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Dissertação

Efeito do acréscimo de nanopartículas radiopacas no desempenho de um sistema adesivo experimental autocondicionante livre de Hema

Cármen Lúcia Rodrigues Macedo

Pelotas, 2014

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...Não importa onde você parou, em que momento da vida você cansou, o que importa é que sempre é possível e necessário recomeçar.
Recomeçar é dar uma nova chance a si mesmo, é renovar as esperanças na vida e, o mais importante, acreditar em você de novo... Recomeçar... Hoje é um bom dia para começar novos desafios...

(Carlos Drummond de Andrade)

NOTAS PRELIMINARES

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A presente dissertação teve seu projeto de pesquisa qualificado em junho de 2013, sendo aprovado pela Banca Examinadora composta pelos Professores Doutores Cesar Henrique Zanchi, Noeli Boscato e Fabrício Aulo Ogliari.

Resumo

MACEDO, Cármen Lúcia Rodrigues. **Efeito do acréscimo de nanopartículas radiopacas no desempenho de um sistema adesivo experimental autocondicionante livre de Hema .** 2014. 74f. Dissertação (Mestrado em Prótese Dentária) – Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Federal de Pelotas, Pelotas, 2014.

O objetivo deste estudo foi desenvolver um sistema adesivo nanoestruturado, do tipo autocondicionante de dois passos, de alto desempenho e radiopaco. Para isso, *primers* autocondicionantes foram formulados a partir da mistura de GDMA-P, HEMA ou UDMA PEG 400, etanol e água. Os adesivos de cobertura foram formulados com Bis-GMA, TEGDMA, HEMA ou UDMA PEG 400, CQ e EDAB, além de nanopartículas de trifluoreto de itérbio (YbF_3) ou sílica (SiO_2). Os adesivos foram avaliados por testes de grau de conversão, radiopacidade, índice de refração, resistência flexural, módulo flexural, sorção e solubilidade em água e resistência de união à dentina após 24h ou 6 meses de imersão em água destilada. Os dados foram analisados estatisticamente com ANOVA e teste complementar de Tukey (α=5%). A concentração mínima de 20% em peso (carga) foi necessária para adesivos contendo UDMA PEG 400 tornarem-se radiopacos, e de 30% em peso (carga) para os adesivos contendo HEMA. De maneira geral, as propriedades físicas avaliadas foram mantidas ou melhoradas a partir da adição das partículas. A adesão à dentina após 6 meses de armazenagem em água foi prejudicada nos grupos contendo sílica, porém o YbF_3 protegeu a degradação da adesão no adesivo contendo HEMA, embora no adesivo contendo UDMA PEG 400, ele resultou em redução da adesão. A adição de partículas nanométrica em adesivos odontológicos é uma estratégia viável para aprimorar o desempenho desses materiais.

Palavras-chave: sistemas adesivos; resistência de união à microtração; autocondicionante; nanopartículas; radiopacidade; trifluoreto de itérbio

Abstract

MACEDO, CármenLúcia Rodrigues. **Effect of addition of radiopaque nanoparticles on performance of an experimental Hema free adhesive system.** 2014. 74f. Dissertation (Master Degree in Prosthetic Dentistry) – Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Federal de Pelotas, Pelotas, 2014.

The aim of this study was to develop a nanostructured adhesive system, two-step self-etch, with high performance and radiopacity property. Selfething-primers were formulated by mixing GDMA-P, HEMA or PEG 400 UDMA, ethanol and water. The resin adhesives were formulated with Bis-GMA, TEGDMA, HEMA or PEG 400 UDMA, CQ and EDAB, besides ytterbium fluoride (YbF_3) or silica (SiO_2) nanoparticles were added. The adhesives were analyzed using degree of conversion, radiopacity, refractive index, flexural strength, flexural modulus, water sorption and solubility tests. The bond strength to dentin after 24h and 6 months of water storage was also performed. Data were statistically analyzed using ANOVA and Tukey test (α=5%). The minimal concentration in mass of 20% was necessary for the PEG 400 UDMA-based adhesives become radiopaque, and of 30% for the HEMA-containing adhesives. In the overall analysis, the physical properties investigated were maintained or improved by addition of nanoparticles. The adhesion to dentin after 6 months of water storage was negatively affected within the SiO_2-based groups, but YbF_3 hampered the adhesion degradation within the HEMA-containing adhesive, although within the PEG 400 UDAM-based adhesive it resulted in decrease of microtensile bond strenght. The incorporation of nanofillers into dental adhesives seems to be an interesting way to achieve enhanced properties, although the domain of this technology still needs further further studies to better understand the effect of incorporation of fillers and/or with their surface treatment.

Key-words: adhesive systems; microtensile bond strength; self-etching; nanofillers; radiopacity; ytterbium fluoride

Lista de Figuras

Artigo 1

Artigo 2

Lista de Tabelas

Artigo 1

Artigo 2

Lista de Abreviaturas

et al.	e outros
Bis-GMA	metacrilato bisfenol-A glicidil
TEGDMA	Dimetacrilato trietilenoglicol
BaO	óxido de bário
SiO_2	dióxido de silício (sílica)
Ta_2O_5	pentóxido de tântalo
Yb_2O_3	óxido de itérbio
HEMA	metacrilato de 2-hidroxietila
PEGDMA	dimetacrilato polietilenoglicol
Bis-EMA 30	dimetacrilato etoxilado bisfenol-Aglicidil
PEG 400 UDMA	Dimetacrilato polietilenoglicol 400 uretano extendido
GDMA-P	glicerol fosfato
CQ	Canforoquinona
YbF_3	trifluoreto de itérbio
EDAB	etil 4-dimetilamino benzoato
ZnSe	seleneto de zinco
SiC	carbeto de silício
LED	luz emissora de diodos
MO	Microscopia Ótica

N	número de espécimes por grupo
ISO	Organização Internacional para Padronização

Lista de Símbolos

≤	Menor ou igual
>	Maior
<	Menor
=	Igual
±	Mais ou menos
+	Mais
%	Percentual
º	Grau
®	símbolo indicativo de marca registrada
ºC	graus Celsius
X	Vezes
~	Aproximadamente
mm^2	Milímetro quadrado
Mm	Milímetro
S	Segundos
Dpi	*dots per inch* (pontos por polegada)
Kv/ mA	quilovoltagem por miliampere
A°	Angstron
Mpa	Mega Pascal

N	Newton
mm/min	milímetro por minuto
g/mol	grama por mol
mW/cm^2	miliwatts por centímetro quadrado
mPa.s	milipascal-segundo
mm/s	milímetro por segundo
µl	Microlitro
Nm	Nanômetro

Sumário

1 Projeto de Pesquisa

1. Introdução

O mecanismo básico de união ao esmalte e dentina consiste na substituição de uma camada mineral por monômeros resinosos que, após a sua polimerização *in situ*, promovem uma união micro mecânica, denominada de “hibridização”(NAKABAYASHI et al., 1982).

A técnica adesiva inicia pela utilização de uma substância ácida (condicionamento ácido) sobre o substrato dentário, cuja função é aumentar a receptividade deste pelo adesivo através da remoção ou modificação da *smearlayer* produzindo microporosidades superficiais, onde ocorrerá a retenção micromecânica do adesivo ao dente. Logo após, uma substância hidrófila (*primer*) é aplicada a fim de infiltrar o substrato previamente condicionado. Por fim, uma resina adesiva de cobertura é então aplicada, completando a hibridização do substrato. Dependendo da seqüência ou ordem de aplicação de cada uma dessas substâncias, os sistemas adesivos podem ser classificados em convencionais, que utilizam a etapa de condicionamento ácido separadamente dos outros passos clínicos, ou autocondicionantes, que por serem constituídos de monômeros ácidos possuem a habilidade de condicionar o substrato simultaneamente à infiltração adesiva (PASHLEY et al., 2011; VAN MEERBEEK et al., 2011).

Uma das maneiras que o cirurgião-dentista dispõe para avaliar o desempenho clínico de uma restauração é o exame radiográfico, o qual permite visualizar desadaptação da restauração e/ou recorrência de processo carioso. Contudo, isso só é possível quando o material a ser avaliado apresenta radiopacidade (WATTS; MCCABE, 1999). Materiais basicamente constituídos por elementos como carbono, hidrogênio, oxigênio e nitrogênio, como é o caso dos adesivos odontológicos, possuem baixa densidade/número atômico, e assim sendo, não são suficientemente radiopacos para serem avaliados por meio do exame radiográfico (JAMES; JAYAKRISHNAN, 2007). Todavia, alguns estudos demonstraram que a adição de partículas de carga em sistemas adesivos resultou em obtenção de radiopacidade (MIYAZAKI et al., 1995; SCHULZ et al., 2008; CONDE et al., 2009). Embora o acréscimo de nanoestruturadas resinas compostas esteja em desenvolvimento avançado e praticamente consolidada (KIM et al., 2005), uma das dificuldades de se incorporar partículas de carga em uma resina adesiva de baixa viscosidade, como o adesivo, é a de manter essas partículas estáveis quando em suspensão na blenda monomérica; mesmo assim, dependendo da concentração e tipo de tratamento superficial das partículas de carga, satisfatórios resultados podem ser obtidos quanto a esta problemática.

O metacrilato de 2-hidroxietila (HEMA), que é um monômero hidrófilo de alta fluidez e de peso molecular de aproximadamente 130 g/mol, é geralmente empregado como redutor de viscosidade (NAKABAYASHI; TAKARADA, 1992), sendo amplamente utilizado na formulação de sistemas adesivos devido, principalmente, a sua capacidade de infiltrar-se facilmente à superfície dentinária desmineralizada, evitando o colapso das fibrilas colágenas (CARVALHO et al., 1996; HITMI et al., 2002). A presença de HEMA também é importante para manter os monômeros resinosos em solução, prevenindo, por exemplo, separação de fases no material (VAN LANDUYT et al., 2005). Apesar de suas vantagens ao processo adesivo, HEMA vem sendo recentemente substituído por outros tipos de monômeros a fim de reduzir-se a hidrofilicidade do adesivo, bem como o comprometimento das propriedades mecânicas da matriz resinosa (COLLARES et al., 2011). Ainda, a presença de HEMA em sistemas adesivos está diretamente relacionada com o desenvolvimento de reações alérgicas/citotóxicas ao tecido pulpar (PARANJPE et al., 2005), podendo comprometer o sucesso do tratamento restaurador. Assim sendo, novas composições de sistemas adesivos livres de HEMA têm sido avaliadas a fim de obter-se um material mais resistente mais estável e com maior expectativa de longevidade .

2. Justificativa

O desenvolvimento de novos sistemas adesivos que incorporem novas características ou que melhorem aquelas atualmente existentes ainda é uma necessidade no mercado odontológico. De fato, a incorporação de partículas de carga pode ser um método interessante já que as propriedades físicas, elétricas e térmicas do material podem ser modificadas. Contudo, a aplicação da nanotecnologia nos sistemas adesivos é ainda recente na Odontologia, sendo necessários estudos laboratoriais que avaliem o efeito da adição de partículas nas propriedades do material, bem como frente a diferentes formulações (mais hidrófilas ou mais hidrófobas), já que os adesivos atuais podem apresentar diversificada composição química.

3 Objetivos

3.1 Objetivo geral:

O objetivo do presente estudo é desenvolver um sistema adesivo odontológico do tipo autocondicionante com ausência de Hema na sua composição, contendo nanopartículas de modo a proporcionar radiopacidade, alto desempenho físico-mecânico.

3.2 Objetivos específicos:

a. Formular *primers* autocondicionantes contendo HEMA ou UDMA PEG 400;
b. Formular resinas adesivas contendo HEMA ou UDMA PEG 400 e diferentes partículas de carga;
c. Avaliar as propriedades físicas das resinas adesivas;
d. Determinar a concentração mínima de partícula de carga que resulte ao adesivo em radiopacidade semelhante à dentina e/ou esmalte dentários;

e. Avaliar a resistência de união dos sistemas adesivos à dentina imediatamente e após 6 meses de armazenamento em água.

4 Hipótese de estudo

A hipótese de estudo do presente projeto a ser investigada é que : a adição de partículas de carga em um sistema adesivo experimental do tipo autocondicionante não afetará negativamente as propriedades físico-mecânicas do material.

5 Metodologia

5.1 Formulação dos sistemas adesivos experimentais

Dois *primers* autocondicionantes foram preparados, conforme estudo previamente realizado (ZANCHI et al., 2013), misturando-se 30% de GDMA-P como monômero ácido (o qual corresponde a uma mistura eqüimolar de dimetacrilato fosfato diidrogênio glicerol e tetrametacrilato fosfato hidrogênio glicerol), 30% de um monômero de variação (HEMA ou dimetacrilato uretano extendido polietilenoglicol 400 – UDMA PEG 400), 20% de etanol e 20% de água.

Após, duas resinas de cobertura (adesivos) foram preparados misturando-se 50% de metacrilato de bisfenol A diglicidil (Bis-GMA), 25% de dimetacrilato de trietilenoglicol (TEGDMA), 25% dos monômeros de variação (HEMA ou UDMA PEG 400), 0,4% de canforoquinona (CQ) e 0,8% de etil 4-dimetilamino benzoato (EDAB). Todos os constituintes foram obtidos da Esstech Inc. (Essington, MA, EUA), com exceção do EDAB, que foi obtido da Fluka (Milwakee, WI, EUA). Cada adesivo foi subdividido em 3 grupos segundo o tipo de partícula de carga adicionado: controle (sem adição de partículas); sílica (SiO_2 – adição de 10% em massa de nanopartículas de sílica, Aerosil, Degussa, Weiss-Fraunenst, Germany); e trifluoreto de itérbio (YbF_3 – adição de 10% em massa de nanopartículas de trifluoreto de itérbio (40-80 nm de tamanho, 99% de pureza).

5.2 Avaliação da cinética e do grau de conversão por FTIR

A cinética de conversão dos adesivos experimentais foi avaliada por meio de um espectrofotômetro infravermelho com Transformada de Fourier (RT-FTIR Shimadzu Prestige 21 Spectrometer, Shimadzu, Japão) equipado com dispositivo de reflectância total atenuada (ATR), composto por um cristal horizontal de seleneto de zinco (ZnSe), com espelhos de angulação de 45º (PIKE Technologies, WI, USA). Um suporte foi acoplado para a fixação da unidade foto-ativadora ao espectrofotômetro, permitindo a padronização de uma distância de 5 mm entre a extremidade da ponteira de fibra ótica e a amostra. Cada amostra foi dispensada diretamente no cristal de ZnSe em pequenas gotas (~3µl) e foto-ativadas por 30 segundos. Para o monitoramento de varredura foi utilizado o software IRSolution, em uma faixa espectral entre 1750 e 1550 cm^{-1}, resolução de 8 cm^{-1} e velocidade de deslocamento de espelho de 2,8 mm/s. Com esta configuração foi possível obter um espectro por segundo durante a foto-ativação. A análise foi realizada em ambiente com temperatura controlada de 23ºC e umidade relativa <60%.

O grau de conversão, por sua vez, foi calculado considerando-se a intensidade da vibração do tipo estiramento da dupla ligação carbono-carbono na frequência de 1635 cm^{-1}. O estiramento simétrico do anel aromático em 1610 cm^{-1} das amostras polimerizadas e não polimerizadas foram utilizado como padrão interno. Posteriormente, os dados obtidos foram plotados em uma curva ajustada pelo parâmetro regressivo não-linear de Hill 1 (OGLIARI et al., 2008).

5.3 Avaliação da viscosidade dos sistemas adesivos

O comportamento reológico dos *primers* e dos adesivos experimentais foi avaliado utilizando-se um viscosímetro rotacional de cilindro coaxial (Brookfield, modelo DV-II+Pro) específico para altas viscosidades. Seis mililitros (mL) de cada amostra foram necessários para a avaliação da viscosidade, que foi expressa em mPas.

5.4 Avaliação da resistência de união à dentina (microtração) e análise do padrão de falha

Incisivos bovinos, com período de extração inferior a três meses, foram utilizados para esta avaliação. Após a extração dentária, os dentes foram armazenados em solução de Cloramina T 0,5% durante sete dias, sendo posteriormente transferidos para água destilada e mantidos congelados em *freezer* até seu uso. Os dentes foram distribuídos aleatoriamente entre os grupos experimentais (n=8). Na face vestibular de cada dente será realizado desgaste com lixas de carbeto de silício (SiC) de modo crescente de aplicação até a exposição de dentina com lixa d'água granulação 600 sob abundante irrigação com água, no sentido de padronizar a espessura de lama dentinária (*smearlayer*) formada. Os dentes foram lavados com água destilada e a superfície de dentina foi inspecionada em microscópio ótico com aumento de 40× para assegurar a ausência de esmalte remanescente na superfície. Então, cada primer foi aplicado vigorosamente sobre a dentina seca durante 30 segundos e em seguida foi aplicado jato de ar a uma distância padronizada de 10 cm durante 10 segundos. O adesivo de cobertura foi aplicado e foto-ativado por 20 segundos com aparelho foto-polimerizador LED (Radii®, SDI, Bayswater, VIC, Austrália). A restauração foi confeccionada utilizando-se três incrementos de até 2 mm de compósito restaurador (Filtek™ Z-250, St. Paul, MN, EUA), sendo foto-ativados por 30 segundos, de acordo com as recomendações do fabricante.

Após armazenagem por 24 horas em água destilada a 37°C, os dentes foram seccionados em cortadeira de precisão (ISOMET 1000, Buheler). Os cortes foram realizados de maneira a produzir palitos com área em sua secção transversa de aproximadamente 0,8 mm^2. As amostras de cada grupo foram divididas em três subgrupos a serem testados em diferentes períodos: 24h após o corte, após 6 meses e após 12 meses de armazenagem em água a 37ºC. Após o tempo de armazenagem, cada palito foi fixado em um dispositivo desenvolvido para ensaios de microtração com o auxílio de um adesivo à base de cianoacrilato (Super Bonder Gel, Loctite). As amostras foram tracionadas a uma velocidade de 1 mm/min até sua falha em uma máquina de ensaio mecânico (DL500, EMIC, São José dos Pinhais, PR, Brasil) com uma célula de carga de 100N e então a resistência de união foi mensurada em MPa.

Todas as amostras fraturadas foram observadas através de microscopia óptica (MO) disponível em Microdurômetro (Futuretech FM700) com um aumento de 100× e 500× para a determinação do modo de falha, sendo classificadas em adesivas, mistas, ou coesivas em resina ou dentina.

5.5 Avaliação da resistência à flexão e módulo de elasticidade

Espécimes medindo 12 mm de comprimento ×2 mm de largura × 2 mm de espessura foram preparados utilizando-se um molde metálico. A matriz foi posicionada sobre uma placa de vidro, previamente isolada com uma lâmina de poliéster, e, posteriormente, preenchida com a resina adesiva, evitando-se a incorporação de bolhas. Outra lâmina de poliéster foi posicionada sobre a matriz e os excessos de material removidos. Finalmente, o conjunto foi foto-ativado por 20 s em cada face, com o mesmo LED descrito anteriormente. Após 24h de armazenagem em água destilada a 37ºC, cada espécime foi submetido ao teste de resistência flexural (DL 500) a uma velocidade de 0,5 mm/min até sua fratura. O módulo de elasticidade dos adesivos foi obtido a partir dos resultados de resistência flexural.

5.6 Avaliação da radiopacidade dos adesivos

Espécimes medindo 6 mm de diâmetro × 1 mm de espessura foram produzidos usando-se um molde de silicone. Cinco espécimes foram preparados para cada adesivo, sendo foto-ativados por 20 s em cada superfície. Cada espécime foi então posicionado sobre um filme oclusal Insight (Kodak, EUA), juntamente com uma escala de alumínio medindo 20 × 10 mm, com 99,6% de alumínio puro, e amostras de esmalte e dentina (da mesma espessura que os materiais experimentais), para fins de comparação radiográfica de radiopacidade. A distância foco-filme foi padronizada em 30 cm com um ângulo de 90° e irradiação com 0,3 s de exposição de um aparelho de radiação (Spectro 70X Selectronic 70Kv/8mA, Dabi Atlante, Brasil). As imagens foram obtidas digitalmente (Artixscan 1100, Microtek, EUA), e então a radiopacidade foi mensurada por análise de pixels usando-se o software Image J (versão 1.42q – Instituto Nacional de Saúde – EUA).

5.7 Avaliação da sorção e solubilidade em água

Para avaliação dos fenômenos de sorção (SO) e solubilidade (SL) foram preparados espécimes medindo 6 mm de diâmetro × 1 mm de espessura (MUNCHOW et al., 2014). Além disso, as especificações da ISO 4049: 2009 (exceto pela dimensão dos espécimes) foi utilizada para a execução deste teste.

Os espécimes foram mensurados quanto à sua massa em uma balança analítica digital (AW220D; Shimadzu, Japão) com precisão de 0,01mg e levados ao dessecador com sílica gel a 37°C. Diariamente, pesagens foram realizadas e anotadas até estabilização da massa seca inicial (variação menor que 0,1mg em 24h), que foi denominada de m_1. Após obtenção da m_1, as medidas de diâmetro (D) e espessura (e) de cada espécime foram obtidas para posterior cálculo do volume (V, em mm³) dos espécimes ($V = \pi R^2 h$, onde π é uma constante igual a 3,1421; R é o raio (D/2); e h é a espessura do espécime).Após, cada espécime foi armazenado em água destilada por 7 dias, novamente mensurado quanto a sua massa (massa úmida m_2), e então, após secagem em dessecador a 37°C, pesados novamente (massa seca final m_3). Logo, a SO e SL foram calculadas através das seguintes fórmulas, sendo expressas em µg/mm³: $SO = (m_2 – m_3)/V$; e $SL = (m_1 – m_3)/V$.

5.8 Análise estatística

Os dados obtidos neste projeto foram analisados com o programa estatístico SigmaPlot versão 12.0 (Systat, San Jose, CA, EUA), considerando-se o valor significativo de $p < 0,05$.

6 Orçamento

REAGENTES E INSUMOS	CUSTO	DESCRIÇÃO	CUSTO
DESCRIÇÃO			
Bis-GMA	450,00	EDAB	375,00
TEGDMA	210,00	Nanopartículas	300,00
GDMA-P	100,00	Clearfill SE Bond	180,00
HEMA	230,00	Solventes	300,00
PEG 400 UDMA	210,00	Resina composta	140,00
Canforaquinona	400,00	Lixas SiC	30,00
TOTAL	**R$ 1.600,00**	**TOTAL**	**R$ 1.325,00**

7 Cronograma

Atividades ano 2012									Set	Out	Nov	Dez
Revisão da Literatura									🟧	🟧	🟧	🟧
Teste Piloto									🟧	🟧	🟧	
Aquisição dos reagentes									🟧	🟧	🟧	🟧
Atividades ano 2013	Jan	Fev	Mar	Abr	Mai	Jun	Jul	Ago	Set	Out	Nov	Dez
Qualificação do Projeto						🟧						
Levantamento bibliográfico	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧	🟧
Execução do Projeto						🟧	🟧	🟧				
Análise dos Resultados								🟧	🟧			
Redação da Disertação									🟧	🟧	🟧	
Atividades ano 2014	Jan	Fev	Mar	Abr	Mai	Jun	Jul	Ago	Set	Out	Nov	
Redação de Resultados			🟧	🟧	🟧	🟧						
Defesa da dissertação											🟧	
Publicação dos resultados											🟧	

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2 Relatório do Trabalho de Campo

O objetivo geral do presente estudo foi desenvolver um sistema adesivo odontológico nanoestruturado, do tipo autocondicionante, e que apresentasse alto desempenho físico-mecânico, além de radiopacidade e ausência de HEMA na sua composição. Para isso, *primers* e resinas adesivas modelos foram preparados, contendo HEMA (controle) ou UDMA PEG 400 (como substituto ao HEMA).

Os materiais experimentais foram formulados conforme previsto no projeto de pesquisa, porém a caracterização e avaliação dos mesmos ocorreram com algumas modificações. Por exemplo, a avaliação da cinética de conversão estava prevista, e apesar de realizada, optou-se por não adicionar os resultados nos artigos, devido à ausência de relevância científica para o contexto dos mesmos. Além disso, a metodologia de grau de conversão das resinas adesivas seria realizada a partir da foto-ativação dos espécimes por 30 segundos; no entanto, adicionou-se o tempo de 60 segundos (como um segundo fator de variação) já que se observou que a polimerização/grau de conversão aumentava; assim, dois períodos de foto-ativação foram utilizados, ao invés de apenas um como previsto no projeto.

A metodologia de viscosidade, que estava também prevista no projeto de pesquisa, não foi realizada devido a problemas técnicos com o equipamento ao longo do período de mestrado. Contudo, a avaliação da propriedade ‘índice de refração’ foi adicionada ao projeto por acrescentar informações pertinentes à caracterização de adesivos contendo partículas de carga, além de tornar-se disponível após realização da qualificação do projeto.

A avaliação da radiopacidade das resinas adesivas, bem como de algumas outras propriedades planejadas, foi realizada apenas com os grupos contendo trifluoreto de itérbio. No projeto, no entanto, planejou-se utilizar os grupos contendo sílica como controle negativo (ao teste de radiopacidade); contudo, nenhuma informação relevante seria adicionada ao estudo, então se optou por remover este grupo de algumas avaliações planejadas. No demais, o projeto foi realizado como previsto.

3 Artigo 1*

Title.

Effect of ytterbium trifluoride concentration on the radiopacity, refractive index and degree of conversion of model dental adhesive resins*

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Running Heads.

Effect of YbF_3 content on physical properties of model adhesives

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Abstract

This study formulated model adhesive resins containing different monomeric systems and different concentrations of ytterbium trifluoride (YbF_3) in an attempt to investigate the effect of these variables on the radiopacity (Ra), refractive index (Ri), and degree of conversion (DC) of the adhesives. Two resins were prepared by mixing Bis-GMA, TEGDMA and a variable monomer – HEMA or PEG 400 UDMA (mass ratio of 2:1:1). YbF_3 was added into the adhesives at different content (0, 5, 10, 20, and 30 wt%). The Ra test was performed using an occlusal In Sight film (n=3); the Ri test using an Abbe refractometer (n=3); and the DC analysis using FTIR (n=3). Data were analyzed using ANOVA and Tukey test (α=5%). The incorporation of 20 wt% of YbF_3 into the UDMA-based adhesive resulted in satisfactory Ra, whereas the HEMA-containing material needed higher content (30 wt%). The addition of YbF_3 progressively reduced the Ri of the HEMA-containing adhesives; high content (20 and 30 wt%) of YbF_3 increased the Ri of the UDMA-based materials. The DC was reduced only for the UDMA-based adhesive containing 30 wt% of YbF_3 compared to the unfilled material. The type of monomeric system and the concentration of YbF_3 influenced on the properties investigated.

Key Words: inorganic fillers, HEMA, monomers, polymerization; optical properties

1. Introduction

Dental adhesives are widely used for restorative purposes, mainly when different materials/surfaces need to be bonded to each other. They are usually constituted by mono- and/or di-methacrylate monomers which sometimes are mixed to solvents in an attempt to obtain low viscous resins able to infiltrate and to adhere to the dental substrates. Even though some current adhesives are constituted by low amounts of inorganic fillers, the incorporation of these compounds into adhesive formulations is uncommon, differing to resin composites which may present higher filler content.[1] Nevertheless, the presence of fillers may interestingly modify the mechanical, biological, optical, thermal and electric properties of the material, as stated by some previous studies.[2-4]

Despite of several potential applications, the addition of fillers into adhesives may result in radiopacity characteristic,[5] which means that the adhesive could be identified during a radiographic exam (i.e., important for diagnosing purposes). Dental adhesives usually lack in radiopacity because they are basically constituted by low density chemical elements, such as carbon, hydrogen, nitrogen and oxygen,[6] and considering that radiopacity is provided by elements with high density/atomic number, the addition of fillers (e.g., metal oxides) with a high atomic number is one approach that would solve this problem.[7] According to a recent review,[8] ytterbium-based compounds were considered the most promising candidates for acting as contrast agents of X-ray radiation, first due to their high atomic number and second due to their K-edge energy situated within the X-ray energy spectra. In this way, ytterbium trifluoride (YbF_3) has already been applied in dentistry in an attempt to impair radiopacity to some materials.[9,10] However, when incorporated into a model adhesive resin, the low concentrations used (e.g., 0.5, 2.5, 5, and 7.5 wt%) were not sufficient to add radiopacity in a similar way to dental substrates (i.e., enamel and dentin).[9] In fact, when incorporated into dental cements, a minimum concentration of 30 wt% of YbF_3 was necessary to obtain satisfactory radiopacity without influencing other properties.[10] Therefore, it seems that the concentration of the filler plays a significant role on the acquisition of radiopacity.

One major concern regarding the incorporation of fillers into adhesives is the filler stability while in suspension. Some studies have been demonstrating that the higher the filler content, the higher the probability of their poor distribution into the resin.[9,11] As a consequence, the physical stability of the material may be affected, resulting in agglomeration of fillers and in the reduction of mechanical strength.[12] Moreover, depending on the type of monomers the stability of the polymer system would change, mainly because different monomers have distinct characteristics that may modify the chemical interaction obtained with the filler.

Hence, the aims of this study were (1) to prepare model adhesive resins containing different monomeric systems and different concentrations of YbF_3, and (2) to investigate the effect of these variables on the radiopacity, refractive index, and degree of conversion of the adhesives. Two hypotheses were tested: (1) the higher the concentration of fillers, the higher the adhesive radiopacity; and (2) the increase in the concentration of fillers would affect negatively the degree of conversion of the adhesive.

2. Materials and methods

2.1. Reagents

2-bis[4-(2-hydroxy-3-methacryloyloxypropyl) phenyl]-propane (Bis-GMA), tryethyleneglycoldimethacrylate (TEGDMA), polyethyleneglycol (400) extended urethane dimethacrylate (PEG 400 UDMA), and camphorquinone (CQ) were purchased from Esstech Inc. (Essington, USA). 2-hydroxyethyl methacrylate (HEMA) and gamma-methacryloxypropyltrimethoxysilane (γ-MPTS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethyl 4-dimethylaminebenzoate (EDAB) was purchased from Fluka (Milwaukee, USA). YbF_3 was obtained from Stanford Materials (Aliso Viejo, USA). Acetone was purchased from Vetec (Duque de Caxias, RJ, Brazil). All reagents were used as received and without further purification.

2.2. Preparation of the experimental adhesive resins

Two different adhesive formulations were prepared in the study: one containing Bis-GMA, TEGDMA and HEMA (2:1:1 mass ratio, ‘H’ group) and other containing Bis-GMA, TEGDMA and PEG 400 UDMA (2:1:1 mass ratio, ‘U’ group); both systems received 0.4 wt% of CQ and 0.8 wt% of EDAB to make the resin light-sensitive. Next, each adhesive was allocated into five subgroups, according to the concentration of YbF_3: 0 (control), 5, 10, 20, or 30 wt%. The composition of all groups is shown in Table 1. Before their incorporation into the adhesive, the YbF_3 fillers were silanized using a 10 wt% γ-MPTS/acetone solution, resulting in a slurry which was stored for 24 h at 37 °C to assure complete solvent removal; next, the fillers were sieved through a 150 µm sieve and then added into the adhesives.

2.3. Radiopacity

Disk-shaped specimens measuring 6 mm in diameter *vs.* 1 mm in thickness were prepared using a metallic mold (n=5). Each adhesive was placed into the mold and light-activated for 20 seconds (s) at the top and bottom surfaces using a light-emitting diode (LED Radii, Bayswater, VIC, Australia) light-curing unit. All specimens were then grouped and placed in an occlusal In Sight film (Kodak, USA); an aluminum (Al) scale measuring 20 × 10 mm (10 steps, varying 0.5 mm per step, with an Al purity of 99.6%) and samples of dentin and enamel (1 ± 0.1 mm in thickness) were used as parameters for radiographic comparison. The set was fixed in an acrylic support, which was developed to standardize the focus-film distance at 35 cm with 90° angle and irradiated with 1.6 s exposures in an X-ray device (Spectro 70X Selectronic 70kV/8 mA, DabiAtlante, Brazil). Three radiographs were taken, which digital images were obtained using a scanner at a resolution of 500 dpi (Artixscan 1100, Microtek, USA). The relative radiopacity densitometry was measured by pixel analysis using Adobe Photoshop software (version 7.0.1, Adobe Systems Incorporated, San Jose, CA, USA). Three points in each sample were measured by means of pixel analysis, and the mean values were used for statistical analysis of data.

2.4. Refractive index

The refractive indices of the adhesives were measured using an Abbe refractometer (model 2WAJ), at 23°C with the measurement accuracy of ±0.0002. Each adhesive was evaluated three times.

2.5. Degree of conversion

The degree of conversion of each adhesive (n=5) was evaluated using Fourier Transform mid-infrared spectroscopy (Prestige21, Shimadzu, Japan). A standard resin volume (~3 µl) was used and a preliminary reading (monomer) for the un-polymerized material was taken. Then, the material was light-activated with the LED light-curing unit for 30 s and another reading was carried out (polymer). The DC was calculated as previously described.[13]

2.6. Statistical analysis

All data were analyzed with the statistical program SigmaPlot (version 12.0, Systat Software Inc., San Jose, CA, USA). While the radiopacity data were analyzed using One Way Analysis of Variance (ANOVA), the refractive index and degree of conversion data were analyzed using Two Way ANOVA (‘monomeric system’ and ‘filler concentration’ factors), and the Tukey test was used for multiple comparison analyses. A linear regression analysis was used to investigate the relationship between YbF_3 concentration and radiopacity. The significance level of all analyses was set at $\alpha=5\%$.

3. Results

3.1. Radiopacity

The radiopacity results and the linear regression model obtained in the study are shown in Figure 1. According to the statistical analyses, a significant linear increase in radiopacity was associated with the increase in YbF_3 concentration (Fig. 1A, $p \leq 0.007$), which can be also observed in the radiograph image showing the experimental and control samples evaluated (Fig. 1B). The minimum YbF_3 content able to produce a similar radiopacity to dentin was 20 wt% in the PEG 400 UDMA-based adhesive ($p = 0.932$) and 30 wt% in the HEMA-containing adhesive ($p = 1.000$) (Fig. 1C). A similar radiopacity to enamel was obtained only with the addition of 30 wt% of YbF_3 in the PEG 400 UDMA-based adhesive ($p = 1.000$). Dentin and enamel demonstrated, respectively, similar radiopacity to the 1.0 mm-thick Al ($p = 0.715$) and to the 2.0-2.5 mm-thick Al samples ($p \geq 0.990$). The highest radiopacity value of the study was obtained with the 3.0 mm-thick Al sample ($p < 0.001$).

3.2. Refractive index

The refractive indices of the adhesive resins are presented in Figure 2. According to the statistical analysis, the factors investigated were not significant, although their interaction was significant ($p \leq 0.001$). Within the HEMA-containing adhesive it was possible to observe that the higher the YbF_3 content the lower the refractive index, although only the most concentrated group showed a significant difference compared to the unfilled adhesive ($p = 0.021$). With regard to the PEG 400 UDMA-based adhesive, the refractive index first decreased until the incorporation of 10 wt% of YbF_3, although without significant differences when compared to the unfilled adhesive ($p \geq 0.053$), and then it progressively increased. U_{30} showed higher refractive index than all the other adhesives ($p \leq 0.025$). At the same filler concentration the adhesives containing HEMA or PEG 400 UDMA showed similar refractive indices ($p \geq 0.069$), except at the 30 wt% loading level, where U_{30} presented higher index than H_{30} ($p < 0.001$).

3.3. Degree of conversion

The results of degree of conversion are shown in Figure 3. According to the statistical analysis the factors 'monomeric system' ($p = 0.036$) and 'filler concentration' ($p = 0.014$) were both significant, although their interaction was not significant ($p = 0.611$). While the addition of 5 to 30 wt% of YbF_3 did not alter the degree of conversion of the HEMA-containing adhesives ($p \geq 0.452$), the unfilled PEG 400 UDMA-based adhesive showed higher degree of conversion than U_{30} ($p = 0.012$). At the same filler concentration the adhesives presented similar degree of conversion ($p \geq 0.054$).

Table 1. Composition of each adhesive resin prepared in the study.

Groups	**Monomers (wt%)**				**CQ / EDAB (wt%)**	**YbF_3 (wt%)**
	Bis-GMA	**TEGDMA**	**HEMA**	**PEG 400 UDMA**		
H_0	50	25	25	0	0.4/0.8	0
H_5	50	25	25	0	0.4/0.8	5
H_{10}	50	25	25	0	0.4/0.8	10
H_{20}	50	25	25	0	0.4/0.8	20
H_{30}	50	25	25	0	0.4/0.8	30
U_0	50	25	0	25	0.4/0.8	0
U_5	50	25	0	25	0.4/0.8	5
U_{10}	50	25	0	25	0.4/0.8	10
U_{20}	50	25	0	25	0.4/0.8	20
U_{30}	50	25	0	25	0.4/0.8	30

Bis-GMA: 2-bis[4-(2-hydroxy-3-methacryloyloxypropyl) phenyl]-propane; TEGDMA: tryethyleneglycoldimethacrylate; HEMA: 2-hydroxyethyl methacrylate; PEG 400 UDMA: polyethyleneglycol (400) extendedurethanedimethacrylate; CQ:

camphorquinone; EDAB: ethyl 4-dimethylaminebenzoate; and YbF_3: ytterbiumtrifluoride.

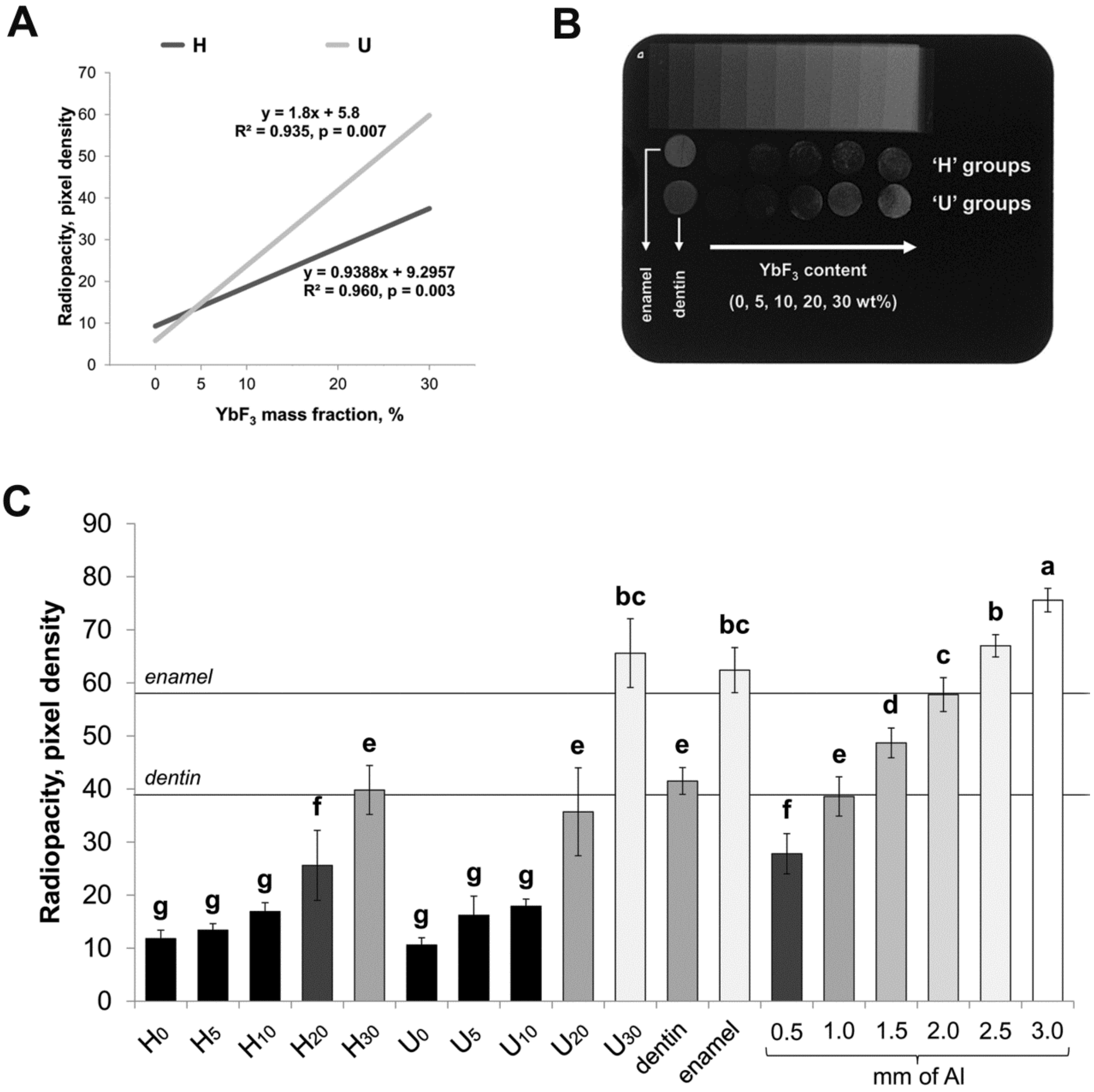
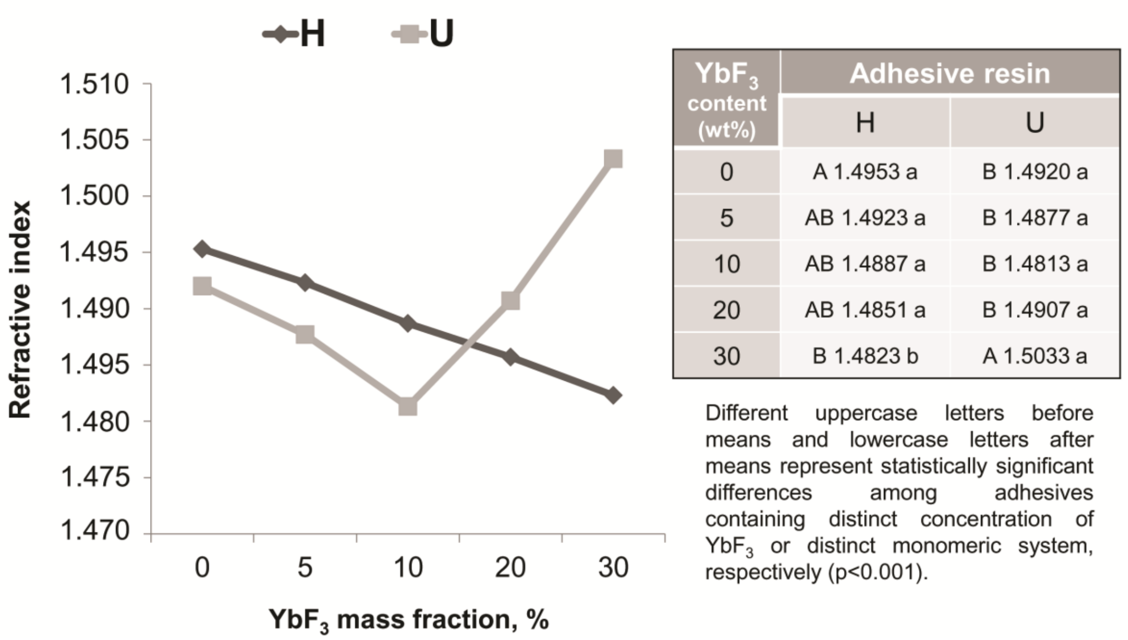


Figure 1. Radiopacity results. (A) Linear regression predictive model that shows a significant relationship between YbF_3 concentration and radiopacity (R^2 = 0.960 and 0.935 for the HEMA-containing and PEG 400 UDMA-based adhesives, respectively;

p ≤ 0.007). (B) Radiograph image comparing the radiopacity of the model adhesives and the controls (aluminum scale, and enamel/dentin samples). (C) Radiopacity means and standard deviations for all adhesives and control samples evaluated; columns marked with different lowercase letters represent statistically significant differences among the groups evaluated ($p < 0.05$).



YbF_3 content (wt%)	Adhesive resin	
	H	U
0	A 1.4953 a	B 1.4920 a
5	AB 1.4923 a	B 1.4877 a
10	AB 1.4887 a	B 1.4813 a
20	AB 1.4851 a	B 1.4907 a
30	B 1.4823 b	A 1.5033 a

Different uppercase letters before means and lowercase letters after means represent statistically significant differences among adhesives containing distinct concentration of YbF_3 or distinct monomeric system, respectively ($p<0.001$).

Figure 2. Graph and table that show the refractive indices of the HEMA-containing and PEG 400 UDMA-based adhesives with different concentrations of YbF_3.

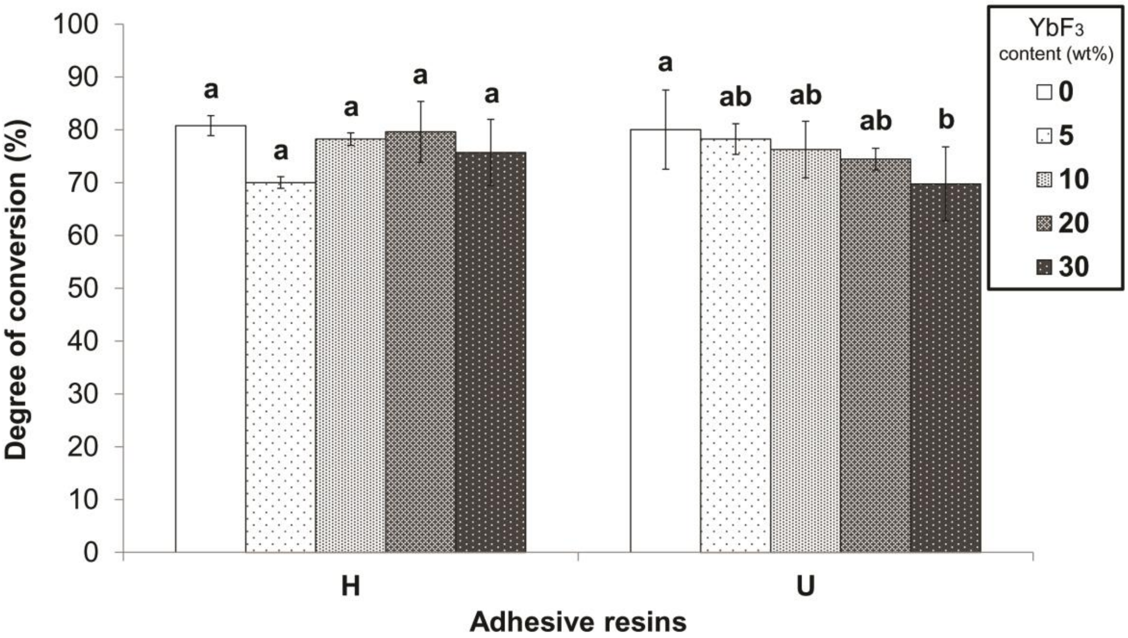


Figure 3. Graph that shows the degree of conversion of the adhesives investigated in the study. Columns marked with different lowercase letters represent statistically significant differences among the groups evaluated ($p < 0.05$).

4. Discussion

The present study demonstrated that YbF_3 fillers impaired radiopacity to the model adhesives formulated, where the higher the concentration added, the higher the radiopacity (Fig. 1A), thus accepting the first hypothesis tested. This can be explained because ytterbium (Yb) is a chemical element with a considerably high atomic number (z = 70),[8] so the YbF_3-based adhesives were able to inhibit X-ray passage by absorption and scattering of the electromagnetic radiation,[12] differently to the unfilled adhesives which showed minimal radiopacity (Fig. 1B). However, only the most concentrated groups presented similar radiopacity to dentin and/or enamel (Fig. 1C), confirming that this property is concentration-dependent. In fact, it was already expected that increasing the mass fraction of fillers into the adhesives would increase the scattering effect of the radiation. Moreover, the most surprising finding of this study is that the type of monomeric system also influenced on the radiopacity of the adhesives: U_{20} and U_{30} were more radiopaque than H_{20} and H_{30} (Fig. 1C), respectively, which can be also easily observed in the radiograph image shown at

Fig. 1B. Considering that the only difference between those adhesive resins was the presence of HEMA or PEG 400 UDMA, it can be suggested that these monomers have some specific characteristics that influenced on the final radiopacity of the material.

HEMA and PEG 400 UDMA have several differences regarding to their molecular weight, structure, hydrophilicity, and viscosity,[14,15] although these characteristics do not seem to have a direct influence on radiopacity. The refractive index, on the other hand, seems to have a more direct relationship to the radiopacity phenomenon since it is a physical property that measures how light or any other radiation propagates or interact within the material.[7] As shown in Fig. 2, the unfilled adhesives demonstrated very close refractive indices (1.4953 and 1.4920, respectively), suggesting that light/radiation was similarly transmitted through these materials.[16] As expected, the incorporation of YbF_3 modified the refractive index of the adhesives, which is explained because inorganic fillers commonly have different refractive indices compared to polymers,[17] and this mismatch can reduce translucency inside the material due to light interaction at the polymer/filler interface,[7] thus affecting the refractive index property. However, it was a surprise that for the HEMA-containing adhesives the refractive index decreased in a progressive way with addition of YbF_3, fact that was also observed for the PEG 400 UDMA-based adhesives containing up to 10 wt% of fillers (Fig. 2); in theory, according to Mädler et al.,[7] the addition of small amounts of ytterbium oxide increased the refractive index of the material, thus differing from the present findings.

It can be suggested that the distribution of YbF_3 particles was influenced by the composition of the adhesives. HEMA has a pendant hydroxyl group on its molecular structure,[14] and considering that YbF_3 is constituted by fluorine, which is an electronegative atom and excellent hydrogen bond acceptor,[18] these molecules could have strongly bind to each other by means of hydrogen bonds; as a consequence, the distribution of YbF_3 within the HEMA-containing adhesives was probably uniform, thus resulting in the progressive reduction of refractive index (Fig. 2), which means that light would be easily transmitted through the material.[7] On the other hand, PEG 400 UDMA do not have hydroxyl group(s) on its molecular structure,[14] thus making the silanized fillers bind to the polymer network only by means of methacrylate bonds (i.e., between the methacrylate group of silane and the methacrylate group of the monomers). This has probably resulted in at least some non-uniform YbF_3 distribution into the adhesives. In fact, U_{20} and U_{30} demonstrated a progressive increase in the refractive index, and according to Mädler et al.,[7] after saturation of the particle within the material, the refractive index follows a linear progression with the atomic content of the component presenting the highest refractive index, which in the present case was the YbF_3.

Interestingly, all the aforementioned discussion regarding the YbF_3 distribution within the adhesives is corroborated by the degree of conversion results, as seen in Fig. 3. The HEMA-containing adhesives demonstrated similar degree of conversion values, independent on the amount of YbF_3 incorporated. This result reaffirms that the particles were uniformly distributed within these adhesives. Additionally, the reduction in the refractive index observed for the filled adhesives compared to the unfilled material allowed proper light propagation, and as a consequence, satisfactory polymerization. In contrast, the PEG 400 UDMA-based adhesive containing the highest YbF_3 content showed lower degree of conversion than the unfilled adhesive, demonstrating that light-activation was affected by this amount of fillers. Once again, when the particles reach the saturation point, light-scattering effect is prone to occur since the filler may absorb and/or reflect light, reducing it availability for excitement/stimulation of the photo-initiator.[19]In this way, the second hypothesis of the study was partially accepted.

The present study demonstrated that the type of monomeric system and the concentration of YbF_3 influenced on the radiopacity, refractive index, and degree of conversion of the model adhesives investigated. While the HEMA-containing adhesive needed the amount of 30 wt% of YbF_3 to produce a similar radiopacity to dentin, the PEG 400 UDMA-based adhesive needed a lower minimum amount (20 wt%). The incorporation of YbF_3 reduced the refractive index of the HEMA-containing adhesives, whereas it increased for the PEG 400 UDMA-based adhesives containing high content of fillers (20 and 30 wt%). The filled adhesives showed similar degree of conversion compared to their unfilled counterparts, except for the PEG 400 UDMA-based adhesive containing 30 wt% of YbF_3, which showed lower conversion of monomers. Therefore, YbF_3 filler particles can be potentially used to impair radiopacity to dental adhesives, although the loading level and the composition of the monomeric system should be considered in an attempt to diminish properties reduction.

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4 Artigo 2*

Title. Incorporation of inorganic fillers into dental adhesives and their influence on physical properties and long-term bond strength to dentin*

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Abstract

This study investigated the effect of different monomeric systems and inorganic fillers on the physical properties of experimental resin adhesives and on their long-term bond strength to dentin. Two self-primers (constituted by GDMA-P, ethanol, water and a variable monomer – HEMA or PEG 400 UDMA) and two resin adhesives (constituted by Bis-GMA, TEGDMA and one the variable monomers) were prepared. Next, the resin adhesives were allocated into three groups according to the type of filler incorporated: unfilled (control), SiO_2 (silica), or YbF_3 (ytterbium trifluoride). Degree of conversion (DC, after 30 and 60 s of light-activation), water sorption (WS) and solubility (SL), and flexural strength (σ) and modulus (E_f) analyses were performed for all resin adhesives. The microtensile bond strength (µTBS) to dentin of all adhesive systems (n=8) was evaluated after 24 h (immediate) and 6 months (long-term) of water storage using a universal testing machine (DL500, EMIC). Data were analyzed using statistical tests (α=5%). The adhesives showed similar DC at a same light exposure time, although light-activation for 60s improved conversion of monomers. The unfilled HEMA-containing adhesive showed higher WS, SL, σ and E_f compared to others. The incorporation of fillers into the adhesives did not affect negatively the immediate µTBS results, although after the long-term evaluation the presence of SiO_2 impaired in complete premature failures and the presence of YbF_3 reduced the µTBS only in the PEG 400 UDMA-based group. The unfilled HEMA-containing group also reduced bond strength after long-term water storage. Depending on the resin matrix composition, YbF_3 seems to be a good option for reinforcing adhesive systems.

Keywords. Self-etch adhesive; Durability; Nanofillers; Ytterbium trifluoride

1. Introduction

Dental adhesive systems are polymer-based biomaterials used to bond restorative materials to the teeth substrates (i.e., enamel and dentin). The adhesion process can be easily achieved by the application of three different components: an acidic substance, a primer solution, and the resin adhesive (bond). The former is used to demineralize the substrate creating micro-porosities on the surface; the second (primer) turns the substrate receptive and/or chemically compatible to the resin adhesive; and the latter is the component that infiltrates and firmly adhere through the substrate [1]. After the application of these materials, a strong adhesive interface is obtained, which is commonly named as ‘hybrid layer’ [2].

Even though adhesive systems have already been long-term evaluated as dental bonding agents by several *in vivo* and *in vitro* studies [3-7], they still present some limitative aspects that should be taken into consideration. For example, the 2-hydroxyethyl methacrylate (HEMA) is a highly hydrophilic monomer commonly present in dental adhesives; nevertheless, studies have been demonstrating the fast bonding degradation that HEMA-containing adhesives undergo when submitted to a wet environment, mainly because this monomer suffers hydrolysis in a more intense way compared to less hydrophilic monomers (e.g., Bis-GMA, TEGDMA, Bis-EMA, UDMA) [8-10]. As a consequence, current research in dental biomaterials has been focused on the replacement of HEMA by more hydrolytically stable monomers. In fact, a recent study has investigated the mechanical and physical properties of experimental adhesives containing alternative dimethacrylates to HEMA [9]. The authors demonstrated that some of these monomers could be potentially used for the development of HEMA-free adhesive systems, and after reviewing some important aspects involved in dental adhesion processes, the polyethyleneglycol (400) extended urethane dimethacrylate (PEG 400 UDMA) was selected as a good option for the replacement of HEMA.

Despite of all the potential benefits that a HEMA-free composition may bring to the durability of the adhesive treatment [11, 12], the decrease in hydrophilicity may on the other hand modify the physical stability and chemical interaction between the adhesive and some inorganic compounds (fillers) that may be present in the material. In fact, the loading of adhesives with different types of fillers has been increasingly evaluated since a significant reinforcement of the material can be achieved [13-16]. In addition, some properties (e.g., radiopacity) that may be absent in unfilled adhesives may be interestingly obtained by the incorporation of fillers into that material [14, 17]. However, there is little information regarding the effect that hydrophilic and/or hydrophobic resin compositions containing or not inorganic fillers may have on the physical properties and also on the bond strength to tooth substrate.

Hence, the aims of this study were: (1) to prepare experimental filled and unfilled adhesive systems containing HEMA or PEG 400 UDMA as variable monomers; and (2) to investigate the effect of different monomers/fillers on the physical properties of the adhesives and on their long-term bond strength to dentin. The following hypotheses were tested: (1) different monomeric systems would result in different physical properties; (2) filled adhesives would present better physical properties compared to the unfilled adhesives; and (3) the PEG 400 UDMA-based adhesives would not decrease the bond strength to dentin after the long-term evaluation.

2. Materials and methods

2.1. Preparation of the experimental adhesive systems

Two-step self-etch adhesive systems were prepared in this study varying the monomeric system and the type of fillers incorporated into the resin matrix. First, two self-primers were formulated by mixing 30 wt% of HEMA (Sigma-Aldrich, St. Louis, MO, USA) or PEG 400 UDMA (Esstech Inc., Essington, PA, USA) (the variable monomers, Fig. 1), 30 wt% of 1,3-glycerol dimethacrylate (GDMA-P), which was synthesized as previously described [18], 20 wt% of ethanol (Vetec, Duque de Caxias, RJ, Brazil) and 20 wt% of distilled water. Next, two resin bonds were formulated based on a 2:1:1 mass ratio of the monomers 2-bis[4-(2-hydroxy-3-methacryloyloxypropyl)phenyl]-propane (Bis-GMA, Evonik, Essen, Germany), triethyleneglycoldimethacrylate (TEGDMA, Esstech), and HEMA or PEG 400 UDMA. To make the resin bonds light-sensitive 0.4 wt% of the photoinitiatorcamphorquinone (CQ, Esstech) and 0.8 wt% of the co-initiator ethyl 4-dimethylamino benzoate (Sigma-Aldrich) were added.

Each resin bond was then separated into three groups according to the type of filler added: unfilled – no fillers added (control); SiO_2 – 10 wt% of silica nanofillers (7 nm average particle size, Aerosil 380, Degussa, Weiss-Fraunenst, Germany); and YbF_3 – 10 wt% of ytterbium trifluoride (Stanford Materials, Aliso Viejo, USA). Prior to their addition into the resin bonds the fillers were silanized using a 10 wt% solution of gamma-methacryloxypropyltrimethoxysilane (γ-MPTS, Sigma-Aldrich) and acetone (Vetec); the resultant slurry was stored for 24 h at 37 °C to assure the complete solvent removal and the fillers were then sieved through a 150 µm sieve. The fillers were finally incorporated into their respective resin bonds, mechanically mixed using a motorized mixer, and ultrasonicated during 1 h.

2.2. Physical properties of the resin bonds

The degree of conversion (DC) was evaluated using Fourier Transform mid-infrared spectroscopy (Prestige21, Shimadzu, Japan). First, a standard resin volume (~3 µl) was used and a preliminary reading (monomer) for the un-polymerized material was taken. Light-activation was then performed with a light-emitting diode (LED) light-curing unit (Radii®, Bayswater, VIC, USA) for 30 and 60 seconds (s) (n = 5); next, another reading was carried out (polymer). The DC was calculated as previously described [19].

The water sorption (WS) and solubility (SL) tests were performed following the ISO 4049/2009 requirements [20]. Each resin bond was placed into a metallic mold and light-activated for 30 s at both top and bottom surfaces. Next, the specimens (n = 5) were placed into a desiccator containing freshly dried silica gel and calcium chloride, weighed after a constant mass ($m1$) was obtained, measured about their diameter and thickness, and immersed in distilled water for seven days at 37°C. All specimens were then blotted dry, weighed ($m2$) again, and placed inside the desiccator until a constant third mass was obtained ($m3$) [9]. For each specimen, the

WS and SL data, in µg/mm^3, were calculated using the following formulas: WS = (m_2-m_3)/V; and SL = (m_1- m_3)/V, where V is the volume (in mm^3) of each specimen, which was calculated using the diameter and thickness measurements.

Bar-shaped specimens (10 × 2 × 2 mm, n = 10) were prepared for each resin bond (n=10) using a metallic mold. Light-activation was performed for 30 s on two consecutive points of the top and bottom surfaces. The specimens were then stored in distilled water for 24 h at 37°C. Next, they were submitted to three-point bend test in a universal testing machine (DL500, EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 1 mm/min. Flexural strength (σ) and flexural modulus (E_f) were calculated in MPa using the following formulas: $\sigma = 3Fl / (2bh^2)$ and $E_f = F1l^3 / 4bh^3d$, where F is the peak load (N); l is the span length (mm); b is the width of the specimen (mm), h is the thickness of the specimen (mm), and d is the deflection of the specimen at load $F1$ during the straight line portion of the load-displacement trace. All data were expressed in MPa.

2.3. Bond strength to dentin

Forty-eight extracted bovine incisors were obtained, stored in 0.5 wt% Cloramine T solution for seven days, and randomly allocated into six groups (n = 8), according to the adhesive systems investigated (2 self-primers × 3 resin bonds). The vestibular enamel of each tooth was removed resulting in flat dentin surfaces, which were wet polished with 600-grit silicon carbide paper to standardize the smear layer. Next, each self-primer was applied for 20 s and gently air-dried for 10 s until a lightly wet and glossy surface was achieved [21]; in the sequence, one coat of the respective resin bond was applied and light-activated for 30 s, followed by two 2 mm-thick increments of resin composite (Filtek™ Z350 XT, 3M ESPE, St. Paul, MN, USA), also light-activated for 20 s each. The samples were then stored in distilled water (24 h at 37°C), sectioned in two perpendicular directions to the adhesive interface using a water-cooled diamond saw at low speed (Isomet 1000, Buheler Ltd, Lake Bluff, IL, USA), resulting in beam-shaped specimens with a cross-sectional surface area of approximately 0.8 mm^2, which were stored in distilled water for 24 h or 6 months, at 37°C. The specimens were finally submitted to microtensile bond strength test (DL500) at a crosshead speed of 1 mm/min [22], and data were expressed in MPa. After testing, the specimens were individually analyzed using a stereomicroscope for determining their failure mode in: adhesive, cohesive in dentin or resin composite, and mixed.

2.4. Statistical analysis

Data were analyzed with the statistical program SigmaPlot version 12.0 (Systat Software Inc., San Jose, CA, USA) using two-way Analysis of Variance (ANOVA) for homoscedastic data (DC, WS, SL, σ, and E_f) and ANOVA on Ranks for heteroscedastic data (bond strength). Tukey test was used for multiple comparisons. Within the degree of conversion and bond strength results, t-tests and Mann-Whitney test were used, respectively, to compare data at the different periods of time tested. The significance level of all analyses was set at $\alpha = 5\%$.

3. Results and discussion

The first property evaluated in this study was the DC of the resin bonds since the incorporation of fillers can affect the optical density of the resin matrix [23], which may lead to higher scattering and light reflection phenomena, hampering the material's polymerization. According to results shown in Fig. 2, and considering them at a same light exposure time, all materials resulted in similar DC ($p \geq 0.090$), corroborating with previous studies that found no detrimental effect on the polymerization of filled adhesives containing 0.5 to 30 wt% of fillers [13, 14, 23]. However, the increase in light-activation time from 30 to 60 s significantly improved the DC of all resin bonds ($p \leq 0.040$); in fact, the two highest gains in conversion were observed with the YbF_3-based adhesives (16.7 and 17.4% for the HEMA-containing and PEG 400 UDMA-based adhesives, respectively), suggesting that the increased light exposure allowed higher excitement of the photoinitiator/CQ, leading to better radical polymerization of monomers.

After the DC analysis, the physical stability of the resin bonds against hydrolysis was evaluated by means of WS and SL tests. According to results displayed in Table 1, the HEMA-containing adhesives demonstrated higher WS than those constituted of PEG 400 UDMA ($p < 0.001$). This confirms that the former were more hydrophilic than the latter, which may be easily explained by the pendant hydroxyl group (–OH) present in HEMA molecule (Fig. 1), which attracts water to the polymer system by hydrogen bonding interaction; in contrast, PEG 400 UDMA has a long chain molecule and no hydroxyls, resulting in a more hydrophobic system [9, 24]. An interesting finding was that the filled adhesives showed similar WS when compared to their unfilled counterparts, suggesting the fillers were uniformly distributed and co-polymerized with the organic matrix [25, 26]. With regard to the solubility results, also shown in Table 1, the unfilled HEMA-containing resin bond demonstrated the highest SL (21.2 µg/mm^3), which was approximately four times higher than the other HEMA-containing groups and almost 20 times higher than those constituted by PEG 400 UDMA ($p < 0.001$) (Table 1). The hydrophilic nature of HEMA has probably turned the polymer system more prone to hydrolysis, resulting in the break of intermolecular chains and consequently in the elution of 'free' components from the adhesive [23, 24]. Surprisingly, the presence of SiO_2 or YbF_3 reduced the SL of the HEMA-containing resin bond, once again suggesting that they were co-polymerized to the organic matrix, protecting the system against the hydrolytic phenomena. In addition, it is important to state that all specimens were light-activated for a total time of 60 s in an attempt to achieve better polymerization (as discussed before) and a more physically stable material [24].

Regarding the mechanical properties investigated, the unfilled HEMA-containing resin bond was stronger than the others ($p < 0.001$) (Table 1), probably due to the formation of strong intermolecular hydrogen bonds between HEMA molecules [9]. Considering that PEG 400 UDMA is not able to form these intermolecular bonds, it impaired lower σ and E_f properties compared to HEMA. In addition, a dual effect was observed regarding to the incorporation of fillers: when they were added into the HEMA-containing adhesive the mechanical properties were significantly reduced, but when added into the PEG 400 UDMA-based adhesive no detrimental effect occurred. Although agglomeration or heterogeneous dispersion of fillers in the resin phase may contribute to reduction in polymer strength [23], it has been already assumed that the fillers were uniformly distributed into the resin bonds since no detrimental effects on the DC, WS and SL properties were observed; therefore, other reasons may have played a role to decreasing the strength of the filled HEMA-containing adhesives. In fact, silanized fillers can co-polymerize with methacrylate monomers via the methacrylate functional group present in silane molecule; this is important to create a stable C=C covalent bond between silane and the polymer [25]. However, considering that HEMA is a mono-methacrylate, it is able to form a C=C bond only one time (Fig. 1), which during the polymerization process may have occured with the silanized fillers, with other HEMA molecules, or with other monomers linked to the main polymeric network. This could have resulted in several un-reacted/unbounded compounds into the polymer, reducing the mechanical strength of the system. On the other hand, PEG 400 UDMA is a dimethacrylate (i.e., it has two methacrylate functional groups per molecule), making it possible to link simultaneously with both the silanized filler and the main polymer network, resulting in a more uniform resin adhesive. These assumptions can therefore explain why the filled PEG 400 UDMA-based adhesives were as strong as the unfilled adhesive and why the incorporation of fillers into the HEMA-containing adhesive decreased the mechanical properties.

Taking together the DC, WS, SL, σ, and E_f results, the first and the second hypotheses of the present study can be only partially accepted.

The main purpose of an adhesive agent in dentistry is bond two surfaces/substrates to each other. Therefore, the bond strength of the formulated adhesive systems to dentin was also investigated in this study. According to Fig. 3-A, the immediate bond strength results showed that the HEMA-containing adhesives produced similar adhesion to each other and independent on the presence of fillers ($p \geq 0.065$); differently, the PEG 400 UDMA-based adhesive reinforced with SiO_2 resulted in higher bond strength than its unfilled and YbF_3-based counterparts ($p \leq 0.002$), although the latter have not differed between each other ($p = 0.996$). The adhesion process to dental substrates involves basically in three steps: surface etching, priming and bonding [1]. Self-etch adhesives are characterized by the simultaneous etching and priming of the substrate, which allows the formation of a homogeneous hybrid layer and uniform monomers infiltration[7]. The self-primers used in this study have already demonstrated efficacy when bonding to dentin [18, 21]; on the other hand, the resin bonds containing SiO_2 or YbF_3 have not been yet investigated.

The presence of SiO_2 or YbF_3 did not affect negatively the bonding ability of the adhesives (Fig. 3-A), which at a first sight could indicate an advantageous characteristic over the unfilled adhesives. Nonetheless, the long-term evaluation demonstrated that a significant reduction in bond strength occurred for some of the groups investigated (Fig. 3-B). In fact, both groups containing SiO_2 resulted in complete premature failures (adhesive failures), suggesting hydrolysis was very aggressive into the hybrid layer formed with these adhesives. According to Antonucci et al. [27], the oxane bond (Si–O–Si) formed between silane and SiO_2 is very prone to hydrolysis, and considering the bond strength specimens were stored in water for 6 months, hydrolytic degradation occurred within these oxane bonds, leaching the fillers out of the polymer; as a consequence, water migration has probably occurred into the debonded interphases, increasing the hygroscopic and hydrolytic phenomena of the polymer [24]. With regard to the unfilled HEMA-containing group, it reduced bond strength after water storage ($p < 0.001$), which was already expected since HEMA makes the polymer hydrophilic due to the pendant hydroxyl group, and thus susceptible to degradation [9, 11, 12, 21]. However, the presence of YbF_3 surprisingly protected the HEMA-containing adhesive against hydrolysis since the bond strength results were stable over time ($p = 0.654$), although the same finding was not seen for the PEG 400 UDMA-based group. It can be suggested that YbF_3, which is constituted by fluorine (i.e., an electronegative atom and excellent hydrogen bond acceptor) [28], formed strong intermolecular hydrogen bonds with the hydroxyl groups of HEMA molecules, impairing lower hydrophilicity to the polymer system containing HEMA. Considering that no hydrogen bond interactions were possible to occur within the PEG 400 UDMA-based polymer since no pendant hydroxyls were present, YbF_3 was not able to protect this polymer system against hydrolysis.

Lastly, the other group that resulted in stable bond strength after 6 months of water storage was the unfilled PEG 400 UDMA-based group (Fig. 3-B), probably because it was less hydrophilic than the HEMA-containing group (Table 1). So, the third hypothesis of the study was also only partially accepted.

4. Conclusions

Silica (SiO_2) and ytterbium trifluoride (YbF_3) fillers were successfully incorporated into resin adhesives, with no detrimental effect over most of the physical properties investigated. The immediate bond strength to dentin was also not affected by addition of fillers, although a significant reduction was achieved after six-month water storage, except for the group containing HEMA and YbF_3. With regard to the unfilled adhesives, the more hydrophobic PEG 400 UDMA-based group showed stable bond strength over time compared to the more hydrophilic HEMA-containing group.

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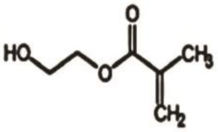
Table 1. Physical properties of all resin bonds investigated in the study with means and standard deviation (SD).

Groups	**WS (in $\mu g/mm^3$)**		**SL (in $\mu g/mm^3$)**		**σ (in MPa)**		**E_f (in GPa)**	
	HEMA	PEG 400 UDMA	HEMA	PEG 400 UDMA	HEMA	PEG 400 UDMA	HEMA	PEG 400 UDMA
Unfilled	A 59.2 a (13.0)	A 36.6 b (4.2)	A 21.2 a (10.5)	A 1.1 b (1.2)	A 168.7 a (23.2)	A 94.2 b (4.8)	A 2.7 a (0.5)	A 1.0 b (0.1)
SiO_2	A 50.9 a(1.9)	A 34.3 b (4.3)	B 5.1 a (4.0)	A 1.6 a (1.2)	B 101.6 a (14.1)	AB 98.5 a (15.1)	B 1.2 a (0.1)	A 1.1 a (0.3)
YbF_3	A 57.3 a(7.9)	A 37.7 b (1.3)	B 4.9 a (3.2)	A 2.3 a (1.2)	B 92.3 a (11.0)	B 79.8 b (4.8)	B 1.1 a (0.1)	A 1.0 a (0.1)

WS: watersorption; SL: solubility; σ: flexuralstrength; E_f: flexuralmodulus; HEMA: 2-hydroxyethyl methacrylate; PEG 400 UDMA: polyethyleneglycol (400) extendedurethanedimethacrylate; SiO_2: sílica; and YbF_3: ytterbiumtrifluoride.

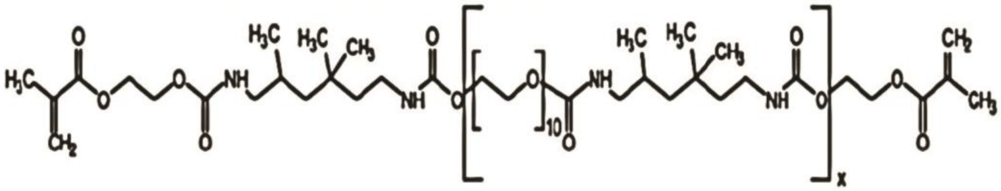
Similar uppercase letters before means and in a same column indicate similarity among filled and unfilled groups ($p > 0.05$); different lowercase letters after means and in a same raw represent statistically significant differences between groups containing HEMA or PEG 400 UDMA ($p < 0.05$).



$C_6H_{10}O_3$
(130 g/mol)

HEMA

2-hydroxyethyl methacrylate

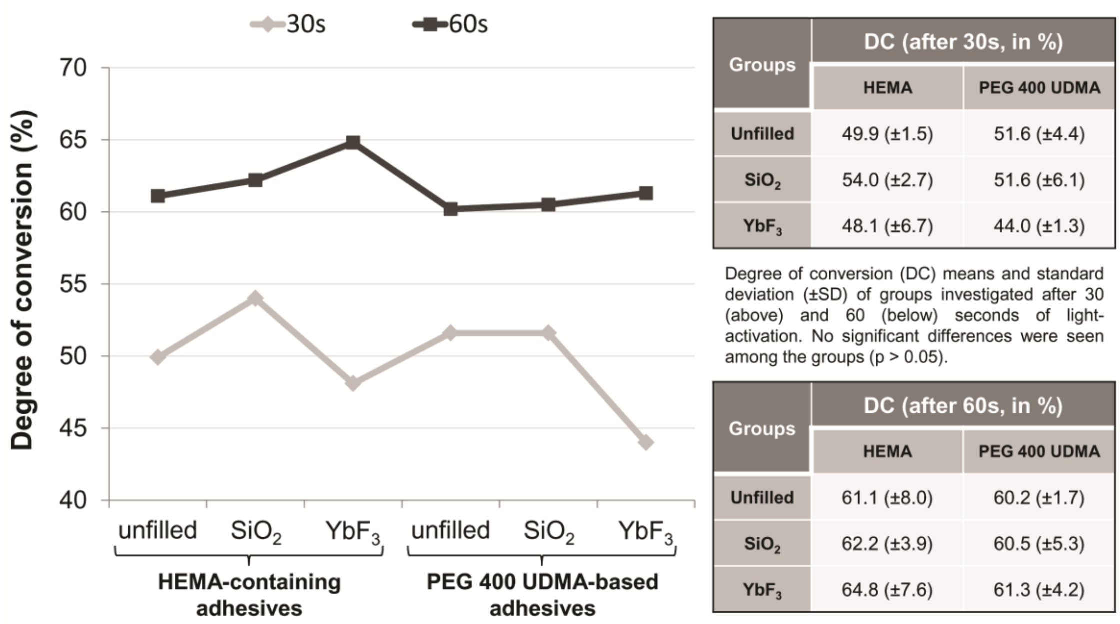


PEG 400 UDMA

$C_{54}H_{98}N_4O_{21}$
(1139 g/mol)

Polyethyleneglycol (400) extended urethane dimethacrylate

Figure 1. Molecular formula, weight and structure of the variable monomers investigated in the study (HEMA and PEG 400 UDMA).



Groups	DC (after 30s, in %)	
	HEMA	PEG 400 UDMA
Unfilled	49.9 (±1.5)	51.6 (±4.4)
SiO_2	54.0 (±2.7)	51.6 (±6.1)
YbF_3	48.1 (±6.7)	44.0 (±1.3)

Degree of conversion (DC) means and standard deviation (±SD) of groups investigated after 30 (above) and 60 (below) seconds of light-activation. No significant differences were seen among the groups ($p > 0.05$).

Groups	DC (after 60s, in %)	
	HEMA	PEG 400 UDMA
Unfilled	61.1 (±8.0)	60.2 (±1.7)
SiO_2	62.2 (±3.9)	60.5 (±5.3)
YbF_3	64.8 (±7.6)	61.3 (±4.2)

Figure 2. Degree of conversion (DC) of the resin bonds after 30 and 60 seconds of light-activation (graph). At the same period of time, no significant differences were seen among the adhesives (tables); however, light-activation for 60 s resulted in higher DC for all adhesives compared to light-activation for 30 s.

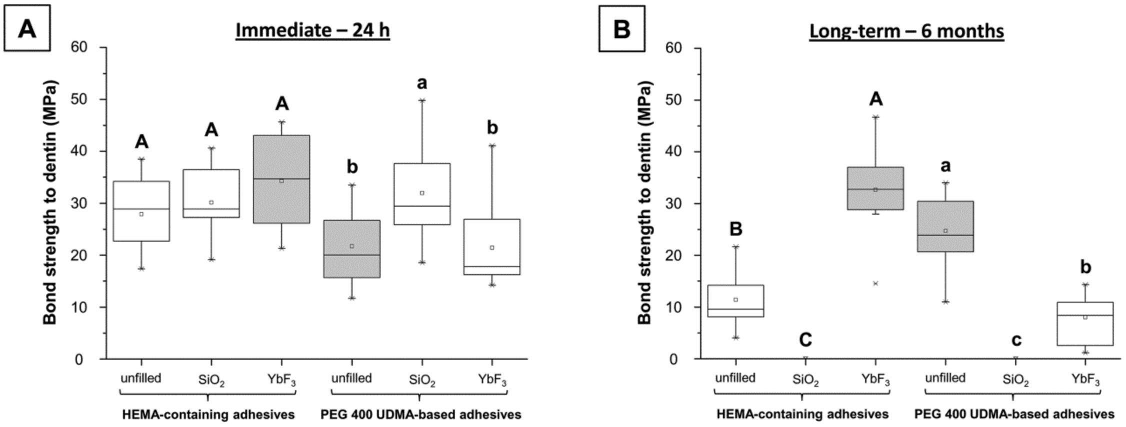


Figure 3. Box-plots showing the immediate (A) and the long-term (B) microtensile bond strength results distribution for the adhesive systems evaluated. The grey shadowed box-plots correspond to similar bond strength results between the immediate and the long-term periods evaluated.

Apêndices

Apêndice A – Termo de Autorização para inserir Trabalho Acadêmico na Base de Dados da UFPel

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