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BERTHYELLE PÁDOVA NYLAND

EFEITO DE VIDROS BIOATIVOS NA ESTRUTURA DENTÁRIA

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do título de Doutor em Odontologia, Área de Concentração em Dentística.

Orientador: Prof. Dr. Sérgio Vieira.
Co-orientadora: Prof. Dra. Andrea Freire

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TERMO DE APROVAÇÃO

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Orientador (a): Prof. Dr. Sérgio Vieira
Programa de Pós-Graduação em Odontologia, PUCPR

Prof. Dr. Rodrigo Nunes Rached
Programa de Pós-Graduação em Odontologia, PUCPR

Prof. Dr. Paulo César Soares Júnior
Programa de Pós-Graduação em Engenharia Mecânica, PUCPR

Profª Drª Stella Kossatz Pereira
Programa de Pós-Graduação em Odontologia, UEPG

Profª Drª Andrea Freire de Vasconcelos
Curso de Odontologia, UFMS

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Espero ter deixado um pedacinho de mim no coração de todos que contribuíram para que este dia chegasse. Muito obrigada

SUMÁRIO

RESUMO

A prevalência da erosão dental é crescente na população de crianças, jovens e adultos em todo o mundo, devido, principalmente, a mudança do estilo de vida e introdução precoce de alimentos ácidos na vida diária. Uma vez que a erosão dental pode levar a perda irreversível de tecido dentário, sendo a principal causa de hipersensibilidade dentinária, o desenvolvimento de estratégias que contenham o processo de desmineralização é essencial para manutenção da saúde bucal. Com este intuito foram desenvolvidas substâncias bioativas, como os vidros ricos em fosfato contendo titânio e/ou magnésio. E, portanto, o objetivo desta tese foi avaliar o efeito destes materiais bioativos em esmalte e dentina humanos, submetidos a desafio erosivo. O primeiro estudo avaliou o efeito de procedimentos de abrasão a ar com agentes dessensibilizantes na permeabilidade dentinária, imediatamente após a aplicação de substâncias bioativas e, após imersão em saliva e ácido cítrico. Foram utilizados cinquenta discos de dentina divididos em 5 grupos (n=10): SBI - bicarbonato de sódio; BAG- Biovidro 45S5 (Sylc); GLI- Clinpro (glicina); 4- Vidro bioativo rico em fosfato contendo titânio (PBG-Ti); 5- Vidro bioativo rico em fosfato contendo magnésio (PBG-Mg). Adicionalmente, os testes de microscopia eletrônica de varredura (MEV) e rugosidade foram realizados. Todos os produtos utilizados reduziram a permeabilidade dentinária e os melhores resultados foram observados nos grupos BAG, GLI e PBG-Ti, entretanto, uma camada mais resistente ao ácido foi observada com o uso do BAG. O segundo estudo avaliou o efeito de vidros ricos em fosfato contendo titânio e magnésio no controle da erosão do esmalte dental. Cinquenta fragmentos de esmalte foram divididos em 5 grupos (n=10): CTRL- controle negativo, BAG- biovidro 45S5, PBG-Ti, PBG-Mg e Vidro bioativo rico em fosfato contendo Ti e Mg (PBG-TiMg). Os espécimes passaram por ciclos de erosão e aplicação de subtâncias bioativas durante 5 dias e, ao final dos ciclos foram realizados os testes de perfilometria óptica 3D, rugosidade, dureza e MEV. Todas as soluções foram efetivas no controle da erosão dental, sendo a menor perda de superfície de esmalte e menor variação da microdureza superficial observada nos grupos PBG-Mg e PBG-TiMg. O grupo PBG-Mg foi o único que não apresentou diferença na rugosidade entre área sadia e área erodida. Foi concluído que todos materiais bioativos testados proporcionaram um efeito

protetor e a taxa de dissolução dos vidros ricos em fosfato foi determinante no controle da desmineralização do esmalte, sendo que a presença do Mg resultou em maior efeito protetor. Com a realização destes estudos concluiu-se que os vidros ricos em fosfatos desenvolvidos na PUCPR podem ser utilizados para desenvolvimento de materiais que controlem a erosão dental e seus sintomas.

Palaras-chave: erosão dental, vidros bioativos, esmalte dental e dentina.

INTRODUÇÃO

A erosão dental é uma condição complexa que afeta a população mundial em diferentes faixas etárias [1,2] e pode ser definida como a perda irreversível de estrutura dentária por dissolução química sem envolvimento de microorganismos [3,4]. Este processo é originado por fatores extrínsecos que estão associados à uma dieta com alto consumo de bebidas e alimentos ácidos ou fatores intrínsecos relacionados a desordens alimentares como bulimia e anorexia nervosa [5]. Inicialmente, a erosão promove alterações nas propriedades do esmalte como redução da microdureza da superfície e aumento da rugosidade [6] e, quando associada a outros fatores como atrição e abrasão, há um desgaste significativo da estrutura dentária que leva a exposição da dentina [5] e hipersensibilidade dentinária [6].

Devido a alta prevalência e severidade dos casos de erosão dental [2,7,8] e hipersensibilidade dentinária [9] é de extrema importância que os clínicos estejam atentos aos fatores de risco que os pacientes estão expostos, a fim de realizar um diagnóstico precoce efetivo [10] e implementar estratégias para prevenção e controle [8]. Desta forma, o aperfeiçoamento de materiais que minimizem os danos às estruturas dentárias em procedimentos rotineiros deve ser incentivado.

Substâncias bioativas podem ser definidas como aquelas que estimulam uma resposta benéfica do corpo [11]. Dentre estas, existem os vidros bioativos, sendo que o primeiro foi desenvolvido por Larry Hench em 1969 e recebeu o nome de Biovidro 45S5 ou *Bioglass*® [11]. Este material apresenta em sua composição 46,1 mol% SiO_2, 24,4 mol% Na_2O, 26,9 mol% CaO e 2,6 mol% P_2O_5 que, quando em contato com meio aquoso libera fosfato para o meio e promove uma troca de cátions Na^+ e/ou Ca^{2+} do vidro com o H^+ da solução. A dissolução destes íons provoca a quebra de ligação Si-O-Si e leva a formação de grupo silanol (Si-OH) que posteriormente são condensados e repolimerizam a camada rica em sílica. Enquanto isto, Ca^{2+} e PO_4^{3-} migram da solução para a superfície formando uma camada amorfa de fosfato de cálcio. Na sequencia, grupos hidroxila e carbonato da solução incorporam-se na camada amorfa de fosfato de cálcio, propiciando a cristalização em hidroxiapatita [11,12].

Vidros a base de sílica como o biovidro 45S5 têm eficiência comprovada em diferentes métodos de aplicação na odontologia [4,13-15], entretanto, a formação de hidroxiapatita foi mais lenta quando comparada a vidros bioativos desenvolvidos recentemente [16,17]. Assim, vidros a base de fosfato podem apresentar alta bioatividade devido ao maior controle da reatividade e formação de hidroxiapatita [16]. A adição de óxidos de titânio e/ou magnésio a vidros a base de fosfato auxilia no controle da solubilidade do vidro [18] e a incorporação destes componentes com diferentes concentrações pode promover resultados promissores a partir de diferentes reações com esmalte e dentina.

Desta forma, o objetivo destes estudos *in vitro* foi avaliar o efeito de vidros bioativos ricos em fosfato contendo titânio e/ou magnésio com diferentes taxas de dissolução em diferentes modos de aplicação e diferentes substratos.

ARTIGO 1 – VERSÃO EM PORTUGUÊS

Página título

Efeito de substâncias bioativas na permeabilidade dentinária

Título Curto: Substâncias bioativas e permeabilidade dentinária

Berthyelle Pádova Nyland[a], Andrea Freire[a], Leticia de Almeida[a], Paulo Soares[b], Denise Stolle da Luz Weiss[b], Sérgio Vieira[a]

[a] Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil

[b] Escola Politécnica, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil

Berthyelle Pádova Nyland,
Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
draberthy@gmail.com

Andrea Freire,
Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
andreavfreire@yahoo.com.br

Leticia de Almeida,
Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
leticia.a@live.com

Paulo Soares,
Escola Politécnica, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
pa.soares@pucpr.br

Denise Stolle da Luz Weiss,
Escola Politécnica, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
denisesluz@bol.com.br

Sérgio Vieira,
Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
s.vieira@pucpr.com.br

Autor Correspondente:
Andrea Freire
Programa de Pós-Graduação em Odontologia
Pontifícia Universidade Católica do Paraná
Escola de Ciências da Vida
R. Imaculada Conceição, 1155, Prado Velho
Curitiba – PR – BRASIL 80215-901
Tel: +55 41 3271-1637; Fax: +55 41 3271-1405
E-mail: andreavfreire@yahoo.com.br

Palavras chave: substâncias bioativas, permeabilidade dentinária, vidros bioativos, bicarbonato de sódio, glicina

Efeito de substâncias bioativas na permeabilidade dentinária

Resumo

Objetivo: Avaliar as mudanças na permeabilidade dentinária após aplicação de diferentes substâncias bioativas e as alterações induzidas pelos tratamentos por meio de microscopia eletrônica de varredura e rugosidade.

Métodos: Cinquenta discos de dentina com aproximadamente 1 mm foram divididos em 5 grupos (n=10): SBI- bicarbonato de sódio; BAG- Biovidro 45S5 (Sylc); GLI- Clinpro (glicina); PBG-Ti -Vidro bioativo rico em fosfato contendo Ti; PBG-Mg - Vidro bioativo rico em fosfato contendo Mg. A permeabilidade dentinária foi quantificada após condicionamento com ácido fosfórico 37% durante 20 s (permeabilidade máxima), após aplicação das substâncias na forma de abrasão a ar e após imersão em saliva e ácido cítrico. A microscopia eletrônica de varredura (MEV) foi utilizada para verificação da obliteração tubular após os diferentes tratamentos e o teste de perfilometria óptica sem contato para avaliação dos parâmetros de rugosidade (n=5). Para análise estatística foram realizados os testes de Análise de Variância por medidas repetidas e o teste Games-Howell, com nível de significância de 5%.

Resultados: Todos os produtos utilizados reduziram a permeabilidade dentinária e os melhores resultados foram observados nos grupos BAG, GLI e PBG-Ti. Após exposição à saliva e ácido cítrico, o grupo PBG-Ti apresentou um aumento da permeabilidade (p=0,001) diferentemente dos demais grupos experimentais e, este grupo apresentou os menores valores de rugosidade. Nas imagens obtidas por MEV foram observados diferentes padrões de obliteração tubular.

Conclusão: Substâncias bioativas podem induzir a obliteração tubular e consequentemente reduzir a permeabilidade dentinária, mesmo sob desafio erosivo.

Significância clínica: Substâncias bioativas podem ser utilizadas para procedimentos de profilaxia como substitutos aos métodos tradicionais e apresentam como vantagem a obliteração tubular e consequente redução da permeabilidade dentinária.

1. Introdução

A hipersensibilidade dentinária (HD) é considerada um problema comum e crescente na clínica odontológica [1-3]. A sua causa está relacionada à perda de esmalte resultante de erosão dental, abrasão, abfração ou recessão gengival [4] e pode ser caracterizada por dor aguda, de intensidade variável, de curta duração, que surge a partir da exposição dentinária em resposta a estímulos físicos ou químicos [5]. Os tratamentos para HD devem ser direcionados para a causa, mais do que para os sintomas, e somente esta abordagem leva ao desenvolvimento de terapias eficazes [6]. Assim, a obliteração dos túbulos dentinários deve ser realizada por meio de substâncias que aumentem significativamente a densidade mineral da dentina exposta, o que reduz a dissolução ácida [7]. Caso a obliteração seja apenas superficial, hábitos diários como escovação e consumo de bebidas ácidas podem facilmente remover os efeitos dessensibilizantes [8,9]. Desta forma, uma nova abordagem está sendo desenvolvida com agentes dessensibilizantes - a abrasão à ar com substâncias bioativas [10-12].

O produto comumente utilizado para remoção de manchas e biofilme é o bicarbonato de sódio aplicado por meio de jateamento, com partículas de aproximadamente 200 µm. Embora este procedimento seja eficiente, podem ocorrer efeitos deletérios sobre as estruturas dentárias expostas, devido a sua alta abrasividade que ocasiona maior rugosidade e perda de estrutura dental sadia [13]. Alguns novos produtos, à base de aminoácido glicina ou biovidros, com tamanho de partículas menores, entre 25 µm e 120 µm, foram desenvolvidos para profilaxia, [10-18]. O aminoácido glicina mostrou-se eficiente na redução da permeabilidade dentinária entretanto, maior efeito remineralizante foi apresentado pelo biovidro 45S5 [15].

O primeiro biovidro a ser desenvolvido foi o 45S5 composto de óxidos de sílica, sódio, cálcio e fósforo [19]. Este vidro libera íons, que formam uma camada amorfa de fosfato de cálcio, à qual posteriormente se cristalizada resultando em hidroxiapatita (HA) [19,20], mineral presente em ossos e dentes. Assim, este material tem demonstrado sucesso em aplicações odontológicas [10,21-23]. Apesar de aceitável obliteração tubular e alívio dos sintomas de HD com o uso do biovidro 45S5, novas formulações foram desenvolvidas com o intuito de otimizar

a bioatividade e estender a funcionalidade [17,24-26]. Biovidros dopados com titânio e magnésio produziram uma camada de HA mais espessa [25,26] entretanto, não foram realizados estudos da capacidade de obliteração tubular com estes novos materiais.

Desta forma, os objetivos deste estudo *in vitro* foram avaliar as mudanças na permeabilidade dentinária após aplicação de diferentes substâncias bioativas e analisar as alterações induzidas pelos tratamentos por meio de microscopia eletrônica de varredura e rugosidade. A hipótese nula testada foi que não haveria diferença entre os materiais avaliados na redução da permeabilidade dentinária.

2. Materiais e Métodos

2.1 Preparo dos espécimes

Para a pesquisa foram utilizados 75 dentes terceiros molares humanos hígidos extraídos (protocolo número: 1.434.128). A superfície oclusal do esmalte foi removida em cortadeira metalográfica (Struers A/S, Ballerup, Dinamarca) equipada com disco diamantado sob refrigeração constante (Extec, Enfield, CT, EUA). Em seguida, um novo corte paralelo ao primeiro foi realizado para obtenção de discos de dentina com aproximadamente 1 mm da porção média de cada dente, e a espessura de todos os discos foi verificada com um paquímetro digital (Modelo CD 6”CS, MitutoyoCorp., Tóquio, Japão). A ausência de esmalte e exposição pulpar foi confirmada por microscopia óptica com ampliação de 50× antes da inclusão dos espécimes no estudo.

2.2 Avaliação da permeabilidade dentinária

Para este teste foram utilizados 50 espécimes, divididos em 5 grupos de acordo com a substância a ser utilizada (n=10), dispostas na Tabela 1. Inicialmente uma *smear layer* padronizada foi criada na superfície oclusal de todos os espécimes utilizando lixa de SiC de granulação 600 (3M do Brasil, Sumaré, SP, Brasil) por 30 segundos. A permeabilidade máxima (Lp Max) foi verificada após o uso de ácido fosfórico 37% por 20 s e lavagem abundante. O tratamento dos espécimes seguiu com a aplicação das substâncias para abrasão a ar descritas na Tabela 1 e então a permeabilidade foi novamente avaliada.

A abrasão a ar utilizou pressão de 500 MPa (5 bars), ângulo de 90° e distância de 5 mm por 15 segundos [10]. A porcentagem (Lp%) do fluxo após o tratamento foi calculada comparando os valores de Lp ao Lp máximo. Após imersão dos espécimes em saliva artificial por 1 hora sob constante agitação (Incubadora Shaker, Marconi MA420, Piracicaba, SP, Brasil) e aplicação de ácido cítrico 0,3% (pH 3,2) durante 2 minutos, obteve-se nova medida de permeabilidade conforme esquematizado na Fig 1. Todas as avaliações da permeabilidade dentinária foram realizadas em triplicata por 5 minutos, utilizando apenas um valor médio para cada amostra.

As medidas de permeabilidade foram obtidas a partir da movimentação de uma bolha de ar no interior de um capilar de vidro e o deslocamento mensurado com um paquímetro digital por meio do aparelho THD 03 (Odeme, Luzerna, SC, Brasil). Para cada espécime, o fluxo de fluído através dos discos de dentina (taxa de filtração) foi calculado a partir da multiplicação da capacidade do capilar (50 µL) pela distância percorrida pela bolha em mm, dividida pelo comprimento do capilar (100 mm) multiplicado pelo tempo em minutos.

$$Q=\frac{50\mu L\ .\ (x)mm}{100\ mm\ .\ \ (z)min}$$

A condutividade hidráulica da dentina, Lp ($\mu L.min^{-1}.\ cm\ H2O^{-1}.\ cm^{-2}$), foi calculada baseada na seguinte equação: Lp = Q / P.A

Onde Q é a taxa de filtração ($\mu L.min^{-1}$), P é a pressão de água (200 mmH_2O) e A é a área de dentina exposta (0,157 cm^2).

Tabela 1: Descrição dos grupos de acordo com o material utilizado, fabricante e sua composição

GRUPO	MATERIAL	FABRICANTE	COMPOSIÇÃO
SBI	Cavitron ®	DentsplyCorp., London, UK	Bicarbonato de sódio
BAG	Sylc ® - Biovidro 45S5	Sspray , London, UK	SiO_2-Na_2O-CaO-P_2O_5
GLI	Clinpro^{TM}Prophy	3MESPE, St. Paul, MN, EUA	Glicina
PBG-Ti	Vidro bioativo rico em fosfato contendo Ti	Experimental	P_2O_5-CaO-Na_2O-SrO-TiO_2
PBG-Mg	Vidro bioativo rico em fosfato contendo Mg	Experimental	P_2O_5-CaO-Na_2O-SrO-MgO

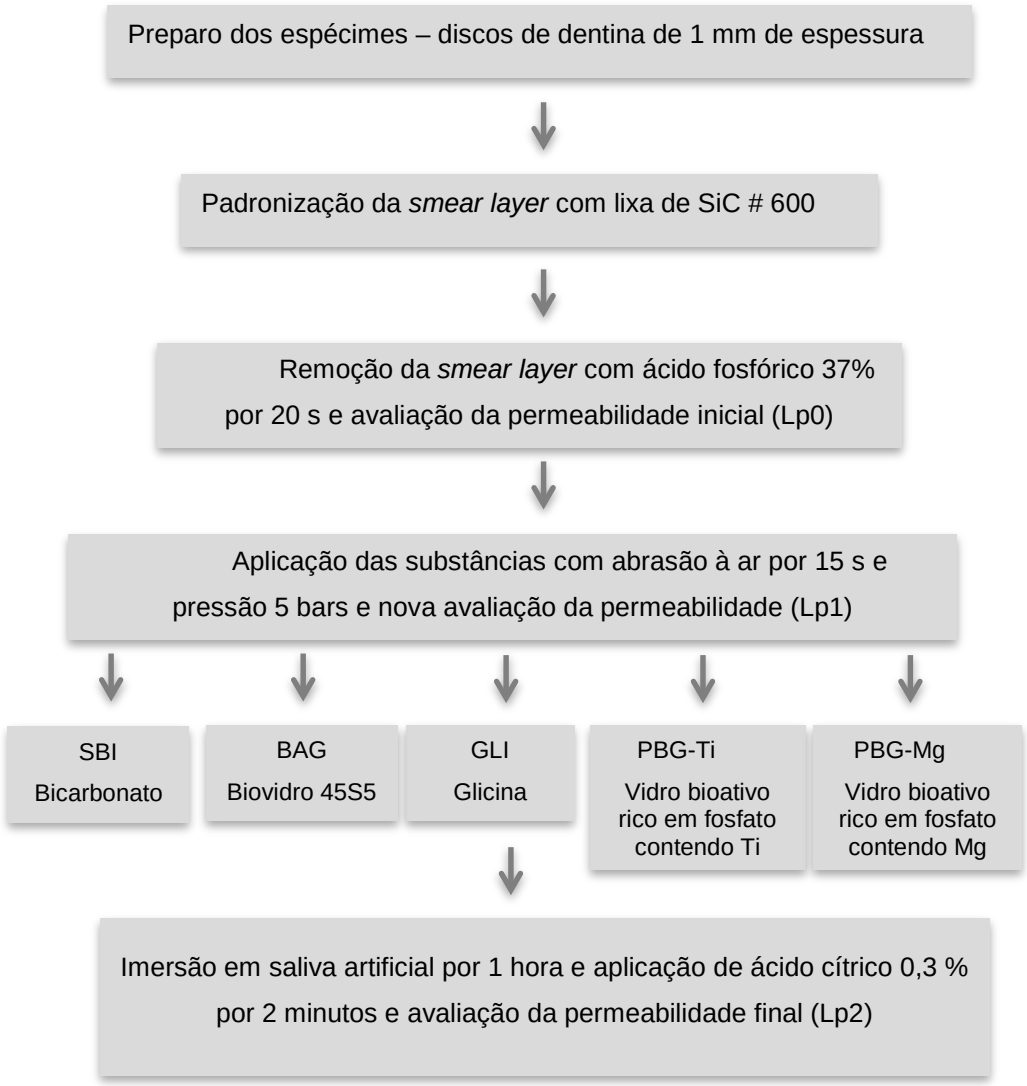

Fig. 1. Representação esquemática da metodologia experimental empregada para avaliação da permeabilidade dentinária

2.3 Síntese dos vidros bioativos

A síntese dos vidros bioativos ricos em fosfato seguiu a metodologia descrita por Weiss [24] e a moagem foi realizada em um moinho de bolas de alta energia (Mixer Mill 5100 – SPEX®SamplePrep, Metuchen, NJ, EUA), onde 10 gramas de vidro foram moídos por vez, durante 8 horas e com uma velocidade de 455 rpm.

2.4 Microscopia Eletrônica de Varredura (MEV)

Discos de dentina (n=5) foram seccionados em 3 partes, permitindo comparações no mesmo espécime, sendo cada parte atribuída a uma das fases do tratamento da dentina: aplicação do ácido fosfórico, abrasão a ar e imersão em saliva artificial e aplicação de ácido cítrico. Após metalização com liga de ouro/paládio (Balzers SCD 050, Balzers, Liechtenstein) os espécimes foram observados em microscópio eletrônico de varredura (TESCAN VEGA3 LMU, Tescan Orsay Holding, Brno-Kohoutovice, República Tcheca) a 20kV e com ampliação de 2500×. Imagens das substâncias utilizadas no estudo também foram obtidas por MEV com aumento de 1000× e 20kV.

2.5 Rugosidade

Após imersão em saliva e ácido cítrico, a superfície da dentina foi analisada por meio de perfilômetro óptico sem contato (Talysurf CCI Lite Non-Contact 3D Profiler, Taylor Hobson, Leicester, Inglaterra) com aumento de 5× (n=5). Obteve-se assim os parâmetros de rugosidade Sa, Sq, Sp, Sv.

2.6 Análise Estatística

Os resultados de permeabilidade dentinária foram verificados quanto a normalidade pelo teste Kolmogorov-Smirnov. e homogeneidade pelo teste Levene. As diferenças estatísticas foram identificadas por Análise de Variância a 2 critérios por medidas repetidas e para as comparações múltiplas utilizou-se o teste Games-Howell.

3. Resultados

A Análise de Variância por Medidas Repetidas detectou diferença nos fatores tratamento e tratamento x momento. A aplicação do ácido fosfórico 37% levou a exposição dos túbulos dentinários (Fig. 2) e a mensuração da permeabilidade foi considerada como 100% (Lp0), assim, cada espécime de dentina condicionada serviu como seu próprio controle.

Após a abrasão com os produtos, houve diferença detectada para todos os grupos, quando comparados com a permeabilidade inicial (Lp0) ($p<0,05$). Dentre os grupos avaliados, o vidro bioativo PBG-Ti e biovidro 45S5 (BAG) juntamente com o Clinpro (GLI) apresentaram maior redução da permeabilidade. Entretanto, após exposição à saliva e ácido cítrico, o grupo PBG-Ti apresentou um aumento da permeabilidade ($p=0,001$) diferentemente dos demais grupos. Desta forma, como houve diferença entre os produtos testados com relação a redução da permeabilidade, rejeitou-se hipótese nula.

Alterações significativas foram observadas após a avaliação da Lp2. A comparação entre os produtos testados (Lp2 – Tabela 2) mostrou uma maior redução da permeabilidade para o GLI, que apresentou diferença com todos os grupos ($p<0,05$), com exceção do BAG, o qual demonstrou semelhança ($p=0,999$).

Nas imagens de MEV (Fig. 2) observou-se o depósito de substâncias, resultante do procedimento de abrasão a ar, o que ocasionou a obliteração tubular em todos os grupos. Entretanto, após a imersão em saliva e ácido cítrico foram observados diferentes padrões de obliteração tubular (Fig 2c), onde o BAG aparentemente manteve todos os túbulos obliterados, enquanto os demais grupos apresentaram túbulos abertos ou parcialmente obliterados. Na Figura 3 é possível observar as diferenças nas partículas das substâncias utilizadas para abrasão a ar.

Tabela 2: Comparação da permeabilidade dentinária (%) e redução da permeabilidade (%) entre as substâncias avaliadas após tratamento e imersão em saliva e ácido cítrico

Produto (Grupo)	%Lp Max	%Tratamento (Lp1)	Redução da permeabilidade %	%Saliva artificial e ácido cítrico (Lp2)	Redução da permeabilidade %
Bicarbonato de sódio (SBI)	100	79,04 ± 9,84 [a] (0,008)	20,96	69,70 ± 6,83[b,c] (0,006)	30,3
Biovidro 45S5 (BAG)	100	58,60 ± 9,75 [b,c] (0,011)	41,4	62,30 ± 10,34[a,b,c,d] (0,007)	37,7
Clinpro (GLI)	100	60,20 ± 6,88 [b,c] (0,011)	39,8	59,40 ± 4,74[d] (0,007)	40,6
Vidro bioativo rico em fosfato contendo Ti (PBG- Ti)	100	55,55 ± 3,95 [b] (0,011)	44,45	68,60 ± 5,62[a,b,c*] (0,008)	31,4
Vidro bioativo rico em fosfato contendo Mg (PBG- Mg)	100	69,40 ± 5,60 [a,c] (0,010)	30,6	71,86 ± 8,36[a,c] (0,007)	28,14

Letras minúsculas diferentes indicam diferença estatística (p<0,05) em coluna, com diferentes produtos no mesmo momento. * Indica diferença estatística em linha, com o mesmo produto em diferentes momentos. Lp após ácido fosfórico 35% foi considerada permeabilidade máxima, descrita na coluna como 100%.

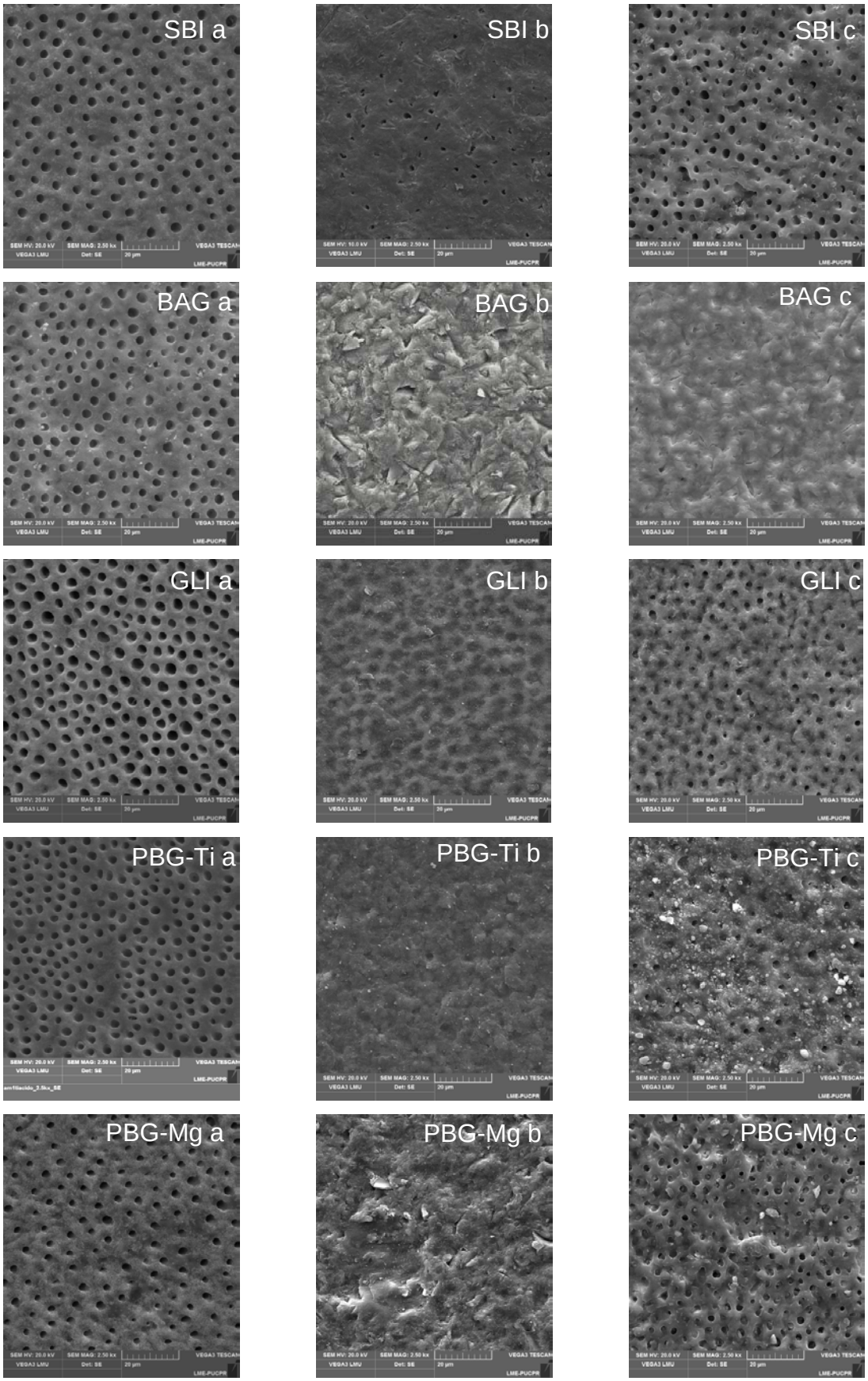

Fig. 2. Fotomicrografias com aumento de 2500× obtidas das diferentes substâncias empregadas, SBI – bicarbonato de sódio, BAG – Sylc (biovidro

45S5), GLI – Clinpro, PBG-Ti – Vidro bioativo rico em fosfato contendo Ti, PBG-Mg – Vidro bioativo rico em fosfato contendo Mg. Onde: (a) após aplicação de ácido, (b) após abrasão a ar e (c) após imersão em saliva e ácido cítrico.

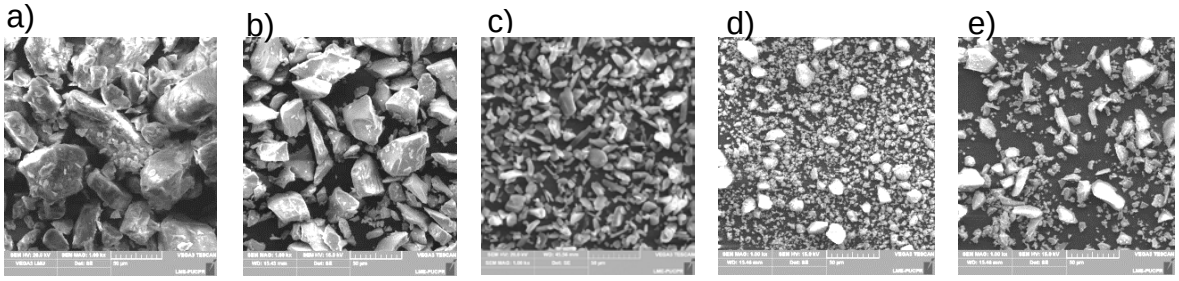

Fig. 3. Imagens de Microscopia Eletrônica de Varredura obtidas das substâncias utilizadas com aumento de 1000×; a) SBI, b) BAG, c) GLI, d) PBG-Ti e e) PBG-Mg.

Os parâmetros de rugosidade avaliados estão descritos na Tabela 3. Os menores valores de rugosidade (Sq, Sa, Sp e Sv) foram apresentados pelo grupo PBG-Ti.

Tabela 3 – Valores médios dos parâmetros de rugosidade (Sq, Sa, Ssk, Sp, Sv) em μm obtidos após tratamento, imersão em saliva e ácido cítrico

Grupos	Sq	Sa	Ssk	Sp	Sv
SBI	4,2	3,06	-0,21	24,9	18,98
BAG	3,98	2,82	0,19	24,66	23,01
GLI	4,24	3,18	-0,37	20,61	20,66
PBG-Ti	2,95	2,09	0,06	17,38	17,35
PBG-Mg	4,64	3,38	-0,60	22,5	26,93

Sq – raiz quadrada da altura média; Ssk- Assimetria do perfil sobre a linha média; Sa – média aritmética da altura; Sp – máxima altura do pico; Sv – máxima altura do vale

4. Discussão

A aplicação das substâncias avaliadas promoveu redução da permeabilidade dentinária em todos os grupos, onde essa variou de 20,96% no bicarbonato a 44,45% no vidro bioativo rico em fosfato contendo Ti (PBG- Ti). Os resultados de maior redução da permeabilidade foram demonstrados pelos grupos BAG, GLI e PBG-Ti.

O Sylc (BAG) apresenta como componente o biovidro 45S5, que é bioativo e foi originalmente desenvolvido como um material osteocondutor [27]. Este vidro libera sódio, cálcio e fosfato que interagem com os fluídos orais e resulta na formação de hidroxiapatita [28]. Este material criou uma camada compacta sobre a superfície dentinária, sem porosidades aparentes (Fig 2b), mesmo após a imersão em ácido cítrico (Fig 2c). Desta forma, a saliva propiciou a deposição de íons que foram efetivos no controle da desmineralização pelo ácido cítrico. A alta resistência da *smear layer* formada pelo BAG demonstrou-se na redução da permeabilidade dentinária que foi semelhante em Lp1 e Lp2 e também nas imagens destes dois momentos (Fig 2). Em um estudo recente [29] que comparou diferentes métodos de aplicação do biovidro 45S5 na efetividade de obliteração tubular, foi observado que o maior contato do material com a dentina, pelo uso de uma moldeira transparente, resultou em um aumento da obliteração tubular, bem como na maior durabilidade do tratamento de dessensibilização dentinária. A manutenção da permeabilidade dentinária, no presente estudo, com o uso do BAG, pode ser justificada pelo método empregado, uma vez que a pressão exercida com o uso do jato propicia maior profundidade de penetração do produto o que facilita a interação com a estrutura por um tempo maior.

O vidro bioativo PBG-Ti, semelhantemente ao biovidro 45S5 apresenta resistência à dissolução. O Ti adicionado ao vidro tem a finalidade de controlar a solubilidade sem comprometer a bioatividade [24,25]. Os íons Ti incorporam-se à camada de fosfato de cálcio amorfo formada sobre a superfície dentinária [25]. Apesar de apresentar um excelente resultado imediatamente após a aplicação do produto, com redução da permeabilidade e formação de uma camada compacta sobre a dentina, após a imersão em saliva e ácido cítrico houve um aumento da permeabilidade. Isto pode estar relacionado ao uso de uma saliva sem proteínas no presente estudo. A presença de proteínas associadas ao biovidro com Ti, sob

condições fisiológicas, favorece a formação de HA assim como propicia uma camada de fosfato de cálcio mais espessa e formada rapidamente [25]. O sódio (Na) é um importante componente para bioatividade do vidro, entretanto o alto conteúdo deste elemento não implica necessariamente em uma maior bioatividade. Foi demonstrado que vidros bioativos com baixo conteúdo de sódio, até 10%, apresentaram alta bioatividade associada a alta dureza [17], o que pode influenciar o poder abrasivo no procedimento clínico de profilaxia. Desta forma, o desenvolvimento de um novo vidro com menor conteúdo de Na e maior de Ti pode ser eficaz, uma vez que o Ti pode contribuir com a manutenção da *smear layer*, já que este elemento auxilia na redução da dissolução do vidro [24], reduz a rugosidade superficial e aumenta as propriedades mecânicas e biológicas [30].

Dentre os parâmetros de rugosidade avaliados, o Sv é representativo da máxima profundidade do vale, sendo um indicativo da estrutura dental perdida. Nesta avaliação o grupo PBG-Ti demonstrou o menor valor. O Ssk representa a assimetria de perfil sobre a linha média. Assim, um Ssk negativo indica que vales acentuados são predominantes e existem poucos picos [30]. A maioria dos grupos apresentou um Ssk negativo, com exceção dos grupos BAG e PBG-Ti, sendo o maior valor positivo apresentado pelo BAG, o que corresponde ao observado pela MEV. A análise qualitativa tem potencial para explicar as características particulares da superfície que associadas a dados quantitativos podem predizer o comportamento do substrato frente a diferentes materiais [31]. A maior rugosidade na superfície dentária está relacionada a um aumento na formação de biofilme [32] e consequentemente desenvolvimento de gengivite. Assim, a utilização de um material que propicie menores valores de rugosidade pode estar associada a maior manutenção da saúde gengival. Entretanto, estudos futuros que avaliem formação de biofilme e inflamação gengival com estes materiais ainda são necessários.

Apesar de vidros a base de sílica terem demonstrado sucesso em aplicações odontológicas [10,23], estes podem necessitar de um tempo mais longo em contato com fluídos orais, como a saliva, para formar hidroxiapatita [29]. No presente estudo, vidros bioativos com maior conteúdo de Ca e PO_4 e agregados com Sr foram desenvolvidos com adição de Mg ou Ti. O Mg presente na estrutura dentária tem a finalidade de regular a cristalização da hidroxiapatita pela estabilização da camada de fosfato de cálcio amorfo [33]. Com relação a

permeabilidade dentinária, o vidro bioativo PBG-Mg obteve resultado semelhante ao biovidro à base de sílica (BAG), tanto na medição imediatamente após a aplicação do produto (Lp1) quanto após imersão em saliva e ácido (Lp2). Contudo, o vidro bioativo PBG-Ti apresentou uma redução mais significativa da permeabilidade que o PBG-Mg, especialmente na leitura Lp1. A Maior dissolução deste material quando comparado ao PBG-Ti, leva a uma rápida liberação do Mg [24], o que pode prejudicar a estabilização da camada de fosfato de cálcio.

O Clinpro é composto por glicina, um aminoácido altamente solúvel em água, com uma dureza menor que o bicarbonato [12] e características anfóteras [10]. Neste estudo, a redução da permeabilidade foi mantida mesmo após a imersão em saliva e ácido cítrico. Apesar deste resultado, não foi detectada uma camada sobre a superfície após imersão em saliva e ácido, diferentemente de alguns grupos que envolveram os vidros. Este fato pode ser devido a uma reação diferente deste produto com a dentina, não pela deposição do produto propriamente dito mas, estímulo de deposição do Ca endógeno.

O bicarbonato de sódio também apresentou redução da permeabilidade dentinária, porém as imagens não detectaram deposição de substâncias após imersão em saliva e ácido cítrico. Este fato pode estar associado ao efeito da saliva na redução da permeabilidade e não ao bicarbonato, uma vez que os componentes inorgânicos da saliva, principalmente Ca e PO_4, podem inferir um efeito protetor na estrutura dentinária, depositando-se sobre a dentina. A presença de saliva artificial pode auxiliar em um delineamento que simule as condições bucais, uma vez que essa é um fluído complexo com várias propriedades que são indispensáveis para uma boa saúde oral e geral [34].

Alguns estudos que avaliaram os danos aos tecidos duros e moles provocados por procedimentos de profilaxia relacionaram maiores defeitos aos métodos convencionais, como bicarbonato de sódio e pedra pomes, do que métodos alternativos como a glicina [12,18,27,35,36]. Entretanto, o presente estudo encontrou semelhança nos parâmetros de rugosidade e MEV entre bicarbonato e glicina quando avaliados após imersão em saliva e ácido cítrico.

O biovidro 45S5 foi comparado ao bicarbonato de sódio em um estudo clínico que avaliou alteração de cor, sensibilidade e conforto dos pacientes. Quando estes apresentavam higiene oral deficiente, e, consequentemente maior manchamento, o biovidro apresentou melhor resultado de limpeza, o que foi

associado às partículas deste material possuírem maior densidade e aspecto mais esférico do que as do bicarbonato, isto pôde ser observado no presente estudo pelas imagens de MEV (Fig. 3). Com relação à sensibilidade, ambos os produtos apresentaram redução significativa imediatamente após o tratamento. Entretanto, em uma avaliação após 10 dias, o biovidro manteve a redução inicial enquanto o bicarbonato de sódio apresentou um aumento médio de 17% na sensibilidade. O conforto do procedimento relatado pelos pacientes foi em média 46% maior quando o biovidro foi utilizado [11]. Estes dados estão de acordo com o presente estudo uma vez que o BAG manteve a obliteração tubular, e esta pode ser um indicativo de ausência ou redução da hipersensibilidade dentinária [3,8,9]. Desta forma, estes resultados incentivam a utilização de métodos alternativos, assim como o desenvolvimento de novos materiais bioativos, que possam causar menor dano ao tecido dentário e ainda protegê-lo frente aos desafios ácidos.

5. Conclusão

Substâncias bioativas podem induzir a obliteração tubular e consequentemente reduzir a permeabilidade dentinária, mesmo sob desafio erosivo. BAG e GLI mostraram maior redução da permeabilidade após desafio erosivo. Entretanto, uma camada ácido resistente foi mais evidente com o uso do BAG.

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ARTIGO 1 – VERSÃO EM INGLÊS

Title Page

Effect of bioactive substances on dentin permeability

Short Title: Bioactive substances and dentin permeability

Berthyelle Pádova Nyland[a], Andrea Freire[a], Leticia de Almeida[a], Paulo Soares[b], Denise Stolle da Luz Weiss[b], Sérgio Vieira[a]

[a] School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil

[b] Polytechnic School, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil

Berthyelle Pádova Nyland,
School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
draberthy@gmail.com

Andrea Freire,
School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
andreavfreire@yahoo.com.br

Leticia de Almeida,
School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
leticia.a@live.com

Paulo Soares,
Polytechnic School, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
pa.soares@pucpr.br

Denise Stolle da Luz Weiss,
Polytechnic School, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
denisesluz@bol.com.br

Sérgio Vieira,
School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
s.vieira@pucpr.com.br

Autor Correspondente:
Andrea Freire
Graduate Program in Dentistry
School of Life Sciences
Pontifícia Universidade Católica do Paraná
R. Imaculada Conceição, 1155, Prado Velho
Curitiba – PR – BRASIL 80215-901
Tel: +55 41 3271-1637; Fax: +55 41 3271-1405
E-mail: andreavfreire@yahoo.com.br
Keywords: bioactive glasses, bioglass 45S5, dentin permeability, sodium bicarbonate, glycine

Effect of bioactive substances on dentin permeability

Abstract

Aim: To evaluate the changes in dentin permeability after application of different bioactive substances and to analyze the changes induced by the treatments by scanning electron microscopy and surface roughness.

Methods: Fifty dentin disks of approximately 1 mm of thickness were divided into 5 groups. (n=10): SBI- sodium bicarbonate; BAG- Bioglass 45S5 (Sylc); GLY- Clinpro (glycine); PBG-Ti – Phosphate based glass Ti-containing; PBG-Mg – Phosphate based glass Mg-containing. The dentin permeability was quantified after etching with 37% phosphoric acid for 20 s (maximum permeability) after application of the substances in the form of air abrasion and after immersion in saliva and citric acid. Scanning electron microscopy (SEM) was used to verify tubular obliteration after different treatments and non-contact optical profilometry test to evaluate the roughness parameters (n=5). For statistical analysis, Analysis of Variance for repeated measurements and Games-Howell test were performed, with a significance level of 5%.

Results: All the products used reduced dentin permeability and the best results were observed in the BAG, GLY and PBG-Ti groups. After exposure to saliva and citric acid, the PBG-Ti group showed increased permeability (p=.001) unlike the other experimental groups, and this group presented the lowest values of roughness. Different patterns of tubular obliteration were observed in the SEM micrographs.

Conclusion: Bioactive substances can induce tubular obliteration and consequently reduce dentin permeability, even under erosive challenge.

Clinical Significance: Bioactive substances can be used for prophylaxis as substitutes for traditional methods and have the advantage of tubular obliteration and consequent reduction of dentin permeability.

Keywords: bioactive glasses, bioglass 45S5, dentin permeability, sodium bicarbonate, glycine

1. Introduction

Dentin hypersensitivity (DH) is considered a common and growing problem in the dental profession [1-3]. Its cause is related to loss of enamel resulting from dental erosion, abrasion, abfraction or gingival recession [4] and may be characterized by acute, variable-intensity, short-duration pain from dentin exposure in response to physical or chemical stimuli [5]. Treatments for DH should be targeted to the cause, rather than to the symptoms, and only this approach leads to the development of effective therapies [6]. Thus, obliteration of the dentinal tubules should be performed by means of substances that significantly increase the mineral density of exposed dentin, which reduces acid dissolution [7]. If the obliteration is only superficial, daily habits such as brushing and consumption of acidic beverages can easily remove the desensitizing effects [8,9]. In this way, a new approach with desensitizing agents is being developed - air abrasion with bioactive substances [10-12].

The commonly used product for stains and biofilm removal is sodium bicarbonate applied by blasting, with particles of approximately 200 µm. Although this procedure is efficient, deleterious effects on the exposed dental structures may occur, due to its high abrasiveness that causes greater roughness and loss of healthy tooth structure [13]. Recently, some glycine or bioglass-based products, with smaller particle size, between 25 µm and 120 µm, have been developed for prophylaxis [10-18]. The aminoacid glycine showed to be efficient in the reduction of the dentin permeability, however, a greater remineralizing effect was presented by the bioglass 45S5 [15].

The first bioglass to be developed was the 45S5, composed by silica, sodium, calcium and phosphorus oxides [19]. This glass releases ions, which form an amorphous layer of calcium phosphate, which is then crystallized resulting in hydroxyapatite (HA) [19,20], a mineral found in bones and teeth. Thus, this material has been shown to have success in dental applications [10,21-23]. Although acceptable tubular obliteration and relief of DH symptoms with the use of 45S5 bioglass, new formulations were developed with the aim of optimizing bioactivity and extending functionality [17,24-26]. Titanium and magnesium doped bioglasses have been shown to produce thicker HA layers [25,26]. However, no

studies of the tubular capacity obliteration using these new materials have been carried out.

In this way, the aims of this in vitro study were to evaluate the changes in dentin permeability after application of different bioactive substances and to analyze the changes induced by the treatments by scanning electron microscopy and surface roughness. The null hypothesis tested was that there would be no difference between the materials evaluated in the reduction of dentin permeability.

2. Materials and methods

2.1 Preparation of specimens

For the research, 75 extracted third molar human teeth were used (protocol #1,434,128). The occlusal surface of enamel was removed in a metallographic cutter (Struers A/S, Ballerup, Denmark) equipped with diamond disk under constant cooling (Extec, Enfield, CT, USA). Then, a new cut parallel to the first one was made to obtain dentin discs with approximately 1 mm thickness of the middle portion of each tooth, and the thickness of all disks was verified with a digital caliper (Model CD 6”CS, MitutoyoCorp., Tokyo, Japan). The absence of enamel and pulp exposure was confirmed by optical microscopy with 50X magnification prior to inclusion of the specimens in the study.

2.2 Evaluation of dentin permeability

For this test, 50 specimens were used, divided into 5 groups according to the substance to be used (n = 10), as shown in Table 1. Initially a standardized smear layer was created on the occlusal surface of all specimens using 600 grit SiC sandpaper (3M, Sumaré, SP, Brazil) for 30 seconds. Maximum permeability (Lp Max) was verified after the use of 37% phosphoric acid for 20 s and abundant rinsing. The treatment of the specimens followed with application of the substances for air abrasion described in Table 1 and then the permeability was again evaluated.

Air abrasion was performed with a pressure of 500 MPa (5 bars), angle of 90° and distance of 5 mm for 15 seconds [10]. The flow percentage (Lp%) after treatment was calculated by comparing the Lp and the maximum Lp values. After immersion of the specimens in artificial saliva for 1 hour under constant agitation (Shaker, Marconi MA420, Piracicaba, SP, Brazil) and application of 0.3% citric acid (pH 3.2) for 2 minutes, a new permeability measurement was obtained (Figure 1). All evaluations of dentin permeability were performed in triplicate for 5 minutes, using only one mean value for each sample.

The permeability measurements were obtained through the displacement of an air bubble inside a glass capillary (THD 03, Odeme, Luzerna, SC, Brazil) and the displacement measured with a digital caliper. For each specimen, the flow

of fluid through the dentin disks (filtration rate) was calculated by multiplying the capacity of the capillary (50 µL) by the distance traveled by the bubble in mm, divided by the length of the capillary (100 mm) multiplied by time in minutes.

$$Q = \frac{50\mu L \,.\, (x)mm}{100\ mm \,.\, (z)min}$$

The hydraulic conductivity of dentin, Lp ($\mu L.min^{-1}.\ cm\ H2O^{-1}.\ cm^{-2}$), was calculated based on the following equation: Lp = Q / P.A

Where Q is the filtration rate ($\mu L.min^{-1}$), P is the water pressure (200 mmH_2O) and A is the area of exposed dentin (0.157 cm^2).

2.3 Phosphate based glasses synthesis

The synthesis of phosphate based glasses followed the methodology described by Weiss [24] and grinding was performed in a high-energy ball mill (Mixer Mill 5100 – SPEX® Sample Prep, Metuchen, NJ, USA), where 10 grams of glass were ground at a time, for 8 hours and with a speed of 455 rpm.

2.4 Scanning Electron Microscopy (SEM)

Dentin disks (n = 5) were sectioned in 3 parts, allowing comparisons in the same specimen, each part being assigned to one of the stages of dentin treatment: application of phosphoric acid, air abrasion and immersion in artificial saliva and application of citric acid. After gold/palladium alloy sputter-coating (Balzers SCD 050, Balzers, Liechtenstein) the specimens were observed under a scanning electron microscope (TESCAN VEGA3 LMU, Tescan Orsay Holding, Brno-Kohoutovice, Czech Republic) at 20kV and with 2500X magnification. Images of the substances used in the study were also obtained by SEM with 1000X magnification and 20 kV.

2.5 Surface roughness

After immersion in saliva and citric acid, the surface of the dentin was analyzed by means of non-contact optical profilometer (Talysurf CCI Lite Non-Contact 3D Profiler, Taylor Hobson, Leicester, England) with 5X magnification (n = 5). This yielded the roughness parameters Sa, Sq, Sp, Sv.

2.6 Statistical analysis

The results of dentin permeability were checked for normality by the Kolmogorov-Smirnov test and homogeneity by Levene's test. Statistical differences were identified by 2-way Analysis of variance for repeated measurements and multiple comparisons Games-Howell test.

3. Results

The Analysis of Variance for Repeated Measures detected difference in the treatment and treatment x moment factors. The application of 37% phosphoric acid led to the exposure of the dentinal tubules (Fig. 2) and the permeability measurement was considered to be 100% (Lp0), thus, each conditioned dentin specimen served as its own control.

After abrasion with the products, there was difference detected for all groups, when compared to the initial permeability (Lp0) ($p<.05$). Among the groups evaluated, PBG-Ti bioglass and 45S5 bioglass (BAG) together associated with Clinpro (GLY) showed greater reduction of permeability. However, after exposure to saliva and citric acid, the PBG-Ti group showed increased permeability ($p=.001$) unlike the other groups. Thus, since there was a difference between the products tested in relation to the reduction of permeability, the first hypothesis was rejected.

Significant changes were observed after Lp2 evaluation. The comparison between the tested products (Lp2 – Table 2) showed greater reduction of permeability for GLY, which presented difference with all groups ($p <.05$), except for BAG, which showed similarity ($p=.999$).

In the SEM images (Fig. 2) the deposition of substances, resulting from the air abrasion procedure, was observed, which caused tubular obliteration in all groups. However, after immersion in saliva and citric acid, different patterns of tubular obliteration were observed, where the BAG apparently kept all the tubules obliterated, while the other groups had open or partially obliterated tubules. In Figure 3 it is possible to observe the differences in the particles of the substances used for air abrasion.

The roughness parameters evaluated are described in Table 3. The lowest roughness values (Sq, Sa, Sp and Sv) were presented by the PBG-Ti group.

4. Discussion

The application of the substances promoted reduction of dentin permeability in all groups, where it ranged from 20.96 in the bicarbonate to 44.45 in the phosphate based glass Ti-containing (PBG- Ti). The results of greater reduction of permeability were demonstrated by the GLY group, and PBG-Ti.

Sylc (BAG) presents as component the 45S5 bioglass, which is bioactive and was originally developed as an osteoconductive material [27]. This glass releases sodium, calcium and phosphate that interact with oral fluids and forms hydroxyapatite [28]. This material created a compact layer on the dentin surface, with no apparent porosity (Fig 2b), even after immersion in citric acid (Fig 2c). Thus, saliva provided the deposition of ions that were effective in the control of citric acid demineralization. The high resistance of the smear layer formed by the BAG was demonstrated in the reduction of the dentin permeability that was similar in Lp1 and Lp2 and in the images of these two moments (Fig 2). In a recent study [29] comparing different application methods of 45S5 bioglass on the effectiveness of tubular obliteration, it was observed that the greater contact of the material with dentin, using a transparent tray, resulted in increased tubular obliteration, as well as in longer durability of the dentin desensitization treatment. The maintenance of the dentin permeability in the present study with the use of the BAG can be justified by the employed method, since the pressure exerted with air abrasion allows a greater depth of penetration of the product which facilitates the interaction with the structure by a longer time.

The PBG-Ti bioactive glass, like the 45S5 bioglass presents resistance to dissolution. The Ti added to the glass has the purpose of controlling the solubility without compromising the bioactivity [24,25]. Ti ions are incorporated into the layer of amorphous calcium phosphate formed on the dentin surface [25]. Although it presented an excellent result immediately after the application of the product, with decreased permeability and formation of a compact layer on the dentin, there was an increase of the permeability after the immersion in saliva and citric acid. This may be related to the use of a protein-free saliva in the present study. The presence of proteins associated to the Ti-containing bioglass, under physiological conditions, favors HA formation as well as provides a thicker calcium phosphate layer which is formed more rapidly [25]. Sodium (Na) is an important

component for glass bioactivity, however the high content of this element does not necessarily imply a higher bioactivity. It was demonstrated that bioactive glasses with low sodium content, up to 10%, showed high bioactivity associated with great hardness [17], which may influence the abrasive power in the clinical procedure of prophylaxis. Thus, the development of a new glass with lower Na and greater Ti contents can be effective, since Ti can contribute to the maintenance of the smear layer, since this element helps to reduce glass dissolution [24], reduces surface roughness and increases mechanical and biological properties [30].

Among the roughness parameters evaluated, Sv is representative of the maximum depth of the valley, being indicative of lost tooth structure. In this evaluation, the PBG-Ti group showed the lowest values. Ssk represents the profile asymmetry on the midline. Thus, a negative Ssk indicates that sharp valleys are predominant and there are few peaks [30]. Most of the groups had a negative Ssk, except for the BAG and PBG-Ti groups, the highest positive value being presented by the BAG, which corresponds to the one observed by SEM. The qualitative analysis has the potential to explain the particular characteristics of the surface that associated with quantitative data can predict the behavior of the substrate against different materials [31]. The greater roughness of the tooth surface is related to an increase in the formation of biofilm [32] and consequently development of gingivitis. Thus, the use of a material that provides lower values of roughness may be associated with greater maintenance of gingival health. However, future studies evaluating biofilm formation and gingival inflammation with these materials are still required.

Although silica-based glasses have shown success in dental applications [10,23], they may require a longer time in contact with oral fluids, such as saliva, to form hydroxyapatite [29]. In the present study, bioactive glasses with higher content of Ca and PO_4 and blended with Sr were developed with addition of Mg or Ti. The Mg present in the tooth structure has the purpose of regulating the crystallization of the hydroxyapatite by the stabilization of the layer of amorphous calcium phosphate [33]. In relation to dentin permeability, the PBG-Mg bioactive glass obtained a similar result to the bioglass based on silica (BAG), both in the measurement immediately after the application of the product (Lp1) and after immersion in saliva and acid (Lp2). However, PBG-Ti showed a more significant reduction of permeability than PBG-Mg, especially in the Lp1 reading. The higher

dissolution of this material when compared to PBG-Ti, leads to a rapid release of Mg [24], which may impair the stabilization of the calcium phosphate layer.

Clinpro is composed of glycine, a highly water soluble amino acid with a hardness lower than bicarbonate [12] and with amphoteric characteristics [10]. In this study, permeability reduction was maintained even after immersion in saliva and citric acid. Despite this result, no layer was detected on the surface after immersion in saliva and acid, unlike some groups that involved the glasses. This fact may be due to a different reaction of this product with dentin, rather than by the deposition of the product itself but by stimulus of deposition of the endogenous Ca.

Sodium bicarbonate also produced reduced dentin permeability, but the images did not detect deposition of substances after immersion in saliva and citric acid. This fact may be associated to the effect of saliva on the reduction of permeability and not to the bicarbonate, since the inorganic components of saliva, mainly Ca and PO_4, can infer a protective effect on the dentin structure, being deposited on dentin. The presence of artificial saliva can help in a design that simulates oral conditions, since this is a complex fluid with several properties which are indispensable for good oral and general health [34].

Some studies evaluating the damage to hard and soft tissues caused by prophylaxis procedures related higher defects to conventional methods, such as sodium bicarbonate and pumice, than alternative methods such as glycine [12,18,27,35,36]. However, the present study found similarity in the parameters of roughness and SEM between bicarbonate and glycine when evaluated after immersion in saliva and citric acid.

The 45S5 bioglass was compared to sodium bicarbonate in a clinical study that assessed patients' color change, sensitivity and comfort. When patients presented poor oral hygiene, and consequently greater staining, bioglass presented a better cleaning result, which was associated to the particles of this material having a higher density and more spherical appearance than the bicarbonate, this could be observed in the present study by SEM pictures (Fig. 3). Regarding sensitivity, both products showed significant reduction immediately after treatment. However, in an evaluation after 10 days, bioglass maintained the initial reduction while sodium bicarbonate presented an average 17% increase in sensitivity. The comfort of the procedure reported by the patients was on average

46% higher when bioglass was used [11]. These data corroborate the present study since the BAG maintained the tubular obliteration, and this may be indicative of absence or reduction of dentin hypersensitivity [3,8,9]. Thus, these results encourage the use of alternative methods, as well as the development of new bioactive materials, which may cause less damage to dental tissue and also protect it against acidic challenges.

5. Conclusion

Bioactive substances can induce tubular obliteration and consequently reduce dentin permeability, even under erosive challenge. BAG and GLY showed greater reduction of permeability after erosive challenge. However, a resistant acid layer was more evident with the use of BAG.

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Tables

Table 1: Description of the groups according to the material used, manufacturer and its compositi

GROUP	MATERIAL	MANUFACTURER	COMPOSITION
SBI	Cavitron ®	DentsplyCorp., London, UK	Sodium Bicarbonate
BAG	Sylc ® - Bioglass 45S5	Sspray , London, UK	SiO_2-Na_2O-CaO-P_2O_5
GLY	Clinpro^{TM}Prophy	3MESPE, St. Paul, MN, EUA	Glycine
PBG-Ti	Phosphate based glass Ti-containing	PUCPR	P_2O_5-CaO-Na_2O-SrO-TiO_2
PBG-Mg	Phosphate based glass Mg-containing	PUCPR	P_2O_5-CaO-Na_2O-SrO-MgO

Table 2: Comparison of dentin permeability (%) and permeability reduction (%) between substances evaluated after treatment and immersion in saliva and citric acid

Product/ Group	%Lp Max	%Tratamento (Lp1)	Redução da permeabilidade %	%Saliva artificial e ácido cítrico (Lp2)	Redução da permeabilidade %
Sodium Bicarbonate (SBI)	100	79,04 ± 9,84 ^{a} (0,008)	20,96	69,70 ± 6,83^{b,c} (0,006)	30,3
Sylc- Bioglass 45S5 (BAG)	100	58,60 ± 9,75 ^{b,c} (0,011)	41,4	62,30 ± 10,34^{a,b,c,d} (0,007)	37,7
Clinpro (GLY)	100	60,20 ± 6,88 ^{b,c} (0,011)	39,8	59,40 ± 4,74^{d} (0,007)	40,6
Phosphate-based glass Ti-containing (PBG- Ti)	100	55,55 ± 3,95 ^{b} (0,011)	44,45	68,60 ± 5,62^{a,b,c*} (0,008)	31,4
Phosphate-based glass Mg-containing (PBG- Mg)	100	69,40 ± 5,60 ^{a,c} (0,010)	30,6	71,86 ± 8,36^{a,c} (0,007)	28,14

Different lower-case letters indicate statistical difference ($p < .05$) within columns, with different products at the same time.
* Indicates statistical difference within lines, with the same product at different times. Lp after phosphoric acid 35% was considered maximum permeability, described in the column as 100%.

Table 3 – Mean values of roughness parameters (Sq, Sa, Ssk, Sp, Sv) in µm obtained after treatment, immersion in saliva and citric acid

Grupos	Sq	Sa	Ssk	Sp	Sv
SBI	4,2	3,06	-0,21	24,9	18,98
BAG	3,98	2,82	0,19	24,66	23,01
GLI	4,24	3,18	-0,37	20,61	20,66
PBG-Ti	2,95	2,09	0,06	17,38	17,35
PBG-Mg	4,64	3,38	-0,60	22,5	26,93

Sq –Root mean square height; Ssk- Skewness; Sa – Arithmetic mean height; Sp – Maximum peak height; Sv – Maximum pit height

Figures

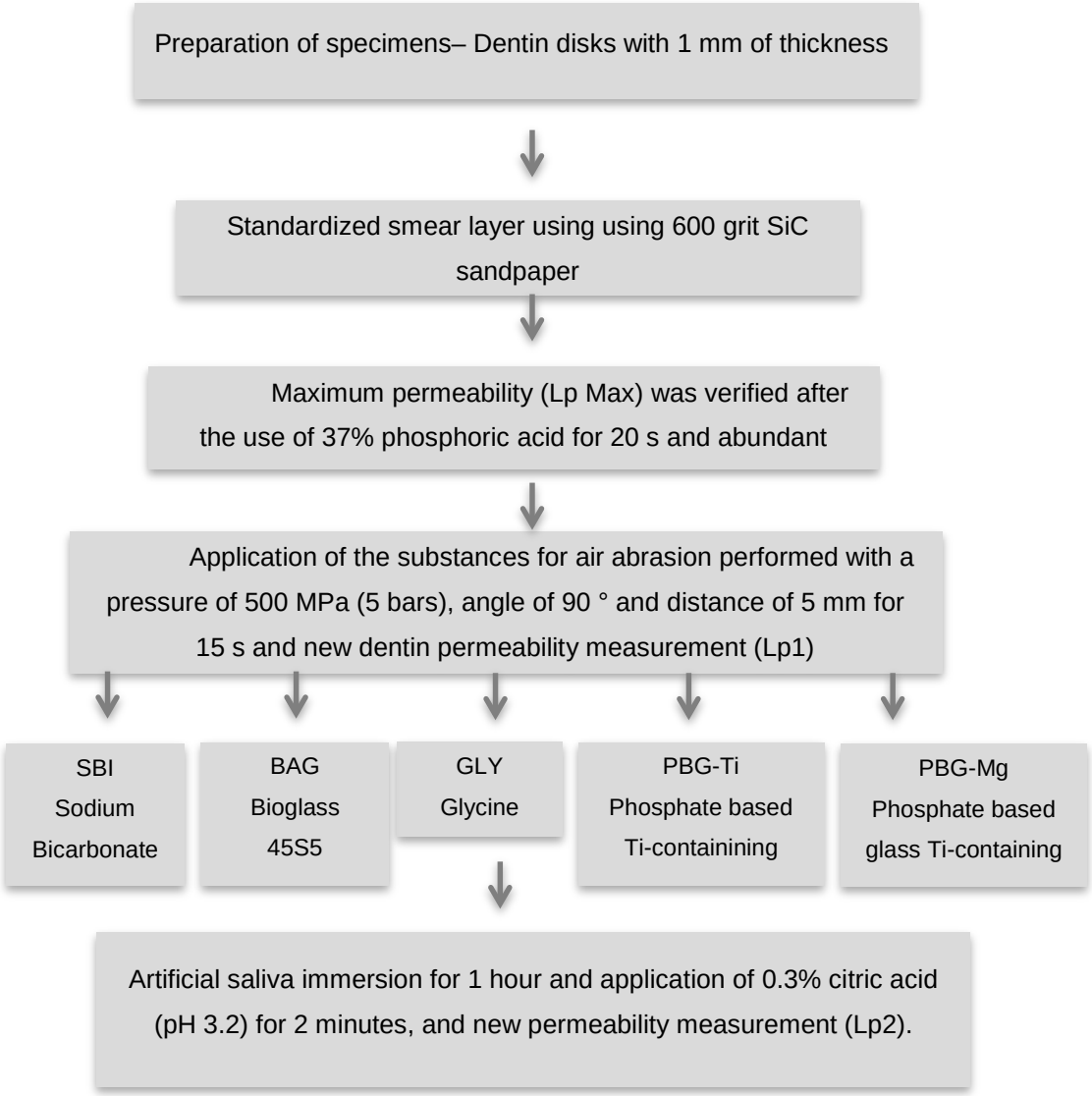

Fig. 1. Schematic representation of the experimental methodology used to evaluate dentin permeability

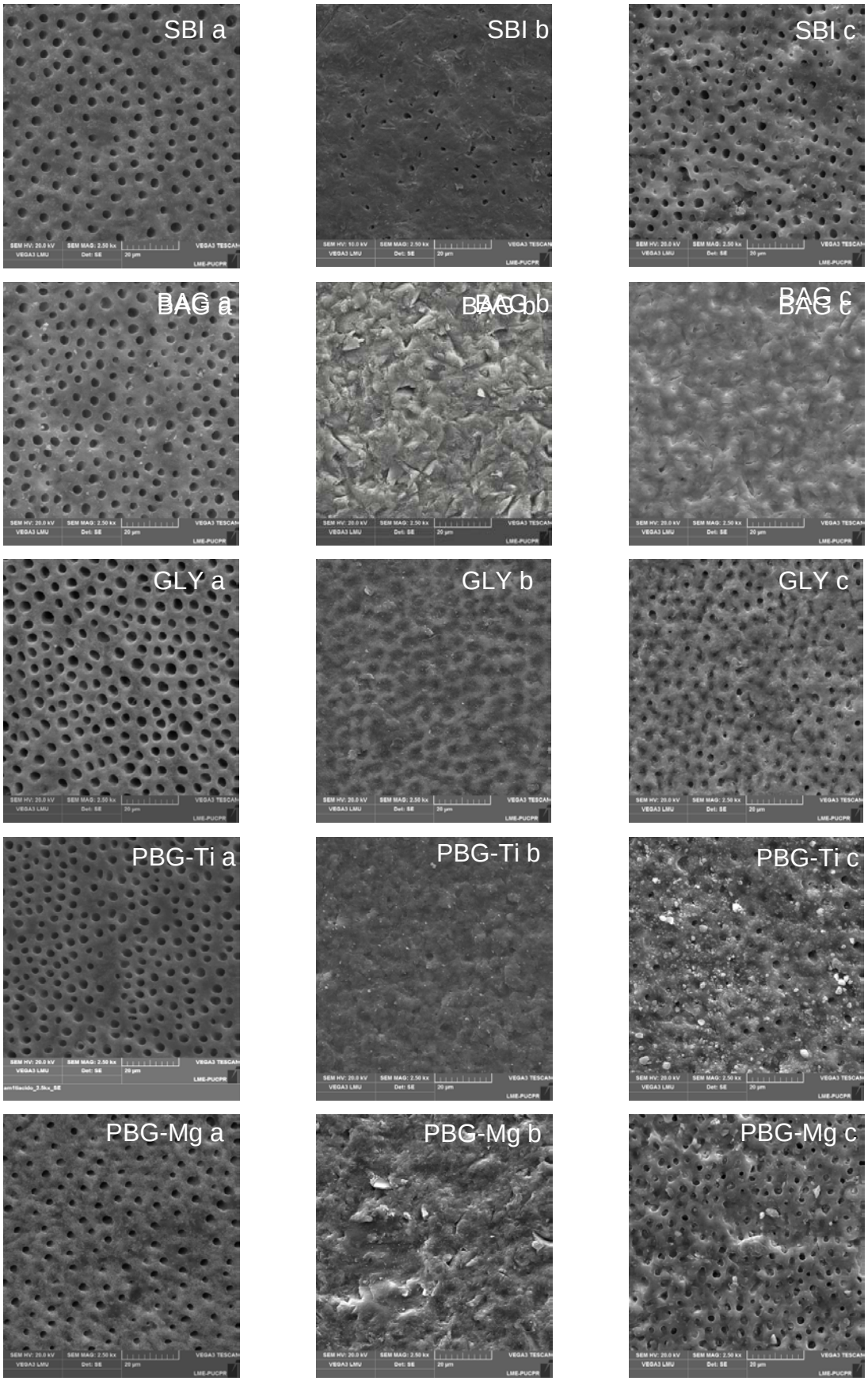

Fig. 2. Photomicrographs with x2500 magnification obtained from the different substances used, SBI – sodium bicarbonate, BAG – Sylc (bioglass 45S5), GLY –

Clinpro, PBG-Ti – Phosphate-based glass Ti-containing, PBG-Mg – Phosphate-based glass Mg-containing. Where: (a) after application of acid, (b) after air abrasion and (c) after immersion in saliva and citric acid.

a) b) c) d) e)

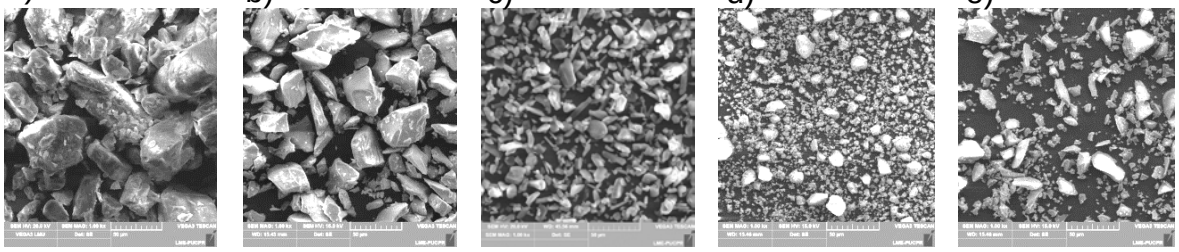

Fig. 3. Images of Scanning Electron Microscopy obtained from substances used with × 1000 magnification; a) SBI, b) BAG, c) GLI, d) PBG-Ti, and e) PBG-Mg.

1 Anexos

2 Parecer do Comitê de ética

Comitê de Ética em Pesquisa da PUCPR

ASSOCIAÇÃO PARANAENSE DE CULTURA - PUCPR

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DE PROCEDIMENTOS DE ABRASÃO À AR NA DESSENSIBILIZAÇÃO DENTINÁRIA

Pesquisador: Andrea Freire de Vasconcelos Eckelberg

Área Temática:

Versão: 1

CAAE: 52821815.0.0000.0020

Instituição Proponente: Pontifícia Universidade Católica do Parana - PUCPR

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.434.128

Apresentação do Projeto:

O objetivo deste estudo in vitro será avaliar as mudanças na permeabilidade dentinária imediatamente após abrasão a ar com diferentes substâncias, após imersão em saliva por 1 hora, após exposição final ao ácido cítrico.´Para isto serão utilizados 90 terceiros molares humanos extraídos, para obtenção de discos de dentina com aproximadamente 1± 0,02 mm da porção média de cada dente. As medidas de permeabilidade serão executadas a partir da movimentação de uma bolha de ar no interior de um capilar de vidro, e o deslocamento será mensurado com um paquímetro digital por meio do aparelho THD 03 (Odeme, Luzerna, SC, Brasil). Para realização deste teste serão utilizados 60 espécimes, divididos em 6 grupos de acordo com a substância a ser utilizada (n=10. As medições serão realizadas em quatro momentos. Cinco molares humanos hígidos serão preparados adicionalmente para cada grupo, totalizando 30 dentes, a fim de obter de imagens da superfície dentinária. Cada parte será atribuída a uma das fases do tratamento da dentina: aplicação do ácido fosfórico, abrasão a ar, armazenamento em saliva artificial por 1 hora e após a aplicação de ácido cítrico.

Objetivo da Pesquisa:

Objetivo Primário:

O objetivo deste estudo in vitro será avaliar as mudanças na permeabilidade dentinária

Endereço: Rua Imaculada Conceição 1155
Bairro: Prado Velho **CEP:** 80.215-901
UF: PR **Município:** CURITIBA
Telefone: (41)3271-2103 **Fax:** (41)3271-2103 **E-mail:** nep@pucpr.br

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imediatamente após abrasão a ar com diferentes substâncias, após imersão em saliva por 1 hora, após exposição final ao ácido cítrico. A microscopia eletrônica de varredura será realizada para avaliar a porcentagem de túbulos obliterados e as mudanças induzidas pelos tratamentos.

Avaliação dos Riscos e Benefícios:

Riscos:

Não há riscos, uma vez que não é utilizado em pacientes, somente in vitro.

Benefícios:

Não há benefícios pois não há participantes na pesquisa.

Comentários e Considerações sobre a Pesquisa:

Sem comentários relevantes

Considerações sobre os Termos de apresentação obrigatória:

Os documentos considerados necessários à formalização do projeto foram anexados à Plataforma Brasil, a saber:

- descritivo do projeto
- folha de rosto
- autorização do banco de dentes da PUCPR

Recomendações:

Sem recomendações relevantes

Conclusões ou Pendências e Lista de Inadequações:

Sem pendências nem inadequações.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_641821.pdf	15/12/2015 16:29:52		Aceito
Declaração de Pesquisadores	declaracaopesquisador.pdf	15/12/2015 16:26:35	Andrea Freire de Vasconcelos Eckelberg	Aceito

Continuação do Parecer: 1.434.128

Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	CEP.pdf	15/12/2015 16:25:04	Andrea Freire de Vasconcelos Eckelberg	Aceito
Projeto Detalhado / Brochura Investigador	PROJETODePESQUISA.docx	15/12/2015 16:24:38	Andrea Freire de Vasconcelos Eckelberg	Aceito
Folha de Rosto	Folhaderosto.pdf	15/12/2015 16:23:17	Andrea Freire de Vasconcelos Eckelberg	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CURITIBA, 02 de Março de 2016

Assinado por:
NAIM AKEL FILHO
(Coordenador)

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1 Análise Estatística

Testes de Normalidade[a]

Tratamento x Momento		Kolmogorov-Smirnov[b]		
		Estatística	gl	Valor p
Permeabilidade (%)				
	Bicarbonato / Substância	,228	10	,152
	Bicarbonato / Saliva + Ácido Cítrico	,181	10	,200*
	Sylc / Substância	,181	10	,200*
	Sylc / Saliva + Ácido Cítrico	,172	10	,200*
	Clinpro / Substância	,188	10	,200*
	Clinpro / Saliva + Ácido Cítrico	,173	10	,200*
	PCNSrTi / Substância	,279	10	,064
	PCNSrTi / Saliva + Ácido Cítrico	,163	10	,200*
	PCNSrMg / Substância	,173	10	,200*
	PCNSrMg / Saliva + Ácido Cítrico	,193	10	,200*

*. Este é um limite inferior da significância verdadeira.

Correlação de Significância de Lilliefors

Descritivas

Permeabilidade (%)

	N	Média	Desvio Padrão	Erro Padrão	Intervalo de confiança de 95% para média		Mínimo	Máximo
					Limite inferior	Limite superior		
Bicarbonato	20	74,37	9,54	2,13	69,91	78,83	57,00	87,00
Sylc	20	60,45	9,97	2,23	55,79	65,11	42,00	80,00
Clinpro	20	59,80	5,76	1,29	57,10	62,50	50,00	70,00
PCNSrTi	20	66,28	13,09	2,93	60,15	72,40	50,00	90,00
PCNSrMg	20	70,63	7,04	1,57	67,33	73,93	58,00	88,00
Total	120	68,81	13,81	1,26	66,32	71,31	42,00	100,00

Teste de Homogeneidade de Variâncias

Permeabilidade (%)

Estatística de Levene	gl1	gl2	Valor p
22,136	5	114	0,0000

Descritivas

Permeabilidade (%)

	N	Média	Desvio Padrão	Erro Padrão	Intervalo de confiança de 95% para média		Mínimo	Máximo
					Limite inferior	Limite superior		
Substância	60	70,47	16,84	2,17	66,11	74,82	42,00	100,00
Saliva + Ácido Cítrico	60	67,16	9,77	1,26	64,63	69,69	45,00	90,00
Total	120	68,81	13,81	1,26	66,32	71,31	42,00	100,00

Teste de Homogeneidade de Variâncias

Permeabilidade (%)

Estatística de Levene	gl1	gl2	Valor p
17,858	1	118	0,0000

Descritivas

Permeabilidade (%)

	N	Média	Desvio Padrão	Erro Padrão	Intervalo de confiança de 95% para média		Mínimo	Máximo
					Limite inferior	Limite superior		
Bicarbonato / Substância	10	79,04	9,84	3,11	72,00	86,08	57,00	87,00
Bicarbonato / Saliva + Ácido Cítrico	10	69,70	6,83	2,16	64,81	74,59	60,00	80,00
Sylc / Substância	10	58,60	9,75	3,08	51,62	65,58	42,00	72,00
Sylc / Saliva + Ácido Cítrico	10	62,30	10,34	3,27	54,90	69,70	45,00	80,00
Clinpro / Substância	10	60,20	6,88	2,17	55,28	65,12	50,00	70,00
Clinpro / Saliva + Ácido Cítrico	10	59,40	4,74	1,50	56,01	62,79	53,00	66,00
PCNSrTi / Substância	10	55,55	3,95	1,25	52,73	58,37	50,00	63,00
PCNSrTi / Saliva + Ácido Cítrico	10	77,00	9,51	3,01	70,20	83,80	61,00	90,00
PCNSrMg / Substância	10	69,40	5,60	1,77	65,39	73,41	63,00	77,00
PCNSrMg / Saliva + Ácido Cítrico	10	71,86	8,36	2,64	65,88	77,84	58,00	88,00
Total	120	68,81	13,81	1,26	66,32	71,31	42,00	100,00

Teste de Homogeneidade de Variâncias

Permeabilidade (%)

Estatística de Levene	gl1	gl2	Valor p
3,351	11	108	0,0005

Fatores dentre-sujeitos

Medida: Permeabilidade

fator1	Variável dependente
1	Permeabilidade Substância
2	Permeabilidade Saliva e Ácido

Fatores entre sujeitos

		Rótulo de valor	N
Tratamento	1	Bicarbonato	10
	2	Sylc	10
	3	Clinpro	10
	4	PCNSrTi	10
	5	PCNSrMg	10

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Testes de efeitos dentre-sujeitos

Medida:

Origem	Tipo III Soma dos Quadrados	gl	Quadrado Médio	F	Valor p	Poder observado[a]
Momento	85,215	1	85,215	1,729	0,1911	0,2565
Momento * Tratamento	9467,358	5	1893,472	38,409	0,0000	1,0000
Erro(Momento)	2662,037	54	49,297			

a. Calculado usando alfa = ,05

Testes de efeitos entre sujeitos

Medida: Permeabilidade

Origem	Tipo III Soma dos Quadrados	gl	Quadrado Médio	F	Valor p	Poder observado[a]
Tratamento	6979,470	5	1395,894	23,119	0,0000	1,0000
Erro	3260,397	54	60,378			

a. Calculado usando alfa = ,05

Comparações múltiplas

Variável dependente: Permeabilidade (%)

Games-Howell

(I) Tratamento		Diferença média (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95%	
					Limite inferior	Limite superior
Bicarbonato	Sylc	13,92000*	3,08433	0,0008	4,6662	23,1738
	Clinpro	14,57000*	2,49168	0,0000	7,0111	22,1289
	PCNSrTi	8,09500	3,62112	0,2480	-2,8210	19,0110
	PCNSrMg	3,74000	2,65074	0,7203	-4,2478	11,7278
Sylc						
	Bicarbonato	-13,92000*	3,08433	0,0008	-23,1738	-4,6662
	Clinpro	,65000	2,57424	0,9998	-7,1725	8,4725
	PCNSrTi	-5,82500	3,67842	0,6143	-16,9005	5,2505
	PCNSrMg	-10,18000*	2,72849	0,0083	-18,4127	-1,9473
Clinpro						
	Bicarbonato	-14,57000*	2,49168	0,0000	-22,1289	-7,0111
	Sylc	-,65000	2,57424	0,9998	-8,4725	7,1725
	PCNSrTi	-6,47500	3,19784	0,3560	-16,2973	3,3473
	PCNSrMg	-10,83000*	2,03471	0,0001	-16,9464	-4,7136
PCNSrTi						
	Bicarbonato	-8,09500	3,62112	0,2480	-19,0110	2,8210
	Sylc	5,82500	3,67842	0,6143	-5,2505	16,9005
	Clinpro	6,47500	3,19784	0,3560	-3,3473	16,2973
	PCNSrMg	-4,35500	3,32327	0,7770	-14,4824	5,7724
PCNSrMg						
	Bicarbonato	-3,74000	2,65074	0,7203	-11,7278	4,2478
	Sylc	10,18000*	2,72849	0,0083	1,9473	18,4127
	Clinpro	10,83000*	2,03471	0,0001	4,7136	16,9464
	PCNSrTi	4,35500	3,32327	0,7770	-5,7724	14,4824

*. A diferença média é significativa no nível 0.05.

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Comparações múltiplas

Variável dependente: Permeabilidade (%)

Games-Howell

(I) Tratamento x Momento		Diferença média (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95%	
					Limite inferior	Limite superior
Bicarbonato / Substância	Bicarbonato / Saliva + Ácido Cítrico	9,34000	3,78864	0,4203	-4,9920	23,6720
	Sylc / Substância	20,44000*	4,38196	0,0076	4,1218	36,7582
	Sylc / Saliva + Ácido Cítrico	16,74000	4,51398	0,0516	-,0749	33,5549

	Clinpro / Substância	18,84000*	3,79670	0,0054	4,4848	33,1952
	Clinpro / Saliva + Ácido Cítrico	19,64000*	3,45470	0,0027	6,1184	33,1616
	PCNSrTi / Substância	23,49000*	3,35322	0,0006	10,1389	36,8411
	PCNSrTi / Saliva + Ácido Cítrico	2,04000	4,32787	1,0000	-14,0791	18,1591
	PCNSrMg / Substância	9,64000	3,58104	0,3174	-4,1493	23,4293
	PCNSrMg / Saliva + Ácido Cítrico	7,18000	4,08399	0,8194	-8,0795	22,4395
Bicarbonato / Saliva + Ácido Cítrico						
	Bicarbonato / Substância	-9,34000	3,78864	0,4203	-23,6720	4,9920
	Sylc / Substância	11,10000	3,76608	0,2110	-3,1367	25,3367
	Sylc / Saliva + Ácido Cítrico	7,40000	3,91890	0,7507	-7,4849	22,2849
	Clinpro / Substância	9,50000	3,06540	0,1576	-1,9153	20,9153
	Clinpro / Saliva + Ácido Cítrico	10,30000*	2,62996	0,0391	,3511	20,2489
	PCNSrTi / Substância	14,15000*	2,49516	0,0020	4,5558	23,7442
	PCNSrTi / Saliva + Ácido Cítrico	-7,30000	3,70300	0,7048	-21,2715	6,6715
	PCNSrMg / Substância	,30000	2,79384	1,0000	-10,1556	10,7556
	PCNSrMg / Saliva + Ácido Cítrico	-2,16000	3,41478	0,9999	-14,9416	10,6216
Sylc / Substância						
	Bicarbonato / Substância	-20,44000*	4,38196	0,0076	-36,7582	-4,1218
	Bicarbonato / Saliva + Ácido Cítrico	-11,10000	3,76608	0,2110	-25,3367	3,1367
	Sylc / Saliva + Ácido Cítrico	-3,70000	4,49506	0,9992	-20,4465	13,0465
	Clinpro / Substância	-1,60000	3,77418	1,0000	-15,8602	12,6602
	Clinpro / Saliva + Ácido Cítrico	-,80000	3,42993	1,0000	-14,2131	12,6131
	PCNSrTi / Substância	3,05000	3,32770	0,9972	-10,1891	16,2891
	PCNSrTi / Saliva + Ácido Cítrico	-18,40000*	4,30813	0,0169	-34,4444	-2,3556
	PCNSrMg / Substância	-10,80000	3,55715	0,1919	-24,4856	2,8856
	PCNSrMg / Saliva + Ácido Cítrico	-13,26000	4,06306	0,1196	-28,4360	1,9160
Sylc / Saliva + Ácido Cítrico						
	Bicarbonato / Substância	-16,74000	4,51398	0,0516	-33,5549	,0749
	Bicarbonato / Saliva + Ácido Cítrico	-7,40000	3,91890	0,7507	-22,2849	7,4849
	Sylc / Substância	3,70000	4,49506	0,9992	-13,0465	20,4465
	Clinpro / Substância	2,10000	3,92669	1,0000	-12,8062	17,0062
	Clinpro / Saliva + Ácido Cítrico	2,90000	3,59707	0,9991	-11,2460	17,0460
	PCNSrTi /	6,75000	3,49972	0,7260	-7,2438	20,7438

	Substância					
	PCNSrTi / Saliva + Ácido Cítrico	-14,70000	4,44235	0,1093	-31,2577	1,8577
	PCNSrMg / Substância	-7,10000	3,71857	0,7381	-21,4879	7,2879
	PCNSrMg / Saliva + Ácido Cítrico	-9,56000	4,20511	0,5257	-25,3078	6,1878
Clinpro / Substância						
	Bicarbonato / Substância	-18,84000*	3,79670	0,0054	-33,1952	-4,4848
	Bicarbonato / Saliva + Ácido Cítrico	-9,50000	3,06540	0,1576	-20,9153	1,9153
	Sylc / Substância	1,60000	3,77418	1,0000	-12,6602	15,8602
	Sylc / Saliva + Ácido Cítrico	-2,10000	3,92669	1,0000	-17,0062	12,8062
	Clinpro / Saliva + Ácido Cítrico	,80000	2,64155	1,0000	-9,1979	10,7979
	PCNSrTi / Substância	4,65000	2,50738	0,7677	-4,9972	14,2972
	PCNSrTi / Saliva + Ácido Cítrico	-16,80000*	3,71124	0,0119	-30,7959	-2,8041
	PCNSrMg / Substância	-9,20000	2,80476	0,1172	-19,6998	1,2998
	PCNSrMg / Saliva + Ácido Cítrico	-11,66000	3,42371	0,0935	-24,4710	1,1510
Clinpro / Saliva + Ácido Cítrico						
	Bicarbonato / Substância	-19,64000*	3,45470	0,0027	-33,1616	-6,1184
	Bicarbonato / Saliva + Ácido Cítrico	-10,30000*	2,62996	0,0391	-20,2489	-,3511
	Sylc / Substância	,80000	3,42993	1,0000	-12,6131	14,2131
	Sylc / Saliva + Ácido Cítrico	-2,90000	3,59707	0,9991	-17,0460	11,2460
	Clinpro / Substância	-,80000	2,64155	1,0000	-10,7979	9,1979
	PCNSrTi / Substância	3,85000	1,95114	0,7040	-3,4467	11,1467
	PCNSrTi / Saliva + Ácido Cítrico	-17,60000*	3,36056	0,0053	-30,7091	-4,4909
	PCNSrMg / Substância	-10,00000*	2,32092	0,0163	-18,6732	-1,3268
	PCNSrMg / Saliva + Ácido Cítrico	-12,46000*	3,04004	0,0325	-24,1703	-,7497
PCNSrTi / Substância						
	Bicarbonato / Substância	-23,49000*	3,35322	0,0006	-36,8411	-10,1389
	Bicarbonato / Saliva + Ácido Cítrico	-14,15000*	2,49516	0,0020	-23,7442	-4,5558
	Sylc / Substância	-3,05000	3,32770	0,9972	-16,2891	10,1891
	Sylc / Saliva + Ácido Cítrico	-6,75000	3,49972	0,7260	-20,7438	7,2438
	Clinpro / Substância	-4,65000	2,50738	0,7677	-14,2972	4,9972
	Clinpro / Saliva + Ácido Cítrico	-3,85000	1,95114	0,7040	-11,1467	3,4467
	PCNSrTi / Saliva + Ácido Cítrico	-21,45000*	3,25615	0,0009	-34,3751	-8,5249
	PCNSrMg / Substância	-13,85000*	2,16699	0,0004	-22,0379	-5,6621

	PCNSrMg / Saliva + Ácido Cítrico	-16,31000*	2,92421	0,0034	-27,7782	-4,8418
PCNSrTi / Saliva + Ácido Cítrico						
	Bicarbonato / Substância	-2,04000	4,32787	1,0000	-18,1591	14,0791
	Bicarbonato / Saliva + Ácido Cítrico	7,30000	3,70300	0,7048	-6,6715	21,2715
	Sylc / Substância	18,40000*	4,30813	0,0169	2,3556	34,4444
	Sylc / Saliva + Ácido Cítrico	14,70000	4,44235	0,1093	-1,8577	31,2577
	Clinpro / Substância	16,80000*	3,71124	0,0119	2,8041	30,7959
	Clinpro / Saliva + Ácido Cítrico	17,60000*	3,36056	0,0053	4,4909	30,7091
	PCNSrTi / Substância	21,45000*	3,25615	0,0009	8,5249	34,3751
	PCNSrMg / Substância	7,60000	3,49030	0,5853	-5,7959	20,9959
	PCNSrMg / Saliva + Ácido Cítrico	5,14000	4,00467	0,9710	-9,8045	20,0845
PCNSrMg / Substância						
	Bicarbonato / Substância	-9,64000	3,58104	0,3174	-23,4293	4,1493
	Bicarbonato / Saliva + Ácido Cítrico	-,30000	2,79384	1,0000	-10,7556	10,1556
	Sylc / Substância	10,80000	3,55715	0,1919	-2,8856	24,4856
	Sylc / Saliva + Ácido Cítrico	7,10000	3,71857	0,7381	-7,2879	21,4879
	Clinpro / Substância	9,20000	2,80476	0,1172	-1,2998	19,6998
	Clinpro / Saliva + Ácido Cítrico	10,00000*	2,32092	0,0163	1,3268	18,6732
	PCNSrTi / Substância	13,85000*	2,16699	0,0004	5,6621	22,0379
	PCNSrTi / Saliva + Ácido Cítrico	-7,60000	3,49030	0,5853	-20,9959	5,7959
	PCNSrMg / Saliva + Ácido Cítrico	-2,46000	3,18288	0,9995	-14,5354	9,6154
PCNSrMg / Saliva + Ácido Cítrico						
	Bicarbonato / Substância	-7,18000	4,08399	0,8194	-22,4395	8,0795
	Bicarbonato / Saliva + Ácido Cítrico	2,16000	3,41478	0,9999	-10,6216	14,9416
	Sylc / Substância	13,26000	4,06306	0,1196	-1,9160	28,4360
	Sylc / Saliva + Ácido Cítrico	9,56000	4,20511	0,5257	-6,1878	25,3078
	Clinpro / Substância	11,66000	3,42371	0,0935	-1,1510	24,4710
	Clinpro / Saliva + Ácido Cítrico	12,46000*	3,04004	0,0325	,7497	24,1703
	PCNSrTi / Substância	16,31000*	2,92421	0,0034	4,8418	27,7782
	PCNSrTi / Saliva + Ácido Cítrico	-5,14000	4,00467	0,9710	-20,0845	9,8045
	PCNSrMg / Substância	2,46000	3,18288	0,9995	-9,6154	14,5354

*. A diferença média é significativa no nível 0.05.

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1 *Normas para publicação*

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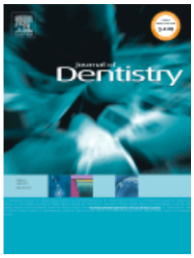

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CF14 4NQ, UK
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ARTIGO 2

Página título

Efeito de vidros bioativos com diferentes taxas de dissolução no controle da erosão do esmalte

Berthyelle Pádova Nyland[a], Andrea Freire[a], Cristiano Porcel Pereira[a], Paulo Soares[b], Denise Stolle da Luz Weiss[b], Sérgio Vieira[a]

[a]Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil

Berthyelle Pádova Nyland,
Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
draberthy@gmail.com

Andrea Freire,
Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
andreavfreire@yahoo.com.br

Cristiano Porcel Pereira,
Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
cristiano_porcel@hotmail.com

Paulo Soares,
Escola Politécnica, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
pa.soares@pucpr.br

Denise Stolle da Luz Weiss,
Escola Politécnica, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
denisesluz@bol.com.br

Sérgio Vieira,
Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
s.vieira@pucpr.com.br

Autor Correspondente:
Andrea Freire
Programa de Pós-Graduação em Odontologia
Pontifícia Universidade Católica do Paraná
Escola de Ciências da Vida
R. Imaculada Conceição, 1155, Prado Velho
Curitiba – PR – BRASIL 80215-901
Tel: +55 41 3271-1637; Fax: +55 41 3271-1405
E-mail: andreavfreire@yahoo.com.br

Resumo

Objetivo: Avaliar o efeito de vidros bioativos a base de fosfato de cálcio e estrôncio contendo óxidos de titânio e magnésio, com diferentes taxas de dissolução, no controle da erosão dental.

Métodos: Cinquenta fragmentos de esmalte foram divididos em 5 grupos: controle negativo (CTRL), biovidro 45S5 (BAG), Vidro bioativo rico em fosfato contendo Ti (PBG-Ti), Vidro bioativo rico em fosfato contendo Mg (PBG-Mg), Vidro bioativo rico em fosfato contendo Ti e Mg (PBG-TiMg). Os espécimes passaram por 5 dias de ciclos que consistiam em desafio erosivo (2x/dia) com ácido cítrico seguido pela aplicação das soluções com substâncias bioativas. Após os ciclos, as análises de perfilometria, rugosidade, microdureza e microscopia eletrônica de varredura (MEV) foram realizadas. Os testes estatísticos utilizados foram Análise de Variância a um critério (perfilometria, rugosidade e variação da microdureza superficial (%VMS)), Tukey HSD (%VMS), Games Howell (perfilometria), teste t de Student (rugosidade) e Correlação de Pearson entre as variáveis.

Resultados: A menor perda de superfície de esmalte e menor %VMS foi observada nos grupos PBG-Mg e PBG-TiMg e somente o grupo PBG-Mg apresentou semelhança na rugosidade entre a área sadia e erodida. Nas imagens obtidas por MEV, PBG-Ti e PBG-Mg demonstraram menor desmineralização aparente.

Significância: Todos os materiais bioativos proporcionaram um efeito protetor ao esmalte dental submetido à erosão. A taxa de dissolução dos vidros ricos em fosfato é um fator determinante no controle da desmineralização do esmalte, sendo que a presença do Mg, nestes vidros bioativos, resultou em maior efeito protetor.

1. Introdução

A erosão dental é definida como a perda irreversível de tecido dental devido a um processo químico, sem o envolvimento de microorganismos [1]. Este processo de desmineralização é caracterizado por um amolecimento da superfície de esmalte, que varia de acordo com o tipo de ácido e o tempo de contato deste com a estrutura dentária [2,3]. A contínua dissolução de esmalte leva à perda permanente deste tecido e em estágios mais avançados pode ocasionar a exposição dentinária [2].

A etiologia da erosão dental está relacionada a ácidos intrínsecos e extrínsecos. A mudança do estilo de vida e a exposição precoce de crianças a produtos ácidos industrializados, como bebidas e doces, tem aumentado a incidência de erosão dental por ácidos extrínsecos [4]. A alta prevalência deste processo de desmineralização em dentes permanentes de crianças e adolescentes foi evidenciada em estudos recentes [5,6] e o crescente aumento da erosão dental indica que esta pode tornar-se um problema de saúde pública [6]. Isto comprova a necessidade do diagnóstico precoce e do tratamento com o objetivo de reduzir ou inibir a progressão dos sinais e sintomas [7]. Estratégias preventivas que envolvem alterações na dieta, estimulação do fluxo salivar e medidas adequadas de higiene oral têm sido utilizadas [8], bem como o desenvolvimento de substâncias que propiciem uma proteção contra erosão [9-13].

Desta forma, houve um aumento no número de pesquisas com substâncias bioativas para conter a erosão dental, como o ACP-CPP (fosfato de cálcio amorfo estabilizado por fosfopeptídeo de caseína) [13,14], o Biovidro 45S5 [11,15,16] e o silicato de cálcio e fosfato [17-19]. Estas substâncias bioativas possuem potencial de prover cálcio e fosfato para a superfície desmineralizada [11] e podem ser utilizadas na forma de dentifrícios [20,21] e pastas [11].

Vidros bioativos contendo titânio e magnésio apresentam alta bioatividade e capacidade de formar cristais de hidroxiapatita [21,22]. Recentemente, foram desenvolvidos vidros bioativos ricos em fosfato, contendo estrôncio e dopados com magnésio ou titânio. A incorporação do titânio ou do magnésio é importante para o controle da solubilidade deste material, uma vez que a presença do estrôncio aumenta a taxa de dissolução dos vidros [23]. O titânio possui uma

menor taxa de dissolução que o magnésio [23] e pode formar sobre a estrutura dentária uma camada hidrófoba e resistente ao ácido [24]. Essas diferentes composições de vidros bioativos ainda não foram testadas no controle da erosão de esmalte. Desta forma, os resultados obtidos serão norteadores para o desenvolvimento de novos produtos odontológicos.

Assim, o objetivo deste estudo foi avaliar o efeito de vidros bioativos ricos em fosfato contendo titânio e magnésio no controle da erosão do esmalte dental.

A hipótese nula a ser testada foi que não haveria diferença entre os vidros bioativos ricos em fosfato contendo óxidos de titânio e magnésio e o biovidro 45S5 no controle da erosão do esmalte dental.

2. Materiais e Métodos

2.1 Preparo dos Espécimes

Para a pesquisa foram utilizados 50 dentes humanos, terceiros molares extraídos, obtidos após aprovação do Comitê de Ética em Pesquisa (protocolo número: 1.824.578). Os dentes foram cortados com a utilização de um disco diamantado em cortadeira metalográfica (Struers A/S, Ballerup, Denmark) sob irrigação constante, obtendo fragmentos de esmalte com 4 x 3 x 2 mm da face vestibular. Os espécimes foram incluídos em resina acrílica, em seguida desgastados com lixa de carbeto de silício nas granulações #800, #1000, #1200 (3M do Brasil, Sumaré, SP, Brasil) e polidos com disco de feltro e óxido de cério em politriz (Struers A/S, Ballerup, Denmark). Após o polimento, os espécimes foram observados em microscópio óptico com aumento de 50x (Olympus, São Paulo, SP, Brasil) para verificar a presença de esmalte em toda a extensão. Em seguida os espécimes foram divididos em 5 grupos (n=10) de acordo com a substância a ser aplicada posteriormente ao desafio erosivo (Tabela 1).

Antes dos ciclos erosão/remineralização todos os espécimes tiveram as áreas de referência delimitadas para realização dos testes de perfilometria, rugosidade e dureza. A delimitação foi realizada com esmalte de unha [16, 25], deixando apenas uma área de 1 x 4 mm exposta [26].

2.2 Síntese dos vidros bioativos

Os vidros bioativos ricos em fosfato contendo Mg e/ou Ti foram obtidos a partir da metodologia descrita por Weiss [23]. A moagem foi realizada manualmente com o auxílio de um almofariz de Ágata. Após a moagem, uma peneira de 50 micrometros foi utilizada para padronizar o tamanho das partículas. Após o término da moagem, o tamanho das partículas de biovidro foi confirmado por Microscopia Eletrônica de Varredura (MEV).

2.3 Desafio Erosivo

Para o desafio erosivo foi utilizada uma solução 0,3% de ácido cítrico com pH de 3,2 ajustado com hidróxido de sódio. Cada espécime recebeu a aplicação de 1 mL desta solução sob agitação (Incubadora Shaker, Marconi MA420, Piracicaba, SP, Brasil) em um período de 2 minutos, sendo 1 mL aplicados a cada minuto. Foram realizadas 2 aplicações por dia, durante 5 dias. Após o desafio erosivo os espécimes foram lavados com 5 mL de água deionizada sob agitação por 1 minuto.

2.4 Aplicação das Soluções

Na sequência, uma solução foi aplicada de acordo com a Tabela 1. Os vidros bioativos foram utilizados em solução na proporção de 1 g de biovidro em 10 mL de água deionizada, sendo 1 mL aplicado por espécime e mantidos sob agitação (Incubadora Shaker, Marconi MA420, Piracicaba, SP, Brasil) durante 3 minutos.

Tabela 1: Descrição dos grupos de acordo com a solução a ser utilizada e sua composição

GRUPOS	SOLUÇÕES	COMPOSIÇÃO
CTRL	Água deionizada	----------
BAG	Biovidro comercial 45S5	SiO_2, Na_2O e CaO e P_2O_5
PBG-Ti	Vidro bioativo rico em fosfato contendo Ti	P_2O_5, CaO, Na_2O, SrO, TiO_2
PBG-Mg	Vidro bioativo rico em fosfato contendo Mg	P_2O_5, CaO, Na_2O, SrO, MgO
PBG-TiMg	Vidro bioativo rico em fosfato contendo Ti e Mg	P_2O_5, CaO, Na_2O, SrO, TiO_2 e MgO

2.5 Armazenamento dos Espécimes

Entre os ciclos de erosão/aplicação das substâncias bioativas os espécimes foram armazenados em saliva humana. Os voluntários para a doação de saliva foram selecionados a partir dos seguintes critérios – inclusão: jovens entre 16 e 30 anos, com boa higiene oral e com mais de 20 dentes na boca; exclusão: fumantes, presença de lesões de cárie ativas, presença de doença periodontal, uso de aparelho ortodôntico, desgaste acentuado por bruxismo, apertamento ou erosão, uso de medicamentos que afetem o fluxo salivar, função salivar anormal, presença de diabetes ou doenças autoimunes, uso de antibióticos com menos de 1 mês da coleta salivar, desordens médicas que necessitem de medição antes da consulta odontológica, desordens gástricas ou alimentares e gestantes. Após análise dos critérios de inclusão e exclusão os pacientes passaram por exame clínico e foram orientados a permanecer no mínimo 1 hora sem consumir alimentos e bebidas além da escovação dentária ser realizada com pelo menos 2 horas de antecedência da coleta. A obtenção da saliva foi estimulada por meio da mastigação de um pedaço de látex estéril com tamanho padrão de 1 cm de comprimento durante 11 minutos ininterruptos, sendo que a saliva produzida no primeiro minuto foi desprezada. O “pool” de saliva foi homogeneizado, separado em recipientes de 100 mL para uso diário e congelado. Ao final dos ciclos as amostras foram armazenadas individualmente com 2 mL de saliva em estufa a 37°C. A saliva foi trocada diariamente após o último ciclo do dia.

O pH, capacidade de tamponamento e presença de proteínas salivares e cálcio foram analisados após a obtenção do “pool” de saliva. A análise do pH foi realizada em triplicata com o uso do pHmetro PHB-500 Benchtop (ION, Curitiba, PR, Brazil) previamente calibrado com solução padrão com pH de 4,0 e 7,0. A capacidade tampão da saliva foi mensurada por meio de titulação com ácido clorídrico, onde utilizou-se uma mistura de 1 mL de saliva estimulada com 3 mL de ácido clorídrico 0,005 M e, a presença de proteínas e cálcio na saliva foi avaliada a partir de dosagens colorimétricas com o espectrofotômetro óptico BIO 200 (Bioplus, Barueri, SP, Brasil).

2.6 Perfilometria Óptica sem contato 3D

Após completar os 5 dias de ciclos, o esmalte aplicado para proteger a área sadia foi removido com acetona e o desgaste dental foi mensurado por meio do teste de perfilometria óptica sem contato 3D (Talysurf CCI Lite Non-Contact 3D Profiler, Taylor Hobson, Leicester, Inglaterra) com ampliação de 5× e abertura de 0,13 mm para todas as áreas escaneadas. Ao término de cada leitura o software do equipamento (Mountains Digital Surf, Besançon, França) forneceu um gráfico com o traçado do perfil, medição da profundidade da lesão em micrometros e obtenção de imagens 3D. Adicionalmente, este equipamento permitiu a obtenção de medidas de rugosidade (Sa) da superfície sadia e erodida. Os testes foram realizados em triplicata e a média dos valores obtidos para cada espécime foi utilizada.

2.7 Microdureza Knoop

A avaliação da microdureza *Knoop* da superfície do esmalte foi realizada em microdurômetro (HMV-2T, ShimadzuCorp., Tóquio, Japão) com carga estática de 10 g por 10 s [27,28]. No total foram realizadas 10 indentações em cada espécime, sendo que 5 foram realizadas na área sadia (microdureza inicial - M_i) e 5 na área submetida aos tratamentos (microdureza final - M_f). A média das indentações em cada região foi calculada para cada espécime, e, utilizando estes valores, a porcentagem de variação da microdureza superficial (%VMS) foi calculada com a seguinte fórmula:

$$\%VMS = [(M_f - M_i)/ M_i]*100.$$

2.8 Microscopia Eletrônica de Varredura (MEV)

A MEV (TESCAN VEGA3 LMU, TescanOrsay Holding, Brno-Kohoutovice, República Tcheca) foi realizada para observar a superfície de esmalte das áreas sadias e das áreas submetidas aos tratamentos com aumento de 1000 × a 20 kV. Para a análise, amostras representativas de cada grupo foram metalizadas com liga ouro/paládio (Balzers SCD 050, Balzers, Liechtenstein).

2.9 Análise Estatística

Os dados foram submetidos à análise estatística e após a verificação da normalidade pelo teste Kolmogorov-Smirnov, a Análise de Variância a um critério foi aplicada para perfilometria, rugosidade e variação da microdureza superficial. O teste de comparações múltiplas aplicado para perfilometria foi o Games-Howell, uma vez que não foi detectada homogeneidade (teste de Levene) e para microdureza aplicou-se o teste Tukey HSD (α=0,05). Os dados de rugosidade foram pareados e analisados pelo teste t de Student (α=0,05). A correlação entre os dados de perfilometria, rugosidade e microdureza foi avaliada pela Correlação de Pearson (α=0,01).

3. Resultados

A análise de perda de esmalte dental revelou diferenças estatísticas entre os diferentes grupos ($p<0,05$) conforme demonstrado na Tabela 2. Todas as soluções foram efetivas no controle da erosão dental, uma vez que houve diferença estatística de todos os grupos quando comparados ao controle negativo, sendo a menor perda de superfície de esmalte observada nos grupos que utilizaram os vidros bioativos PBG-Mg e PBG-TiMg. A diferença entre a perda de estrutura dos diferentes grupos também pode ser observada na Fig 1.

Tabela 2: Perda da superfície de esmalte (μm) e desvio padrão após a realização dos ciclos erosão/vidros bioativos

Grupos	Perda de estrutura
CTRL	0,90 (0,02)[a]
BAG	0,53 (0,08)[b]
PBG-Ti	0,32 (0,10)[c]
PBG-Mg	0,17 (0,03)[d]
PBG-TiMg	0,23 (0,05)[c,d]

Letras minúsculas diferentes indicam diferença estatística entre os grupos ($p<0,05$)

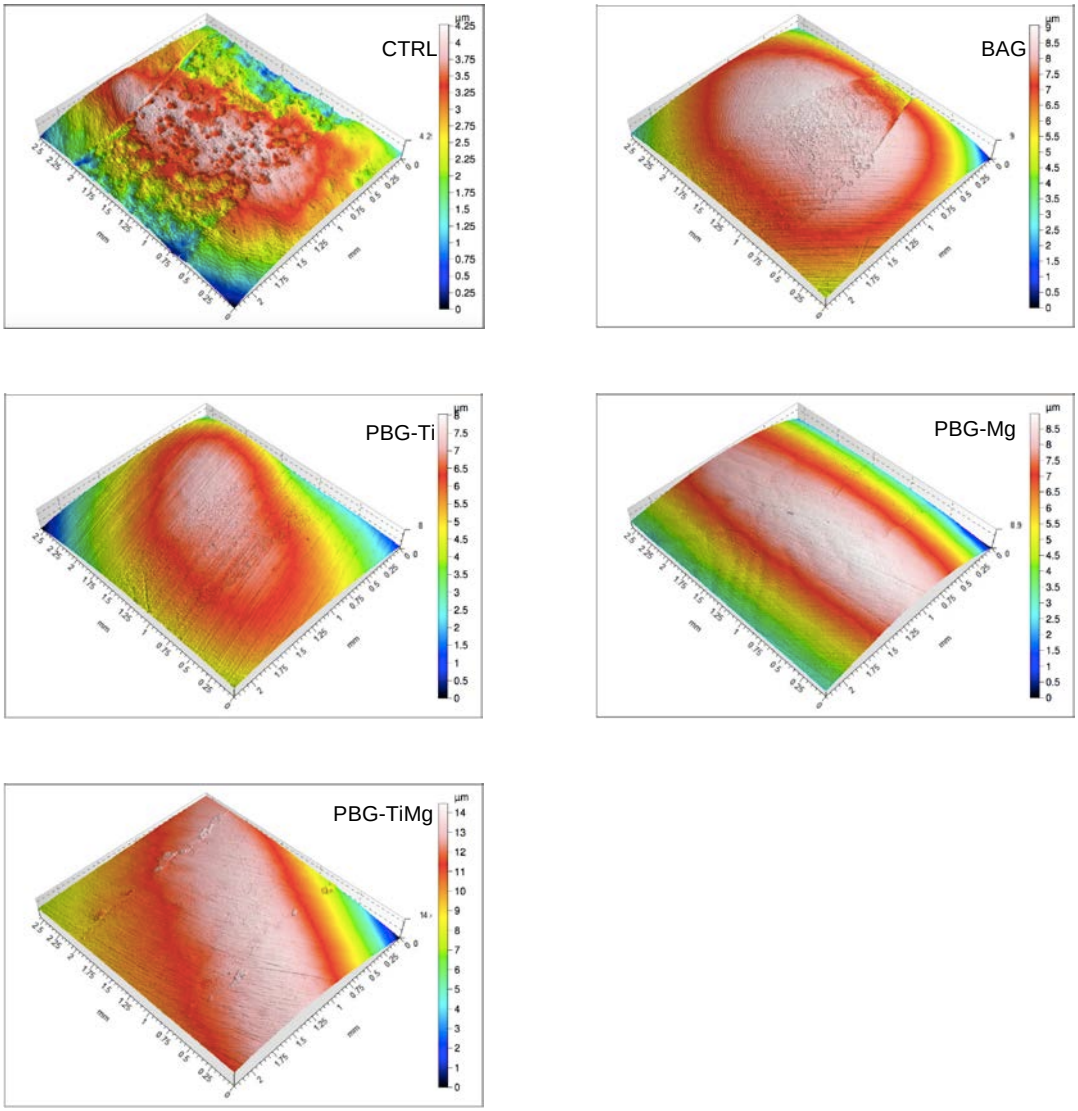

Figura 1. Representação 3D da superfície de esmalte obtida por meio da perfilometria óptica sem contato após a realização dos ciclos. CTRL- controle negativo, BAG- Biovidro 45S5, PBG-Ti – Vidro bioativo rico em fosfato contendo Ti, PBG-Mg - Vidro bioativo rico em fosfato contendo Mg, PBG-TiMg - Vidro bioativo rico em fosfato contendo Ti e Mg

A rugosidade superficial dos espécimes apresentou diferença entre a área protegida e erodida em todos os grupos, exceto no PBG-Mg, conforme descrito na Tabela 3.

Tabela 3: Valores médios de rugosidade (Sa) em μm, e desvio-padrão obtidos das áreas protegidas e das áreas submetidas aos ciclos erosão/vidros bioativos

Grupos	Rugosidade (Sa) área protegida	Rugosidade (Sa) área erodida	Valor de p
CTRL	0,054 (0,02)	0, 124 (0,03)	0,00005
BAG	0,047 (0,02)	0,061 (0,02)	0,00440
PBG-Ti	0,048 (0,01)	0,085 (0,02)	0,00010
PBG-Mg	0,051 (0,02)	0,054 (0,02)	0,19342
PBG-TiMg	0,052 (0,02)	0,067 (0,02)	0,00300

As imagens de MEV correspondentes às áreas protegidas e erodidas, bem como a interface entre as mesmas podem ser visualizadas na Fig 2. As imagens das áreas protegidas mostram uma superfície lisa, confirmando a padronização dos espécimes demonstrada pela rugosidade inicial. Já as áreas erodidas evidenciam os prismas de esmalte com sinais de desmineralização pelo ácido cítrico. Entretanto, diferentes padrões foram observados de acordo com as soluções utilizadas, sendo que os grupos PBG-Ti e PBG-Mg demonstraram menor desmineralização aparente.

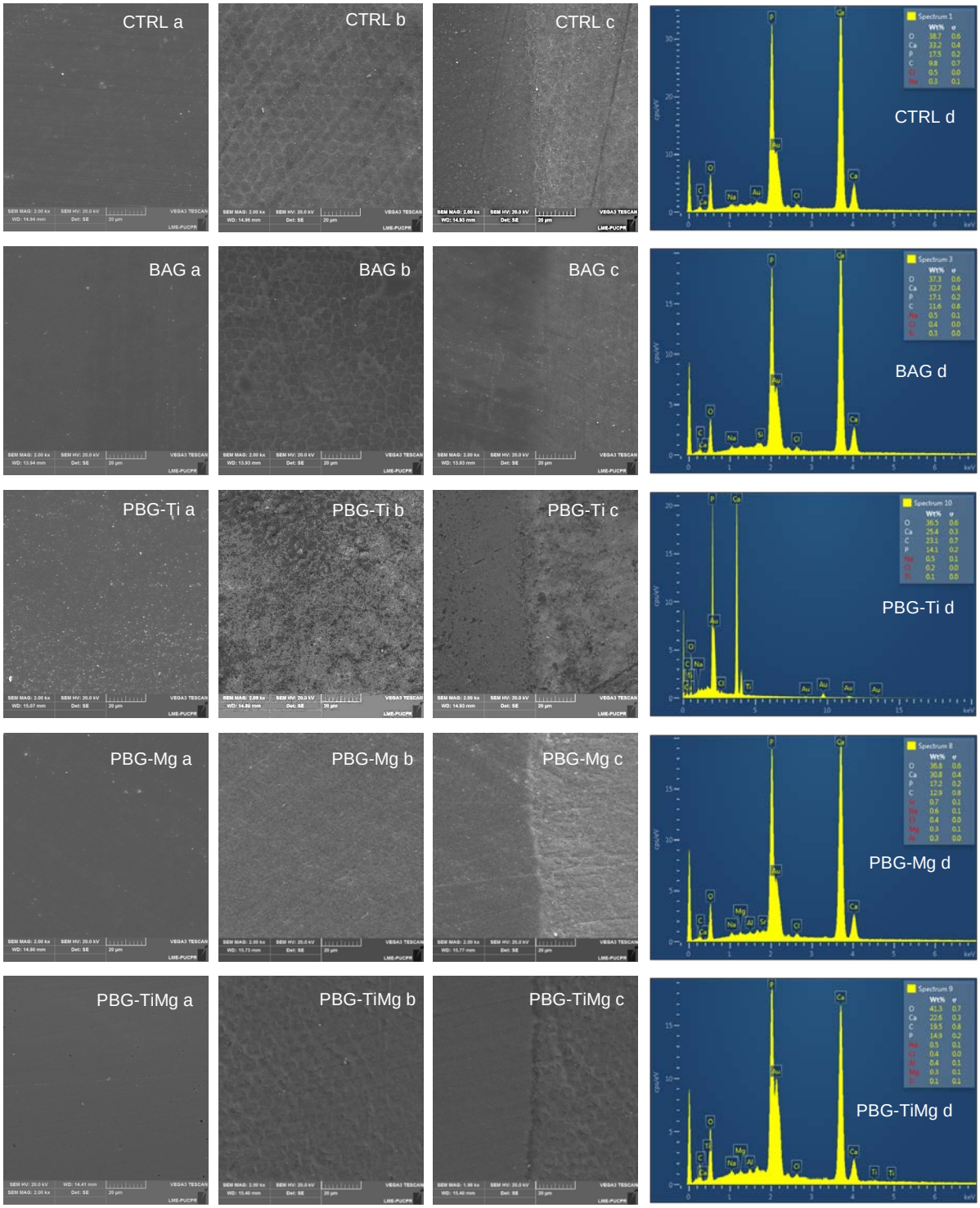

Figura 2. Fotomicrografias das superfícies de esmalte obtidas por MEV com aumento de 2000× e análise EDS. CTRL – Controle Negativo, BAG – Biovidro 45S5, PBG-Ti – Vidro bioativo rico em fosfato contendo Ti, PBG-Mg - Vidro bioativo rico em fosfato contendo Mg, PBG-TiMg - Vidro bioativo rico em fosfato contendo Ti e Mg. (a) área sadia (b) área submetida aos ciclos (c) interface entre

área sadia e área submetida aos ciclos (d) Análise EDS para caracterização do esmalte na região submetida aos ciclos.

Com relação à porcentagem de variação da microdureza superficial houve diferença entre os tratamentos (p<0,05), sendo a menor variação demonstrada pelos grupos PBG-Mg e PBG-TiMg, que foram semelhantes entre si (p=0,5968). O grupo controle apresentou a maior variação e diferiu de todos os outros (p<0,05). Já os grupos PBG-Ti e BAG foram semelhantes entre si (p=0,1069) e mostraram valores intermediários de %VMS, diferindo dos demais grupos (p<0,05). Os valores de %VMS estão expressos na Fig 3.

A hipótese nula foi rejeitada, uma vez que houve diferença entre os materiais avaliados, quanto ao controle da erosão dental.

Tabela 4: % Variação da microdureza da superfície do esmalte.

Grupo	% VMS
CTRL	-60,54 (3,85)[c]
BAG	-41,31 (2,95)[b]
PBG-Ti	-36,74 (5,73)[b]
PBG-Mg	-22,02 (3,71)[a]
PBG-TiMg	-19,37 (3,58)[a]

As letras diferentes indicam diferença estatística significante.

A perfilometria e a microdureza após a realização dos tratamentos obtiveram correlação negativa muito forte (R=-0,903), ou seja, quanto maior a perda de estrutura, menor a microdureza após tratamento. Forte correlação também foi detectada entre perfilometria e rugosidade após tratamento (R=0,643).

As análises do “pool” de saliva demonstraram média do pH igual a 8,09, isto é, alcalino, e a avaliação da capacidade tampão após titulação com ácido clorídrico confirmou a capacidade que o “pool” de saliva tem para neutralizar ácidos. A quantidade de proteínas dosada foi de 1,158 g/dL e cálcio 7,27 mg/dL.

4. Discussão

A erosão do esmalte ocorre inicialmente como uma desmineralização parcial da superfície que resulta no amolecimento da estrutura [19,27]. Quando o desafio ácido é persistente ocorre a dissolução de cristais de hidroxiapatita levando a uma perda permanente do esmalte [2]. No presente estudo, a utilização de substâncias bioativas foi efetiva no controle da erosão dental, uma vez que reduziu significativamente a perda da estrutura de esmalte. Todos os vidros bioativos ricos em fosfato proporcionaram menor perda de esmalte quando comparados ao biovidro 45S5 (BAG).

Os vidros ricos em fosfato, avaliados neste estudo, apresentam 40% de pentóxido de fósforo na sua composição que, durante a sua dissolução, liberam fosfato para o meio. Já o biovidro 45S5 (BAG) tem 6% deste componente, isto justifica o menor efeito protetor deste material. O fosfato pode ser adsorvido na película ou diretamente sobre o esmalte e compete com os íons H^+ em locais específicos na superfície dentária [29]. A variação do conteúdo de fosfato controla a reatividade e formação de hidroxiapatita pelos biovidros, sendo que o alto conteúdo desse permite a formação de HA em baixo pH de forma mais rápida [30].

Os diferentes resultados entre os vidros ricos em fosfato podem ser fundamentados pela diferença nas taxas de dissolução dos mesmos. Os vidros a base de fosfato contendo estrôncio apresentam alta solubilidade [23] que influencia o efeito destes na estrutura dentária, sob desafio ácido. O PBG-Ti apresenta menor taxa de dissolução dos vidros bioativos avaliados. O Ti age como um componente intermediário na estrutura do vidro (modificador e formador) assim, provoca um aumento de ligações com átomos de oxigênio, o que torna a estrutura do vidro mais resistente quimicamente, uma vez que a energia de união Ti-O é maior que de outros modificadores, como CaO, Na_2O, SrO_2 e MgO [23]. Assim, a presença do Mg facilita a lixiviação dos componentes do vidro, disponibilizando Ca e PO_4 rapidamente para agir na estrutura dentária. Desta forma, os biovidros PBG-Mg e PBG-TiMg apresentaram melhores resultados na análise de perda de estrutura, bem como na variação da microdureza.

Além de controlar a taxa de dissolução, o Mg e Ti podem proporcionar efeitos benéficos no controle da erosão dental. Soluções contendo magnésio podem reagir com a superfície externa do esmalte levando a uma dissolução e reprecipitação, reduzindo o tamanho dos cristais na superfície do esmalte. Esta alteração cristalográfica na estrutura do esmalte proporciona uma maior dureza superficial [31], o que está de acordo com os resultados encontrados no presente estudo onde os grupos PBG-Mg e PBG-TiMg apresentaram maiores valores de microdureza após ciclos erosivos. Desta forma, os íons Mg^{+2} incorporados à superfície do esmalte otimizam as propriedades físicas e a estabilidade química desta estrutura [31]. Estes íons também estabilizam a camada amorfa de fosfato da cálcio formada anteriormente à hidroxiapatita e assim, regulam a cristalização desta [32].

Íons Ti após serem dissolvidos do biovidro incorporam-se na camada superficial como fosfato de titânio ou Ti substituído na hidroxiapatita [22]. O Ti, neste caso, substitui o Ca e não o fósforo [33], isto pode ser evidenciado na análise de EDS onde os vidros PBG-Ti e PBG-TiMg apresentaram pequena quantidade de Ti e redução da quantidade de Ca em comparação com os demais vidros bioativos.

Quando comparado ao biovidro 45S5, o vidro dopado com Ti, apresentou reduzida formação de hidroxiapatita, em um meio livre de proteínas. Entretanto, sob condições fisiológicas, com adição de proteínas, constatou-se um aumento da taxa de formação de hidroxiapatita [22]. Assim, a presença de proteínas em uma solução salina contribui para bioatividade dos vidros [22,34,35], bem como pode simular condições bucais [36]. Desta forma, o presente estudo utilizou saliva humana que apresenta na sua composição além de íons minerais também proteínas que têm um papel importante no controle da erosão dental [37]. Os parâmetros salivares analisados, pH, capacidade tampão, dosagem de proteínas e cálcio estavam dentro dos valores referidos na literatura [38-40]. O pH maior que 8 favorece a deposição de íons, entretanto, pode ser uma limitação deste estudo, uma vez que pacientes com erosão dental podem apresentar pH ácido [41,42]. Desta forma, são necessários estudos futuros com estes materiais na presença de saliva com variação dos valores de pH.

Em esmalte, a perda de brilho é um critério de diagnóstico para erosão em estágio inicial e pode estar relacionada a um aumento da rugosidade [2]. Dentre

os grupos avaliados, somente o PBG-Mg não apresentou alteração de rugosidade entre as áreas sadia e erodida, demonstrando assim, maior efeito protetor, que também pode ser observado nas imagens de MEV.

A avaliação da desmineralização por microdureza e rugosidade detecta estágios iniciais de erosão dental, enquanto que a perfilometria quantifica a perda de estrutura em um estágio mais avançado [43]. Entretanto, em um processo de erosão contínua, pode haver perda de estrutura e amolecimento de uma camada subjacente [2]. Devido a isto o presente trabalho associou técnicas com diferentes objetivos e pôde detectar correlação entre as propriedades avaliadas, onde as substâncias bioativas mostraram-se efetivas no controle da erosão dental.

O biovidro 45S5 foi o primeiro a ser desenvolvido e atualmente encontra-se disponível em diferentes produtos comercializados, como cremes dentais e materiais para profilaxia [9,44,45]. A utilização e eficiência deste material contra a erosão do esmalte já foi demonstrada anteriormente [11,16,46], porém, neste estudo o biovidro 45S5 apresentou resultado inferior aos vidros ricos em fostato, o que motiva o desenvolvimento de novos materiais e estratégias clínicas.

A forma de aplicação dos materiais bioativos influencia sua ação contra a erosão dental. Recentemente, foi demonstrado que cremes-dentais têm um maior efeito protetor quando aplicados na ausência da abrasão pela escovação [47]. Desta forma, uma preferência deve ser dada a produtos que não abrasionem a superfície de esmalte no controle da erosão dental, como por exemplo, soluções para bochecho e vernizes.

5. Conclusão

Todos os materiais bioativos utilizados neste estudo proporcionaram um efeito protetor ao esmalte dental submetido à erosão. A taxa de dissolução dos vidros ricos em fosfato foi um fator determinante no controle da desmineralização do esmalte, sendo que a presença do Mg, nestes vidros bioativos, resultou em maior efeito protetor.

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ARTIGO 2 – VERSÃO EM INGLÊS

Title Page

Effect of bioactive glasses with different dissolution for controlling enamel erosion

Berthyelle Pádova Nyland[a], Andrea Freire[a], Cristiano Porcel Pereira[a], Paulo Soares[b], Denise Stolle da Luz Weiss[b], Sérgio Vieira[a]

[a] School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil

[b] Polytechnic School, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil

Berthyelle Pádova Nyland,
School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
draberthy@gmail.com

Andrea Freire,
School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
andreavfreire@yahoo.com.br

Cristiano Porcel Pereira,
School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
cristiano_porcel@hotmail.com

Paulo Soares,
Polytechnic School, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
pa.soares@pucpr.br

Denise Stolle da Luz Weiss,
Polytechnic School, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
denisesluz@bol.com.br

Sérgio Vieira,
School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brasil
s.vieira@pucpr.com.br

Autor Correspondente:
Andrea Freire
Programa de Pós-Graduação em Odontologia
Pontifícia Universidade Católica do Paraná
School of Life Sciences
R. Imaculada Conceição, 1155, Prado Velho
Curitiba – PR – BRASIL 80215-901
Tel: +55 41 3271-1637; Fax: +55 41 3271-1405
E-mail: andreavfreire@yahoo.com.br

Abstract

Objective: To evaluate the effect of calcium phosphate- and strontium-based, titanium- and magnesium oxide-containing bioactive glasses, with different dissolution rates for controlling dental erosion.

Methods: Fifty fragments of enamel were divided into 5 groups: negative control (CTRL), bioglass 45S5 (BAG), Phosphate based glass Ti-containing (PBG-Ti), Phosphate based glass Mg-containing (PBG-Mg), Phosphate based glass Ti and Mg-containing (PBG-TiMg). The specimens underwent 5 days of cycles consisting of erosive challenge 2 times/day with citric acid followed by application of the solutions with bioactive substances. After the cycles, the analyzes of profilometry, roughness, microhardness and scanning electron microscopy (SEM) were performed. The statistical tests used were One-way ANOVA (profilometry, roughness and variation of surface microhardness (%VMS)), Tukey's HSD (%VMS), Games Howell (profilometry), Student's t test (roughness) and Pearson correlation between the variables.

Results: The lower loss of enamel surface and lower VMS was observed in the PBG-Mg and PBG-TiMg groups and only the PBG-Mg group showed similarity in the roughness between baseline and eroded area. In the SEM micrographs, PBG-Ti and PBG-Mg showed lower apparent demineralization.

Significance: All bioactive materials provided a protective effect of enamel against erosion. The dissolution rate of phosphate based glasses is a determinant factor in controlling enamel demineralization, and the presence of Mg in these bioactive glasses resulted in greater protective effect.

Keywords: dental erosion, bioglass 45S5, bioactive glasses, profilometry, roughness, microhardness

Highlights:

- We tested experimental phosphate based glasses in dental erosion
- We examine the changes in enamel with these materials comparing with Bioglass 45S5
- Experimental phosphate based glasses containing Mg presented a consistent remineralizing potential

1. Introduction

Dental erosion is defined as irreversible loss of dental tissue due to a chemical process, without the involvement of microorganisms [1]. This process of demineralization is characterized by softening of the enamel surface, which varies according to the type of acid and the time of contact of this with the tooth structure [2,3]. The continuous dissolution of enamel leads to the permanent loss of this tissue and in more advanced stages can cause dentin exposure [2].

The etiology of dental erosion is related to intrinsic and extrinsic acids. Changing lifestyles and early exposure of children to industrialized acidic products, such as beverages and sweets, have increased the incidence of dental erosion by extrinsic acids [4]. The high prevalence of this process of demineralization in permanent teeth of children and adolescents was evidenced in recent studies [5,6] and the increasing prevalence of dental erosion indicates that it may become a public health problem [6]. This confirms the need for early diagnosis and treatment with the aim of reducing or inhibiting the progression of signs and symptoms [7]. Preventive strategies involving changes in diet, salivary flow stimulation and adequate oral hygiene measures have been used [8], as well as the development of substances that provide protection against erosion [9-13].

Thus, there was an increase in the number of searches with bioactive substances to stop dental erosion, such as ACP-CPP (amorphous calcium phosphate stabilized by casein phosphopeptide) [13,14], Bioglass 45S5 [11,15,16], and calcium and phosphate silicate [17-19]. These bioactive substances have the potential to provide calcium and phosphate to the demineralized surface [11] and may be used in the form of dentifrices [20,21] and pastes [11].

Bioactive titanium and magnesium-containing glasses have high bioactivity and ability to form crystals of hydroxyapatite [21,22]. Recently, phosphate based glass, strontium-containing, magnesium- or titanium-doped have been developed. The incorporation of titanium or magnesium is important to control the solubility of this material, since the presence of strontium increases the rate of dissolution of the glasses [23]. Titanium has a lower dissolution rate than magnesium [23] and can form a hydrophobic, acid-resistant layer on the tooth structure [24]. These different compositions of bioactive glasses have not yet

been tested for controlling enamel erosion. In this way, the results obtained will guide the development of new dental products.

Thus, the aim of this study was to evaluate the effect of phosphate-based glasses, titanium- and magnesium-containing for controlling erosion of dental enamel. The null hypothesis to be tested was that there would be no difference between phosphate based glasses, titanium- and magnesium oxide-containing and 45S5 bioglass for controlling enamel erosion.

2. Materials and methods

2.1 Preparation of Specimens

Fifty human extracted, third molar extracted teeth, obtained after approval of the Research Ethics Committee were used (protocol number 1,824,578). The teeth were cut with the use of a diamond disk in a metallographic cutter (Struers A/S, Ballerup, Denmark) under constant irrigation, obtaining enamel fragments with 4 x 3 x 2 mm of the buccal surface. The specimens were embedded in acrylic resin, then ground with # 800, # 1000, and #1200 grit silicon carbide sandpaper (3M, Sumaré, SP, Brazil) and polished with felt disc and cerium oxide in a polishing wheel (Struers A/S, Ballerup, Denmark). After polishing, the specimens were observed under an optical microscope with 50 × magnification (Olympus, São Paulo, SP, Brazil) for the presence of enamel to the fullest extent. Then the specimens were divided into 5 groups (n = 10) according to the substance to be applied after the erosive challenge (Table 1).

Before the erosion/remineralization cycles, all the specimens had the areas of reference delimited for the tests of perfilometry, roughness and hardness. The delimitation was performed with nail polish [16, 25], leaving only a 1 x 4 mm exposed window [26].

2.2 Synthesis of bioactive glasses

Phosphate based glasses, Mg- and/or Ti-containing were obtained from the methodology described by Weiss [23]. Milling was carried out manually with the aid of an agate mortar. After grinding, a 50-micron sieve was used to standardize the particle size. After the milling, the size of bioglass particles was confirmed by scanning electron microscopy (SEM).

2.3 Erosive Challenge

For the erosive challenge a 0.3% citric acid solution was used, with a pH of 3.2 adjusted with sodium hydroxide. Each specimen received the application of 1 mL of this solution under agitation (Shaker, Marconi MA420, Piracicaba, SP, Brazil) in a period of 2 minutes, with 1 mL being applied every minute. Two applications were performed per day for 5 days. After the erosive challenge the specimens were rinsed with 5 mL of deionized water under agitation for 1 minute.

2.4 Application of Solutions

Subsequently, a solution was applied according to Table 1. The bioactive glasses were used in solution in the proportion of 1 g of bioglass in 10 ml of deionized water, 1 ml applied per specimen, and kept under stirring (Shaker, Marconi MA420, Piracicaba, SP, Brazil) for 3 minutes.

2.5 Storage of Specimens

The specimens were stored in human saliva between the cycles of erosion/application of bioactive substances. The volunteers for saliva donation were selected from the following criteria - inclusion: young people between 16 and 30 years old, with good oral hygiene and with more than 20 teeth; exclusion: smokers, presence of active caries lesions, presence of periodontal disease, use of orthodontic appliance, exagerated wear due to bruxism, clenching or erosion, use of medications that affect salivary flow, abnormal salivary function, presence of diabetes or autoimmune diseases, use of antibiotics with less than 1 month of saliva harvest, medical disorders requiring quantification before dental appointment, gastric or alimentary disorders and pregnant women. After analysis of the inclusion and exclusion criteria, the patients underwent clinical examination and were instructed to stay for at least 1 hour without consuming food and beverages. In addition, toothbrushing should be performed at least 2 hours prior to harvest. Saliva was stimulated by chewing a piece of sterile latex with a standard size of 1 cm in length for 11 uninterrupted minutes, and the saliva produced in the first minute was discarded. The saliva pool was homogenized, separated into 100 mL containers for daily use and frozen. At the end of the cycles the samples were stored individually with 2 mL of saliva at 37 ° C. Saliva was changed daily after the last cycle.

The pH, buffering capacity and presence of salivary proteins and calcium were analyzed after saliva pooling. The pH analysis was performed in triplicate using a pH meter (PHB-500 Benchtop, ION, Curitiba, PR, Brazil) previously calibrated with standard solution (pH range 4.0 and 7.0). The buffer capacity of the saliva was measured by titration with hydrochloric acid, where a mixture of 1 mL of saliva was stimulated with 3 mL of 0.005 M hydrochloric acid and the presence of proteins and calcium in the saliva was evaluated from colorimetric

measurements with an optical spectrophotometer (BIO 200, Bioplus, Barueri, SP, Brazil).

2.6 3D non-contact optical profilometry

After completing the 5 days of cycles, the nail polish applied to protect the healthy area was removed with acetone and dental wear was measured by means of the non-contact 3D optical profilometry test (Talysurf CCI Lite Non-Contact 3D Profiler, Taylor Hobson, Leicester, England) with 5 × magnification and 0.13 mm aperture for all scanned areas. At the end of each reading the proprietary software (Mountains Digital Surf, Besançon, France) provided a graph with the profile tracing, measuring the depth of the lesion in micrometers and obtaining 3D images. Additionally, this equipment allowed to obtain measures of roughness (Sa) of the baseline and eroded surface. The tests were performed in triplicate and the mean values obtained for each specimen were used.

2.7 Knoop microhardness

The evaluation of the Knoop microhardness of the enamel surface was performed in a microdurometer (HMV-2T, Shimadzu Corp., Tokyo, Japan) with a static load of 10 g for 10 sec [27,28]. A total of 10 indentations were performed on each specimen, 5 of which were performed in the healthy area (initial microhardness - Mi) and 5 in the area submitted to the treatments (final microhardness - Mf). The mean indentation in each region was calculated for each specimen, and using these values, the percentage of surface microhardness (% VMS) was calculated using the following formula:

$$\%VMS = [(M_f - M_i)/ M_i]*100.$$

2.8 Scanning Electron Microscopy (SEM))

SEM micrographs (TESCAN VEGA3 LMU, TescanOrsay Holding, Brno-Kohoutovice, República Tcheca) were obtained to observe the enamel surface of the healthy areas and the areas submitted to the treatments with 1000× magnification at 20 Kv. For the analysis, representative samples from each group were sputter-coated with gold/palladium alloy (Balzers SCD 050, Balzers, Liechtenstein).

2.9 Statistical analysis

The data were submitted to statistical analysis and after verification of normality by the Kolmogorov-Smirnov test, and One-way ANOVA was applied for profilometry, roughness and surface microhardness variation. Games-Howell test of multiple comparisons was applied for profilometry, since no homogeneity was detected (Levene's test) and Tukey's HSD test for microhardness (α=.05). The roughness data were paired and analyzed by Student's t-test (α=.05). The correlation between the profile, roughness and microhardness data was evaluated by Pearson's correlation (α=.01).

3. Results

The analysis of tooth enamel loss revealed statistical differences between the different groups ($p < 0.05$) as shown in Table 2. All the solutions were effective for controlling dental erosion, since there was statistical difference for all the groups when compared to the negative control, being the smaller loss of enamel surface observed in the groups that used the bioactive glasses PBG-Mg and PBG-TiMg. The difference between the loss of structure of the different groups can also be observed in Fig 1.

The surface roughness of the specimens showed a difference between the protected and eroded area in all groups except PBG-Mg, as described in Table 3.

SEM micrographs corresponding to the protected and eroded areas, as well as the interface between them, can be visualized in Fig 2. The images of the protected areas show a smooth surface, confirming the standardization of the specimens demonstrated by the initial roughness. However, the eroded areas show the enamel prisms with signs of citric acid demineralization. However, different patterns were observed according to the solutions used, and the PBG-Ti and PBG-Mg groups showed lower apparent demineralization.

Regarding the percentage of variation of the surface hardness was no difference between treatments ($p < 0.05$), the lowest variation shown by PBG-Mg and PBG-TiMg groups and which were similar ($p = 0.5968$). Control group presented the highest variation and differed from all others ($p < 0.05$). PBG-Ti and BAG groups were similar to each other ($p = 0.1069$) and showed intermediate values of % VMS, differing from the other groups ($p < 0.05$). % VMS values are expressed in Table 4.

The null hypothesis was rejected, since there was a difference between the evaluated materials, regarding the control of dental erosion.

The profilometry and the microhardness results after the treatments obtained a very strong negative correlation ($R = -0.903$), that is, the larger the loss of structure the lower is microhardness after treatment. Strong correlation was also detected between perfilometry and roughness after treatment ($R = 0.643$).

Saliva pool analysis showed an average pH of 8.09, that is, alkaline, and the evaluation of the buffer capacity after titration with hydrochloric acid confirmed the ability of the saliva pool to neutralize acids. The amount of protein dosed was 1.158 g / dL and calcium was 7.27 mg / dL.

4. Discussion

Enamel erosion occurs initially as a partial demineralization of the surface resulting in the softening of the structure [19,27]. When acid challenge is persistent, the dissolution of hydroxyapatite crystals leads to a permanent loss of enamel [2]. In the present study, the use of bioactive substances was effective in the control of dental erosion, since it significantly reduced the loss of the enamel structure. All phosphate-rich bioactive glasses produced less loss of enamel when compared to the 45S5 bioglass (BAG).

The phosphate based glasses evaluated in this study present 40% of phosphorus pentoxide in its composition which, during dissolution, release phosphate into the medium. However the bioglass 45S5 (BAG) has 6% of this component, this justifies the minimal protective effect of this material. The phosphate can be adsorbed onto the film or directly onto enamel and competes with the H + ions at specific sites on the dental surface [29]. The variation of the phosphate content controls the reactivity and formation of hydroxyapatite by bioglass, and the high content of this allows a faster formation of HA at low pH [30].

The different results between the phosphate based glasses can be explained by the difference in the dissolution rates of the glasses. Strontium-containing phosphate-based glasses exhibit high solubility [23] which influences their effect on tooth structure under acid challenge. PBG-Ti presents a lower rate of dissolution of the bioactive glasses evaluated. The Ti acts as an intermediary component in the glass structure (modifier and shaper) thus, causing an increase of bonds with oxygen atoms, which makes the structure of the glass more chemically resistant, since the Ti-O bonding energy is greater than other modifiers, such as CaO, Na_2O, SrO_2, and MgO [23]. Thus, the presence of Mg facilitates the leaching of the glass components, making Ca and PO_4 readily available to act on the tooth structure. Thus, PBG-Mg and PBG-TiMg showed better results in the analysis of loss of structure as well as in % VMS.

In addition to controlling the rate of dissolution, Mg and Ti can provide beneficial effects in the control of dental erosion. Magnesium-containing solutions may react with the outer surface of the enamel leading to dissolution and reprecipitation, reducing the size of the crystals on the surface of enamel. This

crystallographic change in the enamel structure produces a higher surface hardness [31], which is in agreement with the results found in the present study, where the PBG-Mg and PBG-TiMg groups showed higher microhardness values after erosive cycles. Thus, Mg^{+2} ions incorporated into the enamel surface optimize the physical properties and chemical stability of this structure [31]. These ions also stabilize the amorphous phosphate layer of calcium formed prior to hydroxyapatite and thus regulate its crystallization [32].

After being dissolved from the bioglass, Ti ions are incorporated into the surface layer as titanium phosphate or Ti replaced on hydroxyapatite [22]. Ti, in this case, replaces Ca and not phosphorus [33], and this can be evidenced in the analysis of EDS where the PBG-Ti and PBG-TiMg glasses presented small amount of Ti and reduction of the amount of Ca in comparison with the other bioactive glasses.

When compared to the 45S5 bioglass, Ti-doped glass showed reduced formation of hydroxyapatite in a protein-free medium. However, under physiological conditions, with the addition of protein, there was an increase in the rate of formation of hydroxyapatite [22]. Thus, the presence of proteins in a saline solution contributes to the bioactivity of the glasses [22,34,35], as well as can simulate oral conditions [36]. In this way, the present study used human saliva that presents in its composition besides mineral ions also proteins that have an important role in the control of dental erosion [37]. The salivary parameters analyzed, pH, buffer capacity, protein and calcium dosage were within the values reported in the literature [38-40]. A pH higher than 8 favors the deposition of ions, however, it may be a limitation of this study, since patients with dental erosion may present acid pH [41,42]. Therefore, future studies with these materials are necessary in the presence of saliva with variation of pH values.

In enamel, loss of gloss is a diagnostic criterion for erosion at an early stage and may be related to increased roughness [2]. Among the groups evaluated, only PBG-Mg did not present any change of roughness between the healthy and eroded areas, thus demonstrating a greater protective effect, which can also be observed in SEM micrographs.

The evaluation of demineralization through microhardness and roughness detects early stages of dental erosion, while the profilometry quantifies the loss of structure in a more advanced stage [43]. However, in a continuous erosion

process, there may be loss of structure and softening of an underlying layer [2]. Due to this the present work associated techniques with different objectives and could detect correlation between the evaluated properties, where the bioactive substances proved to be effective in the control by dental erosion.

The 45S5 bioglass was the first to be developed and is currently available in different commercialized products, such as dentifrices and materials for prophylaxis [9,44,45]. The use and efficiency of this material against enamel erosion has already been previously demonstrated [11,16,46], however, in this study the 45S5 bioglass presented poorer results to the phosphate based glasses, which motivates the development of new materials and clinical strategies.

The application of bioactive materials influences its action against dental erosion. Recently, it has been demonstrated that dentifrices have a greater protective effect when applied in the absence of abrasion from tooth brushing [47]. Therefore, preference should be given to products that do not abrade the enamel surface in the control of dental erosion, such as, for example, mouthwash and varnishes.

5. Conclusion

All bioactive materials used in this study provided a protective effect to enamel subjected to erosion. The dissolution rate of phosphate based glasses was a determinant factor in the control of enamel demineralization, and the presence of Mg in these bioactive glasses resulted in a higher protective effect

6. References

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Tables and Figures

Table 1: Description of the groups according to the solution to be used and their composition

GROUPS	SOLUTION	COMPOSITION
CTRL	Deionized water	----------
BAG	Bioglass 45S5	SiO_2, Na_2O e CaO e P_2O_5
PBG-Ti	Phosphate based glass Ti-containing	P_2O_5, CaO, Na_2O, SrO, TiO_2
PBG-Mg	Phosphate based glass Mg-containing	P_2O_5, CaO, Na_2O, SrO, MgO
PBG-TiMg	Phosphate based glass Ti and Mg-containing	P_2O_5, CaO, Na_2O, SrO, TiO_2 e MgO

Table 2: Loss of enamel surface (µm) and standard deviation after cycles of erosion / bioactive glasses

Groups	Enamel Loss
CTRL	0,90 (0,02)[a]
BAG	0,53 (0,08)[b]
PBG-Ti	0,32 (0,10)[c]
PBG-Mg	0,17 (0,03)[d]
PBG-TiMg	0,23 (0,05)[c,d]

Different lower-case letters indicate statistical difference between groups ($p < 0.05$)

Table 3: Mean values of roughness (Sa) in µm, and standard deviation obtained from protected areas and areas subjected to erosion cycles/bioactive glasses

Groups	Roughness (Sa) healthy area	Roughness (Sa) area submitted to cycles	P value
CTRL	0,054 (0,02)	0, 124 (0,03)	0,00005
BAG	0,047 (0,02)	0,061 (0,02)	0,00440
PBG-Ti	0,048 (0,01)	0,085 (0,02)	0,00010
PBG-Mg	0,051 (0,02)	0,054 (0,02)	0,19342
PBG-TiMg	0,052 (0,02)	0,067 (0,02)	0,00300

Table 4: Results of % Percentage variation of enamel surface microhardness (% VMS)

Grupo	% VMS
CTRL	-60,54 (3,85)[c]
BAG	-41,31 (2,95)[b]
PBG-Ti	-36,74 (5,73)[b]
PBG-Mg	-22,02 (3,71)[a]
PBG-TiMg	-19,37 (3,58)[a]

Different letters indicate significant statistical differences.

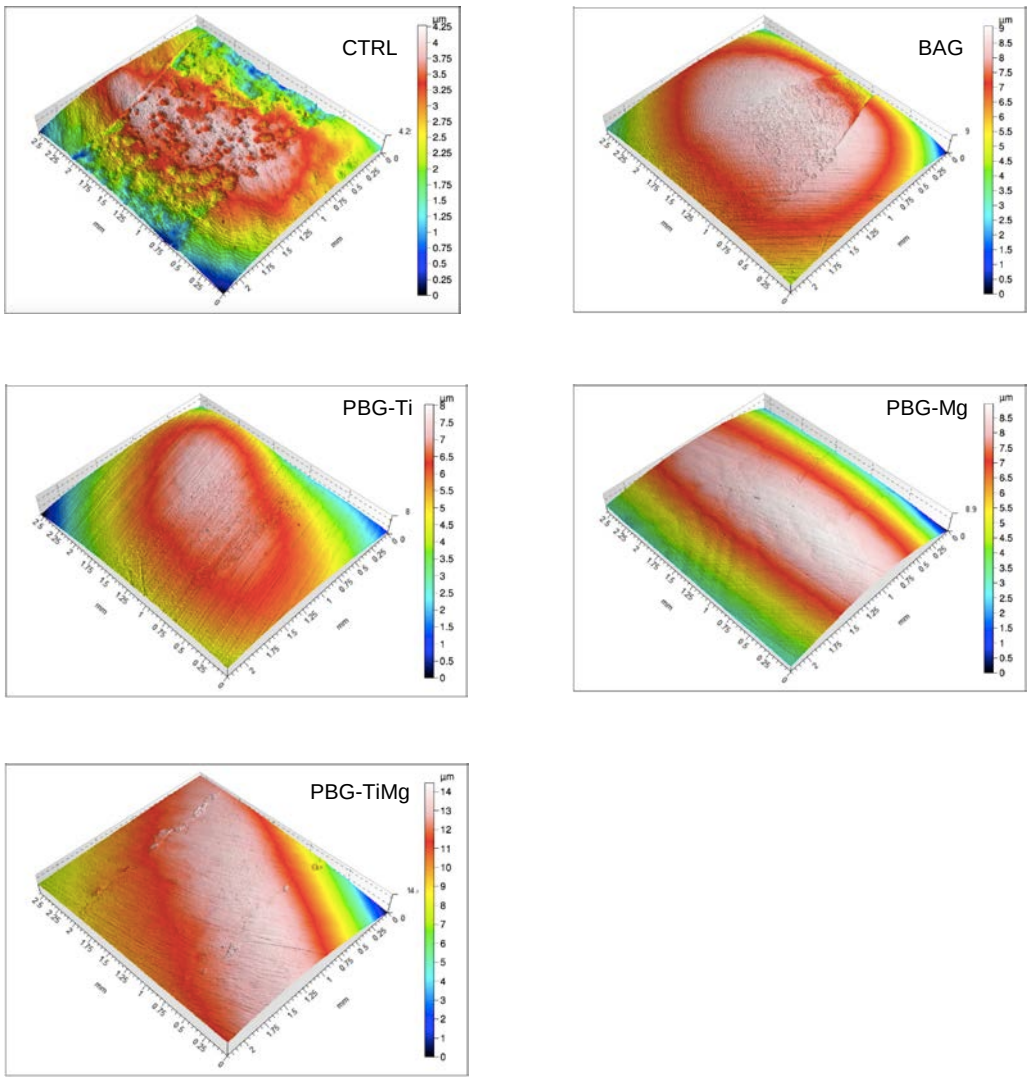


Figure 1. 3D representation of the enamel surface obtained through non-contact optical profilometry after the cycles. CTRL- negative control, BAG- Bioglass 45S5, PBG-Ti – Phosphate based glass Ti-containing, PBG-Mg – Phosphate based glass Mg-containing, PBG-TiMg – Phosphate based glass, Ti- and Mg-containing

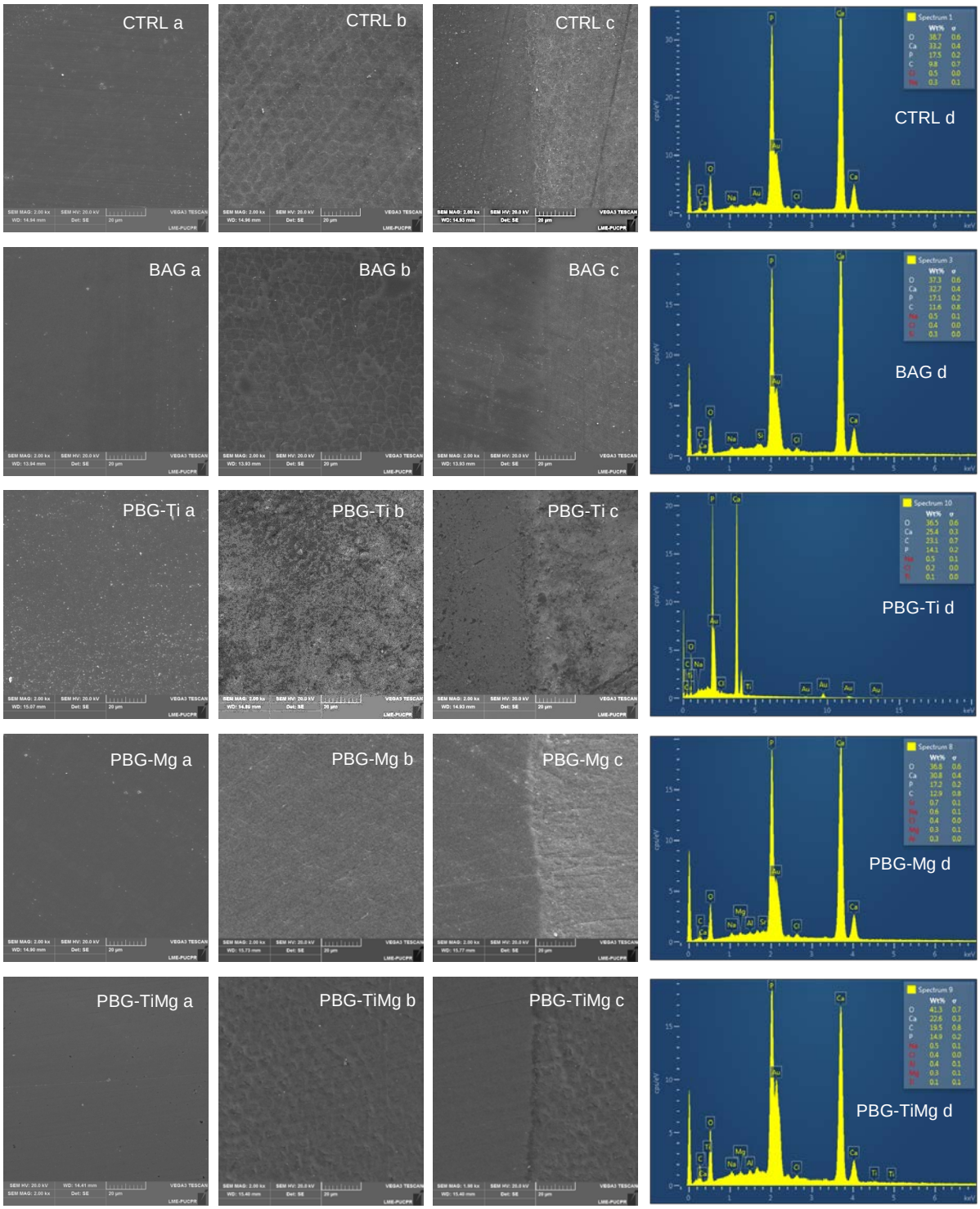


Figure 2. SEM micrographs of enamel surfaces with 2000 × magnification and EDS analysis. CTRL - Negative Control, BAG - Bioglass 45S5, PBG-Ti – Phosphate based glass Ti-containing, PBG-Mg – Phosphate based glass Mg-containing, PBG-TiMg – Phosphate based glass Ti- and Mg-containing. (a) healthy area (b) area submitted to cycles (c) interface between healthy area and area submitted to cycles (d) EDS analysis for characterization of enamel in the region submitted to cycles.

1 Anexos

2 *Parecer de comitê de ética*

Comitê de Ética em Pesquisa da PUCPR

ASSOCIAÇÃO PARANAENSE DE CULTURA - PUCPR

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito da taxa de dissolução de biovidros experimentais no controle da erosão dental

Pesquisador: Andrea Freire de Vasconcelos Eckelberg
Área Temática:
Versão: 1
CAAE: 61827416.5.0000.0020
Instituição Proponente: Pontifícia Universidade Católica do Parana - PUCPR
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.824.578

Apresentação do Projeto:
O objetivo deste estudo in vitro será avaliar o efeito de biovidros experimentais a base de fosfato de cálcio e estrôncio contendo óxidos de titânio e magnésio com diferentes taxas de dissolução no controle da desmineralização do esmalte dental submetido ao desafio ácido. Para este estudo serão utilizados 90 terceiros molares humanos que serão cortados a fim de obter-se fragmentos de esmalte da face vestibular com 4X3x2 mm. Após a inclusão em resina acrílica e padronização com lixas de carbeto de silício os espécimes serão divididos em 6 grupos, de acordo com a substância a ser utilizada: G1- controle, G2- biovidro 45S5, G3- biovidro experimental PCNSrTI, G4- biovidro experimental PCNSrMg, G5- Biovidro experimental PCNSrTiMg e G6 – hidroxiapatita. Os espécimes passarão por desafio erosivo 2 vezes ao dia com ácido cítrico 0,3% durante 5 dias. Após o desafio erosivo as substâncias remineralizantes serão aplicadas por 3 min. Ao final dos 5 dias de ciclos erosão/remineralização serão realizados os testes de perfilometria, microscopia eletrônica de varredura, microdureza knoop e FTIR. A normalidade dos resultados será avaliada pelo teste Shapiro- Wilk e a homogeneidade pelo teste Levene, em seguida será realizada análise de variância e comparações múltiplas pelo teste Tukey HSD e a correlação entre os dados de dureza e perfilometria também será avaliada. Todos os testes utilizarão nível de significância de 0,05.

Endereço: Rua Imaculada Conceição 1155
Bairro: Prado Velho **CEP:** 80.215-901
UF: PR **Município:** CURITIBA
Telefone: (41)3271-2103 **Fax:** (41)3271-2103 **E-mail:** nep@pucpr.br

Objetivo da Pesquisa:

Avaliar o efeito de biovidros experimentais a base de fosfato de cálcio e estrôncio contendo óxidos de titânio e magnésio com diferentes taxas de dissolução no controle da desmineralização do esmalte dental submetido ao desafio ácido.

Avaliação dos Riscos e Benefícios:

Os riscos e benefícios apresentados estão adequados e de acordo com a Resolução 466/2012.

Comentários e Considerações sobre a Pesquisa:

A metodologia e objetivos apresentados estão adequados e em acordo com a Resolução 466/2012.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos necessários para a realização do projeto foram apresentados e estão em conformidade com a Res. 466/12.

Recomendações:

Ver Conclusões ou Pendências e Lista de Inadequações.

Conclusões ou Pendências e Lista de Inadequações:

Projeto aprovado.

Considerações Finais a critério do CEP:

Lembramos aos senhores pesquisadores que, no cumprimento da Resolução 466/2012, o Comitê de Ética em Pesquisa (CEP) deverá receber relatórios anuais sobre o andamento do estudo, bem como a qualquer tempo e a critério do pesquisador nos casos de relevância, além do envio dos relatos de eventos adversos, para conhecimento deste Comitê. Salientamos ainda, a necessidade de relatório completo ao final do estudo. Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEPPUCPR de forma clara e sucinta, identificando a parte do protocolo a ser modificado e as suas justificativas. Se a pesquisa, ou parte dela for realizada em outras instituições, cabe ao pesquisador não iniciá-la antes de receber a autorização formal para a sua realização. O documento que autoriza o início da pesquisa deve ser carimbado e assinado pelo responsável da instituição e deve ser mantido em poder do pesquisador responsável, podendo ser requerido por este CEP em qualquer tempo.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Endereço: Rua Imaculada Conceição 1155
Bairro: Prado Velho **CEP:** 80.215-901
UF: PR **Município:** CURITIBA
Telefone: (41)3271-2103 **Fax:** (41)3271-2103 **E-mail:** nep@pucpr.br

Continuação do Parecer: 1.824.578

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_798941.pdf	01/11/2016 16:17:03		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	justificativa.pdf	01/11/2016 16:16:29	Andrea Freire de Vasconcelos Eckelberg	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_biovidros_Orientador.pdf	25/10/2016 14:44:29	Andrea Freire de Vasconcelos Eckelberg	Aceito
Declaração de Pesquisadores	termo_de_responsabilidade_do_pesquisador.pdf	25/10/2016 14:37:17	Andrea Freire de Vasconcelos Eckelberg	Aceito
Outros	banco_de_dentes.pdf	25/10/2016 14:34:16	Andrea Freire de Vasconcelos Eckelberg	Aceito
Folha de Rosto	folha_de_rosto.pdf	25/10/2016 14:21:25	Andrea Freire de Vasconcelos Eckelberg	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CURITIBA, 18 de Novembro de 2016

Assinado por:
NAIM AKEL FILHO
(Coordenador)

1
2

1 ***Análise estatística***

Testes de Normalidade

variável	Tratamento	Kolmogorov-Smirnov[a]		
		Estatística	gl	Valor p
Perfilometria	Controle Negativo	0,2574	10	0,0611
	SYLK	0,1156	10	,200*
	PBG - Ti	0,2162	10	,200*
	PBG - Mg	0,1908	10	,200*
	PBG - TiMg	0,2300	10	0,1429
Rugosidade Área Sadia	Controle Negativo	0,2126	10	,200*
	SYLK	0,2540	10	0,0667
	PBG - Ti	0,1919	10	,200*
	PBG - Mg	0,2197	10	0,1878
	PBG - TiMg	0,2380	10	0,1143
Rugosidade após Tratamento	Controle Negativo	0,1461	10	,200*
	SYLK	0,2179	10	0,1961
	PBG - Ti	0,2550	10	0,0644
	PBG - Mg	0,1768	10	,200*
	PBG - TiMg	0,2332	10	0,1316
Percentagem de Variação Rugosidade após Tratamento	Controle Negativo	0,2111	10	,200*
	SYLK	0,2536	10	0,0677
	PBG - Ti	0,1489	10	,200*
	PBG - Mg	0,4770	10	0,0000
	PBG - TiMg	0,2254	10	0,1619

*. Este é um limite inferior da significância verdadeira.

a. Correlação de Significância de Lilliefors

Descritivas

variável	Tratamento	N	Média	Mediana	Desvio Padrão	Erro Padrão	Intervalo de confiança de 95% para média		Mínimo	Máximo
							Limite inferior	Limite superior		
Perfilometria	Controle Negativo	10	0,90	0,90	0,02	0,01	0,88	0,91	0,87	0,93
	SYLK	10	0,53	0,52	0,08	0,03	0,47	0,59	0,40	0,66
	PBG - Ti	10	0,32	0,31	0,10	0,03	0,24	0,39	0,15	0,55
	PBG - Mg	10	0,17	0,18	0,03	0,01	0,15	0,19	0,13	0,22
	PBG - TiMg	10	0,23	0,24	0,05	0,02	0,19	0,26	0,13	0,30
Rugosidade Área Sadia	Controle Negativo	10	0,05	0,05	0,02	0,00	0,04	0,07	0,04	0,08
	SYLK	10	0,05	0,04	0,02	0,01	0,03	0,06	0,03	0,08
	PBG - Ti	10	0,05	0,05	0,01	0,00	0,04	0,06	0,03	0,07
	PBG - Mg	10	0,05	0,05	0,02	0,01	0,04	0,07	0,03	0,09
	PBG - TiMg	10	0,05	0,05	0,02	0,01	0,04	0,07	0,03	0,10
Rugosidade	Controle	10	0,12	0,13	0,03	0,01	0,10	0,15	0,08	0,17

após Tratamento	Negativo									
	SYLK	10	0,06	0,06	0,02	0,01	0,05	0,08	0,03	0,10
	PBG - Ti	10	0,09	0,09	0,02	0,01	0,07	0,10	0,05	0,11
	PBG - Mg	10	0,05	0,05	0,02	0,01	0,04	0,07	0,03	0,09
	PBG - TiMg	10	0,07	0,06	0,02	0,01	0,05	0,08	0,04	0,11
Percentagem de Variação Rugosidade após Tratamento	Controle Negativo	10	142,17	136,67	77,76	24,59	86,54	197,79	37,50	300,00
	SYLK	10	33,02	25,00	33,05	10,45	9,38	56,67	0,00	100,00
	PBG - Ti	10	86,12	75,00	50,87	16,09	49,73	122,51	0,00	175,00
	PBG - Mg	10	6,50	0,00	14,15	4,48	-3,62	16,62	0,00	40,00
	PBG - TiMg	10	34,93	22,50	31,06	9,82	12,71	57,15	0,00	100,00

ANOVA

		Soma dos Quadrados	gl	Quadrado Médio	F	Valor p	Poder observado[b]
Perfilometria	Entre Grupos	3,469	4	,867	201,242	0,0000000	1,00000000
	Nos grupos	,194	45	,004			
	Total	3,663	49				
Rugosidade Área Sadia	Entre Grupos	,000	4	,000	,255	0,9052380	0,10061100
	Nos grupos	,015	45	,000			
	Total	,015	49				
Rugosidade após Tratamento	Entre Grupos	,032	4	,008	14,629	0,0000001	0,99999600
	Nos grupos	,024	45	,001			
	Total	,056	49				
Percentagem de Variação Rugosidade após Tratamento	Entre Grupos	116506,077	4	29126,519	13,372	0,0000003	0,99998400
	Nos grupos	98015,635	45	2178,125			
	Total	214521,712	49				

b. Calculado usando alfa = ,05

Teste de Homogeneidade de Variâncias

	Estatística de Levene	gl1	gl2	Valor p
Perfilometria	3,095	4	45	0,02469
Rugosidade Área Sadia	,157	4	45	0,95898
Rugosidade após Tratamento	,324	4	45	0,86045
Percentagem de Variação Rugosidade após Tratamento	3,672	4	45	0,01136

Comparações múltiplas

Variável dependente: Perfilometria

Games-Howell

(I) Tratamento		Diferença média (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95%	
					Limite inferior	Limite superior
Controle Negativo	SYLK	,37000*	,02667	0,0000	,2819	,4581
	PBG - Ti	,57900*	,03336	0,0000	,4681	,6899
	PBG - Mg	,72250*	,01051	0,0000	,6901	,7549
	PBG - TiMg	,67100*	,01772	0,0000	,6137	,7283
SYLK	Controle Negativo	-,37000*	,02667	0,0000	-,4581	-,2819
	PBG - Ti	,20900*	,04197	0,0009	,0814	,3366
	PBG - Mg	,35250*	,02755	0,0000	,2635	,4415
	PBG - TiMg	,30100*	,03103	0,0000	,2055	,3965
PBG - Ti	Controle Negativo	-,57900*	,03336	0,0000	-,6899	-,4681
	SYLK	-,20900*	,04197	0,0009	-,3366	-,0814
	PBG - Mg	,14350*	,03407	0,0114	,0320	,2550
	PBG - TiMg	,09200	,03694	0,1509	-,0238	,2078
PBG - Mg	Controle Negativo	-,72250*	,01051	0,0000	-,7549	-,6901
	SYLK	-,35250*	,02755	0,0000	-,4415	-,2635
	PBG - Ti	-,14350*	,03407	0,0114	-,2550	-,0320
	PBG - TiMg	-,05150	,01902	0,1043	-,1110	,0080
PBG - TiMg	Controle Negativo	-,67100*	,01772	0,0000	-,7283	-,6137
	SYLK	-,30100*	,03103	0,0000	-,3965	-,2055
	PBG - Ti	-,09200	,03694	0,1509	-,2078	,0238
	PBG - Mg	,05150	,01902	0,1043	-,0080	,1110

*. A diferença média é significativa no nível 0.05.

1

Comparações múltiplas

Variável dependente: Rugosidade Área Sadia

Tukey HSD

(I) Tratamento		Diferença média (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95%	
					Limite inferior	Limite superior
Controle Negativo	SYLK	,00700	,00807	0,9073	-,0159	,0299
	PBG - Ti	,00600	,00807	0,9450	-,0169	,0289
	PBG - Mg	,00300	,00807	0,9958	-,0199	,0259
	PBG - TiMg	,00200	,00807	0,9991	-,0209	,0249
SYLK	Controle Negativo	-,00700	,00807	0,9073	-,0299	,0159
	PBG - Ti	-,00100	,00807	0,9999	-,0239	,0219
	PBG - Mg	-,00400	,00807	0,9874	-,0269	,0189

	PBG - TiMg	-,00500	,00807	0,9712	-,0279	,0179
PBG - Ti	Controle Negativo	-,00600	,00807	0,9450	-,0289	,0169
	SYLK	,00100	,00807	0,9999	-,0219	,0239
	PBG - Mg	-,00300	,00807	0,9958	-,0259	,0199
	PBG - TiMg	-,00400	,00807	0,9874	-,0269	,0189
PBG - Mg	Controle Negativo	-,00300	,00807	0,9958	-,0259	,0199
	SYLK	,00400	,00807	0,9874	-,0189	,0269
	PBG - Ti	,00300	,00807	0,9958	-,0199	,0259
	PBG - TiMg	-,00100	,00807	0,9999	-,0239	,0219
PBG - TiMg	Controle Negativo	-,00200	,00807	0,9991	-,0249	,0209
	SYLK	,00500	,00807	0,9712	-,0179	,0279
	PBG - Ti	,00400	,00807	0,9874	-,0189	,0269
	PBG - Mg	,00100	,00807	0,9999	-,0219	,0239

Comparações múltiplas

Variável dependente: Rugosidade após Tratamento

Tukey HSD

(I) Tratamento		Diferença média (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95%	
					Limite inferior	Limite superior
Controle Negativo	SYLK	,06300*	,01038	0,0000	,0335	,0925
	PBG - Ti	,03900*	,01038	0,0043	,0095	,0685
	PBG - Mg	,07000*	,01038	0,0000	,0405	,0995
	PBG - TiMg	,05700*	,01038	0,0000	,0275	,0865
SYLK	Controle Negativo	-,06300*	,01038	0,0000	-,0925	-,0335
	PBG - Ti	-,02400	,01038	0,1598	-,0535	,0055
	PBG - Mg	,00700	,01038	0,9609	-,0225	,0365
	PBG - TiMg	-,00600	,01038	0,9776	-,0355	,0235
PBG - Ti	Controle Negativo	-,03900*	,01038	0,0043	-,0685	-,0095
	SYLK	,02400	,01038	0,1598	-,0055	,0535
	PBG - Mg	,03100*	,01038	0,0350	,0015	,0605
	PBG - TiMg	,01800	,01038	0,4238	-,0115	,0475
PBG - Mg	Controle Negativo	-,07000*	,01038	0,0000	-,0995	-,0405
	SYLK	-,00700	,01038	0,9609	-,0365	,0225
	PBG - Ti	-,03100*	,01038	0,0350	-,0605	-,0015
	PBG - TiMg	-,01300	,01038	0,7208	-,0425	,0165
PBG - TiMg	Controle Negativo	-,05700*	,01038	0,0000	-,0865	-,0275
	SYLK	,00600	,01038	0,9776	-,0235	,0355
	PBG - Ti	-,01800	,01038	0,4238	-,0475	,0115

	PBG - Mg	,01300	,01038	0,7208	-,0165	,0425

*. A diferença média é significativa no nível 0.05.

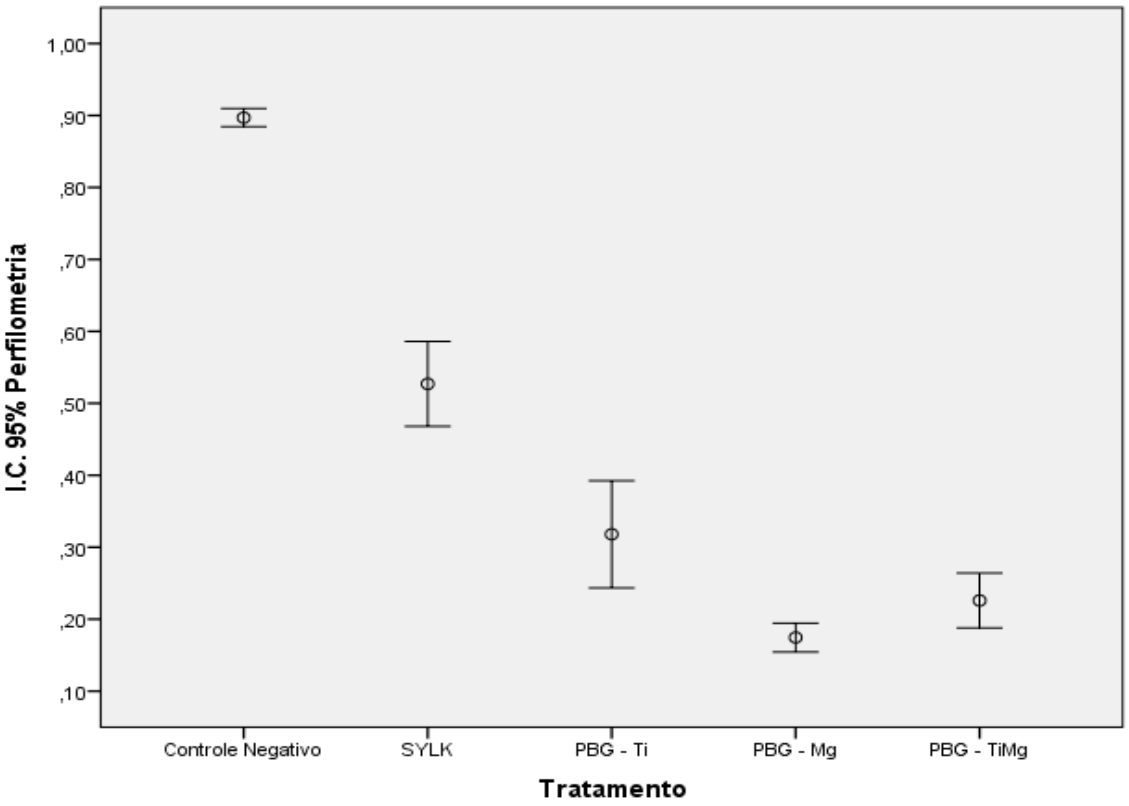
Comparações múltiplas

Variável dependente: Percentagem de Variação Rugosidade após Tratamento

Games-Howell

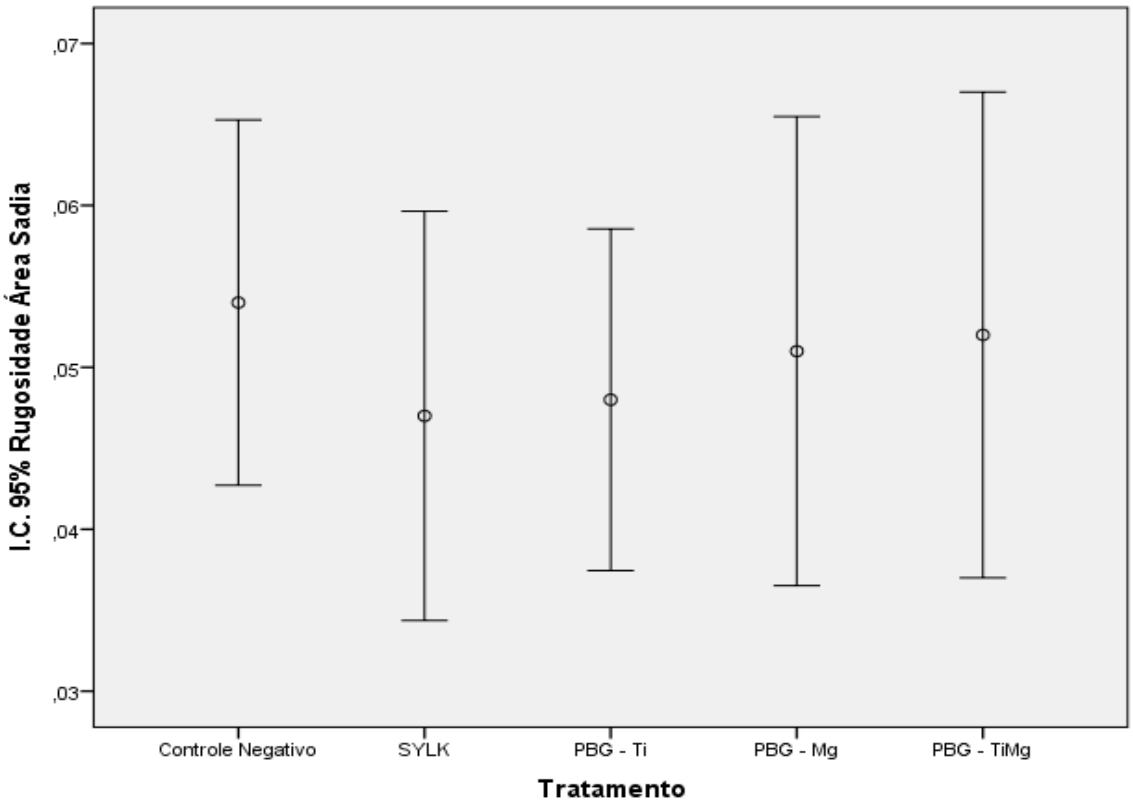
(I) Tratamento		Diferença média (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95%	
					Limite inferior	Limite superior
Controle Negativo	SYLK	109,14286*	26,71721	0,0105	24,1498	194,1359
	PBG - Ti	56,04762	29,38225	0,3537	-34,3063	146,4015
	PBG - Mg	135,66667*	24,99228	0,0024	52,7430	218,5903
	PBG - TiMg	107,23810*	26,47795	0,0117	22,6150	191,8612
SYLK	Controle Negativo	-109,14286*	26,71721	0,0105	-194,1359	-24,1498
	PBG - Ti	-53,09524	19,18210	0,0884	-112,1110	5,9206
	PBG - Mg	26,52381	11,36884	0,1992	-9,6223	62,6699
	PBG - TiMg	-1,90476	14,34288	0,9999	-45,2921	41,4825
PBG - Ti	Controle Negativo	-56,04762	29,38225	0,3537	-146,4015	34,3063
	SYLK	53,09524	19,18210	0,0884	-5,9206	112,1110
	PBG - Mg	79,61905*	16,69605	0,0048	25,0621	134,1760
	PBG - TiMg	51,19048	18,84743	0,0987	-7,0616	109,4425
PBG - Mg	Controle Negativo	-135,66667*	24,99228	0,0024	-218,5903	-52,7430
	SYLK	-26,52381	11,36884	0,1992	-62,6699	9,6223
	PBG - Ti	-79,61905*	16,69605	0,0048	-134,1760	-25,0621
	PBG - TiMg	-28,42857	10,79459	0,1226	-62,5834	5,7263
PBG - TiMg	Controle Negativo	-107,23810*	26,47795	0,0117	-191,8612	-22,6150
	SYLK	1,90476	14,34288	0,9999	-41,4825	45,2921
	PBG - Ti	-51,19048	18,84743	0,0987	-109,4425	7,0616
	PBG - Mg	28,42857	10,79459	0,1226	-5,7263	62,5834

*. A diferença média é significativa no nível 0.05.


1,00
,90
,80
,70
,60
,50
,40
,30
,20
,10
I.C. 95% Perfilometria
Controle Negativo
SYLK
PBG - Ti
PBG - Mg
PBG - TiMg
Tratamento

1

2


I.C. 95% Rugosidade Área Sadia
,07
,06
,05
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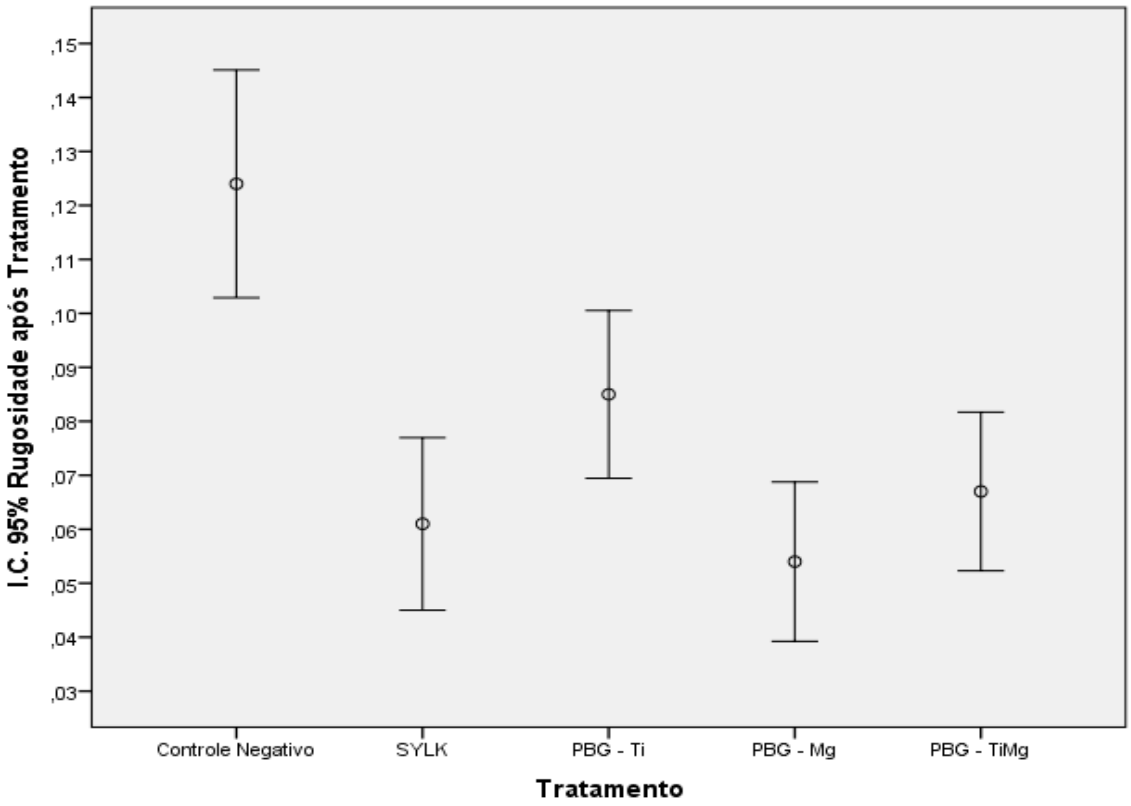
,03
Controle Negativo
SYLK
PBG - Ti
PBG - Mg
PBG - TiMg
Tratamento

1



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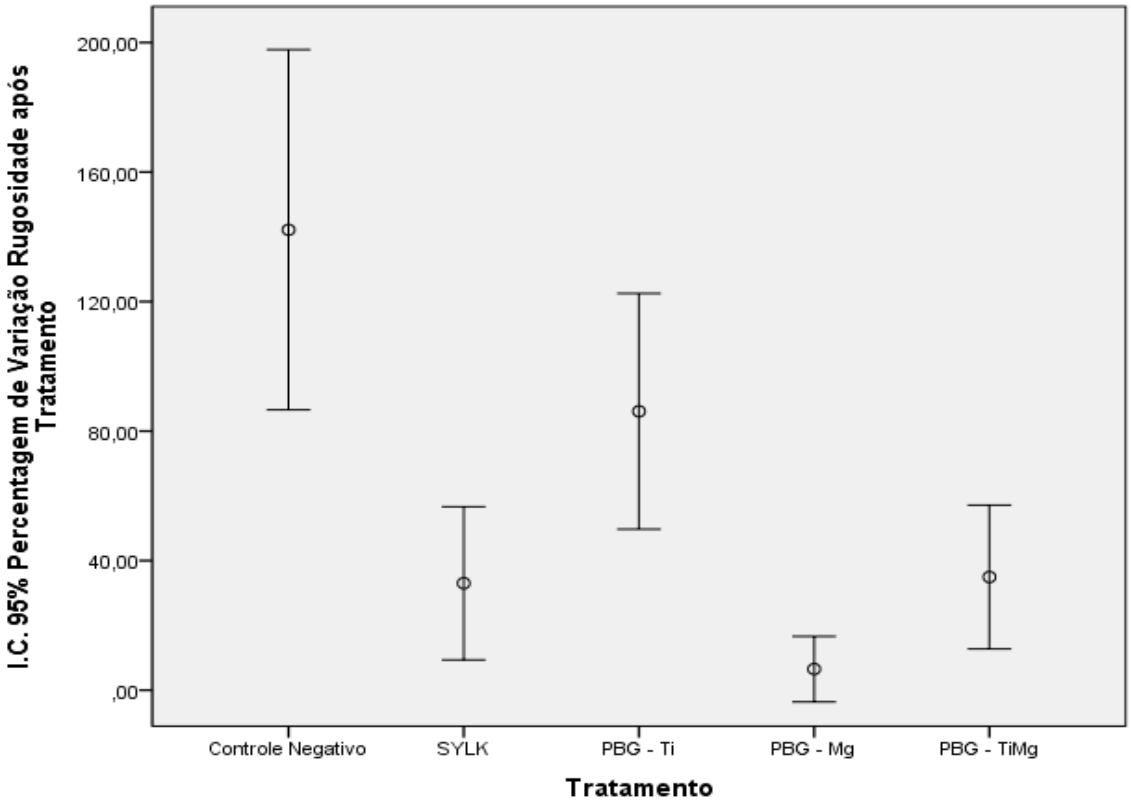


2

COMPARAÇÃO ÁREA SADIA X APÓS TRATAMENTO

Tratamento = Controle Negativo

Teste-T

Estatísticas de amostras emparelhadas

		Média	N	Desvio Padrão	Erro Padrão da Média
Par 1	Rugosidade Área Sadia	,0540	10	,01578	,00499
	Rugosidade após Tratamento	,1240	10	,02951	,00933

Teste de amostras emparelhadas

		Diferenças emparelhadas					t	gl	Valor p
		Média	Desvio Padrão	Erro Padrão da Média	95% Intervalo de Confiança da Diferença				
					Inferior	Superior			
Par 1	Rugosidade Área Sadia - Rugosidade após Tratamento	-,07000	,03055	,00966	-,09185	-,04815	-7,246	9	0,00005

Tratamento = SYLK

Teste-T

Estatísticas de amostras emparelhadas

		Média	N	Desvio Padrão	Erro Padrão da Média
Par 1	Rugosidade Área Sadia	,0470	10	,01767	,00559
	Rugosidade após Tratamento	,0610	10	,02234	,00706

Teste de amostras emparelhadas

		Diferenças emparelhadas					t	gl	Valor p
		Média	Desvio Padrão	Erro Padrão da Média	95% Intervalo de Confiança da Diferença				
					Inferior	Superior			
Par 1	Rugosidade Área Sadia - Rugosidade após Tratamento	-,01400	,01174	,00371	-,02240	-,00560	-3,772	9	0,00440

Tratamento = PBG - Ti

Teste-T

Estatísticas de amostras emparelhadas

		Média	N	Desvio Padrão	Erro Padrão da Média
Par 1	Rugosidade Área Sadia	,0480	10	,01476	,00467
	Rugosidade após Tratamento	,0850	10	,02173	,00687

Teste de amostras emparelhadas

		Diferenças emparelhadas					t	gl	Valor p
		Média	Desvio Padrão	Erro Padrão da Média	95% Intervalo de Confiança da Diferença Inferior	Superior			
Par 1	Rugosidade Área Sadia - Rugosidade após Tratamento	-,03700	,01767	,00559	-,04964	-,02436	-6,622	9	0,00010

Tratamento = PBG - Mg

Teste-T

Estatísticas de amostras emparelhadas

		Média	N	Desvio Padrão	Erro Padrão da Média
Par 1	Rugosidade Área Sadia	,0510	10	,02025	,00640
	Rugosidade após Tratamento	,0540	10	,02066	,00653

Teste de amostras emparelhadas

		Diferenças emparelhadas					t	gl	Valor p
		Média	Desvio Padrão	Erro Padrão da Média	95% Intervalo de Confiança da Diferença Inferior	Superior			
Par 1	Rugosidade Área Sadia - Rugosidade após Tratamento	-,00300	,00675	,00213	-,00783	,00183	-1,406	9	0,19342

Tratamento = PBG - TiMg

Teste-T

Estatísticas de amostras emparelhadas

		Média	N	Desvio Padrão	Erro Padrão da Média

Par 1	Rugosidade Área Sadia	,0520	10	,02098	,00663
	Rugosidade após Tratamento	,0670	10	,02058	,00651

Teste de amostras emparelhadas

		Diferenças emparelhadas					t	gl	Valor p
		Média	Desvio Padrão	Erro Padrão da Média	95% Intervalo de Confiança da Diferença				
					Inferior	Superior			
Par 1	Rugosidade Área Sadia - Rugosidade após Tratamento	-,01500	,01179	,00373	-,02343	-,00657	-4,025	9	0,00300

1
2

ANÁLISE DUREZA

Testes de Normalidade

Tratamento		Kolmogorov-Smirnov[a]		
		Estatística	gl	Valor p
Dureza Área sadia	Controle Negativo	,194	10	,200*
	SYLK	,178	10	,200*
	PBG - Ti	,179	10	,200*
	PBG - Mg	,187	10	,200*
	PBG - TiMg	,177	10	,200*
Dureza após Tratamento	Controle Negativo	,149	10	,200*
	SYLK	,175	10	,200*
	PBG - Ti	,171	10	,200*
	PBG - Mg	,185	10	,200*
	PBG - TiMg	,258	10	0,0585
Percentagem de Variação Dureza após Tratamento	Controle Negativo	,129	10	,200*
	SYLK	,160	10	,200*
	PBG - Ti	,185	10	,200*
	PBG - Mg	,209	10	,200*
	PBG - TiMg	,193	10	,200*

*. Este é um limite inferior da significância verdadeira.

a. Correlação de Significância de Lilliefors

Descritivas

		N	Média	Desvio Padrão	Erro Padrão	média		Mínimo	Máximo
						Limite inferior	Limite superior		
Dureza Área Sadia	Controle Negativo	10	412,96	28,10	8,89	392,86	433,06	361,60	461,60
	SYLK	10	421,71	25,78	8,15	403,27	440,15	350,80	440,40
	PBG - Ti	10	412,86	27,44	8,68	393,23	432,49	369,00	464,20
	PBG - Mg	10	404,92	21,83	6,90	389,31	420,54	374,80	454,60
	PBG - TiMg	10	403,98	27,55	8,71	384,28	423,68	362,20	447,60
	Total	50	411,29	25,97	3,67	403,90	418,67	350,80	464,20
Dureza após Tratamento	Controle Negativo	10	162,46	14,07	4,45	152,40	172,52	143,40	185,80
	SYLK	10	251,64	12,68	4,01	242,57	260,71	232,80	277,20
	PBG - Ti	10	259,98	12,66	4,00	250,92	269,04	242,40	279,80
	PBG - Mg	10	315,06	4,01	1,27	312,19	317,93	308,00	319,60
	PBG - TiMg	10	325,30	19,97	6,31	311,02	339,58	301,20	357,60
	Total	50	262,89	60,07	8,49	245,82	279,96	143,40	357,60
Percentagem de Variação Dureza após Tratamento	Controle Negativo	10	-60,54	3,85	1,22	-63,30	-57,79	-65,63	-54,69
	SYLK	10	-41,31	2,95	0,93	-43,42	-39,20	-44,80	-35,19
	PBG - Ti	10	-36,74	5,73	1,81	-40,84	-32,64	-44,56	-25,47
	PBG - Mg	10	-22,02	3,71	1,17	-24,67	-19,37	-29,70	-17,13
	PBG - TiMg	10	-19,37	3,58	1,13	-21,93	-16,81	-23,33	-13,36
	Total	50	-36,00	15,50	2,19	-40,40	-31,59	-65,63	-13,36

1

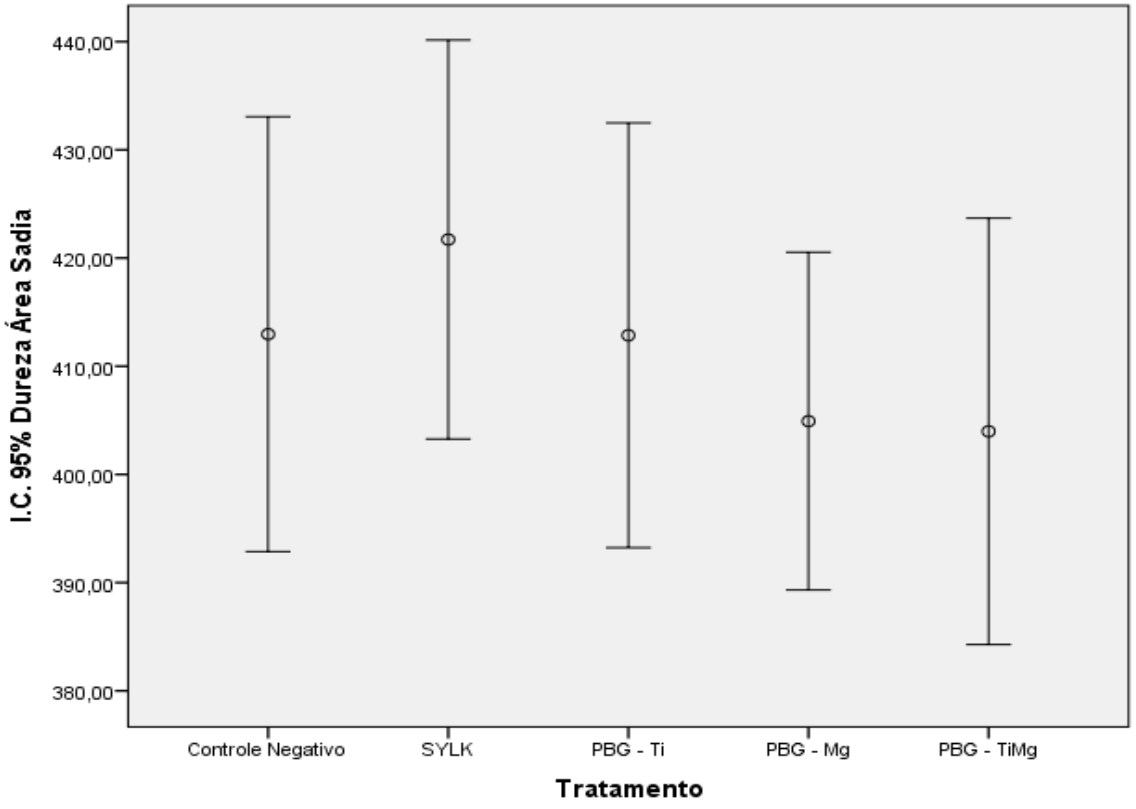
Teste de Homogeneidade de Variâncias

	Estatística de Levene	gl1	gl2	Valor p
Dureza Área Sadia	,273	4	45	0,8940
Dureza após Tratamento	4,516	4	45	0,0038
Percentagem de Variação Dureza após Tratamento	1,305	4	45	0,2825

ANOVA

		Soma dos Quadrados	gl	Quadrado Médio	F	Valor p	Poder observado [b]
Dureza Área Sadia	Entre Grupos	2078,110	4	519,528	,755	0,56025144	0,22359148545
	Nos grupos	30979,753	45	688,439			
	Total	33057,863	49				
Dureza após Tratamento	Entre Grupos	168380,629	4	42095,157	225,401	0,00000000	0,99999999867
	Nos grupos	8404,064	45	186,757			
	Total	176784,693	49				
Percentagem de Variação Dureza após Tratamento	Entre Grupos	11030,342	4	2757,586	166,215	0,00000000	0,99999999231
	Nos grupos	746,573	45	16,591			
	Total	11776,916	49				

b. Calculado usando alfa = ,05



Comparações múltiplas

Variável dependente: Dureza após Tratamento

Games-Howell

(I) Tratamento		Diferença média (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95% Limite inferior	Intervalo de Confiança 95% Limite superior
Controle Negativo	SYLK	-89,18000*	5,98954	0,00000	-107,3113	-71,0487
	PBG - Ti	-97,52000*	5,98431	0,00000	-115,6362	-79,4038
	PBG - Mg	-152,60125*	4,62575	0,00000	-167,6975	-137,5050
	PBG - TiMg	-162,84000*	7,72346	0,00000	-186,4731	-139,2069
SYLK	Controle Negativo	89,18000*	5,98954	0,00000	71,0487	107,3113
	PBG - Ti	-8,34000	5,66700	0,59239	-25,4759	8,7959
	PBG - Mg	-63,42125*	4,20720	0,00000	-77,0748	-49,7677
	PBG - TiMg	-73,66000*	7,48029	0,00000	-96,7114	-50,6086

PBG - Ti	Controle Negativo	97,52000*	5,98431	0,00000	79,4038	115,6362
	SYLK	8,34000	5,66700	0,59239	-8,7959	25,4759
	PBG - Mg	-55,08125*	4,19976	0,00000	-68,7091	-41,4534
	PBG - TiMg	-65,32000*	7,47611	0,00000	-88,3618	-42,2782
PBG - Mg	Controle Negativo	152,60125*	4,62575	0,00000	137,5050	167,6975
	SYLK	63,42125*	4,20720	0,00000	49,7677	77,0748
	PBG - Ti	55,08125*	4,19976	0,00000	41,4534	68,7091
	PBG - TiMg	-10,23875	6,44032	0,53524	-31,5497	11,0722
PBG - TiMg	Controle Negativo	162,84000*	7,72346	0,00000	139,2069	186,4731
	SYLK	73,66000*	7,48029	0,00000	50,6086	96,7114
	PBG - Ti	65,32000*	7,47611	0,00000	42,2782	88,3618
	PBG - Mg	10,23875	6,44032	0,53524	-11,0722	31,5497

*. A diferença média é significativa no nível 0.05.

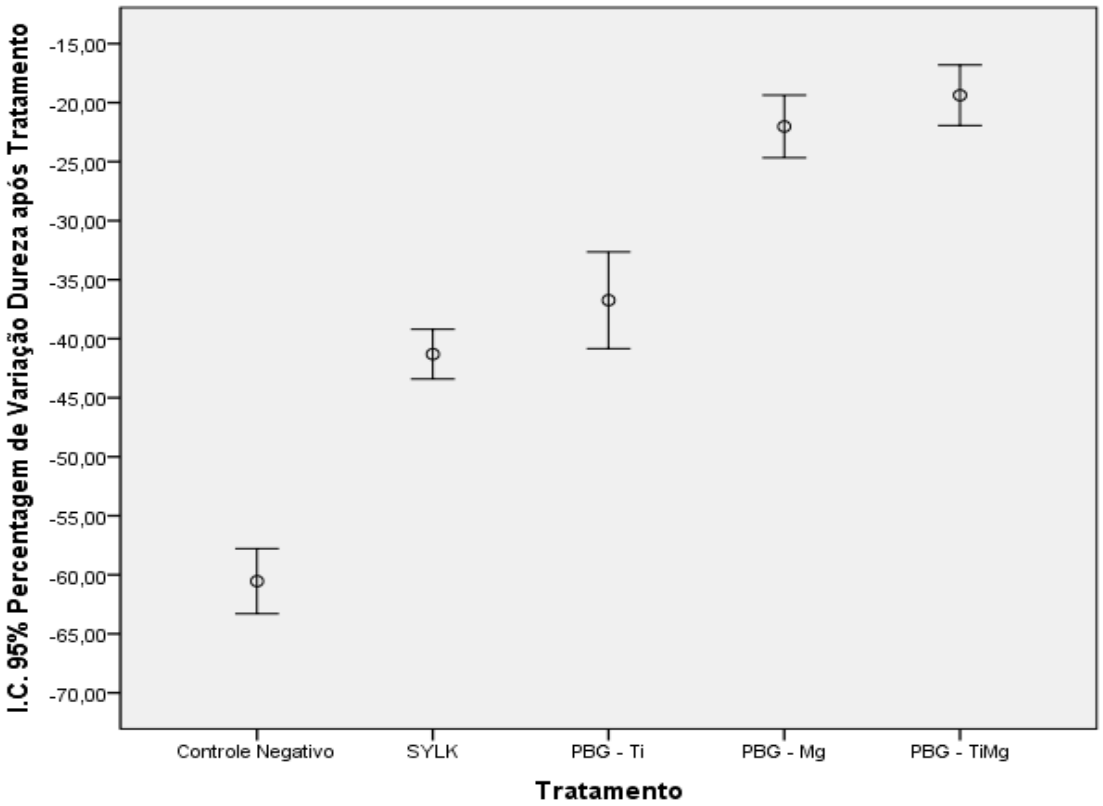
Comparações múltiplas

Variável dependente: Percentagem de Variação Dureza após Tratamento

Tukey HSD

(I) Tratamento		Diferença média (I-J)	Erro Padrão	Valor p	Intervalo de Confiança 95%	
					Limite inferior	Limite superior
Controle Negativo	SYLK	-19,23138*	1,82157	0,00000	-24,4073	-14,0555
	PBG - Ti	-23,79900*	1,82157	0,00000	-28,9749	-18,6231
	PBG - Mg	-38,52221*	1,82157	0,00000	-43,6981	-33,3463
	PBG - TiMg	-41,17100*	1,82157	0,00000	-46,3469	-35,9951
SYLK	Controle Negativo	19,23138*	1,82157	0,00000	14,0555	24,4073
	PBG - Ti	-4,56762	1,82157	0,10696	-9,7435	,6083
	PBG - Mg	-19,29082*	1,82157	0,00000	-24,4667	-14,1149
	PBG - TiMg	-21,93962*	1,82157	0,00000	-27,1155	-16,7637
PBG - Ti	Controle Negativo	23,79900*	1,82157	0,00000	18,6231	28,9749
	SYLK	4,56762	1,82157	0,10696	-,6083	9,7435
	PBG - Mg	-14,72321*	1,82157	0,00000	-19,8991	-9,5473
	PBG - TiMg	-17,37200*	1,82157	0,00000	-22,5479	-12,1961
PBG - Mg	Controle Negativo	38,52221*	1,82157	0,00000	33,3463	43,6981
	SYLK	19,29082*	1,82157	0,00000	14,1149	24,4667
	PBG - Ti	14,72321*	1,82157	0,00000	9,5473	19,8991
	PBG - TiMg	-2,64879	1,82157	0,59680	-7,8247	2,5271
PBG - TiMg	Controle Negativo	41,17100*	1,82157	0,00000	35,9951	46,3469
	SYLK	21,93962*	1,82157	0,00000	16,7637	27,1155
	PBG - Ti	17,37200*	1,82157	0,00000	12,1961	22,5479
	PBG - Mg	2,64879	1,82157	0,59680	-2,5271	7,8247

*. A diferença média é significativa no nível 0.05.



COMPARAÇÃO DUREZA ÁREA SADIA X APÓS TRATAMENTO

Tratamento = Controle Negativo

Teste-T

Estatísticas de amostras emparelhadas

		Média	N	Desvio Padrão	Erro Padrão da Média
Par 1	Dureza Área Sadia	412,9600	10	28,09710	8,88508
	Dureza após Tratamento	162,4600	10	14,06621	4,44813

Teste de amostras emparelhadas

		Diferenças emparelhadas					t	gl	Valor p
		Média	Desvio Padrão	Erro Padrão da Média	da Diferença Inferior	da Diferença Superior			
Par 1	Dureza Área Sadia - Dureza após	250,50000	#######	8,93852	230,27967	270,72033	28,025	9	0,0000

1

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Tratamento = SYLK

Teste-T

Estatísticas de amostras emparelhadas

		Média	N	Desvio Padrão	Erro Padrão da Média
Par 1	Dureza Área Sadia	421,7100	10	25,77908	8,15206
	Dureza após Tratamento	251,6400	10	12,68413	4,01107

Teste de amostras emparelhadas

		Diferenças emparelhadas					t	gl	Valor p
		Média	Desvio Padrão	Erro Padrão da Média	da Diferença Inferior	da Diferença Superior			
Par 1	Dureza Área Sadia - Dureza após	170,07000	#######	7,04588	154,13111	186,00889	24,138	9	0,0000

1
2

Tratamento = PBG - Ti

Teste-T

Estatísticas de amostras emparelhadas

		Média	N	Desvio Padrão	Erro Padrão da Média
Par 1	Dureza Área Sadia	412,8600	10	27,44093	8,67759
	Dureza após Tratamento	259,9800	10	12,65945	4,00327

Teste de amostras emparelhadas

		Diferenças emparelhadas					t	gl	Valor p
		Média	Desvio Padrão	Erro Padrão da Média	da Diferença Inferior	da Diferença Superior			
Par 1	Dureza Área Sadia - Dureza após	152,88000	######	10,29689	129,58682	176,17318	14,847	9	0,0000

1

Tratamento = PBG - Mg

Teste-T

Estatísticas de amostras emparelhadas

		Média	N	Desvio Padrão	Erro Padrão da Média
Par 1	Dureza Área Sadia	404,9225	10	21,82710	6,90233
	Dureza após Tratamento	315, 0613	10	4,01470	1,26956

Teste de amostras emparelhadas

		Diferenças emparelhadas					t	gl	Valor p
		Média	Desvio Padrão	Erro Padrão da Média	da Diferença Inferior	da Diferença Superior			
Par 1	Dureza Área Sadia - Dureza após	89,86127	######	6,40236	75,37813	104,34442	14,036	9	0,0000

2

Tratamento = PBG - TiMg

Teste-T

Estatísticas de amostras emparelhadas

		Média	N	Desvio Padrão	Erro Padrão da Média
Par 1	Dureza Área Sadia	403,9800	10	27,54559	8,71068
	Dureza após Tratamento	325,3000	10	19,96647	6,31395

1

Teste de amostras emparelhadas

		Diferenças emparelhadas					t	gl	Valor p
		Média	Desvio Padrão	Erro Padrão da Média	da Diferença Inferior	da Diferença Superior			
Par 1	Dureza Área Sadia - Dureza após	78,68000	######	5,44779	66,35625	91,00375	14,443	9	0,0000

2

Correlações

		Perfilometria	Rugosidade Área Sadia	Rugosidade após Tratamento	de Variação Rugosidade após Tratamento	Dureza Área Sadia	Dureza após Tratamento	m de Variação Dureza após Tratamento
Perfilometria	Correlação de	1	,102	,643**	,587**	,148	-,903**	-,896**
	Valor p		0,4813	0,0000	0,0000	0,3043	0,0000	0,0000
	N	50	50	50	50	50	50	50
Rugosidade Área Sadia	Correlação de	,102	1	,489**	-,239	-,011	-,047	-,018
	Valor p	0,4813		0,0003	0,0940	0,9414	0,7433	0,9004
	N	50	50	50	50	50	50	50
Rugosidade após Tratamento	Correlação de	,643**	,489**	1	,702**	,090	-,633**	-,605**
	Valor p	0,0000	0,0003		0,0000	0,5358	0,0000	0,0000
	N	50	50	50	50	50	50	50
Percentagem de Variação Rugosidade após	Correlação de	,587**	-,239	,702**	1	,051	-,616**	-,595**
	Valor p	0,0000	0,0940	0,0000		0,7263	0,0000	0,0000
	N	50	50	50	50	50	50	50
Dureza Área Sadia	Correlação de	,148	-,011	,090	,051	1	-,064	-,288*
	Valor p	0,3043	0,9414	0,5358	0,7263		0,6604	0,0427
	N	50	50	50	50	50	50	50
Dureza após Tratamento	Correlação de	-,903**	-,047	-,633**	-,616**	-,064	1	,967**
	Valor p	0,0000	0,7433	0,0000	0,0000	0,6604		0,0000
	N	50	50	50	50	50	50	50
Percentagem de Variação Dureza após Tratamento	Correlação de	-,896**	-,018	-,605**	-,595**	-,288*	,967**	1
	Valor p	0,0000	0,9004	0,0000	0,0000	0,0427	0,0000	
	N	50	50	50	50	50	50	50

3

**. A correlação é significativa no nível 0,01 (bilateral).

*. A correlação é significativa no nível 0,05 (bilateral).

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Bioestatística: Princípios e aplicações - Sídia Callegari-Jacques. Artmed. 2003. 256 p.

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Os vidros bioativos ricos em fosfato contendo Ti e/ou Mg foram efetivos no controle da erosão dental. Entretanto, quando avaliados em esmalte e dentina observou-se comportamento diferente destes materiais. Enquanto o PBG-Ti, dentre os vidros bioativos, foi o que apresentou melhores resultados na obliteração tubular, em esmalte os vidros PBG-Mg e PBG-TiMg controlaram melhor o processo de desmineralização provocado por erosão dental. Logo, a taxa de dissolução destes materiais influenciou a atuação dos vidros frente a diferentes situações. Desta forma, os resultados obtidos com a realização desta tese incentivam o desenvolvimento de novos produtos odontológicos para o controle da erosão dental.

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