

Streptococcus Mutans Biofilm Influences on the Antimicrobial Properties of Glass Ionomer Cements

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The aim of this study was to evaluate the *in vitro* antibacterial and biofilm inhibition properties of glass ionomer restorative cements. Ketac Nano, Vitremer, Ketac Molar Easymix and Fuji IX were analyzed using the following tests: a) agar plate diffusion test to evaluate the inhibitory activity of cements against *S. mutans* (n=8); b) *S. mutans* adherence test by counting colony-forming units after 2 h of material/bacteria exposure (n=10); c) biofilm wet weight after seven days of bacterial accumulation on material disks, with growth medium renewed every 48 h (n=10); d) pH and fluoride measurements from the medium aspired at 48 h intervals during the 7-day biofilm development (n=10). Data from the a, b and c tests were submitted to Kruskal-Wallis and Mann-Whitney tests and the fluoride-release and pH data were submitted to two-way ANOVA and Tukey tests ($\alpha=5\%$). Vitremer followed by Ketac Nano showed the greatest inhibitory zone against *S. mutans* than the conventional ionomers. Vitremer also showed higher pH values than Ketac Nano and Fuji IX in the first 48 h and released higher fluoride amount than Ketac Nano e Ketac Molar Easymix throughout the experimental period. The chemical composition of restorative glass ionomer materials influenced the antibacterial properties. The resin modified glass ionomer (Vitremer) was more effective for inhibition of *S. mutans* and allowed greater neutralization of the pH in the first 48 h. However, the type of glass ionomer (resin modified or conventional) did not influence the weight and adherence of the biofilm and fluoride release.

Introduction

Bacterial biofilms are complex three-dimensional structures in which bacteria are embedded in a matrix made mainly by exopolysaccharides. In the oral cavity, biofilms may be found on dental hard and soft tissues, associated with caries and periodontal diseases, and on the wide array of biomaterials used for the restoration of oral functions(1). Accumulation of bacteria on restorative materials not only degrades the material and roughens its surface, but also causes bacterial reinfection of the interface between the restoration and the tooth, with a recurrence of caries (2). In order to preventing or slow down lesion progression and, consequently, to reduce the rate of restoration replacement, there is an increasing interest in new dental materials capable of attracting less biofilm or releasing antimicrobial compounds.

Glass ionomer cements (GIC) are generally advised where protection against caries is needed, since they potentially reduce microleakage by adhering to tooth structure (3), suppress the growth of caries-related oral bacteria and neutralize acids produced by those bacteria through ion release (4). The fluoride-releasing and neutralizing ability of GIC materials are affected by the nature of the fluoride incorporated in them and also by the nature of

the storage medium (5), particularly its pH. However, these beneficial effects occur at the expense of extensive surface deterioration (2), leading to a negative spiral of events, in which more colonizing organisms will adhere to the degraded material and promote more deterioration.

Different components released from conventional and resin-modified glass-ionomer cements (RMGIC) may modulate the phenotype of cariogenic bacteria. Fluoride, aluminum, and strontium (6) have been associated with a cariostatic activity and reduction of the acidogenicity of *S. mutans* biofilm. On the other hand, some resin monomers; such as hydroxyethyl methacrylate (HEMA), ethyleneglycol dimethylacrylate (EGDMA) and triethyleneglycol dimethylacrylate (TEGDMA) may stimulate the growth of cariogenic bacteria, such as mutans streptococci and lactobacilli, while also enhancing the glucosyltransferase activity in *Streptococcus sobrinus* (7).

There is little information regarding the chemical and biological properties of the nano-filled RMGIC, Ketac™ Nano (3M ESPE) (8). This material has a unique combination of filler content: bonded nanofillers, nanoclusters and fluoroaluminosilcate glass particles (FAS) (3M ESPE Internal Data). In addition, it contains HEMA, bisphenol

glycidyl methacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA) as resin monomers, different from most of the known RMGICs (9). Therefore, it would be interesting to study this material's behavior regarding biofilm-material interaction, since there is less fluoride available for release (27% FAS glass) and a smoother surface is obtained (10,11), potentially modifying biofilm accumulation.

The purpose of the present study was to evaluate four GIC restorative cements with different chemical compositions, including the nano-ionomer, concerning their antibacterial and biofilm inhibition properties.

Material and Methods

Agar Plate Diffusion Test

S. mutans (UA159) was obtained from the culture stock of the Department of Microbiology and Immunology, Dental School of Piracicaba, UNICAMP. The antibacterial activity of each material was evaluated using the agar plate diffusion test. The indicator strain was first grown on Mitis salivarius agar (Difco Laboratories, Detroit, MI, USA) plates at 37 °C for 48 h in a 10% CO₂ incubator (Water-Jacked CO₂ Incubators/Cole Parmer Instruments, Vernon Hills, IL, USA). Subsequently, single colonies were inoculated into 5 mL of Brain Heart Infusion (BHI) broth (Difco Laboratories, Detroit, MI, USA) and incubated at 37 °C for 24 h to form a suspension (inoculum). In each sterilized Petri dish (20x100 mm), a base layer containing 15 mL of BHI agar mixed with 300 µL of each inoculum was prepared. After solidification of the culture medium, five wells with 5 mm diameter were made in each plate and completely filled with one of the testing materials listed in Table 1. Eight wells were filled with each material (n=8). All materials were handled under aseptic conditions and according to the manufacturer's instructions. After

placement, the RMGICs were light-cured. Ten microliters of aqueous 0.12% chlorhexidine digluconate was applied on sterile filter paper discs (n=6), also 5 mm in diameter, placed in the Petri dishes for control.

The plates were maintained for 2 h at room temperature to allow diffusion of the materials. After this, they were incubated at 37 °C for 24 h. Zones of bacterial growth inhibition were recorded in millimeters (mm) using a digital caliper (Mitutoyo, SP, Brazil). Measurements were taken at the greatest distance between two points at the outer limit of the inhibition halo formed around the well. This measurement was repeated three times and the mean was computed for each well (2).

Specimen Preparation

The composition and manufacturing information of the dental restorative materials evaluated are in Table 1. Specimens were prepared with a sterilized custom Teflon mold (5 mm diameter; 2 mm deep) according to the manufacturer's instructions, under aseptic conditions. The materials were mixed by a single operator, packed into the mold, covered and pressed flat with a sterilized glass slide. Vitremer and Ketac™ Nano specimens were polymerized with a curing light unit (Elipar Trilight, 3M ESPE, St. Paul, MN, USA) after checking the intensity of the unit with a curing light meter (Hilux Dental Curing Light Meter, Benliglu Dental Inc., Turkey). Ketac Molar Easymix and Fuji IX specimens were allowed to cure for 5 min.

All the disks were stored in 100% relative humidity at 37 °C for 24 h. Finishing/polishing procedures were not performed in order to avoid surface contamination before the interaction with the *S. mutans* biofilm and, consequently, the need to carry out the sterilization process. Sterilization methods could affect the structure and properties of the studied restorative materials, like

Table 1. Materials used in this study

Material	Composition*	Ratio recommended	Average particle size**
Ketac Nano (3M ESPE)	Paste A: silane treated glass, silane treated zirconia, silane treated silica, glass powder, HEMA, Bis-GMA, TEGDMA, PEGDMA Paste B: silane treated ceramic, water, copolymer of acrylic and itaconic acids, HEMA	1.3:1 paste:paste	1-1.6 µm (cluster) 5-25 nm (nanofiller) 1.0 µm (glass)
Vitremer (3M ESPE)	Powder: fluoroaluminosilicate glass, redox system; Liquid: aqueous solution of a modified polyalkenoic acid, HEMA	2.5:1	3.0 µm
Ketac Molar Easymix (3M ESPE)	Powder: aluminum-calcium-lanthanum fluorosilicate glass, copolymer of acrylic and maleic acid; Liquid: copolymer of acrylic and maleic acid, tartaric acid, water	4.5:1	2.8 µm
Fuji IX (GC Corp.)	Powder: polyacrylic acid, strontium aluminum fluorosilicate glass Liquid: polyacrylic acid, tartaric acid and water	3.6:1	4.4 µm

*Bis-GMA = bisphenol glycidyl methacrylate; HEMA = 2-hydroxyethyl methacrylate; TEGDMA = triethylene glycol dimethacrylate. **Manufacturer information.

altering the polymerization degree, degradation, crack formation or otherwise modifying the surface of the glass ionomers (12). Ten specimens of each material were used for the adherence test and ten for *S. mutans* biofilm analysis, including an analysis of the fluoride releasing and neutralizing effect.

Streptococcus Mutans Adherence Test

To prepare the inoculum, *S. mutans* (UA159) was grown as previously described. Each ionomeric material disk (n=10) was exposed under static conditions to 25 μ L of inoculum adjusted to an optical density (OD) of 0.6 at 550 nm (approximately 8×10^{11} CFU/mL). After 2 h at room temperature, the non-adhering cells were removed by washing two times with 0.9% NaCl solution (saline). Each disk was then inserted into 3 mL of saline solution containing three glass beads and vortexed for 1 min. The suspension was diluted in decimal series from 10^{-1} to 10^{-4} in saline solution and inoculated in triplicate on BHI agar plates. These plates were incubated at 37 °C for 48 h in a 10% supplemented CO₂ environment. The colonies were counted and the number of viable bacteria was determined - CFU/mL corresponding to the cells adhered to the GIC cements after 2 h of *S. mutans* exposure (2).

Streptococcus Mutans Biofilm Analysis

As described above for the adherence test, a *S. mutans* inoculum of 25 μ L (OD of 0.6 at 550 nm) was maintained for 2 h on ten specimens of each material so that the cells would promote an initial adherent biofilm. The non-adhered cells were removed and each biofilm/material disk set was placed in a single well of 24-well polystyrene plates (Multidish 24-well Nunclon) with 2 mL of sterile fresh BHI broth with the addition of 1% (w/v) sucrose. Bacterial accumulation occurred at 37 °C in a 10% supplemented CO₂ environment, developing a 7-day-old biofilm. The medium was renewed at 48 h intervals (2) and the aspired medium was used for pH and fluoride analysis.

Biofilm Wet Weight

At the end of the experimental period (7 days), the biofilm/disk sets were washed twice in sterile 0.9% saline solution to remove loosely bound material. The wet biofilm/disk set was then analytically weighed (± 0.01 mg) on a precision scale (JK 180, Chyo Balance Corp., Tokyo, Japan), in pre-weighed sterilized Petri plates. The disks were ultrasonically washed for 10 min, dried and weighted again to subtract the weight of the specimen from the first value, in order to obtain the biofilm wet weight.

pH Test

The pH of the growth medium aspired from each well at

48 h intervals (1st, 2nd and 3rd exchange - at 48 h, 96 h and 144 h) was determined using a portable pH meter (Orion Model 420A, Analyzer Co., São Paulo, SP, Brazil). The initial pH of the broth medium (prior to microorganism inoculation and cement storage) was 7.26 (standard deviation=0.2). Additionally, were prepared negative control solutions stored under identical conditions containing no cement. Their pH, determined after 1 week, was found to be 3.6 (standard deviation=0.1). In all cases, the pH electrodes were calibrated immediately prior to use with the pH 4.0 and 7.0 standard buffer solutions.

Fluoride Release

The amount of fluoride released by the restorative materials during biofilm growth was analyzed. Fluoride measurements in the medium aspired from each well were made in duplicate using an ion specific electrode (Orion 96-09) connected to a microprocessor ion-analyzer (Orion EA-940, Orion Research, Boston, MA, USA), previously calibrated in triplicate with fluoride standards (0.025 to 4.0 μ g F⁻/mL) in TISAB III (Total Ionic Strength Adjustment Buffer; Thermo Orion, Beverly, MA, USA). Sample readings were in millivolts (mV) and transformed in μ gF⁻/mL (ppm F⁻) by linear regression of the calibration curve.

Statistical Analysis

Data from each material about the inhibition zones (mm), *S. mutans* adherence (CFU/mL) and wet weight of accumulated biofilm (mg) were submitted to Kruskal-Wallis and Mann-Whitney tests ($\alpha=5\%$). Regarding fluoride-release (ppm F⁻) and pH, data were transformed using a log transformation, and two-way ANOVA and Tukey tests were applied ($\alpha=5\%$). The SAS system (version 8.02, SAS Institute Inc., Cary, NC, 1999) software, was used and the level of significance was set at 5%.

Results

The Kruskal-Wallis test did not reveal significant differences among the studied materials concerning the initial streptococci adherence ($p=0.6272$) and the wet weight of the biofilms accumulated for 7 days on the specimen surfaces ($p=0.9612$), as described in Table 2. Regarding the agar plate diffusion test, the RMGIC Vitremer showed the greatest inhibitory effect against *S. mutans* (16.6 mm), which was similar to chlorhexidine (15.8 mm ± 0.59), followed by Ketac Nano (10.4 mm) and finally, the conventional ionomers presented the least inhibitory effect. Ketac Molar Easymix (7.4 mm) and Fuji IX (7.8 mm) presented similar values. Ketac Nano, Ketac Molar Easymix and Fuji IX produced statistically lower inhibition zones than chlorhexidine ($p=0.0008$).

Table 3 shows the pH of the growth medium after

immersion of the test material over 48-h periods, as function of time (1st, 2nd and 3rd exchange). Differences in pH over time were not significant for any tested material. However, at the 1st period evaluated (48 h), there was a significant difference among the materials. Vitremer presented higher pH values (4.8) than Ketac Nano (4.1) and Fuji IX (3.8). In addition, the pH of all studied materials was significantly higher than the negative control ($p < 0.01$).

The results of fluoride release for the same broth medium used for the pH analyses are in Table 4. Vitremer and Fuji IX had the highest fluoride release at the three measured periods. Ketac Nano showed similar values to Ketac Molar Easymix at the 1st exchange and later, the lowest fluoride release. After the initial high rate of release found in the first measurement, the fluoride release rate was significantly lower for all materials. Comparing the first and the last broth change, the fluoride release from Ketac Nano presented a drop in value of about twelve times.

Discussion

Biofilms are diverse and complex aggregates of bacteria that exhibit over 100-fold resistance to antimicrobial agents. Once a biofilm is established, the live cells are

typically buried beneath the surface or between layers of dead cells and encased in an exopolysaccharide matrix, interfering with the diffusion of antibiotics (13). In the oral environment, an established or mature biofilm can accumulate at stagnant sites, as interproximal surfaces, gingival crevices and pits and fissures, in excess of levels compatible with oral health. Additionally, there are novel microenvironments from the formation of marginal gaps around the tooth-restoration interface, contributing to postoperative sensitivity, recurrent caries, pulp inflammation and necrosis (2). Therefore, it would be important to select a restorative material for intraoral sites where biofilm would be protected against dynamic shear forces from saliva, the tongue and a toothbrush.

All of the evaluated GIC showed an antibacterial activity according to the agar-plate diffusion test (Table 2), inhibiting the growth of the selected cariogenic bacteria, probably associated with the solubility of organic and inorganic components. The factors that influence solubility include filler concentration and mean particle size, coupling agents, the nature of the filler particles type of solvent and the monomer conversion degree (14). Vitremer and Ketac Nano produced greater inhibition zones than the conventional ionomer cements. The greater solubility of those materials could be explained by the incomplete formation of a polycarboxylate matrix, since acid-base and polymerization reactions compete with and inhibit one another, and by their lower powder to liquid ratio than in the conventional materials (15). In addition, the pH setting and acid neutralization rate of the RMGICs has been observed to be lower than for the conventional GICs, possibly due to the glass particle silane coatings, water replacement with monomer, and/or lower polyacid levels (15). Sungurtekin et al. (2015) reported that Vitremer presented the most remarkable antibacterial inhibition of *S. mutans*, similar to chlorhexidine. Ketac Nano and Vitremer contain different filler FAS mass fractions (27% and 71.4%, respectively) as an antibacterial ion reservoir.

Table 2. Mean and standard deviation for agar disk-diffusion test results (mm), for adherence test (\log_{10} - CFU/mL) and biofilm wet weight (mg)

Materials	Adhered cells	Biofilm weight	Inhibition zones
Ketac Nano	6.163 (0.19) ^a	6.1 (1.9) ^a	10.4 (0.6) ^b
Vitremer	6.062 (0.29) ^a	5.7 (2.8) ^a	16.6 (0.5) ^a
Ketac Molar Easymix	6.217 (0.15) ^a	6.3 (1.9) ^a	7.4 (0.6) ^c
Fuji IX	6.218 (0.13) ^a	6.2 (2.9) ^a	7.8 (0.6) ^c

Different superscript letters indicate statistically significant difference within the columns ($p < 0.05$).

Table 3. Mean values and standard deviations (in parentheses) of the pH levels of the growth medium at 48-h intervals (three exchanges), for one week (material x time interaction) (initial pH of medium: 7.26, SD: 0.2 and negative control: 3.6, SD: 0.1)

Materials	1 st exchange 48h	2 nd exchange 96h	3 rd exchange 144h
<i>Ketac Nano</i>	4.1 (0.04) ^{aB}	4.01 (0.2) ^{aA}	4.1 (0.4) ^{aA}
<i>Vitremer</i>	4.8 (0.2) ^{aA}	4.6 (0.2) ^{aA}	4.2 (0.5) ^{aA}
<i>Ketac Molar Easymix</i>	4.3 (0.02) ^{aAB}	4.2 (0.2) ^{aA}	3.9 (0.6) ^{aA}
<i>Fuji IX</i>	3.8 (0.8) ^{aB}	4.5 (0.4) ^{aA}	3.9 (0.2) ^{aA}

Different lowercase letters indicate statistically significant difference in rows. Different uppercase letters indicate statistically significant difference in columns ($p > 0.05$).

Table 4. Mean amount of fluoride released (ppm F⁻) by the ionomeric materials during the Biofilm development analyzed at 1st, 2nd and 3rd exchanges at 48-h intervals for one week (material x time interaction)

Materials	1 st exchange 48h	2 nd exchange 96h	3 rd exchange 144h
Ketac Nano	23.9 (1.9) ^{aB}	5.9 (0.8) ^{bC}	1.9 (0.1) ^{cC}
Vitremer	35.4 (0.8) ^{aA}	21.3 (0.4) ^{bA}	7.4 (2.5) ^{cA}
Ketac Molar Easymix	18.8 (2.0) ^{aB}	8.5 (0.2) ^{bB}	3.5 (0.5) ^{cB}
Fuji IX	39.1 (0.5) ^{aA}	17.5 (0.8) ^{bA}	7.7 (0.9) ^{cA}

Different lowercase letters indicate statistically significant difference in rows. Different uppercase letters indicate statistically significant difference in columns ($p > 0.05$).

The development of a complex buffer solution containing mainly calcium and aluminum by GIC materials (5), able to significantly move the pH of the solution closer to a neutral pH, was observed during the severe and persistent adverse condition produced by the biofilm/material interaction (Table 3). In addition, the fluoride derived from the GICs is effective in reducing the acidogenicity of *S. mutans* biofilms (4). Vitremer showed a greater neutralizing effect with the first obtained growth medium (at 48h). This material contains a highly hydrophilic poly-(HEMA) matrix, whose superficial layer remains only partly polymerized due to the oxygen inhibition of polymerization (16). Therefore, its water sorption contributes to a swelling of the resin-based matrix and exposing fillers from the bulk polymer, which are excess unreacted base. However, the OH-groups of the HEMA molecule on the Vitremer surface, whether polymerized or not, could also work to neutralize the filler buffering ability. Otherwise, Ketac Nano contains a less hydrophilic matrix and a smaller FAS filler fraction than Vitremer, providing fewer ions to create an acidic media, and creating either antibacterial (fluoride, aluminum) or buffering (calcium, aluminum) media. Study performed by de Paula and collaborators (2014) submitted Ketac Nano to low pH media (beverages or des-re protocol) and evaluated its surface damages (17), but not the buffering ability of that material. Further investigations will be required to quantify and identify the released components by the nano-ionomer.

Ketac Molar Easymix was more effective than Fuji IX regarding the buffering analysis. First, the former material contains a higher powder:liquid ratio and smaller FAS particles than Fuji IX. The buffering effect is primarily related to the acid attack on the glass particles, which present higher reactivity (oxides) than the ionic polyacrylate matrix (low solubility) (5). Second, the calcium in the Ketac Molar Easymix glass is released in substantial quantities in acidic conditions (5). Calcium salts are less stable than the strontium salts in the Fuji IX composition, producing more dissociated ions due to its smaller pK_b (higher capacity of an ion to dissociate in water). Therefore, one would expect higher buffering ion release from Ketac Molar Easymix during the cariogenic challenge produced in this study.

A greater fluoride release was observed over the first 48 h of the biofilm/GIC interaction in the current study (Table 4). After that time, a progressive and gradual decrease in release rate occurred until the seventh storage day (2nd and 3rd exchange). The high initial level of F^- release may be caused by the superficial rinsing effect and by glass particles reacting with the polyalkenoate acid during the setting reaction. Otherwise, the continuous F^- release during the experimental period occurred because of the fluoride ability to diffuse through cement pores and fractures, which

occurs with a longer cement contact with the storage media. The initial fluoride "burst effect" of Ketac Nano was confirmed (18,19). However, Ketac Nano presented the largest drop in released fluoride values, approximately twelve times, while other materials presented a reduction of about five-six times. The hydrophobic resin matrix and lower incorporation of air bubbles by paste/paste mixing for Ketac Nano certainly reduced the fluid ingress into the structure of resin, decreasing the fluoride/water contact and fluoride movement from the matrix, resulting in a sharply decreasing rate of release over time (20). Markovic et al. (2008) also verified that the fluoride release and ability of taking up fluoride by Ketac Nano was probably restricted to the material surface, since no voids, cracks or microporosities were detected by micrographs, even after 7 days in an acidic environment. Therefore, without the sustainability of F^- release, the anticariogenic effect of Ketac Nano may be questioned. Clinically, Abo-hamar et al. (21) verified increased wear and marginal discoloration with secondary caries after two-year performance of Ketac Nano in Class I primary molars restorations.

Throughout the experimental period in the current study, Vitremer and Fuji IX released significantly higher amounts of fluoride than the other materials. Dionysiopoulos et al. (22) also found that Fuji IX released more fluoride than Ketac Nano for 15 days, yielding lower enamel demineralization surrounding restorations. The F^- release from a restorative material is determined by the matrix of the restorative material, the mechanism by which it sets and the amount of F^- -containing fillers. As discussed above, the hydrophilic HEMA of the Vitremer resin matrix was fundamental for favoring the absorption of enough water to allow for substantial fluoride diffusion, in addition to its greater amount of fluoride-releasing ions than found in Ketac Nano (23).

Comparing the conventional GIC materials, contradictory results were observed with Ketac Molar Easymix releasing a higher amount of buffering ions and lower F^- amounts than Fuji IX. The key point of this comparison is related again to the calcium compounds in the Ketac Molar Easymix, which are replaced by strontium compounds in Fuji IX. This substitution promoted a similar glass structure, with better translucency and anti-cariogenic properties (10). In addition, an enhanced F^- release (by 13-46%) was observed when a similar formulation of FAS glasses had Ca completely replaced by Sr (24). Initially, the intrinsic basic characteristic of Ca (smaller pK_b) makes the CaF_2 salt more basic than SrF_2 , interfering with its solubility. A strongly basic salt (CaF_2) needs a more acidic media to allow the F^- dissociation and diffusion through the bulk cement than does a neutral salt. Still, CaF_2 is a more stable and less soluble salt than SrF_2 , as calcium has a lower ionic

size and higher electro-positivity than strontium. Although both fluoride salts are relatively insoluble, CaF_2 is 15 times less soluble than SrF_2 (24).

Finally, regarding bacterial adhesion and biofilm formation for 7-days, no differences were observed among the studied GICs, regardless of their different physicochemical surface properties. More than surface free energy (SFE), surface roughness is considered an essential factor for the initial attachment of microorganisms, since roughened surfaces increase the area available for adhesion and shelter bacteria against shear and cleaning forces, resulting in a rapid re-growth of the remaining biofilm. It was expected that the nano-ionomer would present a lower amount of adhered cells (CFU/ml values) than the other studied materials. The combination of nanofillers, nanoclusters and FAS fillers that are smaller than the FAS from Vitremer (Table 1) should promote a smoother surface after finishing/polishing procedures (6). In the present study, no surface finishing method was used to avoid contaminating the aseptic surface of the specimens, which could have interacted with the *S. mutans* biofilm. With the migration of organic polymers to the material surface, a matrix-rich surface layer remained covering the fillers and all materials presented a similar initial surface roughness for bacterial colonization (data unpublished). Still, this organic surface, charged by negative elements and with low SFE (hydrophobic character), is less prone to *S. mutans* adherence, since this bacterial strain has high SFE and adheres preferentially to substratum surfaces with high SFE (25).

The biofilm wet weight also presented similar values among the studied materials, regardless of statistically different fluoride releasing and buffering abilities. In general, the attached cells were subjected to similar nutrient conditions for all materials (1% of sucrose every 48 h), sufficient for rapid multiplication and production of stable biofilms, in the absence of detachment forces (static growth conditions). Although different surfaces are related to changes in the physiology and virulence of the immobilized *S. mutans* (4), approximately 80–90% of the weight of biofilm is water; about 70% of the dry weight of biofilm are bacteria and the remainder is a polysaccharide matrix (1). Further studies are required to quantify the biofilm components accumulated on the nano-ionomer and to identify its influence on the virulence factors of *S. mutans* biofilm.

The chemical composition of glass ionomer restorative materials influenced the antibacterial properties. The ionomer cement modified by resin (Vitremer) was more effective in the inhibition of *S. mutans* and allowed greater neutralization of the pH in the first 48 h. However, the type of glass ionomer (resin modified or conventional) did not

influence the weight and adherence of the biofilm and fluoride release.

Resumo

O objetivo neste estudo foi avaliar *in vitro* as propriedades antibacterianas e a inibição do biofilme de cimentos de ionômero de vidro restauradores. Ketac Nano, Vitremer, Ketac Molar Easymix and Fuji IX foram avaliados através dos seguintes testes: a) teste de difusão em ágar para avaliar a inibição de *S. mutans* nos cimentos (n=8); b) adesão de *S. mutans* pela contagem de unidades formadoras de colônia após 2h de exposição material/bactéria (n=10); c) peso do biofilme úmido após sete dias de acúmulo bacteriano nos discos do material, com meio de cultura renovado após 48 h (n=10); d) mensuração do pH e liberação de flúor do meio aspirado nos intervalos de 48 h durante 7 dias de crescimento do biofilme (n=10). Os dados dos testes a, b e c foram submetidos aos testes Kruskal-Wallis e Mann-Whitney e os dados de liberação de flúor e pH a ANOVA dois fatores e Tukey ($\alpha = 5\%$). Vitremer seguido pelo Ketac Nano mostrou maior zona de inibição contra *S. mutans* quando comparados aos ionômeros convencionais. Vitremer também apresentou valores de pH mais elevados do que Ketac Nano e Fuji IX nas primeiras 48 h e liberou maior quantidade de flúor do que Ketac Nano e Ketac Molar Easymix durante todo o período experimental. A composição química dos ionômeros de vidro restauradores influenciou nas propriedades antibacterianas. O ionômero de vidro modificado por resina (Vitremer) foi mais eficaz na inibição de *S. mutans* e permitiu maior neutralização do pH nas primeiras 48 h. No entanto, o tipo de ionômero de vidro (modificado por resina ou convencional) não influenciou no peso e adesão do biofilme e na liberação de flúor.

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