Ana Carolina Rezende Afonso

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Orientador: Prof. Dr. Aline Arêdes Bicalho

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Prof. Dr. Aline Arêdes Bicalho
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Uberlândia-MG, 2020

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Dedico este trabalho,

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Aos meus pais, Nivaldo e Patrícia, e minha irmã, Dany,

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“Aprender é, de longe, a maior recompensa. ”

William Hazlitt

SUMÁRIO

RESUMO

RESUMO

O objetivo foi avaliar o efeito das propriedades mecânicas de diferentes materiais utilizados como selante na contração de tensões e integridade marginal e relacionar os resultados com dois tipos de fadiga mecânica utilizando Microscopia Eletrônica de Varredura (MEV) e Análise de Elementos Finitos (FEA). Os materiais usados neste estudo foram um cimento de ionômero de vidro modificado por resina (RMGIC) Vitremer 3M, um selante resinoso (RS) Prevent, FGM e uma resina de fluida (FR) (Filtek Bulk Fill Flow, 3M. As propriedades analisadas foram resistência à tração (DTS), resistência à compressão (CS), retração pós-gel (Shr), dureza Knoop (KHN) e módulo de elasticidade (E). As tensões de contração foram analisadas usando FEA. Quarenta e cinco molares foram selados e divididos em três grupos de cada material (N = 15), primeiro grupo sem fadiga mecânica (N = 5), segundo grupo com fadiga mecânica com a ponta metálica convencional (N = 5), terceiro grupo com fadiga mecânica simulando o bolo (N = 5). Referente aos resultados obtidos através de imagens SEM e valores de integridade marginal. DTS e CS RMGIC apresentou valores inferiores a RS e FR. Em Shr, RS e FR apresentaram valores maiores quando comparados com RMGIC. RS mostrou maior KHN e E comparado aos outros materiais. Na FEA, observou-se que o RS apresentou altas tensões de retração na região onde o material foi aplicado e principalmente na interface marginal. Quando RMGIC foi submetido à ciclagem mecânica, apresentou a maior falha na interface esmalte e material. Os resultados obtidos foram correlacionados com as imagens SEM e os valores de integridade marginal. Dentre todos os materiais considerando apenas as características biomecânicas, o FR apresentou o melhor desempenho.

PALAVRAS-CHAVE: materiais dentários; selante de fossas e fissuras; fadiga mecânica; tensão de contração

ABSTRACT

ABSTRACT

The purpose of this study was to evaluate the effect of mechanical properties of different materials used as sealant on stress shrinkage and integrity marginal and relation the results with two types of mechanical fatigue using Scanning Electron Microscopy (SEM) and Finite Element Analysis (FEA). The materials used in this study were a Resin-modified glass ionomer cement (RMGIC) Vitremer 3M, a Resin Sealant (RS) Prevent, FGM, and a Flow Resin (FR) (Filtek Bulk Fill Flow, 3M. The analyzed properties were Diametrical Tensile Strength (DTS), Compressive Strength (CS), Post-Gel Shrinkage (Shr), Knoop Hardness (KHN) and Elastic modulus (E). Shrinkage stresses were analyzed using FEA. Forty-five molars were sealed and divided into three groups of each material (N = 15), first group without mechanical fatigue (N=5), second group with mechanical fatigue with the conventional metallic tip (N=5), third group with mechanical fatigue simulating the bolus (N=5). Relating to the results obtained through SEM images and marginal integrity values. In DTS and CS RMGIC it presented values inferior to RS and FR. In Shr, RS and FR showed higher values when compared with RMGIC. RS showed greater KHN and E than other materials. At FEA, it was observed that RS had high retraction stresses in the region where the material was applied and mainly at the marginal interface. When RMGIC was subjected to mechanical cycling, it had the biggest failure in the enamel and material interface. The results obtained were correlated with the SEM images and integrity marginal values. All three materials are suitable considering all of their characteristics, but it is important to note that direct fatigue with the metal tip overestimates the failure values.

KEYWORDS: Pit and Fissure Sealant; Mechanical fatigue; Shrinkage stress

INTRODUÇÃO E REFERENCIAL TEÓRICO

1. INTRODUÇÃO E REFERENCIAL TEÓRICO

A cárie dental é uma doença de etiologia multifatorial que atinge as estruturas dentais, por meio da instalação de microrganismos bacterianos, sendo um processo dinâmico (Machiulskiene *et al*., 2020). A superfície oclusal de molares e pré-molares corresponde a uma região altamente susceptível para o início dessa lesão, com a presença de fossas e fissuras, anatomia complexa, que favorecem a retenção e a maturação da placa bacteriana, que dificulta à higienização nessa área (Mickenautsch *et al*., 2016). Tendo em vista que a prevenção das doenças orais é preferível em relação ao tratamento, uma vez que resulta menos dor e trauma ao paciente, a vedação de fossas e fissuras por meio dos materiais selantes atua no combate à cárie, doença que mais acomete a cavidade oral, principalmente as crianças (Bhushan & Goswami, 2017).

Com o intuito de prevenir e controlar a instalação de lesões cariosas no elemento dental, realiza-se a obliteração mecânica dos defeitos estruturais do esmalte dental, através da aplicação de selante (Liu *et al*., 2019). A indicação desse procedimento visa proteger regiões de fissura e fossas, sendo uma alternativa de tratamento excelente, segura, duradoura e comprovada (Kucukyilmaz & Savas, 2016). Os materiais mais utilizados como selantes dentais são resinas compostas e ionômeros de vidro (Ahovuo-Saloranta *et al*., 2013).

A falha no uso correto dos selantes, pode causar fendas e fraturas com perda parcial ou total (Rahimian-Imam *et al*., 2015). Em relação ao aspecto funcional, o comprometimento do selamento marginal das restaurações de resina composta proporciona a passagem de bactérias, substâncias químicas e fluidas entre as paredes da cavidade e da restauração. Esse mecanismo denominado microinfiltração marginal, proveniente de fendas geradas na interface dente material restaurador, traz como consequências a sensibilidade pós-operatória, cárie secundária, manchamento marginal e injúrias pulpares, originando o fracasso precoce das restaurações (Mjör & Toffenetti, 2000). Sendo assim, diminuir as chances de ocorrência de fendas e fraturas marginais é decisiva para um selamento bem-sucedido (Mehrabkhani *et al*., 2015).

O conhecimento das propriedades mecânicas dos materiais é essencial para a longevidade dos procedimentos odontológicos (Alomari *et al*., 2001). A contração de polimerização de materiais resinosos causa tensões residuais que geram uma mudança de comportamento dos dentes restaurados, mesmo quando não estão em função mastigatória (Versluis & Versluis-Tantbirojn 2011). Os sinais clínicos relacionados com a contração de polimerização das resinas são a adaptação inadequada, microtrincas, fenda marginal, microinfiltração e cáries secundárias (Nedeljkovic *et al*., 2015).

A contração de polimerização dos materiais tem importância clínica devido ao estresse transitório e residual que são introduzidos sobre o dente. No entanto, quando incide uma aplicação direta de carga sobre a estrutura dental, podem ocorrer diversos fenômenos dependentes entre si gerando concentração e/ou dissipação de tensões (Versluis *et al*., 2004).

A dureza e módulo de elasticidade dos materiais resinosos são importantes propriedades que determinam sua resistência à fratura marginal (Bicalho *et al*., 2014). A tração diametral e compressão axial dos materiais são propriedades que se relacionam diretamente à resistência que o material tem à tração e compressão frente às tensões suportadas durante a função (Fonseca *et al*., 2016). Sendo que, os dentes são submetidos constantemente a estas forças durante a mastigação.

No estudo de estruturas dentais e materiais odontológicos, os ensaios mecânicos destrutivos são importantes meios de análise do comportamento do dente e de materiais em situações de aplicação de cargas pontuais e de alta intensidade. No entanto, apresentam limitações para obtenção de informações do comportamento estrutural interno do complexo dente-restauração durante a aplicação de carga, pois são geradas tensões que resultam em deformações estruturais, podendo acentuar, de acordo com a geometria e propriedades mecânicas, ultrapassando o regime elástico até atingir a ruptura da estrutura (Soares *et al*., 2006). Desta forma, para análise da interferência de fatores no processo de intervenção, torna-se necessária a associação de ensaios destrutivos com metodologias não destrutivas experimentais, como o método computacional de análise por elementos finitos, favorecendo cálculo biomecânico sequencial e detalhado do comportamento da amostra (Magne & Belser, 2003).

O Microscópio Eletrônico de Varredura (MEV) permite avaliar possíveis falhas na interface do material e do substrato dental e que podem ser classificadas de acordo com Naves *et al*., 2012, em: falha coesiva no substrato dental (esmalte ou dentina), falha mista envolvendo agente selador e/ou a estrutura do dente e falha de coesão restrita ao selante e, portanto, pode ser uma metodologia utilizada na avaliação de fendas em materiais dentários.

A aplicação de selante é uma intervenção minimamente invasiva que visa a prevenção do processo carioso e consequentemente preservar a estrutura sadia. Afim de obter um bom desempenho do procedimento é necessário que o selante resista às tensões induzidas pelos esforços mastigatórios. Assim, conhecer as propriedades mecânicas desses materiais resinosos parece decisivo para a o sucesso da intervenção (Chuang *et al*., 2011).

A avaliação de propriedades mecânicas de materiais para selamento de fossas e fissuras tem sido pouco relatada na literatura, e quando realizada pouco relacionada com suas falhas. Existem diversos trabalhos que realizam a fadiga mecânica através de uma ponta aplicadora de carga sobre a superfície dental. Entretanto, sabe-se que o selante é um material extremamente friável e pode fraturar com facilidade durante esse teste, resultado de um esforço pontual, porém este teste pode não representar de forma efetiva a sobrevida deste material quando está em função na cavidade oral. Dessa forma, a proposta do trabalho consiste em realizar a ciclagem mecânica com o método convencional e outra ciclagem com uma camada de silicone adaptada na ponta aplicadora de carga, com o objetivo de amortecer as cargas e simular a função do bolo alimentar, sendo uma forma inovadora na literatura e assim simular a realidade clínica analisando o comportamento biomecânico do material.

Capítulo I

2. CAPÍTULO 1

ARTIGO 1

Effects of mechanical properties, types of cycling on the stress shrinkage stress and integrity marginal of different types of materials to sealing Pit and Fissure

***Artigo a ser enviado para o periódico American Journal of Dentistry**

Effects of mechanical properties and type of cycling on the stress shrinkage stress and integrity marginal of different types of materials to sealing Pit and Fissure

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Keywords: Pit and Fissure Sealant; Mechanical fatigue; Shrinkage stress

Effects of mechanical properties and type of cycling on the stress shrinkage stress and integrity marginal of different types of materials to sealing Pit and Fissure

Objective. Evaluate the effect of mechanical properties of different materials used as sealant on stress shrinkage and integrity marginal and relation the results with two types of mechanical fatigue using Scanning Electron Microscopy (SEM) and Finite Element Analysis (FEA).

Methods. The materials used in this study were a Resin-modified glass ionomer cement (RMGIC) Vitremer 3M, a Resin Sealant (RS) Prevent, FGM, and a Flow Resin (FR) (Filtek Bulk Fill Flow, 3M. The analyzed properties were Diametrical Tensile Strength (DTS), Compressive Strength (CS), Post-Gel Shrinkage (Shr), Knoop Hardness (KHN) and Elastic modulus (E). Shrinkage stresses were analyzed using FEA. Forty-five molars were sealed and divided into three groups of each material (N = 15), first group without mechanical fatigue (N=5), second group with mechanical fatigue with the conventional metallic tip (N=5), third group with mechanical fatigue simulating the bolus (N=5). Relating to the results obtained through SEM images and marginal integrity values.

Results. In DTS and CS RMGIC it presented values inferior to RS and FR. In Shr, RS and FR showed higher values when compared with RMGIC. RS showed greater KHN and E than other materials. At FEA, it was observed that RS had high retraction stresses in the region where the material was applied and mainly at the marginal interface. When RMGIC was subjected to mechanical cycling, it had the biggest failure in the enamel and material interface. The results obtained were correlated with the SEM images and integrity marginal values. All three materials are suitable considering all of their characteristics, but it is important to note that direct fatigue with the metal tip overestimates the failure values.

Clinical Significance. The pit and fissure sealant must be correctly indicated, because if applied indiscriminately and if it fails, it will not perform its preventive function and, consequently, will cause risks to the patient.

Introduction

Dental caries is the most prevalent disease in the oral cavity, which mainly affects children and young people, occurs from the installation of cariogenic microorganisms in retentive sites, such as regions of fossae and fissures in the posterior teeth occlusal.[1, 2] The occlusal surface of molars and premolars corresponds to a region highly susceptible to the beginning of the lesion, where its grooves, pits and fissures represent a favorable niche for its development, making this clinical condition aggravated by the difficulty of hygiene in this area.[3]

The pit and fissure sealants allows the mechanical obliteration of the structural enamel defects, functioning as a physical barrier, and thus prevents the installation and advancement of carious lesions, being considered an effective, safe and proven prevention alternative.[4] The preventive sealant should be performed in patients at high risk of caries and with very deep pit and fissures that can be a predisposing factor for food retention and dental plaque accumulation.[5, 6]

Failure to correctly indicate sealants, however, can cause cracks and fractures with partial or total loss of material.[7] Regarding the functional aspect, the compromise of the marginal sealing of restorations allows the passage of bacteria, chemicals and fluids between the cavity and restoration walls, causing the early failure of procedure.[8] Therefore, inhibiting the chances of marginal fractures is decisive for a successful sealing. [9]

The most used materials for sealing pit and fissures are resin sealants or resin-modified glass ionomers. Conventional glass ionomer cement for many years has been the most used material for making pit and fissures sealants, however, several studies have associated it with some negative characteristics, such as restricted finishing and polishing for approximately 24 hours, moisture sensitivity during initial hardening, dehydration, rough surface texture, opacity, low fracture toughness, and low wear resistance.[10, 11] In consequence, the disadvantages of glass ionomers, resinous materials are being most used as the material of choice for sealant pits and fissures by clinicians.[12] These materials have easy handling and less time of procedure because they are photoactivation. In some studies, that analyzed the types of sealing materials in relation to the retention rate, they concluded that the fluid composites present the best results in relation to the others.[13]

The polymerization shrinkage of resinous materials causes residual stresses that result in a change in the behavior of the restored teeth, even when they are not in masticatory function.[14, 15] The clinical signs associated with the polymerization shrinkage of resin materials are inadequate adaptation, marginal failures, microleakage and secondary caries.[16] The results of the unwanted consequences of shrinkage in composite resin restorations are well described in the literature. The effects of shrinkage stresses are also well reported when analyzing composite resin restorations. However, the effects of shrinkage and shrinkage stresses pit and fissure sealing materials are not yet described in the scientific data bases.

The longevity of the preventive procedure with sealants is associated with the retention in dental structures which depends on several factors, including stress induced by polymerization, polymerization contraction, thermocycling, deflection caused by occlusion forces and water sorption.[17] The mechanical cycling of resin materials is well described in the literature to study its survival. [18, 19] However, studies about fatigue mechanical in sealants have also not been found in scientific databases commonly used. The experiments with mechanical cycling use a metallic tip with a high elastic modulus applying force on the material, however, it does not simulate what really exists clinically. Knowledge of the mechanical properties of materials, mastery of the technique and knowledge of its clinical consequences are essential for the longevity of dental procedures.[20]

There are many studies evaluating the adhesion of the sealing material to the enamel and its penetration and marginal infiltration rates, but few studies explain whether there is a direct relationship with its mechanical properties, stresses created and fatigue mechanical. The objective of this paper is to characterize the mechanical properties of different resinous materials indicated as preventive sealants and the biomechanical behavior of posterior teeth sealed with the same materials against two types of mechanical fatigue. The null hypothesis is that the properties mechanical, shrinkage stress and fatigue mechanical will not influence the integrity of occlusal sealants.

Materials and Methods

The materials used in this study were a Resin-modified glass ionomer cement (RMGIC), (Vitremer 3M), a Resin Sealant (RS) (Prevent, FGM) and a Flow Resin (FR) (Filtek Bulk Fill Flow, 3M), described in Table 1.

Compressive strength (CS) and diametral tensile strength (DTS)

Ten of each material were tested samples were made in Teflon matrix and polyester strips to generate surface finishing of the sealants. It was followed the recommendation of manufacturers for each material and the time of ligth-cure. The samples were prepared with 6.0 mm height and 3.0 mm in diameter for the CS test and 4.0 mm in diameter and 2.0 mm height for DTS according to standards ISO 9000: 2000. The light source used (Optilux 501, Kerr Mfg. Co) had 800 mW/cm2 light intensity and was applied for the recommended curing times. was used and the tests performed at 0.5mm /min speed on (DL2000, EMIC). 100 Kgf load cell were used to the DTS and 500 Kgf to the axial CS.

The data were tabulated and then submitted to the initial analysis to detect normal distribution and homogeneity between the obtained values. The values presented requirements that allowed the use of parametric analysis, it was used the factorial ANOVA at 5% probability level to verify if the factors under study presented significant interaction.

Post-gel shrinkage measurements (Shr)

Post-gel linear shrinkage was determined using the strain gauge method. [21] The material (N=10) were shaped into a hemisphere and placed on top of a biaxial strain gauge (PA-06-060BG-350LEN Excel, São Paulo, Brazil) that measured shrinkage strains in two perpendicular directions. The perpendicular strains were averaged since the material properties were homogeneous and isotropic on a macro scale. The hotoactivation unit (Bluephase, Ivoclar Vivadent) was used considering the manufacturer's recommendation time and the deformation in both directions (X and Y) was captured up to 10 minutes after start photoactivation. The data were exported with combinations that are processed in the Fortran language of the finite element analysis software to be used later.

The data obtained were submitted to statistical analysis to determine the difference in post-gel shrinkage. The data were tabulated and then submitted to the initial analysis to detect normal distribution and homogeneity between the obtained values. The values presented requirements that allowed the use of parametric analysis, it was used factorial ANOVA at 5% probability level to verify if the factors under study present significant interaction. As there was a difference between the groups, Tukey test ($p < 0.05$) for means comparison was performed to define between which groups there were significant differences at this level of significance.

Knoop Hardness (KHN) and elastic modulus (E)

Ten cylinders of 2 mm high and 4 mm radius were made with each material. The specimens were embedded with methacrylate resin (Instrumentos de Medição Ltda, São Paulo, SP, Brazil) in circular shapes of a pvc pipe. Prior to testing, the surfaces were polished with silicon-carbide sandpaper (# 600, 800, 1200, 2000; Norton, Campinas, SP, Brazil) and polished with metallographic diamond pastes (6, 3, 1, M ¼; Arotec, Sao Paulo, SP, Brazil).

Hardness measurements were performed on a Future Tech microhardness tester (Microhardness Tester, Future Tech FM-1E, Future Tech Corp., Tokyo 140, Japan) after 24 hours of specimen collection. KWH was performed on the top surfaces at five equidistant points using a 500 grams load for 10 seconds. These test parameters are necessary to determine elastic recovery of the smaller indentation diagonal, thus enabling the modulus of elasticity to be obtained from The Knoop indentations.[15, 22, 23] After each indentation, the recovery of a material is related to the hardness / elastic modulus ratio (H / E) according to the following empirical relationship: $b'/a' = b/a - A\,(H/E)$, where b/a is the ratio of diagonal dimensions a and b in the fully charged state, given by a constant 0.140647. b'/a' is the ratio of changed dimensions when fully recovered, and $A = 0.45$ is a proportionality constant.

The data were tabulated and then submitted to the initial analysis to detect normal distribution and homogeneity between the obtained values. The values presented requirements that allowed the use of parametric analysis, was used the factorial ANOVA at 5% probability level to verify if the factors under study showed significant interaction with respect to the E. A means comparison test, Tukey test ($p < 0.05$) was used to define between which groups significant differences occurred at this level of significance.

3D finite element analysis (FEA)

To calculate the stress distribution, a three-dimensional finite element simulation was performed. A human molar tooth free of caries, cracks, structural defects or restorations was selected. The tooth was scanned on a computerized microtomography machine (SkyScann 1272, Brucker, Belgium) following the parameters described by Magne *et al*.[24] MicroCT is a CT scanner using a microcomputer coupled to a Dell Precision T5600 Intel® Xeon terminal (128GB 1600MHz) and a cluster (Dell Precision Intel® Core 4 Gb, CPU, 2.13 GHz) with NRecon® rebuild software (Skyscan, Belgium).

The DICOM files obtained from the reconstruction of the scanning process were imported into Mimics software (Materialise, Leuven, Belgium), which performed computational segmentation of enamel, dentin and sealant structures using tools automated by the radiodensity levels of each structure.

After the creation of the 'masks' of the enamel, dentin and resin sealant, the original mesh of the scanning process was adjusted to achieve the highest possible level and evenness among the surface elements. These files were imported in STL format for MARC / MENTAT software (MSC Softwares, USA).

The mechanical properties of E and Poisson's ratio Shr, CS and DTS of all material employed were realized and used in post-processing. A *.DAT file was generated to solve the structural problem. [25] In this *.DAT file, the boundary conditions were defined, as well as the designation of the mechanical properties necessary for feeding the models. A simulation analysis of polymerization shrinkage by thermal analogy was employed. To process the model, the DOS environment was applied, resulting in the sequential calculation of the analyzes, a step that was successful when the output number 3004 was expressed at the end of the analysis. To analyze the results of the individually generated models, the modified von Mises criterion was used. [26] Cuts were made in the longitudinal axis of the models, for the analysis and mapping of the stress distribution qualitatively and quantitatively within them.

Selection of teeth and manufacture of sealants

Forty-five extracted intact, caries-free human third molars were used with approval from the University Ethics Committee in Human Research. (Ethics Committee in Human Research approval #1.688.272). The size sample were determined using Easy Sample Size Project (software of public domain ESSP - http:/www.est.ufmg.br/sampling). The roots were covered with a 0.3 mm layer of a polyether impression material (Impregum; 3M ESPE, St Paul, Minn) to simulate periodontal ligament, and embedded in a polystyrene resin (Cristal, Piracicaba, SP, Brazil) up to 2 mm below the cemento-enamel junction to simulate the alveolar bone.[27]

The teeth were cleaned with a rubber cup and fine pumice water paste and distributed in three groups of 15 teeth each. Ten teeth per group were restored with materials and used to measure mechanical fatigue. The other five teeth per group restored and not subjected to mechanical fatigue, were used for SEM analysis.

The teeth were sealed using the three materials. To standardize the process, the acid conditioning system for enamel with 37% phosphoric acid (Condac; FGM, Joinville, SC, Brazil) was used for 30 seconds. Washed for the same application time and dried from the triple syringe.

Each material was applied following the manufacturer's recommendations, with the aid of an exploratory probe and light-curing using the light-curing unit Valo Cordless (Ultradent, South Jordan, UT, USA). After each sample, the intensity of the light emitted by the light-curing was measured.

Mechanical cycling test

Chewing cycles were simulated to induce mechanical fatigue (Biocycle, Biopdi, São Paulo, SP, Brazil) the specimens were immersed in water, kept at approximately 37ºC and fixed in specific devices. Ten samples of each sealant were subjected to fatigue simultaneously under axial compression load at the center of the occlusal surface, with a 50 N load at a frequency of 2 Hz. 120,000 cycles that simulate 6 months of aging were performed.[28]

The mechanical action of the contact tip of the load application and the occlusal surface, in addition to the volume of water in the container were checked periodically to ensure the reliability of the test.

Fatigue was performed in two ways, de according Fig.1:

1) Conventional (N=15): The spherical tip applying the load directly to the restored surface with the three types of material.

2) Adapted with silicone (N=15): The spherical tip adapted with a silicone material applying the load indirectly on the surface restored with the three types of material.

Scanning Electron Microscopy (SEM)

For analysis in a SEM, the teeth were sectioned with a double-sided diamond disc (Extec, USA), mounted on a precision cutter (Isomet 1000; Buehler, Lake Bluff, IL, USA), under refrigeration, in the direction of the enamel-cement junction on the buccal and lingual surfaces, generating two of the crown for each material (n = 10). Then, they were polished with ultrasound. The teeth were analyzed before mechanical fatigue and after mechanical fatigue performed in two ways (with and without adaptation to the metal tip). After vacuum gold plating (MED 010, Balzers, USA), samples were analyzed at 7,000 X magnification (Zeiss EVO MA 10 Scanning Electron Microscopy, Jena, Germany). It was made, in the increase of 7000 X, quantitative analysis to measure the crack size using for this purpose the IMAGE J software (public domain, Java-based image processing and analysis software developed at The National Institutes of Health, Bethesda, MD, USA). The scale was calibrated, using as standard 2 µm corresponding to 1 pixel. Then, 10 segments were drawn line at different points of the crack, measuring the distance from the sealant to the enamel, then the arithmetic mean and standard deviation of the 10 values obtained for each material and the data were tabulated.

Statistical Analysis

CS, DTS, Shr, KHN, E, interface by MEV data were tested for normal distribution (Shapiro-Wilk, $p>0.05$) and equality of variances (Levene's test), followed by parametric statistical tests. One-way analysis of variance (ANOVA) was performed for CS, DTS, Shr, KHN and E. Two-way ANOVA was performed in a split-plot arrangement for interface by MEV. Multiple comparisons were made using Tukey's test. All tests employed $\alpha=.05$ significance level and all analyses were carried out with the statistical package Sigma Plot version 13.1 (Systat Software Inc, San Jose, CA, USA).

Results

CS and DTS

The results obtained were performed using an Excel statistical model and showed in Table. 2. One-way ANOVA showed significant difference among materials tested ($P<0.001$).

After analyzing the results in the CS and DTS test it can be observed that the RS and FR presented similar results in their samples and higher values than the material RMGIC

Shr

The Shr mean values and (standard deviations) of tree materials are presented in Table. 2. One-way ANOVA revealed statistical difference among the materials ($P <0.001$).

Table. 2 represents the Shr values similar between RS and FR, and they have shown a higher result compared to the RMGIC.

KHN and E

One-way ANOVA revealed statistical difference among the materials ($P <0.001$). After analyzing the data, the RS and) FR group presented the KHN values and E values greater RMGIC. Table. 2 presents the average KHN data along with its standard deviation.

FEA

Shrinkage stress distributions after the materials (modified von Mises stress) is shown in Figure 2 and 3. According to the data found in this study using the FEA, it can be observed that the RS presented a high stress concentration in the region where the material was applied and principally in the interface. The RMGIC showed the lowest shrinkage stress concentration.

SEM

The images of all groups were organized in a representative way in Figure 4. The distance in micrometers of the interface between material and enamel obtained are shown in Table 3. Two-way ANOVA revealed statistical difference among the materials and presence or type of fatigue (P <0.001).

In the immediate time after applying the materials, the three showed similar values. In the time after fatigue that simulated six months of aging, both using a metal tip over the occlusal and using silicone between the metal tip and the occlusal one, the RMGIC group showed the highest values of distance at the interface when compared to the RS and FR groups. When the types of fatigue were compared, the RMGIC group showed statistically the highest values of interface distance between material and enamel when compared to the other two groups.

Discussion

The null hypothesis of this study was not accepted, the three materials presented different mechanical properties and shrinkage stress. The type and presence of mechanical fatigue also influenced the marginal integrity of each material used. The polymerization shrinkage process occurs due to the change of state of the material from monomer to polymer. [29] If this process does not occur properly, it can generate cracks between the material and the tooth, and cause infiltrations and even secondary caries. [30]

For the tests CS and DTS there was no significant difference between dados obtained between groups RS and FR, presenting values higher than RMGIC, which has low resistance. These mechanical properties are extremely important in characterizing the possibility of mechanical failure of the material when subjected to occlusal loads.[23]

In one study it was obtained as a result that the bulk-fill resin has less post-gel shrinkage and at the same time low shrinkage, thus increasing the fracture resistance of this material.[19] Bulk fill resins are also used as sealing materials and may have good characteristics in the end result of an occlusal sealant, com high penetration in every irregularity the enamel, due to the fluidity of the material, radio-opaqueness adequate and availability in different colours.[17,31] In a meta-analysis that analyzed the retention rate of resinous materials in pit and fissures, it showed that fluid composite resins proved to be a superior alternative to conventional sealants after 1 year of follow-up; however, no difference between the 2 groups was observed after 2 years of follow-up.[32] Thus, it has become important to investigate the biomechanical characteristics of these materials when used as occlusal sealant.

In the present study, when analyzing the Shr of the sealing materials, the material with the lowest value shrinkage was RMGIC. By shrinkage less, the material will be less likely to generate cracks between sealant and tooth, thus decreasing the likelihood of microleakage and secondary caries.[30] RS and FR showed greater contraction, with no significant difference between groups. The ideal composite should generate the lowest possible shrinkage stress, ensuring a better seal, that is, these groups are more likely to have marginal seal failure compared to RMGIC. [33]

The hardness is a property that directly influence the long resistance of resin materials, since there is a direct link between this property and the wear resistance of these materials.[34] In this study, KHN was characterized not only to analyze the wear resistance of materials, but also as a tool to obtain the E through mathematical formulas. To calculate the E it was used a method based on the elastic recoverability of the material in the indentation walls after removal of the applied load.[35, 36] In test de KHN, RS and FR obtained the highest result, being RMGIC the lowest. Proportionally, the results of the E were also presented in the same sequence, since this property is an important property which describes the relationship between stresses and strains and should be characterized if possible.[37]

With the results of this study we can consider that RS is a harder and more resistant material but presents a high rate Shr which can cause infiltration problems posteriorly. In another study, it has also been shown that a higher E of the material increases the stresses generated by polymerization,[38] and these characteristics were found for the RS material, with a high KHN and E index compared to the other two materials. High shrinkage values of a material can cause cracks and fissures between the material and the tooth. According to the RS material manufacturer, its composition has 50 % load, which can be considered as a factor influencing its hardness with higher value. [33, 37, 39]

In a bibliographic study of works on finite elements, it was found that it is a great method to reach results with greater ease of visualization in relation to the calculation of stress distribution in clinically impossible regions to be evaluated.[40] Mathematical models are performed for finite element analysis, in this study the modified von Mises criterion was used, which allows the tensile and compressive stresses to be characterized as long as the tensile and compressive properties of the materials are embedded in the models processing. The three-dimensional finite element image presented in this research can verify this information, since it can visualize and identify precise regions that suffered stress concentration. In one research, it was found that low shrinkage resins had lower stress distribution by analyzing the FEA.[39] In this study, analyzing and using the same method, the material with the lowest stress distribution values was RMGIC group, which is also a material with low E and low Shr. In the present study, the RS group presented high post-gel retraction values and high E, generating an image with visible stress distribution in the finite element test mainly at the marginal interface when compared to RMGIC and FR.

SEM was considered the gold standard for analyzing sealant retention due to its high sensitivity and specificity.[41] According to Dennison *et al.*[42] most sealant failures occur six months after application, through SEM images after mechanical cycling it is possible to observe more fractures in the group RMGIC which showed lower CS and DTS values. When correlating the SEM images with the data obtained in the FEA, it is possible to affirm that the sealed teeth without mechanical fatigue presented smaller cracks compared to those submitted to occlusal forces. Therefore, RMGIC has a greater interface between material and dental substrate, because it has the lowest CS values (Table.3). It is important to have the knowledge and balance of a material in relation to its mechanical properties,[37] understanding the properties of sealants are extremely important for all professionals, as the biomechanical behavior of materials in the oral cavity will consequently lead to restorative procedures with better quality and predictability

There are challenges when trying to simulate what happens in the oral cavity during chewing, through in vitro studies. When performing the mechanical fatigue test, the metal tip has a high E that during the cycle will easily fracture the material used to seal pits and fissures, so through this study it tried to simulate a food cake with condensation silicone, which is a low E material so that this process tries to reproduce the clinical reality more faithfully. The results obtained were analyzed in the SEM and with the help of the Image J Software to measure the cracks in the enamel and material interface, it showed that the metallic tip compared to the adapted tip failed more, with higher values for all groups.

From the data obtained, we can reflect that all three sealants are adequate considering all their characteristics, but it is noteworthy that the RS group present high shrinkage stress principally in the interface between enamel and sealant. Without mechanical loading, the RMGIC group has better results, but when subjected to direct mechanical fatigue with the metallic tip it overestimates the failure values. The ideal material is one that has low polymerization shrinkage, tensile strength and adequate compression, so that it does not transfer too much tension to the enamel and has a good resistance to occlusal load. Among all materials considering only the biomechanical characteristics, FR presented the best performance. More biological studies must be carried out, as the FR is a new material that has little scientific evidence, and thus compare this fluid resin wit RMGIC, which is considered the gold standard of literature.

The sealing materials must be correctly indicated, because if applied indiscriminately and if it fails, it will not perform its preventive function and, consequently, will cause risks to the patient.

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Table 1 - Dental composites tested in the study

Dental Composites	Code	Comercial Name	Light activation time	Composition	Manufacturer
Resin sealant	RS	Prevent	20 s	Methacrylic monomers such as BisGMA, TEGDMA and acid methacrylic monomers, stabilizer, camphorquinone, co-initiator and fluorine aluminosilicate glass filler.	FGM, Joinville, SC, Brazil
Fluid resin	FR	Filtek Bulk Fill Flow	20 s	BisGMA, UDMA, bisEMA, and Procrylat Fillers: a combination of zirconia/silica with a particle size range of 0.01 to 3.5 microns and ytterbium trifluoride filler with a range of particle sizes from 0.1 to 5.0 microns. The inorganic filler loading is approximately 64.5% by weight (42.5% by volume).	3M-Espe, St. Paul, MN, USA
Resin-modified Glass Ionomer	RMGIC	Vitremer	40 s	Powder: Contains glass of fluoralumino silicate, potassium persulfate and microencapsulated ascorbic acid and pigments; Liquid: Contains aqueous solution with HEMA polycarboxylic acid copolymers and photoinitiators; Primer: Contains copolymers of polycarbixylic acid, HEMA, ethanol and photoinitiators; Enamel: Contains Bis-GMA, TEGDMA	3M-Espe, St. Paul, MN, USA

Bis-GMA, bisphenol-A diglycidyl ether dimethacrylate; HEMA, 2-hydroxyethylmethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

Table 2 - Diametral tensile strength (Mpa); Compression Strength (MPa); Post-Gel Shrinkage (%); Knoop Hardness (KHN); Elastic Modulus (E)

Material	***DTS***	***CS***	***Shr***	***KHN***	***E***
RS	35.6 ± 3.7 A	251.8 ± 36.1 A	0.56 ± 0.09 B	48.1 ± 1.47 A	13.3 ± 1.31 A
FR	38.6 ± 7.5 A	245.1 ± 30.6 A	0.47 ± 0.07 B	39.6 ± 1.75 B	10.4 ± 0.98 B
RMGIC	15.7 ± 8.2 B	121.6 ± 15.7 B	0.18 ± 0.006 A	48.9 ± 1.76 B	10.1 ± 1.13 B

* Capital letters mean statistical difference between columns. Mean values with same superscript letters are not significantly different (significance level 0.05).

Table 3 - Distance between material and enamel (µm)

	RMGIC	***RS***	***FR***
Without fatigue	3,4 ± 0,12 Aa	3,7 ± 0,11 Ba	3,6 ± 0,09 Ca
Fatigue with silicone tip	4,5 ± 0,10 Bb	3,2 ± 0,13 Aa	3,1 ± 0,11 Aa
Fatigue with metalic tip	5,6 ± 0,11 Bc	4,9 ± 0,12 Ab	4,8 ± 0,10 Bb

* Capital letters mean statistical difference between columns and lowercase letters mean statistical difference between lines. Mean values with same superscript letters are not significantly different (significance level 0.05).

Figure 1. Mechanical fatigue test. A) Sample with sealing material and simulation of the food cake with condensation silicone. B) Sample with sealing material. C) Sample ready for fatigue with the metal tip applied to the material. D) Sample ready for fatigue with the metal tip applied directly on the occlusal surface.

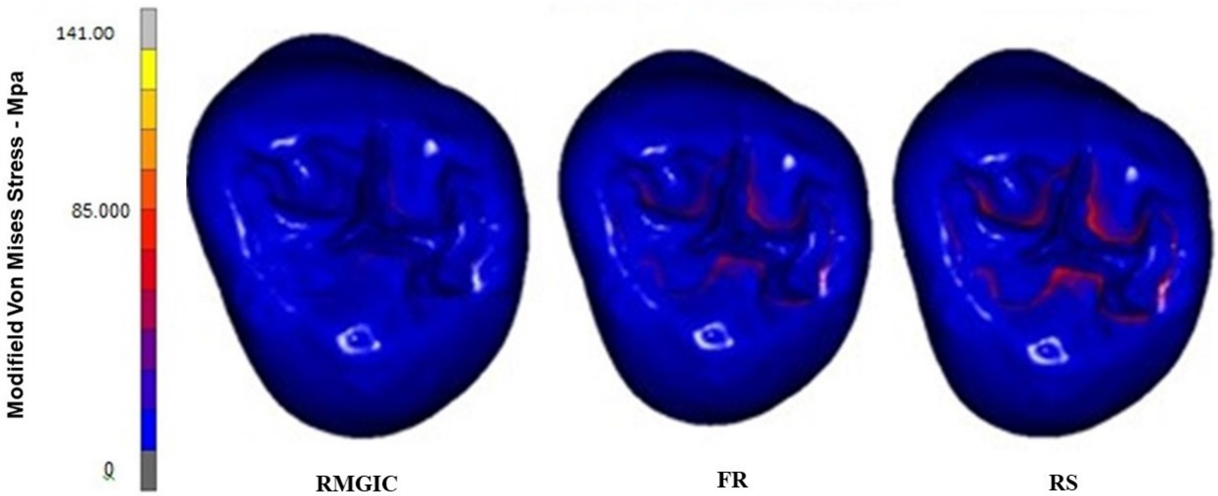

Figure 2. Materials after finite element analysis, with evidence showing regions of red color with concentration of each of the materials on the enamel.

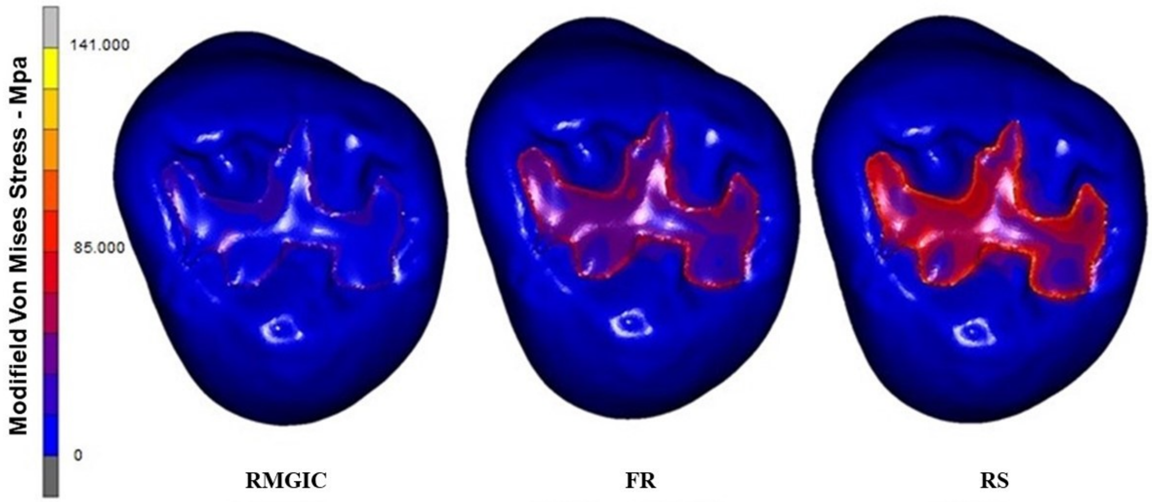

Figure 3. Materials after analysis finite element, with evidence showing red color regions with stress concentration of each of the materials.

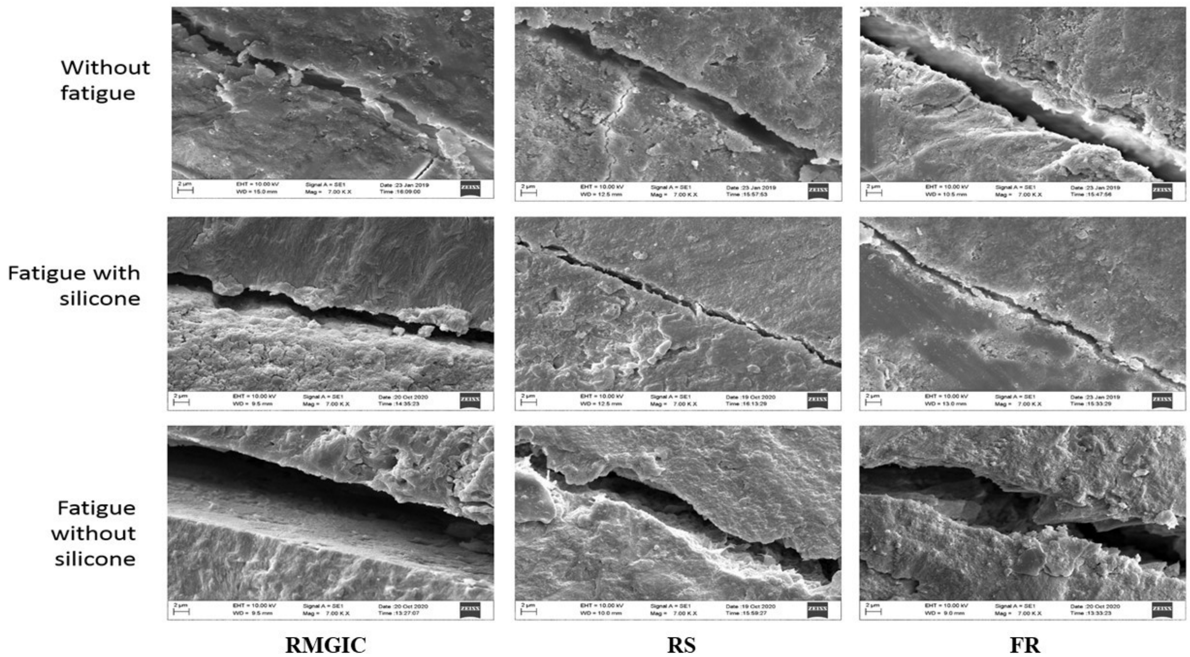

Figure 4. Analysis of SEM images with a magnitude of 7,000K, in columns the three materials, in the first row, material without mechanical fatigue. Second line, material fatigued with silicone and third line, material fatigued without silicone

CONCLUSÃO

3. CONCLUSÃO

As seguintes conclusões podem ser obtidas:

1. O material ideal é aquele que apresenta baixa contração de polimerização, resistência a tração e compressão adequada, para que não transfira de forma demasiada tensões ao esmalte e que tenha uma boa resistência a carga oclusal.
2. Entre todos os materiais considerando apenas as características biomecânicas, a FR apresentou-se com o melhor desempenho.
3. Mais estudos biológicos devem ser realizados, pois FR é um material novo que possui poucas evidências científicas, para compara-lo com o RMGIC, que é considerado o padrão ouro da literatura.
4. Sem carregamento mecânico, o RMGIC tem melhores resultados, mas quando submetido à fadiga mecânica direta com a ponta metálica superestima os valores de falha.

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* De acordo com a Norma da FOUFU, baseado nas Normas de Vancouver. Abreviaturas dos periódicos com conformidade com Medline (Pubmed).

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ANEXO

ANEXO 1

Comitê de Ética em Pesquisa

UNIVERSIDADE FEDERAL DE UBERLÂNDIA/MG

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito das propriedades mecânicas de materiais selantes na penetrabilidade, geração de tensões de contração e formação de fendas marginais em molares

Pesquisador: Aline Aredes Bicalho

Área Temática:

Versão: 2

CAAE: 55948416.5.0000.5152

Instituição Proponente: Universidade Federal de Uberlândia/ UFU/ MG

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.688.272

Apresentação do Projeto:

Trata-se de uma pesquisa da Faculdade de Odontologia da UFU.

A pesquisa se dará em duas etapas. A primeira etapa deste estudo tem como objetivo avaliar a dureza Vickers, módulo de elasticidade, contração de polimerização pós-gel, tração diametral e compressão axial dos três selantes resinosos e quantificação das tensões geradas pela contração em molares por meio do método de elementos finitos.

A segunda etapa deste estudo tem como objetivo avaliar a infiltração marginal e penetrabilidade de molares selados com os três materiais, em tempo imediato e após fadiga mecânica de 300.000 e 600.000 ciclos, por meio de microtomografia computadorizada.

Objetivo da Pesquisa:

Estudar o efeito de três diferentes materiais resinosos na realização de selantes oclusais em molares por meio de microtomografia computadorizada e método de elementos finitos. Avaliar propriedades mecânicas como a dureza, módulo de elasticidade, contração pós-gel, tração diametral e compressão axial dos selantes selecionados:

Materiais selantes:

ANEXO 2

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