

Suzana Beatriz Portugal de Fucio

***Influência da degradação biomecânica sobre
propriedades físico-químicas de cimentos ionoméricos
restauradores com diferentes composições***

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Orientadora: Profa. Dra. Regina Maria Puppim Rontani

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Prof. Dra. REGINA MARIA PUPPIN RONTANI

Prof. Dra. ROSA HELENA MIRANDA GRANDE

Prof. Dr. JOSÉ CARLOS PETTOROSSO IMPARATO

Prof. Dra. MARINÊS NOBRE DOS SANTOS UCHOA

Prof. Dr. SIMONIDES CONSANI

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RESUMO

A interação direta entre restauração e biofilme dentário está presente constantemente na cavidade bucal e suas conseqüências relacionam-se tanto com as características físicas e químicas do material quanto com a virulência da bactéria aderida. O objetivo deste estudo foi avaliar diferentes cimentos ionoméricos restauradores submetidos, *in vitro*, à biodegradação por biofilme de *Streptococcus mutans* e à abrasão por escovação. Cada material selecionado (Ketac N100, Vitremer, Ketac Molar Easymix e Fuji IX) foi utilizado na forma recém-manipulada ou na forma de discos, confeccionados sob condições assépticas. Enquanto o material pré-presa foi inserido em poços para o teste de difusão em ágar e análise dos halos de inibição de crescimento do *S. mutans* UA159 (n=8), os discos foram distribuídos em diferentes testes: a) teste de aderência desta cepa em 2 horas aos materiais, com a contagem das unidades formadoras de colônias (n=10); b) testes relacionados ao acúmulo bacteriano por sete dias (n=10) - peso úmido do biofilme, pH do meio de cultura a cada 48 horas (renovação do meio) e flúor liberado neste mesmo meio. Após os sete dias de biodegradação, os discos foram lavados e avaliados quanto à rugosidade e micro-morfologia de superfície. Como grupo controle, dez discos foram mantidos em umidade relativa pelo mesmo período para avaliação da superfície. A abrasão por escovação (degradação mecânica) foi realizada em seguida, e os espécimes foram reavaliados. Os dados obtidos nos testes de difusão, aderência e peso úmido do biofilme foram avaliados estatisticamente pelos testes de Kruskal-Wallis e Mann-Whitney, enquanto os dados de pH, flúor e rugosidade foram avaliados por ANOVA e Tukey ($\alpha=5\%$). Não houve diferença estatisticamente significativa entre os materiais quanto à aderência inicial

($p=0,6272$) e peso final do biofilme ($p=0,9612$). Entretanto, Vitremer apresentou os maiores halos de inibição, valores de pH nas primeiras 48 horas superiores ao Ketac N100 e Fuji IX, e liberação de flúor superior ao Ketac N100 e Ketac Molar Easymix durante todo o período experimental. Ketac N100 apresentou maior halo de inibição que os materiais convencionais e menor liberação de flúor, com uma diminuição nos valores ao longo do tempo de aproximadamente doze vezes. Quanto à rugosidade, houve interação entre os três fatores: material, meio de armazenamento (umidade x biofilme) e abrasão (antes x depois). Vitremer foi o único material que não apresentou diferença entre os grupos de armazenamento, com similares valores de rugosidade, enquanto os outros materiais apresentaram maiores valores após a biodegradação. Quando a degradação foi cumulativa (biomecânica), Ketac N100 obteve os menores valores de rugosidade. Microscopicamente foi observado um aspecto corroído na matriz biodegradada e a exposição das partículas na superfície dos materiais após os seguidos desafios. Portanto, a incorporação da nanotecnologia ao nano-ionômero auxiliou na obtenção de resistência à degradação biomecânica superior aos outros materiais estudados. Entretanto, suas propriedades químicas anti-cariogênicas foram negativamente influenciadas e consideradas inferiores ao Vitremer.

Palavras chave: cimento de ionômero de vidro, nanotecnologia, *Streptococcus mutans*, biofilme, degradação, pH, flúor

ABSTRACT

The direct interaction between restoration and dental biofilm frequently occurs in the oral cavity. Its consequences are related to physico-chemical characteristics of the restorative material and to virulence of the bacteria adhered. The aim of this study was to evaluate different restorative glass ionomer cements subjected to biodegradation by *Streptococcus mutans* biofilm and to brushing abrasion *in vitro*. Each material studied (Ketac N100, Vitremer, Ketac Molar Easymix e Fuji IX) was tested as recently handled mix or as set discs, prepared under aseptic conditions. At that condition, the material was inserted into wells for agar diffusion test and analysis of growth inhibition zones of *S. mutans* UA159 (n=8), while the discs were distributed in different tests: a) two-hours adherence test of this strain on those ionomeric materials, by counting the colony-forming units (n = 10); b) tests related to bacterial accumulation for seven days (n = 10) - biofilm wet weight, pH of growth medium every 48 hours (medium renewal) and fluoride released in that same medium. After seven days of biodegradation, the discs were washed and evaluated about surface roughness (Ra) and micromorphology. As control group, ten discs were kept in relative humidity for the same period. Then, toothbrush abrasion test (mechanical degradation) was performed, and specimens were reevaluated. Data from inhibition zones, *S. mutans* adherence and wet weight of biofilm were submitted to Kruskal-Wallis and Mann-Whitney tests. ANOVA and Tukey tests were applied to fluoride-released, pH and roughness data. The level of significance was set at 5%. There was no statistically significant difference among the materials regarding the initial adherence (p = 0.6272) and final biofilm weight (p = 0.9612). Vitremer showed

the largest inhibitory zones, higher pH values than Ketac N100 and Fuji IX at the first exchange medium (48h), and higher fluoride release than Ketac N100 e Ketac Molar Easymix throughout the experimental period. Ketac N100 showed greater inhibitory zone than conventional ionomers and the lowest fluoride release, with a fall in values over time about twelve times. Concerning surface roughness, there was significant interaction among factors: material, storage (humidity/biofilm) and abrasion (before/after). Vitremer showed similar Ra values between storage groups, while other materials presented higher Ra values after biodegradation test. Concerning cumulative biomechanical challenge, Ketac N100 presented the lowest Ra values. The corroded aspect after biodegradation and the exposition of fillers after mechanical degradation were visualized at micrographs. Therefore, the nanotechnology incorporation in the nano-ionomer promoted better resistance to biomechanical degradation than other materials studied. However, its anticariogenic chemical properties were negatively influenced and considered inferior to Vitremer.

Key-words: glass ionomer cement, nanotechnology, *Streptococcus mutans*, biofilm, degradation, pH, fluoride

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INTRODUÇÃO

Nos últimos anos, os índices de cárie dental no Brasil foram bastante reduzidos, parte em função principalmente da fluoretação das águas e dos dentífrícos, interferindo no fenômeno de desmineralização e remineralização da estrutura dentária (ten Cate, 1997), e de programas educativos realizados. Entretanto, tais medidas preventivas não foram suficientes para a erradicação dessa patologia, mantendo-se ainda uma alta prevalência de cárie em crianças com idade inferior a seis anos (cárie precoce da infância), variando de 10,1 a 43,4% (Bönecker *et al.*, 2002; Rosenblatt e Zarzar, 2004; Ribeiro *et al.*, 2005, Ferreira *et al.*, 2007, Rihs *et al.*, 2007, Oliveira *et al.*, 2008). Considerando-se que esta população infantil apresentará maior risco de desenvolvimento de cárie no futuro (Sclavos *et al.*, 1988, Peretz *et al.*, 2003), torna-se fundamental a utilização de técnicas e materiais restauradores capazes de restabelecer forma e função dos dentes, assim como proteger permanentemente as estruturas sadias remanescentes contra a infiltração de microrganismos e seus metabólitos, prevenindo, assim, o restabelecimento de novo quadro patológico.

Os cimentos de ionômero de vidro estão entre os materiais restauradores indicados para áreas de difícil higienização e de alto desafio cariogênico, como lesões cervicais (classe V), ocluso-proximais (técnica do sanduíche) e adequação do meio bucal em indivíduos de alta atividade de/risco à cárie (Ermis, 2002; Croll *et al.*, 2001). Além da inerente adesividade química e do coeficiente de expansão térmica similar à estrutura dentária (Xie *et al.*, 2000), propriedades que influenciam positivamente no selamento marginal das

restaurações, os materiais ionoméricos são capazes de liberar íons flúor, alumínio, cálcio, estrôncio, entre outros de potencial protetor ao elemento restaurado contra um biofilme acidogênico e, conseqüentemente, a cárie secundária (Luo *et al.*, 2009).

O biofilme dentário é definido como uma comunidade de diferentes espécies microbianas embebidas em uma matriz de polissacarídeos de origem bacteriana e salivar (Marsh, 2004). Esta organizada estrutura forma-se rapidamente na cavidade bucal, ambiente composto por diferentes superfícies não-descamativas, como os tecidos dentários e os variados materiais utilizados nos tratamentos reabilitadores (Busscher *et al.*, 2010). Desta forma, grandes biomassas e seus metabólitos podem ser acumulados em áreas de retenção, como nas superfícies proximais, oclusais (sulcos, fóssulas e fissuras), cervicais (próximas ao sulco gengival) e mesmo em espaços presentes na interface dente-restauração (Carvalho *et al.*, 1996). Protegido das forças mecânicas de remoção (escovação, língua, fluxo salivar, mastigação), este biofilme torna-se estável e maduro, capaz de produzir cárie dentária e doença periodontal primariamente, além de cárie recorrente e, quando acumulado nas interfaces, sensibilidade pós-operatória e inflamação/necrose pulpar. Portanto, muitos estudos têm sido conduzidos a fim de produzir e testar materiais restauradores capazes de inibir a adesão e/ou desenvolvimento do biofilme sobre a restauração, o elemento restaurado e a interface (Al-Naimi *et al.* 2008; Sousa *et al.*; 2009; Hahnel *et al.* 2010).

Streptococcus mutans é considerado o principal agente etiológico da cárie dentária. Dentre as características de virulência dessa espécie bacteriana, destaca-se a síntese de ácido láctico e a tolerância ao baixo pH,

além da metabolização da sacarose presente na dieta para produzir polissacarídeos extracelulares (Hamada & Slade, 1980). Estes, por sua vez, atuam como fonte reserva de energia e são responsáveis pela integridade estrutural da matriz do biofilme e pela resistência deste aos antimicrobianos. Em espesso biofilme, as bactérias são protegidas do sistema imunológico do hospedeiro, do efeito tampão da saliva e da penetração de agentes antibacterianos pela densa biomassa acumulada (Thurnheer *et al.*, 2003; McNeill & Hamilton, 2003). Íons e moléculas com potencial antibacteriano permanecem adsorvidos e concentrados na porção mais externa do biofilme, região de menor densidade celular e maior área de superfície que em regiões mais profundas, impedidos de proteger os tecidos dentários subjacentes (Hu *et al.*, 2005; Robinson *et al.*, 2006). Desta forma, a seleção de um material restaurador capaz de interagir com estas áreas de privilegiada proteção bacteriana seria relevante a fim de prevenir ou retardar a progressão da lesão cáries, reduzir as substituições de restaurações e, conseqüentemente, diminuir o custo e a necessidade de tratamentos adicionais.

Os materiais restauradores ionoméricos são capazes de interferir na acidogenicidade (Hayacibara *et al.*, 2003) e na viabilidade dos *Streptococcus mutans* em biofilme (Auschill *et al.*, 2002), conseqüências positivas da ação dos íons flúor liberados pelo material, contribuindo na redução da incidência de cárie dentária (Wiegand *et al.*, 2007). Além disso, a interação entre ionômero de vidro e biofilme constituído por cepas acidogênicas resulta na erosão do material, com a conseqüente elevação do baixo pH do meio para níveis mais próximos ao pH neutro (Nicholson *et al.*, 2000). Entretanto, deve-se lembrar que tais efeitos anti-cariogênicos são pH e material-dependentes (Czarnecka

et al., 2002), além de normalmente ocorrerem à custa da degradação da superfície e das propriedades mecânicas do material (Moreau & Xu, 2010).

De uma maneira geral, todos os materiais restauradores inseridos na cavidade bucal estão sujeitos a desafios químicos relacionados à saliva, dieta alimentar e atividade bacteriana (Oilo, 1992). Além disso, a degradação mecânica decorrente da higienização diária realizada pelo paciente poderia ainda intensificar os danos ocorridos na superfície do material previamente degradado (Hotta *et al.*, 1995). A ação abrasiva dos dentífrícios e das cerdas da escova poderia remover a matriz amolecida da superfície e expor e/ou deslocar as partículas de carga do material restaurador (Heintze & Forjanic, 2005), modificando sua rugosidade e morfologia. Alguns estudos avaliam a influência simultânea de métodos corrosivos e abrasivos sobre a superfície do material (Shabaniyan & Richards, 2002; Turssi *et al.*, 2003; Correr *et al.*, 2006). Entretanto, tais estudos utilizaram ácidos semelhantes aos encontradas na dieta alimentar ou produzidos por bactérias acidogênicas, e não a biodegradação diretamente promovida por um biofilme cariogênico.

Considerando-se, então, a importância da inter-relação material restaurador e biofilme dentário na longevidade clínica das restaurações, torna-se interessante avaliar a influência da degradação biomecânica sobre as propriedades físico-químicas de materiais ionoméricos com diferentes composições. Portanto, o objetivo deste estudo¹ foi avaliar quatro cimentos ionoméricos restauradores submetidos, *in vitro*, à biodegradação por biofilme

¹ O presente trabalho encontra-se apresentado no formato alternativo de tese de acordo com as normas estabelecidas pela deliberação 002/06 da Comissão Central de Pós-Graduação da Universidade Estadual de Campinas.

de *Streptococcus mutans* e à abrasão por escovação. Dentre os materiais estudados, um cimento de ionômero de vidro modificado por resina com a incorporação de nanopartículas foi selecionado, o KetacTM N100, o qual poderia apresentar diferenciadas propriedades de lisura de superfície e resistência aos processos de degradação e desgaste.

Capítulo 1

ANTIBACTERIAL AND ANTIBIOFILM PROPERTIES OF A NANO-FILLED RESIN-MODIFIED GLASS IONOMER RESTORATIVE CEMENT

ABSTRACT

Purpose: to evaluate four glass ionomer restorative cements with different chemical compositions concerning their antibacterial and antibiofilm properties against *Streptococcus mutans*, *in vitro*. **Methods:** Ketac N100 (a nano-filled resin-modified ionomer), Vitremer, Ketac Molar Easymix and Fuji IX were analyzed by the following tests: a) agar plate diffusion test (ADT) to analyze the bacterial growth inhibition zones (n=8); b) *S. mutans* adherence test - colony-forming units counting after two hours of materials/cells exposure (n=10); c) biofilm wet weight after seven days of bacterial accumulation on material-disks, with the renewal of growth medium every 48-h (n=10); d) pH and fluoride measurements from the medium aspired at 48-h intervals during 7-days biofilm development (n=10). Data from a, b and c tests were submitted to Kruskal-Wallis and Mann-Whitney tests and, fluoride-released and pH data to two-way ANOVA and Tukey tests ($\alpha=5\%$). **Results:** There was not statistical difference among the materials studied concerning adherence test ($p=0.6272$) and biofilm wet weight ($p=0.9612$). However, Vitremer presented the greatest inhibitory zone, higher pH values than Ketac N100 and Fuji IX at the first exchange medium (48h), and higher fluoride

release than Ketac N100 e Ketac Molar Easymix throughout the experimental period. Ketac N100 showed greater inhibitory zone than conventional ionomers and the lowest fluoride release, with a fall in values over time about twelve times. **Conclusion:** the different chemical composition of restorative ionomeric materials influenced the antibacterial and antibiofilm properties, with Vitremer showing the most effective response against the strain studied.

Key-words: glass ionomer, fluoride, dental biofilm, *Streptococcus mutans*, pH, adherence, nanotechnology.

INTRODUCTION

Bacterial biofilms associated with surfaces are complex three-dimensional structures in which bacteria are embedded in a matrix mainly made of exopolysaccharides.¹ In the oral cavity, biofilms may be found on dental hard and soft tissues, associated with caries and periodontal diseases,² as well as on the diversity of biomaterial surfaces used for the restoration of function. Accumulation of bacteria on restorative materials not only degrades the material and roughens its surface,^{3,4} but also causes colonizing bacteria reinfection of the interface between the restoration and the tooth and the reoccurrence of caries.⁵ In order to preventing or slowing down lesion progression and, consequently, to reduce the rate of restoration replacement, the interest in new dental materials capable of attracting less biofilm or releasing antimicrobial compounds is increasing.

Glass ionomers are generally recommended where protection against caries is needed, since they potentially reduces microleakage by adhering to

tooth structure,⁶ suppresses the growth of caries-related oral bacteria and neutralizes acids produced by those bacteria by ions release.^{7,8} The fluoride-releasing and neutralizing ability of ionomeric materials are affected by the nature of fluoride incorporated into them,⁹ and by the nature of the storage medium,¹⁰ particularly its pH value. However, these beneficial effects occur at the expense of extensive surface deterioration,^{4,7} leading to a negative spiral of events,³ in which more colonizing organisms will adhere to the degraded material and will promote more deterioration.

Different components released from conventional and resin-modified glass-ionomer cements (RMGIC) can modulate phenotype of cariogenic bacteria. Fluoride, aluminum,¹¹ and strontium¹² 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 dimethacrylate (TEGDMA) may stimulate the growth of cariogenic bacteria, like mutans streptococci and lactobacilli,^{13,14} and also enhance the glucosyltransferase activity in *Streptococcus sobrinus*.¹⁵

Little corresponding information exists so far chemical and biological properties of the recent nano-filled RMGIC, Ketac™ N100 (3M ESPE). This material has the unique combination of filler content: bonded nanofillers, nanoclusters, and fluoroaluminosilicate glass particles (FAS) (3M ESPE Internal Data).¹⁶ In addition, it contains HEMA, bisphenol glycidyl methacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA) as resin monomers, differently of the known RMGICs.¹⁷ So, it would be interesting to study the behavior of the nano-ionomers under biofilm-material

interaction, since less amount of fluoride is available for releasing (27% FAS glass) and a smoother surface is possibly obtained,¹⁸ modifying biofilm accumulation. Therefore, the purpose of the present study was to evaluate four glass ionomer restorative cements with different chemical compositions, including the nano-ionomer, concerning their antibacterial and antibiofilm properties against *Streptococcus mutans*.

MATERIALS AND METHOD

Specimen Preparation

Composition and manufacturing information for the dental restorative materials evaluated is presented in Table 1. Specimens were prepared with a sterilized custom Teflon mold (5 mm diameter; 2 mm depth) according to the manufacturer's instructions, under aseptic conditions. The materials were mixed by one operator, packed into the mold, covered and pressed flat with a sterilized glass slide. Vitremer and KetacTM N100 specimens were cured with a curing light unit (Elipar Trilight, 3M ESPE, St. Paul, MN, USA) after the intensity of the unit to be checked by a curing light meter (Hilux Dental Curing Light Meter, Benliglu Dental Inc., Turkey). Ketac Molar Easymix and Fuji IX specimens were allowed to set for 5 min.

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

materials studied, as degree of polymerization alterations, degradation, cracks formation, among others, modifying the surface of glass ionomers.^{19,20,21} Ten specimens of each material were used for adherence test and ten for *S. mutans* biofilm analysis, including fluoride releasing and neutralizing effect. Regarding to agar plate diffusion test, the materials were mixed and inserted before setting into wells from agar/*S.mutans* plate (n=8).

Agar plate diffusion test

S. mutans (UA159) was obtained from the culture stock of the Department of Microbiology and Immunology, Dental School of Piracicaba, Campinas State University. The antibacterial activity of each material was evaluated using the agar plate diffusion test. Indicator strain was first grown in *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, 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 (20x100mm), 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 measuring 5 mm in diameter were made in each plate and completely filled with one of the testing materials listed in Table 1. Eight wells were filled up with each material (n=8). All materials were handled under aseptic conditions according to the manufacturer's instructions and the RMGICs were light-cured. Ten microliters of aqueous 0.12% chlorhexidine

digluconate was applied on sterile filter paper discs, also 5mm in diameter, which acted as a control (n=6).

The plates were kept for 2 h at room temperature for diffusion of the materials. After this time, 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.

***Streptococcus mutans* adherence test**

To prepare the inoculum, *S. mutans* (UA159) was grown as previously described. Each ionomeric material studied (n=10) were exposed under static conditions to 25 μL of inoculum adjusted to an optical density (OD) of 0.6 at 550 nm (approximately 8×10^{11} CFU/mL). After two hours at room temperature, the non-adhering cells were removed by washing two times with 0.9% NaCl solution (saline). Then, each disk was 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 in BHI agar plates. These plates were incubated at 37°C for 48 h in a 10% supplemented CO_2 environment. The colonies were counted and determined the number of viable bacteria - CFU/mL that corresponded to the cells adhered to glass ionomer cements after 2 hours of *S. mutans* exposure.

***Streptococcus mutans* biofilms analysis**

As described above for adherence test, a *S. mutans* inoculum of 25 μL was maintained for two hours on ten specimens of each material in order to that cells would promote an initial biofilm. The non-adhering cells were removed and a single material disk was placed in each well of 24-well polystyrene plates (Multidish 24-well Nunclon) with 2mL of sterile fresh BHI broth with addition of 1% (w/v) sucrose (REFERENCIA). The bacterial accumulation occurred at 37°C in a 10% supplemented CO₂ environment, developing 7-day-old biofilm. Medium was renewed at 48-h intervals.

1. Biofilm wet weight

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

2. pH test

The pH of the growth medium aspired from each well at 48-h intervals (1st, 2nd and 3rd exchange) was determined using a portable pH meter (Orion Model 420A, Analyzer Co., Sao Paulo, Brazil). The initial pH of the broth medium (prior to microorganism inoculation and cement storage) was 7.26 (standard deviation = 0.2). In addition, negative control solutions stored under identical conditions containing no cement were also prepared. 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 standard buffer solutions at pH 4.0 and 7.0.

3. Fluoride release

The amount of fluoride released by the restorative materials during biofilm growth was analyzed too. Fluoride measurements in the medium aspirated from each well were taken in duplicate using an ion specific electrode (Orion 96-09) connected to a microprocessor ion-analyzer (Orion EA-940, Orion Research, Boston, Mass., USA), which had been previously calibrated in triplicate with fluoride standards (2.0 to 40.0 $\mu\text{g F}^-/\text{ml}$) in TISAB III (Total Ionic Strength Adjustment Buffer; Thermo Orion, Beverly, MA, USA). Sample readings were captured in millivolts (mV) and transformed in $\mu\text{gF}^-/\text{mL}$ (ppm F^-) by linear regression of the calibration curve.

Statistical analysis

Data from inhibition zones, *S. mutans* adherence and wet weight of biofilm accumulated on material surface were submitted to Kruskal-Wallis and Mann-Whitney tests. Before applying the two-way ANOVA and Tukey tests, fluoride-released and pH data were transformed using the log transformation. The software SAS system (version 8.02, SAS Institute Inc., Cary, NC, 1999) was used and the level of significance was set at 5%.

RESULTS

Kruskal-Wallis test didn't revealed significant differences among the materials studied concerning the initial streptococcal adherence ($p=0.6272$)

and the wet weight of the biofilms accumulated for 7 days on the specimen surfaces ($p=0.9612$). Regarding to the agar plate diffusion test, the resin-modified glass-ionomer Vitremer showed the greatest inhibitory effect against *Streptococcus mutans* (16.6 mm), similar to chlorhexidine (15.8 mm \pm 0.59), followed by Ketac N100 (10.4 mm) and, finally, by conventional ionomers. Ketac Molar Easymix (7.4 mm) and Fuji IX (7.8 mm) presented similar values between them. Ketac N100, 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 test material over 48 h period, as a function of time (1st, 2nd and 3rd exchange). Differences in pH over time were not significant for any material tested. However, at the 1st period evaluated (48h), there was a significant difference among the materials. Vitremer presented higher pH values (4.8) than Ketac N100 (4.1) and Fuji IX (3.8). In addition, the pH of all materials studied were significantly higher than negative control ($p<0.01$).

For the same broth medium aspired, analyzed for pH previously, the results of fluoride release are presented in Table 4. Vitremer and Fuji IX had the highest fluoride release at the three measured periods. Ketac N100 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 at the first measurement, the rate was significantly low for all materials. Comparing the first and the last broth exchange, the fluoride release from Ketac N100 presented drop in values about twelve times.

DISCUSSION

Biofilms are a diverse and complex aggregate of bacteria that exhibit over 100-fold resistance to antimicrobial agents.²² 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.²³ In the oral environment, this already established or mature biofilm can accumulate at stagnant sites, as interproximal surfaces, gingival crevice and pits and fissures,¹ beyond levels compatible with oral health. In addition, novel microenvironments exist because of the formation of marginal gaps around the tooth-restoration interface, contributing to postoperative sensitivity, recurrent caries, pulp inflammation and necrosis.^{5,24} Then, it would be important to select carefully the restorative material for intra-oral sites where biofilm would be protected against dynamic shear forces from saliva, tongue and toothbrush, stimulating its accumulation and maturation.

All evaluated glass ionomer cements showed antibacterial activity according to the agar-plate diffusion test (table 2), inhibiting the selected cariogenic bacteria growth, likely associated with the solubility of organic and inorganic components. The factors that influence solubility include filler concentration and mean particle size, the coupling agents, the nature of the filler particles,²⁵ type of solvent and the degree of monomer conversion.²⁶ Vitremer and Ketac N100 produced greater inhibition zones than conventional ionomer cements studied. 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,²⁷ and

by their lower powder to liquid ratio than conventional materials.^{28,29} In addition, their pH setting and acid neutralization rate has been observed to be lower than conventional glass ionomers, possibly due to glass particle silane coatings, water replacement with monomer, and/or lower polyacid levels.³⁰ On the other hand, comparing Ketac N100 and Vitremer, they contain different filler FAS mass fraction (27% and 71.4%, respectively) as antibacterial ions reservoir. So, Vitremer presented the best antibacterial activity against *Streptococcus mutans*, similar to the control, chlorhexidine.

The development of a complex buffer solution containing mainly calcium and aluminum by ionomeric materials,^{9,10} able to move significantly the pH of the solution closer to neutral, was observed during the severe and persistent adverse condition produced by the biofilm/material interaction (table 3). In addition, the fluoride derived from ionomers is effective in reducing the acidogenicity of *S. mutans* biofilms.^{11,31} At the first growth media obtained (48h), Vitremer showed a greater neutralizing effect. This material contains a highly hydrophilic poly-(HEMA) matrix, whose superficial layer remains only partly polymerized due to the oxygen inhibition of polymerization.^{32,33} So, its water sorption contributes to swell the network of resin-based matrix and to expose fillers from the bulk polymer, which are excess unreacted base. Still, the OH-groups of the HEMA molecule present at the Vitremer surface could also work to media neutralization apart of fillers buffering ability. Otherwise, Ketac N100 contains a less hydrophilic matrix and less FAS filler fraction than Vitremer, providing fewer ions to acidic media, either antibacterial (fluoride, aluminum) or buffering (calcium, aluminum) ones. Further

investigations will be necessary to quantify and identify the released components from that recent material.

Besides, Ketac Molar Easymix was more effective than Fuji IX regarding buffering analysis. Firstly, that material contains a higher powder:liquid ratio and smaller FAS particles than Fuji IX.³⁴ The buffering effect is primarily related to the acid attack at the glass particles, which present higher reactivity (oxides) than ionic polyacrylate matrix (low solubility).¹⁰ Second, the calcium present just in Ketac Molar Easymix glass is released in substantial quantities under acid conditions.¹⁰ Calcium salts are less stable than strontium salts present in Fuji IX composition, producing more dissociated ions due to its smaller pKb (higher capacity of an ion to dissociate water).²⁵ So, it would really expect the higher buffering ions releasing from Ketac Molar Easymix during cariogenic challenge produced in this study.

The greater fluoride release is observed over the first 48 hours of biofilm/material interaction (table 4). After that time a progressive and gradual decrease in release rate occurs 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 reaction with the polyalkenoate acid, during setting reaction. Otherwise, the continuous F⁻ release during the experimental period occurs because of fluoride ability to diffuse through cement pores and fractures which it occurs with a longer cement contact with the storage media.³⁵ Ketac N100 presented the largest fall in values of fluoride released, about twelve times, while other materials were about five-six times. Its hydrophobic resin matrix and lower

incorporation of air bubbles by paste/paste mixing certainly reduced the fluid ingress into the structure of resin, decrease fluoride/water contact and fluoride movement on the matrix, resulting in a sharply decreasing rate of release over time.³⁶ Markovic *et al.* (2008) also verified that fluoride release and ability of taking up fluoride by Ketac N100 are probably restrict to material' surface, since no voids, cracks and microporosities were detected by micrographs, even after 7 days under acidic environment.¹⁷ So, without sustainability of F⁻ release, its anticariogenic effect could be questioned.

Throughout the experimental period, Vitremer and Fuji IX released significantly higher amounts of fluoride than others. The F⁻ release is determined by the matrix of the restorative material, the mechanism by which it sets, and the amount of F⁻-containing fillers.³⁵ As discussed above, the hydrophilic HEMA of the Vitremer's resin matrix was fundamental to favor the absorption of enough water to allow for a substantial fluoride diffusion, besides its greater amount of fluoride-releasing source than Ketac N100.³⁷

Comparing conventional ionomeric materials, apparently contradictory results were observed with Ketac Molar Easymix releasing higher amount of buffering ions and lower F⁻ amounts than Fuji IX. The key-point of this comparison is related again to calcium compounds present at particles composition from Ketac Molar Easymix, which was replaced by strontium ones in Fuji IX. This substitution promoted a similar essential glass structure, with better translucency and anti-cariogenic properties.¹⁷ In addition, an enhanced F release (by 13-46%) was observed when similar formulation of FAS glasses had Ca completely replaced by Sr.³⁸ Initially, the intrinsic basic characteristic

of Ca (smaller pK_b) makes the CaF₂ salt more basic than SrF₂, interfering with its solubility. A strongly basic salt (CaF₂) needs a more acidic media than neutral salt to allow the F dissociation and diffusion through the bulk cement. Still, CaF₂ is a more stable and less soluble salt than SrF₂ due to calcium has lower ionic size and higher electro-positivity than strontium. Although both fluoride salts are relatively insoluble, CaF₂ is 15 times less soluble than SrF₂.³⁸

Finally, regarding bacterial adhesion and biofilm formation for 7-days, there was not observed difference among ionomers studied regardless of their different physico-chemical surface properties. To a greater extent than Surface Free Energy (SFE), surface roughness is considered an essential factor for initial attachment of microorganisms, since roughening increases the area available for adhesion and shelters them against shear and cleaning forces, resulting in a rapid re-growth of the remaining biofilm.³⁹ Then, it was expected that the nano-ionomer presented lower amount of adhered cells (CFU/ml values) than other materials studied. The combination of nanofillers, nanoclusters and FAS fillers smaller than FAS from Vitremer (Table 1) should promote a smoother surface after finishing/polishing procedures.^{12,18} In the present study, no surface finishing method was used in order to avoid contaminating the aseptic surface of specimens, which would interact with *Streptococcus mutans* biofilm. With the migration of organic polymers to the material' surface,⁴⁰ a matrix-rich surface layer remained covering 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 preferentially adhere to substratum surfaces with high SFE too.⁴¹

The biofilm wet weight also presented similar values among materials studied, regardless fluoride releasing and buffering ability be statistically different. In general, the attached cells were subjected to similar nutrient conditions for all materials (1% of sucrose every 48 hours), sufficient to rapid multiplication and production of stable biofilms, in the absence of detachment forces (growth static conditions). Although different surfaces are related to changes in the physiology and virulence of the immobilized *S. mutans*,^{8,15,16} approximately 80-90% of the weight of biofilm is water; about 70% of the dry weight of biofilm is bacteria, and the remainder is a matrix of polysaccharides.⁴² Further studies are needed to quantify specifically the biofilm's components accumulated on the nano-ionomer and to identify its influence on virulence factors of *Streptococcus mutans* biofilm.

CONCLUSION

The different composition of the nano-filled ionomer (monomers and fillers) negatively influenced on its antibacterial and antibiofilm properties against *Streptococcus mutans* biofilm. Vitremer showed the most effective response against the strain studied.

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Table 1 - Materials used in this study

Materials	Composition*	Ratio recommended	Average particle size**
Ketac N100 (3M ESPE)	Paste A: silane treated glass, silane treated zirconia oxide silica, silane treated silica, HEMA, Bis-GMA, TEGDMA, PEGDMA Paste B: silane treated ceramic, silane treated silica, copolymer of acrylic and itaconic acids, HEMA	1.3:1 paste/paste	1 - 1.6 μm (cluster) 5 - 25nm (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: aluminium-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 aluminium fluorosilicate glass Liquid: polyacrylic acid, tartaric acid and water	3.6:1	4.4 μm

* Abbreviation of monomers in alphabetical order: Bis-GMA = bisphenol glycidyl methacrylate; HEMA = 2-hydroxyethyl methacrylate; TEGDMA = triethylene glycol dimethacrylate.

**Manufacturer` information.

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 N100	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 between averages compared in the same column ($p < 0.05$).

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)

<i>Materials</i>	<i>1st exchange 48h</i>	<i>2nd exchange 96h</i>	<i>3rd exchange 144h</i>
<i>Ketac N100</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

Similar small letters mean no significantly statistical difference between averages on horizontal comparison; Similar capital letters mean no significantly statistical difference between averages on vertical comparisons; (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)

<i>Materials</i>	<i>1st exchange 48h</i>	<i>2nd exchange 96h</i>	<i>3rd exchange 144h</i>
<i>Ketac N100</i>	23.9 (1.9) aB	5.9 (0.8) bC	1.9 (0.1) cC
<i>Vitremer</i>	35.4 (0.8) aA	21.3 (0.4) bA	7.4 (2.5) cA
<i>Ketac Molar Easymix</i>	18.8 (2.0) aB	8.5 (0.2) bB	3.5 (0.5) cB
<i>Fuji IX</i>	39.1 (0.5) aA	17.5 (0.8) bA	7.7 (0.9) cA

Similar small letters mean no significantly statistical difference between averages on horizontal comparison; Similar capital letters mean no significantly statistical difference between averages on vertical comparisons; (p>0.05).

Capítulo 2

BIOMECHANICAL DEGRADATION OF THE NANO-FILLED RESIN-MODIFIED GLASS-IONOMER SURFACE²

ABSTRACT

Purpose: to evaluate *in vitro* the roughness (Ra) and micromorphology surface of the nanofilled resin-modified glass-ionomer (Ketac N100, 3M ESPE) subjected to biomechanical degradation, compared to Vitremer, Ketac Molar Easymix and Fuji IX. **Methods:** Specimens obtained from ionomers were divided into two storage groups (n=10): relative humidity and *S. mutans* biofilm (biodegradation). After 7 days, Ra values and micrographs were obtained. Then, the brushing abrasion test (mechanical degradation) was conducted with dentifrice slurry (third body) and the specimens were reassessed. Data were submitted to repeated measures three-way ANOVA and Tukey tests ($p < 0.05$). **Results:** There was significant interaction among factors: material, storage and abrasion (before/after). Vitremer showed similar Ra values between storage groups, while other materials presented higher Ra values after biodegradation test. Concerning biomechanical challenge, Ketac N100 presented the lowest Ra values. Ketac Molar Easymix and Fuji IX presented the undesirable roughening of their surfaces under the detrimental conditions proposed. The corroded aspect after biodegradation and the exposition of fillers after mechanical degradation were visualized at micrographs.

²Manuscrito aceito para publicação no periódico American Journal of Dentistry.

Clinical significance: The nano-ionomer presented a satisfactory resistance to biomechanical degradation, superior to other materials studied. It should be attributed to the nanotechnology incorporated in this RMGI, with regular, small and silanized fillers.

Keywords: biodegradation, glass ionomer cement, wear, surface roughness, nanotechnology, *Streptococcus mutans*

INTRODUCTION

Glass ionomer cements (GIC), developed by McLean and Wilson,¹ are basically composed of a calcium fluoroaluminosilicate glass powder and an aqueous solution of an acrylic acid homo- or copolymer (polyelectrolyte). These cements present some interesting properties, such as adhesion to moist tooth structure and base metals, anticariogenic properties due to fluoride release, thermal compatibility with tooth enamel, biocompatibility and low cytotoxicity.² Thus, GIC has become a clinically attractive dental restorative material, being used effectively in class V cavity preparations, in erosion and abrasion cavities^{3,4} and in primary teeth,⁵ since composite restorations are more time consuming and require absolute isolation (rubber dam) of the tooth.

In recent years, glass ionomer cements have improved in several aspects, by the increased percentage of filler particle loading, incorporation of a light-polymerizable resin into the cement or both.⁶ The development of resin-modified glass-ionomer cements (RMGICs), one of the major categories of commercial glass-ionomers, has led to easy handling, setting on demand, decreased water sensitivity and improved physical properties.⁷ Recently, 3M

Espe introduced an entirely new category of glass ionomer restorative materials on the dental market: the nano-filled RMGIC, Ketac™ N100, a paste/paste material based on bonded nanofiller technology. This material blended two technologies (fluoroaluminosilicate and nanotechnology), leading to higher wear resistance and smoother surfaces than those provided by other RMGICs, while offering fluoride release similar to that of conventional and RMGIC (3M ESPE Internal Data).⁸

Restorative dental materials capable of resisting biodegradation have a significant influence on the satisfactory performance of dental restorations. In the oral cavity, biodegradation includes disintegration and dissolution in saliva and other types of chemical/physical degradation, wear and erosion caused by food, chewing and bacterial activity.⁹ These mechanisms may operate either alone or in combination with others, promoting surface and subsurface degradation which may involve the resin matrix, filler, or matrix-filler interface, leading to increased surface roughness and wear rate.^{10,11,12} Few studies have simultaneously used corrosive and abrasive wear methods, and normally use acid solutions to simulate acidic diet or regurgitated acid,^{13,14,15} so that little is actually known about the cumulative effects of an acidogenic biofilm and tooth-brushing abrasion on the surface characteristics of restorative materials.

Therefore, the aim of this study was to evaluate *in vitro* the roughness (Ra) and surface micromorphology of the nano-filled resin-modified glass-ionomer (Ketac N100) subjected to *Streptococcus mutans* biofilm degradation (biodegradation) and three-body abrasion (mechanical degradation), in comparison with another three different glass ionomer cements.

MATERIALS AND METHODS

Specimen preparation

Twenty specimens of four different glass ionomer cements, described in Table 1, were fabricated using sterilized Teflon molds (5mm in diameter; 2mm deep) according to the manufacturer's instructions, under aseptic conditions. The materials were mixed, placed in the mold by one operator, covered and pressed flat with a sterilized glass slide. Vitremer (3M ESPE, St. Paul, MN, USA) and Ketac N100 (3M ESPE, St. Paul, MN, USA) specimens were polymerized with a light curing unit (Elipar Trilight, 3M ESPE, St. Paul, MN, USA) after checking the light-curing unit intensity with a curing light meter (Hilux Dental Curing Light Meter, Benliglu Dental Inc., Turkey). Ketac Molar Easymix (3M ESPE, St. Paul, MN, USA) and Fuji IX (GC Corporation, Tokyo, Japan) specimens were allowed to set for 5 min, according to the manufacturers' instructions. After this, all disks were stored in 100% relative humidity at 37°C for 24 hours and the polishing steps were not performed to avoid surface contamination. The specimens were distributed into two groups (n=10): the control group and biodegradation group. The control group was maintained in 100% relative humidity at 37°C for 7 days, while the other group was submitted to biodegradation for the same period of time.

Biofilm Growth

Streptococcus mutans strain UA159 was obtained from the culture of the Department of Microbiology and Immunology, Piracicaba Dental School, University of Campinas. To prepare the inoculum, *S. mutans* was first grown on

Mitis salivarius agar (Difco Laboratories) plates at 37° C for 48 hours in an environment supplemented with 10% CO₂. Subsequently, single colonies were inoculated into 5mL of Brain heart infusion (BHI) broth (Difco Laboratories) and incubated at 37°C for 18 hours. The biodegradation group specimens were exposed to 25µL of *S. mutans* inoculum under static conditions, adjusted to an optical density (OD) of 0.6 at 550nm (approximately 8 x 10¹¹ CFU/mL). After two hours at room temperature, the non-adhering cells were removed by washing two times with 0.9% NaCl solution (saline). Next, a single material disk was placed in each well of 24-well polystyrene plates (Multidish 24-well Nunclon) with 2mL of sterile fresh BHI broth with the addition of 1% (w/v) sucrose. The bacterial accumulation occurred at 37°C in an environment supplemented with 10% CO₂, to develop 7-day-old biofilms. The medium was renewed at 48-h intervals. The purity of the cultures in the media was verified everyday using Gram staining and by plating samples. At the end of experimental period, specimens were ultrasonically washed for 10 min and analyzed for surface roughness.

Surface Roughness Measurements

Before the abrasion test, both experimental groups were analyzed using a Surfscorder SE1700 surface roughness-measuring instrument (Kosaka Corp, Tokyo, Japan). Three readings were taken from each specimen. Additional specimens of each material were taken to compare unbrushed surfaces with abraded surfaces by SEM later.

Three-body Abrasion Test

The tooth-brushing test was conducted at 250 cycles/min, for 30,000 cycles with 200g load. Colgate Total dentifrice (Colgate Palmolive Ind. e Com. Ltda, S. B. Campo, São Paulo, Brazil) diluted in distilled water (1:2) was used as an abrasive third body. Next, samples were washed in an ultrasonic bath for 10 minutes and gently dried. Three final surface roughness readings were taken from each specimen, in the opposite direction to that of the tooth-brushing movement.

Surface Morphology Assessment

After the experimental period, three representative specimens of each group were rinsed, dried and mounted on a holder using double-sided adhesive carbon tape in order to illustrate the effect of tooth-brushing and biodegradation on the material surfaces. The samples were sputter-coated with gold in a vacuum appliance (Balzers-SCD 050 Sputter Coater, Liechtenstein) and examined with a Model JEOL JSM 5600 LV scanning electron microscope (SEM - Tokyo, Japan) operating at 1000x magnification.

Statistical Analysis

First, the data were evaluated to check the equality of variances and normal distribution. After this the data were submitted to repeated measures three-way ANOVA and Tukey tests with at a level of level of significance of 5%, since the specimens used for the abrasion test were the same ones used previously for different storage challenges.

RESULTS

Surface roughness values of all materials tested are described in Table 2. There was significant difference among materials studied ($p < 0.0001$), between storage conditions (humidity/biofilm; $p < 0.0001$) and between tooth-brushing effects (before/after; $p < 0.0001$). Furthermore, a significant interaction was observed among the three factors: materials, storage and abrasion (before x after) ($p = 0.0002$).

When different storage conditions were compared for each material before abrasion, the *S. mutans* biofilm resulted in degradation, i.e., significantly higher roughness values for all tested materials, except for Vitremer. However, after the abrasion test, Ketac N100 presented no significant difference between storage groups, while there was statistically significant difference for the other materials, with higher roughness values for the biodegradation group.

When comparing the roughness values obtained from specimens stored in humidity before and after abrasion, statistically significant difference was observed for all materials, with higher values after abrasion. For the biodegradation group, only Ketac N100 showed similar roughness values before and after abrasion. As regards the other materials, higher values were found after abrasion than before.

When materials within the relative humidity group were compared, all materials presented statistically similar results before and after abrasion. When biodegraded materials were compared before abrasion, Vitremer showed the lowest roughness values, but statistically similar to the Ketac N100 values. The nano-filled resin-modified glass ionomer also presented

similar roughness values to Ketac Molar Easymix, which showed no statistical difference from Fuji IX. In addition, after biodegradation and abrasion, Ketac N100 showed the lowest roughness values, followed by Vitremer, Ketac Molar Easymix and Fuji IX.

The scanning electron micrographs in Fig. 1 show details of the surface morphology of the studied materials, distributed in rows (different materials) and columns (first: humidity group, before abrasion; second: biodegradation group, before abrasion; third: biodegradation plus abrasion). In the humidity group images, a reasonably smooth surface layer was observed, with undetectable fillers for all materials (Fig. 1a, d, g and j). The conventional cements presented a large number of cracks in the microstructures, probably caused by dehydration during preparation for SEM analysis (Fig. 1g and j). After bacteria-surface interaction, changes in the surface texture were very evident in the conventional ionomer samples, revealing numerous surface porosities, exposing some particles and showing the corroded aspect of the matrix (Fig. h and k). The Ketac N100 specimens (Fig. 1b) also had much more severely eroded surface layers than the Vitremer surfaces (Fig. 1e). After the tooth-brushing test of biodegraded specimens, all materials showed a discernible loss of material, leading to irregular surfaces and protruding filler particles (Figs. 1c, f, i and l). There was a clearly visible difference between the particles of Ketac N100 (Fig. 1c) and the other ionomers as regards shape and size.

DISCUSSION

In restorative procedures, surface characteristics such as roughness allow the esthetics and longevity of restorative materials, since surface irregularities can contribute to biofilm accumulation,¹⁶ which may result in gingival irritation, reduction in gloss, superficial staining and secondary caries.^{17,18} The surface roughness of the studied materials is determined by finishing and polishing techniques, but could be affected by mechanical, biological and chemical degradation in the oral environment. In the present study, no surface finishing method was used in order to avoid contaminating the aseptic surface of specimens, which would interact with *Streptococcus mutans* biofilm (biodegradation). Nevertheless, some studies have shown that glass ionomer cements polymerized against a clear matrix or a glass plate presented lower surface hardness and lower abrasion resistance, despite their smoother surfaces,^{19,20} as seen in micrographs 1a, d, g and j.

Before being stored, the specimens were also not sterilized by physical (steam under pressure and gamma rays), chemical (solutions) or physico-chemical methods (ethylene oxide and hydrogen peroxide plasma). While these methods may render the specimen sterile, they probably affect the structure and properties of the restorative materials. Pressure, temperature, post-irradiation, chemical components and vacuum can cause alterations in the degree of polymerization, degradation, crack formation, among others, modifying the surface of conventional and resin-modified glass ionomers.^{21,22,23}

Glass ionomers are often used to restore cervical lesions because they chemically bond to enamel and dentin and can absorb and release fluoride. These restorations are likely to come into contact with acids produced during

biofilm metabolism, and will be exposed to the abrasive action of toothpastes during tooth-brushing. *Streptococcus mutans* can be found on all surfaces in the mouth, irrespective of the nature of the surface,^{24,25} and it causes the pH within the dental biofilm to drop due to its acidogenicity and its aciduric characteristics,²⁶ therefore, this bacterial specie could be responsible for surface damage to restorations. The softening and roughening of materials promoted by lactic acid solutions (the main acid of active caries),^{26,27,28} or acidic biofilms,^{11,29} make their surfaces more susceptible to the physical forces occurring during abrasion and attrition.^{13,30}

Glass-ionomer cement biodegradation is a complex process of absorption, disintegration, and outward transportation of ions.⁹ Interaction of the studied materials with a *S. mutans* biofilm, immersed in an acidic liquid culture medium, certainly resulted in fluid uptake by the matrix, and its solubility. However, the biodegradation rates of different glass-ionomer cements subjected to similar storage conditions depend greatly on their hydrolytic stability, which is related to chemical composition, particularly to the siliceous hydrogel layer peripheral to the glass particles and the hydrophilic functional groups present in the RMGIC network.³¹ In order for GIC to become a photo-polymerized material, 2-hydroxyethyl methacrylate (HEMA) was added to its composition. This feature has provided a less water sensitive material associated with the early stage of the acid-base setting reaction than is the case with conventional glass-ionomers.³² However, HEMA is highly hydrophilic and it is found on the surface of the inadequately polymerized material, due to oxygen inhibition of polymerization.³³ Thus, the water absorption process and the disintegration of the matrix of resin-

modified glass ionomers are heavily dependent on the resin matrix composition and polymerization reactions.

The abrasion resistance can be ascribed to other factors, such as the size, hardness and percentage of surface area occupied by filler particles and the filler/matrix interaction.^{12,14} The degree of conversion of the polymer of the resin matrix³⁴ and powder to liquid ratio³⁵ also are important for this property. The preferential wear of the matrix, exposing the filler particles, is a common situation for all ionomers, since there is a difference between filler and matrix hardness.¹⁹ The resin-based materials without finishing/polishing presented a resin rich surface layer (Fig. 1a and d), due to the migration of organic polymers to the surface of the material.³⁶ This superficial layer remains only partly polymerized due to the oxygen inhibition of polymerization, producing inferior surface properties.²⁰

The nano-filled RMGIC, Ketac N100, contains other resin monomers in addition to HEMA, such as bisphenol glycidyl methacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA). Some studies have shown that in the presence of water, BisGMA/HEMA undergoes micro-phase separation, the hydrophilic tertiary amine and hydrophobic camphorquinone tend to exist in the hydrophilic HEMA phase and hydrophobic BisGMA phase, respectively.^{37,38} This decreases their chances of coming into contact, so that fewer radicals will be generated and a lower degree of conversion could be found in the Ketac N100 matrix than in the Vitremer matrix. It could be a reasonable explanation for the severe biodegradation observed in the micrographs of Ketac N100 specimens, given that a susceptible superficial layer was exposed to biofilm challenge (Fig. 1b). Vitremer did not present an increase in roughness values

when it underwent biodegradation, confirmed by its micrograph (Fig. 1e).

Only Vitremer shows a third polymerization setting reaction initiated through the incorporation of an oxidation-reduction catalyst system composed of micro-encapsulated water soluble potassium persulphate/ascorbic acid. This is similar to the original setting mechanism used in the early composite resins and it is incorporated to ensure that any HEMA, not polymerized through irradiation, will set.³⁹ Therefore, the resin matrix composition and polymerization reactions are extremely important factors in the water absorption process and the disintegration of the matrix of resin-modified glass ionomers. Clinically, the removal of the outermost surface by finishing-polishing procedures would tend to make the resin-modified glass-ionomers more resistant to biodegradation, and thus, more esthetically stable restorative materials.

The micrographs showed that 30,000 strokes, which simulated the use of a tooth-brush in the oral environment for approximately 3 years, removed the resinous surface of both resin-modified glass ionomer cements (Fig. 1c and f). Nevertheless, Ketac N100 presented the lowest surface roughness values when compared with the other materials, when submitted to cumulative challenges. Ketac N100 represents a blend of fluoraluminosilicate technology (40%) and nanotechnology (60%), including silica cluster filler, nonagglomerated silica filler and acid reactive glass fillers smaller than those of others resin-modified glass ionomers.⁸ The aggregated "nanoclusters" are in the 1- μm size range but are composed of 5 to 20nm spherical particles that have been lightly sintered together to form a porous structure interpenetrated with the resin monomers. As the surface of the

"nanocluster"/resin combination is subjected to stress and abrasion, the smaller nanosized particles, which make up the clusters, tend to break apart rather than the entire particle being plucked from the resin matrix.⁴⁰

Other studies verified an enhanced damage tolerance (force fracture and bi-axial flexure strength) for nanocomposites, as a ability of the "nanocluster" to deform and collapse into pre-existing cluster porosities and through progressive fragmentation of the main cluster structure, which subsequently act to absorb and dissipate propagating cracks.^{41,42} Even when the specimens were stored in a wet environment (for 24 hours), it was observed that the hydrolysis and polymerization within the nanocluster silane phase could modify stress transfer both to and within the cluster particles, producing an enhanced capacity to tolerate local stresses. However, the hydrolytic degradation of the silane led to a decrease in fracture toughness, when these materials are aged for 6 months in different media (water, saliva, ethanol), with the fracture occurring around the particles.⁴³ Our study found good results for the nano-filled resin-modified glass ionomer, Ketac N100, after the biomechanical degradation for seven days, regarding surface roughness and morphology. Further studies are necessary to analyze the synergistically influence of degradative processes on mechanical properties.

In a different manner, Vitremer also contains silane treated glass, but the shape and size of particles are different, according to electronic micrographs (Fig. 1f) and manufacturer's information. Larger and irregular filler particles made it more effortless to "pluck out" whole filler particles from the resin matrix,¹⁵ which could act as an additional abrasive agent once it

was detached from the surface and held against the specimen.⁴⁴ Moreover, Ketac N100 is a paste/paste ionomer while Vitremer requires the conventional powder/liquid mixing, which promotes the incorporation of air bubbles (Fig. 1d), and exposure of porosities after abrasion.³⁵ Thus, Ketac N100 was about three times more resistant to biomechanical degradation than other materials (Table 2).

With regard to conventional glass-ionomer cements, Ketac Molar Easymix and Fuji IX presented similar behavior under all conditions proposed. It is well documented that an acidic storage medium has a detrimental and irreversible effect on conventional ionomers, as it erodes the surface of the cement and causes hydrolysis and dissolution mainly of the matrix, as shown in the images of the surfaces observed in the SEM micrographs (Fig. 1h and k).^{29,30,33} The very fine aluminum-calcium-lanthanum fluorosilicate glass of Ketac Molar Easymix⁴⁵ powder promotes a higher powder-liquid ratio than Fuji IX, which could reduce material degradation and abrasion since fillers (powder) protect the cement matrix.³⁵ Nevertheless, this study found that these conventional glass ionomer cements presented similar wear resistance and similar degradation under acidic conditions, as shown in other studies.^{45,46} It is possible that it is an effect of the replacement of calcium by strontium in the Fuji IX particles. The strontium salts of polycarboxylic acids are thought to be more stable than the calcium salts. Moreover, the resultant matrix derived from maleic acid, present in Ketac Molar Easymix, may be more susceptible to attack by acids.⁴⁶

Thus, the nano-filled RMGI Ketac N100 exhibited the best resistance to cumulative challenges (biofilm plus tooth-brushing abrasion), since both

tests promoted exposure of its regular and small particles, differently from particles of the other materials. However, finishing-polishing procedures are important in order to remove the superficial layer of Ketac N100, which is very susceptible to biodegradation. The outermost matrix of Vitremer was the most resistant to biodegradation alone, while the conventional ionomeric materials suffered severe damage from biomechanical degradation in this study. Further clinical studies are necessary to confirm the effectiveness of Ketac N100 as an ionomeric restorative material capable of withstanding all the adverse conditions in the oral environment, as well inhibiting the growth of bacteria and caries progression by means of fluoride release.

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Table 1 - Materials used in this study

Materials	Composition*	Mixture recommended	Mean Filler Size**	Batch #
Ketac N100 (3M ESPE)	Paste A: silane treated glass, silane treated zirconia oxide silica, polyethylene glycol dimethacrylate, silane treated silica, HEMA, Bis-GMA, TEGDMA Paste B: silane treated ceramic, silane treated silica, copolymer of acrylic and itaconic acids, HEMA	Clicker TM technology - paste/ paste	1 μm (cluster) 5 - 25nm (nanofiller) < 3.0 μm (glass)	M3M3
Vitremer (3M ESPE)	Powder: fluoroaluminosilicate glass, redox system; Liquid: aqueous solution of a modified polyalkenoic acid, HEMA	2.5:1	3.0 μm	P: 6LP L: 6FH
Ketac Molar Easymix (3M ESPE)	Powder: aluminium-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	P: 237334 L: 238809
Fuji IX (GC Corp.)	Powder: polyacrylic acid, aluminium fluorosilicate glass; Liquid: polyacrylic acid, tartaric acid and water	3.6:1	4.4 μm	P: 0706061 L: 0706051

* Abbreviation of monomers in alphabetical order: Bis-GMA = bisphenol glycidyl methacrylate; HEMA = 2-hydroxyethyl methacrylate; TEGDMA = triethylene glycol dimethacrylate.

**Manufacturer` information.

Table 2 - Surface roughness values (μm) (mean and standard deviation in parentheses) of ionomeric materials submitted to relative humidity or *Streptococcus mutans* biofilm, before and after tooth-brushing

Brushing	Materials	Storage Conditions	
		Relative Humidity	<i>S. mutans</i> Biofilm
Before	Ketac N100	0.19 (0.08) *Ba	0.57 (0.12) Abc
	Vitremer	0.24 (0.18) *Aa	0.35 (0.08) *Ac
	Ketac Molar Easymix	0.23 (0.11) *Ba	0.72 (0.11) *Aab
	Fuji IX	0.38 (0.24) *Ba	0.85 (0.17) *Aa
After	Ketac N100	0.63 (0.16) Aa	0.46 (0.09) Ac
	Vitremer	0.61 (0.16) Ba	1.01 (0.46) Ab
	Ketac Molar Easymix	0.45 (0.15) Ba	1.04 (0.31) Aab
	Fuji IX	0.64 (0.16) Ba	1.44 (0.29) Aa

Capital letters indicate comparison between storage groups (horizontal). Lower case letters demonstrate comparison among materials (vertical) within each storage condition and each tooth-brushing condition (before or after). Asterisks represent significant statistically difference between tooth-brushing effect (before x after). Groups denoted by the same letter/symbol represent no significant difference ($p>0.05$).

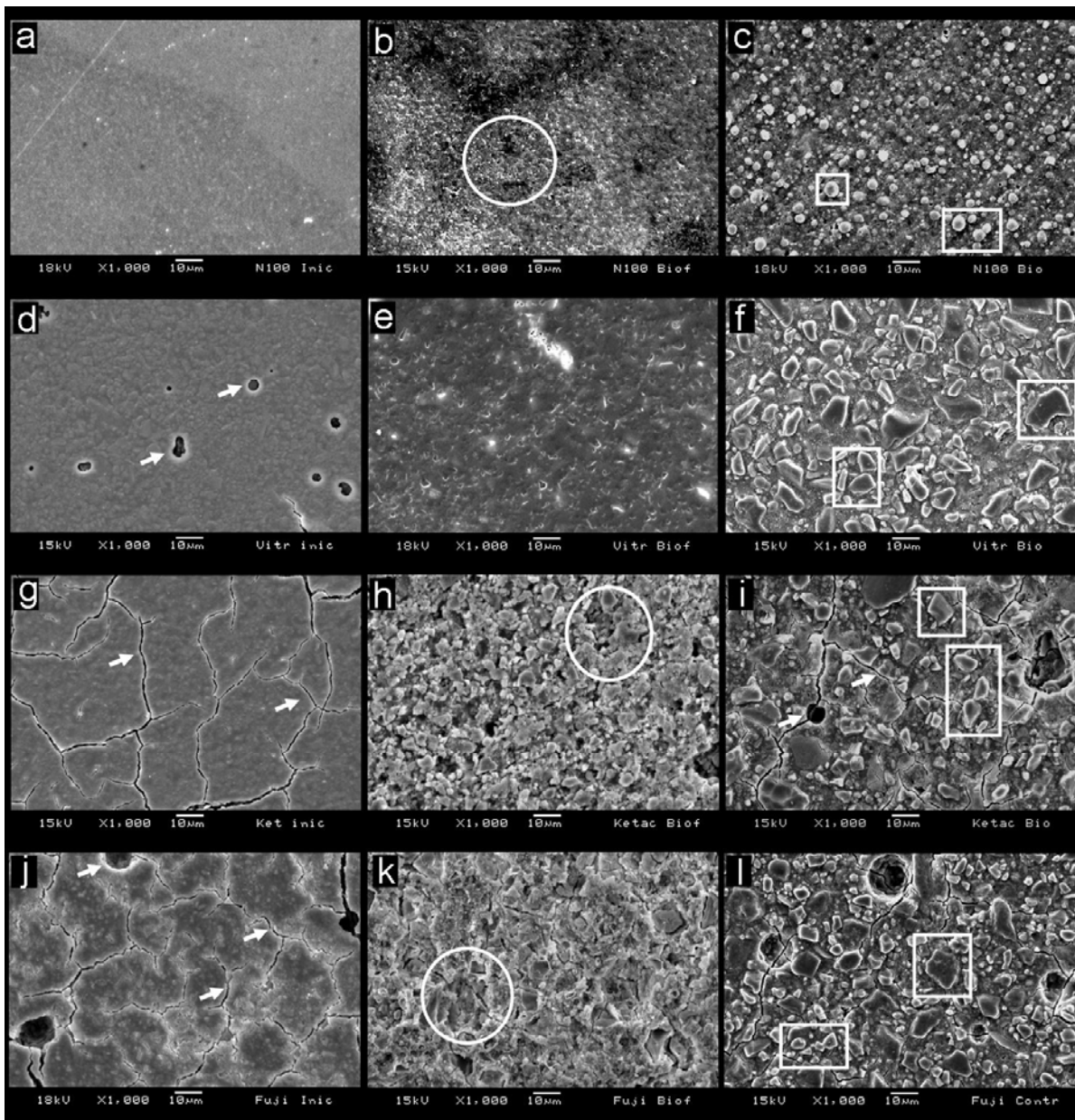


Fig. 1 - Scanning electron micrographs of Ketac N100 (a, b, c), Vitremer (d, e, f), Ketac Molar Easymix (g, h, i) and Fuji IX (j, k, l), at an original 1000X magnification. The first column shows the relative humidity storage groups (a, d, g and j), with cracks (long lines) and porosities (small spherical and irregular shapes) indicated by arrows. The second column represents the *S. mutans* biofilm storage groups (b, e, h and k), with a severely corroded aspect of the matrix pointed out by marking it with circles. The third column corresponds to biofilm storage plus abrasion groups (c, f, i and l), with many exposed particles at the surfaces of materials (squares).

CONSIDERAÇÕES GERAIS

A formação de biofilme sobre cimentos de ionômero de vidro conduz a uma espiral de eventos negativos ao material restaurador e positivos à estrutura dentária restaurada. Primeiramente, as propriedades físicas de superfície do material, como rugosidade e energia de superfície, influenciam na aderência inicial de células bacterianas (Quirynen & Bollen, 1995). Uma superfície mais rugosa disponibiliza maior área para adesão celular, protegendo os microrganismos aderidos das forças mecânicas de remoção e facilitando a rápida multiplicação do biofilme remanescente (Teughels *et al.*, 2006).

A partir do estabelecimento e manutenção de um biofilme acidogênico aderido às superfícies dente/restauração, inicia-se a progressiva desmineralização dentária (ten Cate, 2006), simultaneamente à severa e irreversível deterioração da superfície dos materiais. Este processo conhecido por biodegradação manifesta-se por alteração em propriedades como rugosidade, dureza e micromorfologia do material, resultantes do contato com os metabólitos produzidos por espécies cariogênicas (Willershausen *et al.*, 1999; Hengtrakool *et al.*, 2006; Beyth *et al.*, 2008; Fucio *et al.*, 2008).

Os prejuízos sofridos pelos cimentos de ionômero de vidro (convencionais ou modificados por resina) descritos acima ocorrem a partir de um complexo processo de absorção, desintegração e liberação de íons/monômeros (Oilo, 1992), ainda potencializado pelo baixo pH mantido pelos microrganismos acidogênicos (Czarnecka *et al.*, 2002). Portanto, a intensidade e extensão destes danos, assim como a atividade antibacteriana contra o biofilme intimamente aderido, estariam diretamente relacionadas à composição

química do material restaurador ionomérico. Primeiramente, a composição da matriz resinosa e, conseqüentemente, seu grau de conversão direcionam a estabilidade hidrolítica do material e a lixiviação de monômeros residuais e seus derivados (Kawai & Takaoka, 2002; Finer & Santerre, 2004). Além disso, a composição e quantidade de partículas de vidro e a relação destas com a matriz (silanização) influenciam quantitativa e qualitativamente os íons liberados pelo material (Nicholson, 1998; Sales *et al.*, 2003).

Então, os eventos positivos à estrutura dentária evidenciam-se neste momento pela influência dos íons e monômeros lixiviados sobre o crescimento e virulência bacteriana e o processo desmineralização/remineralização. Entretanto, deve-se observar que monômeros e íons modulam de diferentes formas o desenvolvimento e metabolismo do biofilme cariogênico. Monômeros como TEGDMA (e seu derivado TEG), EGDMA e HEMA mostraram estimular o crescimento e a expressão gênica relacionada a enzimas produtoras de polissacarídeos extracelulares de bactérias cariogênicas em biofilmes e pH ácido (Hansel *et al.*, 1998; Schmalz *et al.*, 2004; Khalichi *et al.*, 2009). Em contrapartida, os monômeros BisGMA e UDMA, assim como os produtos da degradação do Bis-GMA (MA e Bis-HPPP), parecem inibir o crescimento de biofilme de *S. mutans* e a expressão gênica também relacionada à produção de polissacarídeos (Hansel *et al.*, 1998; Singh *et al.*, 2009).

Já os íons provenientes da erosão da matriz ionomérica e da superfície das partículas de vidro, estabelecem a capacidade tampão do material restaurador. Considera-se que, em 30 segundos, o pH encontrado na lesão de cárie ativa (aproximadamente 4,5) poderia ser modificado por discos de ionômero de vidro para valores capazes de paralisar o processo de

desmineralização (Nicholson *et al.*, 2000). Já os íons flúor são liberados ao meio inicialmente pela dissolução dos componentes com fluoreto presentes na matriz da superfície do material e, em longo prazo, pela difusão dos íons flúor ao longo de poros e trincas resultantes da degradação do material (Gao & Smales, 2001; Wiegand *et al.*, 2007). O efeito do fluoreto na inibição do desenvolvimento é essencialmente relacionado ao seu acúmulo e disponibilidade no biofilme dentário, reduzindo a desmineralização e ativando a remineralização (ten Cate, 1999).

Portanto, o presente estudo buscou acompanhar estas etapas da inter-relação material restaurador ionomérico e biofilme dentário, baseando seus resultados na variável composição química dos materiais estudados. Ainda, a disponibilidade de avaliação de um nano-ionômero presente recentemente no mercado odontológico instigou o interesse em evidenciar tal inter-relação. Este cimento de ionômero de vidro apresenta uma composição híbrida de componentes encontrados em compósitos nanoparticuladas (nanopartículas, *nanoclusters*, e monômeros resinosos, como BisGMA e TEGDMA) e em cimento de ionômero de vidro modificado por resina (partículas de flúor-alumino-silicato e HEMA). Portanto, seria importante estabelecer se este material comporta-se de maneira similar ou intermediária às classes compósitos x ionômeros, quanto às propriedades microbiológicas e de resistência à degradação biomecânica, o que poderia influenciar clinicamente em suas indicações e longevidade.

Reunindo os resultados encontrados, observa-se que as modificações em composição do nano-ionômero influenciaram de maneira negativa as propriedades microbiológicas, quando comparado aos resultados encontrados

para o Vitremer, e de maneira positiva a resistência do material aos desafios cumulativos da biodegradação e da abrasão por escovação. Isto, de certa maneira, já poderia ser esperado, já que ambos ocorrem sequencialmente como discutido acima. Ainda, estes resultados evidenciam um comportamento mecânico e químico mais próximo dos compósitos, o que poderia deslocar as indicações do nano-ionômero para áreas de maiores desafios mecânicos, que cariogênicos. Posteriores estudos laboratoriais e clínicos precisam ser realizados com este material a fim de melhor classificá-lo e indicá-lo, garantindo assim ao paciente a durabilidade da restauração.

CONCLUSÕES

Baseado nos resultados obtidos pôde-se concluir que a influência da degradação biomecânica sobre os materiais ionoméricos estudados foi material-dependente, isto é, foi determinada pelas diferentes composições químicas (porção orgânica e inorgânica) desses materiais. Enquanto o Vitremer apresentou melhores propriedades químicas antibacterianas e antibiofilme, o Ketac N100 mostrou-se mais resistente aos desafios cumulativos da biodegradação e abrasão. Dentre os ionômeros convencionais, o Fuji IX apresentou maior liberação de flúor e maior resistência à degradação biomecânica que o Ketac Molar Easymix.

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³ De acordo com a norma da FOP / UNICAMP, baseada na norma do International Committee of Medical Journal Editors - Grupo Vancouver. Abreviatura dos periódicos em conformidade com o MEDLINE.

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APÊNDICE

PRANCHAS DE FIGURAS

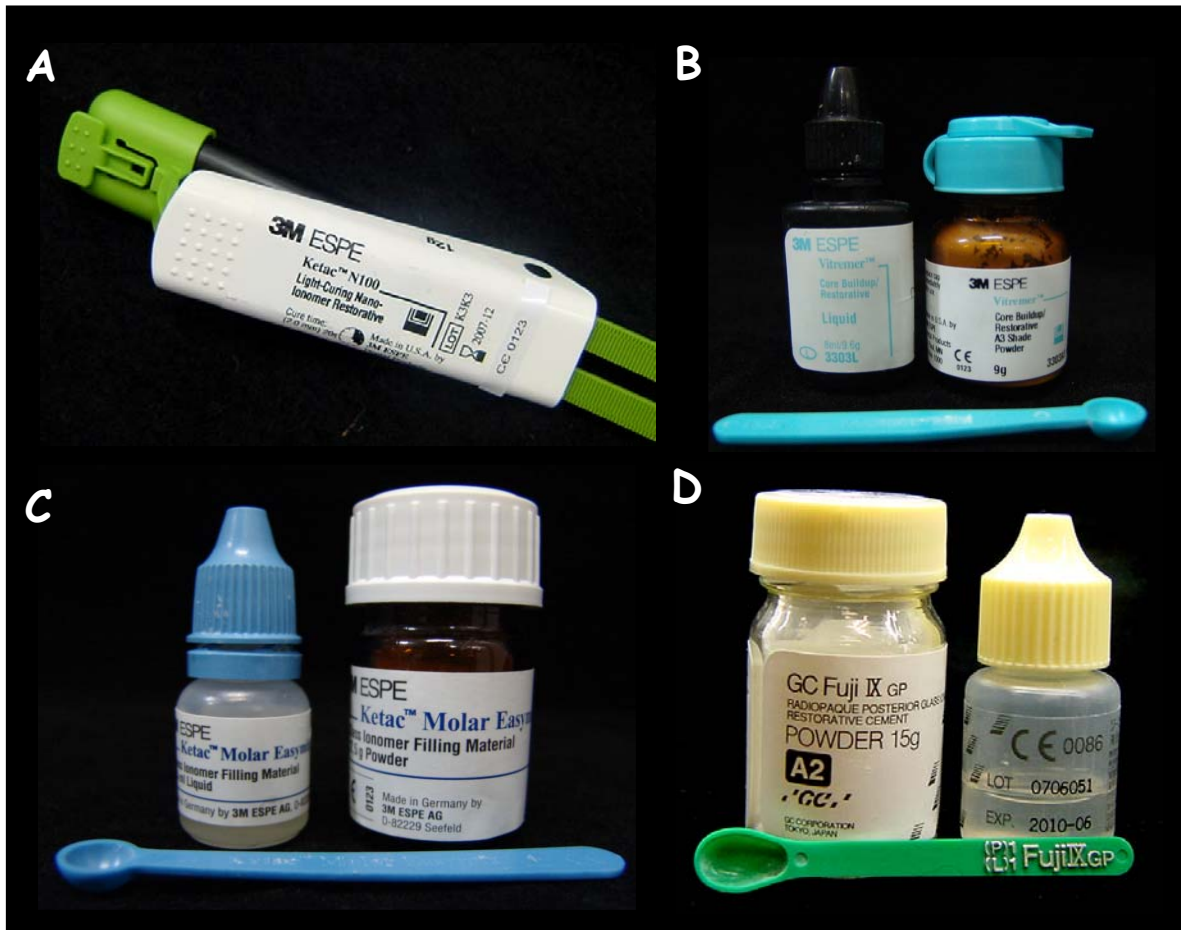


Figura 1 - Materiais estudados nos Capítulos 1 e 2

- A. Ketac™ N100 (3M ESPE, St. Paul, MN, USA) - cimento de ionômero de vidro modificado por resina nanoparticulado
- B. Vitremer™ (3M ESPE, St. Paul, MN, USA) - cimento de ionômero de vidro modificado por resina
- C. Ketac™ Molar Easymix (3M ESPE, St. Paul, MN, USA) - cimento de ionômero de vidro convencional
- D. Fuji IX GP (GC Corporation, Tóquio, Japão) - cimento de ionômero de vidro convencional

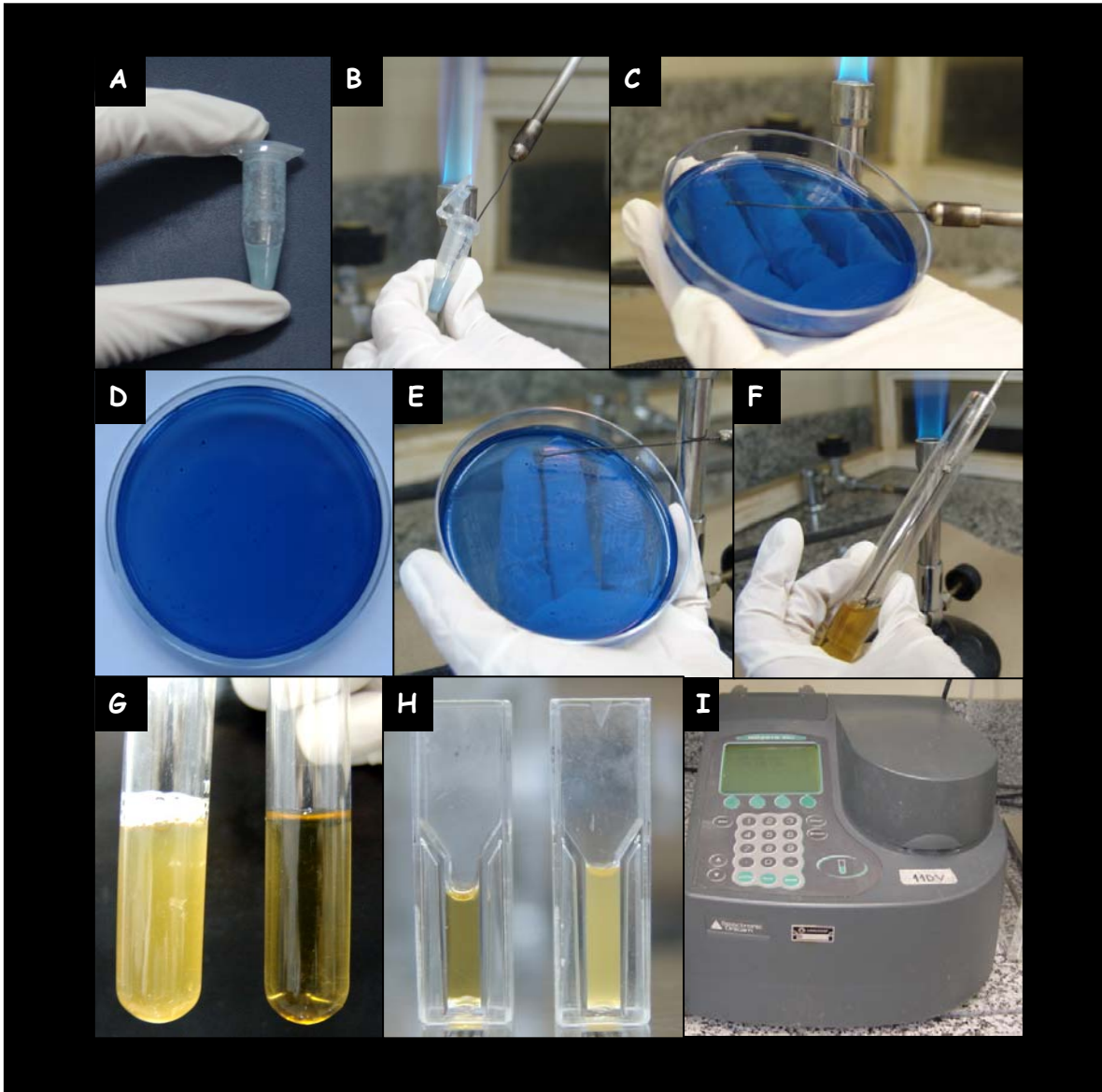


Figura 2 - Reativação da cepa de *Streptococcus mutans* UA159 utilizada em todos os testes microbiológicos e de biodegradação

- A. Cepa de *Streptococcus mutans* UA159 congelada em eppendorf
- B. Retirada de inóculo com alça
- C. Semeadura do inóculo em placa com *Mitis salivarius* ágar
- D. Colônias crescidas após incubação a 37°C em atmosfera suplementada com 10% de gás carbônico por 48 horas
- E. Coleta de algumas colônias da placa
- F. Inoculação das colônias em BHI (Brain-Heart Infusion) caldo
- G. Tubos de ensaio com meios de cultura de turbidez diferentes: o da esquerda após incubação do meio com *Streptococcus mutans overnight* a 37°C em atmosfera suplementada com 10% de gás carbônico, e o da direita apenas meio de cultura
- H. Cubetas para utilização em espectrofotômetro: o da esquerda com alíquota para a determinação do *blank* e o da direita para a determinação da densidade óptica do meio inoculado
- I. Espectrofotômetro utilizado para a padronização das culturas em absorbância de 0,6 em 550 nm (aproximadamente 8×10^{11} UFC/ml)

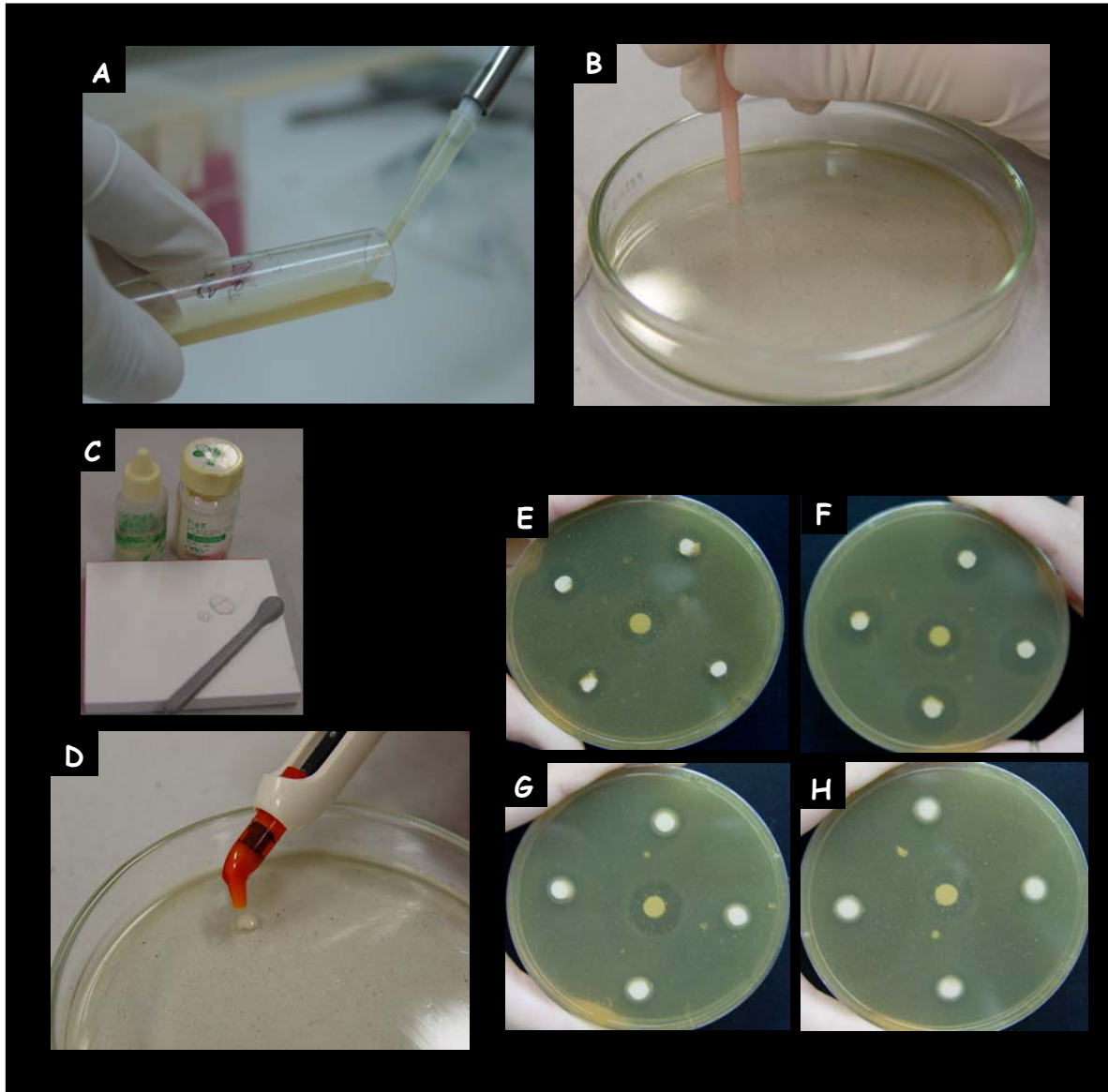


Figura 3 - Teste de difusão em ágar para análise de halos de inibição de crescimento da cepa de *Streptococcus mutans* UA159, com controle positivo central (disco embebido em clorexidina a 0,12%)

- A. Obtenção de inóculo com quantidade calculada do meio incubado
- B. Confecção de poços na camada base (BHI ágar + inóculo)
- C. Manipulação dos materiais seguindo as recomendações do fabricante
- D. Inserção dos materiais manipulados com seringa Centrix para incubação por 24 horas, a 37°C, em estufa bacteriológica em microaerofilia
- E. Ketac N100
- F. Vitremer
- G. Ketac Molar Easymix
- H. Fuji IX

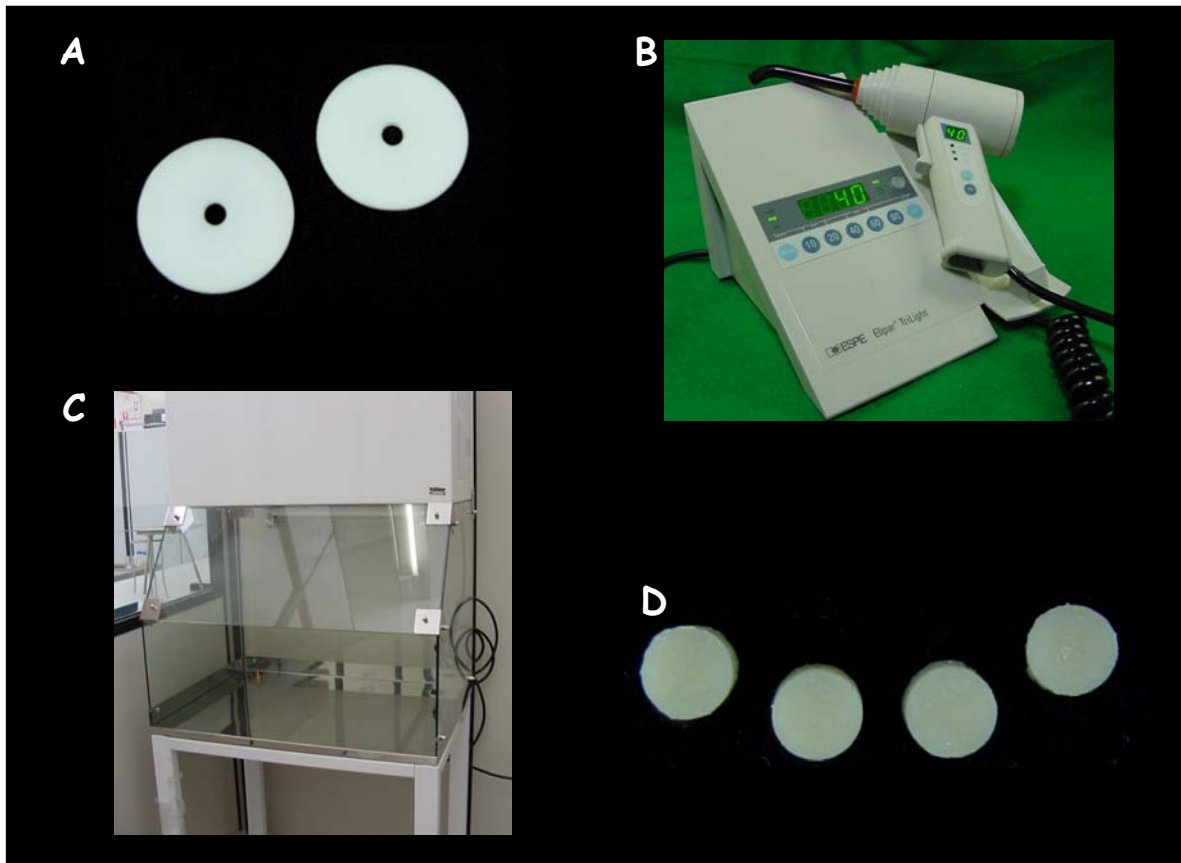


Figura 4 - Confeção dos espécimes para o teste de aderência e posterior desenvolvimento do biofilme de *Streptococcus mutans*

- A. Matriz de teflon com 5 mm de diâmetro e 2 mm de espessura, utilizadas após esterilização em autoclave
- B. Fotoativador Elipar Trilight® (3M ESPE, St. Paul, MN, EUA) utilizado para os cimentos de ionômero de vidro modificados por resina
- C. Câmara de fluxo laminar, utilizado a fim de manter os instrumentais e ambiente asséptico durante a confecção dos discos
- D. Obtenção de 30 discos de cada material ionomérico estudado, assépticos, sem acabamento e polimento para distribuição em diferentes testes

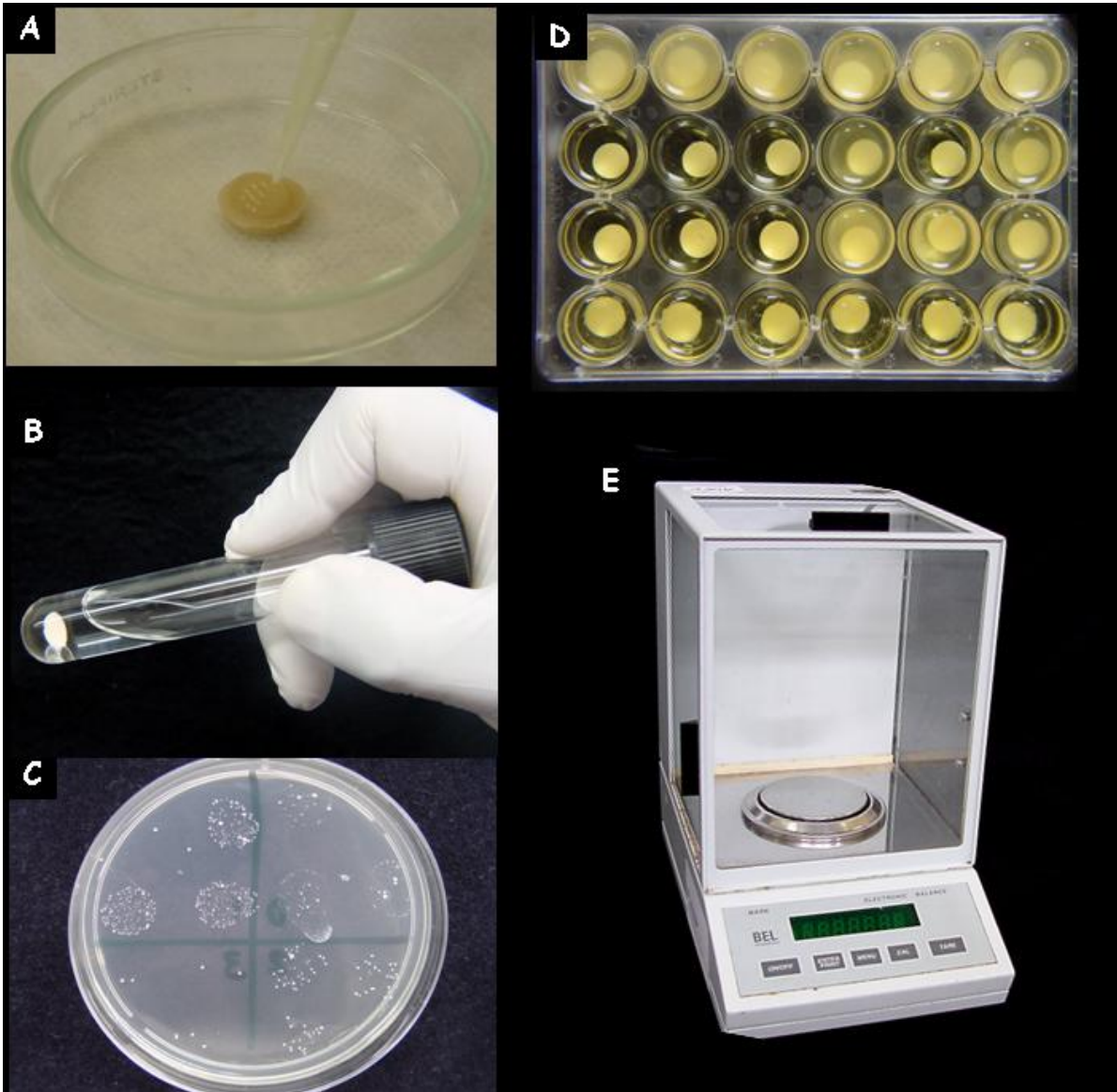


Figura 5 - Teste de aderência bacteriana em duas horas (A, B e C) e acúmulo de biofilme de *Streptococcus mutans* por sete dias para pesagem (A, D e E)

- A. Pipetagem de 25 μ L de cultura com absorvância ajustada sobre a superfície do disco de material restaurador para incubação por 2 horas
- B. Disco com adesão inicial em 3 mL de solução salina e três esferas de vidro para agitação em vórtex
- C. Placa para contagem das unidades formadoras de colônias, após 48 horas de incubação, com diluição de 10^{-1} a 10^{-4}
- D. Placa de cultura de 24 poços (Multidish 24-well Nunclon) nos quais os discos com células aderidas foram mantidos por sete dias, em meio de cultura (BHI + sacarose a 1%), o qual era renovado a cada 48 horas;
- E. Balança de precisão para a pesagem do conjunto placa/disco/biofilme e quantificação final do peso úmido do biofilme



Figura 6 - Análises do pH e do flúor liberado no meio de cultura mantido por 48 horas em contato com o conjunto disco/biofilme (3 análises - 48h, 96h e 144h), durante o desenvolvimento do biofilme de *S. mutans*

- A. pHmetro utilizado para análise do pH do meio de cultura e soluções padrão para calibragem do aparelho (pH 4 e pH 7)
- B. Soluções utilizadas para produzir diluições padronizadas de flúor
- C. Diluições padronizadas utilizadas na calibragem do aparelho medidor de flúor (0.025 a 4 $\mu\text{g F}^-/\text{ml}$)
- D. Fluorímetro utilizado para análise do flúor liberado no meio de cultura pelos materiais ionoméricos

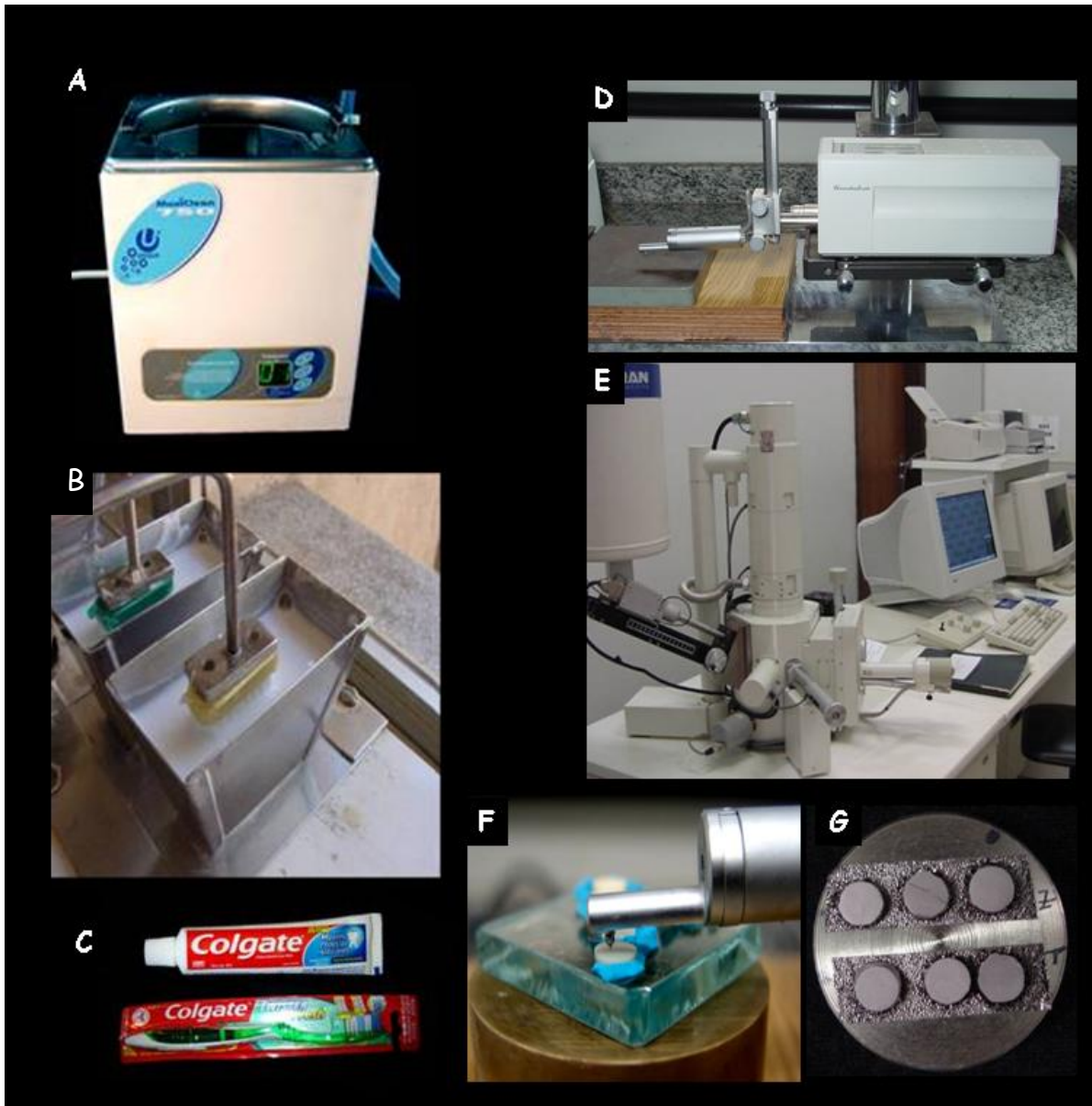


Figura 7 - Análises de rugosidade e micromorfologia da superfície dos materiais após a biodegradação (biofilme de *S. mutans*) e a abrasão por escovação

- A. Aparelho de ultrassom, utilizado para remover o biofilme das superfícies dos discos, previamente às análises
- B. Máquina de escovação, com os discos em posição sendo abrasionados
- C. Dentifrício Colgate Total® e escova de dente com cerdas macias utilizados no teste mecânico
- D. Rugosímetro Surfscorder SE1700 utilizado nas análises de rugosidade de superfície dos discos após a biodegradação e após a degradação biomecânica
- E. Microscópio Eletrônico de Varredura utilizado nas análises micromorfológicas dos mesmos espécimes degradados
- F. Agulha do rugosímetro (Surfscorder SE 1700, Japão) posicionada para leitura na superfície do espécime
- G. Discos degradados preparados (metalizados) para avaliação microscópica

ANEXO



Dr. Franklin García-Godoy, Editor
American Journal of Dentistry
1138 North Germantown Parkway, #360
Cordova, TN 38108
USA
Telephone (901) 752-1225
Fax: (901) 752-1881
E-mail: godoy@amjdent.com

www.amjdent.com

December 15, 2010

Dr Regina M Puppín-Rontani, MS, DDS, PhD
Chair of Pediatric Dentistry Department
Professor at Dental Materials Pre and Post-Graduate Program
Faculdade de Odontologia de Piracicaba
Universidade Estadual de Campinas – UNICAMP
Av Limeira 901 – 13414-018 – Piracicaba – SP
Brasil
Phone: 55 19 21065286/ Fax: 55 19 2106 5218
e-mail: mpuppín@fop.unicamp.br

Re: Biomechanical degradation of the nanofilled resin-modified glass-ionomer surface

Dr. Rontani:

I am pleased to inform you that your manuscript mentioned above has been accepted for publication in 2011 in the *American Journal of Dentistry*. Before publication you will receive page proofs for your approval.

Again, thank you for considering the *American Journal of Dentistry*.

Sincerely,

A handwritten signature in blue ink, appearing to read "Franklin García-Godoy".

Prof. Dr. Franklin García-Godoy
Editor