TASSIANA CANÇADO MELO SÁ

LIBERAÇÃO DE COMPONENTES ORGÂNICOS E RESPOSTA PERIODONTAL EM RELAÇÃO A UM SISTEMA RESTAURADOR RESINOSO BULK- FILL: *ESTUDO IN VIVO*

Faculdade de Odontologia
Universidade Federal De Minas Gerais
Belo Horizonte
2019

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Tese apresentada ao Colegiado de Pós-graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do grau de Doutor em Odontologia - área de Concentração em Clínica Odontológica

Orientador: Prof. Dr. Allyson Nogueira Moreira

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PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

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FOLHA DE APROVAÇÃO

LIBERAÇÃO DE COMPONENTES ORGÂNICOS E RESPOSTA INFLAMATÓRIA FRENTE A UM SISTEMA RESTAURADOR RESINOSO BULK- FILL: Estudo In vivo

TASSIANA CANÇADO MELO SÁ

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Odontologia, como requisito para obtenção do grau de Doutor, área de concentração Clínica Odontológica.

Aprovada em 28 de junho de 2019, pela banca constituída pelos membros:

Prof(a). Allyson Nogueira Moreira - Orientador
FO-UFMG

Prof(a). Claudia Silami de Magalhaes
FO-UFMG

Prof(a). Rodrigo Villamarim Soares
PUC-MG

Prof(a). Rodrigo Richard da Silveira
FO-UFMG

Prof(a). Amanda Beatriz Dadah Aniceto de Freitas Arnaldo

Belo Horizonte, 28 de junho de 2019.

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

ATA DA DEFESA DE TESE DA ALUNA
TASSIANA CANÇADO MELO SÁ

Aos 28 dias de junho de 2019, às 13:30 horas, na sala 3403 da Faculdade de Odontologia da Universidade Federal de Minas Gerais, reuniu-se a Comissão Examinadora composta pelos professores Allyson Nogueira Moreira (Orientador) – FO/UFMG, Claudia Silami de Magalhaes – FO/UFMG, Rodrigo Villamarim Soares – PUC-MG, Rodrigo Richard da Silveira – FO/UFMG e Amanda Beatriz Dadah Aniceto de Freitas – Arnaldo, para julgamento da tese de Doutorado em Odontologia, área de concentração em Clínica Odontológica, intitulada: **Liberação de componentes orgânicos e resposta inflamatória frente a um sistema restaurador resinoso bulk- fill: estudo In vivo.** O Presidente da Banca, abriu os trabalhos e apresentou a Comissão Examinadora. Após a exposição oral do trabalho pela aluna e arguição pelos membros da banca, a Comissão Examinadora considerou:

(X) Aprovada

( ) Reprovada

Finalizados os trabalhos, lavrou-se a presente ata que, lida e aprovada, vai assinada por mim e pelos demais membros da Comissão. Belo Horizonte, 28 de junho de 2019.

Prof(a). Allyson Nogueira Moreira

Prof(a). Claudia Silami de Magalhaes

Prof(a). Rodrigo Villamarim Soares

Prof(a). Rodrigo Richard da Silveira

Prof(a). Amanda Beatriz Dadah Aniceto de Freitas

Dedico esse trabalho aos meus pais, Júlio e Augusta, ao meu marido Ramon que sempre me apoiaram e me ajudaram a tornar esse sonho real.

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Martin Luther King

RESUMO

A maioria das resinas compostas apresenta metacrilatos como principais monômeros em sua composição. A liberação de monômeros de metacrilato, associada aos produtos de polimerização, tem sido considerada como fonte de uma série de reações biológicas como toxicidade ou reações pulpares. Os objetivos deste estudo foram avaliar o desempenho clínico de restaurações em LCNC com resina composta considerando-se também a presença de citocinas IL-1β e IL-6 no fluido crevicular gengival e a liberação de componentes resinosos para a saliva. Utilizou-se o sistema restaurador FL-Bond II, (sistema adesivo) / Beautifil Bulk (resina composta restauradora). Foi feito um estudo clínico longitudinal *in vivo*, no qual foram selecionados pacientes que apresentavam uma lesão cervical não cariosa com necessidade restauradora. Dentes anteriores e posteriores com LCNC e sensibilidade foram designados como grupo experimental e o dente correspondente como grupo controle. Previamente ao tratamento, houve avaliação periodontal, coleta de saliva e de fluido crevicular gengival (FCG). As restaurações foram confeccionadas e, após 10 minutos, 7 dias, 1 mês e 6 meses foi realizada avaliação clínica das mesmas de acordo com o critério Federal Dentist International (FDI) e da resposta periodontal. Adicionalmente, em todos estes períodos de avaliação houve coleta de saliva e de fluido crevicular. As amostras de saliva foram analisadas por LC-EM a fim de identificar eventual presença dos monômeros Bis-GMA e TEGDMA. As amostras de fluido crevicular foram analisadas utilizando-se método ELISA para identificação e quantificação de interleucinas. Para realizar as comparações inter e intragrupo dos parâmetros clínicos foi utilizado o teste de McNemar para as variáveis categóricas e o teste de Wilcoxon para as variáveis numéricas. Para comparar a classificação do critério FDI entre os tempos foi utilizado o teste de Stuart-Maxwell. Para a análise da saliva, os dados de quantidade de monômeros liberados ao longo do tempo foram submetidos ao teste t de Student e a relação com quantidade total liberada foram correlacionados com o número de restaurações e com o volume total coletado de cada restauração por meio da Correlação de Pearson. O desempenho clínico das restaurações de LCNCs de um sistema restaurador resinoso bulk- fill por meio dos critérios FDI foi considerado satisfatório ao longo de 6 meses. Os parâmetros clínicos periodontais Sangramento a Sondagem, Ìndice Gengival e Profundidade de Sondagem foram mais pronunciados em torno dos dentes restaurados. O material restaurador resinoso bulk-fill não causou alteração estatisticamente significante no volume de FCG e as citocinas ao redor de dentes restaurados na amostra avaliada. Não detectou-se Bis-GMA nas amostras de saliva coletadas antes, 01 e 06 meses após. A liberação de TEGDMA média antes da restauração foi estatisticamente menor do que após 10 minutos. Os resultados do presente estudo sugerem que a restauração do LCNC pode afetar os parâmetros clínicos periodontais, porém não foi capaz de afetar a liberação de citocinas e o volume de FCG. A liberação de Bis-GMA não foi considerada significante ao longo de 6 meses, entretanto a liberação de TEGDMA foi expressiva apenas 10 minutos após a execução da restauração.

Palavras-chave: Resinas compostas. Ensaio clínico. Teste de materiais. Interleucinas. Metacrilatos.

ABSTRACT

Release Of Organic Components and Inflammatory Response to a Bulk-Fill Resin Results System: *In Vivo* Study

Most composite resins present methacrylates as the main monomers of their composition. In composite resins, the release of methacrylate monomers, associated with the polymerization products, has been considered as the source of a series of biological reactions such as toxicity or pulp reactions. The objectives of this study were to evaluate the clinical performance of NCCL restorations with composite resin, to determine also the presence of IL-1β and lL-6 cytokines in the gingival crevicular fluid (GCF) and the release of resinous components to saliva. The FL-Bond II restorative system (adhesive system) / Beautifil Bulk (restorative composite resin) was used. A longitudinal clinical study was performed *in vivo*, where patients with a non-carious cervical lesion with a restorative need were selected. Anterior and posterior teeth with NCCL and sensitivity were designated as experimental group and the corresponding tooth as control group. Prior to the treatment, were performed periodontal evaluation, collection of saliva and gingival crevicular fluid. The lesions were restored and, after 10 minutes, 7 days, 1 month and 6 months, there was a clinical evaluation of the restorations according to the FDI criteria and the periodontal response were made. Additionally, in all of these evaluation periods there was collection of saliva and crevicular fluid were colleted. Saliva samples were analyzed by LC-MS in order to identify the possible presence of monomers. Crevicular fluid samples were analyzed using the ELISA method for identification and quantification of interleukins. To perform intra- and inter group comparisons of clinical parameters, the McNemar test for categorical variables and the Wilcoxon test for numerical variables were used. To compare the classification of the FDI criterion between the times, the Stuart-Maxwell test was used. For the analysis of saliva, the data of quantity of monomers released over time were submitted to Student's t-test and the relation with total amount released were correlated with the number of restorations and with the total volume collected from each restoration by means of Pearson's Correlation. The clinical performance of NCCL restorations of a bulk-fill resin restorative system by FDI criteria was considered satisfactory over 6 months. Considering the periodontal response of the surrounding tissue to the NCCL restorations, it was observed that periodontal clinical parameters Bleeding on Probing, Gingival Index and Prohibing on Depth were more pronounced around the restored teeth. The bulk-fill resin restorative material did not cause statistically significant changes in the volume of GCF and in the IL-1β and IL-6 cytokines around restored teeth in the evaluated sample. No Bis-GMA was detected in the saliva samples collected before, 01 and 06 months after. For the Bis-GMA, there was no statistical difference between the analyzed periods. The mean TEGDMA release before the restoration was statistically lower than after 10 minutes. The results of the present study suggest that NCCL restoration may affect periodontal clinical parameters, but it was not able to affect the release of cytokines and the volume of GCF. The release of Bis-GMA was not considered significant over 6 months. However, the release of TEGDMA was significant only 10 minutes after the restoration was performed.

Keywords: Composite resin. Clinical trial. Materials testing. Interleukins. Methacrylates

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LISTA DE ABREVIATURAS E SIGLAS

Bis-GMA	Bisfenol A Glicidil Dimetacrilato
TEGDMA	Trietilenoglicol Dimetacrilato
UDMA	Uretano Dimetacrilato
LCNC	Lesões Cervicais não Cariosas
mm^3	Milímetros Cúbicos
mW/cm^2	Miliwatt Por Centímetro Quadrado
µL	Microlitro
mL/min	Mililitro Por Minuto
mM	Milimolar
mg/ml	Miligrama por Mililitro
pg/µl	Picograma por Microlitro
^oC	Grau Celsius
IL-1β	Interleucina 1Beta
IL-6	Interleucina 6
FCG	Fluido Crevicular Gengival
MDP	Methacryloxydecyl Dihidrogen Phosphat
Rpm	Rotações por Minuto
FDI	Federal Dentist International
USPHS	United States Public Health Service

LED	Light-Emitting Diode
ELISA	Enzyme Linked Immunosorbent Assay
SPSS	Statistical Package For The Social Sciences
LC- EM	Cromatografia líquida Com Espectrometria De Massa
Bis-MPEPP	Bisfenol A Polietoxymetacrilato

SUMÁRIO

1 CONSIDERAÇÕES INICIAIS

As resinas compostas odontológicas apresentam uma matriz polimérica, tipicamente um metacrilato, partículas de carga silanizadas e substâncias que ativam ou modulam a reação de polimerização (FERRACANE, 2011). O monômero-base da matriz orgânica é o Bis-GMA (bisfenol A glicidil dimetacrilato) e possui alto peso molecular e alta viscosidade. O Bis-GMA é normalmente combinado com outros monômeros, como TEGDMA (trietilenoglicol dimetacrilato) que é de baixo peso molecular e baixa viscosidade ou UDMA (uretano dimetacrilato) que possui alto peso molecular e é diluente (WEINMANN; THALACKER, GUGGENBERGER, 2005; ZIMMERLI *et al*., 2010). Os metacrilatos ainda representam a base monomérica predominante das resinas, embora novas formulações tenham sido introduzidas no mercado (PEUTZFELDT, 1997; ZIMMERLI *et al*., 2010). Recentemente modificações associadas aos componentes monoméricos foram realizadas nas resinas compostas para aprimorar as propriedades físico-químicas, reduzir a contração de polimerização (como resinas a base de silorano), e para promover maior biocompatibilidade e funcionalidade (ZIMMERLI *et al*., 2010; FERRACANE, 2011; ILIE, HICKEL, 2011).

No ano de 2000, foi lançada uma categoria de material resinoso chamada Giomer (acrônimo das palavras, em inglês, "ionômero de vidro" e "compósito polimérico"). São materiais que contém partículas pré-reagidas de ionômero de vidro (PRG), que são feitas de vidro de alumíniofluor-borosilicato e são pré-reagidas com ácido poliacrílico antes de serem incorporado na resina (IKEMURA *et al*., 2008). Estas partículas funcionam com base em uma reação entre ácido poliacrílico e alumíniofluor-borosilicato e são capazes de liberar flúor, bem como fazer sua recarga (GURURAJ *et al*., 2013). A resina Beautifil-bulk desta linha é um composto bulk-fill que é principalmente caracterizado pela sua profundidade de polimerização, permitindo que incrementos de espessura (4-5 mm) sejam colocados em uma única etapa se comparado a técnica de estratificação convencional (ALSHALI, SATTERTHWAITE & SILIKAS, 2015).

O tempo de fotoativação recomendado para o material resinoso é de normalmente 40 segundos por incremento, sob temperatura de 37ºC na cavidade bucal (VAN LANDUYT *et al.*, 2011), variando de acordo com o material e aparelho fotoativador utilizado. Nestas circunstâncias, não ocorre total conversão dos monômeros em polímeros (VAN LANDUYT *et al*., 2011). O grau de conversão máximo

das diferentes resinas compostas é variável, oscilando entre 50-75% (AL-AHDAL *et al.*, 2015; NEVES *et al.*, 2005; FERRACANE; CONDON, 1990; HALVORSON; ERICKSON; DAVIDSON, 2002), sendo alcançado o pico máximo após 24 horas do início da reação de polimerização (AL-SHALI, SILIKAS, SATTERTHWAITE, 2013; HALVORSON; ERICKSON; DAVIDSON, 2002; SCOTTI *et al*., 2013; NEVES *et al*., 2005;). Devido ao valor do grau de conversão, especula-se que as resinas compostas podem servir como reservatórios de componentes lixiviáveis (monômeros não convertidos, aditivos e outras substâncias orgânicas), que poderiam induzir inflamação pulpar e, ainda, serem liberados no meio bucal (ANUSAVICE; SHEN; RAWLS, 2013; REICHL *et al*., 2008; JOHN, 2007). A adição do ácido poliacrílico, em materiais como o GIOMER, pode diminuir a eluição dos monômeros devido ao aumento da taxa de conversão dos monômeros (ANDRZEJEWSKA, ANDRZEJEWSKA, ZYCH-TOMKOWIAK, 2003)

Os componentes liberados da reação de fotoativação das resinas compostas têm sido extensivamente estudados (POLYDOROU *et al*., 2009; MICHELSEN *et al*., 2003, 2007, 2012), e a liberação pode ocorrer até 1 ano após a fotoativação (POLYDOROU *et al*., 2009). Adicionalmente, as resinas compostas sofrem processos de degradação no interior do meio bucal por hidrólise ou ação enzimática. Esses processos podem resultar no rompimento das cadeias poliméricas e liberar subprodutos (FINER; SANTERRE, 2004; SANTERRE; SHAJI; TSANG, 1999). A maioria desses subprodutos ainda não foi identificada (ATKINSON *et al*., 2002) mas sabe-se que eles podem gerar efeitos deletérios locais ou até sistêmicos (BAKOPOULOU; PAPADOPOULOS; GAREFIS, 2009; SCHMALZ, 1998; STANLEY, 1993), e ainda serem prejudiciais aos tecidos bucais (SCHMALZ, 1998; STANLEY, 1993).

Os monômeros livres podem aumentar o risco de toxicidade (FILIPOV; VLADIMIROV, 2006) e reações imunoinflamatórias (KUAN *et al*., 2012). Componentes individuais das resinas compostas (como TEGDMA e HEMA) são capazes de gerar lesão em diversos tipos celulares (SPAGNUOLO *et al*., 2004). Dependendo do período de exposição, o TEGDMA induz apoptose ou necrose em células humanas primárias (SPAGNUOLO *et al*., 2004). O monômero Bis-GMA é genotóxico a linfócitos e pode atuar diretamente no DNA humano, causando lesão celular (DROZDZ *et al*., 2011).

Foi constatado que esses monômeros liberados, após confecção de restaurações, podem ser encontrados na saliva de pacientes (MICHELSEN *et al.,* 2012), contudo há poucos estudos *in vivo* que avaliam a quantidade de substâncias liberadas e suas reações, especialmente em relação aos tecidos periodontais (ARENHOLT-BINDSLEV *et al*., 1999; OLEA *et al*., 1996).

Regiões de lesões cervicais não cariosas (LCNC) são muitas vezes restauradas para aliviar a hipersensibilidade, evitar a perda de estrutura dentária e melhorar a estética (KUBO *et al*., 2006) e podendo-se empregar as resinas compostas (WOOD *et al*., 2008).

Devido à localização próxima ao sulco gengival, os procedimentos realizados na região das LCNCs podem alterar o volume e/ou a composição do fluido crevicular gengival (FCG) (GURGEL *et al*., 2016; MORETI *et al*., 2011; PANIZ *et al.,* 2015). O volume e a taxa de fluxo do FCG são indicadores de mudança na permeabilidade vascular, que ocorre em estágios iniciais da inflamação (BARROS *et al*., 2016). Os subprodutos da resina composta poderiam estar presentes no FCG e potencializar a inflamação.

Em 2007, novos critérios clínicos para avaliação de restaurações foram publicados no *Journal of Adhesive Dentistry* e *Clinical Oral Investigation* (HICKEL *et al*., 2010). Segundo esses critérios, a avaliação de uma restauração é categorizada em três grupos: critérios estéticos, funcionais e biológicos. Cada grupo tem subcategorias e a classificação geral é ditada pela pontuação mais grave entre todos os *subscores* (HICKEL *et al*., 2010). Este critério é mais sensível do que os critérios USPHS para avaliação clínica em curto prazo das restaurações (JASSAL, MITTAL e TEWA, 2017).

Dessa forma, é relevante avaliar o desempenho clínico das restaurações de LCNC em resina composta, a resposta inflamatória dos tecidos periodontais e a liberação de componentes lixiviáveis desse material restaurador.

1.1 Objetivos

1.1.1 Objetivo geral

Avaliar *in vivo* o desempenho clínico das restaurações de resina composta em áreas acometidas por LCNC, bem como a presença de marcadores inflamatórios no FCG, a resposta inflamatória dos tecidos periodontais e a liberação de componentes lixiviáveis desse material restaurador, para a saliva.

1.1.2 Objetivos específicos

- Avaliar o desempenho clínico das restaurações em áreas acometidas por LCNC de um sistema restaurador resinoso bulk- fill por meio dos critérios FDI ao longo de 6 meses.
- Avaliar a resposta periodontal do tecido circundante às restaurações de LCNC de um sistema restaurador resinoso bulk-fill e correspondentes não restaurados ao longo de 6 meses.
- Identificar a presença das citocinas IL-1β e IL-6 e quantificá-las no fluido crevicular gengival dos dentes restaurados com um sistema restaurador resinoso bulk-fill e nos dentes correspondentes não restaurados.
- Identificar e quantificar a presença de metacrilatos (Bis-GMA e TEGDMA) na saliva de pacientes ao longo de 6 meses.

2 METODOLOGIA EXPANDIDA

2.1 Desenho do estudo e considerações éticas

Trata-se de um estudo clínico longitudinal prospectivo, qualitativo e quantitativo. Os procedimentos clínicos foram executados na Clínica da Pós-Graduação da FO-UFMG.

Dentes anteriores e posteriores com LCNC e sensibilidade dentinária foram designados como grupo experimental e o dente correspondente como grupo controle. Com o objetivo de aumentar a precisão das comparações, os grupos experimentais e controle foram determinados no mesmo indivíduo.

As variáveis independentes investigadas foram a condição dentária (restaurado e não restaurado) e o tempo de avaliação (24 horas antes e 10 minutos após, 7 dias após, 30 dias após e 180 dias após a restauração). As variáveis–resposta analisadas foram definidas segundo os critérios FDI: brilho de superfície, descoloração de superfície e marginal, cor e translucidez, forma anatômica, fratura e retenção do material, adaptação marginal, hipersensibilidade pós-operatória, vitalidade do dente, recorrência de cárie, erosão ou abfração, integridade dos tecidos dentários, resposta periodontal e mucosa adjacente. Também foram consideradas variáveis-resposta: índice de placa visível, índice gengival, profundidade de sondagem, sangramento a sondagem, volume do FCG, concentração das interleucinas IL-1β e IL-6 no FGC e dos monômeros Bis-GMA e TEGDMA na saliva.

Dois pesquisadores ficaram responsáveis pela avaliação clínica das restaurações, sendo devidamente calibrados por outro pesquisador que já tinha experiência com a utilização do critério da equipe.
Para calibração, os examinadores observaram 40 fotografias representativas de cada pontuação para cada critério e utilizaram a ferramenta online e-calib (www.e-calib.info). Eles avaliaram 10 pacientes cada em dois dias consecutivos. Esses sujeitos que tiveram restaurações cervicais, mas foram excluídos deste projeto. Uma concordância intra e inter examinador de pelo menos 80% foi necessária antes do início. Os períodos de avaliação foram definidos da seguinte forma: 24 horas antes da restauração (T1) e 07 dias após (T2), 30 dias após (T3) e 180 dias (T4) após o procedimento de restauração. O tratamento foi realizado em dentes anteriores e posteriores, tendo um dente correspondente como controle.

Foram também avaliadas as propriedades estéticas, funcionais e biológicas de acordo com os critérios FDI (Anexo 1). Para avaliação FDI das restaurações os tempos foram de 10 minutos (T1), 07 dias (T2), 30 dias (T3) e 180 dias (T4) após o procedimento de restauração.

Os procedimentos laboratoriais foram executados nos laboratórios de pesquisa do Departamento de Odontologia Restauradora da FO-UFMG, no Laboratório de Biologia Celular do ICB-UFMG e no Instituto de Química da Unicamp. Os indivíduos, cuja necessidade de tratamento se enquadraram nos critérios de inclusão, foram convidados a participar da pesquisa e receberam devidos esclarecimentos e explicação sobre os objetivos do estudo. Aqueles que estiveram de acordo em participar do estudo assinaram o Termo de Consentimento Livre e Esclarecido (TCLE) (Apêndice A).

O projeto foi submetido ao Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais (Resolução CNS 466/2012) e aprovado sob número de CAAE: 65909417.0.0000.5149 (Anexo 2). O projeto também foi submetido e aprovado no Registro Brasileiro de ensaios clínicos e no Clinical Trial (NCT03637946-https://clinicaltrials.gov/ct2/show/NCT03637946?term=Noncarious+Cervical+Lesion+Statement. Restorations&rank=2)

2.2 Cálculo amostral

Para calcular o tamanho das amostras necessárias para comparar as variáveis entre os grupos ao longo do tempo, foi utilizada a metodologia proposta por Diggle (2002), sendo a quantidade de dentes em cada grupo dada por:

$$N = \frac{2(z_{\alpha/2} + z_{\beta})^2(1 + (n-1)\rho)}{nd^2}$$

Onde:

z_{α} é o percentil da distribuição Normal correspondente ao nível de significância

z_{β} é o percentil da distribuição Normal correspondente ao poder do teste

n é o número de medidas no tempo

ρ é a correlação entre as medidas repetidas

d é o tamanho do efeito.

Considerando um nível de significância de 5% (α = 0,05), um tamanho do efeito médio (d = 0,50), uma correlação entre as medidas repetidas de 0,50 (ρ = 0,50) e 4

medidas no tempo (n = 4), tem-se que o tamanho amostral mínimo seria de 78 dentes, sendo 39 de cada grupo. O tamanho ideal seria de 104 dentes, sendo 52 de cada grupo. Como nesse estudo foram incluídos 52 dentes do grupo experimental e 52 do grupo controle, o tamanho da amostra foi adequado (Tabela 1).

Tabela 1- Tamanho da amostra de acordo com o poder do teste.

Poder do Teste	**Tamanho da Amostra**	
	Em Cada Grupo	**Total**
80%	39	78
90%	52	104

Fonte: Elaborado pelo autor, 2017

2.3 Seleção dos participantes

A população do estudo foi selecionada por meio de triagem nas clínicas odontológicas da FO-UFMG. Foram excluídos desse estudo indivíduos que utilizaram antibióticos ou anti-inflamatórios nos últimos três meses e que realizaram restaurações em resina composta nos últimos 12 meses. Também foram excluídas gestantes e lactantes. Esses pacientes foram encaminhados para as clínicas de graduação para tratamento baseados nas necessidades de cada um. Foram selecionados 80 pacientes onde 16 foram submetidos a anamnese e exame clínico. Dos 16 pacientes, 3 foram excluídos devido ao uso de antibiótico ou anti-inflamatório nos últimos 3 meses, 2 eram fumantes e 1 desistiu de participar devido a necessidade de retorno.

Foram incluídos 10 pacientes nesse estudo maiores de 18 anos, sistemicamente saudáveis, não fumantes, com ausência de mobilidade dentária e alteração pulpar irreversível. O paciente deveria apresentar ao menos uma lesão cervical não cariosa (LCNCs) supra-gengival com necessidade de restauração. Esta lesão deveria ser não cariada, não retentiva, com profundidade de 1 mm até 3 mm, envolvendo tanto esmalte quanto dentina e apresentar hipersensibilidade. O paciente deveria apresentar também um dente natural e correspondente na mesma posição do

dente que seria restaurado, no arco oposto (controle). Com relação aos parâmetros periodontais, os dentes selecionados ainda deveriam apresentar: 1. Índice de Placa Visível (IPV) igual a 0 (AINAMO E BAY, 1975) após preparo prévio dos pacientes. Esse índice foi registrado utilizando a sonda milimetrada (Hu- Friedy, Chicago, IL, USA) após secagem da superfície dentária com ar comprimido, (ausência escore 0 ou presença escore 1). 2. Índice gengival (GI) igual a zero (LÖE E SILNESS, 1963). Esse índice avalia a existência e severidade de lesões gengivais em uma escala que varia de 0 a 3, onde: 0 é normal, 1 tem inflamação leve com mudança de cor, edema leve, sem sangramento à sondagem, 2 com inflamação moderada com tecido gengival vermelho, brilhante, edemaciado e presença de sangramento à sondagem, 3 com inflamação severa, com grande aumento de cor e edema, ulceração e tendência a sangramento espontâneo. 3 Profundidade à sondagem (PS) de 1 a 3 mm, que é a distância da margem gengival ao fundo do sulco gengival, mensurada manualmente em todos os sítios dos dentes restaurados e controle (CARRANZA E SZNAJDER ,1996; LINDHE, LANG E KARRING, 2014). 4 Sangramento à Sondagem (SS) igual a 0. Esse índice foi avaliado no momento da medida da PS até 60 segundos após a introdução da sonda, ausência de sangramento sendo considerada score 0 ou presença como score 1 (CARRANZA E SZNAJDER, 1996; LINDHE, LANG E KARRING, 2014).

2.4 Procedimentos pré-operatórios

Os dentes a serem restaurados e seus correspondentes foram avaliados individualmente. Antes do procedimento restaurador, avaliaram-se as dimensões da cavidade em milímetros (altura, largura e profundidade) com utilizando a sonda milimetrada (Hu- Friedy, Chicago, IL, USA). A partir disso o volume da cavidade foi calculado. Outras características como a presença de margem cervical em dentina, a presença ou ausência de facetas de desgaste oclusal, presença de sensibilidade dentária pré-operatória a estímulos (espontânea, o jato de água, rajada de ar) e a pressão a partir do explorador também foram conferidas e avaliadas. Fotografias foram realizadas dos dentes antes e após as restaurações e durante todos as consultas subsequentes.

Antes de receber as restaurações, os pacientes foram instruídos sobre higiene bucal para controle da placa supra gengival e receberam também os materiais para

higiene (pasta dental, escova e fio-dental). O índice de placa foi acompanhado durante todo o estudo para que os valores se mantivessem em até 80% dos sítios presentes com Índice de Placa igual a zero. Para cada paciente selecionado, 24 horas antes da restauração, considerado o tempo de controle inicial (*baseline)*, foi realizada profilaxia profissional para remover a película do biofilme dentário utilizando pasta pedra-pomes e água. Em seguida, avaliou-se a condição periodontal, seguida a coleta do FCG do dente a ser restaurado e correspondente e coleta de saliva.

Para coleta das amostras do FCG, os dentes foram isolados com rolete de algodão, a gengiva foi delicadamente seca com seringa de ar e o fluido crevicular gengival foi coletado utilizando uma fita de papel absorvente (Periopaper®, Oralflow, New York, NY, USA). A fita foi introduzida no sulco gengival, pela superfície vestibular, até encontrar resistência do tecido e mantida por 30 segundos (Figura 1). As amostras não deveriam ser contaminadas por sangue. Quando aconteceu a contaminação, a amostra foi descartada (ARIAANS *et al.*, 2015; DEINZER *et al*., 2007; ERTUGRUL *et al*., 2013; ENGEBRETSON *et al*., 2002)**.**

2.5 Materiais utilizados

O sistema restaurador utilizado no estudo está descrito na Tabela 2.

Tabela 2. Materiais que foram utilizados no estudo.

Material (Fabricante)	**Composição básica (volume)**	**Instruções do fabricante**
FL-Bond II (Shofu Inc., Kioto, Japão)	Primer: Etanol (30%), monômeros metacrilatos, água (50%), MDP, outros Adesivo: UDMA (20-30%); TEGDMA (<10%), Hidroxietilmetacrilato(10-20%), pó de vidro (30-40%)	Aplicar o primer em todas as paredes da cavidade com um pincel apropriado durante 10 segundos. Secar com leve jato de ar por mais de 5 segundos até que não mais se note movimento do primer. Aplicar o adesivo em toda a cavidade com aplicador apropriado, de modo a formar uma camada uniforme de material, com o auxílio de leve jato de ar.

		Fotoativar o adesivo por 10 segundos.
Beautifil Bulk-Fill (Shofu Inc., Kyoto, Japão)	Bis-GMA, UDMA, Bis-MPEPP, TEGDMA, S-PRG à base de vidro de fluoroboroaluminossilicato, iniciador de polimerização, pigmentos e outros.	Inserir a resina na cavidade com incrementos de até 4,0 mm. Cada incremento de resina deve ser fotoativado durante 10 segundos.

Fonte: Fabricante, 2019

2.6 Procedimentos operatórios

No dia do procedimento restaurador, a cor da resina composta foi escolhida dentre as opções universal ou A, o dente a ser restaurado foi anestesiado e foi realizado isolamento relativo do campo operatório para controle de umidade. Foi feito condicionamento seletivo do esmalte com ácido fosfórico a 37% por 40 segundos. O tratamento do substrato (esmalte e dentina), a aplicação do sistema adesivo e a inserção da resina composta seguiram as instruções do fabricante (Tabela 1). Todos os procedimentos de fotoativação foram realizados utilizando uma lâmpada LED com o aparelho Radii-Cal (SDI Inc., Victoria, Austrália) a 1200 mW/cm^2. O acabamento e o polimento das restaurações foram realizados no mesmo dia do procedimento restaurador. O acabamento foi realizado com brocas *carbide* multilaminadas, e o polimento com o Kit de desempenho Super-Snap X-Treme Ultra-Gloss (Shofu Inc., Rio de Janeiro, Brasil).

2.7 Avaliação clínica das restaurações e da resposta periodontal

As restaurações foram avaliadas seguindo os critérios FDI (Anexo 1). Avaliaram-se as propriedades estéticas: brilho de superfície, coloração de superfície e marginal, cor e translucidez e forma anatômica. As propriedades funcionais avaliadas foram: fratura e retenção do material e adaptação marginal. As propriedades biológicas avaliadas foram: hipersensibilidade pós-operatória, vitalidade do dente, recorrência de cárie, erosão ou abfração, integridade dos tecidos dentários, resposta

periodontal e mucosa adjacente. A classificação de cada propriedade foi dada pelo maior valor de cada critério e a classificação global pela pontuação mais grave das três propriedades. Para os critérios FDI das restaurações, os tempos de avaliação foram de 10 minutos (T1), 07 dias (T2) 30 dias (T3) e 180 dias (T4) após o procedimento restaurador.

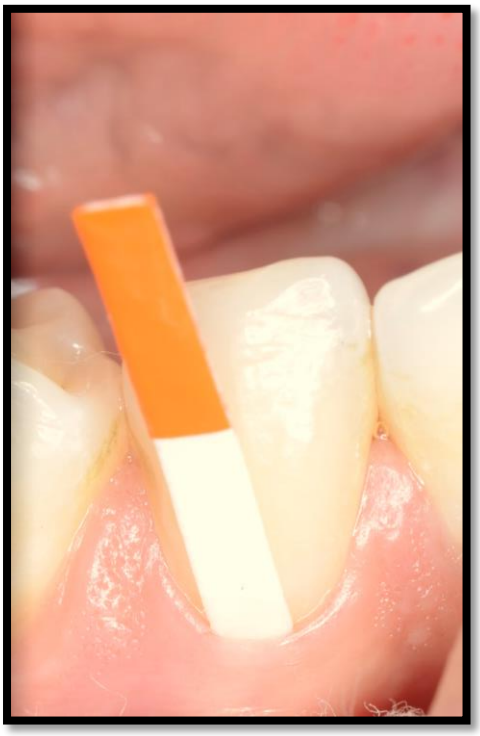
Os parâmetros periodontais adotados para avaliação do estado de normalidade do periodonto foram Ìndice de Placa, Índice Gengival, Profundidade á Sondagem e Sangramento a Sondagem conforme descrito no ítem 2.4. A resposta periodontal foi avaliada 24 horas antes (T1- *baseline*) e 10 minutos (T2), 7 dias (T3), 30 dias (T4) e 180 dias (T5) após o procedimento restaurador.

Foram obtidas imagens fotográficas digitais das restaurações (Máquina Canon™ T6i com lente macro 100 mm Canon™), em cada período de avaliação, para documentação de suas características. Adicionalmente, em todos os períodos de avaliação, amostras de FCG e de saliva não estimulada foram coletadas e armazenadas para análise.

2.7.1 Coleta das amostras do FCG (Fluido Crevicular Gengival) e quantificação do volume

Em todos os períodos de avaliação, amostras de FCG foram coletadas conforme descrito nos procedimentos pré-operatórios no ítem 2.6. Imediatamente após a coleta, o volume do FCG foi quantificado no aparelho Periotron® 8000 (Oralflow, PlainView, New York, NY, USA) (Figura 2) previamente calibrado e o dado obtido anotado para posteriormente ser aplicado a uma curva de calibração e ser convertido em volume (µl) (Figura 3). Em seguida, estas fitas foram armazenadas em tubos de plástico do tipo Eppendorf apropriados com 200 µl de solução inibidora de protease (0,1 mM de fluoreto de fenil metil sulfonil, 0,1 mM de cloreto de benzetônio, 10mM de EDTA e 0,01 mg/ml de aprotinina A) e armazenadas a -80°C para futuramente quantificar as interleucinas (ARIAANS *et al.*, 2015; DEINZER *et al*., 2007; ERTUGRUL *et al*., 2013; ENGEBRETSON *et al*., 2002).

Figura 1- Coleta das amostras do fluido crevicular gengival utilizando uma fita de papel.



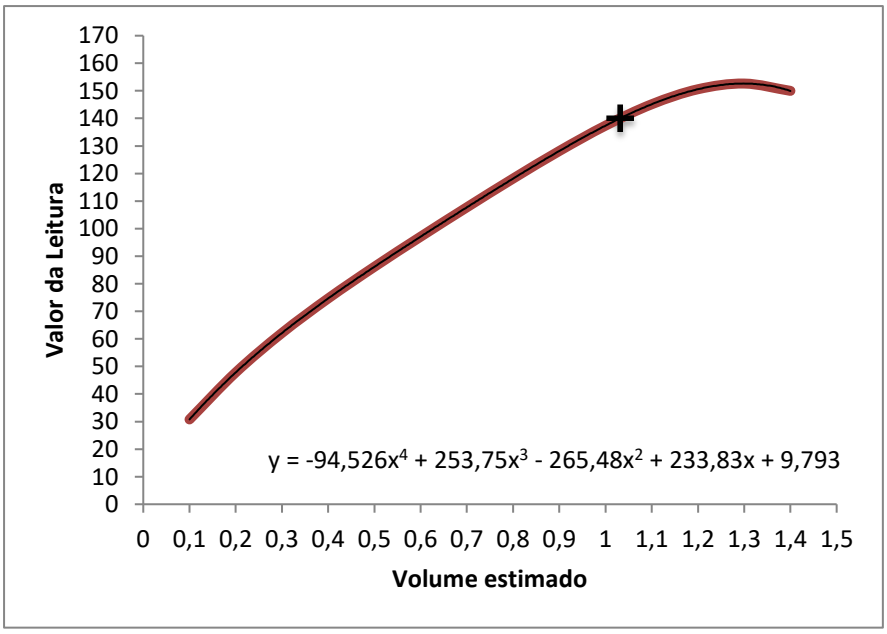
Fonte: Próprio Autor, 2018

Figura 2 - Aparelho Periotron® 8000 utilizado para mensurar o volume do fluido crevicular gengival (FCG).

Fonte: Próprio Autor, 2018

Figura 3- Curva De Calibração utilizada para transformar valores de volume no FCG.

Y = P = -------
X = V = ------- µL



Fonte: Próprio Autor, 2018

2.8 Processamento laboratorial das amostras de FCG para análise da concentração de IL-1β e IL-6

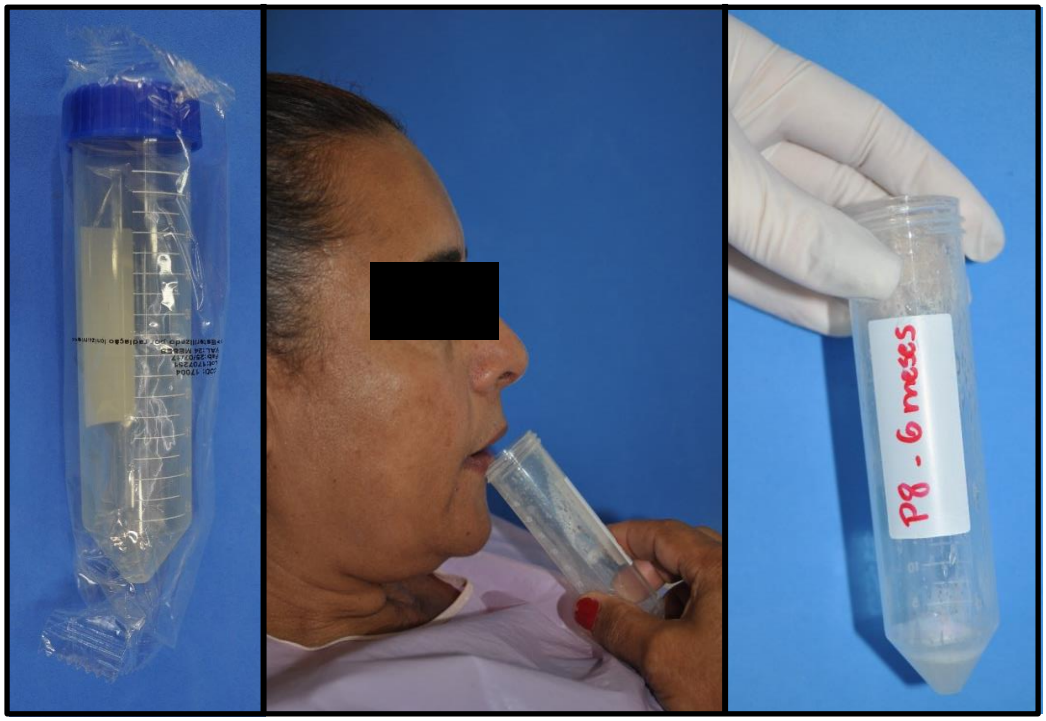
Os níveis de GCF dos kits IL-1β (Kit número: DY201-05, IL-1β / IL-1F2 DuoSet Humano e IL-6 DuoSet Humano; R & D Systems, Minneapolis, MN, EUA) e IL-6 (Número do kit: Os kits DY206-05, IL-6 DuoSet Humano e IL-6 DuoSet Humano, R & D Systems, Minneapolis, MN, EUA) foram avaliados por ELISA. Os mesmos investigadores que coletaram as amostras realizaram os ensaios. A proteína total foi medida usando o método de Bradford (BRADFORD, 1976) e a concentração de proteína total foi usada para corrigir os valores para cada amostra. Após o teste, todas as amostras foram descartadas. As concentrações das citocinas foram expressas em pg / µl (ARIAANS *et al*., 2015; DEINZER *et al.,* 2007; ENGEBRETSON *et al*., 2002ERTUGRUL *et al*., 2013).

2.9 Coleta de saliva não estimulada

As amostras de saliva não estimulada foram obtidas 24 horas antes do tratamento (baseline), e 10 minutos, 7 dias, 30 dias e 180 dias após o tratamento. As amostras foram colhidas sempre pela manhã entre 9 e 11 horas. O protocolo para a coleta e processamento de saliva foi descrito por Michelsen *et al*. (2012). O paciente ficou sentado em uma posição relaxada com a cabeça inclinada para a frente para permitir o acúmulo salivar na região anterior da cavidade bucal. O paciente, em seguida, permaneceu coletando saliva durante 5 minutos num tubo tipo Falcon plástico estéril (Figura 4). Durante a coleta, o paciente foi orientado a evitar deglutição da saliva. No final do período de 5 min, o paciente recolheu o resto da saliva na boca e foi orientado a finalizar a coleta no tubo. Os participantes foram instruídos a não comer ou beber (água era permitido) por um período de 2 horas antes coleta da saliva. Os pacientes foram orientados também a não usar batom, não mastigar goma de mascar, e não comer pastilhas ou doces antes da coleta. Após a coleta, os tubos contendo toda a saliva receberam uma quantidade igual a coletada de solução inibidora de protease (0,1 mM de Fluoreto de Fenil metil sulfonil, 0,1mM de cloreto de benzetônio, 10 mM de EDTA e 0,01 mg/ml de aprotinina A) e colocados num congelador -80˚C até posterior processamento. Foram coletadas amostras de saliva em triplicata de um indivíduo que

não sofreu restaurações dentárias ao longo de sua vida e uma amostra de água ultrapura para controle.

Figura 4- A:Tubo tipo Falcon estéril utilizado para coleta da saliva. B: Durante a coleta, o paciente foi orientado a evitar deglutição da saliva. C: Após a coleta, os tubos contendo toda a saliva foram identificados e receberam uma quantidade igual a coletada de solução inibidora de protease.



Fonte: Próprio Autor, 2018

2.10 Processamento das amostras de saliva (MICHELSEN *et al*., 2012)

2.10.1 Cromatografia líquida com espectrometria de massa

As análises da saliva foram realizadas usando combinado de cromatografia liquida com espectrometria de massa (LC/EM). Para a separação cromatográfica, foi utilizada uma Coluna NovaPack (Waters Corporation, Milford, USA) (C18 Waters 3,9x150 mm x 4 µm) com modo de eluição isocrático, volume de injeção 10 µL, vazão 0,5 mL/min e com fase móvel acetonitrila: acetato de amônio 10 mM 65:35 (v/v). Assim conseguimos determinar a presença de Bis-GMA E TEGDMA na saliva e quantifica-los.

2.10.2 Preparo das amostras

O procedimento de preparo de amostra foi adaptado da referência *"Detection and quantification of monomers in unstimulated whole saliva after treatment with resin-based composite fillings in vivo"* de Michelsen *et al*. 2012 e realizado da seguinte forma:1 mL da amostra e 1 mL de acetato de etila grau HPLC foram transferidos para um tubo falcon de 15 mL (Figura 5), agitou-se o tubo por 1 minuto em vortex (Figura 6) e centrifugou-se por 4 minutos a 3000 rpm (Figura 7). Retirou-se 0,5 mL do sobrenadante e transferiu-se para um frasco de vidro (Figura 8). Repetiu-se esse procedimento por 3 vezes. Ao final, obteve-se 1,5 mL do extrato em acetato de etila, evaporou-se sob fluxo de nitrogênio esse volume e ressuspendeu-se o extrato seco em 0,5 mL de fase móvel. A amostra foi filtrada em filtro de seringa de 0,45 µm para um *vial* e acondicionada no amostrador do cromatógrafo.

Figura 5 - Transferência das amostras para tubo Falcon.

Fonte: Próprio Autor, 2018

Figura 6 - Vórtex utilizado para preparo das amostras.

Fonte: Próprio Autor, 2018

Figura 7 - Processo de centrifugação as amostras.

Fonte: Próprio Autor, 2018

Figura 8 - Transferência para frasco de vidro

Fonte: Próprio Autor, 2018

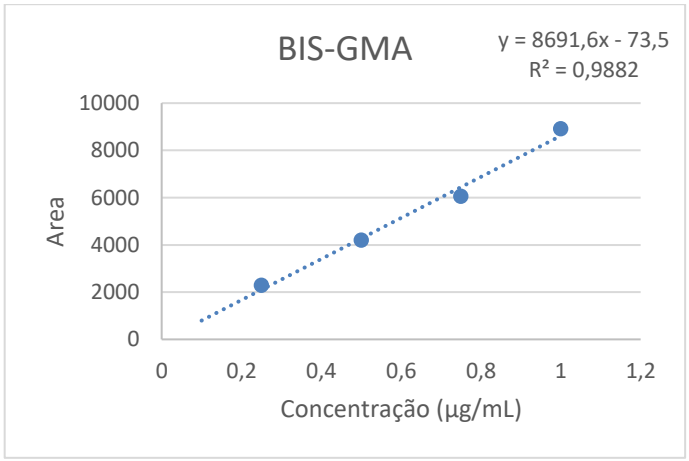
2.10.3 Preparo dos Padrões

Foram preparadas soluções estoque dos padrões de BIS-GMA e TEGDMA, em acetonitrila grau HPLC, na concentração de 10,0 µg/mL e a partir dessas, realizou-se as seguintes diluições para se obter as soluções de trabalho:

BIS-GMA (µg/mL)	TEGDMA (µg/mL)
-	0,10
0,25	0,25
0,50	0,50
0,75	0,75
1,00	1,00

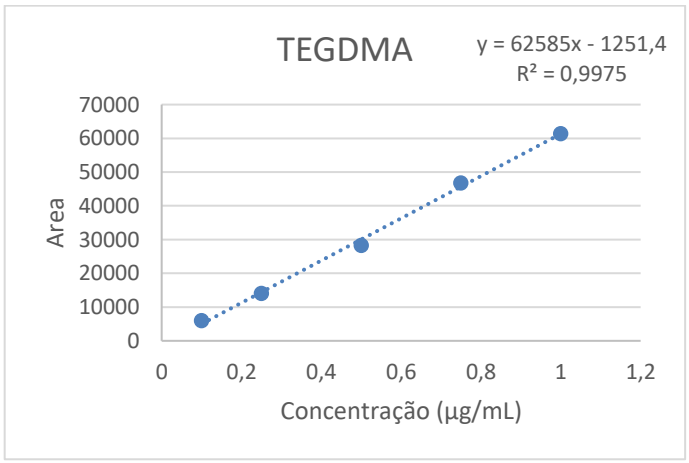
Estas soluções foram filtradas em filtro de seringa de 0,45 µm para um *vial*, acondicionada no amostrador do cromatógrafo e injetadas. Construiu-se a curva analítica utilizada para a quantificação das amostras.

Figura 9 - Curva analítica do BIS-GMA



Fonte: Próprio Autor, 2018

Figura 10 - Curva analítica do TEGDMA



Fonte: Próprio Autor, 2018

2.11 Equipe de trabalho e calibração

A equipe foi formada por cinco pesquisadores: o pesquisador responsável (T.C.M.S) ficou encarregado de selecionar os pacientes, executar os procedimentos

restauradores, avaliar a condição periodontal, coletar o FCG e a saliva. Dois pesquisadores auxiliares (C.S.M) (A.N.M) foram responsáveis por calibrar o pesquisador responsável quanto à avaliação da condição periodontal e os pesquisadores examinadores quanto aos critérios FDI. Os pesquisadores examinadores (2) (F.I.R.L) (C.S.O) foram responsáveis por avaliar os dentes em estudo, segundo os critérios FDI, durante os 4 períodos de acompanhamento.

A avaliação das restaurações (n=52) foi feita de forma independente por dois examinadores (F.I.R.L) (C.S.O) treinados, não envolvidos no processo de confecção das restaurações. Os examinadores participaram de um programa de calibração para padronizar os critérios de avaliação. Na primeira sessão de calibração, foi feito um estudo teórico dos critérios para conhecer a descrição específica das escalas a serem utilizadas. Posteriormente, houve uma demonstração da utilização dos critérios, utilizando-se fotografias de restaurações apresentando todas as categorias de interesse, em triplicata além de utilizar a ferramenta online e-calib (www.e-calib.info). Eles avaliaram 10 pacientes cada em dois dias consecutivos. Esses sujeitos que tiveram restaurações cervicais, mas foram excluídos deste projeto. Uma concordância entre os examinadores e entre o mesmo examinador (intra-examinador) de pelo menos 80% foi necessária antes do início.

2.12 Análise estatística dos resultados

A análise estatística foi realizada por um pesquisador independente. Foram realizados testes de normalidade e de homocedasticidade para determinação dos testes estatísticos. Para descrever os parâmetros clínicos em cada grupo e tempo foram utilizadas as frequências absoluta e relativa para as variáveis categóricas e a média e o erro padrão para as variáveis numéricas. Cabe ressaltar que se optou por categorizar a variável Índice Gengival, uma vez que o percentual de respostas 1, 2 e 3 foram baixos. Sendo assim, se o *score* fosse 0 existia ausência de inflamação, enquanto que se o *score* fosse 1, 2 ou 3 existia a presença de inflamação. Além disso, como a variável Profundidade de Sondagem foi medida nas suas 4 sítios (M, D, L, V), optou-se por utilizar a média das 4 medidas.

Para realizar as comparações inter e intragrupo dos parâmetros clínicos foi utilizado o teste de McNemar (Agresti, 2002) para as variáveis categóricas e o teste de Wilcoxon (Hollander e Wolfe, 1999) para as variáveis numéricas. Para comparar a

classificação do critério FDI entre os tempos foi utilizado o teste de Stuart-Maxwell (Agresti, 2002) que é uma extensão do teste de McNemar. O software utilizado nas análises foi o R (versão 3.5.0).

Para a análise da saliva, a estatística foi realizada por meio do programa SPSS versão 21.0 (Statistical Package for the Social Sciences, IBM Corp). Os dados de quantidade de monômero de Bis-GMA e TEGDMA (ug/mL) liberados ao longo do tempo foram submetidos ao teste t de Student para amostras dependentes com significância de 5% (análise do período inicial comparado aos períodos seguintes e análise de cada período em relação ao período anterior). Os dados de quantidade de monômero de Bis-GMA e TEGDMA (ug/mL) liberados ao longo do tempo e quantidade total liberada foram correlacionados com o número de restaurações e com o volume total coletado de cada restauração (mm^3) por meio da Correlação de Pearson com significância de 5%.

3 ARTIGOS CIENTÍFICOS

3.1 Artigo 1**:**

- Submetido no dia 01 de março de 2019 a revista: *Clinical Oral Investigation* (Quais A1)

Title: Inflammatory Response of the Periodontium to Bulk-fill Resin Composite Cervical Restorations

Short title: Periodontal response to cervical restorations

Authors: Tassiana Cançado Melo **Sa**, DDS, MSc[a]; Elisa Carvalho **Siqueira**, DDS, MSc[b]; Fabrício Tinôco Alvim **de Souza**, DDS, MSc[c]; Ricardo Santiago **Gomez** DDS, MSc, PhD[c] Carolina Cavaliéri **Gomes** DDS, MSc, PhD[b]; Claudia Silami de **Magalhaes**, DDS, MSc, PhD[d] Allyson Nogueira **Moreira** DDS, MSc, PhD[d]; José Eustáquio da **Costa** DDS, MSc, PhD[c], Monica **Yamauti**, DDS, MSc, PhD[e] ✉

Affiliations: a PhD Candidate, Graduate School of Dentistry, Universidade Federal de Minas Gerais, tassianacancado@yahoo.com.br; Av. Pres. Antônio Carlos, 6627 - Pampulha Campus. CEP: 31270-901. Belo Horizonte - MG, Brazil

b Department of Pathology, Biological Sciences Institute, Universidade Federal de Minas Gerais. elisadesiqueira@hotmail.com, carolinacgomes@ufmg.br; Av. Pres. Antônio Carlos, 6627 - Pampulha Campus. CEP: 31270-901. Belo Horizonte - MG, Brazil

c Department of Clinical Dentistry, Pathology and Surgery, Faculty of Dentistry, Universidade Federal de Minas Gerais. rsgomez@ufmg.br, ftadesouza@gmail.com, costa@odonto.ufmg.br; Av. Pres. Antônio Carlos, 6627 - Pampulha Campus. CEP: 31270-901. Belo Horizonte - MG, Brazil

d Department of Restorative Dentistry, Faculty of Dentistry, Universidade Federal de Minas Gerais. dangelogatil@terra.com.br, silamics@yahoo.com.br; Av. Pres. Antônio Carlos, 6627 - Pampulha Campus. CEP: 31270-901. Belo Horizonte - MG, Brazil

e Department of Restorative Dentistry, Graduate School of Dental Medicine, Hokkaido University, myamauti@gmail.com

✉ Corresponding author: Monica Yamauti
Department of Restorative Dentistry, Graduate School of Dental Medicine, Hokkaido University, Sapporo, Kita-ku, Kita 13, Nishi 7, 060-8586, Japan, Telephone: +81-11-7064261, Fax: +81-11-706-4878, Email: myamauti@gmail.com

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Abstract

Objectives: To assess the clinical response to bulk-fill composite resin restorations according to periodontal response and FDI criteria for up to 180 days. *Materials and Methods*: Fifty-two restorations where placed in non-carious cervical lesions using a bulk-fill Giomer-based composite resin. The corresponding teeth were non-restored group. The clinical and biochemical parameters were evaluated 24 hours before the restoration's placement and 7, 30 and 180 days after the procedure. The clinical parameters evaluated were visible plaque index (VPI), probing depth (PD), bleeding on probing (BOP), and gingival index (GI). The volume of the GCF was measured, and ELISA evaluated the levels of IL-1β and IL-6. The results were analyzed using McNemar and Wilcoxon tests. *Results*: Restored and non-restored groups exhibited a significant difference of the VPI between 24 hours before the restoration and the remaining times. The BOP, PD and GI were significantly higher on restored-group after 180 days ($p<0.05$). There was no difference ($p>0.05$) of the GCF volume and the cytokines levels between the restored and the non-restored groups at any of the times ($p > 0.05$). All restorations showed satisfactory esthetic, functional and biological properties. *Conclusion*: The BOP, PD, and GI periodontal clinical parameters seem to be more pronounced around the teeth after restorative treatment using Giomer bulk-fill composite resin comparing with non-restored group. *Clinical relevance*: Giomer cervical restorations did not affect the GCF volume and cytokines IL -Iβ and IL-6 levels but increased BOP, PD, and GI.

Keywords: Composite resins, clinical trials, inflammation, chemotactic cytokines, periodontium.

INTRODUCTION

The term “non-carious cervical lesion” (NCCL) describes the loss of hard dental tissue at the cement-enamel junction (CEJ) without loss of tissue by caries [1]. The NCCL may be restored for protection against loss of more dental structure, aesthetics, or elimination of tooth sensitivity [2]. The use of a composite resin with an appropriate modulus of elasticity (similar to that of the dental structure), together with an adhesive system, may be an effective and nondestructive mean of restoring NCCL [3].

The release of methacrylate monomers, such as TEGDMA, BisGMA, and UDMA [4], associated with by-products generated after polymerization, has been considered as the source of biological reactions such as toxicity and tissue reactions [5]. The results with cell cultures have shown that the methacrylate monomers can affect the recruitment of leukocytes at sites of inflammation [6], inducing the enzymatic activity and the expression of growth factors and cytokines [7]. The release of these monomers and bisphenol A (BPA) [8] has been related from polymerized bulk-fill materials into the oral environment [9-11]. BPA is known to act as an estrogen receptor agonist and can thus cause endocrine disruption in addition to being toxic to cells [8].

The GIOMER-based resin composite contains glass ionomer pre-reacted glass particles (S-PRG) as a source of fluoride release, which releases and recharges fluoride, [12] helping to prevent caries and biofilm formation [13]. However, restorative materials may cause different reactions in the oral soft tissues such as gingiva [14]. No clinical trial related to these reactions about GIOMER bulk-fill resin composite is available. The periodontal parameters may be used as aid in the diagnosis of inflammation in the gingival tissue. The collection and analysis of gingival crevicular fluid (GCF), as well as cytokines’ levels are indicators of the early stages of inflammation [15]. IL-1β and IL-6 play an important role in the regulation and amplification of the inflammatory response in periodontal tissues [16-17]

The objective of this study was to evaluate the clinical response of NCCL restorations using GIOMER bulk-fill composite resin, according to FDI criteria and periodontal response, for up to 180 days (6 months). This study tested the null hypothesis that GIOMER does not affect periodontal clinical parameters, the volume of the GCF, and cytokines IL-Iβ and IL-6 levels.

MATERIALS AND METHODS

This was an interventional, single-center, non-blinded clinical trial. The teeth to be restored were called as the restored group (R) (n = 52), and the control group (corresponding teeth) was called as the non-restored group (N). The evaluation periods were defined as follows: 24 yours before the restoration (T1) and 07 days (T2), 30 days (T3) and 180 days (T4) after the restoration procedure.

The study was approved by the University Ethics Committee (protocol number 2.131.913) and followed the ethical principles established in the Declaration of Helsinki. It was registered as clinical trial (NCT03637946- https://clinicaltrials.gov/ct2/show/NCT03637946?term=Non-carious+Cervical+Lesion+ Statement. Restorations&rank=2).

Sample size

To calculate the sample size to compare the variables between groups over time, the methodology proposed by Diggle (2002) [18] was used, and the number of teeth in each group was given by:

$$N = \frac{2(z_{\alpha/2}+z_{\beta})^2(1+(n-1)\rho)}{nd^2},$$

where z_α is the percentile of the normal distribution corresponding to the level of significance, z_β is the percentile of the normal distribution corresponding to the power of the

test, *n* is the number of measurements in time, ρ is the correlation between the repeated measurements, and *d* is the size of the effect. Considering a significance level of 5% ($\alpha = 0.05$), a mean effect size ($d = 0.50$), a correlation between the repeated measurements and 0.50 ($\rho = 0.50$), and 4 measurements in the time ($n = 4$), it is estimated that the minimum sample size would be 78 teeth, 39 of each group (80 % of test power). The ideal size would be 104 teeth, 52 of each group (90% of test power).

Recruitment

Sixteen individuals who were interested in participating in the research as advertised through posters on the university campus were recruited. All evaluations and procedures were conducted at the Faculty of Dentistry Clinics. Eleven individuals were included. The inclusion criteria were to be over 18 years of age, systemically healthy, nonsmokers, presenting NCCL, of at least 1 mm and up to 3 mm deep and supragingival margins, which needed to be restored because of the presence of dentin hypersensitivity as the main complaint, and have healthy periodontal tissue. The healthy periodontal tissue was described using the following criteria: plaque index - PI (score 0), bleeding on probing – BOP (score 0), probing depth - PD (from 1 to 3 mm at sites that will receive the restoration), and gingival index - GI (score 0). Exclusion criteria included history of systemic diseases, the use of drugs that can influence periodontal health, having received periodontal treatment within the last 6 months or having used antibiotics and anti-inflammatory drugs within the last 3 months, pregnant and lactating women, and who had performed other restorations in the last 12 months.

Clinical procedures

The restorative systems used in the study were the adhesive system FL-Bond II (Shofu Inc., Kyoto, Japan) and the composite resin Beautifil-Bulk (Shofu Inc., Kyoto, Japan). The same operator (T.C.M.S) performed all restorations.

Twenty-four hours before the procedure, the cavity was cleaned with a paste of pumice and water to remove residual dental biofilm. On the day of the restorative procedure, the color of the material was chosen, the area of the tooth to be restored was anesthetized, and the relative isolation of the operative field for moisture control was performed. Cavities were selectively acid-etched by applying FL Bond II Etchant to the enamel margins (10 s) and after, were rinsed with air–water spray for 10 s and dried avoiding over drying. FL-Bond II primer was applied in the all substrates with a micro-brush for 10 seconds, followed by gentle air-drying for more than 5 seconds until no further movement of the primer noticed. The FL-Bond II adhesive was applied throughout the cavity with an appropriate applicator to form a uniform layer of material with the aid of a light jet of air and polymerization for 10 s. The Beautifil-Bulk resin composite was applied following in one increment and polymerized for 10 s. During acid conditioning, it was taken care that it had no contact with the periodontal tissue in the restored teeth. All photoactivation procedures used an LED lamp with the Radii-Cal apparatus (SDI Inc., Victoria, Australia, 1200 mW/cm^2). Excess restorative material was removed with scalpel (blades #15 and #12). Finishing and polishing of the restorations was performed on the same day of the restorative procedure. When necessary, finishing was done using multilayer carbide burs. For polishing, Super- Snap X-Treme Ultra-Gloss Performance Kit was used (Shofu Inc., Rio de Janeiro, Brazil).

Periodontal examination and gingival crevicular fluid sampling

Restored and non-restored sides were examined and scored for clinical parameters including visible plaque index (VPI), probing bleeding (PB), probing depth (PD), gingival

index (GI) (Table 1) and GCF volume. To increase reliability, the same examiner performed all clinical measurements. The clinical parameters were evaluated 24 hours before the restorative treatment and 7 days, 30 days, and 180 days after the restorative treatments in both, restored and non-restored sides. In this way, each tooth from the restored group would also have its initial evaluation for comparison.

For collection of the GCF samples, any clinically detectable biofilm was removed without touching the gingiva to minimize the contamination of the absorbent paper filters with blood. After being isolated with cotton rolls, the gingiva was gently dried with air syringe, and the GCF was collected using an absorbent paper strip (Periopaper®, Oraflow Inc., Plainview, NY, USA). The strip was carefully and gently introduced inside the gingival margin until soft resistance was felt, and it was kept in place for 30 seconds. In case of blood-contamination, strips were discarded.

The GCF volume was quantified in the Periotron® 8000 device (Oraflow Inc., Plainview, NY, USA). These tapes were then be stored in appropriate Eppendorf type plastic tubes with 200 μl of protease inhibitor solution (0.1 mM phenyl methyl sulfonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA, and 0.1 mM, 01 mg / ml aprotinin A) and stored at -80°C for subsequent assays. Periotron calibration (Periotron® 8000, Oraflow Inc., Plainview, NY, USA) was performed for the determination of the GG volume using bovine fetal fluid as vehicle. They were expressed by the unit (μl).

IL-1β and IL-6 cytokine levels assessment

The GCF levels of IL-1 β (Kit number: DY201-05, Human IL-1β / IL-1F2 DuoSet and Human IL-6 DuoSet kits; R&D Systems, Minneapolis, MN, USA) and IL-6 (Kit number: DY206-05, Human IL-6 DuoSet and Human IL-6 DuoSet kits; R&D Systems, Minneapolis, MN, USA) were assessed by ELISA. The same investigators that collected the samples

performed the assays. Total protein was measured using the Bradford method [19] and the total protein concentration was used to correct the values for each sample. After testing, all samples were discarded. The concentrations of the cytokines were expressed in pg/µl.

Clinical analysis of restorations

Restorations were evaluated according to FDI criteria [20] following aesthetic, functional and biological properties of the restorations. The aesthetic criteria were surface and brightness, coloring surface, color of margin, color and translucency, and anatomical shape. The functional parameters were material fracture and marginal adaptation. The biological criteria included hypersensitivity, enamel and dentin caries, tooth integrity, and adjacent mucosa. The most severe score among all the variables that compose it formed the overall classification of each criterion. Digital photographic images of the restorations were obtained in each evaluation period to document their characteristics.

Statistical analysis

The normality of the data distribution and the homogeneity of groups' variances were verified by the Kolmogrov-Smirnov and Levene tests, respectively. For the categorical variables, the McNamar test was used, and for the numerical variables, Wilcoxon test was employed. For all tests, $p<0.05$ was considered statistically significant, and calculations were performed using R software (version 3.5.0) (R Inc., Vienna, Austria)

RESULTS

Clinical parameters

Figure 1 illustrates the patient flow in the course of the study. Ten individuals who were interested in participating were recruited and 52 restoration were performed. All evaluations

and procedures were conducted at the dentistry faculty clinics. Participants' age ranged from 30 to 60 (n=10; age average 47.9 years). Clinical periodontal measurements are outlined in Tables 2 and 3.

Both restored and non-restored groups exhibited a significant difference ($p<0.05$) of the VPI between 24 hours before the restoration and the remaining times (Figure 2).

Tables (2-3) and figures (2-3) showed the non-restored group with a difference ($p<0.05$) in the presence BOP and GI between 24 hours before the restoration and 6 months and between 6 months and the other times. Additionally, there was a significant difference in non-restored group ($p<0.05$) in PD 24 hours before with 1 month and 6 months.

Tables (2-3) and figures (2-3) presents additionally the restored group with a significant difference ($p<0.05$) in the presence of BOP between 24 hours before the restoration and 6 months, and between 6 months and other times. In the restored group, there was a significant difference ($p<0.05$) in PD between 24 hours before and 6 months, between 7 days and 6 months and 1 month and 6 months.

There was a significant difference between restored and non-restored groups on BOP ($p<0.001$), PD ($p=0.010$) and GI ($p<0.001$) after 6 months (Figures 2 and 3). The BOP, PD and GI were higher on restored-group after 6 months.

The non-restored group showed no significant difference ($p>0.05$) in the GCF volume between the times. However, the restored group shows a difference in the GCF volume between the times between 7 days and 1 month. No significant differences ($p>0.05$) within different group (restored *versus* non-restored) at any of the times (Table 3).

Table 4 presents the comparison of the FDI criteria according to each evaluation period.

Cytokines IL-1β and IL-6

The non-restored group showed a significant reduction ($p<0.05$) on IL-1β cytokines levels from 24 hours before the restoration and the other times. In the restored group, there was a significant reduction in IL-1β cytokines between 24 hours and 6 months and between the times 7 days and 6 months and 1 month and 6 months. The non-restored group showed a progressive reduction ($p<0.05$) in IL-6 cytokines from 24 hours before and 6 months. In the restored group, there was a significant reduction in IL-6 cytokines between the times 24 hours before and 1 month and between 1 month and 6 months. Nevertheless, there was no significant difference ($p>0.05$) for cytokines levels between the restored *versus* non-restored at any time (Table 3, Figure 3).

DISCUSSION

Leachable components released from composite resin can induce inflammation [21-22] and may cause different reactions in the gingiva [14]. The GIOMER-based materials have demonstrated antiplaque effect [13], and it seems that the elution of S-PRG fillers may be associated to decreased inflammation of gingival tissue. It is attributed to this category of materials a suppressive effect on *P. gingivalis* and disturbance of the formation of advanced multi-strain bacterial communities in the periodontal environment [13]. Even so, a negative effect of composite resin has been associated with subgingival biofilm composition [23].

The dental biofilm and the irritation caused by restorations may lead to inflammation of the gingival tissue [24]. The present results show that the restored group presented higher VPI and a significant difference of the VPI between 24 hours before the restoration and 7 days, 1 and 6 months after the restorative procedures. On the other hand, the non-restored group also showed an increase in VPI in the same periods of evaluation. Nevertheless, most of the non-restored teeth had NCCLS and has already been described a high prevalence of plaque NCCL

compared to adjacent teeth [1]. There are also reports in literature about VPI enlargement [25] or decrease [26-27] after NCCLs restorations.

Another important factor about restorations in close contact with the gingival tissues is that they may accelerate and increase the biofilm formation in case of the absence of good polishing or smoothness state [28]. Furthermore, the results of this study at 6-months evaluation showed that BEAUTIFIL-bulk restorations resulted in acceptable clinical performance, and all resin restorations presented an excellent or good classification concerning the aesthetic, functional, and biological properties according to the FDI criteria. However, a recent article using USPHS criteria in class V has shown a fail of one restoration due to loss of retention after 6 months. However, after 1 year, both bulk-fill flowable and regular nanofilled composites showed good clinical results and the frequency of clinically unacceptable rates was 3.3% for anatomical form and 1.1% for retention, and 2.2% for marginal adaptation [29].

Although resin composite restorations are described as factors that induce inflammation [21-22], it has been reported the non-association between periodontal parameters (PD, VPI and GI) and restorative materials (resin-modified glass-ionomer cement and composite resin) [30-31]. The results of the present study suggest that the restored teeth showed an increase of presence of bleeding, PD, and presence of GI (score 1, 2, or 3) when compared to the non-restored teeth. It is important to emphasize that despite the increase of PD; the average values were considered normal (1 to 3 of depth) [24]. In the current study, following 6 months of the restorative procedures, there were signs of gingival inflammation in the restored group. On the other hand, an increase in GI and plaque index has been reported after 15 days the placement of the restorations using silorane-based composite resin [25]. This increase has been attributed to the chemical content of the composite resin and increase of the biofilm [25]. Differently in other study, a significant decrease on plaque index and gingival index has been found, as well

as similar results in the non-restored control group [27]. This decrease has been attributed to the insertion of the restoration and the successful education about oral hygiene [27].

The measurement of GCF volume is used as an indicator of change in vascular permeability [32]. An increase in the volume of GCF is positively associated with the degree of gingival inflammation [33]. GCF increase has been related to patients without restorations [34], with NCCL [35], with cervical caries [36], with restorations with resin [25,27] or glass ionomer [27,37] or ceramics [38,39]. Another study reported that composite resins for Class V had no effect on GCF volume [40]. In our study, there was no significant difference of the GCF between the restored and the non-restored groups at any of the evaluation periods. There has been a small increase in restored-group between 07 days and 01 month, but our results suggest that neither Beautifil-Bulk nor the presence of biofilm were able to generate an increase in GCF after 6 months.

Cytokines are mediators of cellular functions and inflammatory responses. IL-1β and IL-6 play an important role in the regulation and amplification of the inflammatory response in periodontal tissues [16-17]. Changes in cytokines IL-1β or IL-6 levels have been reported in patients presenting innumerous situations: without restorations [16], with restorations using composite resin [25,27,37,40], using glass ionomer [27,37] and with ceramics [41]. Even though dental resin monomers are toxic to human gingival fibroblasts and keratinocytes, they cannot induce IL-1β release from these cells [42]. Furthermore, it has been reported that Class V composite resins restorations had no effect on IL-1β levels from the GCF [40]. In the present study, there was no significant difference for cytokines IL-1β and IL-6 levels between the restored and the non-restored groups at the evaluation periods. However, high IL-6 levels in the GCF were reported in the restored teeth [25,27]. The authors suggested that the release of inflammatory mediators may lead to not only plaque but also compounds released from the

materials and that the differential release of cytokines may be associated with differences in the composition of the GCF or the individual's biological response [25,27].

According to our results, it is still precocious to attribute the responsibility of a negative biological periodontal response only to the GIOMER-based restorative system. A long-term evaluation in which the association between the release of cytokines and the presence/quantity of monomers and other components should be conducted

CONCLUSIONS

The GIOMER Beautifil-Bulk resin composite used to restore NCCL did not affect the GCF volume, cytokines IL-1β and IL-6 levels in the sample evaluated. However, the periodontal clinical parameters BOP, PD, and GI seem to be more pronounced around the restored teeth. The results of the present study suggest that restoration of NCCL may affect the periodontal clinical parameters.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

No funding was received. Shofu Dental Corporation only provided the restorative materials and polishing systems used in the study.

Ethical approval

All procedures performed in this study followed the ethical principles and standards of the Helsinki Declaration of 1964, and was approved by the University Ethics Committee (Process nº 2.131.913). The study was registered as a clinical trial (NCT03637946).

Informed consent

Informed consent was obtained from all individual participants included in the study

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Figure captions

Fig. 1 Diagram of participants flow

Fig. 2 Periodontal clinical parameters results. (a) Percentage of presence of plaque index; (b) bleeding on probing; (c) gingival index. Different lower-case letters indicate significant differences within the same group in different times ($p<0.05$). Different upper-case letters indicate significant differences between groups (Restored *versus* Non-Restored) in different times

Fig. 3 Periodontal clinical parameters results (a) mean of probing depth (mm); (b) gingival grevicular fluid volume (µl); (c) concentration of IL-1β (pg/µl); (d) concentration of IL-6 cytokine (pg/µl). Different lower-case letters indicate significant differences within the same

group in different times ($p<0.05$). Different upper-case letters indicate significant differences within different group (Restored *versus* Non-Restored) in different times

Table 1 Periodontal clinical parameters used to control and experimental sides

CRITERIA	MEANS OF EVALUATION
Visible Plaque Index (VPI)	Presence (score 1) or absence (score 0)
Bleeding on Probing (BOP)	Until 30 to 60 seconds after the introduction of the probe with dichotomic values by presence (score 1) or absence (score 0)
Probing on Depth (PD)	Distance from the gingival margin to of the gingival sulcus (manual circumferential probing)
Gingival index (GI) of Löe and Silness (1963) [43] modified	Gingival index (GI) of Löe and Silness from 0 to 3 considering 0 as normal. The score 1 with mild inflammation with color change, mild edema, no bleeding at probing. The score 2 with moderate inflammation with red, shiny, swollen gingival tissue and presence of bleeding on probing. The score 3 with severe inflammation, with great increase of color and edema, ulceration and tendency to spontaneous hemorrhage. The values were dichotomous by presence of inflammation (score 1) or absence (score 0)

Table 2 Inter and intragroup comparison of the categorical clinical parameters

Clinical Parameters			Non-Restored					Restored					C x E
			N	%	Valor-p*			N	%	Valor-p*			Valor-p*
					24 h	7 d	1 m			24 h	7 d	1 m	
Visible Plaque Index (VPI)	24 yours before	Absence	52	100,0%	-	-	-	52	100,0%	-	-	-	1,000**
		Presence	0	0,0%				0	0,0%				
	7 days after	Absence	9	17,3%	**0,000**	-	-	5	9,6%	**0,000**	-	-	0,386
		Presence	43	82,7%				47	90,4%				
	1 month after	Absence	2	3,8%	**0,000**	**0,023**	-	3	5,8%	**0,000**	0,480	-	1,000
		Presence	50	96,2%				49	94,2%				
	6 months after	Absence	4	7,7%	**0,000**	0,182	0,683	2	3,8%	**0,000**	0,450	1,000	0,683
		Presence	48	92,3%				50	96,2%				
Bleeding on Probing (BOP)	24 yours before	Absence	52	100,0%	-	-	-	48	92,3%	-	-	-	0,134
		Presence	0	0,0%				4	7,7%				
	7 days after	Absence	52	100,0%	1,000**	-	-	48	92,3%	1,000**	-	-	0,134
		Presence	0	0,0%				4	7,7%				
	1 month after	Absence	51	98,1%	1,000	1,000	-	48	92,3%	1,000**	1,000**	-	0,371
		Presence	1	1,9%				4	7,7%				
	6 months after	Absence	39	75,0%	**0,001**	**0,001**	**0,001**	20	38,5%	**0,000**	**0,000**	**0,000**	**0,000**
		Presence	13	25,0%				32	61,5%				
Gingival Index (GI)	24 yours before	Absence	52	100,0%	-	-	-	48	92,3%	-	-	-	0,134
		Presence	0	0,0%				4	7,7%				
	7 days after	Absence	52	100,0%	1,000**	-	-	48	92,3%	1,000**	-	-	0,134
		Presence	0	0,0%				4	7,7%				
	1 month after	Absence	51	98,1%	1,000	1,000	-	48	92,3%	1,000**	1,000***	-	0,371
		Presence	1	1,9%				4	7,7%				
	6 months after	Absence	39	75,0%	**0,001**	**0,001**	**0,001**	13	25,0%	**0,000**	**0,000**	**0,000**	**0,000**
		Presence	13	25,0%				39	75,0%				

*McNemar test; ** The p-value was equal to 1000 since the values were the same

Table 3 Intergroup and intragroup comparison of the numerical clinical parameters

Clinical Parameters		Non-Restored group					Restored group					N x R
		Mean	S.E***	Valor-p			Mean	S.E***	Valor-p*			Valor-p*
				24 h	7 d	1 m			24 h	7 d	1 m	
Probing Depth (PD)	24 hours before	2,161	0,041	-	-	-	2,248	0,044	-	-	-	0,122
	7 days after	2,214	0,044	0,076	-	-	2,250	0,044	1,000	-	-	0,559
	1 month after	2,243	0,042	**0,011**	0,343	-	2,248	0,044	1,000**	1,000	-	0,980
	6 months after	2,238	0,040	**0,004**	0,412	0,802	2,399	0,044	**0,012**	**0,015**	**0,012**	**0,010**
Gingival Crevicular Fluid Volume (GCF)	24 hours before	1,136	0,104	-	-	-	1,180	0,095	-	-	-	0,838
	7 days after	0,917	0,115	0,600	-	-	1,214	0,096	0,253	-	-	0,204
	1 month after	1,052	0,100	0,265	0,659	-	1,045	0,091	0,311	**0,026**	-	0,992
	6 months after	1,176	0,108	0,699	0,231	0,389	1,126	0,095	0,809	0,191	0,074	0,533
Concentrations of Cytokine IL1-β	24 hours before	5,399	1,795	-	-	-	1,865	0,301	-	-	-	0,172
	7 days after	1,691	0,255	**0,004**	-	-	1,625	0,276	0,267	-	-	0,563
	1 month after	1,957	0,444	**0,001**	0,753	-	1,625	0,335	0,207	0,695	-	0,409
	6 months after	0,069	0,013	**0,000**	**0,000**	**0,000**	0,045	0,007	**0,000**	**0,000**	**0,000**	0,061
Concentrations of Cytokine IL-6	24 hours before	0,535	0,128	-	-	-	0,173	0,029	-	-	-	0,213
	7 days after	0,142	0,027	**0,028**	-	-	0,157	0,028	0,216	-	-	0,601
	1 month after	0,155	0,039	**0,022**	0,105	-	0,099	0,020	**0,009**	0,087	-	0,611
	6 months after	0,130	0,022	0,108	0,563	**0,030**	0,118	0,014	0,486	0,895	**0,047**	0,859

*Wilcoxon test; **The p-value was equal to 1000 since the values were the same, ***Standard Error.

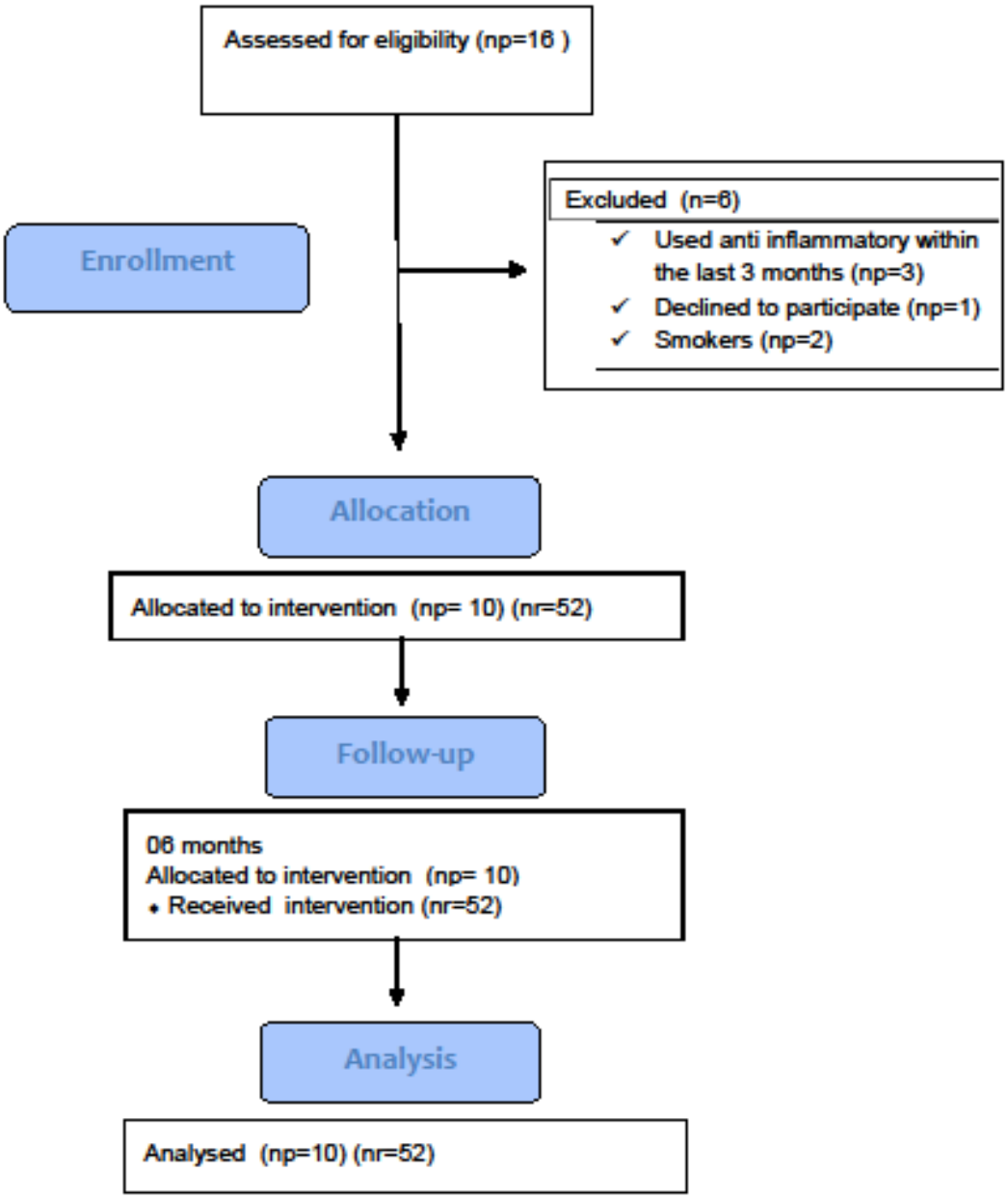
Table 4 Comparison of the classification of the complete FDI criteria between times

FDI Criteria		Excellent		Good		Satisfactory		Insatisfactory		Poor		General (%)		Times
		N	%	N	%	N	%	N	%	N	%	Satis.	Fail	p-value*
Esthetic Properties	10 minutes after	15	28,8%	32	61,5%	5	9,6%	0	0,0%	0	0,0%	100,0%	0,0%	-
	7 days after	19	36,5%	33	63,5%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,244
	1 month after	24	46,2%	27	51,9%	1	1,9%	0	0,0%	0	0,0%	100,0%	0,0%	**0,023**
	6 months after	12	23,1%	40	76,9%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,149
Functional Properties	10 minutes after	47	90,4%	5	9,6%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
	7 days after	47	90,4%	5	9,6%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
	1 month after	45	86,5%	7	13,5%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,736
	6 months after	34	65,4%	18	34,6%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	**0,011**
Biological Properties	10 minutes after	22	42,3%	30	57,7%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
	7 days after	20	38,5%	32	61,5%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,736
	1 month after	15	28,8%	37	71,2%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,136
	6 months after	12	23,1%	40	76,9%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,080

* Stuart-Maxwell test; ** The p-value was equal to 1000 since the values were the same.

Figure captions

Fig.1 Diagram of participants flow



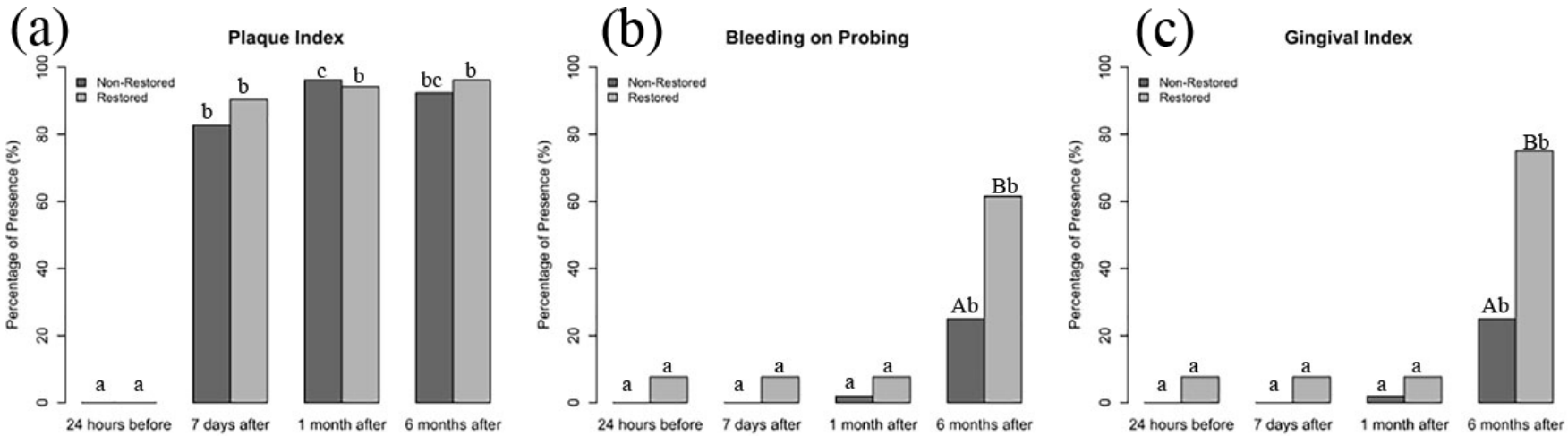


Fig. 2 Periodontal clinical parameters results. (a) Percentage of presence of plaque index; (b) bleeding on probing; (c) gingival index. Different lower-case letters indicate significant differences within the same group in different times ($p<0.05$). Different upper-case letters indicate significant differences between groups (Restored *versus* Non-Restored) in different times

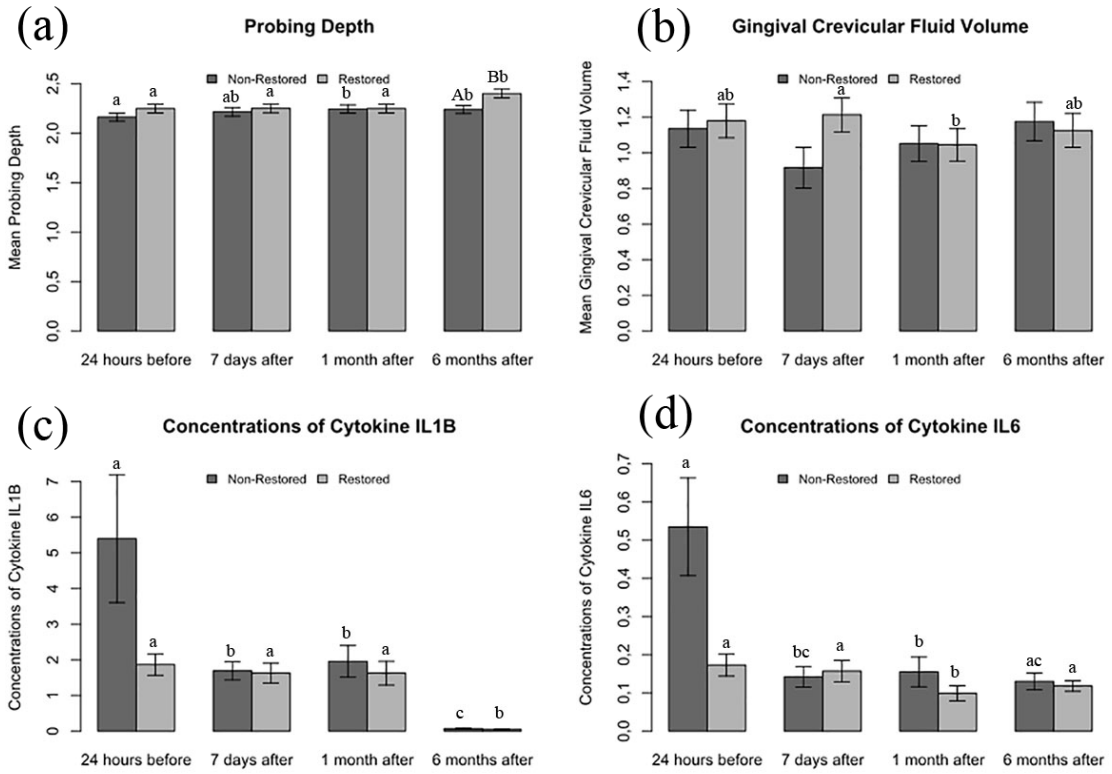


Fig. 3 Periodontal clinical parameters results (a) mean of probing depth (mm); (b) gingival grevicular fluid volume (µl); (c) concentration of IL-1β (pg/µl); (d) concentration of IL-6 cytokine (pg/µl). Different lower-case letters indicate significant differences within the same group in different times ($p<0.05$). Different upper-case letters indicate significant differences within different group (Restored *versus* Non-Restored) in different times.

3.2- Artigo 2

Os dados obtidos foram compilados na forma de artigo a ser submetido ao periódico Operative Dentistry (Qualis A1). O texto a seguir encontra-se nas normas desse periódico. Após as correções sugeridas pela banca examinadora durante a defesa, o artigo será alterado e enviado a revista. As normas do periódico podem ser acessadas em: https://www.jopdent.com/authors/authors.php.

Title: Clinical performance of a bulk-fill composite resin in non-carious cervical lesion: long-term monomer elution

Running title: Clinical performance of bulk-fill resin restorations

Clinical Relevance: The clinical performance of LCNC restorations of a bulk-fill resin restorative system was considered satisfactory over 6 months. Monomers can be elution from a long-term period follow-up. Long-term elution and subsequent chronic exposure to monomers should be observed when assessing the overall human health risks.

Summary

Objective: Assess the performance of restorations according to the FDI criteria up to 6 months and to quantify the Bis-GMA and TEGDMA monomers of uncured resin release.

Methods and Materials*:* Fifty-two restorations where placed in non-carious cervical lesions (NCCLs), using a bulk-fill Giomer-based composite resin. The clinical parameters were evaluated 10 minutes, 7 days, 30 days, and 180 days after the procedure. For saliva, there was an extra evaluation 24 hours before the procedure. The saliva was collected for 5 minutes in a sterile plastic falcon type tube. The results were analyzed using Stuart-Maxwell test ($p < 0.05$) for FDI criteria. For monomers, the amount of Bis-GMA and TEGDMA monomer released over time were subjected to Student's t-test and Pearson Correlation ($p < 0.05$) was carried to monomer released over time and total amount released were correlated with the number of restorations and total volume.

Results: At 6-months follow-up, the GIOMER-based bulk-fill resin composite used for restoring NCCLs resulted in acceptable clinical performance. No Bis-GMA was detected in saliva samples collected before, 01 and 06 months after and there was no statistical difference between the periods analyzed. There was no correlation between the number

of restorations and time and the elution of Bis-GMA and TEGDMA over the 6-month analysis period ($p < 0.05$). The mean TEGDMA elution before the restoration was statistically lower than after 10 minutes. The TEGDMA monomers were detected in the saliva in 08 samples collected after 10 minutes, 06 samples after 07 days and 30 days , and in 02 samples after 06 months.

Conclusion: The clinical performance of LCNC restorations of a bulk-fill resin restorative system was considered satisfactory over 6 months. The mean TEGDMA release before the restoration was (0.0052 μg / mL) was statistically lower than after 10 minutes (0.0365 μg / mL).

KEYWORDS: composite resins, methacrylates, periodontium, clinical trial.

INTRODUCTION

Non-carious cervical lesions (NCCLs) may need professional intervention due to the possibility of further caries development, fractures, hypersensitivity, aesthetic and biofilm retention. The clinical resolution of NCCLs is very challenging due to their inner features and etiological factors. Interventions can be performed through periodontal surgeries, non-restorative protocols - such as occlusal therapies, use of desensitizing agents and lasertherapy - or restorative protocols[1]. The last one can be done using resin composites, ceramics or glass ionomer cement. The glass ionomer cements present the benefit of being a rechargeable reservoir that releases fluoride in month[2] and similar elastic modulus to dentin. However, it has some drawbacks such as limited aesthetic and low wear resistance.[3] Restorations with composite resin is the most frequently treatment, based on their excellent esthetic properties, improved adhesive capacity, and good mechanical properties.[2] However, composite resin materials can cause adverse reactions in some patients[4] and failures on bond strength.[5] Yet, they show characteristics as polymerization shrinkage and abrasion coefficient.[6] Those poor properties can cause subsequent decay and low longevity of the restorations.

A category of hybrid aesthetic restorative adhesive material called GIOMER has been introduced (Shofu Dental Corp., Kyoto, Japan, 2000). This type of materials is a bulk-fill composite that are mainly characterized by their enhance depth of cure allowing thick increments (4–5 mm) to be placed in a single step instead of the conventional layering technique.[7] It contains a stable glass-ionomer phase on a glass core, which

induces an acid-base reaction between fluoride containing glass and polycarboxylic acid in the presence of water.[8] Differently from other fluoride-releasing restorative materials, the presence of these pre-reacted glass filler (S-PRG) in resin matrix, allows the material to release and recharge fluoride.[8] They also provide the release of many ions, such as sodium ions, silicate ions, aluminum ions, fluoride ions, borate ions and strontium ions. The ions release implicates in multiple biological functions like anti-biofilm effect and modulation of saliva pH. Moreover, the aesthetic, light transmission, diffusion and fluorescence properties are well comparable to natural teeth.[8-9]

Resin-based dental materials are not inert in the oral environment, and may release components, initially due to incomplete polymerization, and later due to degradation[10]. The elution of methacrylate monomers, associated with the by-products generated after the polymerization, has been considered as the source of biological reactions such as toxicity and tissue reactions.[11] Released monomers can increase antibody production and alter the immune system in oral tissues[12] negatively affecting the biocompatibility of restorative systems. Small low molecular weight monomers, such as TEGDMA, diffuse easily and at a higher rate compared to bulkymolecules with a rigid structure, such as Bis-GMA and Bis-EMA monomers[13]. Long-term elution and subsequent chronic exposure to monomers from resin-based dental materials should not be neglected when assessing the overall human health risks[14]. The addition of the polyacrylic acid, i.e. Giomer materials, can decrease elution of the monomers due to the increase of the conversion rate of the monomers[15].

As different materials are introduced, i.e. GIOMER, it is important to assess the biological response of oral tissues. However, no clinical study has yet reported the clinical effectiveness GIOMER-based resin composites concerning the elution of monomers. The aims of the present study were to assess the performance of restorations according to the FDI criteria up to 6 months and to quantify the monomers of uncured resin release. The first null hypothesis was that the restorations would fill all the FDI criteria. The second null hypothesis was that there would be no release of monomers from the GIOMER-based bulk-fill resin composite.

METHODS AND MATERIALS

This was an interventional, single-center, non-blinded clinical trial. The evaluation times were defined as follows: 10 minutes (T1), 7 days (T2), 1 month (T3) and

6 months (T4) after treatment. For saliva, there was an extra evaluation 24 hours before the procedure (T0). The study was approved by the University Ethics Committee (protocol number 2.131.913) and followed the ethical principles established in the Declaration of Helsinki. It was registered as clinical trial (NCT03637946-https://clinicaltrials.gov/ct2/show/NCT03637946?term=Non-carious+Cervical+Lesion+Statement. Restorations&rank=2).

Sample size

To calculate the sample size to compare the variables between groups over time, the methodology proposed by Diggle (2002)[16] was used, and the number of teeth in each group was given by:

$$N = \frac{2(z_{\alpha/2}+z_{\beta})^2(1+(n-1)\rho)}{nd^2},$$

where z_α is the percentile of the normal distribution corresponding to the level of significance, z_β is the percentile of the normal distribution corresponding to the power of the test, n is the number of measurements in time, ρ is the correlation between the repeated measurements, and d is the size of the effect. Considering a significance level of 5% ($\alpha = 0.05$), a mean effect size ($d = 0.50$), a correlation between the repeated measurements and 0.50 ($\rho = 0.50$), and 4 measurements in the time ($n = 4$), it is estimated that the minimum sample size would be 78 teeth, 39 of each group (80 % of test power). The ideal size would be 104 teeth, 52 of each group (90% of test power) (Table 1).

Eligibility Criteria

A total of sixteen individuals were examined by two calibrated dental students to determine if they met the inclusion and exclusion criteria. The evaluations were performed using a mouth mirror, an explorer, and a periodontal probe. We included 10 individuals. Participants had to be over 18 years of age, systemically healthy, nonsmokers, presenting NCCL, of at least 1 mm and up to 3 mm deep and with gingival supragingival margins. Exclusion criteria included history of systemic diseases, the use of drugs that can influence periodontal health, having received periodontal treatment within the last 6 months or having used antibiotics and anti-inflammatory drugs within the last 3 months, pregnant and lactating women, and who had performed other restorations in the last 12 months.

Clinical procedures

The restorative system used in the study was the 2-step self-etching fluoride releasing adhesive FL-Bond II (Shofu Dental Corp., Kyoto, Japan), and the composite resin Beautifil-Bulk (Shofu Dental Corp., Kyoto, Japan). The materials are described in Table 2. The same operator (T.C.M.S) performed all restorations.

Before restoration, teeth were cleaned with a suspension of pumice and water. To restorative procedure, the color of the material was chosen, the area of the tooth to be restored was anesthetized and the relative isolation of the operative field for moisture control was performed. The treatment of the substrate (enamel and dentin), the application of the adhesive system and the insertion of the composite resin followed the manufacturer's instructions (Table 2). No cavity preparation and just etching of the enamel[17] with a phosphoric acid was performed. All photoactivation procedures used an LED lamp with the Radii-Cal apparatus (SDI Inc., Victoria, Australia, 1200 mW/cm^2). Excess restorative material was removed with scalpel (blades #15 and #12). When necessary, finishing and polishing of the restorations was performed on the same day of the restorative procedure. Finishing was done using multilayer carbide burs. For polishing, Super- Snap X-Treme Ultra-Gloss Performance Kit was used (Shofu Inc., Rio de Janeiro, Brazil).

Collection of non-stimulated saliva after the restorative procedures

Samples of non-stimulated saliva were always collected in the morning between 9 and 11 hours. The protocol for the collection and processing of saliva was described by Michelsen *et al*. (2012).[18] The patient then continued to collect saliva for 5 minutes in a sterile plastic falcon type tube. After collection, tubes containing all saliva were covered with a thin layer of aluminum foil and placed in a -80˚C freezer until further processing. Samples of saliva were collected in an individual who did not undergo dental restorations throughout their life for control.

Clinical evaluation of restorations

Two experienced and calibrated dentists that were not involved with the restoration procedures performed the evaluation. For calibration, the examiners observed 40 photographs that were representative of each score for each criterion and used the online e-calib tool (www.e-calib.info). They evaluated 10 patients each on two consecutive days. These subjects that had cervical restorations but were excluded of this

project. An intra-examiner and inter-examiner agreement of at least 80% was necessary before the beginning.

The clinical analysis of restorations was performed according to FDI criteria (n = 52)[19]. The overall classification of each criterion was formed by the most severe score among all the variables that compose it. Digital photographic images of the restorations were obtained in each evaluation period to document their characteristics. The volume of the restorations was calculated based on the height versus the width and depth of the cavity.

Processing of saliva samples

The sample preparation procedure was adapted from the reference *"Detection and quantification of monomers in unstimulated whole saliva after treatment with resin-based composite fillings in vivo"* by Michelsen *et al*. 2012.[18] It was carried out as follows 1 mL of the sample and 1 mL of HPLC grade ethyl acetate were transferred to a 15 mL falcon tube, the tube was vortexed for 1 minute and centrifuged for 4 minutes at 3000 rpm. 0.5 mL of the supernatant was removed and transferred to a glass vial. This procedure was repeated 3 times. At the end, 1.5 ml of the ethyl acetate extract was obtained, the volume was evaporated under nitrogen flow and the dried extract was resuspended in 0.5 ml of mobile phase. The sample was filtered through a 0.45 μm syringe filter into a vial and packed in the chromatograph sampler.

Liquid Chromatography with mass spectrometry

Saliva analyzes were performed using combined liquid chromatography with mass spectrometry (LC / MS). For the chromatographic separation, a NovaPack (Waters Corporation, Milford, USA) column (C18 Waters 3.9x150 mm x 4 μm) was used with isocratic elution mode, 10 μL injection volume, 0.5 mL / min flow and with mobile phase acetonitrile: ammonium acetate 10 65:35 (v / v). Thus we were able to determine the presence of Bis-GMA and TEGDMA in the saliva and quantify them. Stock solutions of the BIS-GMA and TEGDMA standards were prepared in HPLC grade acetonitrile at the concentration of 10.0 μg / mL and from these the following dilutions were performed to obtain the working solutions:

BIS-GMA (µg/mL)	TEGDMA (µg/mL)
-	0.10
0.25	0.25
0.50	0.50
0.75	0.75
1.00	1.00

These solutions were filtered on a 0.45 µm syringe filter into a vial, wrapped in the chromatograph sampler and injected. The analytical curve used for the quantification of the samples was constructed (Figures 1 and 2).

Statistical analysis

To FDI criteria, data was analyzed using R software (version 3.5.0) (R Inc., Vienna, Austria). The normality of the data distribution and the homogeneity of groups' variances were verified by the Kolmogrov-Smirnov and Levene tests, respectively. It was used the Stuart-Maxwell test. To saliva, data were entered SPSS software version 21.0 (Statistical Package for the Social Sciences, IBM Corp.). The amount of Bis-GMA and TEGDMA monomer released over time were subjected to Student's t-test for dependent samples. Pearson Correlation was carried out to explore the amount monomer released over time and total amount released were correlated with the number of restorations and total volume. All tests were carried out at $\alpha = 0.05$.

RESULTS

Ten 10 patients were selected, and 52 restorations were performed. Participants' age ranged from 30 to 60 (47.9 years). All patients returned for 6-month and the recall rate was 100%. The tests of both intra-examiner and inter-examiner agreement resulted in Cohen's Kappa statistics of 0.81 and 0.82 respectively.

At baseline (10 minutes after the restorative procedure) for esthetic criteria 28.8% of the restorations were classified as excellent and 61.5% as good and 9.6% as satisfactory and after 6 months 23.1% were classified as excellent and 76, 9% as good. For the functional criteria, 90.4% were classified as excellent and 9.6% as good, and after 6 months 65.4% were classified as excellent and 34.6% as good. For the biological criteria

and baseline 42.3% were classified as excellent and 57.7% as good. After 6 months, for the biological criteria, 23.1% were classified as excellent and 76.9% as good.

Table 3 presents the comparison properties 'performance between the evaluation times. The properties were evaluated as excellent or good classification. The aesthetic criteria: there was a significant difference (p-value = 0.023) from the classification of between 10 minutes after restoration and 1 month after restoration, with 1 month after the percentage of excellent scores increased from 28.8% to 46.2%. In addition, there was also a significant difference (p-value = 0.0273) between the evaluation periods of 1-month and 6-months. At 6-months evaluation, the percentage of excellent scores went from 46.2% to 23.1% while the percentage of good ratings went from 51.9% (1 month) to 76.9% (6-months). Related to functional criteria there was a significant difference (p-value = 0.011) between the evaluation of 10-minutes and 6-months after the restoration's placement. At 6-months, the "excellent" percentage went from 90.4% to 65.4%, while the percentage of "good scores" went from 9.6% to 34.6%. In addition, there was also a significant difference (p-value = 0.0113) between the evaluation periods of 7-days and 6-months; and at 6 months, the percentage of "excellent scores" went from 90.4% to 65.4%, while the percentage of "good scores" went from 9.6% to 34.6%. Related to biological criteria: there was no significant difference (p-value> 0.050) between the scores at different evaluation periods (Figure 3).

The Bis-GMA and TEGDMA were quantified in the samples (ug/mL) and the results obtained are presented in the Tables 4 and 5. Samples of saliva were collected in an individual who never did any dental restorations throughout their life and all controls were negative. The samples were stored in the same conditions and periods of time as the samples of the patients of the study. When the elution of Bis-GMA was evaluated during the 6-month period of analysis, no statistical difference was observed between the periods analyzed (p >0.05). There was no correlation between the number of restorations and the elution of Bis-GMA over the 6-month analysis period (p >0.05). No monomers were detected in the saliva samples collected before, 01 and 06 months after, and only 1 sample taken after 10 minutes and 2 samples after 7 days. TEGDMA elution was evaluated over the 6-month analysis period, the mean TEGDMA before the restoration (0.0052 μg/mL) was statistically lower than after 10 minutes (0.0365 μg/mL) (p = 0.042). For the comparison between the other periods, no statistical difference was observed (p <0.05). Regarding the correlation there was no correlation between the number of restorations and the elution of TEGDMA in the other periods of analysis (p <0.05). The TEGDMA

monomers were detected in the saliva in 08 samples collected after 10 minutes, 06 samples after 07 days and 30 days, and in 02 samples after 06 months.

DISCUSSION

The proper material choice according to the need of patients associated with the characteristics of the materials is crucial for the restorations´ longevity. The composite resin showed good clinical performance over 22 years with 1.5% and 2.2% annual failure rate and for in posterior restorations show a good survival, with annual failure rates of 1.8% at 5 years and 2.4% after 10 years of service.[20] The NCCL can be restored using resin composites, ceramics or glass ionomer cement.[3] When choosing a restorative material, it is very important to consider its potential to reduce the risk of new caries lesions.[21] Fluoride-releasing materials demineralization rate is reduced and the remineralization process is promoted.[22] Furthermore, the operator should consider either the benefit/loss ratio of the restorative materials, especially in shallow and/or non-retentive cavities. [23] The clinical performance of resin composite and glass ionomer cements is controversial regarding the restoration of NCCLs. Some studies report superiority of glass-ionomer,[2,24] others advocate for composite resin,[25-26] and a recent systematic review describes similar clinical performance between both materials. [27]

The 6-months clinical evaluation showed that Beautifil-Bulk restorations resulted in acceptable clinical performance, and all resin restorations presented an excellent or good classification concerning the aesthetic, functional, and biological properties according to the FDI criteria. Concerning the bulk type of resin composite, a systematic review indicates similar clinical performances of that material and conventional resin composite over 72 months[28]. The results of the present study showed that Beautifil-Bulk restorations resulted in no failure on retention up to 6-months evaluation, which agree with ADA guidelines. According to the American Dental Association guidelines, retention rates at six months must be at least 95% for provisional acceptance and the ideal is 5 years of follow-up for retention.[29] Similar retention rate of other GIOMER-based material (Beautifil II) has been reported after 1-year evaluation; none of the restorations were completely lost.[30] Differently, composite resin restorations of non-carious cervical lesions have shown failure of one restoration due to loss of retention after 6 months using regular nanofilled composite.[30] In addition, when self-etch adhesives are used, it is

recommended to selectively acid-etch the enamel to improve retention of resin composite materials and consequently increase restorations longevity. [17,31] Therefore, no cavity preparation but enamel selective etching was performed in this study.

Usually, the use of rubber dam is recommended when resin composite restorations are placed. However, in the present study, no rubber dam was used. The reason for not using rubber dam was to avoid compromising the periodontal evaluation. The use of cotton rolls /retraction cord showed to be more favorable than the use of rubber dam in terms of patient's acceptance, gingival damage, chairside time, and retention rates of adhesive restorations in NCCLs.[32] Occurrence of gingival damage has been reported due to the retention of the rubber dam into the gingival sulcus.[33,34] Moreover, some very low-quality evidence suggesting that rubber dam usage in dental direct restorative treatments may lead to a lower failure rate of the restorations, compared with the failure rate for cotton roll usage.[35]

Concerning monomers elution, the results from this clinical evaluation is coherent with some studies.[10,18,36-39] Elution from bulk-fill resin-composites, as GIOMER-bulk, is comparable to that of conventional materials despite their increased increment thickness. Monomer elution is highly dependent on the hydrophobicity of the base monomers and the final network characteristics of the resin-matrix.[37] Elution from bulk-fill resin-composites is also highly dependent on the molecular weight of the eluted compounds .[37] Bis-GMA (hydrophobic high molecular weight molecule) was not detected neither in saliva samples collected before the restorative procedure nor in those samples collected 01 and 06 months after restorations. Bis-GMA was only detected in three samples collected at 10 minutes and 7 days. The low/non-detectable mount of released Bis-GMA is in accordance to previous studies *in vitro* .[40,41] The result of low Bis-GMA elution could be attributed to the accuracy and sensibility of the measuring instrument (HPLC) or to the high molecular weight of this monomer, that could hinder and delay the elution. [13,42-44] TEGDMA was the predominant eluted monomer on this study, presenting significant amounts after 10 minutes of the restoration. TEGDMA is potentially harmful to human health as it affects production of proinflammatory cytokines and may interfere with the homeostasis between the immune system and the indigenous microflora in the oral cavity.[45]

In contrast, *In vivo*, monomers from the investigated resin-based composite (Z250, 3M) were present in saliva shortly after treatment (Bis-GMA, HEMA, TEGDMA, and UDMA).[18] Monomers were not detected in saliva samples collected before treatment,

or 24 h or 7 d after treatment. The composition of the resin part of the material, as given by the manufacturer, was (V/V): TEGDMA 1– 5%, Bis-GMA 1–5%, Bis-EMA 5–10%, and UDMA 5–10% .[18] However, our results were different. The main reason is because the composition of material used on this studies is different. Basically, according to the manufacture the Giomer bulk composite has in their composition Bis-GMA, UDMA, Bis-MPEPP and TEGDMA.

Composite materials continued to release certain monomers after a long incubation period *in vitro* .[10,14] The effect of residual monomer on degradation shown to accelerate the reduction of molecular weight and mass loss of the material.[45] Clearly, the *in vivo* presence of biofilm is just one of the factors that may stimulate surface degradation, other factors being acidic fluid intake, temperature fluctuations, or simply the presence of an aqueous environment.[46] Neutrophils can also degrade methacrylate resin monomer, cured resin composite and demineralize dentin, thus potentially affecting the resin-dentin interface.[47] Hansel *et al*. 1998 suggested that especially the release of TEGDMA from composite resins may enhance the growth of cariogenic bacteria, like *mutans streptococci*, organisms found mostly along the margins of composite fillings.[48] The nature of the salivary enzymes as esterase activity is also potentially involved in the biochemical breakdown of composite resins used in the production of dental composite fillings.[49] Several researchers demonstrated that in human saliva, the enzymatic activity of hydrolase reaches levels high enough to degrade composite resin monomers .[50-51]

Some of the limitations of this study were short evaluation period (6-months), reduced number of participants, assessment of only two monomers and lack of evaluation of ions-release from the material. Therefore, long-term follow-ups with a large sample size are needed to allow more understanding on the clinical behavior of bulk-fill composites materials as well as to assess the release of components from resin composites to saliva.

Conclusion

At 6-months follow-up, the GIOMER-based bulk-fill resin composite used for restoring NCCLs resulted in acceptable clinical performance. There was no correlation between the number of restorations and time and the elution of Bis-GMA and TEGDMA over the 6-month analysis period .The elution of Bis-GMA was not considered significant

over 6 months, however the release of TEGDMA was significant only 10 minutes after the restoration was performed.

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Table 1: Sample size according to the power of the test.

Test Power	Sample Size	
	In Each Group	Total
80%	39	78
90%	52	104

Table 2: Materials Compositions, and Application Mode.

Materials	Composition	Application Mode
FL-Bond II (Shofu Inc., Kyoto, Japão)	Primer: Ethanol (30%), methacrylate monomers, water (50%), MDP, others Adhesive: UDMA (20-30%); TEGDMA (<10%), Hydroxyethylmethacrylate (10-20%), glass powder (30-40%)	Apply primer to all cavity walls with an appropriate brush for 10 seconds. Dry with a light jet of air for more than 5 seconds until no further movement of the primer is noticed. Apply the adhesive throughout the cavity with an appropriate applicator to form a uniform layer of material with the aid of a light jet of air. Photoactivate the adhesive for 10 seconds.
Beautifil Bulk (Shofu Inc., Kyoto, Japão)	Bis-GMA, UDMA, Bis-MPEPP, TEGDMA, S-PRG filler based on fluoroboroaluminosilicate glass, polymerization	Insert the composite resin into the cavity in increments up to 4.0 mm. Each resin increment should be photoactivated for 10 seconds.

	initiator, pigments and others.	

Table 3: Comparison of the classification of the complete FDI criteria between time.

	FDI CRITERIA		Excellent		Good		Satisfactory		Insatisfactory		Poor		General (%)		Times
			N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**Satis.**	**Fail**	**Valor-p***
Esthetic Properties	**Surface gloss/luster and roughness**	10 minutes after	43	82,7%	4	7,7%	5	9,6%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	47	90,4%	5	9,6%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,244
		1 month after	48	92,3%	4	7,7%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,736
		6 months after	41	78,8%	11	21,2 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,184
	Surface staining	10 minutes after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		1 month after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		6 months after	46	88,5%	6	11,5 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,199
	Marginal staining	10 minutes after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		1 month after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		6 months after	44	84,6%	8	15,4 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,092
	Color match/stability and translucency	10 minutes after	48	92,3%	4	7,7%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	50	96,2%	2	3,8%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,736
		1 month after	47	90,4%	5	9,6%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,995
		6 months after	44	84,6%	8	15,4 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,736
	Anatomic form	10 minutes after	18	34,6%	34	65,4 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	21	40,4%	31	59,6 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,558
		1 month after	25	48,1%	26	50,0 %	1	1,9%	0	0,0%	0	0,0%	100,0%	0,0%	0,168
		6 months after	26	50,0%	26	50,0 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,255
Functional Properties	**Fracture of restorative material**	10 minutes after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**

	and restoration retention	1 month after	50	96,2%	2	3,8%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,736
		6 months after	44	84,6%	8	15,4 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,092
	Marginal adaptation	10 minutes after	47	90,4%	5	9,6%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	47	90,4%	5	9,6%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		1 month after	47	90,4%	5	9,6%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		6 months after	39	75,0%	13	25,0 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,092
Biological Properties	**Postoperative sensitivity and tooth vitality**	10 minutes after	51	98,1%	1	1,9%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	49	94,2%	3	5,8%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,736
		1 month after	51	98,1%	1	1,9%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		6 months after	48	92,3%	4	7,7%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,558
	Enamel secondary caries	10 minutes after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000[2]
		1 month after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		6 months after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
	Dentin secondary caries	10 minutes after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		1 month after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		6 months after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
	Tooth integrity	10 minutes after	22	42,3%	30	57,7 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	21	40,4%	31	59,6 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,910
		1 month after	15	28,8%	37	71,2 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,136
		6 months after	13	25,0%	39	75,0 %	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	0,118
	Localized reactions of soft tissue	10 minutes after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	-
		7 days after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		1 month after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**
		6 months after	52	100,0%	0	0,0%	0	0,0%	0	0,0%	0	0,0%	100,0%	0,0%	1,000**

*Stuart-Maxwell's test; ** The p-value was equal to 1,000 due to the fact that the values were exactly the same;

Table 4: Bis-GMA monomer in samples (ug/mL), Total Volume (mm^3) and number of restorations in each patient.

Patient	n	Total Volume (mm^3)	Before	10 min	7 days	1 month	6 months
1	05	100,000	0,000	0,000	0,000	0,000	0,000
2	02	21,500	0,000	0,000	0,000	0,000	0,000
3	07	114,000	0,000	0,309	0,000	0,000	0,000
4	07	35,500	0,000	0,000	0,000	0,000	0,000
5	05	50,000	0,000	0,000	0,051	0,000	0,000
6	04	38,000	0,000	0,000	0,000	0,000	0,000
7	04	36,000	0,000	0,000	0,000	0,000	0,000
8	08	93,500	0,000	0,000	0,040	0,000	0,000
9	03	13,000	0,000	0,000	0,000	0,000	0,000
10	07	33,000	0,000	0,000	0,000	0,000	0,000

Table 5: TEGDMA monomer in samples (ug/mL). Total Volume (mm^3) and number of restorations in each patient

Patient	n	Volume Total (mm^3)	Before	10 min	7 days	1 month	6 months
1	05	100,000	0,013	0,039	0,010	0,000	0,013
2	02	21,500	0,013	0,015	0,014	0,015	0,023
3	07	114,000	0,000	0,056	0,014	0,000	0,000
4	07	35,500	0,000	0,130	0,000	0,000	0,000
5	05	50,000	0,000	0,020	0,020	0,044	0,000
6	04	38,000	0,000	0,012	0,014	0,028	0,000
7	04	36,000	0,014	0,076	0,000	0,016	0,000
8	08	93,500	0,000	0,000	0,012	0,011	0,000
9	03	13,000	0,012	0,000	0,000	0,000	0,000
10	07	33,000	0,000	0,017	0,000	0,010	0,000

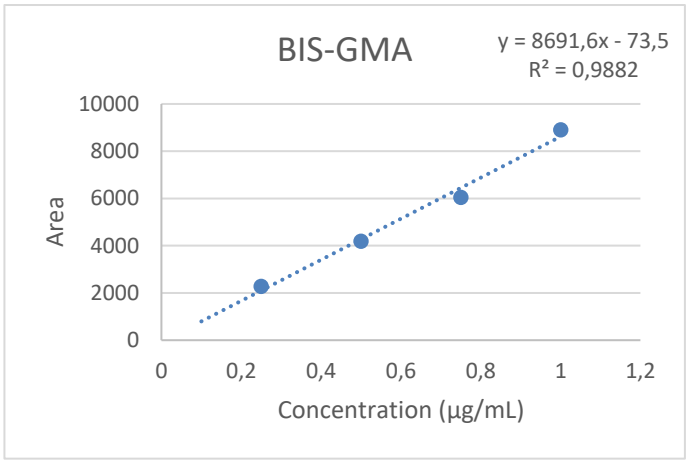

Figure 1: BIS-GMA analytical curve

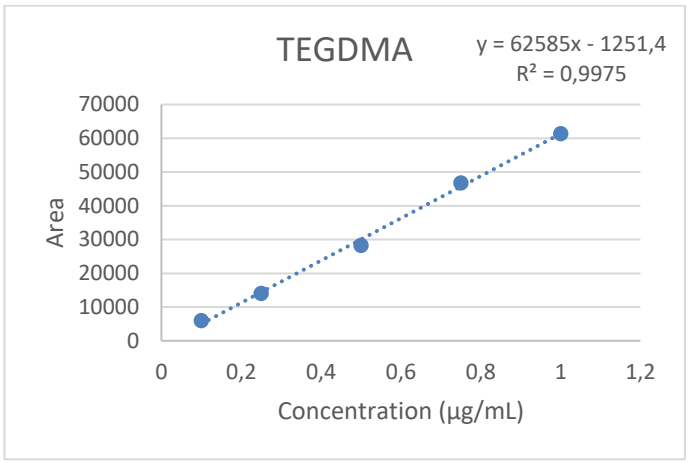

Figure 2: TEGDMA analytical curve

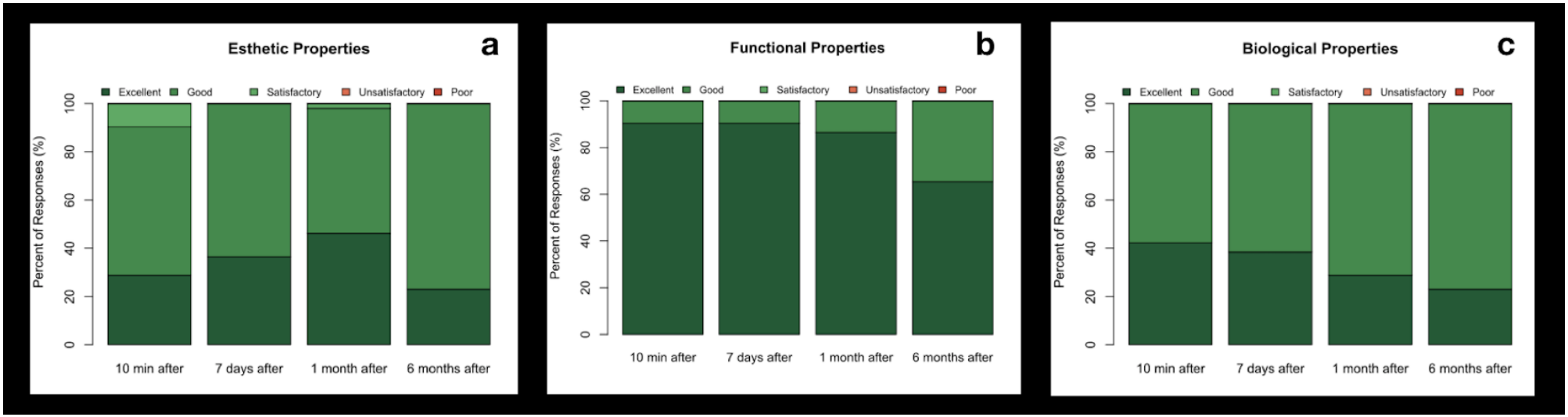

Figure 3: Comparison of the classification of the FDI criteria between times; a: esthetic properties, b: functional properties and c: biological properties

4 CONSIDERAÇÕES FINAIS

Dentro das limitações do estudo, pode-se considerar os seguintes aspectos: o desempenho clínico das restaurações de LCNC de um sistema restaurador resinoso bulk- fill por meio dos critérios FDI foi considerado satisfatório ao longo de 6 meses. Considerando a resposta periodontal do tecido circundante frente às restaurações de LCNC, os resultados do presente estudo sugerem que a restauração de LCNC pode afetar os parâmetros clínicos periodontais. Além disso, foi observado que o material restaurador resinoso bulk-fill não causou alteração estatisticamente significante no volume de FCG e nas citocinas IL-1β e IL-6 ao redor de dentes restaurados na amostra avaliada. A liberação de Bis-GMA não foi considerada significante ao longo de 6 meses, entretanto a liberação de TEGDMA foi expressiva apenas 10 minutos após a execução da restauração.

Algumas das limitações deste estudo e dificuldades encontradas foram: período curto de avaliação e análise de apenas dois monômeros devido ao elevado custo. Portanto, *follow-ups* de longo prazo se fazem necessários para maximizar a compreensão sobre o comportamento clínico de resinas compostas GIOMER bulk-fill. A restauração depende também da habilidade e do conhecimento do operador em indicar e manipular o material, e controlar os fatores relacionados a restauração. O paciente, por sua vez, é responsável pela manutenção da higiene oral e por isso precisa ter acompanhamento constante para evitar que as restaurações sejam comprometidas.

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APÊNDICE - Termo de Consentimento Livre e Esclarecido

Convido o/a Sr.(a):_____, a participar da pesquisa "LIBERAÇÃO DE COMPONENTES ORGÂNICOS DE UM SISTEMA RESTAURADOR À BASE DE METACRILATOS: Estudos *In vitro* e *In vivo"* sob a responsabilidade dos pesquisadores: Mônica Yamauti , Allyson Nogueira Moreira e Tassiana Cançado. Nesta pesquisa vamos avaliar as substâncias liberadas por um tipo de resina usada para restaurar os dentes. **A pesquisa contribuirá para que a resina e a reação que provoca nos tecidos sejam melhor entendidas e assim novas medidas possam melhorar a qualidade de vida dos pacientes e beneficiar pacientes futuramente de acordo com os resultados.** A avaliação será feita ao longo do tempo por meio da coleta de sua saliva e do fluido da gengiva **para serem estudados em laboratório**. Em consulta, que será marcada com antecedência, o seu dente será anestesiado, quando necessário, e preparado para receber a restauração, de acordo com as técnicas adequadas. A sua saliva e fluido da gengiva será coletada durante 5 minutos em um frasco, 24 horas antes do início do tratamento e 7 dias, 1 mês e 6 meses após o tratamento. **Os riscos envolvidos na pesquisa envolvem o risco de desconforto e constrangimento durante a coleta dos dados. Porém, a coleta será feita em local reservado e tranquilo e você pode deixar de responder a qualquer pergunta se não se sentir à vontade.** Para participar dessa pesquisa, você deve possuir boa higiene bucal e dentes pré-molares ou molares com desgaste na base (lesão cervical não-cariosa) com necessidade de restauração. Um pesquisador da equipe fará o exame de sua boca confirmando o estado dos dentes. A pesquisa será realizada nas clínicas odontológicas da Faculdade de Odontologia da Universidade Federal de Minas Gerais (UFMG). Você poderá sentir desconforto durante o tratamento durante a anestesia ou isolamento do dente. Para minimizar o desconforto será empregado anestésico tópico previamente à aplicação da anestesia. Trata-se de um material já disponível no mercado. Após a anestesia, procedimentos restauradores serão executados por profissional capacitado e treinado. Há o risco de você ter um desconforto no dente após o tratamento. Caso isso ocorra, você deverá entrar em contato com algum dos pesquisadores para que este desconforto seja averiguado e tratado. Todas as medidas serão tomadas para minimizar as possibilidades de risco, como a padronização dos procedimentos e utilização de um material confiável. Com essa pesquisa você terá o benefício de restaurar seu dente e com isso protege-lo contra a perda de mais estrutura dentária, melhora da estética, além de poder eliminar ou diminuir a sensibilidade do dente. Em nenhum momento você terá seu nome divulgado, e mesmo com a publicação dos resultados a sua identidade será preservada. Você não terá qualquer ônus ou ganho financeiro por participar da pesquisa, porém será beneficiado recebendo o tratamento. Sua participação é voluntária e você é livre para desistir de participar da pesquisa a qualquer momento, sem nenhum prejuízo**. Seus dados e sua saliva coletados serão utilizados somente para a finalidade dessa pesquisa, ficarão arquivados com o pesquisador responsável por um período de 5 (cinco) anos, e após esse tempo serão destruídos.** Você, concordando com a participação nesta pesquisa, assinará este termo em duas vias de igual teor e forma, ficando uma via consigo e outra via com os pesquisadores.

Rubrica do pesquisador: _____

Rubrica do participante*:*_____

Eu_____,
portador do documento de Identidade _____ declaro que fui informado (a) dos objetivos da pesquisa LIBERAÇÃO DE COMPONENTES ORGÂNICOS DE UM SISTEMA RESTAURADOR À BASE DE METACRILATOS: Estudos *In vitro* e *In vivo*'"', de maneira clara e detalhada e esclareci minhas dúvidas. Sei que a qualquer momento poderei solicitar novas informações e modificar minha decisão de participar, se assim o desejar.
( ) Concordo que o meu material biológico seja utilizado somente para esta pesquisa.
Declaro que concordo em participar. Recebi uma via original deste termo de consentimento livre e esclarecido e me foi dada à oportunidade de ler e esclarecer as minhas dúvidas.

_____

Nome completo do participante:
Data:

_____

Assinatura do (a) participante:

Belo Horizonte, _____, de 201

Dados para contato:

Nome do Pesquisador Responsável: Monica Yamauti
Endereço: Av. Presidente Antônio Carlos, 6627, sala 3.342, Pampulha - Belo Horizonte, MG.CEP: 31.270-901 – MG.Fone: (31) 3409-2456
E-mail: myamauti@gmail.com

Assinatura do pesquisador responsável
Data
Nome do Pesquisador Responsável: Allyson Nogueira Moreira
Endereço: Av. Presidente Antônio Carlos, 6627, sala 3.342, Pampulha - Belo Horizonte, MG.CEP: 31.270-901 – MG.Fone: (31) 3409-2456
E-mail: dangelogatil@terra.com.br

Assinatura do pesquisador responsável
Data

Nome completo do Pesquisador: Tassiana Cançado Melo Sá
Endereço: Av. Presidente Antônio Carlos, 6627, sala 3.310, Pampulha - Belo Horizonte, MG. CEP: 31.270-901 – MG.Fone: (31) 3409-2456
E-mail: tassianacancado@yahoo.com.br

_____

Assinatura do pesquisador (doutoranda)

Em caso de dúvidas éticas o Comitê de Ética em Pesquisa (COEP – UFMG) poderá ser contactado.
Av. Presidente Antonio Carlos, 6627 – Unidade Administrativa II – 2º andar – Sala 2005 – Telefax: 3409 4592 – Belo Horizonte – MG

ANEXO 1 – Critérios FDI para avaliação das restaurações (Hickel *et al*. 2010)

ANEXO 1 – CRITERIOS FDI PARA AVALIAÇÃO DE RESTAURAÇÕES

PROPRIEDADES ESTÉTICAS	SUPERFÍCIE/ BRILHO	COLORAÇÃO A.SUPERFÍCIE B.MARGEM	COR E TRANSLUCIDEZ	FORMA ANATÔMICA
Clinicamente excelentes/ muito boas	**Brilho comparado ao esmalte.**	**Sem coloração na superfície. Sem coloração marginal.**	**Não há diferença na tonalidade e/ou translucidez.**	**Forma é ideal.**
Clinicamente boas (Após o polimento provavelmente muito boas)	**Com um pouco de perda, mas não detectável a distância. Alguns poros isolados.**	**Menor superfície com coloração, fácil de remover com polimento. Menor superfície com coloração, fácil de remover com polimento.**	**3.2 pequeno desvio na cor e/ou translucidez**	**Forma é levemente alterada do normal.**
Clinicamente satisfatórias	**Superfície opaca, porém aceitável se coberta com filme de saliva. Mais de 1/3 das superfícies opacas.**	**Coloração moderada na superfície que pode estar presente também em outros dentes, porém não é inaceitável esteticamente. Coloração marginal moderada, porém não é inaceitável esteticamente.**	**Pequeno desvio, mas a estética é preservada: Mais opaco Mais translúcido Mais escuro Mais brilhante**	**A forma desvia do normal, porém é esteticamente aceitável.**
Clinicamente insatisfatória (Mas podem ser reparadas)	**Superfície áspera, que não pode ser mascarada pela saliva, polimento não é suficiente. Vazios.**	**Inaceitável a coloração da superfície da restauração sendo necessária uma intervenção para melhorar. Coloração marginal pronunciada; uma maior intervenção é necessária para aprimorar.**	**Desvio que pode ser corrigido por reparo: Muito opaco Muito translúcido Muito brilhante**	**Forma é afetada e esteticamente inaceitável. Intervenção é necessária.**

Clinicamente ruins (Precisam ser trocadas)	**Muito rugoso, com a superfície retentiva para placa.**	**Coloração da superfície severa generalizada ou localizada, não acessível para intervenção. Coloração marginal profunda, não acessível para intervenção.**	**Inaceitável Precisa ser trocada.**	**Forma é insatisfatória e ou/perdida. Precisa ser refeita.**

PROPRIEDADES FUNCIONAIS	FRATURA DO MATERIAL E RETENÇÃO	ADAPTAÇÃO MARGINAL
Clinicamente excelentes/ muito boas	**Sem fraturas ou fendas**	**Linha de adaptação harmoniosa, sem gaps sem linhas de descoloração**
Clinicamente boas (Com dano que pode ser ajustado)	**Pequena linha ou fenda**	**Gap marginal (< 150µm), linhas brancas. Fratura marginal removível com polimento. Ligeiro afundamento ou degrau/ menores irregularidades**
Clinicamente satisfatórias	**2 ou mais ou linhas grandes de fendas, mas a integridade marginal ou contato proximal**	**Gap < 250 µm não removível. Pequenas fraturas marginais severas. Irregularidades menores, afundamentos ou degraus**
Clinicamente insatisfatória (Mas podem ser reparadas)	**Fendas atinge a qualidade marginal ou contato proximal**	**Gap > 250 µm ou dentina/ base expostas. Severo afundamento ou fratura marginal**
	Fraturas com a perda parcial (menos da metade da restauração)	**Grandes irregularidades ou degraus (o reparo é necessário)**
Clinicamente ruins (Precisam ser trocadas)	**Parcial ou completa perda da restauração ou múltiplas fraturas**	**A restauração está perdida, porém no local**

PROPRIEDADES BIOLÓGICAS	HIPERSENSIBILIDADE PÓS-OPERATÁRIA E VITALIDADE DO DENTE	RECORRÊNCIA DE CÁRIE, EROSÃO OU ABFRAÇÃO	INTEGRIDADE DO DENTE	RESPOSTA PERIODONTAL	MUCOSA ADJACENTE
Clinicamente excelentes/ muito boas	Sem hipersensibilidade; vitalidade normal	Sem cáries secundárias ou primárias	Integridade completa	Sem placa, inflamação ou bolsa	Mucosa saudável adjacente a restauração
Clinicamente boas (Após correção podem ficar muito boas)	Menor hipersensibilidade por um período de tempo limitado, vitalidade normal	Pequena e localizada: 1 Desmineralização 2 Erosão 3. Abfração	Fratura marginal do esmalte pequena (<150 µm) Linha de fratura no esmalte (< 150 µm)	Pequena placa, sem inflamação (gengivite), nenhuma bolsa desenvolvida Gaps marginais ou forma anatômica inadequada	Saúde após remover a irritação mecânica (placa, calculo, etc)
Clinicamente satisfatórias (Pequena deficiência com nenhum efeito adverso, mas não são ajustados sem gerar dano ao dente)	Hipersensibilidade moderada Sem reclamações Não precisa de tratamento	Grandes áreas de: 1 Desmineralização 2 Erosão 3 Abrasão/ abfração, dentina não exposta Apenas medidas preventivas são necessárias	Defeito marginal do esmalte < 250 µm Fratura < 250 µm Lascas no esmalte Múltiplas fraturas	Diferenças acima de um gral de severidade no PBI comparado ao baseline e dente controle. Com gaps marginais ou forma anatômica inadequada	Alterações na mucosa, porém sem suspeitas de relação causal com o material
Clinicamente insatisfatória (Reparo por razões profiláticas)	Hipersensibilidade intensa Dano com sintomas subjetivos menores Sensibilidade não detectável clinicamente Intervenção necessária	Cárie com cavitação e suspeita de cárie abaixo da restauração Erosão em dentina Abrasão/abfração em dentina	Defeito marginal de esmalte maior; gap > 250 µm ou dentina ou base exposta Grande lasca no esmalte ou	Diferenças acima de um gral de severidade no PBI comparado ao baseline e dente controle ou bolsa > 1mm que precisa de intervenção	Suspeita de reação alérgica branda ou reação tóxica

		Localizado e acessível pode ser reparado	**fratura da parede**	**Com gaps marginais ou forma anatômica inadequada**	
Clinicamente ruins (Precisam ser trocadas)	**Intensa, pulpite aguda ou não vitalidade do dente Tratamento endodôntico é necessário e a restauração precisa ser trocada**	**Caries profundas ou com dentina exposta que não foi acessível para reparo da restauração**	**Cúspide ou dente fraturado**	**Gengivite ou periodontite severa ou aguda Com gaps marginais ou forma anatômica inadequada**	**Suspeita de alergia severa ou tóxica**

ANEXO 2- Parecer consubstanciado do Comitê de Ética em Pesquisa

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: LIBERAÇÃO DE COMPONENTES DE UM SISTEMA RESTAURADOR RESINOSO
Pesquisador: Monica Yamauti
Área Temática:
Versão: 2
CAAE: 65909417.0.0000.5149
Instituição Proponente: UNIVERSIDADE FEDERAL DE MINAS GERAIS
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.131.913

Apresentação do Projeto:
A maioria das resinas compostas apresenta metacrilatos como principais monômeros de sua composição. Idealmente, um material odontológico deveria ser inerte aos tecidos dentários e de suporte e à mucosa bucal, e não deveria conter substâncias tóxicas difusíveis capazes de atingir a circulação sanguínea, causando respostas sistêmicas. Nas resinas compostas, a liberação de monômeros de metacrilato, associada aos produtos de polimerização, tem sido considerada como fonte de uma série de reações biológicas como toxicidade, reações pulpares e efeitos alergênicos. A resina composta é comumente utilizada para restaurar lesões cervicais não cariosas (LCNC), o que coloca o material em íntimo contato com o tecido gengival. O estudo será divido em duas partes. A parte 1 (Laboratorial) corresponde a um estudo quantitativo, do tipo investigação experimental em laboratório ou "in vitro". A parte 2 (Clínica) corresponde a um estudo clínico longitudinal prospectivo, quali- e quantitativo, in vivo. Os procedimentos laboratoriais serão executados nos laboratórios de pesquisa e os procedimentos clínicos serão executados na Clínica da Pós-Graduação da Universidade Federal de Minas Gerais (UFMG). A primeira etapa é laboratorial. Cada amostra contenrá aproximadamente 3 L de adesivo ou 2 mm de resina composta (matiz A2) será depositada diretamente no cristal de ZnSe. A segunda etapa: Trata- se de um estudo clínico longitudinal prospectivo, simples cego.

Endereço: Av. Presidente Antônio Carlos,6627 2º Ad SI 2005
Bairro: Unidade Administrativa II **CEP:** 31.270-901
UF: MG **Município:** BELO HORIZONTE
Telefone: (31)3409-4592 **E-mail:** coep@prpq.ufmg.br

PRODUÇÃO CIENTÍFICA (2015-2019)

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Title Page:

Clinical performance of GIOMER restorative composites in comparison to different types of dental restorative materials: a systematic review and meta-analysis

Clóvis Ciryllo Limonge Neto[1], André Martins das Neves[1], Diandra Costa Arantes[2,] Tassiana Cançado Melo Sa[2], Monica Yamauti[3,4], Cláudia Silami de Magalhães[5,] Lucas Guimarães Abreu[6], Allyson Nogueira Moreira[5]

[1]DDS, MS student. Department of Restorative Dentistry, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

[2]DDS, MS, PhD student. Department of Restorative Dentistry, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

[3]DDS, MS, PhD. Adjunct professor, Department of Restorative Dentistry, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

[4]Associate Professor, Department of Restorative Dentistry, Faculty of Dental Medicine, Hokkaido University, Sapporo, Japan.

[5]DDS, MS, PhD. Titular professor, Department of Restorative Dentistry, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

[6] DDS, MS, PhD. Adjunct professor, Department of Pediatric Dentistry and Orthodontics, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

Author for correspondence

Dr. Lucas Guimarães Abreu

E-mail: lucasgabreu01@gmail.com

Telephone: 55 31 34092470

Av. Antônio Carlos, 6627. Campus Pampulha, CEP 31270-091, Belo Horizonte, MG, Brazil.

Contribution to the paper: all listed authors have made substantial contributions to the paper. Clóvis Ciryllo Limonge Neto contributed to the design of the study, bibliographic search, data analysis and drafting of the paper. André Martins das Neves contributed to the design of the study, bibliographic search, data analysis and drafting of the paper. Diandra Costa Arantes contributed to the design of the study, data analysis and drafting of the paper. Tassiana Cançado Melo Sá contributed to the design of the study, data analysis and drafting of the paper. Monica Yamauti contributed to the design of the study, critical review and proofread the paper. Claudia Silami de Magalhães contributed to drafting of the paper and proofread the paper. Lucas Guimarães Abreu contributed to the design of the study, bibliographic search, data analysis, drafting of the paper and proofread the paper. Allyson Nogueira Moreira contributed to the design of the study, critical review and proofread the paper.

Clinical performance of GIOMER restorative composites in comparison to different types of dental restorative materials: a systematic review and meta-analysis

Abstract

Purpose: To analyze the clinical effectiveness of GIOMER restorative composites in permanent teeth. *Methods:* A systematic review with a meta-analysis was conducted based on the PRISMA Statement. Clinical trials that evaluated the clinical performance of GIOMER restorative composites in permanent teeth compared to any other dental material were included. A meta-analysis was performed for the marginal adaptation and surface roughness criteria between GIOMER restorative composite and resin-modified glass ionomer cement (RMGIC) at the 6- and 12-month follow-ups. The quality of available evidence was evaluated by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE). *Results:* Six studies fulfilled the inclusion criteria. In these studies, GIOMER restorative composites were compared with different types of dental restorative materials, such as composite resin, glass ionomer cement (GIC), and RMGIC. Dental restorations were evaluated by the modified USPHS (the United States Public Health Service) criteria in all included studies. Two studies were suitable for the meta-analysis, which showed a significant difference between GIOMER and RMGIC surface roughness at the 6-month (OR = 6.56, IC = 2.38–18.13) and 12-month (OR = 8.76, IC = 3.19–24.07) follow-up. No significant difference between GIOMER restorative composites and RMGIC for marginal adaptation was found at the 6-month (OR = 1.54, IC = 0.59–4.02) and 12-month (OR = 1.36, IC = 0.51–3.60) follow-up. The certainty of the evidence was low for marginal adaptation outcome and moderate for surface roughness outcome. In conclusion, the GIOMER restorative composites presented similar performance to RMGIC concerning the marginal adaption and better surface roughness when compared to RMGIC.

Clinical relevance: GIOMER restorative composites could be an alternative for direct restorations in permanent teeth with better surface roughness when compared to RMGIC.

Clinical performance of GIOMER restorative composites in comparison to different types of dental restorative materials: a systematic review and meta-analysis

Introduction

Due to the demand for esthetic procedures and the advocacy for the minimum removal of dental tissues during dental preparation, composite resins have been widely used in Restorative Dentistry.[1,2] Several studies have demonstrated the clinical evidence of the survival of this restorative material.[3-5] On the other hand, composite resins are susceptible to failures due to their physical and chemical properties,[6] as well as the risk of secondary carious lesions adjacent to the restorations.[7]

Materials through which fluoride is released, such as glass ionomer cements (GICs), have the capacity to neutralize the pH of the saliva with proven efficacy for control and the reduction of bacterial growth.[8] The release of fluoride and strontium ions forms an acid-resistant layer and reinforces the dental structure, converting hydroxyapatite into fluorine-apatite and strontium-apatite with proven anti-cariogenic efficacy.[8] GICs are widely used for restorations in deciduous teeth or as temporary restorations in permanent teeth due to their properties of low resistance and weight loss over time, which, ultimately, lead to the increase of roughness on their surface and the consequent accumulation of plaque.[4]

GIOMER is a new class of restorative material introduced by Shofu Inc, which combines the fluoride-releasing properties of glass ionomer cement and the strength and aesthetics of composite resins.[9-11] The main difference between GIOMER materials and compomers is the presence of pre-reacted glass ionomer particles (S-PRG) incorporated into the resin matrix.[10,12] S-PRG particles enable the mechanical strength, durability, and aesthetics of a composite material ,[13] as well as the release of various ions (fluorine ions, sodium ions, silicate ions, aluminum ions, borate ions, and strontium ions)[14] that provide multiple biological functions including the release and recharge of fluoride, an anti-plaque effect, an anti-biofilm effect, and pH modulation.[10]

There are several studies in the literature on the clinical efficacy of composite resins, however there are few studies comparing this clinical efficacy with fluoride-releasing materials as GIOMER. Thus, the objective of the present systematic review and meta-analysis was to compare the clinical performance of GIOMER restorative composites with the clinical performance of restorations performed with other types of direct restorative materials. The null hypothesis of this study is that the clinical performance of GIOMER restorative composites is similar to the clinical performance of restorations with other types of direct restorative materials.

Material and Methods

Protocol and registration

This systematic review and meta-analysis were registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42018110634. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was followed.[15]

Eligibility criteria

This systematic review and meta-analysis aimed to answer the following question: Is there any difference in the clinical performance of dental restorations with GIOMER restorative composite in permanent teeth compared to other direct restorative materials? The PICO question described below was applied.

(P) Population - adults or adolescents with restorations in anterior or posterior permanent teeth.

(I) Intervention - restorations with GIOMER restorative composite.

(C) Comparison - restorations with other restorative material.

(O) Outcome - primary outcome: clinical performance of dental restorations evaluated by postoperative sensitivity, color match, marginal adaptation, anatomic form, surface roughness,

staining, marginal staining, secondary caries, retention criteria, and the influence of isolation (rubber dam or cotton roll) on the primary outcome.

Randomized clinical trials comparing the clinical performance of dental restorations with GIOMER restorative composite and restorations with other restorative materials performed in permanent teeth of adults or adolescents were included. Letters, literature reviews, case reports, case series, non-randomized clinical trials, *in vitro*, and *in situ* studies were excluded. Restrictions on language or date of publication was not imposed in any way.

Information sources and search strategy

In September 2018, electronic searches were performed in the following databases: PubMeb, Web of Science, Scopus, Medline Ovid, and the Cochrane Library. A grey literature search in Google Scholar, limiting the search to the first 300 hits was performed. Manual searches of the reference list of the included articles were also carried out. In May 2019, an update of the search was carried out to verify if there were any new potential publications.

The search strategy for PubMed, Web of Science, Medline Ovid, and Cochrane Library was as follows: [giomer OR s-prg OR pre-reacted glass ionomer OR s-prg filler OR beautifil]. For Scopus, the search strategy was tailored according to the characteristics of the database.

Study Selection

Endnote Web (Clarivate Analytics, Philadelphia, US) was used to manage the bibliographic references. Any duplicated references were removed upon identification. Titles and abstracts were evaluated independently by two review authors (CCLN and AMN), who applied the eligibility criteria. The full text of the references with insufficient information in the titles and abstracts was also evaluated by the two review authors. The references that fulfilled the eligibility criteria were included. If any divergence between the review authors took place, a third review author (MY) decided if the reference should be included or otherwise.

Data collection process and data items

Data collection of the included articles was carried out by two review authors (CCLN and AMN). Divergences were also resolved by a third review author (MY). The following data were collected from the included articles: identification of the study (last name of the first author and year of publication), study design, period of follow-up, age of participants, total number of restorations, and total number of the participants at study's onset, type of dental materials assessed and number of restorations per group at the end of the study, isolation method, evaluation criteria, outcomes evaluated, and results.

Risk of bias in individual studies

The risk of bias assessment of the included articles was performed by two review authors (CCLN and AMN) according to the Cochrane Risk of Bias Tool for Randomized Clinical Trials (http://handbook.cochrane.org). The aspects of bias were evaluated individually in order to assess the selection, performance, attrition, reporting, and detection bias.

Six domains were assessed: random sequence generation, allocation concealment, blinding of outcome assessors, blinding of participants and personnel, incomplete outcome data, and selective outcome reporting. A low risk, an unclear risk, or a high risk of bias were used to classify each domain. Divergences between the two review authors were resolved by a third review author (DCA).

Synthesis of results

Included studies with methodological homogeneity were incorporated into the meta-analysis. The results of the meta-analysis were reported as odds ratio (OR) and 95% confidence intervals (CI). In the meta-analysis, statistical heterogeneity was assessed by means of the I^2 statistics.[16] Analyses with a value of I^2 greater than or equal to 40% were classified as having a high statistical heterogeneity and the random effect model would be used. Analyses with a value of I^2 lower than 40% were classified as having a low statistical heterogeneity and the fixed effect model would be used. The meta-analysis was performed using the software Review Manager (Rev.Man), version 5.3 software

(Review Manager. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014).

Additional analysis

Depending on the outcomes evaluated in the included studies, subgroup analyses were carried out considering the results of two clinical performance criteria (surface roughness and marginal adaptation) at two follow-up periods (six and twelve months). For studies that analyzed other clinical performance criteria, only these two criteria were incorporated into the meta-analysis.

Quality of evidence rating and strength of recommendations grading

The Grading of Recommendations, Assessment, Development, and Evaluation (GRADE)[17] system was used to analyze the quality of the evidence and the strength of recommendations using the GRADEpro GDT online software. The selected outcomes were marginal adaptation and surface roughness six and twelve months after restoration with GIOMER and RMGIG. For each one, the GRADE evaluated the number of studies included in the meta-analysis, the studies' design, risk of bias, inconsistency, indirectness, imprecision, and other considerations, such as publication bias. The evidence could be downgraded in one or two levels according to the seriousness of the limitation. The certainty of the assessment of the outcome could be classified as high, moderate, low, or very low.

Results

Study selection

The searches across the five electronic databases retrieved 910 references. After the removal of duplicates, 552 references remained. Titles and abstracts were assessed, and ten articles were selected for the evaluation of the full text. Among the ten articles, four studies were excluded because the focus of the analysis was on periodontal outcomes and not outcomes related to the clinical performance of the restorative material.[18-21] Therefore, six studies fulfilled the eligibility criteria and were included in this systematic review and meta-analysis.[22-27] No study meeting the eligibility

criteria was identified in Google Scholar or in the reference list of the included studies. Figure 1 displays the process of the study selection of this systematic review and meta-analysis.

Characteristics of included studies

The characteristics of the included studies are described in Table 1. Two studies compared composite resin and GIOMER.[22,25] One study used two types of GIOMER.[23] One study compared RMGIC and GIOMER.[24] In one study,[26] composite resin and GIOMER were compared with bases pre-filled with RMGIC or compomer. One study compared restorations with RMGIC, GIC, and GIOMER.[27]

In two included studies, rubber dam isolation was used.[22,23] In three studies, cotton rolls were used.[24,25,27] In one study, no information on the isolation method was provided.[26] In four included studies, Class IV restorations were evaluated.[22-24,27] In one study[23], both carious and non-carious cervical lesions were evaluated, and Class I restorations with only one type of GIOMER were assessed. In two studies, Class II restorations were evaluated [25,26]. The minimum period of the follow-up period was six months, and the maximum was 72 months. The participants' age ranged from 16 to 75 years. The total number of restorations evaluated in the included studies was 547.

Risk of bias within studies

In all included studies, information on the blinding of participants and personnel was unclear.[22-27] In one study, the random sequence generation and the allocation concealment were of a low risk of bias and the authors reported that the cards were sequentially numbered and placed in opaque and sealed envelopes.[27] Three included articles presented a low risk of bias[24,25,27] (Jyothi *et al.*, 2011; Dijken *et al.*, 2013; Priyadarshini *et al.*, 2017) and three presented an unclear risk of bias[22,23,26] for the blinding of the outcome assessor. In regards to incomplete outcome bias, only one included study showed an unclear risk of bias[26] and five showed low risk of bias. [22-25,27] For selective reporting, two included studies presented a low risk of bias[24,27], three presented a high risk of bias[22,23,25], and one presented an unclear risk of bias.[26] No other bias was observed in any of the

included studies. Figure 2 displays the assessment of the risk of bias for each included study, and Figure 3 shows a summary of the risk of bias assessment.

Synthesis of results and subgroup analysis

Two included studies were incorporated into the subgroup analyses.[24,27] One subgroup analysis compared marginal adaptation between GIOMER and RMGIC, six months and twelve months after the restoration placement. The subgroup analysis showed no difference with respect to marginal adaptation between GIOMER and RMGIC at 6 months (OR = 1.54, CI = 0.59–4.02, I^2 = 38%) and 12 months (OR = 1.36, CI = 0.51–3.60, I^2 = 31%) after restoration placement (Figure 4). One subgroup analysis compared surface roughness between GIOMER and RMGIC, six and twelve months after restoration placement. RMGIC was 6.56 times more likely to present Bravo scores six months after restoration placement than GIOMER (OR = 6.56, CI = 2.38–18.13, I^2 = 0%). RMGIC was 8.76 times more likely to present Bravo scores twelve months after restoration placement than GIOMER (OR = 8.76, CI = 3.19–24.07, I^2 = 0%) (Figure 5). In all subgroup analyses, the fixed effect model was used.

Quality of evidence rating and the strength of recommendation grading

The certainty of the evaluation of the outcomes marginal adaptation and surface roughness, after dental restoration with GIOMER materials and RMGIG, was low for marginal adaptation (6- and 12-month follow up) and moderate for surface roughness (6- and 12-month follow up) (Table 2).

Discussion

The present systematic review and meta-analysis was conducted due to the lack of evidence of the clinical efficacy of GIOMER restorative composites. One of the most important properties of GIOMERS is the ability to release and recharge fluoride to prevent secondary caries.[9,28,29] RMGIC materials were also associated with a higher reduction of demineralization in adjacent hard tooth tissue under caries challenge than composite resins without fluoride.[30] There are no other systematic

reviews comparing the clinical efficacy of GIOMER restorative composites with different types of restorative materials.

In the studies included in this systematic review and meta-analysis, dental restorations were evaluated by the United States Public Health Service (USPHS) criteria.[22-27] However, those studies did not explicitly mention if CONSORT recommendations were followed. It would have been very helpful if the studies had used those recommendations to write, review, or assess reports. The CONSORT statement collaborates to improve the quality of randomized clinical trials. In addition, no study cited the protocol registration number for *in vivo* trials in any specific database.

The present systematic review and meta-analysis included six studies that evaluated the clinical performance of materials in the short- and long-term periods. [22-27] In general, the present results demonstrated that the clinical performance of GIOMER restorative composite was like that of RMGIC, concerning marginal adaption and surface roughness. The included studies employed different designs regarding the type of cavity (Class I, II, V) and restorative material (GIOMER, composite resin, GIC, and RMGIC). Thus, it was only possible to perform the meta-analysis of two outcomes using two studies.[24,27] The subgroup analyses of both outcomes compared GIOMER with RMGIC in non-carious Class V restorations at the same time periods (6- and 12-months).

In the included studies, the GIOMER restorative composites used were Beautifil, Beautifil II, and Reactmer. This class of material has properties of GIC related to fluoride release and fluoride recharge along with better esthetics, resistance, and easy polishing.[23] PRG-technology is classified into two categories: F-PRG (full reaction type), with which the entire filler particle is attacked by polyacrylic acid, and the S-PRG (surface reaction type), with which only the surface of the glass filler is attacked by polyacrylic acid, and a glass core remains. In fact, S-PRG has replaced F-PRG. A previous Reactmer (Shofu, Kyoto, Japan) used F-PRG technology, but this material was indicated only for cervical cavities.[23] Current versions of the Beautifil resins and FL-Bond adhesive system (Shofu, Kyoto, Japan) developed using S-PRG technology are indicated for Class I through Class VI cavities.[23] Beautifil II is considered a second-generation GIOMER introduced into the market.[27]

Two studies were suitable for meta-analysis. One subgroup analysis showed a significant difference between GIOMER and RMGIC with respect to the surface roughness at the 6- and 12-month follow-up. In both studies, the restorations were submitted to polishing procedures to get the surfaces as smooth as possible.[22-27] Several factors related to the restorative procedures, the characteristics of composites, and the operator may affect the surface roughness. According to some authors, increased surface roughness enlarges the area available for bacterial adhesion[31] and biofilm formation. This could happen in the case of the absence of good polishing or a smooth state, which could lead to secondary caries[31] and inflammation of gingival tissue.[10,18,31-34] The presence of biofilm is one of the factors that may stimulate surface degradation,[31] compromising the longevity of resin composite restorations.

Moreover, no significant difference between the marginal adaptation of GIOMER and RMGIC restorations was found at the 6- and 12-month follow-up. Jyothi *et al*.[24] and Priyadarshini *et al*.[27] also reported that GIOMER presented a better color match and worse retention than GIC.

The GIOMER restorative composites were considered suitable as definitive restorative materials.[22-24,26,27] Dijen *et al*.[25] found a higher failure rate in GIOMER than in composite resin due to fracture or secondary caries. However, Matis *et al.*[22] have not found a significant difference between GIOMER and composite resins in all the evaluated periods and outcomes. Saveanu & Dănilă[26] reported that GIOMER restorative composite presented an inferior quality for marginal staining and color match when compared with composite resin. This was the only study included in this systematic review and meta-analysis that used the compomer and RMGIC as a restorative base, using the composite resin or GIOMER as a restoration for enamel in class II cavities.[26]

There is still poor information on this type of material. The major limitation of this study was the scarce number of non-randomized clinical trials using GIOMER materials. Therefore, it was unfeasible to perform subgroup analyses with other outcomes. For future research, it would be convenient to follow the CONSORT's recommendations for designing and reporting studies, in particular regarding the blindness of operators and evaluators. A detailed report of the results is highly

relevant to describe the gross values of the analysis of each outcome in each period for the eventual meta-analyses.

Conclusion

The GIOMER restorative composite presented a similar performance to that of RMGIC restorations concerning marginal adaption. However, GIOMER presented better surface roughness when compared to RMGIC. It is still premature to assert that the clinical behavior of GIOMER restorative composites is similar to the clinical performance of restorations with other types of direct restorative materials. Randomized clinical trials with long-term follow-ups are still necessary to compare the clinical performance of GIOMER restorative composites and other materials.

Acknowledgements

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Conflicts of interest

The authors do not have conflicts of interest.

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Table 1. Characteristics of the included studies.

Author Year (Country)	Study Design	Follow-up (months)	Age of participants	Initial number of restorations and participants	Type of dental materials and final number of restorations per group	Isolation method	Evaluation criteria	Outcomes	Results
Matis *et al.* 2004 (USA)	Split mouth Randomized Clinical Trial	6, 18, and 36	Mean age = 45 years Range = 30–75 years	80 restorations (Class V) 30 individuals	**Microfield Composite** (Scotchbond Multi-Purpose Plus Dental Adhesive + Silux Plus[a]) = 39 **GIOMER** (FL-Bond + Beautifil[b]) = 39	Rubber dam and retraction cord when necessary	Modified USPHS	**POS, MA, AF, SR, S, MS, SC,** and **R**	**POS, MA, AF, SR, S, SC, R:** There were no significant differences in the evaluated periods among all the evaluated outcomes. **MS:** 7 teeth exhibited margin with discoloration restored with Beautifil and 4 with Silux Plus, but without significant differences. Neither material was significantly different from each other in the outcomes evaluated. Both materials meet the clinical portion of the Acceptance Program Guidelines for Dentin and Enamel Adhesives Materials established by the American Dental Association.
Sunico *et al.* 2005 (Phillippines)	Split mouth Randomized Clinical Trial	6 and 24	Mean age = 35 years Range = 20–50 years	62 restorations (42 Class V and 20 Class I) 15 individuals	**GIOMER** (Imperva FluorBond[b] + Beautifil[b]) = 20	Rubber dam	Modified USPHS	**POS, CM, MA, AF, SR, MS, SC,** and **R**	**POS, CM:** There was no significant differences in the evaluated periods for both materials.

					Class V and 20 Class I) (Reactmer[b] + Reactmer Bond[b]) = 21 Class V				**MA:** There were significant differences for MA in CL V restorations with Beautifil at periods evaluated ($p < 0.05$). **MA, MS:** Both GIOMER materials presented failures in marginal adaptation, marginal discoloration, and wear in the evaluated periods. **MA, AF, MS** were the criteria that had the most Charlie and Delta ratings at both six months and two years for the Reactmer CL V restorations. **SC:** 20% of restorations with Reactmer showed secondary caries at the 24-month evaluation. **SR:** Not reported. **R:** At 6 months, 19% (n = 4) of the restorations with Reactmer dislodged and after 24 months another restoration was lost. While for Beautifil, any restoration lost retention. Beautifil CL V restorations were better retained then Reactmer CL V restorations in the evaluated periods. At 24 months, there was an 80% success rate for Beautifil and only a 71% success rate for Reactmer.

Saveanu & Dănilă 2010 (Romania)	Split mouth Randomized Clinical Trial	6, 12, 24, and 36	Mean age = not reported Range = 16–55 years	90 restoration (Class II)	**Composite Resin with RMGIC** (Filtek Supreme[a] + Vitrmer[a]) = 24 **GIOMER with RMGIC** (Beautifil[b] + Vitremer[a]) = 23 **GIOMER with Compomer Flow** (Beatutifil[b] + Dyract Flow[e]) = 21 **Composite Resin with Compomer Flow** (Filtek Supreme[a] + Dyract Flow[d]) = 22	Not reported	Modified USPHS	**POS, CM, MS,** and **R**	**POS:** There was higher for composite resin restorations compared to restorations made with GIOMER, but without significant difference. **CM:** There were no significant differences at 6 and 12 months. But with 24 months, restorations with composite resin showed 33,33 (8) score Alpha, while restorations with GIOMER showed only 4.34 (1) score Alpha with significant differences ($p< 0.05$). **MS:** There were significant differences at 12 months for restorations with $p = 0037$ with favor to composite Resin with rating Alpha (95.65%) and GIOMER with rating Alpha (73.91%), the rest is bravo. **R:** None of restorations lost in the evaluated periods.
Jyothi *et al.* 2011 (India)	Split mouth Randomized Clinical Trial	15 days, 6 and 12 months	Mean age = not reported Range = 20–60 years	80 restorations (Class V) 32 individuals	**RMGIC** (Fuji II LC [c]) = 40 **GIOMER** (FL-Bond II[b] + Beatufil II[b]) = 40	Cotton Rolls, saliva ejector and gingival retraction cords	Modified USPHS	**POS, MA, SR, S, MS,** and **R**	**SR:** There were significant differences in the evaluated periods. GIOMER-exhibited a superior surface finish compared to RMGIC. **MA, R:** There were no significant differences in the evaluated periods.

									POS, S, MS: There was no marginal discoloration, staining, and postoperative sensitivity for all the restorations.
Dijken 2013 (Sweden)	Split mouth Randomized Clinical Trial	12, 24, 36, 48, 60, and 72	Mean age = 57.1 years Range = 24–77 years	115 restorations (Class II) 54 individuals	**Hybrid Resin** (G-Bond[c] + Gradia Direct Posterior[c]) = 58 **GIOMER** (FL-Bond[b] + Beautifil[b]) =53	Cotton Rolls and suction device	Modified USPHS	**POS, CM, AF, MA, SR, MS,** and **SC**	**POS:** No post-operative sensitivity was reported. **CM:** There was a significant decrease in color match at the period evaluated for both materials ($p < 0.05$). **AF:** There were no significant differences in the evaluated periods. **MS:** There were changes for both materials, but this was significantly higher for the GIOMER material **MA, SC:** During the total period evaluated, 5 (8,5%) restorations with composite resin and 9 (17,7%) restorations with GIOMER failed due to receding caries or fracture ($p <0.05$). **SR:** Not reported.
Priyadarshini *et al.* 2017 (India)	Split mouth Randomized Clinical Trial	6 and 12	Mean age = not reported Range =35–65 years	120 restorations (Class V) 20 individuals	**RMGIC** (Self Conditioner[c] + Fuji Filling LC[c]) = 40 **GIC**	Cotton rolls, saliva ejector, and gingival retraction cords	Modified USPHS	**POS, CM, MA, SR, MS,** and **R**	There was a significant reduction for some outcomes such as **CM** and **SR** for RMGIC, **R** for GIOMER, **MS,** and **CM** for GIC, after 12 months with $p < 0.05$.

					(Ketac N100 Nano Ionomer Primer[a] + Ketac N100[a]) = 40 **GIOMER** (FL Bond II LC[b] + Beautifil II[b]) = 40				**CM, SR, MS, R:** There was a significant difference with $p < 0.05$ from 6 to 12 months for all materials. **R:** GIV and RMGIC restorations were better retained than GIOMER restorations in the evaluated periods with significance differences. **MS:** It was higher for GIC than others material with significance differences. **CM:** GIOMER was better than GIC and RMGIC in the evaluated periods. **SR:** GIOMER was better than RMGIC with a significance difference. **POS, MA:** There were no significant differences in the evaluated periods.

RMGIC = Resin-Modified Glass Ionomer Cement; GIC = Glass Ionomer Cement; POS = Postoperative Sensitivity; CM = Color Match; MA = Marginal Adaptation; AF = Anatomic Form; SR = Surface Roughness; S = Staining; MS = Marginal Staining; SC = Secondary Caries; R = Retention. [a] = 3M Dental Products, St. Paul, Minn; [b] = Shofu, Kyoto, Japan; [c] = GC Corp., Tokyo, Japan; [d] = 3M ESPE, St. Paul, USA; [e] = Dentsplay Sirona, Sidney, Australia.

Table 2. GRADE quality of evidence.

Outcome	Nº of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Relative (95% CI)	Quality
Marginal adaptation 6 months	2	randomized trials	serious [a]	not serious	not serious	serious [b]	none	OR 1.54 (0.59 to 4.02)	⊕⊕◯◯ LOW
Marginal adaptation 12 months	2	randomized trials	serious [a]	not serious	not serious	serious [b]	none	OR 1.36 (0.51 to 3.60)	⊕⊕◯◯ LOW
Surface roughness 6 months	2	randomized trials	serious [a]	not serious	not serious	not serious	none	OR 6.56 (2.38 to 18.13)	⊕⊕⊕◯ MODERATE
Surface roughness 12 months	2	randomized trials	serious [a]	not serious	not serious	not serious	none	OR 8.76 (3.19 to 24.07)	⊕⊕⊕◯ MODERATE

CI: Confidence interval; **OR:** Odds ratio.

a. The evidence has been downgraded by one level because of serious concern regarding the risk of bias. According to the Cochrane Tool, most information is from studies at moderate risk of bias.

b. The evidence has been downgraded by one level because confidence intervals cross threshold.

Fig 1. Flowchart of study selection

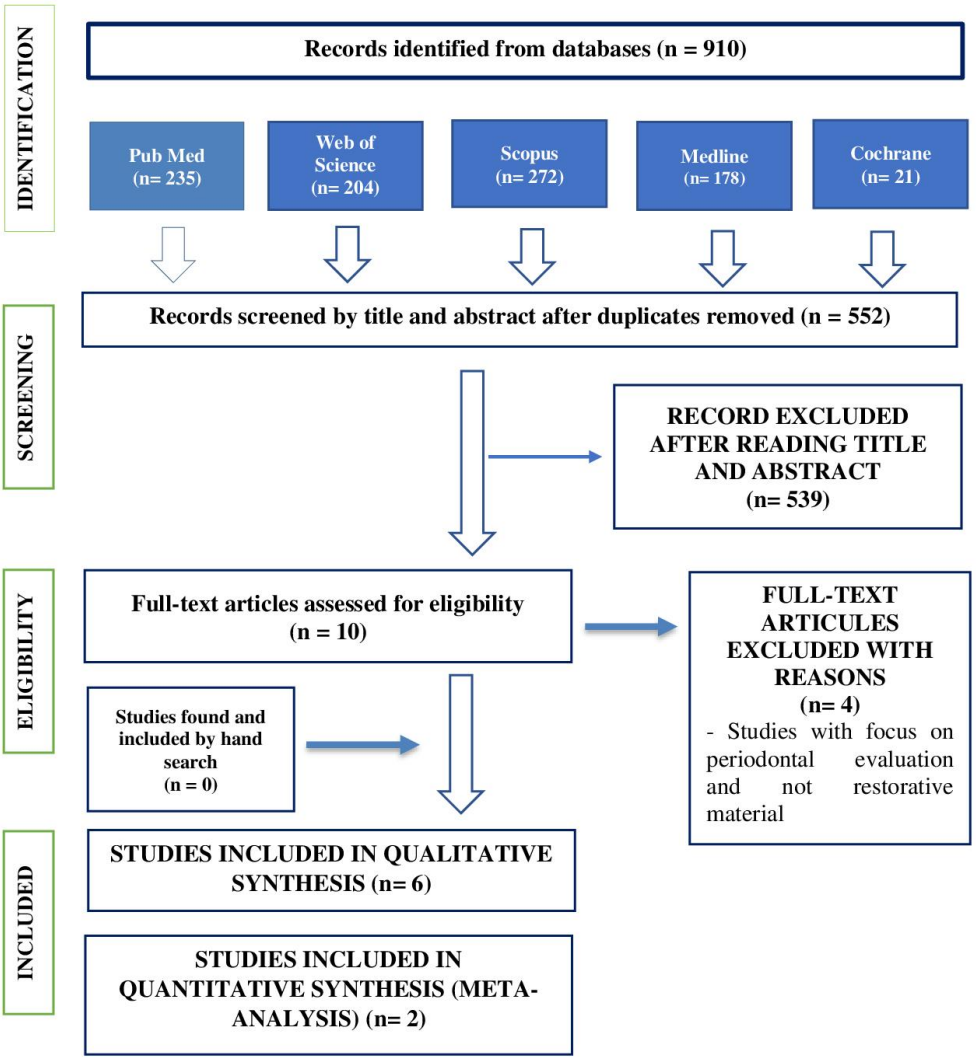


Fig 2. The assessment of risk of bias for each included study

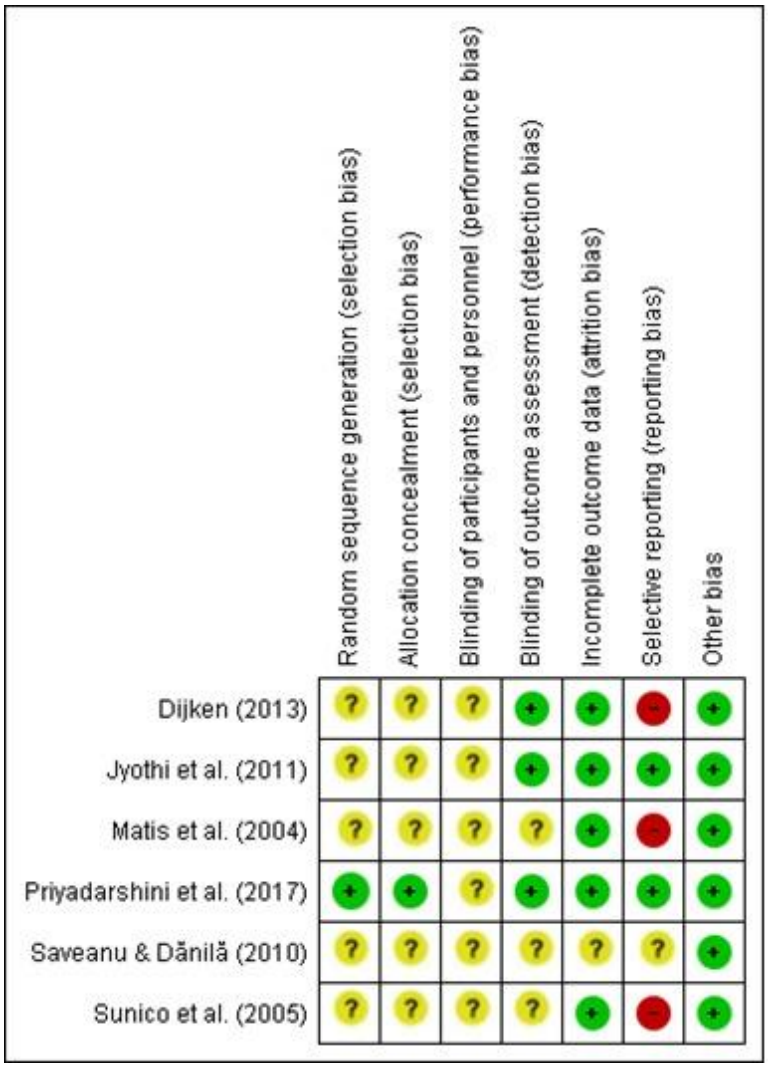


Fig 3. Summary of the risk of bias of the include studies

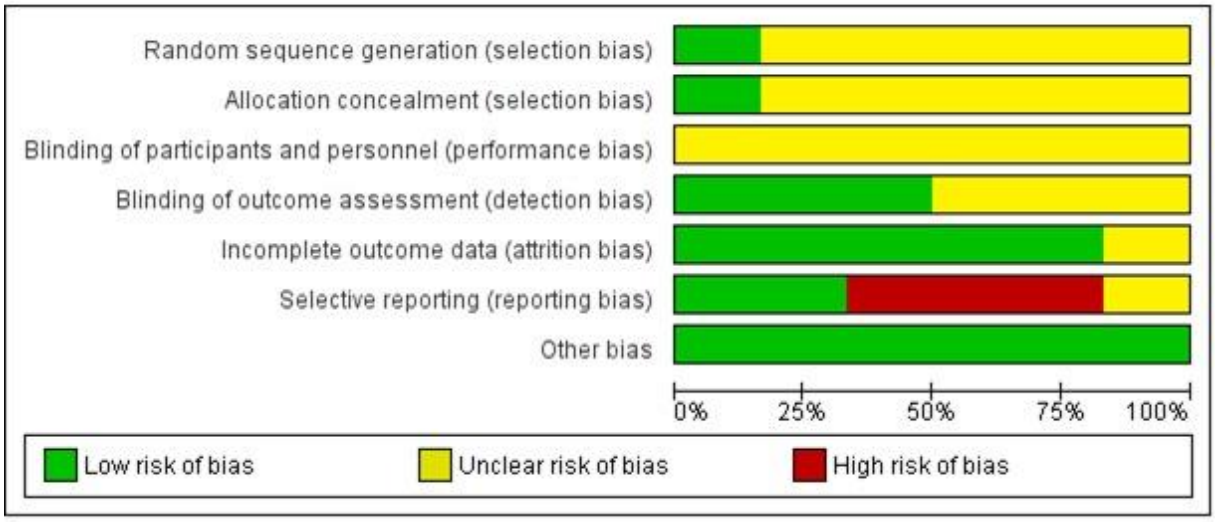


Fig 4. Forest Plot for marginal adaptation between GIOMER and RMGIC at six- and 12-months

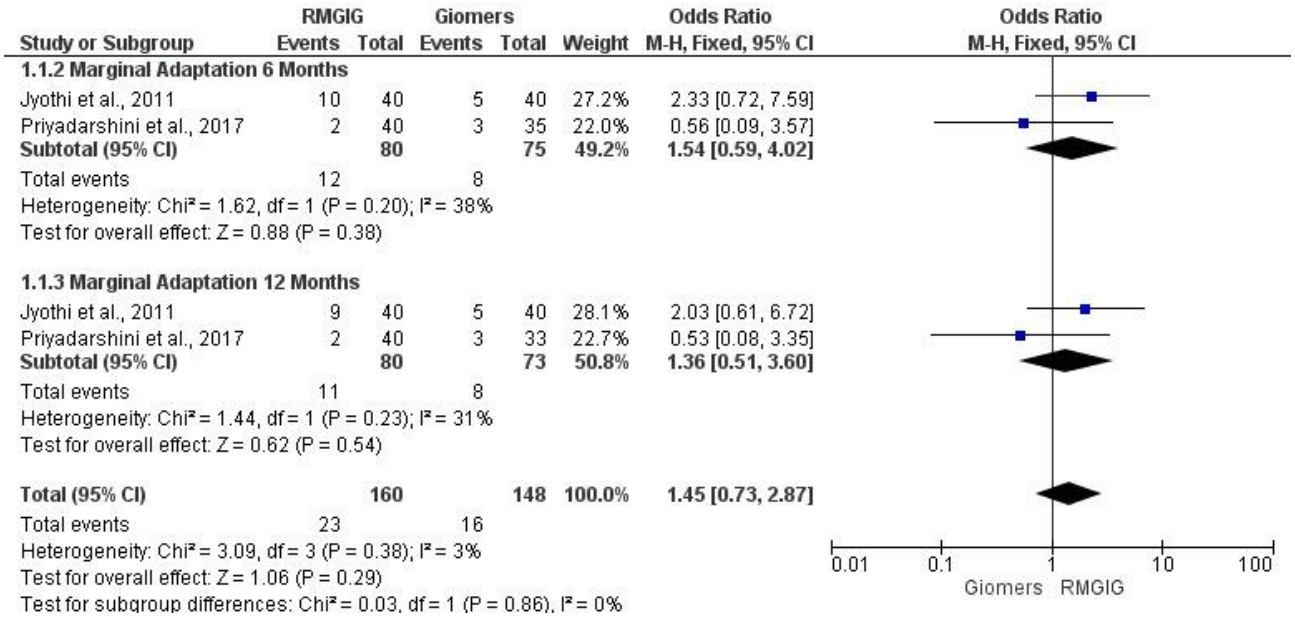
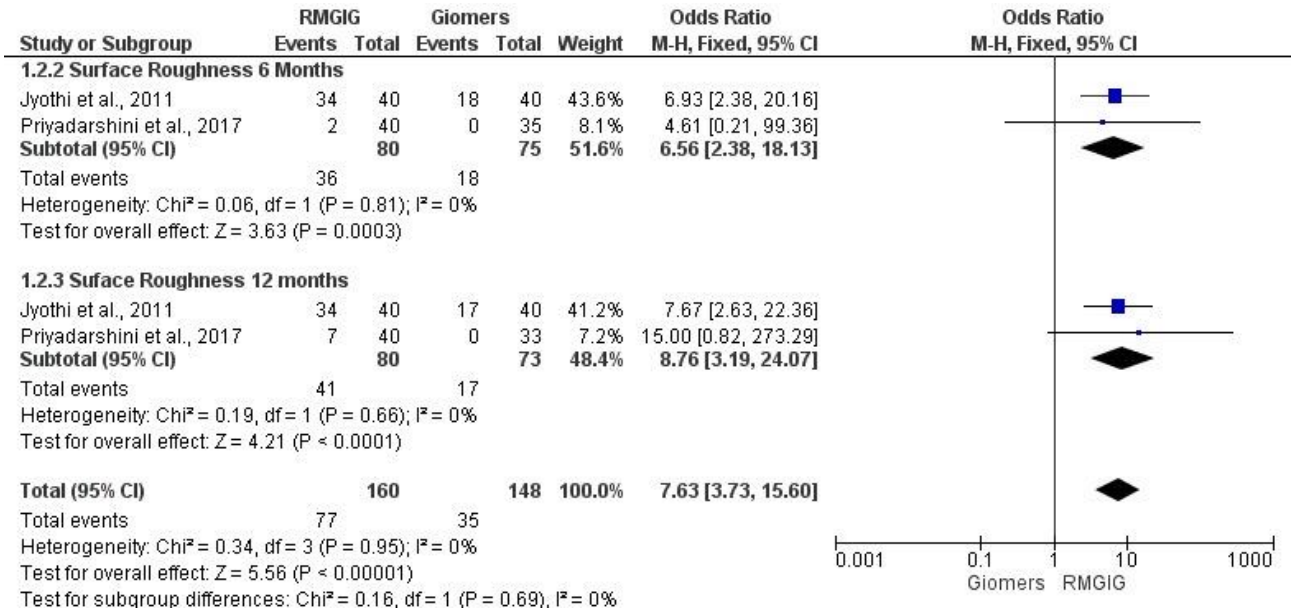


Fig 5. Forest Plot for surface roughness between GIOMER and RMGIC at six- and 12-months



Formação Acadêmica

2018 -2019 Doutorado- Período sanduíche em University of Toronto (Orientador : Grace Mendonça de Souza)

Artigos completos

1. **SÁ, TASSIANA MELO**; LIMEIRA, F. I. R.; RODRIGUES, 3. A. A.; SA, J. C. M.; MAGALHAES, C. S.; MOREIRA, A. N.; YAMAUTI, M.

Rehabilitation with fixed prosthodontics associated with removable partial prosthesis: a 5-year follow-up clinical evaluation. CONTEMPORARY CLINICAL DENTISTRY, 2019.

2. **SÁ, T. C. M.**; CARVALHO, M. F.; SA, J. C. M.; MOREIR, A. N.; MAGALHAES, C. S.; YAMAUTI, M.

Esthetic rehabilitation of anterior teeth with different thicknesses of porcelain laminate veneers: An 8-year follow-up clinical evaluation. European Journal of Dentistry (Online). , v.2, p.590 - 593, 2018.

3. ANKLI, V.; YAMAUTI, M.; LIMEIRA, F. I. R.; **SÁ, T. C. M.**

Gingival veneer used as prosthetic solution for esthetic-compromised malpositioned dental implant. CONTEMPORARY CLINICAL DENTISTRY. , v.9, p.123 - 127, 2018.

4. LACERDA, V. G.; SA, J. C. M.; SOUSA, G. R.; RODRIGUES, C. N.; ASSUNCAO, E. L. F.; **SÁ, T. C. M.**

Utilização do laser de alta intensidade para aumento de retenção e estabilidade de prótese total removível - relato de caso. FULL DENTISTRY IN SCIENCE. , v.8, p.75 - 80, 2017.

5. AMARAL, FABRÍCIO RESKALLA; ASSUNÇÃO, ÉLIDA LÚCIA FERREIRA; VIDIGAL, BRUNO CÉSAR LADEIRA; **SÁ, TASSIANA MELO**; OLIVEIRA, BRUNO CANÇADO; DE FARIA, ADRIANA ALVES; AKAKI, EMILIO; DE CAMPOS, EDSON ALVES

Applicability of Knowledge of Graduates in Dentistry: Use of Irreversible Hydrocolloid. International Journal of Odontostomatology., v.9, p.519 - 524, 2015.

Artigos aceitos para publicação

1. **SÁ, TASSIANA MELO**; LIMEIRA, F. I. R.; RODRIGUES, 3. A. A.; SA, J. C. M.; MAGALHAES, C. S.; MOREIRA, A. N.; YAMAUTI, M.

Rehabilitation with fixed prosthodontics associated with removable partial prosthesis: a 5-year follow-up clinical evaluation. CONTEMPORARY CLINICAL DENTISTRY. , 2019.

Atividades de ensino

Universidade Federal de Minas Gerais - UFMG

2017 - 2018 Vínculo: Professora substituta do Departamento de Odontologia Restauradora da UFMG. 20 horas-aula no período.

2017 – 2017 Vínculo: Professor Visitante , Enquadramento funcional: Professor Voluntário de Prótese , Carga horária: 20, Regime: Parcial

2015 - 2015 Vínculo: Professor Visitante , Enquadramento funcional: Professora de Prótese fixa , Carga horária: 5, Regime: Parcial Professora bolsista junto ao PROTATEC/COLTEC-UFMG

Trabalhos publicados em anais de congresso

1. **SÁ, T. C. M.**; SIMOES, C. F.; SIQUEIRA, E. C.; GOMES, R. S.; COELHO, C. G.; MOREIR, A. N.; YAMAUTI, M.
Efeito biológico de um material restaurador contendo partículas vítreas pré-reagidas no fluido crevicular gengival In: Sociedade Brasileira de Pesquisa Odontológica, 2018, Campinas.
Brazilian Oral Research. São Paulo: Brazilian Oral Research, 2018. v.32. p.508 - 508

2. HORTA, M. C.; YAMAUTI, M.; **SÁ, T. C. M.**; LIMA, M. A.; MAGALHAES, L.; DUTRA, W. O.; GOLLOB, K. J.; SOUZA, P. E.
Dental resins affect differently the expression of chemokines and chemokine-receptors In: Academy Dental Materials, 2017, Nuremberg.
Dental Materials. , 2017. v.33. p.e84 - e84

3. **SÁ, T. C. M.**; CARVALHO, M. F.; LIMEIRA, F. I. R.; SANTA-ROSA, C. C; MAGALHAES, C. S.; YAMAUTI, M.; MOREIR, A. N.
Resistência de união na fixação de pinos intrarradiculares de acordo com diferentes pré-tratamentos dentinários In: Sociedade Brasileira de Pesquisa Odontológica, 2017, Campinas.
Brazilian Oral Research. , 2017. v.31. p.309 - 309

4. **SÁ, T. C. M.**; LIMA, M. A.; MAGALHAES, L.; DUTRA, W. O.; GOLLOB, K. J.; HORTA, M. C.; SOUZA, P. E.
Efeito das resinas para a técnica indireta Belle Glass® e Ceramage® na expressão de quimiocinas por leucócitos humanos in vitro In: 52 Encontro do Grupo Brasileiro de Materiais Dentários, 2016, Uberlândia.
52 Encontro do Grupo Brasileiro de Materiais Dentários. , 2016. p.202 - 202

5. **SÁ, T. C. M.**; QUEIROZ, C. M. F.; AYALA, A. P.; SOUSA, F. F. O.; MENDONCA, J. S.; RICARDO, N. M. P. S.; SANTIAGO, S. L.; YAMAUTI, M.
Preparo de solução de ácido anacárdico para utilização em Odontologia como agente de limpeza cavitária In: 33 Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica, 2016, Campinas.
Brazilian Oral Research. , 2016. v.30. p.438 - 438

Apresentação de trabalho e palestra

1. FERREIRA, L. A. Q.; PEIXOTO, R. T. R. C.; LIMEIRA, F. I. R.; **SÁ, T. C. M.**; YAMAUTI, M.; SILAMI, F. D. J.
ANÁLISE DA ALTERAÇÃO DE COR DE RESINAS COMPOSTAS SUBMETIDAS À IMERSÃO EM DIFERENTES AGENTES PIGMENTANTES, 2018. (Outra, Apresentação de Trabalho)

2. FERREIRA, L. A. Q.; PEIXOTO, R. R. C.; LIMEIRA, F. I. R.; **SÁ, T. C. M.**; YAMAUTI, M.; SILAMI, F. D. J.
Avaliação da alteração de cor de resinas compostas submetidas à ação de diferentes agentes pigmentantes, 2018. (Outra, Apresentação de Trabalho)

3. **SÁ, TASSIANA MELO**; SIMOES, C. F.; SIQUEIRA, E. C.; TINOCO, F.; GOMES, R. S.; COELHO, C. G.; MOREIR, A. N.; YAMAUTI, M.
EFEITO BIOLÓGICO DE UM MATERIAL RESTAURADOR CONTENDO PARTÍCULAS VÍTREAS PRÉ-REAGIDAS NO FLUIDO CREVICULAR GENGIVAL, 2018. (Congresso, Apresentação de Trabalho)

4. LIMONGE NETO, C. C.; **SÁ, T. C. M.**; LIMEIRA, F. I. R.; MAGALHAES, C. S.; YAMAUTI, M.
RESISTÊNCIA FLEXURAL DE UMA RESINA COMPOSTA CONTENDO PARTÍCULAS PRÉ-REAGIDAS DE IONÔMERO DE VIDRO (S-PRG), 2018. (Outra, Apresentação de Trabalho)

5. **SÁ, T. C. M.**; CARVALHO, M. F.; LIMEIRA, F. I. R.; SANTA-ROSA, C. C; MAGALHAES, C. S.; YAMAUTI, M.; MOREIR, A. N.
Resistência de união na fixação de pinos intrarradiculares de acordo com diferentes pré-tratamentos dentinários, 2017. (Congresso, Apresentação de Trabalho)

6. **SÁ, T. C. M.**; QUEIROZ, C. M. F.; AYALA, A. P.; SOUSA, F. F. O.; RICARDO, N. M. P. S.; SANTIAGO, S. L.; YAMAUTI, M.
Caracterização morfológica da dentina tratada com ácido anacárdico, 2016. (Congresso, Apresentação de Trabalho)

7. **SÁ, T. C. M.**
Diagnóstico e plano de tratamento em prótese e/ou reabilitação oral, 2016. (Conferência ou palestra,Apresentação de Trabalho)

8. **SÁ, T. C. M.**; LIMA, M. A.; MAGALHAES, L.; DUTRA, W. O.; GOLLOB, K. J.; HORTA, M. C.; SOUZA, P. E.
Efeito das resinas para a técnica indireta Belle Glass® e Ceramage® na expressão de quimiocinas por leucócitos humanos in vitro, 2016. (Outra,Apresentação de Trabalho)

9. **SÁ, T. C. M.**; QUEIROZ, C. M. F.; AYALA, A. P.; SOUSA, F. F. O.; MENDONCA, J. S.; RICARDO, N. M. P. S.; SANTIAGO, S. L.; YAMAUTI, M.

Preparo de solução de ácido anacárdico para utilização em Odontologia como agente de limpeza cavitária, 2016. (Outra,Apresentação de Trabalho)

Orientações e supervisões concluídas

Monografias de conclusão de curso de aperfeiçoamento/especialização

1. VANESSA GONTIJO LACERDA. **APROFUNDAMENTO DE VESTÍBULO COM UTILIZAÇÃO DE LASER DE ALTA INTENSIDADE: RELATO DE CASO**. 2016. Monografia (Especialização em Prótese Dentária) - Faculdade de Estudos Administrativos de Minas Gerais

2. VIVIANE ANKLI. **EPÍTESE GENGIVAL COMO ALTERNATIVA ESTÉTICA PARA IMPLANTES MAL POSICIONADOS: RELATO DE CASO**. 2016. Monografia (Especialização em Prótese Dentária) - Faculdade de Estudos Administrativos de Minas Gerais

3. WOLFGANG LUIZ. **PRINCÍPIOS DE OCLUSÃO APLICADOS A PRÓTESES FIXAS UNITÁRIAS IMPLANTOSSUPORTADAS**. 2016. Monografia (Especialização em Prótese Dentária) - Faculdade de Estudos Administrativos de Minas Gerais

Eventos
Participação em eventos

1. **35 encontro da sociedade Brasileira de Pesquisa Odontológica**, 2018. (Encontro)
2. Avaliador no(a) **VIII SEMINÁRIO DE INICIAÇÃO CIENTÍFICA JÚNIOR**, 2018. (Seminário)
VIII SEMINÁRIO DE INICIAÇÃO CIENTÍFICA JÚNIOR.
3. **34 Reuniao Anual da Sociedade Brasileira de Pesquisa Odontológica**, 2017. (Congresso) Período de 3 a 6 de setembro.
4. Avaliador no(a) **CIOMIG**, 2017. (Congresso)
Trabalhos de alunos de graduação e pós-graduação.
5. **CIOMIG**, 2017. (Congresso)
6. **33 Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica**, 2016. (Outra)
7. **52 Encontro Do Grupo Brasileiro de Materiais Dentários**, 2016. (Encontro)
8. **IX Congresso Brasileiro de Microscopia dos Materiais**, 2016. (Congresso)
9. **XIII ENCONTRO CIENTÍFICO DA FACULDADE DE ODONTOLOGIA – UFMG**, 2016. (Encontro)
10. Avaliador no(a) **XIII Encontro Científico da Faculdade de Odontologia da Universidade Federal de Minas Gerais**, 2016. (Encontro) Trabalhos dos alunos de Graduação.

Bancas

Participação em banca de trabalhos de conclusão

Curso de aperfeiçoamento/especialização

1. **SÁ, T. C. M.**; SOUZA, L. C.; SANTOS, B. F.
Participação em banca de Danilo Viegas da Costa. **Preservação alveolar pós-exodontias com o objetivo de instalação de implantes osseointegrados**, 2018
(Especialização em Periodontia e Capacitação em Cirurgia Plástica Periodonta) São Leopoldo Mandic

2. **SÁ, T. C. M.**; SANTOS, B. F.; SOUZA, L. C.
Participação em banca de Ana Paula Lopes Seabra. **Restauração dento alveolar imediata- RDI**, 2018
(Especialização em Periodontia e Capacitação em Cirurgia Plástica Periodonta) São Leopoldo Mandic

3. **SÁ, T. C. M.**; SOUZA, L. C.; SANTOS, B. F.
Participação em banca de Eustáquio Ramos Bonarki Júnior. **Tratamento cirúrgico de periimplantite através de regeneração óssea guiada-relato de caso clínico**, 2018
(Especialização em Periodontia e Capacitação em Cirurgia Plástica Periodonta) São Leopoldo Mandic

4. SOUZA, L. C.; **SÁ, T. C. M.**; SANTOS, B. F.
Participação em banca de Alan Rodrigues De Andrade. **Uso da terapia fotodinâmica coadjuvante ao tratmento da periimplantite**, 2018
(Especialização em Periodontia e Capacitação em Cirurgia Plástica Periodonta) São Leopoldo Mandic

5. **SÁ, T. C. M.**; SOUZA, M. T.; MOURA, M. F.
Participação em banca de Gustavo de Azevedo. **A correlação da periodontite agressiva e dos herpes vírus: alternativa clínica ao cirurgião dentista**, 2017
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

6. **SÁ, T. C. M.**; SOUZA, M. T.; MOURA, M. F.
Participação em banca de Thais dos Anjos. **Síndromes Genéticas e a relação com a doença periodontal**, 2017
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

7. **SÁ, T. C. M.**; SOUZA, M. T.; MOURA, M. F.
Participação em banca de Emanuela Pereira. **Uso de matriz de colágeno(mucograft) como substituto de enxertos gengivais livres: relato de caso**, 2017
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

8. **SÁ, T. C. M.**; SOUZA, M. T.; MOURA, M. F.
Participação em banca de Marcos Vidigal. **Uso dos biomateriais bio-oss e gen-orx no preenchimento de seio maxilar em implantodontia**, 2017
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

9. **SÁ, T. C. M.**; SA, J. C. M.; SOUZA, E. L.

Participação em banca de Vanessa Gontijo Lacerda. **Aprofundamento do Vestíbulo com utilização de laser de alta intensidade: relato de caso**, 2016
(Especialização em Prótese Dentária) Faculdade de Estudos Administrativos de Minas Gerais

10. SOUZA, M. T.; SOUZA, L. C.; **SÁ, T. C. M.**
Participação em banca de Igor Oliveira Cardoso. **Cimento cirúrgico Periodontal: Revisão de literatura e a opção do Gingi DAm como alternativa de curativo periodontal**, 2016
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

11. **SÁ, T. C. M.**; SA, J. C. M.; SOUZA, E. L.
Participação em banca de Viviane Ankli. **Epítese gengival como alternativa para implantes mal posicionados: relato de caso**, 2016
(Especialização em Prótese Dentária) Faculdade de Estudos Administrativos de Minas Gerais

12. SA, J. C. M.; **SÁ, T. C. M.**; GRECO, G. D.
Participação em banca de Bruno Emérito Campos Maciel. **Facetas Odontológicas**, 2016
(Especialização em Prótese Dentária) Faculdade de Estudos Administrativos de Minas Gerais

13. SOUZA, E. L.; **SÁ, T. C. M.**; SA, J. C. M.
Participação em banca de Arthur Nogueira Hickson. **Guia Anterior**, 2016
(Especialização em Prótese Dentária) Faculdade de Estudos Administrativos de Minas Gerais

14. SOUZA, M. T.; SOUZA, L. C.; **SÁ, T. C. M.**
Participação em banca de José Campos Filho. **Periimplantite: Avaliação sistemática da metodologia diagnóstica**, 2016
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

15. SOUZA, M. T.; SOUZA, L. C.; **SÁ, T. C. M.**
Participação em banca de Fábio Oliveira Cardoso. **Periimplantite: Avaliação sistemática dos protocolos de tratamento**, 2016
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

16. SA, J. C. M.; **SÁ, T. C. M.**; GRECO, G. D.
Participação em banca de Tiago Sávio Saraiva Lopes. **Planejamento Protético: Filosofia DATO**, 2016
(Especialização em Prótese Dentária) Faculdade de Estudos Administrativos de Minas Gerais

17. SOUZA, L. C.; SOUZA, M. T.; **SÁ, T. C. M.**
Participação em banca de João Neves Dos Santos Junior. **Princípios básicos de ajuste oclusal em pacientes periodontalmente comprometidos**, 2016
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

18. **SÁ, T. C. M.**; SA, J. C. M.; GRECO, G. D.
Participação em banca de Wolfgang Luiz Morais de Oliveira. **Princípios de Oclusão aplicados a Próteses Fixas Unitárias**, 2016

(Especialização em Prótese Dentária) Faculdade de Estudos Administrativos de Minas Gerais

19. **SÁ, T. C. M.**; SA, J. C. M.; GRECO, G. D.
Participação em banca de Diego Candido da Silva. **Protocolo de cimentação passiva de Prótese Fixa Extensa**, 2016
(Especialização em Prótese Dentária) Faculdade de Estudos Administrativos de Minas Gerais

20. **SÁ, T. C. M.**; CUNHA, F. A.; SOUZA, L. C.
Participação em banca de Fábio Naves Melo. **Alternativas do Tratamento de Periimplantite**, 2015
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

21. **SÁ, T. C. M.**; SOUZA, L. C.; CUNHA, F. A.
Participação em banca de Marcos Ferraz de Oliveira. **Decisão quanto a escolha da técnica de recobrimento radicular: estudo de revisão**, 2015
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

22. **SÁ, T. C. M.**; SOUZA, L. C.; CUNHA, F. A.
Participação em banca de Décio Geraldo dos Santos. **Utilização de Osso autógeno no Tratamento de Periimplantite**, 2015
(Especialização em periodontia) Faculdade de Odontologia São Leopoldo Mandic

Graduação

1. YAMAUTI, M.; **SÁ, T. C. M.**
Participação em banca de Felipe Soares Pereira. **Efeito da Cor na propriedade de Absorção/Solubilidade de um Polímero Odontológico contendo prtículas de vidro bioativo**, 2018
(Odontologia) Universidade Federal de Minas Gerais

2. YAMAUTI, M.; **SÁ, T. C. M.**
Participação em banca de Carla Ferreira Simões. **Lesões Cervicais não cariosas: desafios de diagnóstico e tratamento**, 2018
(Odontologia) Universidade Federal de Minas Gerais

3. VAZ, R. R.; **SÁ, T. C. M.**
Participação em banca de Bruno Cesar de Castro Penna. **Lava Ultimate Processada no Sistema CEREC**, 2017
(Odontologia) Universidade Federal de Minas Gerais