

Antibacterial, chemical and physical properties of sealants with polyhexamethylene guanidine hydrochloride

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Abstract: The aim of this study was to evaluate the influence of polyhexamethylene guanidine hydrochloride (PHMGH) in the physico-chemical properties and antibacterial activity of an experimental resin sealant. An experimental resin sealant was formulated with 60 wt.% of bisphenol A glycol dimethacrylate and 40 wt.% of triethylene glycol dimethacrylate with a photoinitiator/co-initiator system. PHMGH was added at 0.5 ($G_{0.5\%}$), 1 ($G_{1\%}$), and 2 ($G_{2\%}$) wt.% and one group remained without PHMGH, used as control (G_{CTRL}). The resin sealants were analyzed for degree of conversion (DC), Knoop hardness (KHN), and softening in solvent (Δ KHN), ultimate tensile strength (UTS), contact angle (θ) with water or α -bromonaphthalene, surface free energy (SFE), and antibacterial activity against *Streptococcus mutans* for biofilm formation and planktonic bacteria. There was no significant difference for DC ($p > 0.05$). The initial Knoop hardness ranged from 17.30 (± 0.50) to 19.50 (± 0.45), with lower value for G_{CTRL} ($p < 0.05$). All groups presented lower KHN after immersion in solvent ($p < 0.05$). The Δ KHN ranged from 47.22 (± 4.30) to 57.22 (± 5.42)%, without significant difference ($p > 0.05$). The UTS ranged from 54.72 (± 11.05) MPa to 60.46 (± 6.50) MPa, with lower value for $G_{2\%}$ ($p < 0.05$). PHMGH groups presented no significant difference compared to G_{CTRL} in θ ($p > 0.05$). $G_{2\%}$ showed no difference in SFE compared to G_{CTRL} ($p > 0.05$). The groups with PHMGH presented antibacterial activity against biofilm and planktonic bacteria, with higher antibacterial activity for higher PHMGH incorporation ($p < 0.05$). PHMGH provided antibacterial activity for all resin sealant groups and the addition up to 1 wt.% showed reliable physico-chemical properties, maintaining the caries-protective effect of the resin sealant over time.

Keywords: Pit and Fissure Sealants; Methacrylates; Polymerization; Anti-Bacterial Agents.

Introduction

Caries recurrence is one of the major causes of restoration failure and restoration replacement in the long term.¹ The incorporation of antibacterial agents in restorative materials have been studied, aiming

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to develop therapeutic materials with improved biological properties.^{2,3,4,5,6,7} Resin sealants for pits and fissures, applied to prevent new caries lesions⁸ and to arrest non-cavitated lesions,⁹ wear and detach over time¹⁰ with consequent biofilm formation around the sealant/enamel interface, increasing the risk of recurrent caries.¹¹

To overcome this issue, the incorporation of fillers in resin sealants have been used to improve the acid- and caries-resistance of dental tissue. Sodium monofluorophosphate,¹² nylon-6 and chitosan,¹³ fluoroboroaluminosilicate glass,¹⁴ and Bioglass 45S5^{15,16} were already tested. In addition, enamel pre-treatments, such as 45S5 bioactive glass air-abrasion, done before sealant application, have been evaluated, improving enamel etchability and reducing microleakage.¹⁶ Nevertheless, the incorporation of inorganic fillers may negatively affect the rheological properties of resins,¹⁷ compromising polymer chain mobility and sealing properties,¹² and decreasing the degree of conversion.¹⁸

Polyhexamethylene guanidine hydrochloride (PHMGH) is an organic compound from guanidine family with cationic charge¹⁹ and broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria^{19,20,21} and fungi.²² Compared to other disinfectants, such as chlorhexidine digluconate, PHMGH presents higher antimicrobial activity against ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Actinobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species*), which are clinically important antibiotic-resistant microorganisms.²⁰ PHMGH has shown to be an effective sporicidal at low concentration, killing spores of Gram-positive bacteria at 0.52% (w/v) in 90 seconds and 0.36% (w/v) in three minutes.¹⁹ Therefore, it has been used as one of the major components of Akacid plus®, a disinfectant widely recommended for hospital and household use, besides its usage in the food and drug industries, due to its colorless and odorless qualities.^{19,21} However, there is no report about the use of PHMGH against *Streptococcus mutans* or in dental materials. Polyhexamethylene guanidine may have toxic effects, such as causing pulmonary fibrosis, when used in humidifier disinfectants.²³ Other studies showed that PHMGH presented lower

cytotoxic effects against human cells than commonly used antimicrobial agents such as chlorhexidine and quaternary ammonium compounds.²⁴

The incorporation of PHMGH into resin sealants could result in a material with antibacterial properties without affecting other properties.^{2,7} The aim of this study was to evaluate the influence of PHMGH in the physico-chemical properties and antibacterial activity of an experimental resin sealant. The null hypothesis tested for the present study was that the addition of PHMGH does not influence the resin sealant properties.

Methodology

Formulation of experimental resin sealants

Bisphenol A glycol dimethacrylate (BisGMA, Aldrich Chemical Company, St. Louis, Missouri, USA) at 60 wt.% and triethylene glycol dimethacrylate (TEGDMA, Aldrich Chemical Company, St. Louis, USA) at 40 wt.% were hand-mixed for 5 min, sonicated for 180 s and hand-mixed again for 5 min²⁵. A photoinitiator /co-initiator system composed by Camphorquinone (CQ, Aldrich Chemical Company, St. Louis, USA) and ethyl 4-dimethylaminobenzoate (EDAB, Aldrich Chemical Company, St. Louis, USA) added at 1 mol%, according to BisGMA and TEGDMA moles¹³. Polyhexamethylene guanidine hydrochloride (PHMGH), Figure 1, was incorporated at 0.5 (G_{0.5%}), 1 (G_{1%}), and 2 (G_{2%}) wt.% in the resin sealant for test groups and one group, without PHMGH, was used as control (G_{CTRL}). For experimental resin sealants formulation, all components were weighed with an analytical balance (AUW220D, Shimadzu, Kyoto, Kyoto, Japan). A light-emitting diode (Radii Cal, SDI, Australia) at 1200 mW/cm² was used for photoactivation.

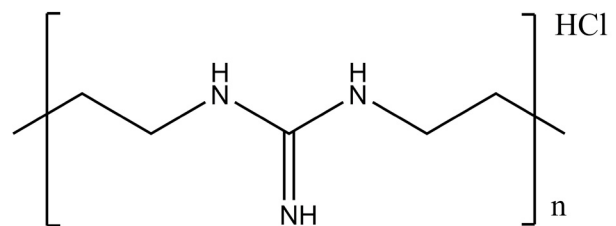


Figure 1. Polyhexamethylene guanidine hydrochloride (PHMGH) representation.

Degree of conversion

For degree of conversion (DC) assessment, three samples per group, $n = 3$, were evaluated by FTIR-ATR (Vetrex 70, Bruker Alpha, Ettingen, Germany). The resin sealants were dispensed onto the ATR crystal in a polyvinylsiloxane matrix with 1 mm thickness measured using a digital caliper. The uncured resin sealant was positioned on ATR in the polyvinylsiloxane matrix. The resin sealants were photoactivated for 50 s with the tip of the LED unit fixed by a device at 1 mm from the top of each specimen. The polymerized samples were then measured using a digital caliper. Data were evaluated with Opus 6.5 software (Bruker Optics, Ettlingen, Germany) with Blackman-Harris 3-Term apodization, in 4000-400 cm^{-1} range with 64 scans at 4 cm^{-1} resolution. Spectra were obtained before and after polymerization and the DC was calculated considering the intensity of carbon-carbon double bond stretching vibration (peak at 1640 cm^{-1}) using the aromatic carbon-carbon double bond stretching vibration (peak at 1610 cm^{-1}) from the polymerized and unpolymerized samples as internal standard.²⁶

Softening in solvent

The softening in solvent of experimental resin sealants was evaluated with five samples per group ($n = 5$, 1.0 mm of thickness x 4.0 mm of diameter) photoactivated for 30 s on each side. The samples were embedded in an acrylic resin to be polished (Model 3v, Arotec, Cotia, Brazil) with silicon carbide sandpapers (1000, 1200, and 2000-grit) and a felt disc saturated with 0.5- μm alumina suspension. After 24 h, five indentations (10 g for 5 s) were performed on each sample using a microhardness tester (HMV 2; Shimadzu, Tokyo, Japan) to obtain the initial Knoop hardness number (KHN1). The samples were immersed in a solution of ethanol:water (70:30) for 2 h, washed with distilled water, and evaluated to obtain the final Knoop hardness number (KHN2). The percentage difference between KHN1 and KHN2 was calculated ($\Delta\text{KHN}\%$) for each group.²⁷

Ultimate tensile strength

For the ultimate tensile strength (UTS), ten samples per group, $n = 10$, were prepared using a metallic

matrix (hourglass-shaped with 8.0 mm long x 2.0 mm wide x 1.0 mm thickness) with a cross-sectional area of 1 mm^2 at the constriction. The samples were photoactivated for 30 s on each side. After 24 h, the samples were fixed in a metallic device with cyanoacrylate resin and submitted to microtensile strength in a universal testing machine (EZ-SX Series, Shimadzu, Kyoto, Japan) at a crosshead speed of 1 mm/min ; the values were reported in MPa .²⁸

Contact angle and surface free energy

For contact angle and surface free energy evaluation, three samples per group, $n = 3$, were prepared (1.0 mm of thickness x 5.0 mm of diameter) with photoactivation for 30 s on each side. The samples were analyzed by an optical tensiometer Theta (Biolin Scientific, Stockholm, Sweden) to evaluate the contact angle (θ) between the samples' surface and a drop of distilled water (polar liquid) or α -bromonaphthalene (non-polar liquid). The surface free energy (SFE) was assessed by the sessile drop method. The drop out size was 3.0 μL , the drop rate was 2.0 $\mu\text{L}/\text{s}$, the displacement rate was 20.0 $\mu\text{L}/\text{s}$, and the speed dispersion of the liquids was 50 mm/min . The evaluation was performed during 20 s and the static θ between each drop and the polymer surface was measured at 10 s. The SFE was achieved using the Owens-Wendt-Rabel-Kaelble (OWRK) method as previously reported³ and OneAttension software (Biolin Scientific, Stockholm, Sweden).

Evaluation of antibacterial activity against biofilm formation

To evaluate the antibacterial activity against biofilm formation, a direct contact inhibition analysis was performed using three samples ($n = 3$, 1.0 mm thickness x 4.0 mm diameter) per group, photoactivated for 30 s on each side. The samples were attached on the lid of a test plate and the assembly was submitted to hydrogen peroxide plasma (58%) sterilization for 48 min at 56°C. Each well of a 48-well plate contained 900 μL of brain-heart infusion broth (Aldrich Chemical Co., St. Louis, Missouri, USA), 1 wt.% sucrose, and 100 μL of *Streptococcus mutans* (NCTC 10449) at 10^7 CFU/mL suspension from an overnight broth culture. The 48-well plate was incubated with the assembly (lid

and samples) at 37°C for 24h. Three wells with broth and *Streptococcus mutans* but without samples were used as negative control. The samples were removed from the lid and vortexed for 1 min in 1 mL of saline solution (0.9%) to be diluted until 10⁻⁶ dilution. Two 25-μL drops of each dilution were plated in brain-heart infusion agar Petri dishes and incubated at 37°C for 48h. The number of colony forming units (CFUs) was visually counted and transformed to log CFU/mL.^{4, 29,30,31}

Evaluation of antibacterial activity against planktonic bacteria

For the evaluation of antibacterial activity against planktonic bacteria, 100 μL of each well from the direct contact inhibition assay (n=3) were vortexed in 900 μL of saline solution (0.9%), diluted until 10⁻⁶ dilution, and plated in brain-heart infusion agar Petri dishes as previously described. The number of CFUs was visually counted and transformed to log CFU/mL.

Statistical analysis

Statistical analysis was performed using SigmaPlot (version 12.0, Systat Software Inc., USA). Data distribution was evaluated by Shapiro-Wilk test.

Paired t-test was used to compare KHN1 and KHN2 in each group at a level of 0.05 of significance. One-way ANOVA and Tukey's post-hoc test were used to compare groups in softening in solvent, DC, UTS, contact angle, SFE, antibacterial activity against biofilm and against planktonic bacteria among groups at a level of 0.05 of significance.

Results

Table 1 presents the DC, KHN1, KHN2, and ΔKHN% results for the experimental resin sealants. The values of DC ranged from 60.29 (± 3.50) to 63.04 (± 0.65) %, without significant difference among groups (p > 0.05). The groups with PHMGH incorporation reached higher values of KHN1 compared to control (p < 0.05). All groups presented a decrease in Knoop hardness after 2 h immersed in an ethanolic solution (p < 0.05). The ΔKHN% ranged from 47.22 (± 4.30) to 57.22 (± 5.42) %, without significant difference among groups (p > 0.05).

Table 2 presents the values of UTS, contact angle with water or α-bromonaphthalene, and SFE. The UTS ranged from 48.40 (± 11.00) to 60.46 (± 6.50) MPa, without significant difference up to 1 wt.% of

Table 1. Mean and standard deviation of degree of conversion (DC) after 50 s of photoactivation, initial Knoop hardness number (KHN1), final Knoop hardness number (KHN2) and percentage of microhardness variation (ΔKHN%) of experimental resin sealants.

Groups	DC (%)	KHN1	KHN2	ΔKHN (%)
G _{CTRL}	61.69 (± 0.72) ^A	17.30 (± 0.50) ^{Ba}	7.40 (± 0.86) ^b	57.22 (± 5.42) ^A
G _{0.5%}	60.73 (± 1.82) ^A	18.81 (± 0.90) ^{Ab}	9.60 (± 1.40) ^b	49.10 (± 7.20) ^A
G _{1%}	60.29 (± 3.50) ^A	19.50 (± 0.45) ^{Aa}	10.30 (± 0.93) ^b	47.22 (± 4.30) ^A
G _{2%}	63.04 (± 0.65) ^A	18.61 (± 0.73) ^{Ab}	9.00 (± 1.42) ^b	52.00 (± 6.32) ^A

Different capital letters indicate statistical difference in the same column (p < 0.05). Different small letters indicate statistical difference in the same row (p < 0.05). p-value of normality test of DC p = 0.343; KHN1 p = 0.219; KHN1 and KHN2 of G_{CTRL}- p = 0.282; G_{0.5%}- p = 0.739; G_{1%}- p = 0.807; G_{2%}- p = 0.319.

Table 2. Mean and standard deviation of ultimate tensile strength (UTS), contact angle (θ) with water and α-bromonaphthalene, and surface free energy (SFE) of experimental resin sealants.

Groups	UTS (MPa)	Contact angle		SFE (mN/M)
		Water	α-bromonaphthalene	
G _{CTRL}	60.46 (± 6.50) ^A	67.90 (± 6.03) ^{AB}	18.11 (± 6.33) ^A	50.00 (± 2.04) ^A
G _{0.5%}	54.72 (± 11.05) ^{AB}	74.20 (± 4.54) ^A	19.80 (± 4.83) ^A	46.95 (± 1.83) ^B
G _{1%}	57.43 (± 7.09) ^{AB}	74.80 (± 2.90) ^A	19.23 (± 2.80) ^A	46.82 (± 1.20) ^B
G _{2%}	48.40 (± 11.00) ^B	64.80 (± 2.33) ^B	21.64 (± 7.10) ^A	50.60 (± 1.23) ^A

Different letters indicate statistical difference in the same column (p < 0.05). p-value of normality test of UTS p = 0.484; contact angle with water p = 0.478; contact angle with α-bromonaphthalene p = 0.356; surface free energy with water p = 0.664.

PGMGH compared to G_{CTRL} ($p > 0.05$). There was no significant difference for PHMGH groups compared to G_{CTRL} for the contact angle with water ($p > 0.05$) and α -bromonaphthalene ($p > 0.05$). The SFE ranged from 46.82 (± 1.20) to 50.60 (± 1.23) mN/M, without significant difference between $G_{2\%}$ and G_{CTRL} ($p > 0.05$); lower values were found for $G_{0.5\%}$ and $G_{1\%}$ compared to control and $G_{2\%}$ ($p < 0.05$), which did not differ between them ($p > 0.05$).

Table 3 shows the results of antibacterial activity against biofilm formation on polymerized samples and against planktonic bacteria. With the increase of PHMGH concentration, higher antibacterial activity against biofilm formation was found ($p < 0.05$). The evaluation in planktonic bacteria showed that the G_{CTRL} presented no significant difference to the negative control ($p > 0.05$). $G_{1\%}$ and $G_{2\%}$ showed less planktonic bacteria than G_{CTRL} and $G_{0.5\%}$ ($p < 0.05$).

Discussion

In the last major study about prevalence and incidence of oral health conditions, the 2015 Global Burden of Disease (GBD) Study, it was observed that untreated caries in permanent teeth affected more than 2.5 billion people (95% uncertainty interval (UI): 2.4–2.7 billion) and 573 million children, making caries the most prevalent disease in the world.³² In addition, in the last estimation for direct and indirect costs due to dental diseases, untreated caries was the cause of 12% of global productive losses and considered one of the major global public health challenges³². Resin sealants are effective in preventing caries lesions.^{8,33} However, the rate of intact sealants decreases over time (73.3% after 5 years),³⁴ negatively

impacting their protective effect.¹¹ In the present study, PHMGH was incorporated as an antibacterial agent in an experimental resin sealant. The addition of up to 1 wt.% PHMGH showed reliable physicochemical properties and all PHMGH groups showed antibacterial activity against biofilm formation and planktonic bacteria of *Streptococcus mutans*. Thus, the null hypothesis proposed for the study was rejected.

The experimental resin sealants were evaluated regarding DC, determined as the conversion of unsaturated carbon-carbon double bonds in saturated bonds of monomers. High DC is associated with better mechanical properties²⁶ and the addition of different compounds in polymers may decrease the DC by altering chain mobility,¹² light transmission,¹⁸ or degree of functionality (number of carbon double bonds).²⁶ PHMGH is a polymer with a short alkyl chain composed by seven carbon atoms with saturated bonds. There was no significant difference in the DC among groups even with lower degree of functionality in PHMGH groups (there was less C=C per volume in PHMGH groups compared to G_{CTRL}). In addition, all groups achieved more than 60% DC, which is in accordance with the values of commercial resin sealants.³⁵ PHMGH presents structural analogies with polymers and quaternary ammonium compounds, which have already been tested in dental resins, especially adhesive resins. Compounds such as 12-methacryloyloxydodecylpyridinium bromide,³⁵ 1,3,5-triacryloylhexahydro-1,3,5-triazine,⁷ 2-methacryloyloxy ethyl trimethyl ammonium chloride,² and dimethylaminododecyl methacrylate³⁶ generally present no influence in the DC at low concentrations (up to 10 wt.%, generally being tested up to 5 wt.%). The short alkyl chain

Table 3. Mean and standard deviation of direct contact inhibition assay in logarithmic transformation of colony forming units per milliliter (log CFU/mL) and planktonic bacteria inhibition assay in log CFU/mL.

Groups	Direct contact inhibition assay	Planktonic bacteria inhibition assay
G_{CTRL}	5.99 (± 0.06) ^A	7.15 (± 0.11) ^{AB}
$G_{0.5\%}$	5.69 (± 0.04) ^B	6.74 (± 0.35) ^B
$G_{1\%}$	4.57 (± 0.14) ^C	6.15 (± 0.03) ^C
$G_{2\%}$	2.06 (± 0.15) ^D	6.05 (± 0.14) ^C
Negative control	-	7.32 (± 0.12) ^A

Different capital letters indicate statistically significant difference in the same column ($p < 0.05$). p-value of normality test of direct contact inhibition assay $p = 0.499$ and planktonic bacteria inhibition assay $p = 0.407$.

of PHMGH contributes to a suitable DC due to the greater chain mobility of the base resin compared to resins with quaternary ammonium compounds with longer alkyl chain.³⁷ Furthermore, the refractive index of PHMGH (poly (hexamethylene biguanide) hydrochloride -1.5486³⁸) is similar to the index of the co-monomer blend (mixture of BisGMA, TEGDMA, and HEMA - 1.47 to 1.59 (monomer) and 1.50–1.62 (polymer)¹⁸). Because of the similar refractive index between the base resin and the material incorporated, the light energy availability is less susceptible to reduction, which probably contributed to the similar DC among groups.³⁹ This is an advantage of PHMGH over the incorporation of antibacterial oxides/inorganic fillers,¹⁸ which usually have different refractive index, decreasing the DC.²⁵ PHMGH did not affect the DC regardless the concentration evaluated. Besides having great mechanical properties, the sealants incorporated with PHMGH should provide stability to the material over time, as high DC also decreases the leaching of uncured monomers from the polymer matrix, improving biocompatibility.⁴⁰

Although high DC may indicate satisfactory physico-chemical properties,²⁶ the evaluation of mechanical and stability properties after solvent storage are necessary. The UTS analysis indicated no significant difference up to 1 wt.% of PHMGH. With 2 wt.% of PHMGH powder, agglomerates may form, which are not well-bonded to the organic matrix, decreasing the mechanical properties.¹⁷ Agglomerates in polymers do not constrain the surrounding matrix of deforming under mechanical load,¹⁷ which may have led to the lower values for $G_{2\%}$ compared to G_{CTRL} . The lower concentrations of PHMGH promoted a better dispersion of the compound in the organic matrix, with less agglomeration of PHMGH, resulting in the non-difference among G_{CTRL} , $G_{0.5\%}$ and $G_{1\%}$. However, despite the lower UTS observed for $G_{2\%}$, there was no difference for softening in solvent among the experimental resin sealants, and PHMGH groups reached higher KHN1 values. PHMGH powder could be pressed into the softer matrix rather than being plastically deformed, leading to the higher values of KHN1. After ethanolic solution storage, all experimental resin sealants showed lower values of Knoop hardness. This can be explained by the higher

interaction between the solvent molecules and polymer chains on the resin surface rather than the covalent bonds in the polymer.⁴¹ One could expect that the increase in PHMGH incorporation would increase ΔKHN due to the hydrochloride in its structure by increasing water sorption and solubility as occurred when hydrophilic monomers are incorporated in resins at higher concentration, increasing resin degradation.²⁶ The addition of PHMGH did not increase the interaction with solvent molecules during immersion in the ethanolic solution and the high DC observed for all experimental resin sealants probably positively influenced these results.²⁶ Thus, besides not having influenced the DC, the PHMGH incorporation probably did not negatively affect the crosslinking density,²⁶ as there was no influence in the softening by the solvent regardless the concentration tested.

The contact angle and the SFE of polymers may also change due to the incorporation of fillers, as boron nitride nanotubes in dental adhesives,⁴² or different monomers, as quaternary alkylammonium in dental composites.⁴³ A lower value of contact angle with water was obtained for $G_{2\%}$ compared to $G_{0.5\%}$ and $G_{1\%}$, with no significant differences between each PHMGH group and G_{CTRL} . Higher amounts of PHMGH would lead to lower contact angle values compared to G_{CTRL} due to PHMGH hydrophilicity.¹⁹ Also, there was a slightly but significant difference for SFE among groups, without significant difference between G_{CTRL} and $G_{2\%}$. Previous studies indicate higher cell attachment in surfaces with higher wettability.⁴⁴ This theme is controversial, since another research shows no linear correlation between these properties and bacterial adhesion,⁴⁵ turning the antibacterial activity evaluation indispensable. Even with the results observed for surface properties, the hydrophilic character of PHMGH did not influence the stability after the storage in ethanol:water solution compared to G_{CTRL} . The non-difference in the softening in solvent, associated with the high values observed for DC up to 2 wt.% and the non-difference for UTS up to 1 wt.% may assist in the preservation of the polymer against hydrolytic degradation over time.^{2, 27, 28}

The experimental resin sealants with PHMGH were evaluated by direct contact inhibition and planktonic bacteria viability assays. The higher the

PHMGH incorporation, the higher the antibacterial activity against biofilm formation on polymerized samples, with more than 60% of biofilm reduced with $G_{2\%}$ compared to G_{CTRL} . Dimethacrylates (TEGDMA, BisGMA and urethane dimethacrylate (UDMA)) commonly used for resin sealants composition do not present antibacterial activity.⁵ With the same purpose of antibacterial agents incorporation in adhesive systems,^{2,4,7} composite resins³ and glass ionomer cements,⁴⁶ resin sealants with PHMGH could prevent bacterial attachment and biofilm formation at tooth/resin interface, where recurrent caries commonly occur, and act as an additional strategy against disease development. The planktonic bacteria inhibition assay showed no difference between G_{CTRL} and $G_{0.5\%}$, while $G_{1\%}$ and $G_{2\%}$ showed lower values of planktonic bacteria compared to G_{CTRL} and $G_{0.5\%}$. The decrease of planktonic bacteria in broth may be associated with the leaching of PHMGH from the polymer.⁴⁷ It is also possible that the decrease of planktonic bacteria occurred due to the contact of the cells in broth with the surface of resin sealants,⁴⁴ similar to a previous study with antibacterial monomers (quaternary ammonium compounds) that copolymerized with the base resin, inducing planktonic bacteria reduction in broth around the polymerized samples.^{2,7}

The antibacterial activity of PHMGH observed in direct contact inhibition and planktonic bacteria viability assays occurred due to the increase of the cytoplasmic membrane permeability after adsorption and bonding to the negative charge of bacteria's surface, leading to leakage of intracellular constituents and cell death.²¹ In addition to the membrane disorganization and pore formation,²¹ guanidine compounds have shown to affect DNA and cellular proteins in Gram-positive and Gram-negative bacteria.²¹ The selective DNA binding between guanidine molecules and

bacteria chromosomes⁴⁸ differentiates the antibacterial action of guanidine compounds from classical quaternary ammonium compounds, which commonly act only in bacteria wall and membrane.⁴⁹ Regarding human cells, a previous study investigated the cytotoxicity of antiseptics, including guanidine molecules (as polyhexamethylene biguanide (PHMB) and octenide dihydrochloride (OCT)), chlorhexidine digluconate, and cetylpyridinium chloride against human fibroblasts.²⁴ Guanidine molecules showed low cytotoxicity and high antibacterial activity.²⁴ However, it is suggested that polyhexamethylene guanidine may be associated to toxic effects, including pulmonary fibrosis, when used in humidifier disinfectants.²³ Thus, the results of this study should be used with caution and more cytotoxic tests should be performed.

The development of restorative materials with antibacterial activity is desirable for the improvement of the therapeutic effect. The present study presented the formulation of a new resin sealant with PHMGH as an antibacterial agent with reliable physico-chemical properties. In this way, PHMGH may be an alternative for long-lasting caries prevention of resin sealants.

Conclusion

PHMGH provided antibacterial activity for all resin sealant groups and the addition up to 1 wt.% showed reliable physico-chemical properties, maintaining the caries-protective effect of the resin sealant over time.

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