FACULDADE DE ODONTOLOGIA DE PIRACICABA

UNIVERSIDADE ESTADUAL DE CAMPINAS

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Resistência à degradação biológica, química e mecânica de materiais resinosos nanoparticulados

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do título de Doutor em Materiais Dentários.

Orientadora: Profa. Dra. Regina Maria Puppin-Rontani

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A Deus, por sempre iluminar meu caminho.

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"Aquilo que desejamos já nos foi
concedido e está dentro de nós".
Masuharu Taniguchi

RESUMO

O avanço da Odontologia Estética tem sido impulsionado pela introdução de novos materiais restauradores, tais como os nanoparticulados, os quais têm demonstrado bom desempenho mecânico e excelentes propriedades ópticas. Entretanto, deve-se considerar que os materiais restauradores estão constantemente sujeitos aos desafios térmicos, mecânicos e químicos na cavidade bucal que podem acarretar danos a estes materiais, num processo denominado degradação. Dessa forma, os objetivos nesta tese, composta por 2 capítulos, foram: (1) avaliar a morfologia e a rugosidade de superfície de materiais nanopartículados quando submetidos a biodegradação e abrasão por escovação e (2) avaliar os efeitos do armazenamento em diferentes soluções simuladoras da dieta ácida na rugosidade e dureza de superfície de materiais restauradores. No capítulo 1, vinte espécimes obtidos de cada material (Vitremer, Ketac N100, TPH Espectrum e Filtek Z350) foram divididos em dois grupos de armazenamento (n=10): umidade relativa e bioflime de *S. mutans*. Após sete dias, todos os espécimes foram lavados em ultrassom para a mensuração da rugosidade de superfície (Ra) e avaliação em microscopia eletrônica de varredura. Seguidamente, o teste de abrasão por escovação foi conduzido no grupo biodegradado e os espécimes reavaliados. Os dados foram submetidos aos testes ANOVA 3 fatores para medidas repetidas e Tukey ($p<0.05$). Após a biodegradação o ketac N100 apresentou os maiores valores de Ra. Quando as amostras biodegradadas foram submetidas à abrasão por escovação, o compósito Z350 apresentou os menores valores de Ra. No capítulo 2, foram confeccionados espécimes (5mm-diâmetro; 2mm-espessura), dos materiais utilizados no Capítulo 1, os quais foram divididos em 4 grupos (n=30). Após 24h, foram realizados os procedimentos de acabamento e polimento e os testes de dureza Knoop

(KHN) e rugosidade de superfície. Seguidamente, cada grupo foi dividido em 3 subgrupos (n=10) de acordo com o meio de armazenagem: saliva artificial, suco de laranja e Coca-Cola®. A rugosidade de superfície e dureza Knoop dos espécimes foram reavaliadas após 30 dias de armazenamento e os dados submetidos aos testes ANOVA 3 fatores para medidas repetidas e Tukey ($p<0,05$). Os compósitos apresentaram menor Ra e maior KHN que os materiais ionoméricos em todas as situações estudadas. Após a degradação química, a diminuição significativa da KHN foi observada para todos os materiais, enquanto o aumento da Ra foi observado nos materiais ionoméricos armazenados em Coca-Cola® e suco de laranja. O compósito Z350 apresentou valores similares de KHN após a erosão qualquer que fosse a solução de armazenamento. Para os outros materiais, a Coca-cola e o suco de laranja provocaram maiores reduções nos valores de KHN. Com base nos resultados obtidos pode-se concluir que nanopartículas incorporadas aos materiais estudados exerceram influência significativa para a obtenção de maior resistência frente à degradação biomecânica. No entanto, a presença de nanopartículas não influenciou na resposta à erosão quanto à rugosidade e dureza Knoop.

Palavras chave: compósito resinoso, cimento de ionômero de vidro, biofilme, degradação química, rugosidade de superfície, dureza Knoop

ABSTRACT

Esthetic Dentistry has increasingly advanced with the introduction of new restorative dental materials, such as nanofilled materials, which have demonstrated better mechanical behavior and excellent optical properties. However, restorative materials are constantly subjected to thermal, mechanical and chemical challenges in the oral cavity that leads to damage of these materials in a process called degradation. The objectives of this dissertation were to evaluate: (1) the morphology and surface roughness of nanofilled materials subjected to biodegradation and brushing and (2) the effects of storage in different acid diet simulating solutions on roughness and surface hardness of resin based restorative materials. In the study 1, twenty specimens obtained from each material (Vitremer, Ketac N100, TPH Espectrum e Filtek Z350) were divided into two storage groups (n=10): relative humidity (control) and Streptococcus mutans biofilm (biodegradation). After 7 days of storage, roughness values (Ra) and micrographs by Scanning Electron Microscopy (SEM) were obtained. In a second experimental phase, the specimens previously subjected to biodegradation were abraded via toothbrushes (mechanical degradation). Next, these specimens were washed, dried, and reassessed by roughness and SEM. The data were submitted to repeated measures three-way ANOVA and Tukey's tests ($p<0.05$). After biodegradation, Ketac N100 presented the highest Ra values. Concerning bio plus mechanical challenges the nano composite Filtek Z350 exhibited the best resistance to cumulative challenges proposed. In the study 2, disc-shaped specimens (5mm-diameter; 2mm-thick) of the same materials used on study 1 were obtained according to the manufacturers' instructions, thereby forming four groups (n=30). After 24h, polishing procedures were performed and initial hardness(KHN) and roughness(Ra) measurements were realized. Next,

each group was divided into three subgroups (n=10) according to storage media: artificial saliva, orange juice and Coca-Cola®. After 30 days of storage, the specimens were reevaluated about Ra and KHN. Data were tested for significant differences by three-way ANOVA and Tukey ($p<0.05$). It was observed that composites presented lower roughness values and higher hardness values than ionomeric materials under all storage conditions. After erosion, KHN of all experimental samples dropped significantly, while only the Ra of ionomeric materials increased, depending on the media, with a markedly negative impact of Coca-Cola® and orange juice. There was not difference among storage media for Filtek Z350 regarding to KHN values. Based on the results it can be concluded that nanofillers incorporated into the studied materials was important to obtain greater resistance against biomechanics degradation. However, the presence of nanofillers did not influence the surface roughness and Knoop hardness when the tested materials underwent to erosion.

Keywords: composite resin, glass ionomer cement, biodegradation, wear, surface roughness, erosion, hardness

SUMÁRIO

INTRODUÇÃO

A nanotecnologia, desenvolvida a partir de conceitos de engenharia molecular, possibilita manipular a estrutura de materiais por métodos físicos e químicos na escala de 0,1 a 100 nanômetros. Essa tecnologia pode proporcionar melhorias significativas nas propriedades elétricas, químicas, mecânicas e ópticas, de modo a desenvolver materiais com novas características. No caso dos polímeros, a interação com as nanopartículas pode resultar num nanocompósito polimérico, com propriedades diferentes daquelas dos compósitos convencionais (Mitra et al., 2003; Zhang et al., 2005; Beun et al., 2007). Por definição do Instituto de Padrões e Tecnologia de Gaithersburg, MD, Estados Unidos, nanocompósito polimérico é um sistema multicomponente no qual o maior constituinte é um ou mais polímeros, e o menor é uma partícula de carga com dimensões inferiores a 100 ηm, isto é, uma nano partícula.

Na Odontologia considera-se como carga nanométrica aquelas com tamanho médio de 40 ηm. Entretanto, não é exatamente o tamanho das partículas que inova estes materiais e sim a possibilidade de aumentar o conteúdo de carga incorporada a eles e, consequentemente, a diminuição do volume de matriz orgânica, o que implicaria em melhora no comportamento mecânico e nas propriedades ópticas dos materiais resinosos (Beun et al., 2007).

Estudos tem mostrado a importância das partículas de carga para o compósito em relação às propriedades mecânicas e ópticas. A incorporação de partículas nanométricas aos compósitos resinosos acarretaria no aumento da dureza de superfície e da resistência mecânica ao desgaste, quando comparados aos compósitos híbridos (Suzuki et al., 2009; Rodrigues et al., 2008). Além disso, maior lisura de superfície e

manutenção do polimento também têm sido constatados (Endo et al., 2010). Entretanto, Mitra (2003) verificou que os compósitos nanoparticulados apresentam desempenho equivalente ou superior aos demais compósitos em relação às propriedades mecânicas. A relevância desta informação reside no comportamento dos compósitos híbridos, os quais revelaram não ser primordial a presença da nanotecnologia para alcançar resultados satisfatórios. Sendo assim, além do tamanho das partículas de carga outros fatores devem ser considerados para determinar as características de superfície e as propriedades mecânicas das restaurações, como por exemplo, a união das partículas à cadeia polimérica. A resistência dessa união evita o deslocamento da partícula e as alterações das características físico-químicas da superfície, como aumento da resistência ao desgaste, rugosidade e diminuição da dureza (Wilson et al., 2005).

Os cimentos de ionômero de vidro têm sido utilizados na Odontologia há mais de 20 anos. A preferência por esses materiais se deve a algumas de suas características como a liberação de flúor, biocompatibilidade, adesão química à estrutura dentária, além do uso diversificado na pratica clínica, podendo ser utilizado como material restaurador temporário, forrador e fixador de peças protéticas (Xie et al., 2000).

Recentemente foi lançado no mercado o Ketac™ N100, um cimento de ionômero de vidro modificado por resina (CIVMR), apresentado na forma pasta/pasta e desenvolvido com nanotecnologia, contendo partículas silanizadas variando de 5-25 ηm. Segundo dados técnicos do fabricante (3M ESPE), a presença de nanopartículas acarretaria melhorias na estética e no polimento (maior lisura superficial), tornando a superfície

de acabamento semelhante ao do compósito microhíbrido. Além disso, possibilitaria o aumento da resistência ao desgaste quando comparado aos cimentos ionoméricos e modificados por resina convencionais, os quais devido às características e ao tamanho das partículas de carga apresenta maior rugosidade de superfície e acúmulo de biofilme, com prejuízo a estética (Oxman et al., 2008).

Neste sentido, apesar de ser possível a melhoria na microestrutura e morfologia da superfície, pela incorporação de nanopartículas aos materiais restauradores estéticos, deve-se considerar que esses materiais estão constantemente sujeitos aos desafios térmicos, mecânicos e químicos na cavidade bucal que acarretam danos à superfície, num processo denominado degradação.

A degradação química pode ser causada por ácidos, incluindo aqueles produzidos pelo biofilme cariogênico (Asmussen, 1984), dieta ácida (Yap et al., 2001 e 2002) e enzimas salivares (Larsen & Munksgaard, 1991; de Gee et al., 1996). A formação do biofilme dental ocorre por meio da fixação de bactérias sobre as superfícies dentárias e do material restaurador. O desenvolvimento do biofilme acarreta no amolecimento e aumento da rugosidade superficial de materiais restauradores resinosos (Fucio et al, 2008, Yap et al., 2000a; Turssi et al., 2002).

Clinicamente, os materiais restauradores são submetidos simultaneamente ao acúmulo de biofilme dentário e ao dano produzido pela dieta ácida, ocasionando associação de efeitos que induzem alterações nas superfícies das restaurações. Dentre estas, a diminuição da dureza (Asmussen, 1984; Yap *et al.*, 2000a) e o aumento da rugosidade de materiais restauradores resinosos (Yap *et al.*, 2000a; Turssi *et al.*, 2002) são os mais evidentes. Dessa forma, a superfície das restaurações torna-se mais susceptível ao desgaste e, consequentemente, à perda de componentes

que resulta na alteração da forma anatômica e afeta o desempenho clínico destes materiais. Além disso, o aumento da rugosidade de superfície dos materiais restauradores está diretamente relacionado à retenção de biofilme. Bollen et al. (1997) demonstraram que o aumento na rugosidade de superfície promove rápida colonização e maturação do biofilme, aumentando a susceptibilidade ao manchamento e corrosão dos materiais restauradores, além de aumentar o risco de desenvolvimento de cárie e doença periodontal (Bagheri *et al.*, 2005).

Frente ao processo de degradação, os monômeros que compõe a matriz orgânica é um dos fatores que pode influenciar as características superficiais dos materiais resinosos. O bisfenol glicidil metacrilato (Bis-GMA) é o componente mais comumente utilizado nos compósitos resinosos, apresentando alto peso molecular e baixa contração de polimerização (Rueggeberg, 2002). No entanto, devido à alta viscosidade desse monômero, a matriz orgânica necessita ser diluída com monômeros mais fluidos como, por exemplo, o trietileno glicol dimetacrilato(TEGDMA). O processo de diluição melhora as características de manipulação industrial do compósito e permite a incorporação de maior quantidade de carga o que poderia aumentar a resistência desses materiais frente ao processo de degradação. Entretanto, tal diluição monomérica ocasiona determinadas limitações, uma vez que o TEGDMA aumenta a absorção de água e principalmente a contração de polimerização do material (Dulik et al., 1981; Anseth, et al., 1996).

Mais recentemente, o bisfenol A glicidil dimetacrilato etoxilado (Bis-EMA) tem sido utilizado como componente alternativo ao Bis-GMA para compósitos odontológicos. Este monômero é estruturalmente semelhante ao Bis-GMA, sem, entretanto, apresentar os radicais hidroxil pendentes, que por sua vez são responsáveis pela sorção de água e principalmente pela

alta viscosidade do Bis-GMA. Dessa forma, o Bis-EMA poderia minimizar ou mesmo eliminar o uso do TEGDMA como diluente, e consequentemente aumentar a longevidade clínica do material (Achilias & Sideridou, 2004; Sideridou, et al., 2004a).

Segundo Smith (1988), a matriz de polissais dos cimentos ionoméricos modificados por resina é facilmente degradável em condições ácidas. Acredita-se, ainda, que a liberação de flúor dos cimentos ionoméricos, quando expostos em ambiente ácido, possa acarretar lixiviação das partículas de vidro, degradando a camada superficial do cimento e consequentemente aumentando a rugosidade superficial e diminuindo a dureza (Nomoto et al, 2003).

A remoção do biofilme da superfície dentária é um fator importante na prevenção da doença cárie e periodontal, sendo a limpeza por meio da escovação, um dos métodos de higiene mais utilizados, possibilitando a abrasão da superfície. Frente a esse fato, um aspecto a ser considerado na avaliação das características de superfície dos materiais restauradores é a capacidade de resistir ao desgaste, principalmente aquele determinado pela abrasão (Johannsen et al., 1989)

No entanto, quando restaurações degradadas são escovadas, alterações de contorno e aumento da rugosidade de superfície podem ocorrer, ocasioando desgaste, alteração de cor do material e maior acúmulo do biofilme, sendo essas conseqüências consideradas potencialmente prejudiciais para a longevidade clínica das restaurações (Sulong & Aziz, 1990). Em estudo realizado por Senawonge e Pongprueska, (2007), compósitos nanoparticulados apresentaram maior resistência ao desgate abrasivo quando comparados aos microhibridos e nanohibridos.

Estudos que enfoquem o desempenho de cimentos de ionômero de vidro em condições desafiantes são importantes, considerando que estes

materiais restauradores são adequados a pacientes com alto risco/atividade de cárie, portanto representando alto desafio para o material. Entretanto, para o cimento ionomérico nanoparticulado poucos estudos enfatizam o comportamento deste material frente ao desgaste abrasivo, quando comparado aos cimentos ionoméricos com partículas convencionais (Oxman, et al, 2008). Neste contexto torna-se importante estudar os possíveis efeitos sinérgicos da degradação química e biológica, pela ação do biofilme e dieta ácida, sobre a estrutura desses materiais.

A dieta é a fonte externa mais comum de ácidos relacionados à degradação de materiais restauradores na cavidade bucal. A agitação da vida moderna leva cada vez mais as pessoas a adotarem dieta pouco saudável, porém mais rápida, incluindo a ingestão de refrigerantes, sucos de fruta industrializados e condimentos prontos para o consumo. Os ácidos mais frequentemente consumidos são fosfórico, presente nos refrigerantes, e cítrico, presente em alguns sucos de frutas (West et al., 2001).

Estudos tem demonstrado que o potencial erosivo das bebidas pode ser influenciado por alguns fatores relacionados às características das soluções ácidas, como o tipo e concentração do ácido, pH e capacidade tampão (Hanning et al., 2009; Zero & Lussi, 2005). Freqüência de consumo, tempo de contato entre o liquido e o material e propriedades do material são outros fatores que podem influenciar na degradação química.

Considerando estes fatores, e com o intuito de presumir o desempenho dos materiais restauradores nanoparticulados, observa-se a importância de determinar o comportamento desses materiais quando submetidos aos desafios ácidos e desgaste abrasivo. Assim, os objetivos da presente tese[1], composta de 2 capítulos foram: 1) avaliar a

morfologia e a rugosidade de superfície de materiais nanopartículados quando submetidos à biodegradação e abrasão por escovação; 2) avaliar os efeitos do armazenamento em diferentes soluções simuladoras da dieta ácida na rugosidade e dureza de superfície de materiais resinosos.

[1] Esta tese foi apresentada no formato alternativo de acordo com as normas estabelecidas pela deliberação 002/06 da Comissão Central de Pós-Graduação da Universidade Estadual de Campinas.

Capítulo 1*

Biodegradation and abrasive wear of nano restorative materials

Short Title: Degradation of nano restorative materials

Clinical relevance: This study demonstrated that the nanotechnology incorporated in restorative materials, as composite resin and resin-modified glass ionomer, was important for the superior resistance to biomechanical degradation.

* Trabalho aceito pelo periódico Operative Dentistry (Anexo 1)

Abstract

The purpose of this study was to evaluate the biomechanical degradation of two nanofilled restorative materials (a resin-modified glass-ionomer - Ketac N100) and a composite - Filtek Z350), compared with conventional materials (Vitremer and TPH Spectrum). Twenty specimens obtained from each material were divided into two storage groups (n=10): relative humidity (control) and Streptococcus mutans biofilm (biodegradation). After 7 days storage, roughness values (Ra) and micrographs by Scanning Electron Microscopy (SEM) was obtained. In a second experimental phase, the specimens previously subjected to biodegradation were fixed to the tooth-brushing device and abraded via toothbrushes, using dentifrice slurry (mechanical degradation). Next, these specimens were washed, dried, and reassessed by roughness and SEM. The data were submitted to repeated measures three-way ANOVA and Tukey's tests ($p<0.05$). There was statistically significant interaction among factors: material, storage (humidity/biofilm) and abrasion (before/after). After biodegradation (S. mutans biofilm storage), Ketac N100 presented the highest Ra values. Concerning bio plus mechanical challenge, TPH Spectrum, Ketac N100 and Vitremer presented the undesirable roughening of their surfaces, while the nano composite Filtek Z350 exhibited the best resistance to cumulative challenges proposed. The degraded aspect after biodegradation and the exposure of fillers after mechanical degradation were visualized in micrographs.

Keywords: composite resin, biodegradation, glass ionomer cement, wear, surface roughness

1. Introduction

Developed from concepts of molecular engineering, nanotechnology has enabled the structure of materials to be manipulated, to provide significant improvements in electrical, chemical, mechanical and optical properties, and to develop materials with new features.[1,2] In Dentistry, nanoscale fillers or nanofillers correspond to primary particles about 40 nm or 0.04 μm in size. However, the material innovations are not exactly related to particles size, but to the possibility of increasing the nanofiller load in restorative materials,[2] leading to better mechanical behavior of materials.[3] Some studies have shown that nanocomposites presented higher surface hardness values and lower brushing abrasive wear than microfilled and hybrid composites.[2,4] In addition, resin-based nanocomposites offer high translucency, high polish and polish retention, with a vast range of shade and opacity options.[5] Thus, the manufacturers indicate nanocomposites for both posterior and anterior restorations, since these materials show high mechanical properties and superior esthetics.

Recently, a new resin-modified glass ionomer cement (RMGIC) has been introduced for operative dentistry. Ketac™ N100 light polymerizing nano-ionomer (3M ESPE) includes fluoroaluminosilicate glass, nanofillers, and nanofiller "clusters" combined to improve mechanical properties, such as three-body wear resistance (3M ESPE Internal Data).[6] In addition, this material contains 2-hydroxyethyl methacrylate (HEMA), bisphenol glycidyl methacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA) as resin monomers, differently of the known RMGICs. So, it would be interesting to compare this material with a traditional resin-modified glass-ionomer, as Vitremer, and with a nano composite, as Filtek Z350, which has similar filler characteristics and greater diversity of resin monomers. Thus, it could establish if the nano-ionomer shows a behavior similar to or intermediary

between ionomeric and composite classes, predicting its mechanical and chemical properties.

Although it is possible to obtain improvement in the microstructure and surface morphology with the incorporation of nanofillers into restorative materials, one should consider that the restorative materials are constantly subject to thermal, mechanical and chemical challenges in the oral cavity. The chemical challenges can be caused by acids produced by cariogenic biofilm,[7] acidic diet[8,9] and salivary enzymes,[10,11] leading to softening and increased surface roughness of resin-based materials.[11,13] When brushed with dentifrice daily, these damaged surfaces gradually would loose softened material (matrix and filler), causing loss of contour, change of color and roughening the restoration surface again, influencing its esthetic and clinical longevity.[14] Nevertheless, little is actually known about the cumulative effects of an acidogenic biofilm and tooth-brushing abrasion on the surface characteristics of nano-filled restorative materials.

Therefore, the aim of this study was to evaluate in vitro the surface roughness and micromorphology of nano restorative materials, a nano ionomer and a nano composite, subjected to Streptococcus mutans biofilm degradation (biodegradation) and three-body abrasion (mechanical degradation), when compared with other resin-modified glass ionomer cement and composite.

2. Materials and Methods

2.1 Specimen Preparation

Twenty specimens of each resin-modified glass ionomer cement and composite tested (described in Table 1) were fabricated using sterilized Teflon molds (5 mm in diameter; 2 mm deep) according to the manufacturer's instructions, under aseptic conditions. The materials were

manipulated, placed in the mold by one operator, covered and pressed flat with a sterilized glass slide. All specimens were polymerized with a curing light unit (Elipar Trilight, 3M ESPE, St. Paul, MN, USA), according to the manufacturer's instructions, after the intensity of the light-curing unit was checked with a curing light meter (Hilux Dental Curing Light Meter, Benliglu Dental Inc., Turkey). 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. Initially, the specimens were distributed into 2 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 one group was submitted to biodegradation for the same period of time.

2.2 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% CO_2. Subsequently, single colonies were inoculated into 5 mL of Brain heart infusion (BHI) broth (Difco Laboratories) and incubated at 37°C for 18 hours. The biodegradation group specimens were exposed under static conditions to 25 µL of S. mutans 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). After this, a single material disk was placed in each well of 24-well polystyrene plates (Multidish 24-well Nunclon) with 2 mL of sterile fresh BHI broth with addition

of 1% sucrose (w/v). The bacterial accumulation occurred at 37°C in an environment supplemented with 10% CO_2, developing 7-day-old biofilms. The medium was renewed at 48-h intervals. The purity of the cultures in the media were 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.

2.3 Surface Roughness Measurements

Before the biodegraded disks were submitted to abrasion, both groups (control and biodegradation) were analyzed using a Surfcorder SE1700 surface roughness-measuring instrument (Kosaka Corp, Tokyo, Japan). Three readings from each specimen were taken. Additional specimens from the biodegradation group were taken to compare unbrushed surfaces with abraded surfaces by SEM later.

2.4 Three-body Abrasion Test

The tooth-brushing test was conducted at 250 cycles/min, for 30,000 cycles with a 200gf load on the biodegradation group specimens only. 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 from this group (biomechanical degradation), in the opposite direction to that of the tooth-brushing movement.

2.5 Surface Morphology Assessment

After the experimental period, three representative specimens of each group (control, biodegradation and biomechanical degradation)

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 under vacuum (Balzers-SCD 050 Sputter Coater, Liechtenstein) and examined with a Model JEOL JSM 5600 LV scanning electron microscope (Tokyo, Japan) operating at 1000x magnification.

2.6 Statistical Analysis

First, the data were evaluated to check the equality of variances and normal distribution. Then, the data were submitted to repeated measures three-way ANOVA and Tukey's tests with a significance limit of 5%, since the specimens used for the abrasion test were the same ones used previously for the biodegradation procedure (biomechanical degradation).

3. 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; $p<0.0001$). Moreover, a significant interaction was observed among the three factors: materials, storage and abrasion (before/after) ($p<0.0001$).

When different storage conditions were compared for each material before abrasion, the S. mutans biofilm provided degradation, i.e., significantly higher roughness values for Ketac N100 specimens. The other materials tested presented similar values between the control (humidity) and biodegradation groups. However, the cumulative effect of biodegradation plus abrasion roughened the specimens of all materials, except the Filtek Z350 surface. Under these conditions (biomechanical

degradation), the roughness values of TPH Spectrum and Vitremer almost tripled, while the nano-ionomer became about two times rougher. With regard to the effects of abrasion on the biodegradation group surface, only Ketac N100 showed similar roughness values before and after brushing. The other materials presented higher values after abrasion than before it.

When the materials were compared within the control group (relative humidity), both composites presented similar roughness values. Ketac N100 values showed no statistical difference from those of composites and Vitremer, while the latter material presented the roughest surface. When biodegraded materials were compared before abrasion, the composites retained the smoothest surfaces, followed by Vitremer, then Ketac N100. Moreover, after biomechanical degradation, Vitremer showed higher roughness values than the other materials.

The scanning electron micrographs in Figure 1 show details of the surface morphology of the studied materials, distributed in rows (different materials) and columns (different conditions). In the control group images, a smooth surface layer with undetectable fillers was observed for all materials (Fig. 1a, d, g and j). Only Vitremer presented a large number of porosities on its surface, probably caused by the incorporation of air bubbles during conventional powder/liquid mixing (Fig. 1j). After bacteria-surface interaction (biodegradation group), changes in the surface texture were very evident for resin-modified ionomer samples, particularly for the nano-ionomer, which presented a degraded aspect of the matrix (fig. 1h). After the abrasion test of biodegraded specimens (biomechanical degradation group), all materials showed a discernible loss of organic matrix, leading to irregular surfaces and protruding filler particles (Fig. 1c, f, i and l). The difference in particle shapes and sizes among the studied materials (nano x conventionals) was clearly visible.

4. Discussion

Corrosive wear or biomechanical degradation results from the joint action of chemical and mechanical forces, and is associated with the mechanical removal of degraded layers that form on the surface of a material by reaction with its environment.[15] Since it is a continuous process occurring during the lifetime of the restoration, degradation characteristics of restorative materials are related to their long-term clinical performance. While resin-based materials undergo the cleavage of polymer chains to form oligomers and monomers, the ionomeric cements present a complex process of absorption, disintegration, and outward transportation of ions.[16] The present study evaluated the biomechanical degradation resistance of two composites and two resin-modified glass ionomers with important differences in their chemical composition, as discussed bellow.

Initially, the interaction of the studied materials with a Streptococcus mutans biofilm promoted the biodegradation process. The organic acids produced by bacterial metabolism can change the environment pH,[17] which started at about 7.3 and fell to about 4.0 for composites and nano-ionomer and 4.5 for Vitremer during the experimental period (seven days). According to Sarkar,[15] corrosive wear begins with water absorption that diffuses internally through the resin matrix, filler interfaces, pores, and other defects, accelerated by low pH. Thus, the biodegradation rates of different restorative materials depend greatly on their hydrolytic stability, which is related mainly to resin matrix composition and polymerization reactions in this study. It is important to remember that the present study did not use any method of surface finishing in order avoid contamination of the aseptic surface of the specimens, since all available sterilization methods may affect the structure and properties of the restorative materials.[18,19] Therefore, the outermost surface subjected to the biodegradation process was a resin

rich surface layer (Fig. 1a, d, g and j), due to organic polymer migration to the surface of the material.[20] Furthermore, this superficial layer remains only partly polymerized due to the oxygen inhibition of polymerization, producing inferior surface properties of glass ionomer[21] and composite resins.[22]

In this study, resin-modified glass ionomer and composite resins behaved differently with regard to their surface roughness and morphology, as result of the 7-day biodegradation period. The ethoxylated version of the Bis-GMA (Bis-EMA) existing in composition of Filtek Z350 and TPH Spectrum matrixes probably contributed to their hydrolytic and biochemical stability, due to the hydrophobicity of this monomer.[12] Whereas, the nano-filled resin-modified glass ionomer, Ketac N100, significantly presented the highest roughness value in comparison with the other materials subjected to biodegradation and it was the only material with higher roughness values when compared with the control group (humidity). A reasonable explanation for this severe biodegradation, also observed in the micrographs of Ketac N100 specimens (Fig. 1h), is the other resin monomers in addition to 2-hydroxyethyl methacrylate (HEMA) present in this material, 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 camphoroquinone tend to exist in the hydrophilic HEMA phase and hydrophobic BisGMA phase, respectively.[23,24] This decreases the chance of their coming into contact, therefore fewer radicals will be generated and a lower degree of conversion could be found in the Ketac N100 matrix than in Vitremer matrix. Moreover, Vitremer shows a third polymerization setting reaction that ensures that any HEMA not polymerized by irradiation, will set.[25] Clinically, the removal of the

outermost surface by finishing-polishing procedures would tend to make the nano-filled RMGICs more resistant to biodegradation, and therefore, more esthetically stable restorative materials.[21,22,26]

As regards relative humidity storage, Vitremer presented the highest surface roughness value in comparison with the other materials. Composites and Ketac N100 exhibit an external hydrophobic film,[20] arranged by different monomers, with different molecular structure and chemical characteristics (Bis-GMA, TEGDMA, Bis-EMA, UDMA, among others). The hydroxyl groups of HEMA give a hydrophilic property to the polymer matrix of Vitremer.[27] At 100% relative humidity, the water vapor can adsorb to Vitremer surface through hydrogen bridges with the hydroxyl (OH) of HEMA[28] and promote a roughening of its superficial layer. Thus, the protection of Vitremer surface with varnishes, adhesives systems or petroleum jelly is fundamental in order to avoid premature contact with water and the filling of small surface voids and defects, reducing the uptake of stains, the loss of calcium and aluminum ions, surface erosion and loss of translucency.[29] Moreover, Ketac N100 is a paste/paste ionomer while Vitremer requires the conventional powder/liquid mixing, which promotes the incorporation of air bubbles (Fig. 1j).

The wear resistance can be ascribed to several factors, such as the size, hardness and percentage of surface area occupied by filler particles and the filler/matrix interaction,[13] as well as the degree of conversion of the polymer resin matrix.[29] The selective abrasion of the resin matrix and exposure of filler particles were observed for all materials studied (Fig. 1c, f, i and l). It is known that there is a difference between filler and matrix hardness in resin-based materials,[30] mainly when this surface is a resin-rich layer, partially polymerized (oxygen inhibition) and softened by the biodegradation process. As regards three-body wear (tooth-brushing), it is

necessary to establish two evaluations: the effect of wear alone (biodegradation x biomechanical degradation groups) and the cumulative effect of biofilm plus abrasion (control x biomechanical degradation groups).

When previously biodegraded, only Ketac N100 specimens presented no increase in roughness values after tooth-brushing abrasion. Whereas, only Filtek Z350 specimens retained similar roughness values before and after biomechanical degradation. In the micrographs the removal by abrasion of the superficial exposed layer of all materials was observed, as soon as it reached a critical degree of softening as a result of the biodegradation process. However, the surface roughness value of Ketac N100 was already high after this process, due to its matrix composition, as related above. Therefore, there was probably no statistical difference between the abrasion values obtained from the biodegraded specimens of Ketac N100. Further clinical studies are necessary to confirm the effectiveness of this recent nano-ionomer as a restorative material able to withstand all adverse conditions of the oral environment, as well to inhibit the growth of bacteria and caries progression by means of fluoride release.

The biomechanical degradation resistance of nanocomposite Filtek Z350 is basically related to its chemical composition. As regards filler particles, this material is formulated using a combination of nanomer sized particles with the nanocluster formulations.[5] The higher filler loading with smaller particle size provides a reduction in the interstitial spacing, which effectively protects the softer matrix, reduces the incidence of filler exfoliation and enhances the overall resistance of the material to abrasion.[31] When the nanocomposite undergoes toothbrush abrasion, only nanosized particles are plucked away, leaving the surfaces with defects smaller than the wavelength of light.[5] Whereas, the larger and irregular filler

particles of Vitremer (Fig.1l) made it easier to "pluck out" a whole filler particle from the resin matrix, which could act as an additional abrasive agent once it was detached from the surface and held against the specimen.[32] Thus, Vitremer was the roughest material after biomechanical degradation.

As regards the resin system of Filtek Z350, the greater part of TEGDMA was replaced with a blend of UDMA (urethane dimethacrylate) and Bis-EMA (ethoxylated bisphenol-A dimethacrylate).[33] The low strength of TEGDMA-rich resin mixtures could be caused by low crosslink density and cyclization, since TEGDMA is a small and flexible molecule.[34] In addition, the absence of a phenol ring in the monomer chain of UDMA leads to higher flexibility and toughness in comparison with BisGMA, making the UDMA resins more reactive, with higher conversion and crosslink density than the Bis-GMA polymers. Thus, Filtek Z350 showed the best biomechanical degradation resistance, since TPH Spectrum does not contain the nanotechnology and UDMA in its matrix composition. Vitremer presented the highest roughness values after the cumulative changes, probably due to larger and irregular filler visualized in the micrographs of its specimens (Fig. 1l).

5. Conclusion

The nano-filled composite Filtek Z350 exhibited the best resistance to cumulative challenges (biofilm plus tooth-brushing abrasion), since both tests promoted the exposure of its regular and small particles. Although Ketac N100 contains the nanotechnology, its outermost matrix was fragile under biodegradation process, suggesting the requirement of finishing-polishing procedures after restoration treatment. Differently, Vitremer presented a satisfactory resistance to biodegradation alone, but the

exposure of its particles after abrasion promoted the highest roughness values in this study. It would be interesting to select carefully the restorative material for intra-oral sites where there are frequent accumulation of dental biofilm and brushing abrasion, giving special attention to shape and size of fillers, since these will certainly be exposed and will determine the surface characteristics of tooth-colored restoratives.

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Table 1 – Materials used in this study

Materials	Composition*	Mean Filler Size**	Manufacturer (batch)
Filtek™ Z350	Zirconia / silica cluster filler; nonagglomerated silica filler; Bis-GMA; Bis-EMA; UDMA; TEGDMA	0.6 - 1.4 µm (cluster) 5 - 20nm (nanofiller)	3M/ESPE. St. Paul, MN, USA (8NU)
TPH Spectrum	Ba-Al-borosilicate glass; coloidal silica; Bis-GMA; Bis-EMA; TEGDMA	0.8 µm	Dentsply Ind. E Com.Ltda., Petropolis, RJ, Brazil (L797977)
Ketac™ N100	**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	1 µm (cluster) 5 - 25nm (nanofiller) < 3.0 µm (glass)	3M/ESPE. St. Paul, MN, USA (M3M3)
Vitremer™	**Powder:** fluoroaluminosilicate glass, redox system; **Liquid:** aqueous solution of a modified polyalkenoic acid, HEMA	3.0 µm	3M/ESPE. St. Paul, MN, USA - (P: 6LP / L: 6FH)

* Abbreviation of monomers in alphabetical order: Bis-EMA, ethoxylated bisphenol-A dimethacrylate; Bis-GMA, bisphenol glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.
** Manufacturers' information.

Table 2 – Surface roughness values (μm) (mean and standard deviation in parentheses) of restorative materials submitted to relative humidity (control), biodegradation or biomechanical degradation (biodegradation + abrasion).

GROUPS	**MATERIALS**			
	Filtek Z350	TPH Spectrum	Ketac N100	Vitremer
Control	0.10 (0.02) **Ba**	0.08 (0.02) ***Ba**	0.19 (0.08) ***ABb**	0.24 (0.20) ***Aa**
Biodegradation	0.08 (0.02) #**Ca**	0.08 (0.05) #**Ca**	0.57 (0.12) **Aa**	0.36 (0.08) #**Ba**
Biomechanical degradation	0.24 (0.05) **B**	0.29 (0.03) **B**	0.46 (0.09) **B**	1.01 (0.46) **A**

Capital letters indicate comparison among materials (horizontal) within each group. Lower case letters demonstrate comparison between storage groups (control x biodegradation). Symbols represent the differences between biomechanical degradation and control groups (asterisks) / biodegradation group (hash). Groups denoted by the same letter/symbol represent no significant difference (p>0.05).

Figure 1

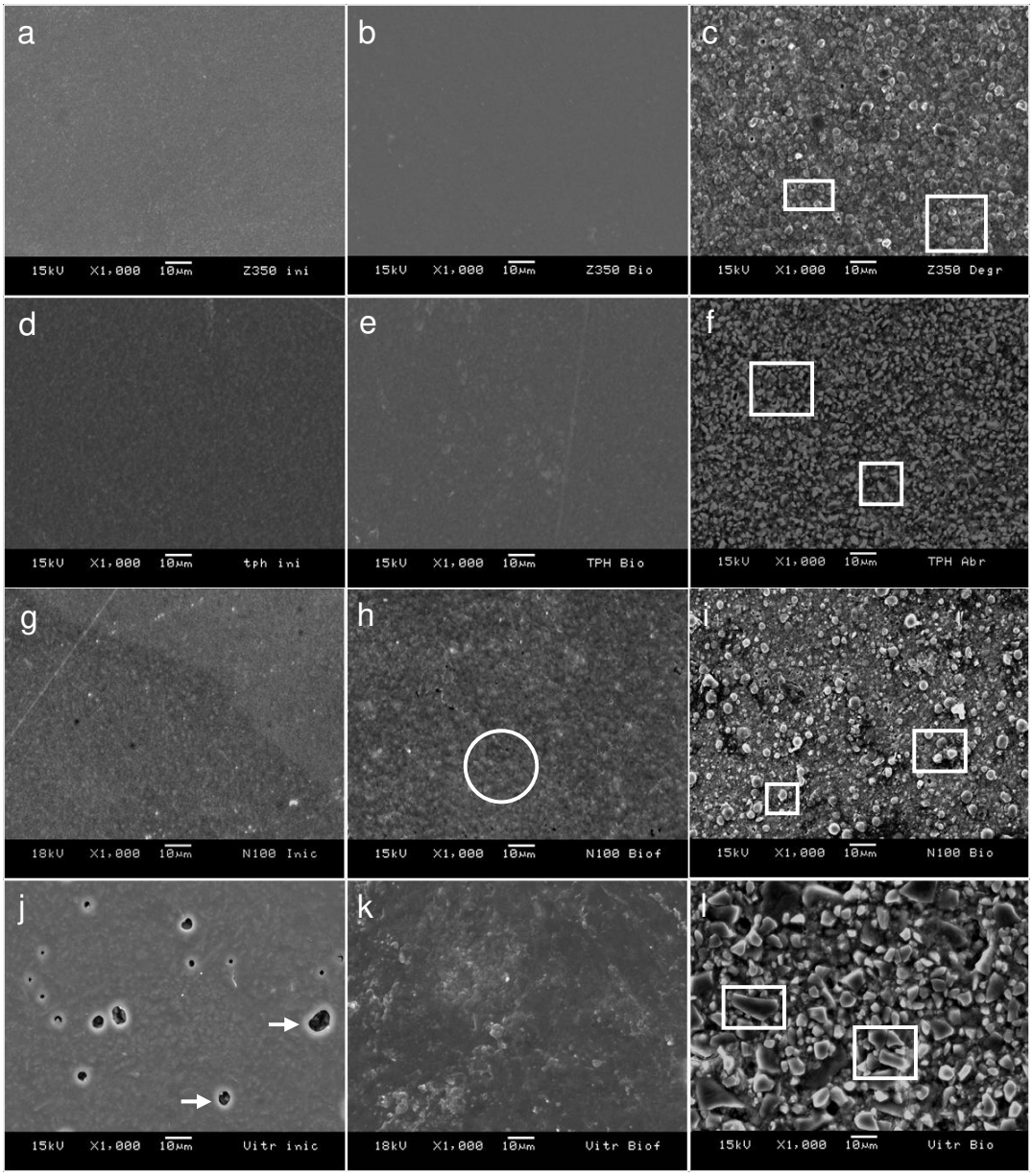
a
15kV X1,000 10µm Z350 ini
b
15kV X1,000 10µm Z350 Bio
c
15kV X1,000 10µm Z350 Degr
d
15kV X1,000 10µm tph ini
e
15kV X1,000 10µm TPH Bio
f
15kV X1,000 10µm TPH Abr
g
18kV X1,000 10µm N100 Inic
h
15kV X1,000 10µm N100 Biof
i
15kV X1,000 10µm N100 Bio
j
15kV X1,000 10µm Vitr inic
k
18kV X1,000 10µm Vitr Biof
l
15kV X1,000 10µm Vitr Bio

Figure 1 – Scanning electron micrographs of Filtek Z350 (a, b, c), TPH Spectrum (d, e, f), Ketac N100 (g, h, i) and Vitremer (j, k, l), at an original magnification of x1000. The first column shows the relative humidity storage groups (a, d, g and j), with 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 severe 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 surface of materials (squares).

Capítulo 2

Influence of erosive challenge on surface properties of nano restorative materials

Abstract

The aim of this *in vitro* study was to investigate the effect of chemical degradation (erosion) on surface roughness (Ra) and hardness (KHN) of nano restorative materials. Disc-shaped specimens (5mm-diameter; 2mm-thick) of Filtek Z350™ and TPH Spectrum™ composites and the Vitremer™ and Ketac Nano™ light-curing restoratives glass ionomer cements were obtained according to the manufacturers' instructions. After 24h, polishing procedures were performed and initial measurements of Ra and KHN were taken. Specimens were divided into 12 groups (n=10) according to material and storage media: artificial saliva, orange juice and Coca-Cola®. After 30 days of storage, the specimens were reevaluated about Ra and KHN. The pH values of storage media were measured weekly. Data were tested for significant differences by repeated measures three-way ANOVA and Tukey's tests ($p<0.05$). It was observed that composites presented lower roughness values and higher hardness values than ionomeric materials under all storage conditions. After erosion, KHN of all experimental samples decreased significantly, while the Ra of ionomeric materials increased, depending on the media, with a markedly negative impact of Coca-Cola® and orange juice. There was not difference among storage media for Filtek Z350 regarding to KHN values. Nanofillers did not show influence on roughness and hardness of RMGIC and resin composites studied concerning erosive resistance.

Keywords: composite resin, resin modified glass-ionomer cements, storage media, surface hardness, surface roughness.

Introduction

The ability of restorative dental materials to withstand the functional force and exposure to various substances in the mouth is an important requirement for their clinical performance for a considerable period of time. The chemical factors known to cause deleterious effects include low pH due to cariogenic biofilm,[1] consumption of acidic drinks or foodstuffs, [2,3] and action of enzymes,[4] which can soften the outermost layers and roughen restorative materials. Glass-ionomer cement degradation is a complex process of extracting metal cations from the cement matrix and incorporated glass particles,[5] certainly resulted from fluid uptake by the matrix, and its solubility. Still, the highly hydrophilic HEMA present in RMGI becomes this material also susceptible to the disintegration of its matrix, in a variable performance heavily dependent on the resin matrix composition and polymerization reactions.[6]

The application of nanotechnology to composite resins was firstly introduced by Filtek Supreme (3M-ESPE), which contains a unique combination of nanofillers (5-75nm) and nanoclusters embedded in an organic polymer matrix. Additionally to improved optical properties, nano materials present better mechanical behavior,[7] since the particles size was able to increasing the nanofiller load in restorative material.[8] Some studies have shown that nanocomposites presented higher surface hardness values and lower brushing abrasive wear than microfilled and hybrid composites.[9,10] Recently, a new RMGI has been introduced for operative dentistry. Ketac™ Nano light curing nano-ionomer (3M ESPE) includes fluoroaluminosilicate glass, nanofillers, and nanofiller "clusters" combined with HEMA, bisphenol glycidyl methacrylate (Bis-GMA) and triethylene glycol dimethacrylate (TEGDMA) as resin monomers, (3M ESPE Internal Data).[11] So, it would be important to compare this material with a

traditional RMGI (Vitremer) and a nano composite (Filtek Z350), in an attempt to establish if the nano-ionomer shows a similar behavior to or intermediary between ionomeric and composite classes, predicting its mechanical and chemical properties.

Therefore, the aim of this study was to evaluate *in vitro* the effects of different beverages on the surface roughness and hardness of nano restorative materials, a nano-ionomer and a nanocomposite, when compared with other resin-modified glass ionomer cement and composite.

Material and Methods

Specimen Preparation and Initial Analysis

Four different types of tooth-colored restoratives materials were used in this study (Table 1). They are namely: two resin-modified glass ionomers (Vitremer and Ketac Nano - 3M ESPE) and two composites (Filtek Z350 – 3M ESPE and TPH Spectrum - Dentsply Ind e Com Ltda). Thirty specimens of each material were handled according to manufacturers' instructions and inserted into plastic molds with internal dimensions of 5mm diameter by 2mm thickness. The surface of each specimen was covered by a polyester strip and pressed flat by a glass slab. The top surface of all materials was cured using an Elipar Trilight curing light unit (3M ESPE, St. Paul, MN, USA) with mean intensity about 800mW/cm^2, according to manufacturers' cure times, after the intensity of the light-curing unit was checked with a curing light meter (Hilux Dental Curing Light Meter, Benliglu Dental Inc., Turkey). The surface of Vitremer was protected with Finishing Gloss (3M ESPE). All specimens were maintained at 100% relative humidity and 37°C for 24 hours. Next, the surfaces were wetground with water-proofed silicon carbide discs of decreasing abrasiveness (600, 1200 and 2000) and ultrasonically cleaned (Ultrasonic Cleaner, model USC1400, Unique Co, São

Paulo, SP, BR) in distilled water for 10 minutes to remove polishing debris. Then, specimens were randomly distributed into 3 groups (n=10), according to the test storage media: artificial saliva (control), orange juice and Coca-Cola® (Table 2).

Before erosion, specimens were analyzed about surface roughness and Knoop hardness. Regarding surface roughness-measuring, specimens were analyzed using a Surfcorder SE1700 instrument (Kosaka Corp, Tokyo, Japan). Three successive readings from the center of each disk in different directions were taken; the mean of surface roughness values (Ra, mm) was obtained and considered to be baseline measurements. Next, hardness tests were carried out with a hardness tester (Shimatzu, Tokyo, Japan) using a Knoop indenter and a load of 50g, with a dwell time of 15 s. Three readings were taken for each specimen, and the mean KHN was calculated

Erosion - Storage in acidic drinks

Finally, all specimens were immersed individually in 4mL of storage solutions: Coca-Cola® (pH 2.49), orange juice (pH 3.23) and artificial saliva (pH 7), for 30 days.[12] The solutions were weekly exchanged and its pH was weekly determined using a portable pH meter (Orion Model 420A, Analyzer, São Paulo SP 03638-030, Brazil). In all cases, the pH electrodes were calibrated immediately prior to use with the aid of standard buffer solutions at pH 4.0 and 7.0.

At the end of the storage period, the specimens were ultrasonically washed for 10min, dried and revaluated about roughness and hardness. Representative specimen of each group was also observed by Scanning Electron Microscopy (model Jeol JSM 5600 LV, Tokyo, Japan) to illustrate the effect of erosive challenge on materials. Additional specimens from

each material were taken as baseline to compare baseline surfaces to chemical degraded surfaces.

Statistical analysis

Data were evaluated using the PROC LAB from statistical software SAS in order to check the equality of variances and normal distribution. Hardness and roughness data were submitted to repeated measures three-way ANOVA and Tukey test with a significance limit of 5%. Hardness data were transformed using root square to attend ANOVA rules.

Results

There was significant interaction between the factors "materials" and "erosion effect" (before x after) ($p=0.0439$) and between "storage solution" and "erosion effect" ($p=0.0074$). A significant interaction was not observed between "materials" and "storage solution" ($p=0.4733$) and among the three factors ($p=0.0699$). Means and standard deviations of surface roughness of each material under different storage conditions are displayed in Table 3. There was no significant difference among storage solutions (saliva/orange juice/Coca; $p=0.2010$) and between erosion effect (before/after; $p=0.2251$); however, there was statistical difference among materials studied ($p<0.0001$).

Regardless of the storage solution, both composites (Filtek Z350 and TPH Spectrum) presented similar roughness values ($p>0.05$) and significantly lower roughness values than ionomers, before and after erosive challenge. There was not statistical difference on roughness values between Ketac Nano and Vitremer, in all storage conditions. In addition, when different

storage solutions were compared concerning each material after erosive challenge, it was observed that there was not statistically significant difference among them. Concerning erosion effects on each material' surface, the exposure to acidic drinks (orange juice and Coca-Cola®) resulted in chemical degradation, i.e., significantly higher roughness values for both ionomeric materials tested. Artificial saliva did not produce any difference on roughness results of all materials. Composites surfaces were not rough by any storage solution, inside the conditions studied.

Table 4 shows means and standard deviations of Knoop Hardness of each material under storage conditions proposed. There was significant interaction between the factors "materials" and "storage solution" ($p=0.0009$), "materials" and "erosion effect" ($p<0.0001$), between "storage solution" and "erosion effect" ($p<0.0001$), as well among the three factors ($p=0.0022$). Besides, there was significant difference among materials studied ($p<0.0001$), among storage solutions (saliva/juice/Coca-Cola®; $p<0.0001$) and between erosion effects (before/after; $p<0.0001$).

Before erosive challenge, it was observed that both composites (Filtek Z350 and TPH Spectrum) presented similar hardness values and significantly higher values than ionomers studied, which also presented similar values between them. Regarding to erosion effects on each material' surface, the exposure to any storage solutions produced statistical lower hardness values for all materials tested. It was also observed that there was influence of the storage solution for each material: The acidic drinks (Coca-Cola® and orange juice) were more aggressive than artificial saliva for Vitremer and Keta Nano. Orange juice was also very detrimental to TPH Spectrum, while it was not observed difference among solutions for Filtek Z350. In addition, composites presented significant higher hardness values than ionomeric materials after chemical degradation by artificial

saliva and Coca-Cola®. However, after juice storage, Filtek Z350 showed the highest hardness values, followed by TPH Spectrum and, finally, by both ionomeric materials.

Discussion

Despite the notable improvement in the composition and characteristics of modern esthetic restorative materials, all of them will be subjected to a great number of unfavorable conditions that challenge their integrity and durability over time. Consumption of certain beverages, such as coffee, tea, soft drinks, fruit juices and alcoholic beverages may affect the aesthetic and physical properties of resin-based materials.[13] In addition, the severity of the effects depends on the intrinsic and extrinsic features of the restorative materials, as chemical composition[2] and finishing/polishing procedures[14] respectively, the amount and frequency of intake of drinks and its pH and the buffering capacity.[15]

Under acidic conditions all dental resin-based restorative materials have shown degradation over time, such as increased surface roughness, decreased of hardness and color change.[16] Acid beverages may contain several different types of acid that contribute to the low pH value.[15] Clinical studies have found carbonated drinks, especially carbonated cola drinks, to be associated to erosion. [17,18] In addition, in vitro studies have show that fruit juices may also be potentially erosive, due their high content of titratable acid. [15,19]

This study evaluated the effects of acidic beverages on the surface roughness and hardness of nano restorative materials and conventional. It was selected three storage media: orange juice and Coca-Cola®, due to their potential to cause erosion and artificial saliva as positive control. Coca-Cola® has in its composition phosphoric acid and has low

titratablility. Orange juice contain citric acid high titratable ability and buffer capacity.[20] Overall both storage solutions caused a significant increase on surface roughness values for RMGIC and a significant decrease on hardness for all materials, confirming their potential to degrade resin based restorative materials.

Before erosion challenge it was observed higher roughness values for RMGIC than composite resins. The differences observed at the baseline conditions among materials regarding their means of surface roughness on baseline are mainly related to differences in their filler particles size, shape, volume, and distribution and its interaction with organic matrix, what allows better polishing characteristics for composite. [21] Besides, those results have occurred due to the handling of RMGIC, since they are in the powder:liquid or paste:paste formulation and air can be entrapped on the material structure, resulting in surface bubbles and exposure of porosities after finishing/polishing procedures.

It was observed that before erosion similar roughness values between the nanofilled and conventional materials, both for composite as for the ionomer groups. In a different way, Mitra et al (2003)[7] and Cavalcante et al (2009)[22] have showed that nanofilled composite present lower roughness values and better polishing characteristics than hybrid composites due to the presence of nanofiller. Probably, in this study resinous matrix was not totally removed by initial finishing/polishing procedures leaving a matrix layer over the fillers.

Composite resins and RMGICs performed differently with regard to their surface roughness, as result of the 30-day chemical degradation period in this study. The chemical challenge caused no effect on roughness values for both composites. The ethoxylated version of the Bis-GMA (Bis-EMA) existing in composition of Filtek Z350 and TPH Spectrum matrixes

probably contributed to their hydrolytic and biochemical stability, due to the hydrophobicity of this monomer. Yap et al. (2000)[23] also showed that surface roughness of a Bis-EMA-based composite were not affected by acidic beverages. Bis-EMA shows decreased flexibility and increased hydrophobicity due to the elimination of the hydroxyl groups, when compared to composites formulated with BisGMA.[24] Hence, the reduction in water uptake may be partially responsible for the chemical stability of composites that have Bis-EMA on the formulation.

Regarding RMGIC, it has been shown that acid environment has a severe effect on surface degradation.[20] The erosive challenge with orange juice or Coca-Cola caused a significant increase on roughness surface for Ketac N100 and Vitremer (Figure 1, Table 3). Actually, the presence of hydroxyethyl methacrylate (HEMA), a highly hydrophilic monomer in the organic matrix from RMGICs can increase its solubility.[25] Rogalewicz et al. (2006)[26] observed HEMA, TEGDMA, and additive decomposition products eluted from RMGIC after 7 days immersion in acidic media. It is possible that loss of components from two Vitremer and Ketac N100 matrixes (polyacrylate–inorganic and polymer–organic) leads to changes in surface roughness and hardness. In this way, it could be speculate that acidic environment corroded the RMGICs matrix, promoting the increasing the roughness (Figure 1, Table 3). According with these results, it is evident that the composition of matrix influenced the surface roughness of materials before and after the erosive challenge and that the incorporation of nanoparticles in the composite and glass ionomer cement did not interfere in their erosive resistance.

Hardness is a property that is used to predict the wear resistance of a material and was the parameter most affected by the erosive challenge. According to the results of this study, when compared the Knoop hardness

of the materials before erosion, we can see that both composites (Filtek Z350 and TPH Spectrum) presented higher values hardness than RMGICs. The different content of organic matrix and higher filler loading, as well as the highest degree of conversion resin composites could explain the behavior of these materials. In addition, it can be observed that the initial characteristics of hardness are not affected by the presence of nanofillers in the different materials studied.

After erosion, all materials showed significant reduction of hardness, and RMGICs showed a greater loss of hardness than resin composite. The decreased hardness observed for all solutions storage seems to have been originated from hydrolysis, since the more hydrophilic the organic matrix, the higher hydrolysis.[27] According Sakar,[28] corrosive wear begins with water absorption that diffuses internally through the resin matrix, filler interfaces, pores, and others defects, accelerated by low pH of the solution. Thus, the chemical degradation rates of different materials depend firstly on their hydrolytic stability, which is related mainly to resin matrix in the study. Since the resin matrix of composites is known to absorb a small percentage of water,[29] composites were more degradation resistant than hydrophilic materials such as RMGICs.[30] In addition, the storage solutions promoted dissolution peripheral to the glass particles which could be the result of dissolution of the siliceous hydrogel layer of RMGICs.[16] On the other hand, the acid could also attack the resin (in a lesser extent) softening of methacrylate based polymers which could be caused by leaching of the comonomers such as triethylene glycol dimethacrylate (TEGDMA) decreasing the surface hardness of these materials.[20,31]

We can also observe that the composite Z350 was not influenced by storage medium. This result can be supported again by the hypothesis that the prime deleterious action resulted from the water and not from de

acidic environment.[32] Despite a minor difference in the percentage load of the composites tested, the higher filler loading with smaller particle size provides a reduction in the interstitial spacing (less matrix exposition) and enhances the overall resistance of the Filtek Z350 to chemical degradation,[19] comparing to TPH Spectrum. Moreover, the greater part of TEGDMA from that resin composite was replaced with a blend of UDMA (urethane dimethacrylate) and Bis-EMA (ethoxylated bisphenol-A dimethacrylate). Pearson and Longman (1989)[33] determined that UDMA has lower water sorption than BisGMA due to higher conversion and crosslink, evidencing the importance of the type of resin matrix in chemical degradation resistance.

Concerning the resin composite TPH, it was observed a significant loss of surface hardness after storage in orange juice. This could be related to the type of its inorganic fillers, as suggested by Soderholm et al (1984).[34] It was showed that materials containing barium glass fillers are more susceptible to acid attack. Moreover, the erosive effect of storage solutions does not depend only on its intrinsic pH value but also on its buffering effect. According to Owens (2007)[35] and Cheng (2009)[36] the orange juice has greater buffering capacity and erosive effect than the Coca-Cola, explaining the lower hardness values of TPH composite when stored in orange juice (Figure 2, Table 4).

Although all the materials have degraded with storage in all solutions, the Coca-Cola and orange juice produced greater reductions in the hardness values for materials TPH and RMGICs, with or without nanofillers inclusion.

Conclusion

It was concluded that different beverages (Coca-Cola® and orange juice) provided great changes on surface roughness for RMGIC regardless nanofillers added; Overall, Coca-Cola and orange juice provide decrease on hardness for all materials studied. The most intense decrease on hardness was observed for RMGICs immersed in both and TPH in orange juice. Nanofillers did not show influence on roughness and hardness of RMGIC and resin composites studied concerning erosive resistance.

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Table 1- Materials used in this study

Materials	Composition	Mean Filler Size (µm)	Manufacturer/Batch #
Ketac™ N100 (3M ESPE)	**Paste A:** silane treated glass, silane treated zirconia oxide silica, polyethylene glycol dimethacrylate(5-15%), silane treated silica, HEMA, Bis-GMA(<5%), TEGDMA (<5%), HEMA(1-10%) **Paste B:** silane treated ceramic, silane treated silica, copolymer of acrylic and itaconic acids, HEMA(1-10%)	5-25nm	3M/ESPE. St. Paul, MN, USA M3M3
Vitremer (3M ESPE)	**Powder:** fluoroaluminosilicate glass; redox system **Liquid:** aqueous solution of a modified polyalkenoic acid, HEMA(15-20%)	3.0µm	3M/ESPE. St. Paul, MN, USA P: 6LP L: 6FH
Filtek Z350 (3M ESPE)	58-60 vol% (78.5 wt%) combination of aggregated zircônia/sílica cluster filler with primary particles size of 5-20 nm, and non-agglomerated 20nm silica filler , Bis-EMA, Bis-GMA; UDMA; TEGDMA	5-20nm 0.6-1.4 µm (clusters)	3M/ESPE. St. Paul, MN, USA 8NU
TPH (Dentsply)	Polymer matrix: Bis-GMA, Bis-EMA and TEGDMA; Filler: 57 vol%of Ba-Al-borosilicate glass and coloidal sílica with mean particle size of 0.8 µm	4.4 µm	Dentsply Ind. E Com.Ltda., Petropolis, RJ, Brazil L797977

Bis-GMA=bisphenol glycidyl methacrylate; TEGDMA=triethylene glycol dimethacrylate; HEMA=2hydroxyethyl methacrylate; Bis-EMA= . bisfenol A glicidil dimetacrilato etoxilado;UDMA=dimetacrilaro de uretano.

Table 2 – Main ingredients in the storage solutions studied

Food/Drink	Main Ingredients	pH
Coca-Cola®	Carbonated water, sugar, caramel color, phosphoric acid, natural flavors, caffeine	2.49
Orange Juice (Minute Maid®)	Water, orange juice, sugar, citric acid, natural flavor and antioxidant ascorbic acid	3.23
Artificial Saliva	Calcium (0.1169 g of calcium hydroxide/liter of deionized water); 0.9 mM of phosphorus and potassium (0.1225 g potassium phosphate monobasic/liter of deionized water); 20 mM TRIS buffer (2.4280 g TRIS buffer/liter deionized water)	7

Table 3 – Surface roughness values (μm) (mean and standard deviation in parentheses) of restorative materials submitted to different storage solutions.

Erosion	Materials	Storage Solutions		
		Artificial saliva	Coca-Cola®	Orange juice
Before	Filtek Z350	0.14 (0.07) Ab	0.13 (0.03) Ab	0.13 (0.03) Ab
	TPH Spectrum	0.18 (0.04) Ab	0.18 (0.03) Ab	0.20 (0.04) Ab
	Ketac Nano	0.33 (0.12) Aa	0.37 (0.13) *Aa	0.31 (0.11) *Aa
	Vitremer	0.47 (0.19) Aa	0.39 (0.17)*Aa	0.34 (0.09) *Aa
After	Filtek Z350	0.11 (0.01) Ab	0.11 (0.01) Ab	0.11 (0.01) Ab
	TPH Spectrum	0.17 (0.02) Ab	0.17 (0.05) Ab	0.19 (0.05) Ab
	Ketac Nano	0.32 (0.14) Aa	0.48 (0.16) Aa	0.40 (0.11) Aa
	Vitremer	0.40 (0.09) Aa	0.48 (0.16) Aa	0.49 (0.15) Aa

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

Table 4 - Knoop Hardness Number (KHN) (mean and standard deviation in parentheses) of restorative materials submitted to different storage solutions.

Erosion	Materials	Storage Solutions		
		Artificial saliva	Coca-Cola®	Orange juice
Before	Filtek Z350	78.11 (8.55)*Aa	84.17 (10.79)*Aa	82.06 (12.31)*Aa
	TPH Spectrum	81.84 (11.15)*Aa	79.93 (9.11)*Aa	79.43 (10.97)*Aa
	Ketac Nano	41.16 (5.29)*Ab	39.65 (5.79)*Ab	39.64 (6.83)*Ab
	Vitremer	39.12 (4.53)*Ab	40.31 (7.83)* Ab	39.41 (8.43)*Ab
After	Filtek Z350	65.33 (5.80) Aa	57.97 (6.60) Aa	65.13 (7.46) Aa
	TPH Spectrum	64.30 (5.22) Aa	52.35 (5.76) ABa	42.85 (4.96) Bb
	Ketac Nano	27.38 (4.18) Ab	18.92 (2.18) Bb	15.53 (2.69) Bc
	Vitremer	28.91 (2.76) Ab	16.29 (4.19) Bb	19.45 (4.27) Bc

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

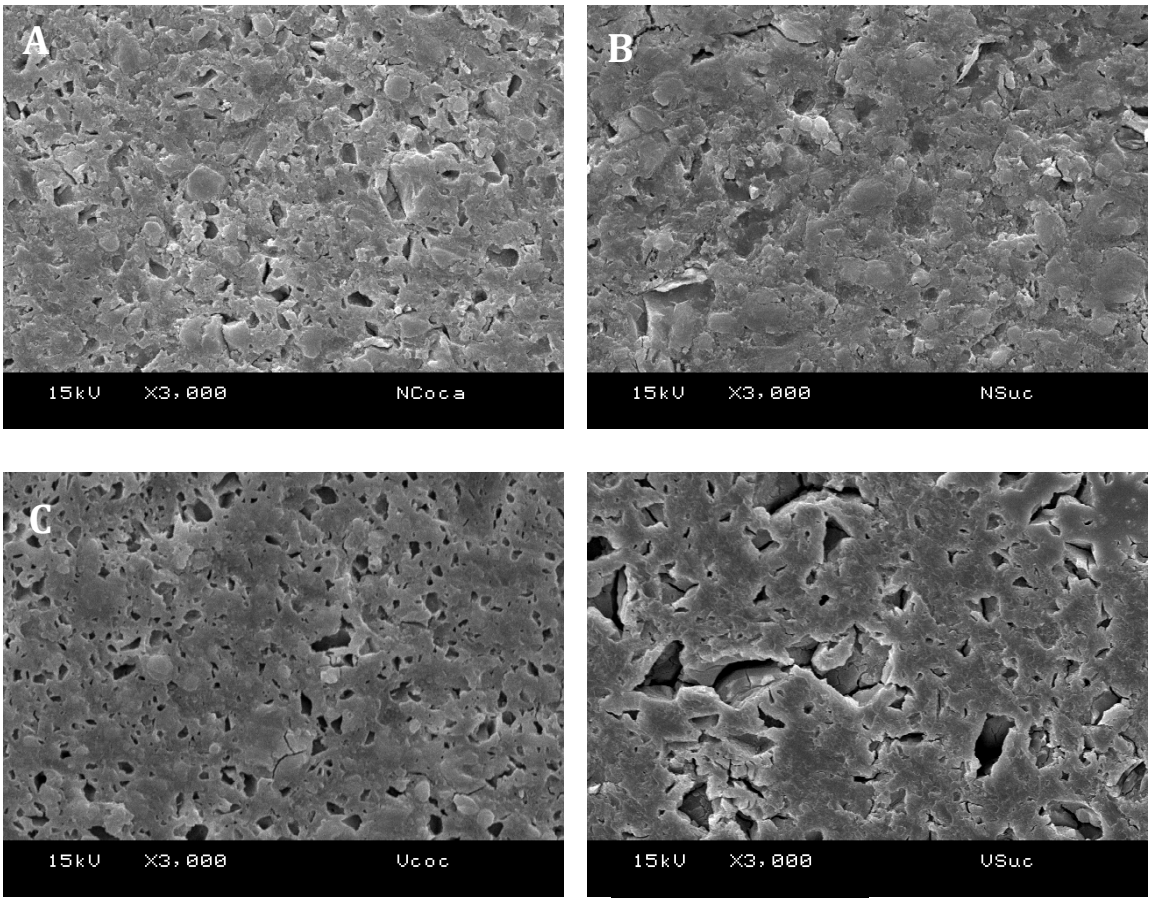

Figure 1 – Representative SEM micrographs of glass ionomers cement after erosion. Ketac N100/Coca-Cola® (A), ketac N100/orange juice (B), Vitremer/Coca-cola (C) and Vitremer/orange juice (D). Note corroded resin matrix provided by chemical erosion; extrusion fillers. Original magnification x3000.

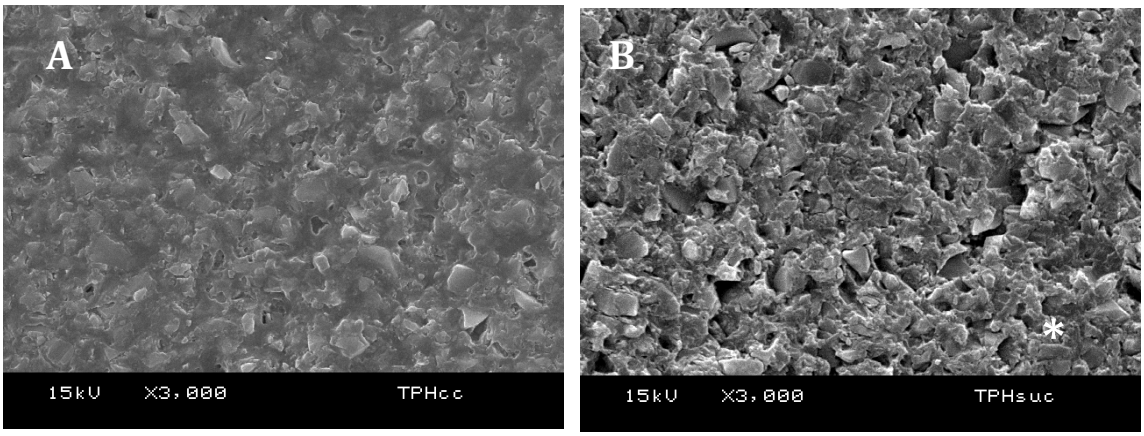

Figure 2 – Representative SEM micrographs of TPH Spectrum after storage in Coca-Cola® (A) and Orange juice (B). Severe corroded aspect of the resin matrix pointed out by marking it with after storage in Orange juice (B) . Original magnification x3000.

CONCLUSÕES GERAIS

Baseado nos resultados obtidos pôde-se concluir que:

1. A biodegradação com biofilme de *Streptococcus mutans* em materiais nanoparticulados apresenta-se material-dependente, afetando negativamente o cimento de ionômero de vidro, porém não o compósito resinoso. O compósito Filtek Z350 apresentou-se mais resistente frente aos desafios cumulativos da biodegradação e abrasão por escovação.

2. A incorporação de nanopartículas exerceu influência negativa nas propriedades dos materiais restauradores quando submetidos à erosão pelas soluções simuladoras da dieta ácida (Coca-Cola e suco de laranja). Estas soluções produziram aumento da rugosidade de superfície dos CIVMRs e promoveram a diminuição da dureza Knoop de todos os materiais estudados. Diminuição da dureza foi observada para CIVMRs em ambas as soluções e para o TPH quando armazenado em suco de laranja.

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APÊNDICE

Legendas e Figuras

Figura 1 – Ilustrações dos materiais de consumo utilizados na tese e confecção das amostras do Capítulo 1.

A. Cimento de Ionômero de Vidro Modificado por Resina Vitremer® cor A3 (3M/ESPE, St Paul, MN EUA)

B. Cimento de ionômero de Vidro Modificado por Resina nanoparticulado Ketac N100® cor A3 (3M/ESPE, St Paul, MN EUA)

C. Compósito restaurador Filtek™ Z350 (3M/ESPE, St Paul, MN EUA)

D. Compósito restaurador TPH Spectrum (Dentsply Ind. E Com.Ltda., Petropolis, RJ)

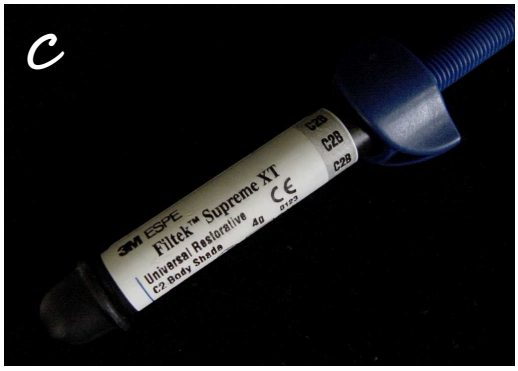
E. Matriz plástica (5mm de diâmetro X 2mm espessura)

F. Fotoativador Elipar Trilight® (ESPE, St. Paul, MN, EUA)

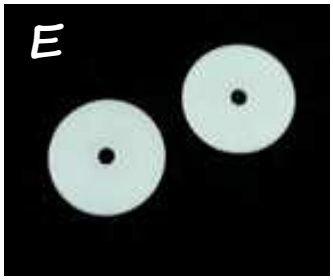
G. Discos de Compósito restaurador e de Cimento de Ionômero de Vidro Modificado por Resina


A
3M ESPE
Vitremer
Core Buildup/
Restorative
B2 Shade
Powder
9g
3M ESPE
Vitremer
Liquid
2.5ml


B
3M ESPE
Ketac™ N100
Light-Curing Nano-
Ionomer Restorative


C
3M ESPE
Filtek™ Supreme XT
Universal Restorative
C2 Body Shade
C2B
C2B


D
Dentsply
TPH
spectrum
A3


E


F
ESPE Elipar TriLight

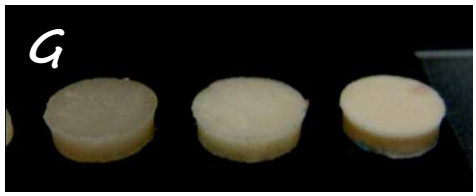

G

Figura 2 – Reativação do *S. mutans* e desenvolvimento do biofilme

Reativação do *S. mutans*

A. Seleção da Cepa de S. mutans UA159

B. Semeadura de inóculo em placa com MAS

C. Coleta de colônias crescidas após incubação a 37°C / 10% CO_2 / 48 horas em estufa de CO_2

D. Inoculação em BHI caldo e incubação a 37°C / 10% CO_2 / 18 horas em estufa de CO_2

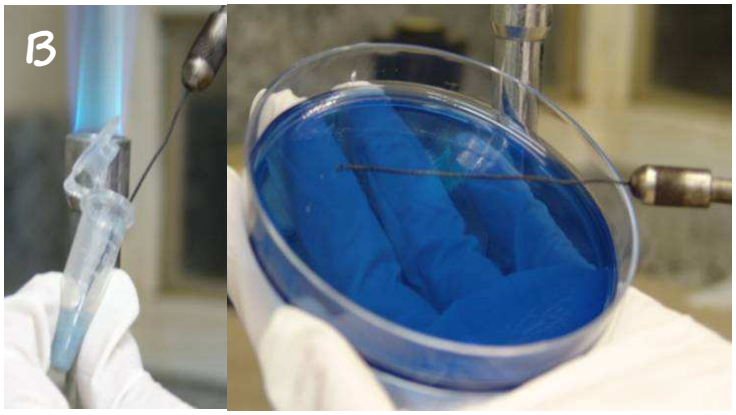
Desenvolvimento do biofilme

E. Inóculo de 20 µL sobre a superfície do material para adesão inicial de células

F. Placa de cultura de 24 poços contendo o disco com o biofilme aderido imerso em meio de BHI com 1% sacarose. Troca do meio a cada 48 horas.

G. Estufa de CO_2, na qual as amostras foram armazenadas


A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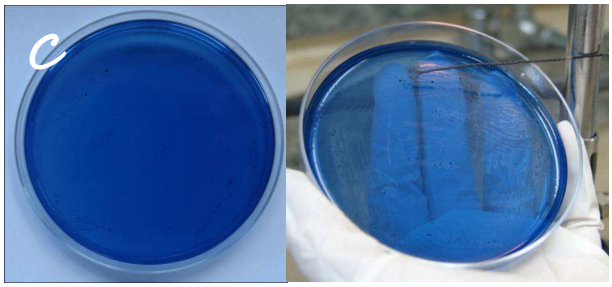
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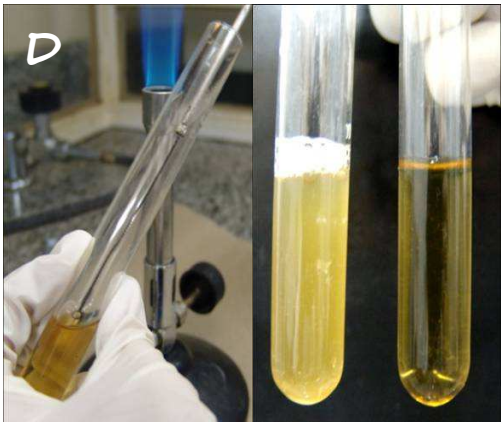
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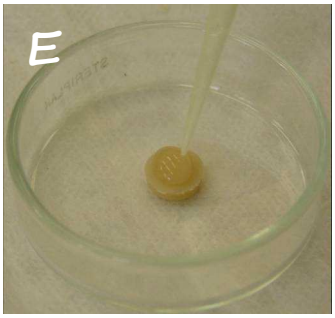

E


F


G

Figura 3 – Abrasão por escovação, Rugosidade de superfície e Microscopia Eletrônica de Varredura

A. Aparelho de ultrassom (Ultrasonic Cleaner, Model USC1400, UNIQUE Ind. e Com. Ltda., São Paulo, SP, Brazil) utilizado para remover o biofilme das superfícies dos discos

B. Dentifrício Colgate Total 12 ® (Colgate Palmolive Ind. e Com. Ltda, S. B. Campo, São Paulo, Brazil)

C. Escova de dente com cerdas macias (Colgate Palmolive Ind. e Com. Ltda, S. B. Campo, São Paulo, Brazil);

D. Máquina de escovação, com as amostras em posição sendo abrasionadas;

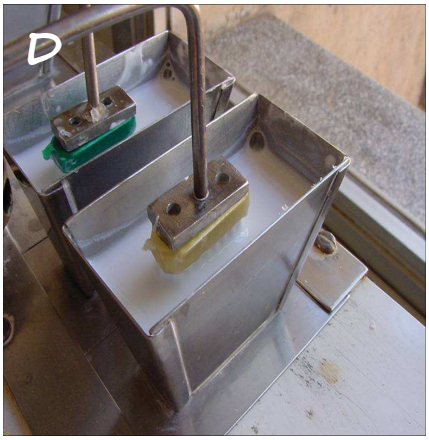
E. Rugosímetro Surfcorder SE1700 (Kosaka Corp, Tokyo, Japan);

F. Microscópio Eletrônico de Varredura (Jeol JSM 5600 LV, Tokyo, Japan)


A


B
Colgate


C
Colgate
TWISTER
Fresh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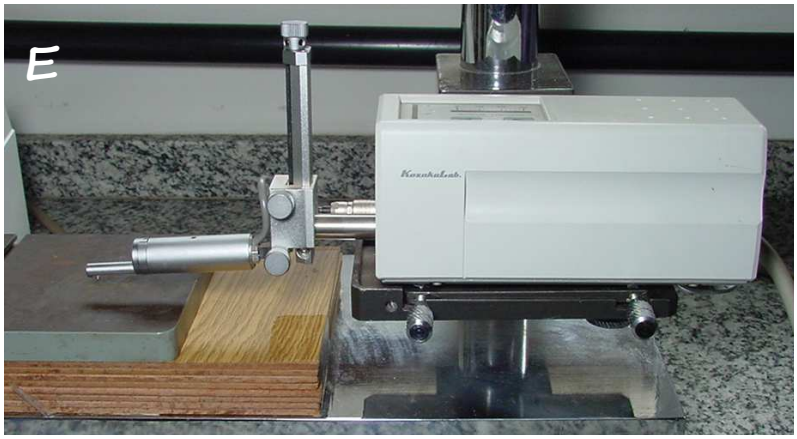

E


NORAN
F

Figura 4 – Confecção das amostras e Soluções de armazenamento do Capítulo 2

A. Matriz plástica (5mm de diâmetro X 2mm espessura)

B. Fotoativador Elipar Trilight® (ESPE, St. Paul, MN, EUA)

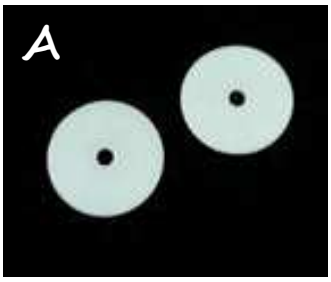
C. Discos de Compósito e de Cimento de Ionômero de Vidro Modificado por Resina

D. Lixas de Carbeto de Silício (Saint-Gobain, Recife, Pernambuco, Brasil), nas granulações 600, 1200 e 2000

E. Suco de Laranja Minute Maid ®

F. Refrigerante Coca-Cola ®

G. Saliva artificial


A


B
ESPE Elipar TriLight


C


D


E
Minute Maid
Maçã
Feito com carinho
Laranja Caseira
Com Gominhos da Laranja
1 litro
Néctar de Laranja


F
600 ml
Coca-Cola

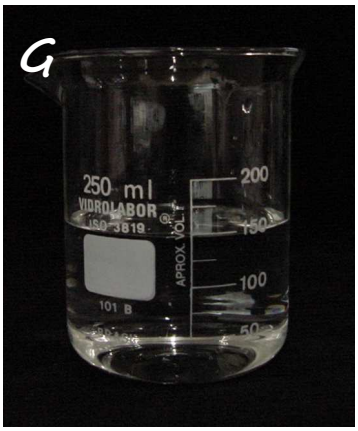

G
250 ml
VIDROLABOR
ISO 3819
APROX. VOL. F
200
150
100
50
101 B

Figura 5 – Armazenamento das amostras, análises de dureza Knoop e Rugosidade de superfície

A. Frascos de plásticos utilizados para armazenar as amostras;

B. Seringa de plástico utilizada para mensurar a quantidade de solução dispensada no frasco plástico (4 mL),

C. Frascos plásticos com as diferentes soluções de armazenamento;

D. Aparelho de ultrassom, utilizado na limpeza das amostras após o polimento;

E. Microdurômetro modelo HMV2 Shimadzu Microhardness Tester (Shimadzu, Tóquio, Japão);

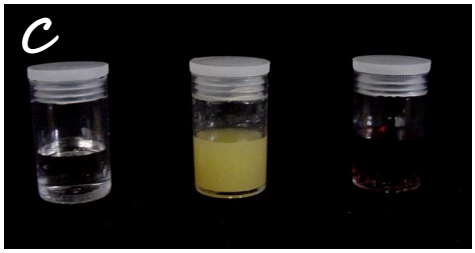
F. Amostras posicionadas no microdurômetro. Foi utilizada carga de 50gf durante 15s para a realização das endentações,

G. Amostras posicionadas no rugosímetro. Foram realizadas 3 leituras em direções diferentes;

H. Rugosímetro Surfcorder SE1700(Kosaka Corp, Tokyo, Japan);

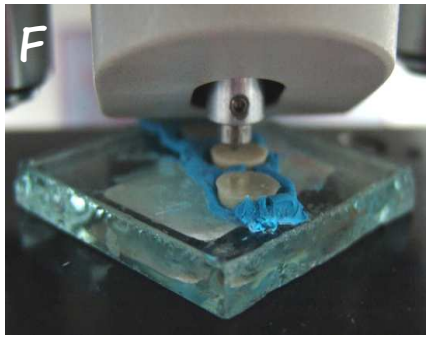

A


B


C


D
MaxiClean 750


E
HMV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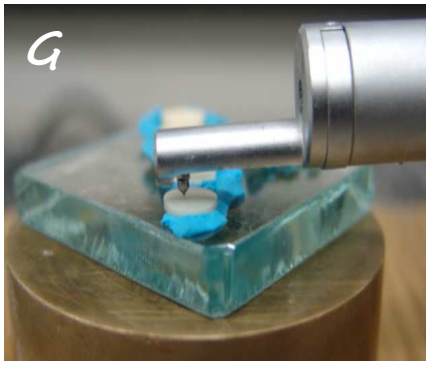
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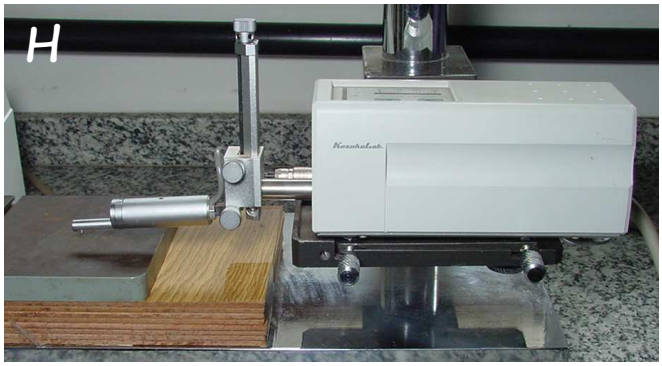
H

ANEXO 1

Fw: Operative Dentistry Manuscript #10-221-LR

From: editor@jopdent.org
Sent: Saturday, September 11, 2010 11:59 AM
To: rmpuppin@fop.unicamp.br
Subject: Operative Dentistry Manuscript #10-221-LR

September 11, 2010

Dear Regina Puppin-Rontani,

The referees' comments regarding your manuscript, "Biodegradation and abrasive wear of nano restorative materials", have been received. On the basis of these reviews I am pleased to inform you that your paper is accepted for publication in Operative Dentistry.

Please be sure to fill in the copyright agreement form by following the link below. We will begin our initial editing of the paper within one month of its receipt. The paper will be first published electronically on our online journal site at www.jopdentonline.org and then published in the Journal as determined by the Editor (usually within 6-8 months from acceptance). The DOI will remain the same for both print and electronic versions.

Thank you for considering Operative Dentistry for publication of this excellent manuscript.

Sincerely yours,
Jeffrey Platt
Editor
Operative Dentistry
http://jopdent.allentrack.net/cgi-bin/main.plex?el=A7DR3Wo1B1oq6G4E9fPjPNWPQxvDBtRoT046DgZ