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Linha de Pesquisa: Biomateriais e Técnicas Terapêuticas em Odontologia

DESENVOLVIMENTO E CARACTERIZAÇÃO DE ADESIVO ORTODÔNTICO CONTENDO TRIAZINA E VIDRO BIOATIVO NIÓBIO-FOSFATO

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Dissertação apresentada como requisito obrigatório para obtenção do título de **Mestre em Odontologia** na área de Concentração em Clínica Odontológica.

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"O desejo profundo da humanidade pelo

conhecimento é justificativa suficiente

para nossa busca contínua."

(Stephen Hawking)

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Nota Preliminar

Esta dissertação foi redigida de acordo com a Resolução 093/2007 do Conselho de Ensino, Pesquisa e Extensão da Universidade Federal do Rio Grande do Sul, estando enquadrada nas formas descritas nos itens "b" e "c", do artigo 3° da resolução: "Tese, Dissertação ou Trabalho de Conclusão de Curso que contenham artigo(s) pronto(s) para submissão à publicação"; e "Tese, Dissertação ou Trabalho de Conclusão de Curso que contenham artigo(s) já publicado(s)".

Resumo

Pacientes ortodônticos apresentam grande prevalência de lesões de mancha branca devido ao maior acúmulo de biofilme em torno dos *brackets*. O objetivo deste estudo foi desenvolver um adesivo ortodôntico que apresentasse atividade antibacteriana e que estimulasse a deposição mineral para evitar o aparecimento e/ou a evolução destas lesões. Adesivos experimentais foram formulados contendo 75% de BisGMA e 25% de TEGDMA por peso; sistema fotoiniciador a base de canforoquinona e 5% de sílica coloidal. Os compostos 1,3,5-triacriloilhexahidro-1,3,5-triazina (TAT) e vidro fosfato invertido contendo pentóxido de nióbio (PIG-Nb) foram adicionados ao adesivo base, como agente antibacteriano e remineralizante, respectivamente. Foram analisados grupos contendo somente TAT nas concentrações de 10, 15 e 20% por peso; grupos contendo somente PIG-Nb nas concentrações de 1, 2,5 e 5% por peso e um grupo contendo 20% TAT e 5% PIG-Nb, por peso. Utilizou-se um grupo sem a adição de TAT e PIG-Nb como Grupo Controle, dentre os experimentais. O adesivo ortodôntico comercial Transbond XT foi utilizado para comparação. Avaliou-se a atividade antibacteriana, capacidade de deposição mineral, variação de pH, grau de conversão, amolecimento em solvente e resistência de união dos adesivos ortodônticos. Os adesivos desenvolvidos apresentaram atividade antibacteriana e capacidade de estimular deposição mineral sem prejudicar as propriedades dos adesivos. Os resultados obtidos a partir dos ensaios realizados neste trabalho permitem concluir que se trata de material com propriedades favoráveis e potencialidade de, após ajustes necessários, tornar-se viável para uso na prática odontológica.

Palavras-chave: antibacteriano; deposição mineral; adesivo; Ortodontia; lesão de mancha branca.

Abstract

White spot lesions are a concern for orthodontic patients due to biofilm accumulation around brackets. The aim of this study was to develop an antibacterial and remineralizing orthodontic adhesive to overcome these lesions. Experimental orthodontic adhesives were formulated containing 75wt% BisGMA and 25wt% TEGDMA; camphorquinone-based photoinitiator system and 5wt%fummed silica. The compounds 1,3,5-tryacryloylhexahydro-1,3,5-triazine (TAT) and phosphate invert glass containing niobium pentoxide (PIG-Nb) were added to the adhesive as antibacterial and remineralizing agents, respectively. Experimental groups obtained containing only TAT at 10, 15 and 20wt% concentration; groups containing only PIG-Nb at 1, 2.5 and 5wt%, and one group containing 20wt% TAT and 5wt% PIG-Nb. A groups without TAT and PIG-Nb was used as Control and Transbond XT was used as a commercial control. Antibacterial activity, mineral deposition, changes in pH, degree of conversion, softening in solvent and shear bond strength of orthodontic adhesives was assessed. The experimental adhesives presented antibacterial activity and were capable to induce mineral deposition with no negative effect on adhesive's properties. The results of this study allow the conclusion that this material has favorable properties and potential, after necessary adjustments become viable for use in dental practice.

Keywords: antibacterial; mineral deposition; adhesive; Orthodontic; white spot lesion.

Sumário

1. Introdução

O maior acúmulo de biofilme em torno dos *brackets*, em pacientes ortodônticos tem como conseqüência o aumento do desafio cariogênico (ZACHRISSON, 1975; OGAARD; ROLLA; ARENDS, 1988; CHAPMAN *et al.*, 2010). O aumento na colonização oral por *Streptococcus mutans* leva a uma diminuição do pH nesses sítios, provocando um processo de desmineralização e o desenvolvimento de lesões de mancha branca (MILLETT *et al.*, 1999). A implementação de adequada higiene oral é a melhor maneira de se evitar o desenvolvimento destas lesões, porém, em muitos casos, a higienização dos pacientes ortodônticos é deficiente (BISHARA; OSTBY, 2008).

A utilização de sistemas adesivos com liberação contínua de flúor na base do *bracket* é uma maneira de tentar evitar a desmineralização do esmalte nos sítios mais suscetíveis. Cimentos de ionômero de vidro foram testados para colagem de *bracket*s, visando a liberação contínua de flúor, mas a sua baixa resistência de união limitou o seu uso na Ortodontia (MILLER *et al.*, 1996; NKENKE *et al.*, 1997; ORTENDAHL; THILANDER, 1998). Na tentativa de aumentar a resistência de união, foram desenvolvidos ionômeros de vidro modificados por resina para uso odontológico (RIX; FOLEY; MAMANDRAS, 2001; COUPS-SMITH; ROSSOUW; TITLEY, 2003; SUMMERS *et al.*, 2004).

Alguns compostos têm sido adicionados a adesivos ortodônticos como agentes antimicrobianos. Tais compostos, como cloreto de benzalcônio, triclosan, clorexidina e prata, entre outros, apresentam atividade antimicrobiana contra bactérias orais, especialmente contra os *Streptococcus mutans* (OTHMAN *et al.*, 2002; KARAMAN; UYSAL, 2004; MALKOC *et al.*, 2005; POLAT; UYSAL; KARAMAN, 2005; SEHGAL *et al.*, 2007; SHARMA; YNGARD; LIN, 2009). Apesar de se ter evidencias de que a adição de agentes antimicrobianos a adesivos ortodônticos não afeta a resistência de união (ALTMANN *et al.*, 2015), alguns autores observaram uma redução nas propriedades dos adesivos, como baixa resistência de união (KARAMAN; UYSAL, 2004; SINGH *et al.,* 2013, SOKOKU *et al.,*, 2010). A adição do monômero antibacteriano MDPB leva a uma redução no crescimento bacteriano (IMAZATO *et al.*, 1999), sem comprometer significativamente a resistência de união (IMAZATO, et al., 2007) devido a copolimerização do seu radical metacrilato com outros monômeros presentes no adesivo (IMAZATO *et al.*, 2003).

Apesar das melhorias das propriedades dos materiais adesivos ortodônticos, ainda se faz necessário considerar o desenvolvimento de sistemas adesivos ortodônticos que apresentem atividade antibacteriana e capacidade remineralizante, buscando evitar a formação de lesões de mancha branca e sua progressão na superfície do esmalte em torno dos *brackets*, visto que sua prevalência em pacientes ortodônticos é alta, sendo reportada na literatura de 11 a 97% (Tabela 1, JULIEN; BUSCHANG; CAMPBELL, 2013).

Table 1. Prevalence of White Spot Lesions (WSLs) Reported in Studies Based on Comparisons of Pre- to Posttreatment Differences or on Posttreatment Evaluation Only[a]

Study	Design	Index	Sample	Fluoride	Prevalence	Control
Gorelick et al.[3]	DVA	GI	121	No FI	50%	CG
Mizrahi[4]	DVA	MI	269	No FI	12%	CG
Artun and Brobakken[5]	DVA	GI	60	FI rinse	59%	CG
Geiger et al.[6]	DVA	GI	34	FI rinse	34%	CG
Ogaard[2]	DVA	GI	51	FI rinse	11%	CG
Sonis and Snell[7]	DVA	MI	22	FI adhesive	0–13%	CG
Boersma et al.[8]	QLF	QLF	64	No FI	97%	CG
Strateman and Shannon[9]	DVA and P	No. of WSL	99 (no FI) 110 (with FI)	FI gel	27% (FI)– 58% (no FI)	I
Zachrisson and Zachrisson[10]	DVA	CI	174	FI rinse	89%	I
Lovrov et al.[11]	P	GI	53	FI rinse	26%	I
Chapman et al.[12]	DVA and P	% of surface	332	Unknown	36%	I
Tufekci et al.[13]	DVA	GI	35 at 6 mo 37 at 12 mo	Unknown	38% 6 mo 46% 12 mo	I

[a] DVA indicates direct visual assessment; QLF, quantitative light-induced fluorescence; P, photographic evaluation; GI, Gorelick Index; MI, Mizrahi Index; CI, caries index; FI, fluoride; CG, used control group for comparisons; I, individual posttreatment status compared with pretreatment status.

Compostos de Triazina têm sido sintetizados e avaliados na Medicina como agentes antibacterianos, antivirais, antimaláricos, antiprotozoáricos, anticancerígenos e também têm sido utilizados no tratamento da depressão (SHANMUGAN *et al.*, 2013; LV *et al.*, 2014). O composto 1,3,5-triazina pode reduzir o crescimento bacteriano por ser um pequeno composto que mimetiza os padrões hidrofóbico e de carga presentes no farmacóforo de peptídeos antibacterianos catiônicos curtos. É mais seletivo contra bactérias gram-positivas, por meio da ruptura da integridade da sua membrana, e apresenta baixa atividade hemolítica (ZHOU, *et al.*, 2008).

Vidros bioativos são materiais que estimulam uma resposta biológica específica, resultando na formação de uma ligação entre os tecidos vivos e o material (HENCH, 2006). Ainda, vidros bioativos podem agir como fonte de grandes quantidades de cálcio e fosfato (MILLY *et al.*, 2014), cuja disponibilidade pode permitir a remineralização de tecidos, dependendo das condições do meio. Na Odontologia, este material tem sido usado para correção de defeitos periodontais, como preenchimento de cavidades ósseas, e tratamento de dentina hipersensível (HENCH, 1998). Vidros fosfatos estão sendo pesquisados para também serem utilizados como materiais bioativos, pois sua composição química é naturalmente

semelhante a dos tecidos ósseos (CARBONARI *et al.*, 2011; BIH *et al.*, 2013), apresentando alto teor de fosfato (ALKEMPER, 1997). O potencial de remineralização destes vidros bioativos em lesões de mancha branca tem ganhado atenção, por serem capazes de liberar íons necessários para depositar minerais semelhantes aos do esmalte e, ao mesmo tempo, se difundirem através da lesão. MILLY *et al.* (2014) observaram que o esmalte tratado com vidro bioativo, submetido a processo de des-remineralização, apresentou melhores propriedades mecânicas e maior conteúdo de fosfato quando comparado com esmalte sem tratamento, e ainda apresentou deposição mineral na superfície da lesão. Em outro estudo (MANFRED *et al.,* 2013), a associação de um biovidro a um adesivo ortodôntico apresentou maior liberação de íons cálcio e fosfato para o meio oral, quando comparado com adesivos ortodônticos convencionais, mantendo a dureza superficial do esmalte em torno de *brackets.* Vidros fosfatos que apresentam menos de 40%mol de fosfato são chamados de “Vidro Fosfato Invertido”, uma vez que suas propriedades estão mais relacionadas aos íons modificadores do arranjo estrutural, do que ao arranjo estrutural em si (BRAUER *et al.*, 2010; WALTER *et al.,* 2010). Vidros fosfato invertidos contendo óxido de nióbio tem ganhado atenção pela possibilidade de serem utilizados numa ampla gama de aplicações (CARBONARI *et al*., 2009). O pentóxido de nióbio (Nb_2O_5), quando em contato com saliva, é capaz de estimular a deposição mineral (KARLINSEY; HARA*,* 2006), e quando presente em vidro fosfato invertido, aumenta sua durabilidade química e suas propriedades mecânicas, sem apresentar citotoxicidade (CHENU *et al.,* 2012).

Até o presente momento, não foram encontrados estudos utilizando triazina, como agente antibacteriano e vidro niobio-fosfato invertido, como agente remineralizante, associados em adesivos ortodônticos.

2. Objetivo

O objetivo deste estudo foi desenvolver um adesivo ortodôntico que apresentasse atividade antibacteriana e que estimulasse a deposição mineral.

Desta forma, o trabalho dividiu-se nos seguintes objetivos:

- desenvolver um adesivo ortodôntico que apresentasse atividade antibacteriana contendo triazina, e avaliar suas propriedades;

- desenvolver um adesivo ortodôntico que estimulasse a deposição mineral contendo vidro bioativo nióbio-fosfato, e avaliar suas propriedades;

- desenvolver um adesivo ortodôntico que apresentasse atividade antibacteriana e que estimulasse a deposição mineral, contendo as concentrações de triazina e vidro bioativo nióbio-fosfato que apresentaram melhor desempenho nos testes prévio, e avaliar suas propriedades.

3. Artigos

O corpo dessa dissertação é composto por três artigos:

Artigo 1: **Effect of methacrylated-based antibacterial monomer at orthodontic adhesive system properties.** Publicado no periódico *American Journal of Orthodontics and Dentofacial Orthopedics* (doi: 10.1016/j.ajodo.2015.01.015) (ALTMANN, *et al.,* 2015*).*

Artigo 2: **Phosphate invert glass containing niobium pentoxide as a mineralizing agent in an experimental orthodontic adhesive.** Enviado para publicação no periódico *Journal of Biomedical Materials Research Part A.*

Artigo 3: **Novel Antibacterial and Remineralizing Orthodontic Adhesive containing Triazine and Niobium Pentoxide Phosphate Invert Glass.** Enviado para publicação no periódico *Journal of Dentistry.*

Os manuscritos, na formatação exigida pelos periódicos correspondentes, encontram-se a seguir:

3.1. ARTIGO 1

Effect of methacrylated-based antibacterial monomer at orthodontic adhesive system properties

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Effect of methacrylated-based antibacterial monomer on orthodontic adhesive system properties

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Introduction: Antibacterial adhesives were developed to reduce the incidence of white spot lesions in orthodontic patients. Compounds that contain triazine are known as effective antibacterial agents. The aims of this study were to develop an experimental orthodontic adhesive containing 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT) and to characterize it. **Methods:** TAT was added in 3 concentrations (10%, 15%, and 20%) to the experimental orthodontic adhesive. Antibacterial activity was assayed by brain-heart infusion broth dilution against *Streptococcus mutans*. The degree of conversion was measured using Fourier transform infrared spectroscopy, and solvent degradation was evaluated by Knoop microhardness before and after immersion in ethanol for 2 hours. The shear bond strength of metal brackets bonded to bovine enamel surface was assessed. **Results:** All experimental adhesives reduced bacterial growth. The addition of 15% and 20% TAT increased the degree of conversion compared with the control group (0%) and the 10% group. All groups showed a decrease in hardness after ethanol immersion, and there was also a decrease in the percentage of variation of Knoop hardness in the experimental adhesives containing TAT, whereas the shear bond strength increased. **Conclusions:** Orthodontic adhesives containing TAT are promising antibacterial materials, especially those with 15% and 20% TAT. (Am J Orthod Dentofacial Orthop 2015;147:S82-7)

Orthodontic patients experience a higher cariogenic challenge because of bacterial biofilm accumulation around their brackets. An increase in oral colonization by *Streptococcus mutans* lowers the pH, and the demineralization process that takes place leads to the development of white spot lesions.[1] Proper oral hygiene is the foremost mode to prevent lesions, but many patients are inefficient at this.[2] Thus, the development of antibacterial adhesives could help to overcome this problem. However, the incorporation of antibacterial compounds should not decrease material properties.

Seeking the improvement of orthodontic adhesives, some authors have performed studies using different antibacterial agents such as MDPB,[3] chlorhexidine,[4,5] nanoparticle silver,[6] triclosan,[7] zinc oxide,[8] titanium dioxide,[9] and others. A reduction in bacterial growth is observed with a decrease in the adhesive's properties, such as lower bond strength.[4,5,10] Addition of MDPB monomer causes reliable antibacterial growth without significantly compromising the bond strength because of the copolymerization of the methacrylate radical with other monomers of the adhesive. Studies have shown that other methacrylated-based monomers have antibacterial properties. Triazine compounds have been synthesized and evaluated in the medical field as antibacterial, antiviral, antimalarial, antiprotozoal, and anticancer agents. They are also used in the treatment of depression.[11,12] 1,3,5-triazine can decrease bacterial growth because it is a small compound that mimics the hydrophobic and charge patterns detected in the pharmacophore of short cationic antimicrobial peptides. It is more selective against gram-positive bacteria, by means of membrane integrity disruption, and shows low hemolytic activity.[13]

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All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.
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To date, no study has been performed with triazine compounds as the antibacterial agent in orthodontic adhesive systems. The influence of triazine incorporation in orthodontic adhesive materials properties (eg, degree of conversion, antibacterial activity, and bond strength) should be investigated. The aims of this study were to develop an experimental orthodontic adhesive and to evaluate the influence of the addition of 1,3,5-triacryloylhexahydro-1,3,5-triazine (TAT) on the adhesive's properties.

MATERIAL AND METHODS

The experimental orthodontic adhesives were formulated: 75 wt% bisphenol A glycidyl methacrylate and 25 wt% of triethylene glycol dimethacrylate, 1% mol of camphorquinone, ethyl-4-dimethylamino benzoate, and diphenyliodonium hexafluorophosphate as the photoinitiator system, and 0.1 wt% of butylated hydroxytoluene, all from Sigma-Aldrich (St Louis, Mo). Also, 5% of colloidal silica (AEROSIL 200; Evonik, Piscataway, NJ) was added. TAT (Sigma-Aldrich) was added in 3 concentrations: 10%, 15%, and 20%; for the control, a group with no TAT was used. The light source device used for photoactivation was Radii cal (1200 mW/cm^2; SDI, Bayswater, Victoria, Australia).[14]

The specimens of each group were prepared (2 × 2 × 3 mm), photopolymerized for 40 seconds, and submitted to ethylene oxide sterilization. Each specimen was placed in a well of a sterile 96-well plate. Each well contained 300 μL of brain-heart infusion broth (Sigma-Aldrich) with 1% sucrose and 20 μL of a suspension of an overnight broth culture of *S mutans* UA 159 adjusted to optical density of 0.67 (550 nm). The 96-well plate was incubated at 37°C for 24 hours, and 100 μL of each well was diluted in 900 μL of saline solution until the 10^{-6} dilution. Two 25-μL drops of each dilution were platted in brain-heart infusion agar Petri dishes and incubated for 48 hours at 37°C. The number of colony forming units (CFUs) was visually counted by optical microscopy and transformed to log CFU per milliliter.

The degree of conversion was evaluated through Fourier transform infrared spectroscopy (Vertex 70; Bruker Optics, Ettlingen, Germany). The spectrometer was equipped with an attenuated total reflectance device (Platinum ATR-QL; Bruker Optics). A support device was used to hold the light-curing unit at a standard distance of 5 mm from the sample for photopolymerization of 40 seconds. The absorbance spectra were obtained before and immediately after polymerization using software (Opus 6.5; Bruker Optics), transferred to ImageJ software (Image J 1.47; National Institutes of Health, Bethesda, Md), and the degree of conversion was calculated.[15,16]

For the solvent degradation, the specimens from each group were prepared (6 mm in diameter × 1 mm in thickness) and embedded in acrylic resin (VIPIFlash; Vipi Industry, Pirassununga, São Paulo, Brazil). The samples were polished with silicon carbide sandpapers (600, 1200, and 2000 granulation) and felt disks saturated with alumina suspension (alumina, 6 μm; Arotec, Cotia, São Paulo, Brazil). Three indentations were made (at 10 g for 5 seconds) in each specimen with a microhardness tester (HMV 2; Shimadzu, Tokyo, Japan).

Knoop hardness values before and after the immersion in ethanol (Labsynth, Diadema, São Paulo, Brazil) for 2 hours were recorded. The percentage of variation of Knoop hardness was calculated for each specimen.

For the shear bond strength test, 48 crowns of extracted bovine incisors without fractures or cracks stored in distilled water at 4°C for less than 3 months were used in this study.[17] The teeth were embedded in acrylic resin, and the facial surfaces were cleaned with fluoride pumice for 10 seconds and etched with 37% phosphoric acid gel (Atacktec; CaiTECH Indústria, Rio do Sul, Santa Catarina, Brazil) for 30 seconds, rinsed with water for 30 seconds, and dried with oil-free compressed air. Transbond XT Primer (3M Unitek, Monrovia, Calif) was applied to the bonding surface and photoactivated for 20 seconds. Maxillary central incisor metal brackets (Roth Max; Morelli, Sorocaba, São Paulo, Brazil) with a base area of 11.65 mm^2 were bonded to the teeth with each experimental orthodontic adhesive.

The experimental orthodontic adhesives were applied to the bracket base, and it was placed on the facial tooth surface. To standardize the thickness of the adhesive, brackets were placed under a load of 300 g, and the excess adhesive was removed. The adhesives were light-cured for 40 seconds (10 seconds for each side of the bracket). After 24 hours of storage in distilled water at 37°C, the specimens were submitted to a shear bond strength test in a universal testing machine (EMIC DL200; Equipamentos de Ensaio, São José dos Pinhais, Paraná, Brazil) using a knife-edged chisel with a crosshead speed of 1 mm per minute, and the results were recorded in megapascals.

The analysis of the residual adhesive on the tooth surface with the adhesive remnant index was realized with a stereoscopic microscope (10-times magnification), and the scores were recorded.[18]

Statistical analysis

Statistical analysis was done with Sigma Plot for Windows (version 12.0; Systat Software, San Jose, Calif). The sample size calculation for each assay was based on previous studies.[19,20]

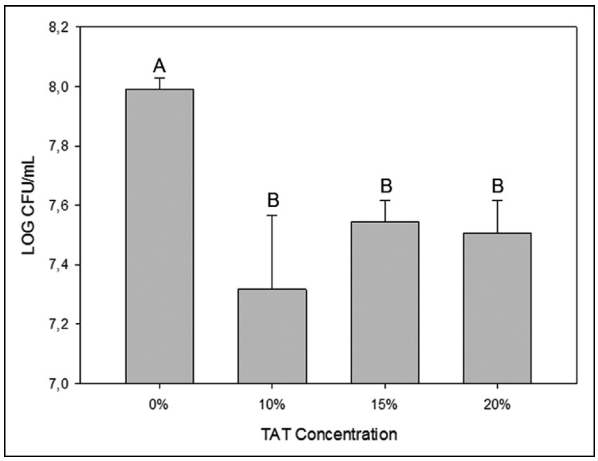

Fig 1. Bacterial growth. Different letters indicate significant statistical difference ($P < 0.05$).

Comparisons of antibacterial activity, degree of conversion, percentage of variation of Knoop hardness, and shear bond strength data were performed with 1-way analysis of variance and Tukey tests. Comparisons of initial and final Knoop hardness values were performed with paired *t* tests for each group.

RESULTS

The addition of TAT to the experimental orthodontic adhesives showed a significant decrease in bacterial growth in comparison with the control group (7.99 ± 0.04 log CFU/mL) ($P < 0.05$). There was no statistical difference in bacterial growth as the concentration of TAT increased (7.32 ± 0.25, 7.54 ± 0.07, 7.51 ± 0.11 log CFU/mL, respectively, for 10%, 15%, and 20%) (Fig 1).

The degrees of conversion of the groups are shown in Table I. There were significant increases in the degree of conversion of the experimental adhesives containing 15% (62.06% ± 1.26%) and 20% (62.70% ± 2.52%) TAT when compared with the control (57.08% ± 0.97%) ($P < 0.05$).

For the solvent degradation, all experimental adhesives had similar initial Knoop hardness values and significant decreases in the final Knoop hardness after 2 hours of immersion in ethanol. There was a significant decrease of 18% in the percentage of variation of Knoop hardness of all experimental adhesives containing TAT ($P < 0.05$) compared with the control experimental adhesive (67.99 ± 1.97), and no statistical difference was observed among them (Table II).

There were increases in the shear bond strength in all experimental adhesives containing TAT ($P < 0.05$) (Table I). The control group had a shear bond strength value of 11.15 ± 2.58 MPa, and the experimental adhesives had values of 15.00 ± 2.91, 15.51 ± 3.36, and 14.44 ± 2.72 MPa, respectively, for the 10%, 15%, and 20% concentrations. No significant difference was observed in the debond pattern between adhesives. The adhesive remnant index scores were mainly 0 and 1 for the adhesives containing 0%, 10%, and 15% TAT, meaning that no adhesive or less than 50% of the adhesive remained on the enamel surface after debonding, and they were mainly score 1 for adhesives containing 20% TAT, meaning that less than 50% of the adhesive remained on the enamel surface after debonding (Fig 2).

Table I. Degree of conversion by Fourier transform infrared spectroscopy (n = 3) and shear bond strength (n = 12) of experimental orthodontic adhesives containing different concentrations of TAT

Concentration	*Degree of conversion (%), mean ± SD*	*Shear bond strength (MPa), mean ± SD*
0% (Control)	57.08 ± 0.97^{B}	11.15 ± 2.58^{B}
10%	57.59 ± 1.27^{B}	15.00 ± 2.91^{A}
15%	62.06 ± 1.26^{A}	15.51 ± 3.36^{A}
20%	62.70 ± 2.52^{A}	14.44 ± 2.72^{A}

Different letters indicate significant statistical difference ($P < 0.05$) in the column.

Table II. Microhardness values of experimental orthodontic adhesives containing different concentration of TAT before (KNH1) and after (KNH2) ethanol immersion and percentage of variation of Knoop hardness (ΔKHN%)

Concentration	*KHN1*	*KHN2*	*ΔKHN%*
0% (Control)	$23.35 \pm 3.36^{A,a}$	7.46 ± 1.06^{b}	67.99 ± 1.97^{B}
10%	$24.76 \pm 1.38^{A,a}$	11.04 ± 0.88^{b}	55.11 ± 3.03^{A}
15%	$20.94 \pm 3.35^{A,a}$	9.35 ± 2.69^{b}	55.72 ± 5.83^{A}
20%	$20.97 \pm 2.46^{A,a}$	9.11 ± 1.64^{b}	56.97 ± 3.16^{A}

Different uppercase letters indicate a significant statistical difference in the column, and different lowercase letters indicate a significant statistical difference in the lines.

DISCUSSION

The main causes of white spot lesions in orthodontic patients are biofilm formation around brackets and poor oral hygiene, leading to a white spot lesion prevalence range of 11% to 46%.[2,21] Also, there was an increase in the oral colonization of *S mutans* after bracket bonding.[22] Therefore, if the orthodontic adhesive shows the capacity to inhibit or reduce bacterial growth, especially gram-positive bacteria growth, these lesions could be prevented or minimized. In this study, TAT was used as the antibacterial agent chemically bounded to the polymer matrix by copolymerization.

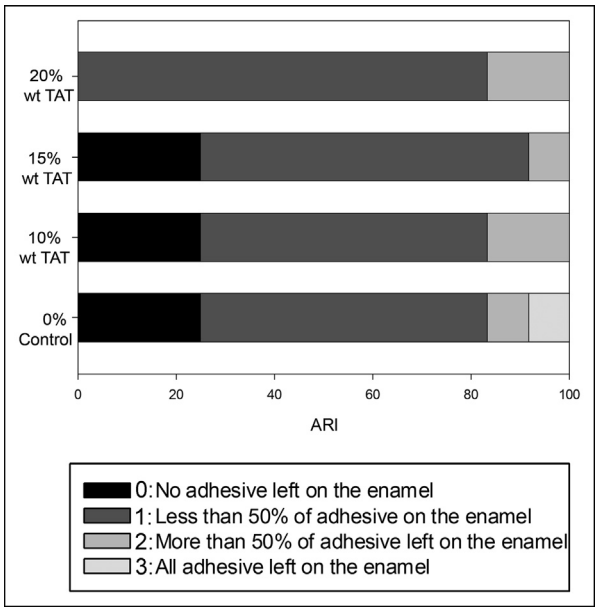

Fig 2. Adhesive remnant index scores.

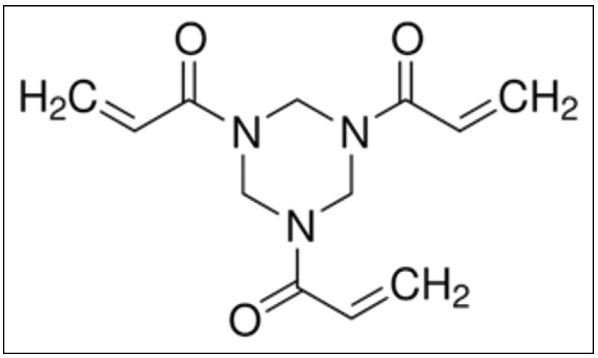

Fig 3. TAT structure.

The antibacterial activity was evaluated by broth dilution that measures bacterial growth inhibition by direct contact of bacteria with the cured adhesives specimens.[23] The TAT concentration added was able to reduce *S mutans* growth by direct contact. Imazato et al[3] also observed a reduction in bacterial growth by contact using an antibacterial monomer (MDPB) that copolymerizes with other monomer components in an adhesive resin. However, the adhesive system containing MDPB is available in the market only as a dentin adhesive, containing a self-etching primer with MDPB and a bonding agent. When it was used to bond brackets to enamel surfaces, some researchers found reductions in the bond strength.[10,24]

An orthodontic adhesive must be able to bond to an enamel surface with bond strength high enough to resist masticatory loads, lasting for an average of 2 years, but not so high that it damages the enamel during debonding.[25] However, the literature includes great variations regarding a threshold of megapascals to fracture enamel.[25-27] In-vitro bond strength data of different studies should be interpreted with caution because of the many variables involved. One attempt to investigate which variables influence bond strength values has already been done, and adhesive area and storage time showed significant influences on the bond strength results.[28] Another variable that could influence the results is the type of tooth (bovine or human). Although bovine enamel acts similarly to human enamel, the bond strength is lower in the bovine probably because of the larger crystal grain sizes from the rapid formation of the bovine teeth.[29] Ruttermann et al[30] observed no difference in the fracture analysis between bovine and human enamel when orthodontic brackets were debonded. However, those authors found high bond strength values ranging from 11.15 to 15.51 MPa. Furthermore, the addition of TAT to the experimental orthodontic adhesives significantly increased the bond strength, with no enamel fracture observed. Other researchers evaluating the influence of antibacterial agents to orthodontic adhesives, such as Karaman and Uysal[4] and Singh et al,[5] observed reductions of shear bond strength when chlorhexidine was used as the antibacterial agent. This agent does not copolymerize with a resin adhesive such as MDPB and TAT.[3] Even though a slight bacterial growth was observed, TAT may play an important a role in the maintenance of adhesion to the tooth surface. In this way, TAT prevents adhesion degradation by reducing the bacterial products that degrade orthodontic adhesives.[31,32] Antibacterial agents that copolymerize with other monomers should be the first choice for antibacterial materials.

The Fourier transform infrared spectroscopy allows the assessment of the degree of conversion of polymerized resins: ie, the percentage of carbon double bond conversion. A higher degree of conversion is related to higher mechanical properties,[33] less unreacted monomers,[34] and reduced degradation.[35] Unreacted monomers raise the risk of allergic reactions caused by leaching of these free monomers.[33,36] TAT increased the degree of conversion in the experimental orthodontic adhesives containing 15% and 20% TAT. This increase was probably due to the 3 functional groups in the TAT molecule (Fig 3) that led to a higher carbon double bond conversion. No difference was found in the initial Knoop hardness values between the groups, evidencing that TAT did not influence the initial hardness in the experimental adhesives. All groups

underwent degradation after immersion in ethanol because of permeation in the polymer network. Organic solvent sorption reduces the secondary molecular interactions and could lead to structural expansion, allowing leaching of uncured monomers[35] and causing some softening.[37] There was a reduction in the percentage of variation of Knoop hardness in the groups containing TAT, demonstrating that these adhesives became more resistant to degradation. This fact may be due to a higher degree of crosslinking, once TAT is a cyclic azine compound with 3 branches in the 1,3,5 positions (Fig 3). This configuration facilitates the crosslinking during polymerization, leading to a tougher polymer, with fewer spaces in the polymer network, thus hindering degradation.[35] This degradation resistance observed in TAT adhesives is also related to the higher degree of conversion with less elution of unreacted components.[33]

Orthodontic adhesives containing TAT are promising antibacterial materials, especially those containing 15% and 20% TAT. It was possible that TAT improved the experimental orthodontic adhesives, and these improvements are interconnected, since the higher degree of conversion and less degradation resulted in a tougher adhesive, with greater bond strength within the suggested values, and mainly with a reduction in bacterial growth.

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Nostalgia Advertisement from a 1921 issue of the Journal

3.2. ARTIGO 2

Niobium pentoxide phosphate invert glass as a mineralizing agent in an experimental orthodontic adhesive.

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Niobium pentoxide phosphate invert glass as a mineralizing agent in an experimental orthodontic adhesive.

Abstract:

White spot lesions (WSL) are an important issue for orthodontic patients. Materials containing remineralizing agents could act as a synergic approach to overcome this issue. The aim of this study was to develop an experimental orthodontic adhesive and evaluate the influence of the addition Phosphate Invert Glass containing Niobium Pentoxide (PIG-Nb) in adhesive's properties. PIG-Nb was added at 1, 2.5 and 5wt% to experimental adhesive (75wt% BisGMA, 25wt% TEGDMA, 5wt% colloidal silica and photo-initiator system). The adhesives were evaluated for mineral deposition, degree of conversion (DC), softening in solvent, pH changes and shear bond strength (SBS). The addition of PIG-Nb to orthodontic adhesives induced the deposition on its surface associate to a constant neutral pH. The SBS of PIG-Nb adhesives was improved after immersion in artificial saliva and the PIG-Nb5 presented less softening. The incorporation of PIG-Nb into orthodontic adhesives induced mineral deposition and may act as a source of ions to prevent or remineralize WSL, presenting no negative effects on the adhesive's properties. The PIG-Nb5 seemed to present the best properties.

Keywords: mineral deposition; orthodontic; adhesive; bioactive glass; niobium pentoxide.

Introduction

White spot lesions (WSL) around brackets are an important issue during orthodontic treatment. This fact occurs due to a deficient hygiene in these sites[1]. An increase in oral colonization by *Streptococcus mutans* lows the pH and the demineralization process takes place[2]. To reduce the decalcification around these susceptible sites, fluoridated orthodontic adhesives, sealants and varnishes have been suggested as solutions, however none of them seemed to have been effective[3]. The development of an orthodontic adhesive that stimulates mineral deposition could act against the development of WSL.

Bioactive glass could stimulate a specific biological response, resulting in a bond between living tissue and material[4]. Also, bioactive glasses can be a source of calcium and phosphate [5] and their availability could allow tissue remineralization, depending on the environment conditions. In Dentistry, this material is being used to correct periodontal defects, as bone void filler, and in the treatment of dentin hypersensitive[6]. Phosphate glasses are also considered biomaterials due to its chemical composition, which is naturally similar to bone tissues[7,8], presenting a high content of phosphate on its composition[9]. There is an increasing interest in the remineralizing potential of these materials to reduce WSL, once they can leach ions necessary for mineral deposition resembling those from enamel and, at the same time, can diffuse through the lesion[5]. Phosphate glasses presenting less than 40mol% of P_2O_5 are reffered as phosphate invert glasses (PIG), once their properties are more related to the network modifier ions than on the network former[10,11]. PIGs containing niobium oxide has been brought to attention due to its ability to be used in a wide range of applications[8]. Niobium pentoxide (Nb_2O_5) can stimulate mineral deposition when in contact with saliva[12] and when present in dental adhesives[13], and when present in PIG, it increases its chemical durability and its mechanical properties, showing no cytotoxicity[14].

Up to this time, no study has been performed incorporating PIG containing niobium pentoxide (PIG-Nb) as a mineralizing agent in orthodontic adhesive. Thus, the aim of this study was to develop an experimental orthodontic adhesive and evaluate the influence of the addition PIG-Nb in adhesive's properties.

Material and Methods

PIG-Nb Preparation and Characterization: Phosphate invert glass containing 10% mol of Nb_2O_5 (PIG-Nb) was prepared by melt-quenching method using the following precursors: $CaCO_3$, H_3PO_4, NbO_5. The precursors' mixture was melted at 1400°C in an electric furnace for 30 minutes and then it was quenched by pouring onto a stainless-steel plate at room temperature[15]. The PIG-Nb composition in mols was 60% CaO, 30% P_2O_5 and 10% Nb_2O_5. The PIG-Nb was milled and the particles were selected for use by the Granutest® sieve system with a final mesh size of 74μm (Telastem, São Paulo, Brazil).

To determine the surface area of PIG-Nb, a Quantachrome NOVA1000 Autosorb Automated Gas Sorption System (Boynton Beach, USA) was used through the Brunauer-Emmett-Teller (B.E.T) method. The particle size was assessed using a laser diffraction particle size analyzer (CILAS 1180; Orleand, France).

Formulation of experimental orthodontic adhesives: the experimental orthodontic adhesives were formulated with 75wt% of bisphenol A glycidyl methacrylate (BisGMA) and 25wt% of triethylene glycol dimethacrylate (TEGDMA); 1mol% of camphorquinone (CQ), ethyl-4-dimethylamino benzoate (EDAB) and diphenyliodonium hexafluorophosphate (DPIHFP) as the photo-initiator system and 0.1wt% of hydroxytoluene butylated (BHT), (Sigma-Aldrich, St. Louis, MO, USA). Also 5% of colloidal silica (AEROSIL 200, Piscataway, NJ, USA) was added to adjust the viscosity. The prepared PIG-Nb was added in three different concentrations: 1, 2.5 and 5 wt%, and for control, a group with no PIG-Nb was used. The light source device used for photo-activation was Radii cal (1200mW/cm^2, SDI, Bayswater, Victoria, Australia).

Artificial saliva preparation: The artificial saliva was prepared according to Karlinsey et al. [12]. The reagents ($CaCl_2.H_2O$, KH_2PO_4, KCl and NaCl) were dissolved in distilled water, buffered with trishydroxymethyl aminomethane to a pH ~ 9 and then the pH was adjusted to 7.04 using concentrated hydrochloric acid.

Sample preparation: adhesive discs were prepared and photo-activated for 40 seconds for each one of the four groups (Control-0%, PIG-Nb1, PIG-Nb2.5 and PIG-Nb5), measuring 5mm ± 0.05 in diameter by 1mm ± 0.02 in thickness. These samples were used for the mineral deposition, pH and solvent degradation assays.

Mineral Deposition Assay: Three samples of each group were immersed in 20ml of artificial saliva for 7, 14 and 28 days at 37°C. After the storage period, the samples were washed with distilled and dried.

Raman Spectroscopy: four samples from each group were analyzed by Senterra Raman microscope (Bruker Optics, Ettlingen, Germany): one sample not immersed in artificial saliva and the other three samples according to the immersion periods. An area of 10x10µm in the center of the sample was irradiated five times for 3 seconds by a 100mW diode laser with 785

nm wavelength on 100 equidistant points. Spectra were obtained at 440 to 1800cm^{-1} Raman band. Post-processing was performed in Opus 6.5 (Bruker Optics, Ettlingen, Germany) by integration of the 960 cm^{-1} peak (reference to phosphate) to map the chemical changes on the sample surface.

Scanning Electron Microscopy - Energy Dispersive X-Ray (SEM-EDS): the samples not immersed and immersed in artificial saliva for 28 days were carbon-sputtered and analyzed by JSM 5800 scanning electron microscope (JEOL, Tokyo, Japan) at 10kV, followed by energy dispersive X-ray analysis to determine the elemental composition of the mineral deposits on the samples' surfaces.

pH Analysis: three samples from each group were immersed in 20 ml of artificial saliva and its pH was measured before immersion, 30 minutes, 1, 2, 3, 4, 5, 6, 18, 24 hours, 2, 3, 4, 5, 6 and 7 days of immersion.

Degree of Conversion (DC): The DC was evaluated through Fourier Transform Infrared spectroscopy (FTIR) (Vertex 70, Bruker Optics, Ettlingen, Germany). The spectrometer was equipped with attenuated total reflectance (ATR) device (Platinum ATR-QL, Bruker Optics, Ettlingen, Germany). A support device was used to uphold the light-curing unit at standard distance of 5mm from the sample for photo-polymerization of 40 seconds. The absorbance spectra were obtained before and immediately after polymerization using Opus 6.5 software (Bruker Optics, Ettlingen, Germany), transferred to Image J 1.47 software (National Institutes of Health, USA) and DC was calculated[16,17].

Softening in Solvent: Three samples from each group were embedded in acrylic resin (VIPIFlash, Vipi Industry, Pirassununga, SP, Brazil). The samples were polished with SiC sandpapers (600, 1200 and 2000 granulation) and felt disc saturated with alumina suspension (Alumina, 6 µm, Arotec, Cotia, SP, Brazil). Three indentations were made (10g for 5s) in each specimen (HMV 2, Shimadzu, Tokyo, Japan) to determine Knoop hardness values (KNH).

KNH values before (KNH1) and after (KNH2) the immersion in ethanol (Labsynth LTD, Diadema, SP, Brazil) for 2 hours were recorded. The percentage of variation of Knoop hardness (ΔKNH%) was calculated for each specimen.

Shear Bond Strength Test (SBS): Ninety-six crowns of extracted bovine incisors free of fractures and cracks stored in distilled water at 4°C[18] within less than 3 months were used in this study. The teeth were embedded in acrylic resin and the facial surface was cleaned with no fluoride pumice for 10 seconds and etched with 37% phosphoric acid gel (Atacktec, CaiTECH Indústria LTDA, Rio do Sul, SC, Brazil) for 30 seconds, rinsed with water for 30 seconds and dried with oil-free compressed air. The Transbond XT Primer (3M Unitek, Monrovia, CA, USA) was applied to the bonding surface and photo-activated for 20 seconds. Maxillary central incisor metal brackets (Roth Max, Morelli, Sorocaba, SP, Brazil), presenting a base area of 11.65mm^2, were bonded to the teeth using each one of the experimental orthodontic adhesives.

Teeth were randomly divided into four groups and the experimental orthodontic adhesives were applied to the bracket base and it was placed on the facial tooth surface. In order to standardize the thickness of the adhesive, brackets underwent a 300gF and the excesses were removed. The adhesives were light-cured for 40 seconds (10 seconds for each side of the bracket). Then each group was divided into two subgroups (n=12): immediate (24 hours) and 28 days of storage in artificial saliva, at 37°C. Specimens were submitted to shear bond strength (SBS) after each storage period at an universal test machine (Shimadzu EZ Test EZ-SX, Kyoto, Japan) using a knife-edge chisel with a crosshead speed of 1mm/min and results were recorded in MPa.

The analysis of the residual adhesive on the tooth surface (Adhesive Remnant Index - ARI) was realized with a stereoscopic microscope (10x) and the ARI[19] were recorded.

Statistical analysis: statistical analysis was done on Sigma Plot for Windows version 12.0 (Systat Software Inc, San Jose, CA, USA). Sample size calculus for each assay was performed based on previous studies[20,21]. Comparisons degree of conversion, percentage of variation of Knoop hardness data were performed with One-way ANOVA and Tukey. Shear bond strength data was compared by Two-way ANOVA and Student-Newman-Keuls Method. pH data was compared with Repeated Measures ANOVA. Comparison of initial and final Knoop hardness values were performed with paired t-test for each group.

Results

The PIG-Nb powder presented a specific surface area of 3.16 m^2/g and the mean particle size of 67.55 µm. The artificial saliva initial pH was 7.27. The pH in all groups varied from 6.29 to 7.29, presenting no difference between the groups and times (Fig. 1).

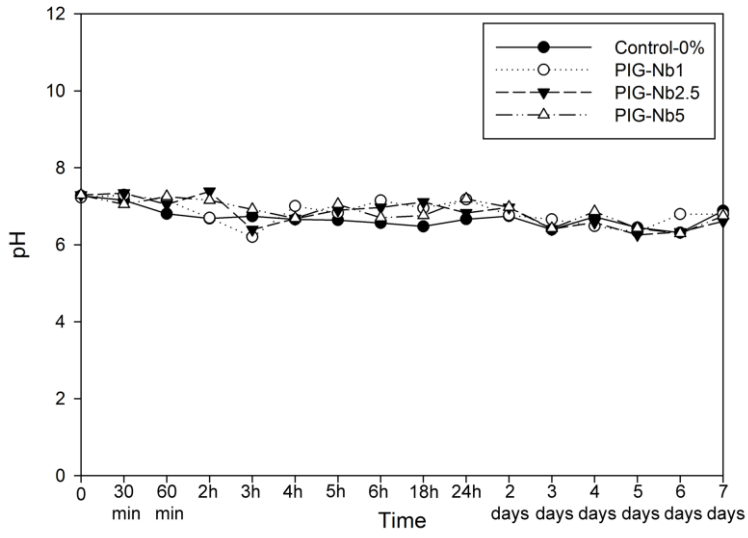

Figure 1. pH changes in the artificial saliva with immersed samples in function of time.

Phosphate content changes over the samples' surfaces after distinct periods immersed in artificial saliva, assessed by variation in the absorbance of the phosphate peak ($960cm^{-1}$) by Raman spectroscopy are shown in Figure 2. No phosphate content was observed on the surface of all groups at the first two periods (not immersed and 7 days), also on the Control-0% group at the remaining two periods (14 and 28 days). Phosphate content was observed in all experimental groups containing PIG-Nb after 14 and 28 days of immersion in artificial saliva; and the intensity of the phosphate peaks seems to increase from the PIG-Nb1 towards PIG-Nb5.

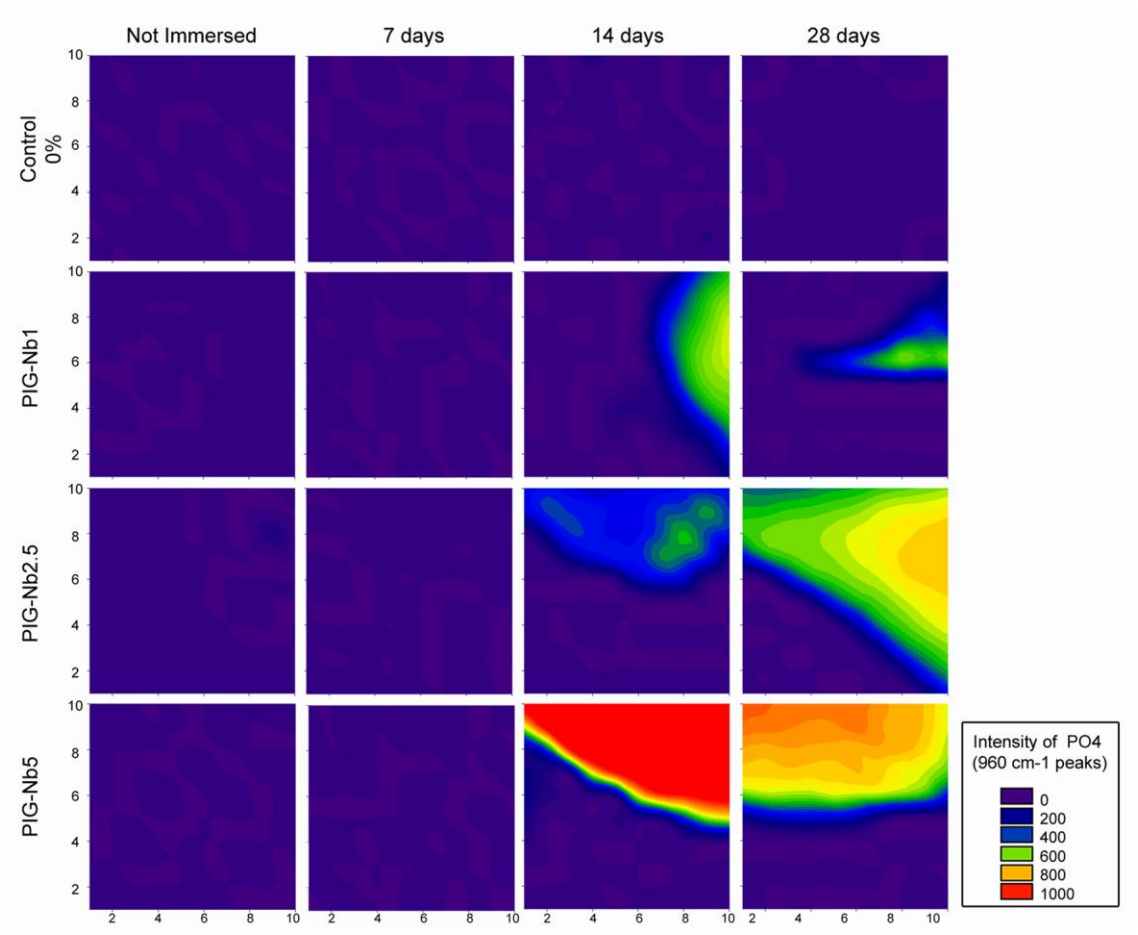

Figure 2. Raman map of phosphate deposition. The intensity is given by the integration of 960cm^{-1} peaks.

Samples from all groups, not immersed and immersed in artificial saliva for 28 days, were analyzed by SEM. Figure 3 contains SEM images acquired at different magnifications (95, 1500 and 5500x) and it reveals that all samples present a similar surface morphology before immersion. No mineral deposition was observed on the surface of Control-0% group after 28 days of immersion. On the other hand, all PIG-Nb groups presented mineral deposits on its surfaces. At the 1500x magnification images was possible to observe a similar structure of mineral deposits; the 5500x magnification images denote the plate-like crystals with sharp edges structures of these mineral deposits.

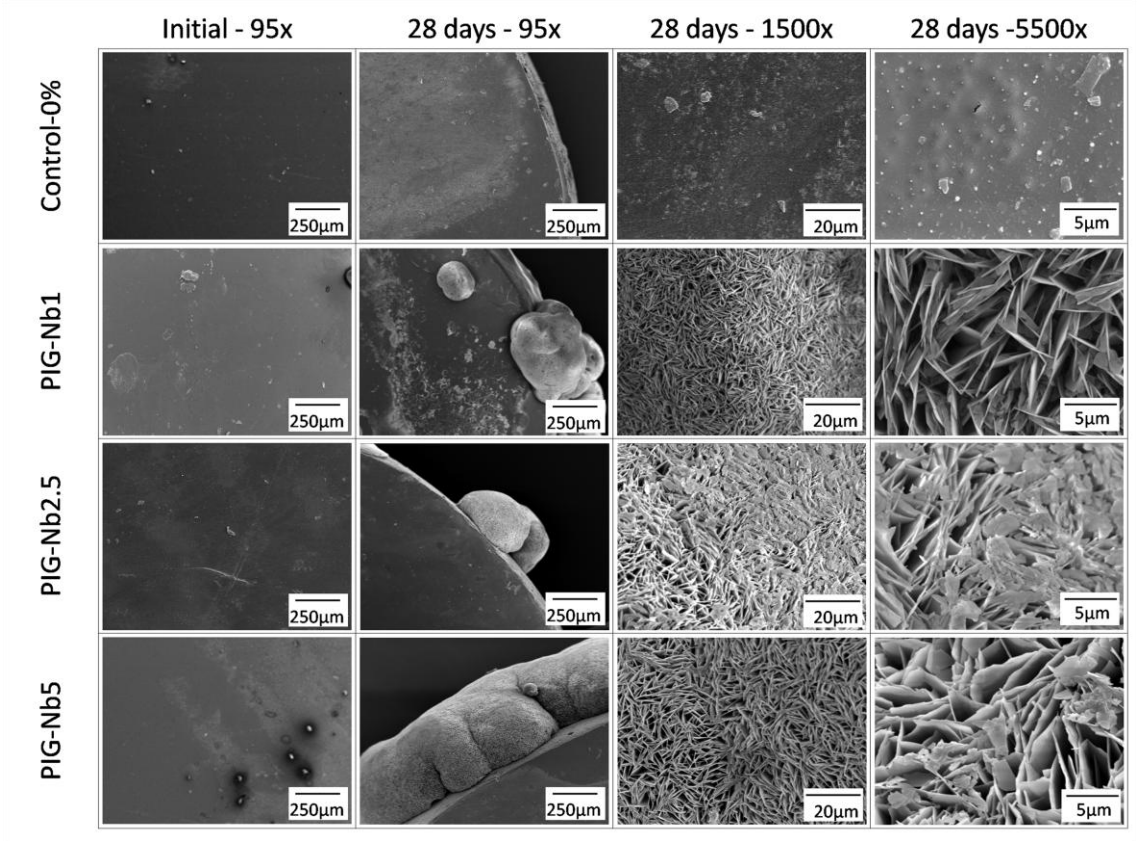

Figure 3. SEM images of samples' surfaces not immersed and after 28 days of immersion in artificial saliva at 95x, 1500x and 5500x magnifications.

The samples' surface composition was assessed by SEM-EDS (Table 1). The analysis of the samples not immersed showed that all samples presented a similar composition, mostly of Carbon (C) and Silicon (Si); the Control-0% group presented no Niobium (Nb), while the PIG-Nb groups presented an increasing content on its surface. After 28 days of immersion in artificial saliva, it was possible to observe a great amount of Calcium (Ca) in all PIG-Nb groups, whereas little Ca was observed on the Control-0% group; Phosphorus (P) was present in all groups but in different amounts.

Table 1. Samples' surface composition assessed by SEM-EDS.

	Initial			28 days Immersion				
Groups	**% C**	**% Si**	**% Nb**	**%P**	**%Ca**	**%Nb**	**%C**	**%O**
Control-0%	97.74	2.26	0	1.84	3.23	0	94.8	0.12
PIG-Nb1	69.18	1.44	29.38	30.44	33.99	16.81	11.71	7.05
PIG-Nb2.5	60.34	1.35	38.31	13.29	79.48	6.09	0.30	0.84
PIG-Nb5	54.68	1.68	43.64	5.97	76.04	16.58	0.70	0.71

The DC, assessed by FTIR, presented a significant difference between Control-0% and all PIG-Nb groups ($p<0.001$) (Table 2). The Control-0% group presented a DC of 57.05%, while the PIG-Nb1, PIG-Nb2.5 and PIG-Nb5 groups presented 52.32%, 51.95% and 51.87% respectively.

Table 2. Degree of conversion (%DC) and Knoop hardness values of experimental orthodontic adhesives containing different concentrations of PIG-Nb. Hardness values before (KHN1) and after solvent immersion (KHN2) and the variation of Knoop hardness values (∆KHN%).

Groups	DC (Mean ± SD)	KHN1 (Mean ± SD)	KHN2 (Mean ± SD)	∆KHN% (Mean ± SD)
Control 0%	57.05 ± 0,08^{A}	17.43 ± 0.99^{AB,a}	4.46 ± 0.53^{b}	74.50 ±1.64^{A}
PIG-Nb1	52.32 ± 0.80^{B}	14.71 ± 2.02^{B,a}	4.06 ± 0.67^{b}	72.33 ± 3.58^{A}
PIG-Nb2.5	51.95 ± 0,51^{B}	19.47 ± 0.21^{A,a}	5.68 ± 0.69^{b}	70.84 ± 3.47^{AB}
PIG-Nb5	51.87 ± 0,93^{B}	18.30 ± 1.96^{AB,a}	6.47 ± 0.58^{b}	64.61 ± 1.25^{B}

Different uppercase indicate significant statistical difference in the column and different lowercase indicate significant statistical difference in the lines.

The PIG-Nb groups presented initial Knoop hardness values (KNH1) near to control group ($p>0.05$). The PIG-Nb2.5 group presented initial Knoop hardness values higher than the PIG-Nb1 group ($p<0.05$), but no difference to the other groups was observed (Table 2). After two hours of immersion in ethanol all groups presented a reduction in the final Knoop hardness values (KNH2) ($p<0.05$). The Control-0% group showed a degradation (%∆KNH) of 74.50% and the PIG-Nb1 and PIG-Nb2.5 groups had a degradation of 72.33% and 70.84%, respectively, showing no statistical difference among them. The PIG-Nb5 group presented a significant reduction in the %∆KNH when comparing to the Control-0% and PIG-Nb1 groups, showing %∆KNH value of 64.61% ($p<0.05$) (Table2).

Table 3. Shear bond strength of metallic brackets bonded to bovine teeth with experimental adhesives. Immediate and 28 days after immersion in artificial saliva.

Groups	Immediate	28 days
Control 0%	18.30 ± 3.74^{Aa}	16.03 ± 1.17^{Aa}
PIG-Nb1	13.38 ± 3.52^{Ba}	19.68 ± 1.23^{Ab}
PIG-Nb2.5	13.78 ± 3.31^{Ba}	17.45 ± 1.17^{Ab}
PIG-Nb5	14.15 ± 2.53^{Ba}	18.96 ± 1.12^{Ab}

Different uppercase indicate significant statistical difference in the column and different lowercase indicate significant statistical difference in the lines.

The SBS results are presented in Table 3. In the immediate SBS evaluation, the Control-0% group presented statistically higher values (mean 18.30 MPa) than PIG-Nb groups (PIG-Nb1 13.38 MPa; PIG-Nb2.5 13.78MPa; PIG-Nb5 14.15 MPa). In the 28 days analysis, there was no statistical difference between the Control-0% and PIG-Nb groups. When

comparing the SBS values from immediate and 28 days analysis, no significant change was observed in the values of Control-0% group (mean 16.03 MPa). However, all PIG-Nb groups (PIG-Nb1, PIG-Nb2.5, PIG-Nb5) presented higher SBS values after the immersion (19.68MPa; 17.45MPa; 18.96MPa respectively). The ARI scores for immediate and 28 days of immersion in artificial saliva were mainly 0 and 1 for all groups, meaning that no adhesive or less than 50% of the adhesive remained on the enamel surface after debonding, presenting the same pattern in the fracture analysis (Figure 4).

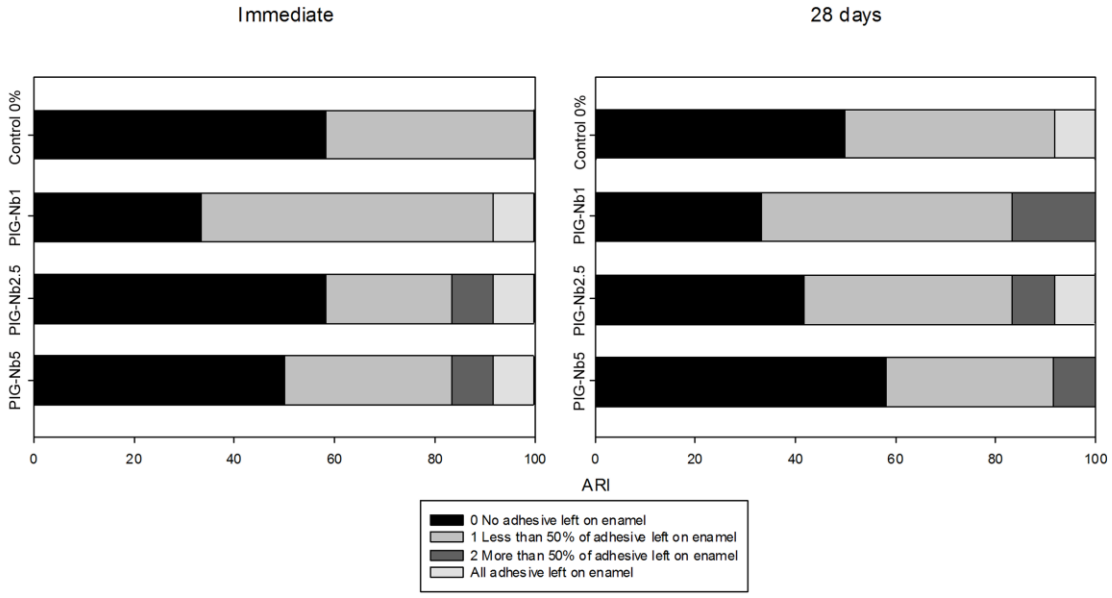

Figure 4. ARI scores of debonded interfaces after immediate and 28 days SBS test.

Discussion

Subsurface enamel demineralization are clinically detectable as WSL and represent the first stage of caries development[22]. The prevalence is high, ranging from 11 to 97% in orthodontic patients[23,24]. The biofilm accumulation around the brackets lows the pH and tends to promote a demineralization process[24]. The variety of fluoride-containing materials, and the lack of predictability related to patient compliance on his own dental hygiene have shown to be insufficient in preventing the WSL[25]. A source of minerals or the induction of mineral deposition on these sites could inhibit the demineralization process or stimulate remineralization process. Therefore, an orthodontic adhesive presenting these characteristics could avoid or minimize the

WSL. This study showed that orthodontic adhesive containing PIG-Nb can induce mineral deposition in artificial saliva.

A material that is capable to promote apatite deposition on its surface when implanted in a living body may be called a bioactive material [26]. Niobium pentoxide has shown the ability to promote nucleation of apatite when immersed in supersaturated solutions such as simulated body fluid (SBF)[13], artificial and human saliva[12]. Phosphate invert glasses (PIG) have been capturing the attention of medical and dental field for use as a biomaterial since their structure and mechanical properties are close to that of the mineral phase of bone and dental tissues[27]. In addition, their dissolution can be controlled[9], releasing ions in aqueous media with a neutral constant pH [28,29], as it was observed in this study, where the pH remained close to 7 throughout the period analyzed.

The PIG are glasses that present a low content of phosphate (less than 40%mol)[11] and the increasing content of calcium gives a decrease in solubility[28,30]. The PIG-Nb prepared in this study contains 30mol% of phosphate and 60mol% calcium, leading to a less soluble glass. In neutral solutions, Nb_2O_5 strongly influences the solubility of PIG. Its addition to the glass induces dramatic changes in the glass structure, increasing its chemical stability, thus also reducing its dissolution[29]. The niobium pentoxide was added at 10%mol in this glass, in order to improve its chemical durability[14]. The development of WSL is due to a lowered pH for a extended period of time[31]. In this acid pH, the PIG-Nb may present a smaller dissolution rate than other glasses, thus its mineral deposition capacity may last longer.

The constant pH indicates that the mineral deposits are induced by the biomaterial and not by artifacts of precipitation caused by pH change[32]. These glasses are known to deposit apatite in its surfaces when immersed in SBF[33], and the addition of niobium oxide can improve this ability to promote crystal growth and mineralization of the surrounding tissues[8,12,29]. The resulting PIG-Nb presented a regular surface area of 3.16 m^2/g. The surface area is related to the rate of mineral deposition, where higher the surface area, more reactive the glass is, thus leading to a higher mineral deposition rate[34].

The ability of the orthodontic adhesives containing PIG-Nb to induce mineral deposition was evaluated by Raman spectroscopy and SEM-EDS. Until 7 days of immersion in artificial

saliva, no mineral deposits were observed on all groups; however, after 14 and 28 days it was possible to observe deposits of minerals on PIG-Nb groups, through the 960cm-^{1} Raman peak that has been used as a reference for phosphate[12,21]. SEM-EDS initial analysis confirmed the presence of the major components of the orthodontic adhesives, as Carbon and Silicon for all groups and Niobium in the PIG-Nb groups. The analysis after 28 days showed that mineral deposits were highly selective to Ca an P (Table 1). Since the artificial saliva used in this study was constructed based on the inorganic content found in human stimulated saliva, and it is a supersaturated medium for mineral deposition. It is likely that Ca and P are the major comprised components in the deposits[12]. The plate-like crystals structure observed indicate that the mineral deposits are octacalciumphosphate (OCP), in accordance with the literature[35-38]. Also, it was confirmed by the presence of 1010cm^{-1} and 955cm^{-1}peaks (Fig.5) in all PIG-Nb containing samples immersed for 14 and 28 days, which are considered the most characteristics OCP Raman bands[32,39]. Octacalcium phosphate is considered the precursor of apatitic biominerals, such as hydroxyapatite (HA), and it seems to play an important role in enamel mineralization process[40]. The OCP conversion into HA has been observed *in vivo,* due to its dissolution by hydrolysis[41,42].

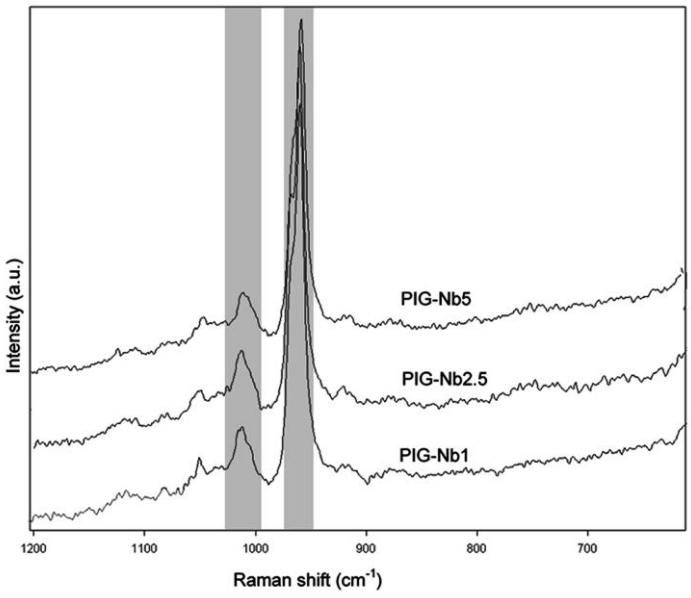

Figure 5. Raman spectrums of mineral deposits on PIG-Nb adhesives after 28 days immersed in artificial saliva. Absorbance band with 1010 and 955 cm^{-1} peaks related to octacalcium phosphate (OCP) are highlighted.

The FTIR spectroscopy allow the assessment of the DC of polymerized resins, that is the percentage of carbon double bonds (C=C) conversion. In this study, the DC of the PIG-Nb groups was reduced when compared to the Control-0%. The DC is related to the mechanical properties[43], unreacted monomers[44] and degradation[45]. The unreacted monomers raise the risk of allergic reactions due to leaching of these free monomers [43,46]. The lower DC observed in this study may be caused mainly by the limitation of the mobility of reactive groups imposed by the rapid formation of cross-linked polymeric network[47]. Also the DC of light-cured composites depends on a series of factors, including the structure of monomer and filler characteristics. The orthodontic adhesives were formulated with 75wt% of BISGMA, which is a viscous monomer that may have a great impact on the mobility, and the PIG-Nb particles may have increased the viscosity of the adhesive[48]. In addition, the filler used produces light diffraction into orthodontic adhesives, resulting in a reduced degree of conversion. Commercial orthodontic adhesives present inorganic fillers and show comparable results of degree of conversion to PIG-Nb adhesive groups[47]. The DC values achieved here (higher than 50%) are in accordance with the literature for orthodontic adhesives. Eliades et al.[49] and Jagdish, et al. [50] observed FTIR-DC values lower than 50% for light-cured orthodontic adhesives associated to a higher cell viability[49].

The PIG-Nb orthodontic adhesives presented no difference on the KNH1 values when compared to the Control-0%. All groups underwent a reduction in the Knoop hardness after immersion in ethanol, evincing a degradation due to permeation in the polymer network. Organic solvent sorption reduces the secondary molecular interactions and could lead to a structural expansion, allowing leaching of uncured monomers[45] and causing some degree of softening[51]. The PIG-Nb5 group presented a significant lower variation in hardness (%KNH) when comparing to the remaining groups. This finding is related to the higher content of filler in this group (5%wt PIG-NB), since the addition of fillers reduces the amount of organic matrix in the same volume material. Presenting less organic matrix and considering that PIG-Nb particles are less prone to degradation[28,52], a lower variation in the %KNH is observed, resulting in a softening resistance.

Also, the lower DC may result in inferior physico-mechanical properties and clinical performance, such as poor bond strength[47]. In spite of the low DC values presented in this

study, an increase in the bond strength after 28 days of immersion in artificial saliva was observed in the PIG-Nb groups, regardless the concentration of PIG-Nb. An orthodontic adhesive must present a bond strength as high as it will resist to masticatory loads, lasting for an average of two years, but not so high, that could damages the enamel during the debonding[53]. However, literature present a great variation regarding a threshold of MPa to fracture enamel[54-56]. Data of *in vitro* bond strength studies should be interpreted with caution due to the many variables that are involved. In this study the values for bond strength varied from 13.38 to 19.68MPa and only one enamel fracture was observed in the PIG-Nb1 group at 28 days of immersion in artificial saliva.

The enamel subsurface demineralization occurs due to a loss of calcium and phosphate from its matrix during local ion concentration imbalances[57]. The critical pH for enamel dissolution is inversely related to the content of calcium and phosphate from the oral environment; the higher the content, lower critical pH must to be achieved in order to occur demineralization[58]. The experimental mineralizing orthodontic adhesives developed can act as a source of calcium and phosphate for the susceptible sites, as the bracket surroundings, since the PIG-Nb and the dissoluble OCP are composed mainly by these elements, avoiding WSL development. Also, there is some clinical evidence that WSL can be remineralized by the use of bioactive glasses[5]. As long as the surface of the lesion remains undisturbed and the environment adjacent to the WSL is supersaturated, the calcium and phosphate can penetrate in the subsurface and remineralize the lesion[58].

The results of this study indicate that the incorporation of PIG-Nb into orthodontic adhesives induces mineral deposition *in vitro* and may act as a source of ions to prevent or remineralize WSL, associated to sound properties. The experimental orthodontic adhesive containing 5%wt of PIG-Nb seemed to present the best properties.

Acknowledgments

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3.3. ARTIGO 3.

Novel Antibacterial and Remineralizing Orthodontic Adhesive containing Triazine and Niobium Pentoxide Phosphate Invert Glass.

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Keywords: mineral deposition; WSL; orthodontic; adhesive; antibacterial;

Abstract

Objectives: White spot lesions are still a concern for orthodontic patients. The aim of this study was to develop an orthodontic adhesive that presents antibacterial activity and is capable to induce mineral deposition containing triazine and a bioactive glass to overcome these lesions, and evaluate the influence of their addition in the adhesive's properties.

Methods: The compounds 1,3,5-tryacryloylhexahydro-1,3,5-triazine (TAT) and phosphate invert glass containing 10% mol of niobium pentoxide (PIG-Nb) were added at 20wt% and 5wt%, respectively, to an experimental adhesive (75wt% BisGMA, 25wt% TEGDMA, 5wt% colloidal silica and photo-initiator system) and called TPN. A group without the addition of these compounds was used as Control and the orthodontic adhesive Transbond XT (TXT) was used for comparison. All three adhesives were evaluated for antibacterial activity, mineral deposition, degree of conversion (DC), softening in solvent and shear bond strength (SBS).

Results: TPN group presented a reduction on bacterial growth when compared to Control and TXT. Mineral deposits were observed on the surface of TPN adhesive after 14 and 28 days of immersion in artificial saliva. There was an increase in DC after 28 days, whereas TPN group presented the highest DC. All groups underwent some degree of softening. No significant changes were observed on SBS after 28 days of immersion in artificial saliva.

Conclusion: This study successfully developed an orthodontic adhesive that presents antibacterial activity and has the ability to induce mineral deposition *in vitro,* maintaining its properties.

Clinical Significance: The use of orthodontic adhesives, that are capable to reduce bacterial growth and induce mineral deposition, may prevent the development of white spot lesions due to availability of minerals around brackets.

Introduction

White spot lesions (WSL) are still a concern in orthodontic patients[1]. An increase in *Streptococcus mutans* oral colonization occurs at the beginning of treatment due to biofilm accumulation around bracket and hampered hygiene[2]. As a result, the pH at these sites is lowered and a demineralization process occurs, leading to a porous enamel subsurface calcium- and phosphate-deficient, with a chalky opaque appearance, called WSL[3].

In order to reduce bacterial growth around brackets, antibacterial agents have been added to orthodontic adhesives such as antibacterial monomers (including methacryloyloxydodecylpyridinium bromide (MDPB)[4], dimethylaminododecyl methacrylate (DMADDM)[5], 1,3,5-tryacryloylhexahydro-1,3,5-triazine (TAT)[6]), chlorhexidine[7], nanoparticle silver[8], triclosan[9], zinc oxide[10], titanium dioxide[11], and others. Despite the fact that some authors have observed a reduction on adhesive's bond strength when antibacterial agents were added[9], there is evidence that this procedure does not influence the bond strength to enamel[12]. The addition of antibacterial monomers, as MDPB, presents reliable antibacterial activity without significantly compromise the bond strength due to the copolymerization of methacrylate radical with other monomers of adhesive[13]. Triazine compounds have been synthesized and evaluated in the medical field as antibacterial, antiviral, antimalarial, antiprotozoal and anticancer agents [14]. 1,3,5-triazine compounds can decrease bacterial growth because it is a small compound that mimic the hydrophobic and charge pattern detected in the pharmacophore of short cationic antimicrobial peptides. It is more selective against gram-positive bacteria, by means of membrane integrity disruption and show low hemolytic activity[15]. When TAT was added at 20wt% to an experimental orthodontic adhesive, it presented a significant reduction on bacterial growth, with improved properties[6].

To reduce the decalcification around the susceptible sites, fluoridated orthodontic adhesives, sealants and varnishes have been suggested as solutions, however none of them seemed to have been effective[16]. Phosphate glasses are considered biomaterials since they can stimulate a specific biological response, resulting in a bond between living tissue and material[17] and due to its chemical composition, which is naturally similar to bone tissues[18], presenting a high content of phosphate in its composition[19]. There is an increasing interest in

the remineralizing potential of these materials to reduce WSL, once they can be instrumental in this process, leaching ions necessary for mineral deposition resembling those from enamel and, at the same time, can diffuse through the lesion.

Phosphate glasses presenting less than 40mol% of phosphate are referred as phosphate invert glasses (PIG), once their properties are more related to the network modifier ions than on the network former[20]. PIGs containing niobium oxide have been brought to attention due to its ability to be used in a wide range of applications[21]. Niobium pentoxide (Nb_2O_5) can stimulate mineral deposition when in contact with saliva[22] and when present in dental adhesive[23]. The addition of niobium in PIGs, results in increased chemical durability and mechanical properties, showing no cytotoxicity[24]. In a previous study, PIG containing niobium pentoxide (PIG-Nb) was added to an experimental orthodontic adhesive and mineral deposits were observed at the adhesive's surfaces (Data not published). These mineral deposits can act as a source of ions that can leach and remineralize WSL[25].

The development of an orthodontic adhesive that reduces bacterial growth and stimulates mineral deposition could act as a synergic approach to overcome the development of WSL. However, these features should not decrease material properties (e.g., degree of conversion, softening in solvent and bond strength). The aim of this study was to develop an orthodontic adhesive that presents antibacterial activity and is capable to induce mineral deposition containing TAT and PIG-Nb and evaluate their influence on the adhesive's properties.

Material and Methods

The experimental orthodontic adhesives were formulated with 75wt% of *bisphenol A glycidyl methacrylate* (BisGMA) and 25wt% of triethylene glycol dimethacrylate (TEGDMA); 1mol% of camphorquinone (CQ), *ethyl-4*-dimethylamino benzoate (EDAB) and diphenyliodonium hexafluorophosphate (DPIHFP) as the photo-initiator system and 0.1wt% of hydroxytoluene butylated (BHT), (Sigma-Aldrich, St. Louis, MO, USA). Also 5% of fummed silica (AEROSIL 200, Piscataway, NJ, USA) was added to adjust the viscosity. One group was used as prepared (*Control*), and the other experimental group (*TPN*) received 20% of 1,3,5-tryacryloylhexahydro-1,3,5-triazine (TAT) (Sigma-Aldrich, St. Louis, MO, USA) and 5% of a

phosphate invert glass containing 10% mol of Nb_2O_5 (PIG-Nb), prepared as described previously[26]. Transbond XT orthodontic adhesive system (3M Unitek, Monrovia, CA, USA) (*TXT*) was used as a commercial control, as it is world widely used and frequently used in Orthodontic research as a standard for light cure adhesive [8, 11]. The light source device used for photo-activation was Radii cal (1200mW/cm^2, SDI, Bayswater, Victoria, Australia) .

Artificial saliva was prepared according to Karlinsey, Hara, Yi and Duhn (2006)[22]. The reagents ($CaCl_2.H_2O$, KH_2PO_4, KCl and NaCl) were dissolved in distilled water, buffered with trishydroxymethyl aminomethane to a pH ~ 9 and then the pH was adjusted to 7.04 using concentrated hydrochloric acid.

Adhesive discs were prepared for each one of the three groups (Control, TPN and TXT), using a mould, two slide glasses and polyester strips. The discs were photo-activated for 40 seconds and resulted in samples measuring 5mm ± 0.05 in diameter by 2,05mm ± 0.02 in thickness. These samples were used for the mineral deposition, antibacterial activity and solvent degradation assays.

For antibacterial activity evaluation, five samples from each groups were fixed on teflon matrixes on the lid of a 48-well plate and sterilized by hydrogen peroxide gas plasma. In a sterile 48-well plate, 800µl of brain heart infusion (BHI) broth (Sigma-Aldrich, St Louis, MO, USA) with 1% sucrose and 80µl of a suspension of an overnight broth culture of *S. mutans* UA 159, adjusted to optical density of 0.3 (550nm) were added to each one of the 15 wells used. The plate was closed and incubated at 37°C for 24 hours. The samples from each group were then removed from the lid's teflon matrixes and placed inside a micro-tube containing 900µl of saline and vortexed. Dilutions were made until the 10^{-6}. Two 25µl-drops of each dilution were platted in BHI agar Petri dishes and incubated for 48 hours at 37°C. The number of colony forming units (CFU) was visually counted by optical microscopy and transformed to logCFU/ml.

The mineral deposition was analyzed by Senterra Raman microscope (Bruker Optics, Ettlingen, Germany). Four samples from each group were used: three samples were immersed in 20 ml of artificial saliva for 7, 14 and 28 days at 37°C, and one sample was not immersed. An area of 10x10µm in the center of the sample was irradiated five times for 3 seconds by a 100mW diode laser with 785 nm wavelength on 100 equidistant points. Spectra were obtained

at 440 to 1800cm^{-1}. Post-processing was performed in Opus 7.2 (Bruker Optics, Ettlingen, Germany) by integration of the 960 cm^{-1} peak (reference to phosphate content) to map the chemical changes on the sample surface.

The degree of conversion (DC) was evaluated by Senterra Raman microscope (Bruker Optics, Ettlingen, Germany). The monomers were irradiated five times for 3 seconds in three points by a 100mW diode laser with 785 nm wavelength. Spectra were obtained at 440 to 1800cm^{-1}. The photo-polymerized spectra were obtained *in situ,* from the enamel-adhesive interface analysis at two different times: immediate and 28 days after bonding. Post-processing was performed in Opus 7.2 (Bruker Optics, Ettlingen, Germany), transferred to Image J software (Image J 1.47, National Institutes of Health, USA) and DC was calculated[27].

For the softening in solvent assay, three samples from each group were embedded in acrylic resin (VIPIFlash, Vipi Industry, Pirassununga, SP, Brazil) and three indentations were made (10g for 5s) in each specimen (HMV 2, Shimadzu, Tokyo, Japan). Knoop hardness values before (KNH1) and after (KNH2) the immersion in ethanol (Labsynth LTD, Diadema, SP, Brazil) for 2 hours were recorded. The percentage of variation of Knoop hardness (ΔKNH%) was calculated for each specimen.

Seventy-two bovine incisors crowns, cleaned, free of fractures and cracks, stored in distilled water at 4°C within less than 3 months were used for shear bond strength test. The teeth were embedded in acrylic resin and the facial surface was cleaned with fluoride-free pumice for 10 seconds and etched with 37% phosphoric acid gel (Atacktec, CaiTECH Indústria LTDA, Rio do Sul, SC, Brazil) for 30 seconds, rinsed with water for 30 seconds and dried with oil-free compressed air. The Transbond XT Primer (3M Unitek, Monrovia, CA, USA) was applied to the bonding surface and photo-polymerized for 20 seconds. Maxillary central incisor metal brackets (Roth Max, Morelli, Sorocaba, SP, Brazil), presenting a base area of 11.65mm^2, were bonded to the teeth using each one orthodontic adhesives (Control, TPN, TXT). Teeth were randomly divided into three groups and the orthodontic adhesives were applied to the bracket base and it was placed on the facial tooth surface. In order to standardize the thickness of the adhesive, brackets underwent a 300gF and the excesses of adhesive were removed. The adhesives were light-cured for 40 seconds (10 seconds for each side of the bracket). Then each

group was divided into two subgroups (n=12): immediate (24 hours) and 28 days of storage in artificial saliva, at 37°C. Specimens were submitted to shear bond strength (SBS) after each storage period at an universal test machine (Shimadzu EZ Test EZ-SX, Kyoto, Japan) using a knife-edge chisel with a crosshead speed of 1mm/min and results were recorded in MPa. The analysis of the residual adhesive on the tooth surface (Adhesive Remnant Index - ARI) was realized with a stereoscopic microscope (10x) and the ARI [28] were recorded.

A mapping was performed on TPN bracket bases after immediate and 28 days SBS test to characterize the distribution of TAT and PIG-Nb on the adhesive interface. The entire bracket base was mapped by the irradiation of 400 points (five times for 3 seconds by a 100mW diode laser with 785 nm wavelength) by Senterra Raman microscope. Spectra were obtained at 440 to 1800cm^{-1}. Post-processing was performed in Opus 7.2 (Bruker Optics, Ettlingen, Germany) by integration of the 835 cm^{-1} peak (deformation of C_2N_3 ring[29] - present in TAT) and 1082 cm^{-1} peak (phosphate ions present in phosphate glasses[30] - as reference for PIG-Nb).

Statistical analysis was done on Sigma Plot for Windows version 12.0 (Systat Software Inc, San Jose, CA, USA). Sample size calculus for each assay was performed based on previous studies [6, 31]. Comparison of antibacterial activity, degree of conversion (within groups) and percentage of variation of Knoop hardness data were performed with One-way ANOVA and Tukey. Shear bond strength data were compared by Two-way ANOVA and Student-Newman-Keuls Method (adhesive and time). Comparison of immediate and 28 days degree of conversion and initial and final Knoop hardness values were performed with paired t-test for each group.

Results

Table 1 presents the results for antibacterial activity test, degree of conversion and shear bond strength. A significant reduction on bacterial growth was observed in TPN group after 24 hours of incubation at 37°C, when comparing to Control and TXT groups (p<0,001).

Table 1. Antibacterial activity (LOG CFU/ml), degree of conversion (DC - %) and shear bond strength (MPa) of orthodontic adhesives.

	Antibacterial Activity (Mean ± SD)	DC Immediate (Mean ± SD)	DC 28 days (Mean ± SD)	SBS Immediate (Mean ± SD)	SBS 28 days (Mean ± SD)
Control	5.62 ± 0.16^{A}	70.84 ± 2.45^{Aa}	74.80 ± 0.55^{Ba}	16.33 ± 5.06A^{Ba}	12.93 ± 5.08^{Ba}
TPN	4.22 ± 0.30^{B}	66.94 ± 2.81^{Aa}	79.33 ± 0.92^{Ab}	14.68 ± 3.93^{Ba}	15.81 ± 2.97^{Ba}
TXT	5.51 ± 0.42^{A}	53.17 ± 2.99^{Ba}	64.09 ± 2.44^{Cb}	20.44 ± 6.23^{Aa}	21.13 ± 6.31^{Aa}

Different uppercase letters indicate significant statistical difference within each column (adhesive). Different lowercase letters indicate significant statistical difference within the same row (time).

Phosphate content changes over the samples' surfaces after distinct periods immersed in artificial saliva, assessed by variation in the phosphate peak (960cm^{-1}) by Raman spectroscopy are shown in Figure 1. No phosphate content was observed on the surface of all groups at the first two periods (not immersed and 7 days). Phosphate content was observed only on TPN group after 14 and 28 days of immersion in artificial saliva.

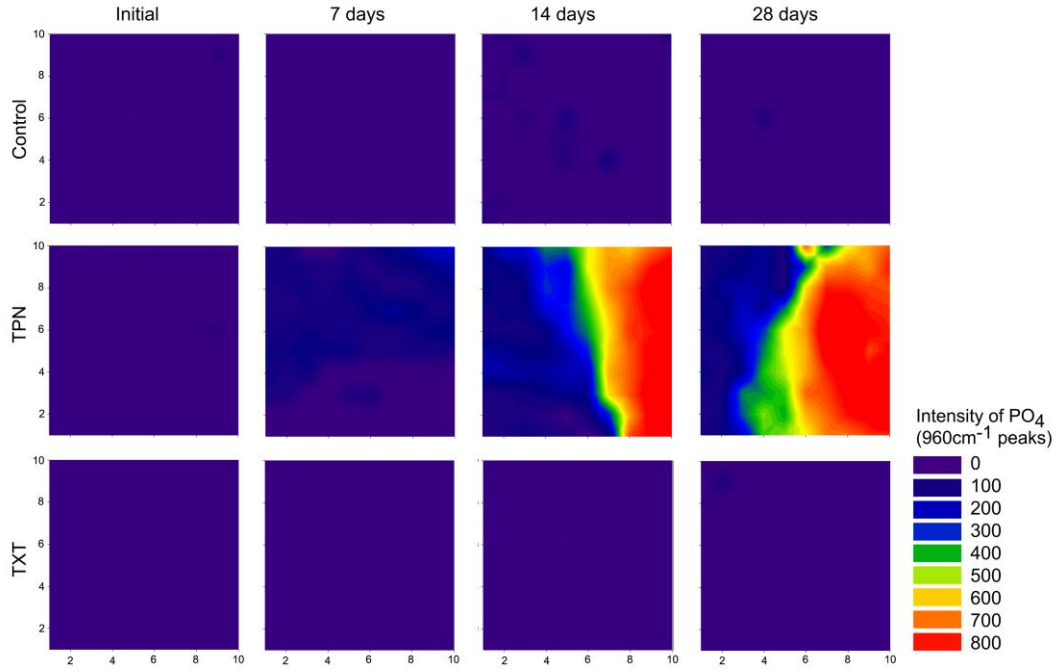

Figure 1: Raman map of phosphate deposition. The intensity is given by the integration of 960cm^{-1} peak. *Control:* experimental orthodontic adhesive without addition of compounds; *TPN*: orthodontic adhesive containing triazine and phosphate invert glass with niobium pentoxide; *TXT:* orthodontic adhesive Transbond XT.

The *in situ* DC was affected by both factors Adhesive (Control, TPN and TXT) and Time (Immediate and 28 days) ($p<0.05$). The interactions between groups is shown in Table 1. At immediate DC, Control and TPN presented statistically higher values than TXT, whereas at 28 days DC, the TPN had the highest value, statistically different from Control and TXT ($p<0.05$). Comparing the different times for the same adhesive, TPN and TXT had an statistical increase in DC values after 28 days ($p<0.05$), while Control showed no statistical difference ($p>0.05$).

Knopp hardness values, determined before (KNH1) and after (KNH2) the immersion of adhesives in ethanol, as well as the variation in these hardness values (ΔKHN%), are presented in Table 2. The TXT presented the highest KNH1 value (32.37), followed by TPN (19.30) and

Control (15.21) ($p<0.05$). All groups presented a significant reduction on hardness values (KNH2) ($p<0.05$). Control had the highest ΔKHN% (79.98%) followed by TPN (67.49%) and TXT (16.04%), which were all statistically different from each other.

Table 2. Knoop hardness values before (KHN1) and after solvent immersion (KHN2) and the variation of Knoop hardness values (ΔKHN%).

	KHN1 (Mean ± SD)	KHN2 (Mean ± SD)	ΔKHN% (Mean ± SD)
Control	$15.21 \pm 2.77^{C,a}$	3.15 ± 1.41^{b}	79.98 ± 5.02^{A}
TPN	$19.30 \pm 1.82^{B,a}$	6.40 ± 2.22^{b}	67.49 ± 4.34^{B}
TXT	$32.37 \pm 2.64^{A,a}$	27.2 ± 2.58^{b}	16.04 ± 4.68^{C}

Different uppercase letters indicate significant statistical difference in the column and different lowercase letters indicate significant statistical difference in the lines.

The results from SBS test are presented in Table. 1. In the immediate SBS evaluation within groups, TXT had the highest value (20.44MPa), which was statistically higher than TPN (14.68MPa), however, no statistical difference was observed between TXT and Control (16.33MPa) and TPN and Control. In the SBS analysis after 28 days of immersion in artificial saliva, TXT and TPN presented higher values than the immediate SBS (21.14MPa and 15.81MPa, respectively) and Control presented a reduction (12.93MPa), although these changes were not statistically significant. As at immediate test, TXT presented the highest bond strength, statistically different from Control and TPN. The ARI scores for all groups at immediate and 28 days of immersion in artificial saliva were mainly 0 and 1, meaning that no adhesive or less than 50% of the adhesive remained on the enamel surface after debonding, presenting the same pattern in the fracture analysis (Figure 2).

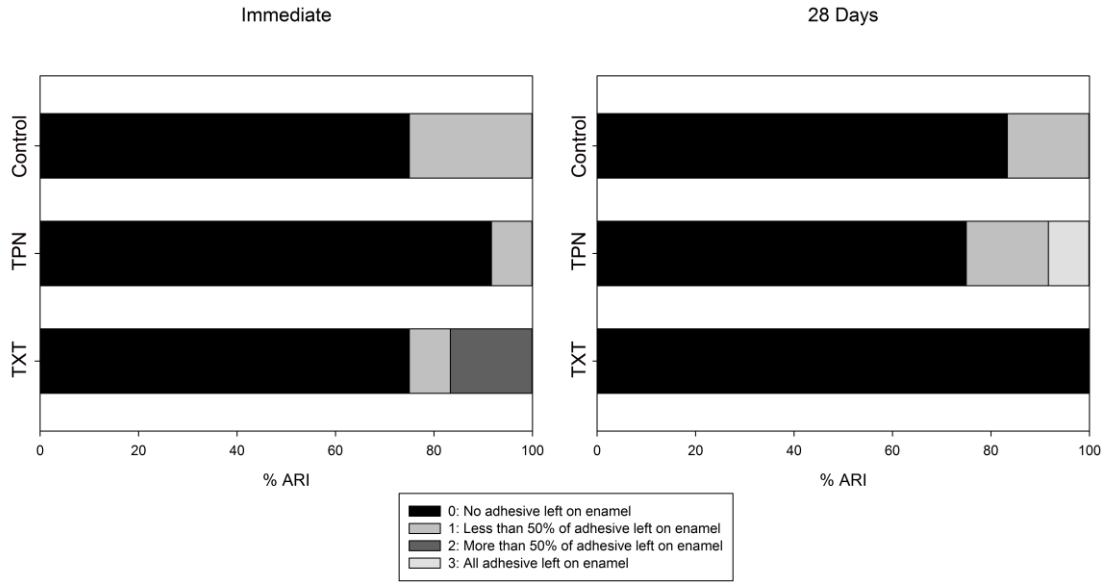

Figure 2: ARI scores for immediate and 28 days of immersion in artificial saliva SBS test.

Control: experimental orthodontic adhesive without addition of compounds; *TPN*: orthodontic adhesive containing triazine and phosphate invert glass with niobium pentoxide; *TXT:* orthodontic adhesive Transbond XT.

The figure 3 illustrates the distribution of TAT and PIG-Nb on the bracket base of TPN brackets after SBS test. It is possible to observe that TAT is unifformally distributed throug the immediate base. It appearss that TAT is more present at the edges after 28 days of immersion in saliva. PIG-Nb is concentrated at the edges of bracket base, both immediate and 28 days after artificial saliva immersion.

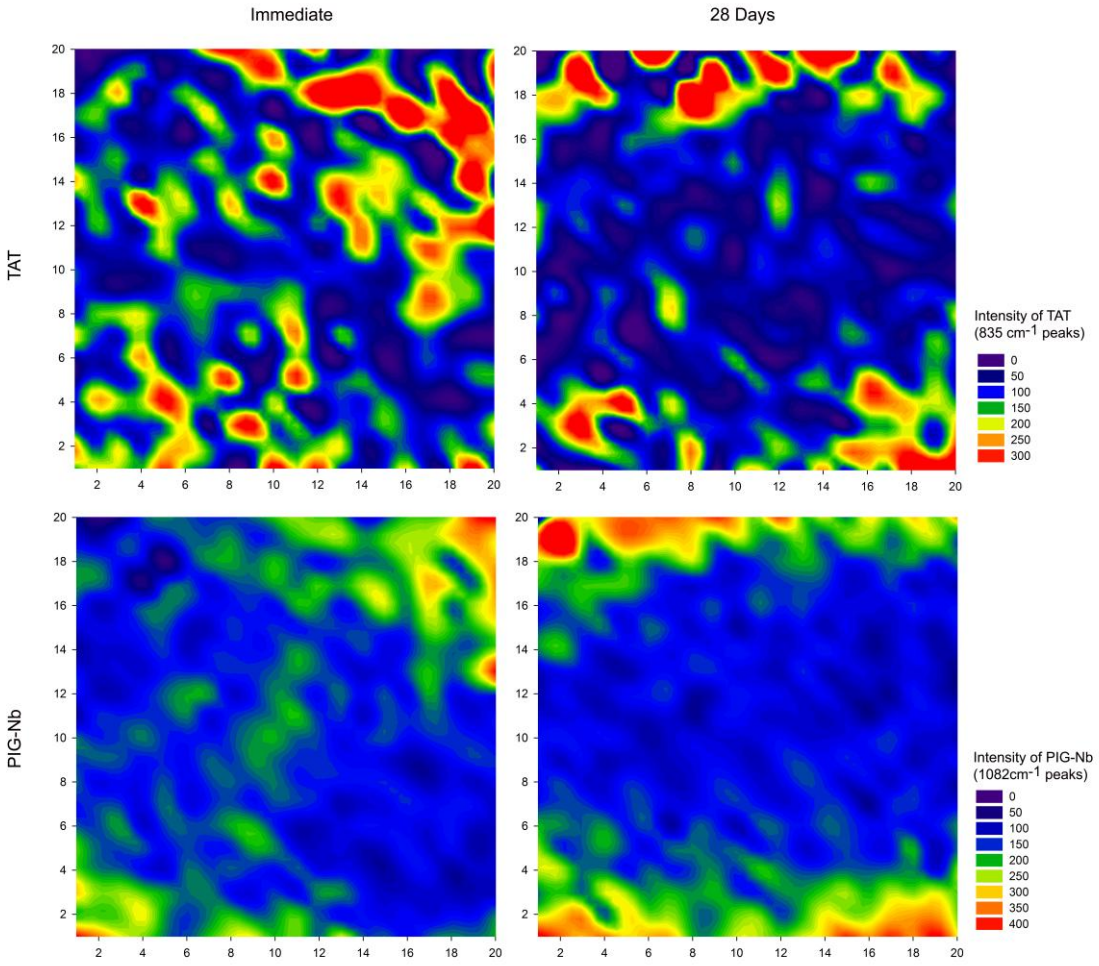

Figure 3: Bracket base mapping by Raman microscopy for TAT ($835cm^{-1}$) and PIG-Nb ($1082cm^{-1}$).

Discussion

White spot lesions prevalence is high, being reported as high as 97% in orthodontic patients. It is a concern since their negligent management can lead to cavitated carious lesions and aesthetic compromise[32]. Not only the increase in oral bacterial colonization and hampered hygiene make the enamel surface around brackets susceptible sites for WSL development[2], but also the phosphoric acid etching due to mineral loss, that leads to a decreased enamel surface hardness around brackets[33]. For these reasons, the experimental orthodontic adhesive TPN was developed in this study. It showed the ability to reduce bacterial growth and induce mineral deposition, indicating that it may be able to reduce the cariogenic challenge and remineralize the enamel surface around brackets, thus avoiding or minimizing WSLs.

. The addition of antibacterial monomers to dental materials has shown a decrease in bacterial growth[34, 35], as it was shown in this study. TAT has three functional groups (Figure 4) that copolymerize with other adhesive's monomers, leading to a direct contact antibacterial agent, since it is immobilized in the polymer matrix and not leached or lost over the time[35]. The broth dilution method used for antibacterial activity evaluation assess the direct contact of bacteria with the cured adhesives, inhibiting bacterial adherence[36]. A reduction of 20 times on CFU count in TPN group was observed after 24 hours of incubation, when compared to Control and TXT groups. Up to this time, only three antibacterial monomers have been tested in orthodontic adhesives for bracket bonding: MDPB and DMADDM have shown reduction on bacterial growth, however some authors found a reduction on shear bond strength when it was used to bond brackets to enamel surface[5, 37]; TAT also presented a reduction on bacterial growth, however it was associated to a increased bond strength[6]. Nevertheless, no orthodontic adhesive containing antibacterial agent is present on market.

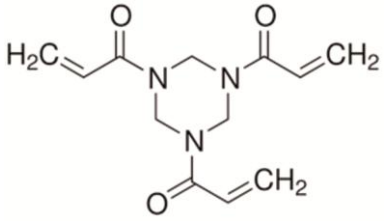

Figure 4: 1,3,5-tryacryloylhexahydro-1,3,5-triazine (TAT) structure.

A material that is capable to promote apatite deposition on its surface when implanted in a living body may be called a bioactive material[38]. Phosphate invert glasses (PIG) have been capturing the attention of medical and dental field for use as a biomaterial since their structure and mechanical properties are close to that of the mineral phase of bone and dental tissues[39]. These glasses present a low content of phosphate (less than 40%mol)[20] and the increasing content of calcium gives a decrease in solubility[40]. The PIG-Nb used in this study contains 30mol% of phosphate and 60mol% calcium, leading to a less soluble glass. In neutral solutions, Nb_2O_5 strongly influences the solubility of PIG. Its addition to the glass induces dramatic changes in the glass structure, increasing its chemical stability, thus also reducing its dissolution[41]. The niobium pentoxide was present at 10mol% in this glass, in order to improve its chemical durability[24]. The development of WSL is due to a lowered pH for a extended period of time[42]. Even in this acid pH, the PIG-Nb can induce mineral deposition and may present a smaller dissolution rate than other glasses due to its composition, thus its mineral deposition capacity may last longer.

The ability of the orthodontic adhesives to induce mineral deposition was evaluated by Raman spectroscopy, which has been widely used to gather information about mineral content within a substrate[43-45]. The artificial saliva used in this study was constructed based on the inorganic content found in human stimulated saliva, and it is a supersaturated medium for mineral deposition[22]. No mineral deposits were present in Control and TXT groups. After 14 days of immersion in saliva, mineral deposits were observed in the TPN group, through the 960cm-^{1} Raman peak, that has been used as a reference for phosphate[22, 46]. The spectra of mineral deposits from TPN group were analyzed and the peaks 1010cm^{-1} and 955cm^{-1} were identified (Figure 5), characterizing the mineral deposits as octacalcium phosphate (OCP), once these peaks are considered the most characteristics OCP Raman bands[47]. Octacalcium phosphate is considered the precursor of apatitic biominerals, such as hydroxyapatite (HA), and it seems to play an important role in enamel mineralization process[48]. The OCP conversion into HA has been observed *in vivo,* due to its dissolution by hydrolysis[49].

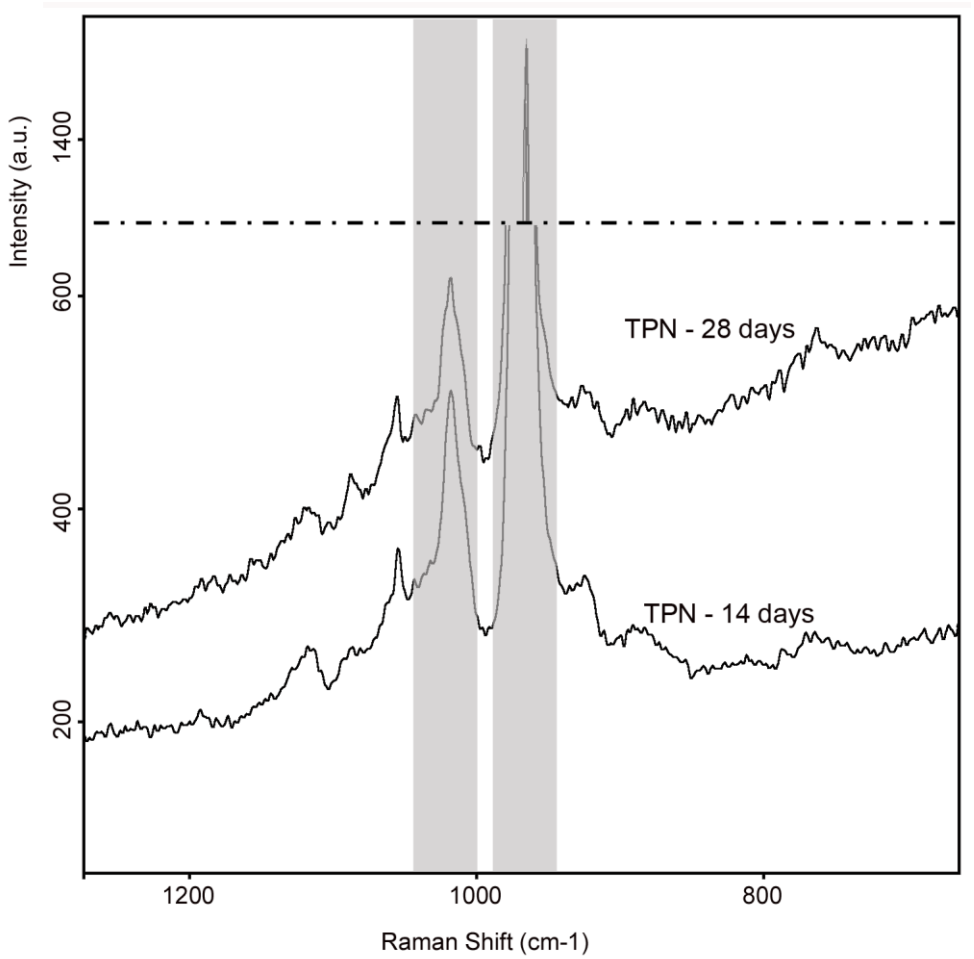

Figure 5: Raman spectrums of mineral deposits on TPN adhesives after 14 and 28 days immersed in artificial saliva. Raman bands with 1010 cm^{-1} and 955 cm^{-1} peaks related to octacalcium phosphate (OCP) are highlighted.

The degree of conversion (DC) was performed using Raman spectroscopy, which allows the assessment of the DC of polymerized adhesives *in situ,* at bracket base. It is a precise, water insensitive and non destructive method that supplies comparable results to FTIR method[50]. A higher DC is related to higher mechanical properties[51], less unreacted monomers[52] and reduced degradation[53]. The unreacted monomers raise the risk of allergic reactions due to leaching of these free monomers[51]. It was possible that the presence of TAT in the adhesive would increase the DC due to the presence of three functional groups in the TAT molecule (Figure 4), which led to a higher C=C conversion and crosslinking [6]. However, at immediate DC, TPN did not present higher DC than Control. This may be due to the fact that DC of light-cured composites depends on a series of factors, including the viscosity and filler characteristics[54].

The presence of PIG-Nb particles may have increased the viscosity of the adhesive[55] and produced light diffraction[43], resulting in a reduced degree of conversion. Transbond XT presents a different composition from the formulated orthodontics adhesives in this study, especially regarding the monomer composition: 10-20wt% BisGMA; 5-10wt% BisEMA; <2wt% silane treated silica; 70-80wt% silane treated quartz. This composition may explain the lower DC on TXT group, since composites composed by BisGMA and BisEMA present lower DC than those composed by BisGMA and TEGDMA[56] and the high content of filler can obstruct the passage of light, thus resulting in a lower DC[43]. The increase in DC after 28 days is related to the gradual post-irradiation polymerization after light exposure[57]. TPN group presented higher DC than Control at this time, indicating that the presence of TAT may have increased the conversion of C=C.

Also, the composition of Transbond XT can explain the higher KHN1 values observed for this group, once it is a highly loaded adhesive (70-80wt% quartz filler) and the hardness of adhesives is related to its filler content[33]. In this way, the higher initial hardness of TPN group, when compared to Control, is explained by the presence of PIG-Nb particles that act as a filler. All groups underwent some degree of degradation after immersion in ethanol due to its permeation into the polymer network. Organic solvent sorption reduces the secondary molecular interactions and could lead to a structural expansion, allowing leaching of uncured monomers[53] causing some degree of softening[58]. There was a reduction in the %ΔKNH in TPN group, comparing to control, demonstrating that these adhesives became more resistant to degradation. This fact may be due to a higher degree of crosslinking, once TAT is a cyclic azine compound with three branching in the 1,3,5 positions (figure 4). This configuration facilitates the crosslinking during polymerization, leading to a tougher polymer, with less spaces in the polymer network, thus hindering the degradation[53]. This degradation resistance observed in TPN adhesives is also related to the higher degree of conversion where there was less elution of unreacted components[51]. TXT group presented the least %ΔKNH since it has high filler content and less polymer matrix, that is susceptible to softening in solvent.

An orthodontic adhesive must present a bond strength as high as it will resist to masticatory loads, lasting for an average of two years, but not so high, that could damages the enamel during the debonding[59]. However, literature present a great variation regarding a

threshold of MPa to fracture enamel[60, 61]. Data of *in vitro* bond strength studies should be interpreted with caution due to the many variables that are involved. In this study the values for bond strength varied from 12.93 to 21.13 MPa. Control and TPN presented similar SBS values for immediate and 28 days. TXT group presented the highest SBS values both at immediate and 28 days assessment. Only TXT group presented enamel fractures (three) at 28 days of immersion in artificial saliva. These fractures may be related to its higher values[60] and attention must be paid to excessive bond strength. No significant changes in SBS were observed after 28 of immersion in all groups, indicating that time immersed did not affect the bond strength (e.g. hydrolytic degradation). The fracture pattern was similar among groups (ARI score 0 and 1) and did not change after 28 days in artificial saliva, indicating that no adhesive or less than 50% of it remained at the enamel surface.

The distribution of TAT and PIG-Nb at bracket base indicate that these components are well distributed according to the amount that they were added to the adhesive (20 and 5wt% respectively). Both TAT and PIG-Nb are present at the edges of the bracket base, being available to perform their functions: antibacterial effect by direct contact and induction of mineral deposition, respectively.

The increase in the oral colonization of *Streptococcus mutans* after brackets bonding may lead to enamel subsurface demineralization[2]. It occurs due to a loss of calcium and phosphate from its matrix during local ion concentration imbalances[62]. The critical pH for enamel dissolution is inversely related to the content of calcium and phosphate from the oral environment; the higher the content, lower critical pH must to be achieved in order to occur demineralization[63]. Even though a slight bacterial growth was observed, TAT may play a important a role in the maintenance of the adhesion to the tooth surface. In this way, TAT avoids adhesion degradation by reducing the bacterial products that degrades the orthodontic adhesives [64]. Antibacterial agent that copolymerizes with others monomers should be the first choice for antibacterial materials. The PIG-Nb and the dissoluble OCP are composed mainly by calcium and phosphate and can act as a source of minerals for the susceptible sites, as the bracket surroundings, avoiding WSL development. Also, there is some clinical evidence that WSL can be remineralized by the use of bioactive glasses [25, 44]. As long as the surface of the

lesion remains undisturbed and the environment adjacent to the WSL is supersaturated, the calcium and phosphate can penetrate in the subsurface and remineralize the lesion[63].

Conclusions

This study successfully developed an orthodontic adhesive that reduces bacterial growth and induces mineral deposition *in vitro*, without affecting its properties. This material may be a promising material to avoid or minimize WSL, that does not depends only on patients' compliance.

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4. Considerações Finais

O desenvolvimento de um adesivo ortodôntico que apresente atividade antibacteriana e remineralizante é de grande importância, uma vez que a prevalência de lesões de mancha branca em pacientes ortodônticos ainda é alta. O maior acúmulo de biofilme em torno dos *brackets* associado à dificuldade de higienização, faz com que estas lesões se desenvolvam de maneira rápida, sendo possível observá-las em até 4 semanas após a instalação da aparatologia ortodôntica. A redução do crescimento bacteriano por contato direto pode levar a um menor acúmulo de biofilme na interface adesivo/esmalte, o que pode levar a uma redução da incidência de lesões de mancha branca. Ainda, a presença de um agente que induza deposição mineral no adesivo servirá como uma fonte de íons, que poderão se difundir pelas lesão remineralizando-a, caso a redução do crescimento bacteriano por si só não seja capaz de inibir totalmente o seu desenvolvimento.

Os objetivos deste trabalho foram alcançados e seus resultados estão apresentados na forma de três artigos. No primeiro artigo, obteve-se um adesivo ortodôntico capaz de reduzir o crescimento bacteriano com a adição de TAT. Este composto é um monômero antibacteriano capaz de copolimerizar com os demais monômeros presentes no adesivo, ficando imobilizado e agindo por contato direto. Sua ação antibacteriana causa a ruptura da membrana da parede celular bacteriana, devido ao seu padrão de carga catiônico, sendo seletivo para bactérias gram positivas, como o *Streptococcus mutans.* Sua associação ao adesivo ortodôntico proporcionou melhoria das propriedades, como resistência de união, grau de conversão e resistência à degradação.

No segundo artigo, desenvolveu-se um adesivo ortodôntico capaz de estimular a deposição mineral na sua superfície pela presença do vidro bioativo nióbio-fostato invertido. Os depósitos minerais identificados são compostos de fosfato octacálcico, que é um dos precursores da hidroxiapatita, que está presente na mineralização do esmalte, durante seu desenvolvimento. O fosfato octacálcico, é uma fonte de fosfato e cálcio, bastante solúvel, sendo capaz de liberar íons que podem se difundir pelas lesões de mancha branca, remineralizando-as. As propriedade deste adesivo foram mantidas após a adição das partículas de vidro bioativo. Observando-se os resultados deste estudo, é provável que a adição de uma

maior concentração de vidro possa resultar em melhores propriedades, como por exemplo, uma maior resistência à degradação.

Já, no terceiro artigo, foi possível atingir o principal objetivo desta dissertação, unindo as melhores concentrações de TAT e de vidro bioativo testadas previamente, resultando em um adesivo ortodôntico com atividade antibacteriana e capacidade de deposição mineral *in vitro*. Associado a isto, este adesivo ortodôntico apresentou adequado grau de conversão e resistência de união, porém ao compará-lo a uma adesivo ortodôntico comercial, observou-se que ajustes ainda necessitam ser feitos referentes à resistência à degradação.

O adesivo ortodôntico desenvolvido neste trabalho poderá levar a uma prática Odontológica com maior controle de efeitos adversos, como as lesões de mancha branca em pacientes ortodônticos, uma vez que não dependerá somente da colaboração do paciente com a adequada higienização oral.

Os resultados obtidos dos ensaios *in vitro,* nos três artigos apresentados, mostraram-se favoráveis. Estudos de citotoxicidade, estudos *in situ* e ensaios clínicos certamente poderão sugerir ajustes necessários para que este material se torne viável para uso na prática odontológica.

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