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EFEITO DO BROMETO DE MIRISTIL TRIMETIL AMÔNIO NAS PROPRIEDADES FÍSICO-QUÍMICAS E BIOLÓGICAS DE SELANTES RESINOSOS EXPERIMENTAIS

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RESUMO

Este estudo tem como objetivo avaliar a influência do brometo de miristiltrimetilamônio nas propriedades físico-químicas e biológicas de um selante resinoso experimental. O selante de resina experimental foi formulado com 50% em peso de metacrilato de bisfenol A-glicidilo e 50% em peso de dimetacrilato de trietilenoglicol com um sistema fotoiniciador / co-iniciador. Tungstato de cálcio (30% em peso) e silica coloidal (0,7%) foram adicionados. Adicionou-se brometo de miristiltrimetilamio a 0,5 ($G_{0,5\%}$), 1 ($G_{1\%}$) e 2 ($G_{2\%}$)% em peso e permaneceu um grupo sem este composto para ser utilizado como controlo (G_{Ctrl}). Os selantes de resina foram analisados quanto à cinética de polimerização e grau de conversão (DC), resistência coesiva (UTS), atividade antibacteriana contra Streptococcus mutans e citotoxicidade contra queratinócitos humanos. Diferenças na cinética de polimerização foram observadas e as DC variaram de 57,36 (± 2,50) para $G_{2\%}$ a 61,88 (± 1,91) para $G_{0,5\%}$, sem diferença estatisticamente significante entre os grupos ($p > 0,05$). A UTS variou de 32,85 (± 6,08) MPa para $G_{0,5\%}$ a 35,12 (± 5,74) MPa para G_{Ctrl} ($p > 0,05$). Os grupos com o composto apresentaram atividade antibacteriana contra a formação de biofilme a partir de 0,5% em peso ($p < 0,05$) e contra bactérias planctônicas a partir de 1% em peso ($p < 0,05$). Quanto maior a incorporação do composto quaternário, maior foi o efeito citotóxico. $G_{1\%}$ e $G_{2\%}$ tiveram diferença comparado ao G_{Ctrl} ($p<0.05$), mas não houve diferença entre G_{Ctrl} e $G_{0.5\%}$ ($p > 0,05$). Em conclusão, a adição de 0,5% em massa de brometo de miristiltrimetilamônio não alterou as propriedades físico-químicas do selante resinoso e proporcionou atividade antibacteriana sem efeito citotóxico.

PALAVRAS-CHAVE: Selantes de fossas e fissuras; Antibacterianos; Propriedades químicas.

ABSTRACT

This study aims to evaluate the influence of myristyltrimethylammonium bromide on the physico-chemical and biological properties of an experimental resin sealant. The experimental resin sealant was formulated with 50 wt.% of bisphenol A-glycidyl methacrylate and 50 wt.% of triethylene glycol dimethacrylate with a photoinitiator/co-initiator system. Calcium tunsgtate (30 wt.%) and colloidal silica (0.7 wt.%) were added. Myristyltrimethylammonium bromide was added at 0.5 ($G_{0.5\%}$), 1 ($G_{1\%}$), and 2 ($G_{2\%}$) wt.% and one group remained without this compound to be used as control (G_{Ctrl}). The resin sealants were analyzed for the polymerization kinetics and degree of conversion (DC), ultimate tensile strength (UTS), antibacterial activity against *Streptococcus mutans* and cytotoxicity against human keratinocytes. Differences in the polymerization kinetics were observed and the DC ranged from 57.36 (±2.50) for $G_{2\%}$ to 61.88 (±1.91) for $G_{0.5\%}$, without statistically significant difference among groups ($p>0.05$). The UTS ranged from 32.85 (±6.08) MPa for $G_{0.5\%}$ to 35.12 (±5.74) MPa for G_{Ctrl} ($p>0.05$). The groups with the compound showed antibacterial activity against biofilm formation from 0.5 wt.% ($p<0.05$) and against planktonic bacteria from 1 wt.% ($p<0.05$). The higher the quaternary ammonium compound addition, the higher the cytotoxic effect. $G_{1\%}$ and $G_{2\%}$ showed statistically significant difference compared to G_{Ctrl} ($p<0.05$), without difference between G_{Ctrl} e $G_{0.5\%}$ ($p>0,05$). In conclusion, the addition of 0.5 wt% of myristyltrimethylammonium bromide did not alter the physico-chemical properties of the resin sealant and provided antibacterial activity without cytotoxic effect.

KEY WORDS: pit and fissure sealants, anti-bacterial agents, chemical properties.

SUMÁRIO

1. ANTECEDENTES E JUSTIFICATIVA

A cárie dental é considerada um dos problemas de saúde bucal com maior incidencia e prevalência a nível mundial. Em 2016, o *Global Burden of Diasese Study* (Estudo Global da Carga de Doenças) revelou que a incidência de cáries em dentes permanentes era de 7,26 bilhões e em dentes decíduos, 1,76 bilhão, assim ocupando o segundo e quinto lugares, respectivamente, entre as doenças mais comuns (LIANG et al., 2018). Outras pesquisas evidenciaram que 95% da população mundial já padece destas doenças e que das lesões encontradas, 30% são originadas em faces oclusais (sulcos e fissuras); enquanto que 50% tem origem em zonas interproximais (CORTÉS et al., 1989; RIVAS et al., 2002).

A cárie dental constitui uma doença multifatorial, crônica e progressiva. Essas lesões são causadas por bactérias presentes no biofilme ao fermentarem açúcares e produzirem ácidos que desmineralizam esmalte e dentina, podendo se tornar um processo irreversível com a formação de cavidades nos tecidos dentários (LYNCH et al., 2012). A teoria acidogênica permite entender o processo carioso com base na ação de vários fatores como: hóspede suscetível, tipo de dieta, presença de microrganismos, tempo do processo. Dentro desse processo, o biofilme complexo devido a higiene bucal deficiente favorece a adesão e multiplicação bacteriana, desempenhando um importante papel Assim, as enzimas bacterianas degradam os carboidratos, formando os ácidos responsáveis pela desmineralização, dissolvendo íons de fosfato e cálcio da hidroxiapatita, ocasionando a perda de minerais (FEJERSKOV, 2004).

A perda de minerais é considerada um desequilíbrio no processo de desmineralização-remineralização e faz com que as lesões de cárie tenham várias fases, podendo dizer que nos estágios iniciais as lesões cariosas têm características subclínicas. Porém, se a diminuição do pH continua, a cárie dental se torna clinicamente visível por meio, inicialmente, de uma lesão de mancha branca (MARTIGNON et al., 2011). Sob estas condições fisiológicas, a saliva age como um agente de remineralização e tenta manter o equilíbrio entre o tecido dental e o biofilme (BARDOW et al., 2008). Posteriormente ao ataque ácido, quando o pH é superior a 5,5, o fluido salivar reduz a concentração de H+ produzidos pelas bactérias e leva à remineralização porque a saliva está supersaturada de Ca^{+2}, PO_4^{-3} e F^{-9} (Castellanos et al., 2013).

Atualmente, a Odontologia baseada em evidências mudou o entendimento sobre cárie dental e o desenvolvimento dos materiais odontológicos, estimulando a detecção precoce das lesões de cárie dental. Assim, são visados novos sistemas de diagnóstico que permitem identificar o risco cariogênico de um paciente, quantidade de lesões que apresenta e a possibilidade de realizar tratamentos minimamente invasivos, os quais preservam a estrutura dental dentro de um manejo integral do paciente, detectando de forma precoce a perda de minerais (PITTS, 2009). Neste contexto, os clínicos precisam entender e gerenciar critérios atuais de diagnóstico que permitem realizar a tomada correta de decisões. O Sistema Internacional de Detecção e Valorização de Cáries (*ICDAS*) identifica as lesões de cáries desde estágios iniciais e as categoriza segundo os descobrimentos visuais com uma alta correlação histológica, de acordo com a gravidade ou profundidade da lesão

(MULLER et al., 2018). As lesões iniciais de cáries que o ICDAS categoriza com códigos 1 e 2 podem ser paralisadas com tratamentos conservadores. Códigos ICDAS mais severos, nos quais existem um maior compromentimento da estrutura dentária, códigos de 3 a 6, requerem maiores tratamentos restauradores conforme cada caso. A Odontologia minimamente invasiva maneja técnicas remineralizantes nas quais o flúor resulta ser muito efetivo, no entanto novos agentes remineralizantes demonstraram ser eficazes no tratamento de pacientes com alto risco cariogênico, sendo considerados junto aos selantes dentários uma excelente alternativa ao uso de fluoretos (AHOVUO et al., 2017).

O termo selante se refere a um material que forma uma camada protetora de união micromecânica e que cobre a estrutura dentária previamente tratada com ácido. Os selantes podem ser de cura química ou física, com eficácia semelhante. Esses materiais podem ter partículas de carga, flúor e corantes. A eficácia de um selante depende da sua capacidade de penetração, resistência ao desgaste, manipulação e ausência de solubilidade no meio bucal (GÖRKEM et al., 2018).

Estes antecendentes levaram a várias pesquisas a fim de adicionar compostos que não alterem as propriedades do material e produzam efeito antibacteriano. Os compostos quaternários de amônio (QACs) têm sido utilizados em formulação de materiais com o objetivo de reduzir ou impedir a formação de biofilmes microbianos nas superfícies desde 1990 (LIANG et al., 2018), sendo um composto estável com boa permeabilidade, baixa toxicidade, baixa corrosão, efeitos biológicos duradouros entre outras propriedades em comparação com outros agentes antimicrobianos. As propriedades bactericidas dos QACs são atribuídas à ligação da membrana citoplasmática e à difusão por meio da parede celular, do aumento da pressão osmótica da bactéria e da liberação de compostos citoplasmáticos (COCCO et al., 2015). Os últimos estudos têm mostrado que QACs possuem atividade antimicrobiana contra fungos e bactérias, incluindo *S. mutans*, *l. acidophilus*, *C. albicans*, entre outros (ZHANG et al., 2018). Levando em consideração esses resultados, resinas com QAC foram previamente desenvolvidas , conseguindo obter uma redução da atividade metabólica do biofilme sem reduzir as propriedades mecânicas do material. QACs foram incorporados a resinas compostas, sistemas adesivos, resinas acrílicas, cimentos ósseos, materiais de revestimento de celulose, selantes, desinfetantes de cavidades, cimento de fosfato de zinco e cimento de policarboxilato de zinco, obtendo excelentes resultados antibacterianos sem alterar as características dos materiais (COCCO et al., 2015).

A eficácia dos compostos quaternários de amônio está associada ao comprimento da cadeia alquílica, onde quanto maior a cadeia, melhor as propriedades bactericidas do material são consideradas (ZHANG et al., 2018). Neste sentido, e fazendo uma revisão da literatura, o brometo de miristil trimetil-amônio, composto o qual possui 14 carbonos na cadeia alifática, ainda não foi testado em materiais dentários. Portanto, decidiu-se incorporá-lo a um selante resinoso experimental a fim de avaliar sua propriedade antibacteriana e observar se o composto influencia nas propriedades físico-químicas e citotoxicidade do polímero.

2. OBJETIVO

O objetivo desse trabalho foi incorporar brometo de miristil trimetil amônio em 0,5%, 1% e 2%, em peso, a um selante resinoso experimental e avaliar as propriedades físico-químicas e biológicas dos materiais formulados.

3. MANUSCRITO

Essa tese de doutorado se apresenta na forma de um artigo, escrito na língua inglesa e que segue as normas referentes ao periódico *Brazilian Oral Research*, para o qual será submetido.

3.1 MANUSCRIPT

*Dental Materials

Myristyltrimethylammonium bromide effect on physico-chemical and biological properties of experimental resin sealants

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Myristyltrimethylammonium bromide effect on physico-chemical and biological properties of experimental resin sealants

ABSTRACT

This study aims to evaluate the influence of myristyltrimethylammonium bromide on the physico-chemical and biological properties of an experimental resin sealant. The experimental resin sealant was formulated with 50 wt.% of bisphenol A-glycidyl methacrylate and 50 wt.% of triethylene glycol dimethacrylate with a photoinitiator/co-initiator system. Myristyltrimethylammonium bromide was added at 0.5 ($G_{0.5\%}$), 1 ($G_{1\%}$), and 2 ($G_{2\%}$) wt.% and one group remained without this compound to be used as control (G_{Ctrl}). The resin sealants were analyzed for the polymerization kinetics and degree of conversion (DC), ultimate tensile strength (UTS), antibacterial activity against *Streptococcus mutans* and cytotoxicity against human keratinocytes. Differences in the polymerization kinetics were observed and the DC ranged from 57.36 (±2.50) for $G_{2\%}$ to 61.88 (±1.91) for $G_{0.5\%}$, without statistically significant difference among groups ($p>0.05$). The UTS ranged from 32.85 (±6.08) MPa for $G_{0.5\%}$ to 35.12 (±5.74) MPa for G_{Ctrl} ($p>0.05$). The groups with the compound showed antibacterial activity against biofilm formation from 0.5 wt.% ($p<0.05$) and against planktonic bacteria from 1 wt.% ($p<0.05$). The higher the quaternary ammonium compound addition, the higher the cytotoxic effect. $G_{1\%}$ and $G_{2\%}$ showed statistically significant difference compared to G_{Ctrl} ($p<0.05$), without difference between G_{Ctrl} e $G_{0.5\%}$ ($p>0,05$). In conclusion, the addition of 0.5 wt% of myristyltrimethylammonium bromide did not alter the physico-chemical properties of the resin sealant and provided antibacterial activity without cytotoxic effect.

KEY WORDS: pit and fissure sealants, anti-bacterial agents, chemical properties.

1. Introduction

The current understanding of tooth decay and the new advances in dental materials promote the preservation of dental structure and the application of minimally invasive techniques that allow the treatment of initial carious lesions[1,2]. In this way, it is possible to maintain dental tissues and to avoid the advance of the lesion. The minimum intervention protocols in current dentistry use fluorides as the first option for management of caries lesions in enamel[3]. Other way to achieve reliable treatment and prevention of lesions is by the placement of dental sealants on vulnerable pits and fissures[4].

Dental sealants are used in initial caries lesions with the purpose of covering pits and fissures, which are areas of difficult hygiene and may favor biofilm formation[5]. In this way, sealants successfully inhibit the development of dental caries due to its ability to seal the demineralized tissue and form a mechanical barrier, inhibiting bacterial growth and hampering lesion progression[6]. Among dental sealants, resin sealants are preferred because of their better penetration depth, greater retention and less wear[7].

Resin sealants consist a material that can be cured by chemical or photo-activation, presenting inorganic fillers and, sometimes, pigments and fluorine[8]. The use of resin sealant in children and adolescents allows to decrease the development of carious lesion in occlusal surfaces of permanent molars compared to people with no sealed teeth[9]. Taking into consideration the composition of the materials available in the market, it is observed that these materials do not present antibacterial agents in their composition[10]. Therefore, researches have been attempting to formulate and to evaluate new resin sealants with antibacterial compounds aiming to improve the therapeutic activity of these materials.

Quaternary ammonium compounds (QACs) have been added in dental materials composition to provide them antibacterial activity[11]. This property is attributed for QACs due to their interaction and bonding to bacteria membrane and wall, besides their diffusion into the cytoplasmic membrane, increase of osmotic pressure and release of some cytoplasmic constituents[12,13]

The myristyltrimethylammonium bromide is a quaternary ammonium compound (QAC) with 14 carbons in the aliphatic chain and has not been evaluated in dental materials so far. Due to its long alkyl chain, it is possible that this compound shows reliable antibacterial effect. Thus, this study aims to evaluate the influence of myristyltrimethylammonium bromide on the physico-chemical and biological properties of an experimental resin sealant.

2. Methodology

Experimental resin sealants formulation

To formulate the resin sealants, two dimethacrylate monomers were mixed: bisphenol A glycol dimethacrylate (BisGMA, 50 wt.%) and triethylene glycol dimethacrylate (TEGDMA, 50 wt.%). As photoiniciator/co/initiator system, camphorquinone and ethyl 4-dimethylaminobenzoate were incorporated into the resin of BisGMA and TEGDMA at 1 mol% each one. Butylated hydroxytoluene was also added into the resin at 0.01 wt.%. Calcium tungstate was added into the resin at 30 wt.% as a radiopacifier filler and silica was added at 0.7 wt.% to adjust the resin’s viscosity. All reagents were purchased from Aldrich Chemical Company (St. Louis, Missouri, USA) and after being hand-mixed for 5 min, they were sonicated for 180s and hand-mixed for more 5 min. This experimental resin sealant was divided to form four groups: three test groups with 0.5% ($G1_{0.5\%}$), 1% ($G_{1\%}$) and 2% ($G_{2\%}$) of the QAC myristyltrimethylammonium bromide, and one control group (G_{Ctrl}) that presented no addition of this QAC. All samples used in this study were photoactivated during 20 s on each side (light-emitting diode, Radii Cal, SDI, Australia, 1200 mW/cm^2), with exception of samples for polymerizaztion kinetics and degree of conversion tests, which were photoactivated during 40 s on the top. The samples were prepared and kept in distilled water at 37 °C for 24 h before being tested, with exception of polymerization kinetics and degree of conversion tests because the samples were evaluated while they were prepared.

Polymerization kinetics and degree of conversion (DC)

Fourier Transform Infrared Spectroscopy (FTIR, Vetrex 70, Bruker Optics, Ettingen, Germany) was used to evaluate the polymerization kinetics and the DC of the experimental resin sealants. For this test, three samples per group were analyzed by dispensing them on the attenuated total reflectance (ATR) device in the polyvinylsiloxane matrix measuring 1 mm thickness and 4 mm diameter. To perform the photoactivation of each sample, the light-cured unit was fixed using a support to keep 1 mm between the tip of the light-cured unit and the top of each sample. During 40 s of photoactivation, two spectra were obtained per second in absorbance mode (10 kHz velocity, 4 cm^{-1} resolution, Opus 6.5 software, Bruker Optics, Ettlingen, Germany) in the range of 4000 to 400 cm^{-1}. To calculate the DC, the first spectrum obtained was used as “uncured resin sealant”, and the last spectrum, as “cured resin sealant”. The peak at 1610 cm^{-1} from aromatic carbon-carbon double bond was used as internal standard, and the peak at 1640 cm^{-1} was used as aliphatic carbon-carbon double bond to calculate the conversion in percentage[14]. The polymerization rate was calculated according to previous study[15].

Ultimate tensile strength (UTS)

Ten samples per group with hourglass shape were prepared in a metallic matrix with hourglass shape with 8.0 mm long, 2.0 mm wide, 1.0 mm thickness and a cross-sectional area of ±1 mm^2. The samples were fixed in metallic jigs with cyanoacrylate resin and submitted under tensile strength test in a universal testing machine (EZ-SX Series, Shimadzu, Kyoto, Japan) at 1mm/min of crosshead speed until fracture. To calculate the maximum value of tensile strength, it was divided the maximum force value (N) for the constriction area of each sample previously measured (mm), obtaining the result in MPa[16].

Antibacterial activity evaluation

To evaluate the antibacterial activity of the experimental resin sealants, two tests were performed: against biofilm formation on the polymerized samples and against planktonic bacteria. The bacteria used in both tests were *Streptococcus mutans* (NCTC 10449). Three samples per group were prepared for biofilm test and other three samples per group were used in the test against planktonic bacteria. *Streptococcus mutans* were prepared according to previous study[15] and the initial inoculum used for the tests were assessed by serial dilution method and colonies counting, which indicated an inoculum at 7.8 x 10^7 CFU/mL.

To evaluate the antibacterial activity against biofilm formation, the polymerized samples (1 mm thickness and 4 mm diameter) were fixed on teflon matrixes fixed on the lid of a 48-well plate and this assembly was sterilized with hydrogen peroxide plasma (58%, 48 min, 56 °C)[15,17]. 100 μL of the initial inoculum was added in each well of a 48-well plate with 900 µL of brain-heart infusion (BHI) broth with 1 wt.% of sucrose. The sterile assembly of lid and samples was joint with this 48-well plate and kept for 24 h under 37 °C for biofilm formation on the samples. After this period, each sample was detached from the lid and vortexed for 1 min in 1 mL of sterile saline solution. The solution was serial diluted up to 10^{-6} mL and plated on petri dishes containing BHI agar to count the colonies and to calculate the colonies forming units per milliliter (CFU/mL).

To evaluate the antibacterial activity against planktonic bacteria, it was evaluated a BHI broth that was in contact with the polymerized samples along the 24 h mentioned above for the test against biofilm formation. From each well of the 48-well plate, 100 µL were collected after this 24 h of bacteria-samples contact and it was inserted in eppendorf tubes with 900 µL of saline solution to be vortexed, diluted until 10^{-6} and plated on BHI agar Petri dishes. As negative control, three wells were kept with BHI broth and *Streptococcus mutans* at the same proportion (10% of initial inoculum of bacteria in each well) without samples' contact and they were compared to the other groups in the planktonic bacteria analysis. Colonies were visually counted and expressed in CFU/mL.

Cytotoxicity evaluation

To evaluate the possible cytotoxic effect of the experimental resin sealants, human keratinocytes (HaCaT, CLS Cell Lines Service GmbH, Eppelheim, Germany) were used in this test[18]. The keratinocytes were kept in contact ($5x10^3$ cells/per well) with 100 µL of Dulbecco's Modified Eagle Medium (DMEM) in 96-well plates for 24 h at 37 °C. In the same day, five samples per group (1 mm thickness and 4 mm diameter) were placed separately in eppendorf tubes containing 1 mL of DMEM and kept for 24 h at 37 °C. Thus, eluates from possible leaching from the samples were formed. These eluates (100 µL) were kept in contact with the keratinocytes in the 96-well plates for 72 h at 37 °C. There was one group that was maintained without eluates with 100 µL of pure DMEM that was used as control for the test. In addition to using five samples per group, the eluates were applied in five replications, totalizing one hundred wells containing eluates from the samples. After 72 h, 50 µL of trichloroacetic acid/distilled water solution (50:50) was added in each well and kept at 4 °C for one hour to fix the cells on the bottom. Running water (30 s) was used to wash the 96-well plates six times. The 96-well plates were kept at room temperature until drying. Sulforhodamide B at 0.4% (50 µL) was added in each well and the 96-well plates were kept at room temperature for 30 min. The 96-well plates were washed with acetic acid at 1% four times and kept at room temperature until drying. Trizma solution at 10 mM (100 µL) was added in each well and the 96-well plates were incubated for 1 h at room temperature. The absorbance of plates' wells was analyzed at 560 nm and the cell viability of the wells that contained eluates were compared to the wells without eluates. The results were expressed in percentage of cell viability using the cells without eluates as 100%.

Statistical Analysis

Data normality was evaluated by Shapiro-Wilk test. One-way ANOVA and Tukey *post hoc* test were used to compare groups for all tests at a level of 0.05 of significance.

3. Results

The results of polymerization kinetics are presented in Figure 1 and the results of DC are shown in Table 1. The experimental resin sealants had different polymerization behavior along the 40 s of photoactivation. The DC ranged from 57.36 (±2.50)% for $G_{2\%}$ to 61.88 (±1.91)% for $G_{0.5\%}$, without statistically significant difference among groups ($p>0.05$). Figure 1b, which presents the polymerization rate versus time, shows that the higher the concentration of myristyltrimethylammonium bromide, the higher the delay to achieve the maximum polymerization rate and the lower the maximum polymerization rate. Figure 1c evidences these differences among groups, showing that at the same DC among groups, the polymerization rate of G_{Ctrl} was higher than $G_{1\%}$ and $G_{2\%}$ and lower than $G_{0.5\%}$.

Table 1 also presents the results of UTS expressed in MPa. UTS ranged from 32.85 (±6.08) MPa for $G_{0.5\%}$ to 35.12 (±5.74) MPa for G_{Ctrl}. There was no statistically significant difference among groups ($p>0.05$).

Table 2 shows the results of biological properties tested. In the test against biofilm formation, the values ranged from 4.58 (±0.08) log CFU/mL for $G_{2\%}$ to 7.21 (±0.08) log CFU/mL for G_{Ctrl} ($p<0.05$). The higher the myristyltrimethylammonium bromide addition in the experimental resin sealant, the lower the CFU/mL identified ($p<0.05$). The test against planktonic bacteria showed values ranging from 6.68 (±0.58) log CFU/mL for $G_{2\%}$ to 8.28 (±0.05) log CFU/mL for G_{Ctrl} ($p<0.05$). From 1 wt.% of myristyltrimethylammonium bromide addition, there was statistically significant difference compared to G_{Ctrl} ($p<0.05$). In the cytotoxicity test, expressed as percentage of viable cells, the results ranged from 45.26 (±14.11)% for $G_{2\%}$ to 110.16 (±14.64)% for G_{Ctrl} ($p<0.05$). $G_{0.5\%}$ presented no statistically significant difference compared to G_{Ctrl} ($p>0.05$) regarding cytotoxicity against human keratinocytes.

4. Discussion

Resin based sealants are a reliable material to prevent carious lesions in pit and fissures[19]. In this study, the experimental resin based sealant with myristyltrimethylammonium bromide at 0.5 wt.% showed antimicrobial effect without compromising physico-chemical and biological properties.

In the present study, the experimental resin sealants formulated were evaluated for polymerization kinetics and DC. The polymerization kinetics were different among groups, mainly with the addition from 1 wt.% of myristyltrimethylammonium bromide in the resin (Figure 1). From this concentration, the polymerization process delayed, and the groups reached the gel point later compared to G_{Ctrl} and $G_{0.5\%}$. On the other hand, $G_{0.5\%}$ showed higher maximum polymerization rate compared to all groups (Figure 1b). It is possible that 0.5 wt.% of myristyltrimethylammonium bromide decreased the viscosity of the resin sealant, since QACs act as cationic surfactants and may increase monomer chains mobility[20]. A higher monomer chains mobility of the resin sealant may lead to the higher maximum polymerization rate. However, increasing myristyltrimethylammonium bromide incorporation, the spaces among monomer chains may increased further, leading to lower polymerization rate for $G_{1\%}$ and $G_{2\%}$. However, even with the addition of 2 wt.% of myristyltrimethylammonium bromide, there was no statistically significant difference for DC among groups, achieving values of DC compatible with commercial resin sealants[21].

The suitable DC values observed for the experimental resin sealants is desired, since this chemical property is related to mechanical properties of the polymers[22]. Even though all groups presented reliable DC, the delay during the polymerization kinetics observed for $G_{1\%}$ and $G_{2\%}$ may induce the formation of more linear matrix, with lower crosslinking density. Therefore, the mechanical evaluation of the formulated materials was performed via UTS analysis. The specimens of resin sealants were tensile until fracture in a universal testing machine and, despite the fact that the polymerization process was different among groups, there was no statistically significant difference. This is a promising result because the incorporation of antibacterial agents could lead to lower mechanical properties[23]. Resin sealants are composite resins commonly used in occlusal areas, being daily tested under great mechanical stress. Thus, the maintenance of their mechanical properties besides the therapeutic activity provided to an originally inert material is necessary to keep the resin in the tooth site and maybe increase the protective effect against caries.

It is desired that materials used in oral environment present antibacterial effect. In this context, QACs have shown to be effective antibacterial agents. In the current study, the higher the QAC addition, less CFU/mL was found, as observed in previous studies with similar compounds added in resins for dentistry. The studies of PAMAM (polyamidoamine) and DMADDM (dimethylaminododecyl methacrylate) in adhesive systems showed that these QAC have the ability to inhibit the formation of lactic acid and the growth of biofilms[24]. Other studies indicated the efficacy of QAC in resins by testing urethane methacrylate quaternary ammonium compound (UDMQA-12), which has shown to have excellent antimicrobial properties without being cytotoxic[25]. In this study, the experimental resin sealant with 2 wt.% of myristyltrimethylammonium bromide showed the highest antibacterial effect against biofilm formation and growth of *Streptococcus mutans* compared to G_{Ctrl}. The antibacterial activity observed may could assist in the reduction of demineralization process of tooth.

The cytotoxicity test was performed against human keratinocytes via SRB method. The higher the incorporation of the QAC, the higher the cytotoxic effect observed. This result corroborates with the previous studies of (Wu et al., 2019), which show that QAC with long-alkyl chains could lead to cytotoxic effects. However, with the lowest concentration of myristyltrimethylammonium bromide tested, there was no effects on the cell viability compared to G_{Ctrl}. G_{Ctrl} and $G_{0.5\%}$ showed values higher than 70% of viability, which is used by ISO as a cutoff: values higher than 70% indicate that the material presents no cytotoxicity. Therefore, the addition of 0.5 wt.% of myristyltrimethylammonium bromide is a promising method to provide antibacterial activity for a resin sealant without compromise its physico-chemical properties and cytotoxicity[26].

5. Conclusion

The experimental resin sealant formulated with myristyltrimethylammonium bromide at 0.5% ($G_{0.5\%}$) did not alter the physico-chemical properties of the experimental resin sealant. In addition, there was antibacterial effect against biofilm formation and no cytotoxic effect with this concentration.

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Figures

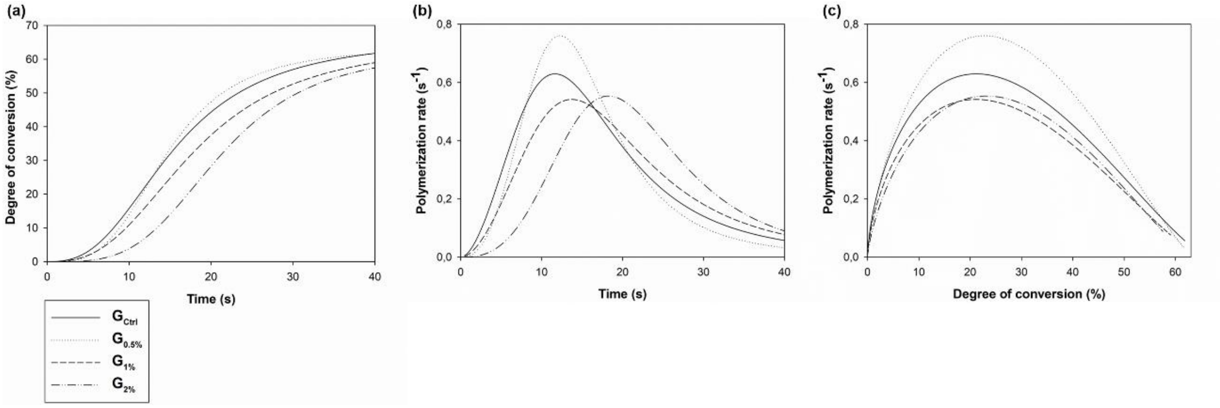

Figure 1. Graphs of polymerization kinetics during photoactivation for 40 s of the experimental resin sealants. DC versus photoactivation time **(a)**. Polymerization rate versus photoactivation time **(b)**. DC versus polymerization rate **(c)**.

Tables

Table 1. Mean and standard deviation values of degree of conversion (DC) in percentage (%) after 40 s of photoactivation and ultimate tensile strength (UTS) in megapascals (MPa) of the experimental resin sealants.

Groups	DC	UTS
G_{Ctrl}	61.73 (±0.55) ^{A}	35.12 (±5.74) ^{A}
$G_{0.5\%}$	61.88 (±1.91) ^{A}	32.85 (±6.08) ^{A}
$G_{1\%}$	58.93 (±1.59) ^{A}	32.86 (±6.29) ^{A}
$G_{2\%}$	57.36 (±2.50) ^{A}	34.29 (±6.36) ^{A}

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

Table 2. Mean and standard deviation values of biofilm formation (log CFU/mL) and planktonic bacteria viability (log CFU/mL) analyses and human cells viability in percentage (%) of cytotoxicity test of the experimental resin sealants.

Groups	Biofilm formation	Planktonic bacteria viability	Human cells viability
G_{Ctrl}	7.21 (±0.08) ^{A}	8.28 (±0.05) ^{A}	110.16 (±14.64) ^{A}
$G_{0.5\%}$	6.80 (±0.20) ^{B}	7.57 (±0.28) ^{AB}	91.82 (±12.17) ^{A}
$G_{1\%}$	5.11 (±0.12) ^{C}	7.00 (±0.16) ^{BC}	50.87 (±6.63) ^{B}
$G_{2\%}$	4.58 (±0.08) ^{D}	6.68 (±0.58) ^{C}	46.26 (±14.11) ^{B}
$G_{negative}$	-	8.21 (±0.06) ^{A}	-

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

4. CONSIDERAÇÕES FINAIS

Os tratamentos realizados com selantes resinosos são considerados uma das primeiras opções terapêuticas em Odontologia minimamente invasiva. Nesse contexto, vários estudos (COLOMBO et al., 2018; LIANG et al., 2018; AHOVUO et al., 2017) mostram sua eficácia em parar ou prevenir lesões iniciais de cárie. No entanto, estes materiais carecem de agentes antimicrobianos em sua composição, sendo o flúor e a clorexidina os únicos componentes introduzidos em selantes no mercado (DIONYSOPOULOS et al., 2015; AGGARWAL et al., 2018). Por isso, esse estudo visou a formulação e avaliação de selantes resinosos com um composto quaternário de amônio.

Os QACs são uma excelente opção a serem incorporados a materias dentários, pois graças à sua estrutura, são eficazes como agentes antibacterianos (MAKVANDI et al., 2018). O efeito dos QACs esta relacionado ao comprimento da cadeia alquila e a carga positiva do grupamento com nitrogênio (CHERCHALIA et al., 2017). Com estas considerações e analisando-se os resultados do presente trabalho, determinou-se que ao adicionar brometo de miristil trimetil amônio em 0,5% em massa, as propriedades físico-químicas do selante resino experimental não foram alteradas e foi observado efeito antibacteriano contra *Streptococcus mutans* sem reduzir a viabilidade celular.

Na presente investigação, a longevidade do agente antimicrobiano não foi avaliada, o que pode ser avaliado em investigações futuras. E ainda, sugere-se a investigação desse composto com grupamento metacrilato para a copolimerização na matriz resinosa (ZHANG et al., 2018). Com base nos resultados desse estudo, outros materiais dentários tais como cimentos resinosos para cimentação de peças protéticas ou selamento de canais radiculares, bem como resinas compostas e sistemas adesivos, poderiam ser avaliados com diferentes concentrações de brometo de alquil trimetil amônio.

Nesse estudo, foi possível formular e avaliar um selante resinoso em que, por meio da adição de um QAC, foi possível fornecer mais uma propriedade biológica ao material (MELO et al., 2018). Apesar de sabermos que o processo de lesão de cárie está associado aos hábitos do paciente, estimula-se o desenvolvimento de materiais dentários com atividade antimicrobiana para talvez ser possível postergar ou prevenir o surgimento de lesões cariosas.

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