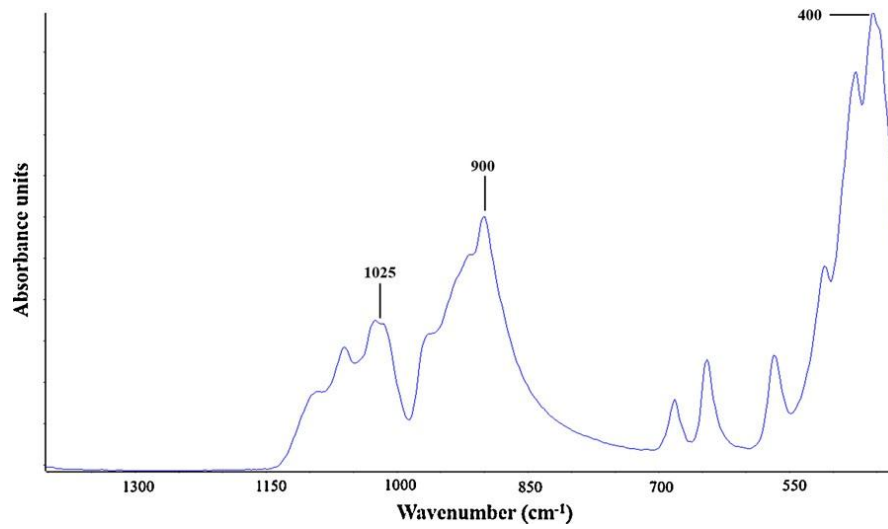

Fig.3. Chemical composition of CaSiO$_3$. The FTIR spectrum shows the band near 450 cm^{-1} related to Si–O bond.
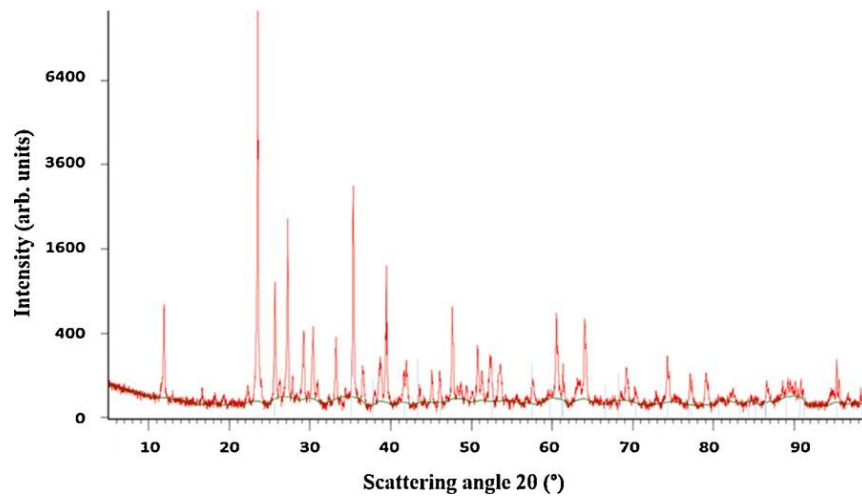
Fig.4. Characterization of phase composition of $CaSiO_3$ the X-ray diffraction analysis of $CaSiO_3$ evidences the β-$CaSiO_3$ phase.
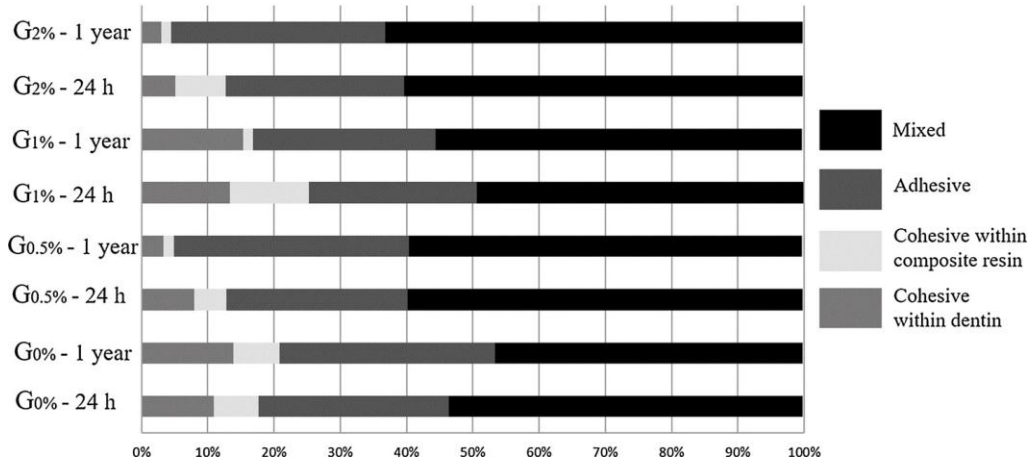
Fig.5. Failure pattern analysis of μTBS after 24 h or 1 year of storage in SBF showing that mixed and adhesive failures were the most predominant among groups.
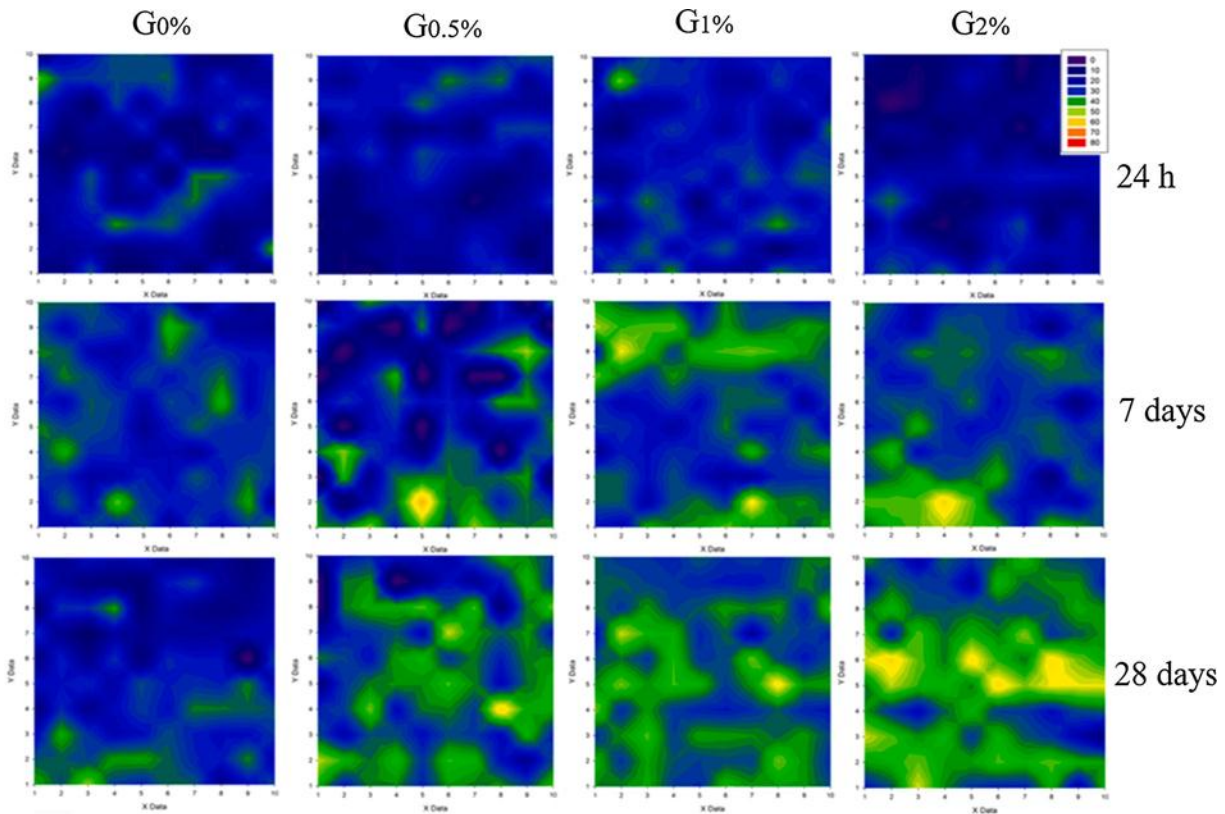
Fig.6 Micro-Raman analysis of the adhesives' surface after 24 h, 7 days, and 28 days of storage in SBF. The images show the mineral deposition on the adhesives' surface based on the intensity of the phosphate group (peak at 960 cm^{-1}). The higher the mineral deposition on the adhesives' surfaces, the more greenish areas are observed.

Table 1

Mean and standard deviation values of radiopacity, degree of conversion (DC%), initial Knoop hardness (KHN1), final Knoop hardness (KHN2), and difference of Knoop hardness in percentage (ΔKHN%) of the experimental adhesives.

Table 1	Radiopacity, DC (%), KHN1, KHN2 and Δ (KHN%)				
Groups	Radiopacity (pixels)	DC (%)	(KHN1)	(KHN2)	Δ (KHN %)
0%	31.9 (±4.6)^A	60.3 (±1.0)A	21.5 (±1.6)$^{B, a}$	8.2 (±1.4)^b	62.5 (±5.5)^A
0.5%	32.5 (±5.0)^A	60.9 (±1.6)A	21.9 (±0.5)$^{B, a}$	8.4 (±1.6)^b	61.5 (±5.9)^A
1%	35.9 (±6.0)^A	61.6 (±2.6)A	22.6 (±1.2)$^{B, a}$	8.5 (±1.4)^b	61.7 (±4.9)^A
2%	35.4 (±5.8)^A	59.1 (±1.0)A	25.6 (±2.7)$^{A, a}$	9.8 (±1.6)^b	61.5 (±5.3)^A

Different capital letters indicate a statistically significant difference in the same column ($p < 0.05$).
Different lowercase letters indicate a statistically significant difference in the same row ($p < 0.05$).

Table 2

Mean and standard deviation values of ultimate tensile strength (UTS) in MPa and microtensile bond strength (μTBS) in MPa of the experimental adhesives.

Table B	UTS (MPa) and μTBS (MPa)		
Groups	UTS (Mpa)	μTBS (Mpa)	
		24 h	1 year
0%	34.9 (±7.1)^B	61.0(±8.0)^{A,a}	43.4(±9.7)^{A,b}
0.5%	39.5 (±4.5)^{AB}	44.1(±12.9)^{AB,a}	40.4(±9.7)^{A,a}
1%	36.8 (±4.3)^{AB}	46.7(±9.1)^{AB,a}	47.7(±9.8)^{A,a}
2%	46.0 (±4.0)^A	40.6(±9.3)^{B,a}	47.4(±8.7)^{A,a}

Different capital letters indicate a statistically significant difference in the same column ($p < 0.05$).
Different lowercase letters indicate a statistically significant difference in the same row ($p < 0.05$).

4. CONSIDERAÇÕES FINAIS

Aumentar a longevidade clínica das restaurações de resina é uma preocupação para os dentistas clínicos e um dos objetivos dos pesquisadores da área odontológica. Para tanto, muitas pesquisas foram realizadas na tentativa de criar adesivos dentais com propriedades físico-químicas e biológicas melhoradas que possam resistir à degradação hidrolítica e permanecer estáveis ao longo do tempo. Com relação a esta questão, o reforço de resina adesiva à base de metacrilato com carga à base de silicato demonstrou resultados positivos, especialmente no aumento das propriedades físico-químicas e biocompatíveis de materiais à base de polímero (Profeta et al., 2013, Martins et al., 2014, Sfalcin et al., 2017 e Balbinot et al., 2020).

No presente estudo, a incorporação do $CaSiO_3$ é ilustrada como um obstáculo na propagação de fissuras, conforme demonstrado em resultados de resistência coesiva. Além disso, a sua capacidade de interagir com a matriz polimérica orgânica contribui com as propriedades elásticas gerais da matriz, reforçada a rigidez adesiva dentária (Sauro et al., 2012), sem dificultar a fotopolimerização adequada necessária para manter a resistência físico-mecânica de a matriz de polímero.

A avaliação da resistência da união adesiva após um ano de envelhecimento obtida para grupos de adesivos com wollastonita em comparação com o controle mostrou uma maior estabilidade. Isso pode estar relacionado à liberação de íons Ca^{2+} pela primeira troca com H^+, levando à formação de silanol $(Si-OH)$. Essa reação possui liberação de hidróxido de cálcio (Liu et al., 2004), que apresentou efeito na redução da atividade das MMPs na interface resina-dentina devido ao aumento da alcalinidade do meio (Breschi et al., 2008). Enquanto, a sílica hidratada carregada negativamente $(Si-O^-)$ formada na superfície fornece um local favorável para a nucleação da apatita (Liu et al., 2004). Íons de Ca^{2+} liberados foram inicialmente atraídos na camada rica em sílica antes dos íons de fósforo (PO_4^{-3}) da mídia circundante ou no substrato de dentina. Além disso, a formação de novos depósitos minerais ilustrados em imagens de espectroscopia Raman indicou mais deposição mineral na superfície das amostras adesivas preenchidas após 7 e 28 dias, que têm efeitos remineralizantes terapêuticos observados na interface resina-dentina. A formação de apatita na interface de dentina colada pode ter aumentado a formação de novos depósitos minerais sobre os constituintes minerais existentes na matriz dentinária (biocatalisação) (Gandolfi et al., 2010). Os quais, podem desempenhar um papel na proteção da interface resina-dentina dos ácidos produzidos, reduzir a nanoinfiltração e o

efeito desmineralizante das bactérias nos meios orais (Profeta et al., 2013 and Degrazia et al., 2018).

A aplicação de $CaSiO_3$ como nova carga inorgânica adicionada ao adesivo dentário promoveu deposição mineral e sendo na interface resina-dentina pode auxiliar na estabilidade da união adesiva, sem impedimento de fotopolimerização adequada, nem efeito na tonalidade da cor.

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