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Ionic liquid as antibacterial agent for an experimental orthodontic adhesive

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ABSTRACT

Objective. The aim of this study was to formulate and evaluate experimental orthodontic adhesives with different concentrations of 1-*n*-butyl-3-methylimidazoilium bis(trifluoromethanesulfonyl)imide (BMIM.NTf$_2$).

Methods. The experimental orthodontic adhesives were formulated with methacrylate monomers, photoinitiators and silica colloidal. The ionic liquid BMIM.NTf$_2$ was synthesized and characterized. BMIM.NTf$_2$ was added at 5 ($G_{5\%}$), 10 ($G_{10\%}$) and 15 ($G_{15\%}$) wt.%. One group contained no BMIM.NTf$_2$ to function as control (G_{Ctrl}). The adhesives were evaluated for polymerization kinetics, degree of conversion (DC), Knoop hardness and softening in solvent, ultimate tensile strength (UTS), shear bond strength (SBS), thermogravimetric analysis (TGA), antibacterial activity and cytotoxicity.

Results. BMI.NTf$_2$ showed the characteristic chemical peaks. The polymerization kinetics were different among the groups. $G_{10\%}$ and $G_{15\%}$ showed higher DC ($p < 0.05$). $G_{5\%}$ and G_{Ctrl} had no differences for softening in solvent ($p > 0.05$). There were no differences for UTS ($p > 0.05$) and SBS ($p > 0.05$). TGA showed one different peak for $G_{15\%}$. All groups with BMIM.NTf$_2$ showed antibacterial activity compared to G_{Ctrl} ($p < 0.05$) without cytotoxicity ($p > 0.05$).

Significance. To reduce biofilm formation around brackets and to prevent demineralization at susceptible sites, materials have been developed with antibacterial properties. In this study,

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a new experimental orthodontic adhesive was formulated with an imidazolium ionic liquid (BMIM.NTf_2) as antibacterial agent. The incorporation of 5 wt.% of ionic liquid decreased biofilm formation without affecting the physico-chemical properties and cytotoxicity of an experimental orthodontic resin.

1. Introduction

Fixed orthodontic appliances act as biofilm retention sites and increase caries lesions, compromising the treatment [1]. Inefficient hygiene around orthodontic brackets leads to higher *Streptococcus mutans* colonization [2], decreasing pH due to lactic acid production from bacterial metabolism and promoting enamel demineralization and white spot lesions (WSL). WSL are observed in 68.4% of orthodontic patients after one year of treatment [3]. To reduce its incidence, fluoridated resins have been proposed [4]. However, fluoridated resins showed no success in reducing decalcification process [4]. The development of orthodontic adhesives with antibacterial property is necessary to decrease *S. mutans* adhesion at bracket-enamel interface and to reduce demineralization at susceptible sites [5].

To create antibacterial orthodontic adhesives, quaternary ammonium compounds (QACs) [6–9], chlorhexidine gluconate [10], silver nanoparticles [11], zinc oxide [12], titanium dioxide nanoparticles [13] and triclosan-loaded halloysite [14] were previously added to resins. However, the release of antibacterial particles and tooth discolouration leading to anaesthetics appearance are reported for most of them [15]. In addition, there is a concern about antimicrobial resistance with these common agents. As a strategy to overcome these issues, ionic liquids have been proposed due to their versatile chemical structure, which is necessary for the design of drugs to accompany the growing challenge of antimicrobial resistance [16].

Ionic liquids are organic salts with low melting points (below 100 °C) and big cationic nuclei linked to an aliphatic chain associated to an anion. Despite being salts, ionic liquids present molecular properties such as geometry and dipole moment, and they can be presented in liquid phase even at room temperature [17], which occurs with the colourless ionic liquid 1-*n*-butyl-3-methylimidazoilium bis(trifluoromethanesulfonyl)imide (BMIM.NTf_2) [18]. The antimicrobial property of ionic liquids has been documented since the 1990s, indicating broad-spectrum antimicrobial activity and affecting gram-positive and gram-negative bacteria, mycobacteria and fungi [17]. Ionic liquids have been associated with polymers for drug delivery systems [19] and have been used to synthesize antibacterial polymer coatings [20] and antibacterial particles [21]. These salts have been studied as pharmaceutical solvents for drug delivery, especially those that are poorly soluble in water [22], besides being tested as active pharmaceutical compounds to overcome classical issues related to common salts (such as the polymorphism problem associated with salt drugs) [22]. Dicationic imidazolium-based ionic liquids with bis(trifluoromethylsulfonyl)imide (NTf_2) were tested to coat titanium implants and showed superior adhesion to the implants compared to imidazolium ionic liquids with other anions (amino acid-based), which was attributed to NTf_2's higher hydrophobicity [23]. Following this study, imidazolium ionic liquids were suggested to protect titanium surfaces against bacterial colonization while the conditions for cell survival and differentiation (human gingival fibroblasts and pre-osteoblasts) were maintained [24]. Furthermore, titanium implants lubrication and anticorrosion property were achieved with imidazolium ionic liquids [25]. Imidazolium ionic liquids were also used with silver nanoparticles, and it was suggested for endodontic disinfection due to its bactericidal effect against *Enterococcus faecalis* accompanied to higher cytocompatibility to fibroblasts (L929 cells) compared to 2.5% of sodium hypochlorite and 0.2% of chlorhexidine [21]. In a recent study, titania quantum dots involved by the ionic liquid BMIM.BF_4 were incorporated in an experimental adhesive resin, showing antibacterial activity with no cytotoxicity against pulp fibroblasts [26]. However, there are no reports about the effects of pure ionic liquids in dental resins. The aim of this study was to formulate and evaluate experimental orthodontic adhesives with different concentrations of BMIM.NTf_2. The null hypothesis to be tested is that the addition of BMIM.NTf_2 does not influence the orthodontic adhesives' properties.

2. Materials and methods

2.1. Ionic liquid formulation

The ionic liquid was formulated according to a previous study [18]. In a first step, butyl methanesulfonate was synthesized. Butanol (74.5 g, 1.0 mol), triethylamine (101.0 g, 1.0 mol) and CH_2Cl_2 (940 mL) were mixed in a 3 L flask. An external ice-water bath was used, and 114.5 g of methanesulfonyl chloride (1.0 mol) was slowly added under constant stirring. At the end of the addition, the reaction was placed at room temperature for 2 h. After the mixture was washed twice with water (190 mL and 125 mL, respectively), and the organic phase was dried with anhydrous $MgSO_4$. The dichloromethane was evaporated on the rotary evaporator, and the resulting ester was distilled under reduced pressure ($\tilde{1}$ mmHg, 84 °C) to give a colourless liquid (138.2 g, 0.91 mol, 91% yield).

In a second step, 1-butyl-3-methylimidazolium methanesulfonate was synthesized. Butyl methanesulfonate (138.2 g, 0.91 mol) was mixed with 1-methylimidazole (74.7 g, 0.91 mol), and the reaction mixture was kept at room temperature. After 24 h, one crystal of 1-butyl-3-methylimidazolium methanesulfonate was added to induce crystallization. After 72 h, the product was recrystallized using an acetone volume identical

to the reaction mass, and the mixture was kept in the freezer overnight. The supernatant yellow solution was decanted from the almost colourless crystals, and the recrystallization procedure was repeated. After drying under vacuum, colourless crystals of 1-butyl-3-methylimidazolium methanesulfonate were obtained (209.9 g, 96% yield, melting point at 77.2 °C).

The third step was the synthesis of the ionic liquid 1-*n*-butyl-3-methylimidazoilium bis(trifluoromethanesulfonyl)imide (BMIM.NTf$_2$). Lithium trifluoromethanesulfonimidate (50.0 g, 174 mmol) was dissolved in water (25 mL), and 1-butyl-3-methylimidazolium methanesulfonate (38.6 g, 165 mmol) was also dissolved in water (65 mL). Both solutions were mixed, vigorously stirred for 30 min, and dichloromethane (200 mL) was added. The organic phase was separated, washed with water (30 mL) and dried with MgSO4. Solvent evaporation afforded the desired BMIM.NTf$_2$ as a colourless liquid with a yield of 67.6 g (98%).

2.2. Ionic liquid characterization

The ionic liquid synthesized was analysed by Nuclear Magnetic Resonance (NMR) Spectroscopy and Infrared Spectroscopy. ^{1}H and ^{13}C NMR spectra were obtained (Gemini 2000 NMR Spectrometer, Varian, Paolo Alto, CA, USA) using 20 mg of sample dissolved in deuterated chloroform as solvent. The chemical shifts (δ) were related in parts per million (ppm) in relationship to the internal standard tetramethylsilane. It was used at 400 MHz for ^{1}H and 101 MHz for ^{13}C. For Fourier Transform Infrared (FTIR) Spectroscopy (Bruker, Alpha-P, Ettingen, Baden-Württemberg, Germany), one drop of the ionic liquid was dispensed on the attenuated total reflectance (ATR) device, and it was analysed from 400 to 4000 cm^{-1} with resolution of 4 cm^{-1}.

2.3. Experimental orthodontic adhesives formulation

The experimental orthodontic adhesives were formulated by mixing 75 wt.% of bisphenol A glycidyl methacrylate (BisGMA) and 25 wt.% of triethylene glycol dimethacrylates (TEGDMA). Camphorquinone, diphenyliodonium hexafluorophosphate and ethyl 4-dimethylaminobenzoate at 1 mol% according to monomer moles were added as photoinitiator system. As a polymerization inhibitor, 0.01 wt.% of hydroxytoluene butylated was added. Monomers, photoinitiators and hydroxytoluene butylated were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Colloidal silicon dioxide (Aerosil 200, Evonik, Essen, Germany) was added at 5 wt.% [5–7] to adjust the viscosity and improve the resins' properties. BMIM.NTf$_2$ was added at 5 ($G_{5\%}$), 10 ($G_{10\%}$) and 15 ($G_{15\%}$) wt.% to formulate three test groups. One group contained no BMIM.NTf$_2$ to be used as control (G_{Ctrl}). To formulate the experimental resin, BisGMA and TEGDMA were mixed for 5 min and ultrassonicated (L100; Schuster, Santa Maria, RS, Brazil) for 480 s. BMIM.NTf2 was added and hand-mixed for 5 min and ultrassonicated for 480 s. Afterwards, silica was incorporated and hand-mixed for 5 min and 480 s sonicated. All formulations were weighed using an analytical balance (AUW220D; Shimadzu, Kyoto, Japan). To perform photoactivation, a light-emitting diode unit was used (Radii Cal, SDI, Bayswater, Victoria, Australia) at 1200 mW/cm^2. The specimens for all tests were prepared and stored in distilled water for 24 h at 37 °C before testing, except for specimens of polymerization kinetics and degree of conversion (DC), which were prepared during the assay itself.

2.4. Polymerization kinetics and DC

To evaluate the polymerization behaviour of the experimental orthodontic adhesives, three samples per group were tested via FTIR Spectroscopy (Vertex 70, Bruker Alpha, Ettingen, Germany). The software Opus 6.5 (Bruker Optics, Ettlingen, Germany) was used to obtain the data with Blackman Haris 3-Term apodization in the range of 400 to 4000 cm^{-1} with 2 scans per second at a 10 kHz velocity and resolution of 4 cm^{-1}. Each sample was placed on the diamond crystal of ATR device using a silicon matrix ofpoly (vinyl siloxane) with light-body density (Express, 3 M/ESPE, St. Paul, MN, USA) with 4 mm diameter and 1 mm thickness to certify that all specimens would present the same thickness. The samples were analysed for 40 s of photoactivation. Data obtained via the spectrometer were plotted, and curve fit was used to acquire the graphs. Formula 1 was used to calculate the DC after 40 s of photoactivation and the results were expressed as percentage [27]. To calculate the polymerization rate (Rp), the DC at time *t* was subtracted from the DC at time *t*-1 [26].

$$DC\,(\%) = 100 \times \left(\frac{peak\ height\ of\ cured\ aliphatic\ \ C{=}C / peak\ height\ of\ cured\ aromatic\ \ C{=}C}{peak\ height\ of\ uncured\ aliphatic\ C{=}C / peak\ height\ of\ uncured\ aromatic\ C{=}C} \right) \quad (1)$$

2.5. Softening in solvent

To evaluate the softening in solvent, five specimens per groups were prepared (n = 5, 1.0 mm thickness ×4.0 mm diameter) with photoactivation for 20 s on each side. The specimens were embedded in self-cure acrylic resin and polished (Model 3v, Arotec, Cotia, SP, Brazil) with silicon carbide sandpapers (1000, 1200 and 2000 grit) followed by a felt disc saturated with alumina suspension (Alumina, 0.5 μm, Arotec, Cotia, SP, Brazil). The specimens were washed with distilled water for 15 min in ultrasonic bath. After 24 h, the specimens were tested to obtain the initial Knoop hardness (KHN1) with five indentations (10 g for 10 s) on each surface (HMV2, Shimadzu, Tokyo, Japan). The specimens were immersed for 2 h in 50:50 ethanol:water solution [28], and the final Knoop hardness (KHN2) was analysed. The difference between KHN1 and KHN2 was expressed as a percentage (ΔKHN%).

2.6. Ultimate tensile strength (UTS)

The experimental orthodontic adhesives were evaluated for UTS using fifteen specimens per group (n = 15). The unpolymerized orthodontic adhesives were inserted into an hourglass-shaped metallic matrix (8 mm long, 2 mm wide, 1 mm thickness, cross-sectional area of 1 mm^2) and photoactivated for 20 s on each side. The specimens were submitted to microtensile strength test using a universal testing machine

(Shimadzu EZ-SX, Shimadzu Co., Kyoto, Japan) at a crosshead speed of 1 mm/min until failure. Data were recorded in newton (N) and divided for the area of each specimen to achieve values in megapascals (MPa).

2.7. Shear bond strength (SBS)

To evaluate the SBS, human pre-molars were used. The teeth were obtained under a protocol approved by the local Ethics Committee (no 19687), and informed consent was obtained from all participants included in the research. Forty-eight premolar teeth (n = 12) were stored up to 3 months in distilled wa Please check the presentation of Tables 1 and 2, and correct if necessary. ter at 4 °C. The teeth were fixed with the buccal face perpendicular to an acrylic resin base. The buccal surface of each tooth was conditioned with phosphoric acid at 37% for 30 s, which was rinsed with running water for 30 s and dried with oil-free compressed air for 5 s. A thin layer of each orthodontic adhesive resin was placed on the bracket base to completely cover it. One bracket per tooth (Roth Max, Morelli, Sorocaba, SP, Brazil) was fixed with the experimental orthodontic adhesives and kept under pressure (with 300 g applied on the bracket surface) [7]. The excess resin around the brackets was removed in each tooth with a sharp dental probe [14] and then photoactivated for 10 s on each face of the bracket [7]. The specimens were stored in distilled water for 24 h at 37 °C before being tested for SBS. Using a knife-edge chisel (0.1 mm), the specimens were subjected to SBS test using a universal testing machine (Shimadzu EZ Test EZ-SX, Kyoto, Japan) with 1 mm/min until the moment that the bracket was detached. The results were recorded in N and divided per each area to achieve in MPa.

2.8. Thermogravimetric analysis (TGA)

For TGA analysis, one specimen per group (4 mm diameter, 1 mm thickness) was prepared after 20 s of photoactivation on each side. The specimens from each group were analysed (10 mg) via SDT Q600 (TA Instruments, New Castle, Delaware, USA) operated under nitrogen gas and were heated until 800 °C at a rate of 20 °C min^{-1}. TGA and derivative thermogravimetric (DTG) curves were measured by Advantage Software v5.5.20 (Universal Analysis, TA Instrument, New Castle, Delaware, USA) and plotted in Origin 9.0 Software (OriginLab, Northampton, Massachusetts, USA).

2.9. Antibacterial activity against biofilm formation and planktonic bacteria

To evaluate the antibacterial activity, three specimens per group of the experimental orthodontic adhesives were prepared (n = 3, 1.0 mm thickness ×4.0 mm diameter) with photoactivation for 20 s on each side. The specimens were attached on the lid of a 48-well plate, and this assembly was submitted to hydrogen peroxide plasma (58%) sterilization for 48 min at 56 °C. In each well of the 48-well plate, 900 μL of brain-heart infusion broth (BHI) with 1 wt.% of sucrose and 100 μL of a suspension of an overnight broth culture of *S. mutans* (NCTC 10449) at 10^6 CFU/mL were added. The 48-well plate containing the specimens was incubated for 24 h at 37 °C [26].

To evaluate the antibacterial activity against biofilm formation on orthodontic adhesive surface, the specimens were used that were in contact for 24 h with the BHI broth containing *S. mutans*. The specimens were removed from the lid of the 48-well plate and vortexed for 1 min in 1 mL of sterile saline solution (0.9%) in an Eppendorf tube. The solution of the first tube was diluted until 10^{-6} mL of saline solution (serial dilution). Two drops of 25 μL each from each Eppendorf tube were plated on BHI-agar Petri dishes and incubated for 48 h at 37 °C [26,29].

To evaluate the antibacterial activity against planktonic *S. mutans*, 100 μL of each well from the 48-well plate were removed and inserted in Eppendorf tubes containing 900 μL of sterile saline solution in each (0.9%). The solutions were diluted until 10^{-6} mL of saline solution (serial dilution) and plated and incubated on BHI-agar Petri dishes as described above [26,30]. The number of colonies was visually counted in all plates for biofilm formation analysis and for planktonic bacteria viability analysis. With the number of colonies observed, the dilution factor for each specimen (for biofilm analysis) and for each well (for planktonic bacteria analysis) was used to calculate the colony forming units per millilitre (CFU/mL). The values of CFU/mL were represented via logarithmic transformation of CFU/mL.

2.10. Cytotoxicity

For the cytotoxicity evaluation of experimental orthodontic adhesives, three specimens per group (n = 3, 1.0 mm thickness ×4.0 mm diameter) were prepared after 20 s of photoactivation on each side. The specimens were stored in 1 mL of Dulbecco's Modified Eagle Medium (DMEM) for 24 h to prepare the eluates. Human keratinocytes—HaCaT (CLS Cell Lines Service GmbH, Eppelheim, Germany)—were placed at 5×10^3 per well in 96-well plates to be treated with 100 μL of eluate from each specimen. After 72 h of incubation, cells were fixed on the bottom of the wells by trichloroacetic acid (Sigma-Aldrich Chemical Co, St. Louis, Missouri, USA) at 10%. The plates were incubated for 1 h at 4 °C, washed six times with running water during 30 s and dried at room temperature. Sulforodamide B (Sigma-Aldrich Chemical Co, St. Louis, Missouri, USA) at 0.4% was added in each well. The plates were incubated for 30 min at room temperature. Plates were washed with acetic acid at 1% four times and dried at room temperature. Trizma solution was added in each well, and the plates were incubated for 1 h at room temperature. The 96-well plates were read at 560 nm, and the cell viability was normalized against cell viability achieved in wells with no eluates from specimens. The cell viability was expressed in percentage [26].

2.11. Statistical analysis

The normality of the data was analysed using Shapiro–Wilk test. The data were analysed by one-way ANOVA for all evaluations, except for the comparisons between KHN1 and KHN2 for each group, which were analysed by paired t-test. If there were statistically significant differences among groups,

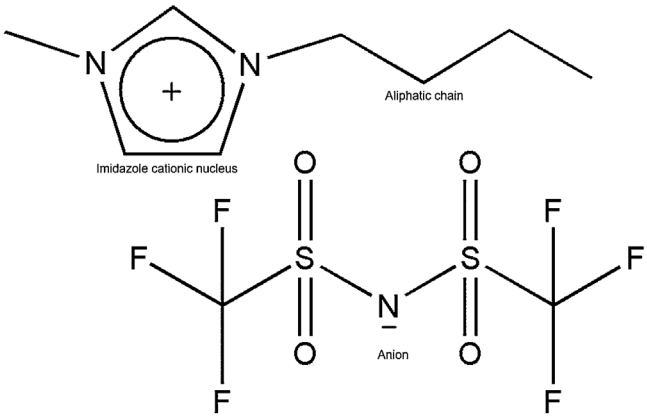

Fig. 1 – Chemical structure of the ionic liquid 1-*n*-butyl-3-methylimidazoilium bis(trifluoromethanesulfonyl)imide (BMIM.NTf$_2$) used in the study to formulate the experimental orthodontic adhesives. The three main components of the ionic liquid are indicated in the figure: imidazole cationic nucleus, aliphatic chain and anion.

Tukey's multiple comparisons test was applied. Significance level at 5% was used.

3. Results

Fig. 1 shows the molecular structure of BMIM.NTf$_2$, and Fig. 2 shows the results of RMN and FTIR spectroscopies. The analysis of ^{1}H in the RMN spectrum (Fig. 1a) showed the following peaks: 8.64 (s, 1 H), 7.39 (t, J =1.8 Hz, 1 H), 7.36 (t, J =1.8 Hz, 1 H), 4.17 (t, J = 7.5 Hz, 2 H), 3.93 (s, 3 H), 1.86 (p, J = 7.6 Hz, 2 H), 1.37 (dq, J = 14.8, 7.4 Hz, 2 H), 0.95 (t, J = 7.4 Hz, 3 H). The analysis of ^{1}C in the RMN spectrum (Fig. 1b) showed the following peaks: 135.61, 124.49, 123.66, 123.38, 122.34, 122.28, 121.30, 118.10, 114.91, 77.46, 77.34, 77.15, 77.00, 76.83, 49.72, 36.13, 36.02, 31.91, 31.75, 31.57, 19.29, 19.13, 18.95, 12.97, 12.92. The FTIR spectroscopy showed band characteristics of the stretching of the C–N bond of the imidazole ring around 1351 cm^{-1}, and the bands of the bistrifluorosulfonylimide anion around 1137 and 1182 cm^{-1} for the S=O bonds of sulfone and at 1055 cm^{-1} for the C–F bond. The main bands observed were 1568 cm^{-1} related to C=C, 1351 cm^{-1} related to C–N, 1182 and 1037 cm^{-1} related to S=O, 1055 cm^{-1} to C–F and 3154 cm^{-1} related to Csp2-H.

Fig. 3 shows the polymerization kinetics of the experimental orthodontic adhesives. $G_{10\%}$ and $G_{15\%}$ began the polymerization process before G_{Ctrl} and $G_{5\%}$ (Fig. 3a), and they achieved the maximum Rp earlier than G_{Ctrl} and $G_{5\%}$ (Fig. 3b). The DC (Table 1) ranged from 64.04 (±1.06)% for $G_{5\%}$ to 70.67 (±0.41)% for $G_{15\%}$, with higher values for $G_{10\%}$ and $G_{15\%}$ compared to G_{Ctrl} ($p < 0.05$).

The results of KHN1 ranged from 25.12 (±1.59) for $G_{15\%}$ to 32.24 (±0.93) for G_{Ctrl}, with lower values found for $G_{10\%}$ and $G_{15\%}$ compared to G_{Ctrl} ($p < 0.05$) and no statistically significant difference between G_{Ctrl} and $G_{5\%}$ ($p > 0.05$). All groups had decreased Knoop hardness values after being immersed for 2 h in solvent ($p < 0.05$). The groups $G_{10\%}$ and $G_{15\%}$ had statistically significant higher ΔKHN compared to G_{Ctrl}. There was no statistically significant difference between G_{Ctrl} and $G_{5\%}$ ($p > 0.05$) (Table 1).

The results of UTS and SBS (Table 1) show that UTS ranged from 24.31 (±7.78) MPa to 32.89 (±8.70) MPa, without statistically significant differences among groups ($p > 0.05$). The SBS ranged from 13.90 (±3.95) MPa to 18.14 (±5.14) MPa, without statistically significant differences among groups ($p > 0.05$).

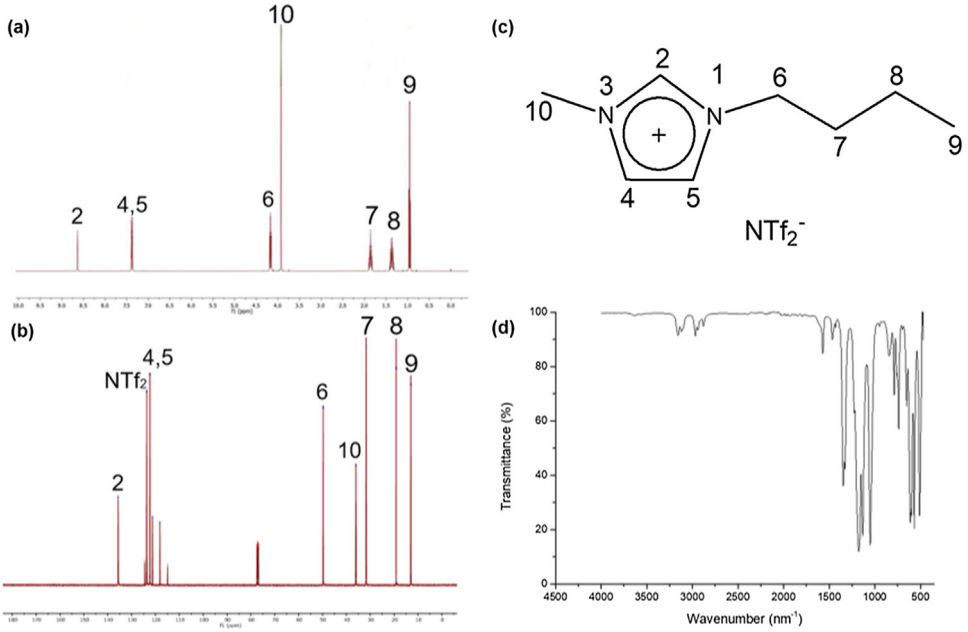

Fig. 2 – Results of RMN Spectroscopy and FTIR Spectroscopy of BMIM.NTf$_2$ synthesized. Image (a) shows spectrum of RMN analysis of ^{1}H and image (b) shows spectrum of RMN analysis of ^{1}C. Image (a) and (b) present numbers in each peak of spectra related to the same numbers in the molecular structure of BMIM.NTf$_2$ represented in image (c). Image (d) shows the spectrum of FTIR Spectroscopy.

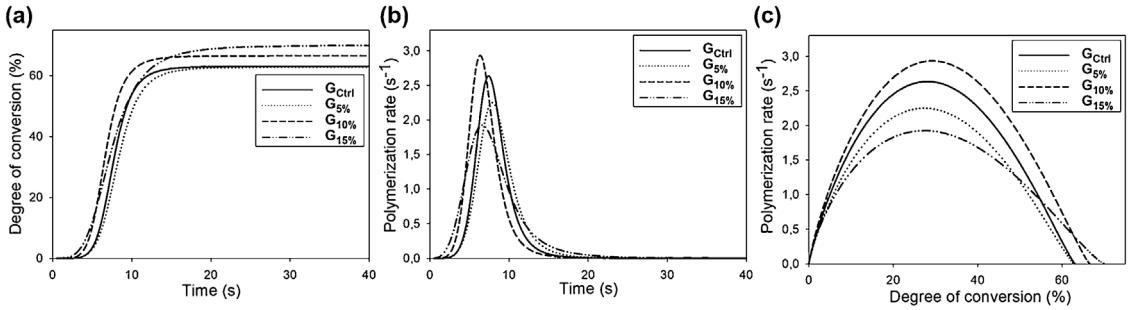

Fig. 3 – Polymerization kinetics graphs during photoactivation for 40 s of the experimental orthodontic adhesives. DC *versus* photoactivation time (a). Rp *versus* photoactivation time (b). DC *versus* Rp (c).

Table 1 – Mean and standard deviation values of degree of conversion (DC) after 40 s of photoactivation, initial Knoop hardness number (KHN1), final Knoop hardness number (KHN2), percentage of Knoop hardness variation (ΔKHN), ultimate tensile strength (UTS) and shear bond strength (SBS) of experimental orthodontic adhesives.

	DC (%)	KHN1	KHN2	ΔKHN (%)	UTS (MPa)	SBS (MPa)
G_{Ctrl}	64.56 (±0.68)^{C}	32.24 (±0.93)^{Aa}	25.57 (±0.51)^{b}	20.98 (±2.75)^{B}	32.89 (±8.70)^{A}	17.98 (±7.47)^{A}
$G_{5\%}$	64.04 (±1.06)^{C}	31.62 (±1.04)^{Aa}	22.79 (±1.32)^{b}	27.68 (±4.05)^{B}	32.13 (±9.80)^{A}	14.64 (±2.96)^{A}
$G_{10\%}$	68.24 (±0.44)^{B}	27.76 (±1.57)^{Ba}	17.69 (±0.90)^{b}	36.45 (±4.03)^{A}	31.65 (±10.14)^{A}	13.90 (±3.95)^{A}
$G_{15\%}$	70.67 (±0.41)^{A}	25.12 (±1.59)^{Ca}	14.37 (±0.90)^{b}	41.69 (±4.64)^{A}	24.31 (±7.78)^{A}	18.14 (±5.14)^{A}

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

TGA analysis (Fig. 4) shows 2 mainly decreased areas for each group with low or no differences of decomposition temperature among them. It is possible to observe that $G_{15\%}$ had one more DTG peak at 488.24 °C compared to other groups.

Table 2 shows the results of antibacterial activity and cytotoxicity tests. The test against biofilm formation showed lower values for all groups with BMIM.NTf$_2$ compared to G_{Ctrl} ($p < 0.05$). $G_{15\%}$ showed the lowest log CFU/mL (1.40 ± 0.17) among all groups ($p < 0.05$). In the evaluation against planktonic bacteria, all orthodontic adhesives with BMIM.NTf$_2$ presented antibacterial activity compared to G_{Ctrl} ($p < 0.05$), without statistically significant difference between $G_{10\%}$ and $G_{15\%}$ ($p > 0.05$). In the cytotoxicity analysis, all groups achieved mean values of keratinocytes viability higher than 90%. The viability ranged from 93.10 (±6.87)% for $G_{10\%}$ to 97.01 (±1.43)% for $G_{5\%}$ without statistically significant difference among groups ($p > 0.05$).

4. Discussion

The prevalence of white spot lesions in enamel increases during orthodontic treatment [1]. In an attempt to reduce biofilm formation around brackets and prevent demineralization at susceptible sites, materials for fixed orthodontic appliances have been developed with antibacterial properties [5–911,14,31]. In this study, the ionic liquid BMIM.NTf$_2$, was added to an experimental orthodontic adhesive at different concentrations. The incorporation of any tested concentration provided antibacterial activity without cytotoxic effect for human keratinocytes. Higher concentrations of BMIM.NTf$_2$ were shown to negatively affect the softening in solvent behaviour of the orthodontic adhesive. However, the addition of 5 wt.% of BMIM.NTf$_2$ presented no differences for physicochemical properties compared to G_{Ctrl}. Therefore, the null hypothesis proposed must be rejected.

Although described more than a century ago [32], ionic liquids have been increasingly studied due to the significant advances in understanding their chemical, physical and biological properties, promoting ionic liquids' wide application for biomedical materials [19,21–26]. The higher the BMIM.NTf$_2$ addition, the higher the DC that was achieved. One could think that the charges of the ionic liquid could interact with the monomers via hydrogen bond or electron transference [17], leading to these higher values for $G_{10\%}$ and $G_{15\%}$. However, electronic resonance occurs in BMIM.NTf$_2$, decreasing chances of protons (H^+) acquisition from tertiary amine or electrons providing for C=C of BisGMA and TEGDMA. The polymerization kinetics evaluation showed details of the chemical process that supported the elucidation of this result. The polymerization kinetics indicated that with 10 and 15 wt.% of BMIM.NTf$_2$, the polymerization reaction started before G_{Ctrl} and $G_{5\%}$. In addition, with about 10 s of photoactivation, G_{Ctrl} and $G_{5\%}$ had achieved almost the maximum DC, while it continued increasing for $G_{10\%}$ and $G_{15\%}$, which presented higher DC at the end of the photoactivation. With the incorporation of 10 and 15 wt.% of BMIM.NTf$_2$, the viscosity of the orthodontic adhesive may have decreased, increasing monomers' chain mobility and increasing the DC [33]. It is possible to observe that $G_{15\%}$ presented lower maximum Rp compared to other groups, which may be related to the incorporation of a higher amount of a compound with no C=C chemical bonds (BMIM.NTf$_2$), since previous study indicated that blends with lower C=C bonds have lower degrees of functionality and lower maximum Rp [34].

The DC achieved by all groups were in accordance with commercial adhesives [35], and higher values were obtained

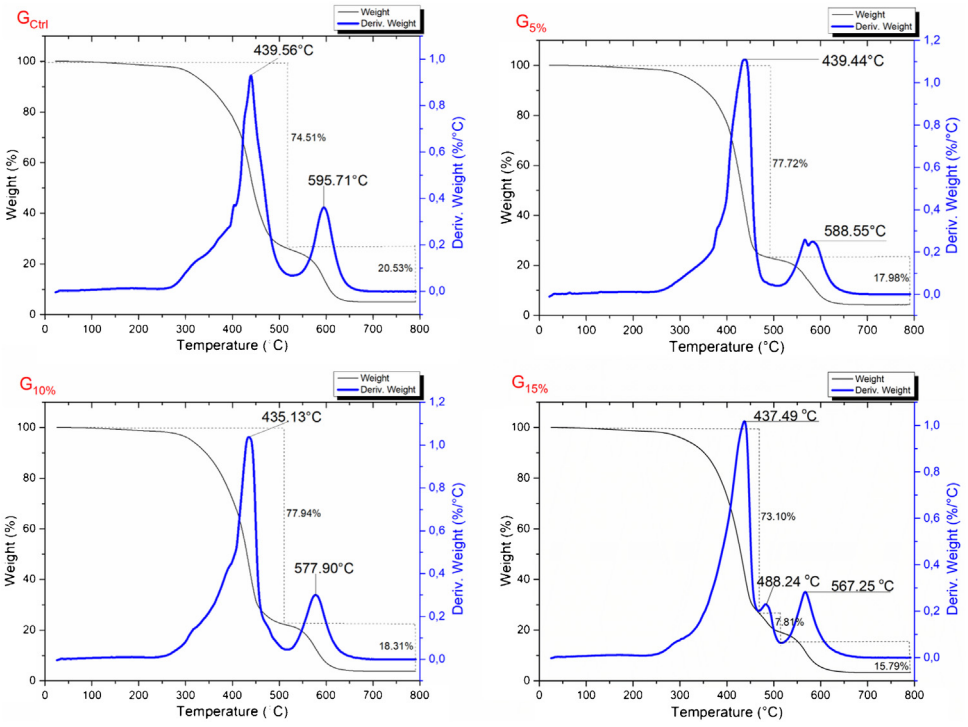

Fig. 4 – Graphs of thermogravimetric analysis of each experimental orthodontic adhesive. A previous study showed that BisGMA presented stronger degradation peak at around 415 °C, while TEGDMA showed peaks at 306 °C and 400 °C. In composite resins of BisGMA and TEGDMA with higher concentration of BisGMA, it was observed that the peak of TEGDMA almost disappears and the peaks change to one single strong peak upper to 424 °C [56]. In our study, there is a high intensity peak at around 435 °C probably related to BisGMA degradation and a shoulder probably related to TEGDMA degradation. This result indicates that the composition contains aromatic and aliphatic chains and that the monomer with aromatic component (BisGMA) is at higher concentration than aliphatic monomers (TEGDMA) [56]. All groups present a peak higher than 500 °C that is probably related to the silanols condensation at silica's surface [57,58]. An additional peak is present in $G_{15\%}$ graph at 488.24 °C.

Table 2 – Mean and standard deviation values of bacteria viability in colony forming units per milliliter (CFU/mL) after logarithmic transformation and human keratinocytes viability in percentage (%) after cytotoxicity test.

	Biofilm analysis (log CFU/mL)	Planktonic bacteria analysis (log CFU/mL)	Human keratinocytes (%)
G_{Ctrl}	4.12 (±0.02)^{A}	8.35 (±0.02)^{A}	95.04 (±1.05)^{A}
$G_{5\%}$	2.53 (±0.04)^{B}	8.12 (±0.09)^{B}	97.01 (±1.43)^{A}
$G_{10\%}$	1.79 (±0.20)^{C}	7.25 (±0.08)^{C}	93.10 (±6.87)^{A}
$G_{15\%}$	1.40 (±0.17)^{D}	7.20 (±0.06)^{C}	96.20 (±1.48)^{A}

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

with more BMIM.NTf_2. However, a possible increase in monomers' mobility and plasticization with 10 and 15 wt.% of BMIM.NTf_2 was reflected in the Knoop hardness and softening in solvent. It is well-known that DC and plasticization are not correlated because it is possible to achieve high DC with lower crosslinking density [36]. $G_{10\%}$ and $G_{15\%}$ showed lower KHN1 and higher ΔKHN compared to G_{Ctrl}, which may be explained by increased linear areas with increasing amounts of ionic liquid. Because BMIM.BF_4 has no C=C bond and probably increased monomers' mobility, less crosslinking density may be formed with higher BMIM.NTf_2 addition, favouring the penetration of Knoop diamond in the surface and decreasing KHN1 values [36]. Furthermore, linear polymers are more prone to softening when in contact with alcohol [36]. Thus, the attraction inside the polymer is overtaken by the attraction of polymer and solvent molecules [36]. On other hand, with the addition of the lowest BMIM.NTf_2 concentration tested, there was no differences either in the DC or in the KHN1 and ΔKHN compared to G_{Ctrl}. This suggests that $G_{5\%}$ may had no different resin matrix formation, supporting the stability of the material after 2 h in contact with the alcoholic solution.

Despite the differences observed for softening in solvent analysis and the higher DC with higher amounts of BMIM.NTf_2, the analyses of UTS and SBS showed no differences among groups. Despite the fact that no difference was observed for UTS, this test evaluates only the resistance to failure of the pure material, without regard to adhesion process. In this way, the SBS was evaluated using brackets

adhered to human pre-molars' enamel [8]. The incorporation of BMIM.NTf$_2$ up to 15 wt.% also did not change the SBS, which is in accordance to a previous meta-analysis showing that the addition of antibacterial agents to orthodontic adhesives does not influence the SBS [31]. Even though there is no consensus about values predicting clinical outcome [37,38] due to the variation of test parameters [31], probably higher bond strength leads to higher longevity of bracket retention as observed with dental restorations [39,40]. Interestingly, TGA analysis showed one more peak of DTG compared to other groups (488.24 °C), which may be related to the formation of a polymer area different from the major part of the specimen. Considering the observed results of softening in solvent and polymerization kinetics, this DTG peak may have been formed by a more linear area surrounded by more crosslinked network. Differences in polymer formation can be observed via dynamic mechanical analysis [41] and DTG evaluation via TGA analysis [42], which may indicate the heterogeneity of the material. Therefore, even with no differences for UTS and SBS, we can expect that higher amounts of BMIM.NTF$_2$ indeed decrease the crosslinking of the polymer. The use of 5 wt.% of BMIM.NTf$_2$ was shown to be the more suitable concentration tested for the experimental orthodontic adhesive.

During orthodontic treatment, inefficient hygiene around brackets leads to higher oral colonization by *S. mutans* [2], bacteria positively correlated with caries [43]. Orthodontic adhesives with antibacterial property that are able to reduce *S. mutans* adhesion and biofilm formation around brackets can minimize or prevent enamel demineralization [5], thereby decreasing caries development. Some ionic liquids were previously tested and have already shown activity against *S. mutans*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus gordonii* and *Streptococcus uberis* [24]. The specimens of experimental orthodontic adhesives with BMIM.NTf$_2$ had lower biofilm formation of *S. mutans*. The higher the concentration of BMIM.NTf$_2$, the lower the biofilm formation. In addition, the orthodontic adhesives with BMIM.NTf$_2$ showed activity against planktonic bacteria. The reduced viability of planktonic *S. mutans* in broth is related to the leaching of compounds [44] and/or due to the direct contact of antibacterial agents on the polymer's surface with the bacteria in broth [45,46]. Because we observed via softening in solvent, polymerization kinetics and TGA analysis, it is possible the BMIM.NTf$_2$ was released due to the formation of linear areas in the polymer with the addition of higher amounts of this ionic liquid. Quantifying the BMIM.NTf$_2$ leached from the polymer may help explain the mechanism of the ionic liquid in the network regarding the physico-chemical and biological properties. However, it is possible to conclude with this study that researches focused on the development of copolymerizable ionic liquids or different ways to carry them in the resin matrix are promising areas to be studied in an attempt to improve polymers formation.

In this study, the addition of BMIM.NTf$_2$ to an experimental orthodontic adhesive inhibited biofilm formation and decreased the viability of planktonic *S. mutans* in broth due to its cationic group (imidazole) associated to an aliphatic chain and a hydrophobic anion [16]. The imidazole group presents positive charge responsible for the electrostatic attraction with negative compounds from bacteria's wall and membrane, as teichoid acid, lipoteichoic acid and *N*-acetylmuramic acid [16,47], which were previously illustrated [26]. The hydrophobic elements of the IL (alkyl chain and NTf$_2$) may assist membrane disorganization. Moreover, as occurs with QACs, it is possible that ionic liquids may coagulate and precipitate constituents of the cytoplasmic matrix, such as nucleic acids, and cause denaturation of enzymes, which should be further investigated [48,49]. Critical cellular functions such as ATP synthesis, energy transfer and substrate oxidation may be also affected by the inhibition of enzymes related to cellular respiration [50]. In addition to the presented antibacterial activity, ionic liquids have been suggested as promising alternatives to face antimicrobial resistance due to their chemical versatility. Due to the wide range of cations and anions possible pairing, it was estimated there are 10^{18} accessible ionic liquids [51]. Thus, the wider use of ionic liquids in other biomaterials is expected.

The characteristics of a more hydrophilic organic group positively charged with one or more hydrophobic chains observed in ionic liquids and cationic surfactants, as QACs, lead to structural and functional analogies shared for these materials [16]. In addition to the similar antibacterial activity mechanism, cytotoxic effects are reported for both substances depending on chain length. With a longer alkyl chain, higher hydrophobic property is achieved, leading to higher antimicrobial activity and higher cytotoxicity for ionic liquids and QACs [24,52,53]. All experimental orthodontic adhesives showed no cytotoxicity, as the cell viability was higher than 70% for all groups, suggesting that there was no leaching of cytotoxic compounds for broth [54]. The cytotoxicity evaluation was based on SRB colorimetric test for cell density determination, which is a reliable method widely used with better predictive power over MTT assay [55] recommended by ISO [54]. The choice for BMIM.NTf$_2$ probably positively influenced the non-cytotoxicity. The ionic liquid used in the experimental orthodontic adhesive presents a short alkyl chain, which may assist the non-cytotoxicity against mammalian cells. Moreover, previous authors have suggested that while prokaryotic cells' membranes are mainly constituted by negative charges, facilitating the interaction with ionic liquids, mammalian cells' membrane is composed mainly of lecithins, sphingolipids and sterols, making its charge net neutral [47]. Consequently, the electrostatic attraction between mammalian cells and the positive imidazole group is hindered, assisting the biocompatibility property [16].

In this study, new antibacterial orthodontic adhesives were formulated. Despite the fact that higher concentrations tested (10 and 15 wt.%) increased the softening in alcohol solution, the addition of 5 wt.% maintained the physico-chemical properties evaluated of the orthodontic adhesive and induced antibacterial activity without cytotoxicity for human keratinocytes. Because ionic liquids present versatile chemical structures, they could be promising in other biomaterials to reduce bacterial viability and biofilm formation.

5. Conclusion

The addition of BMIM.NTf$_2$ at 5 wt.% did not change the physico-chemical properties of the orthodontic adhesive

and provided antibacterial activity with no cytotoxic effects against human keratinocytes.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.dental.2019.05.010.

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