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Faculdade de Odontologia de Piracicaba

ALEJANDRA DEL CARMEN BRENES ALVARADO

LIBERAÇÃO DE FLUORETO DE CIMENTOS DE IONÔMERO DE VIDRO SIMULANDO O PROCESSO DE DES-REMINERALIZAÇÃO DE CÁRIE

FLUORIDE RELEASE FROM GLASS IONOMER CEMENTS MIMICKING THE CARIES PROCESS OF DE-REMINERALIZATION

PIRACICABA

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Orientador: Prof. Dr. Jaime Aparecido Cury

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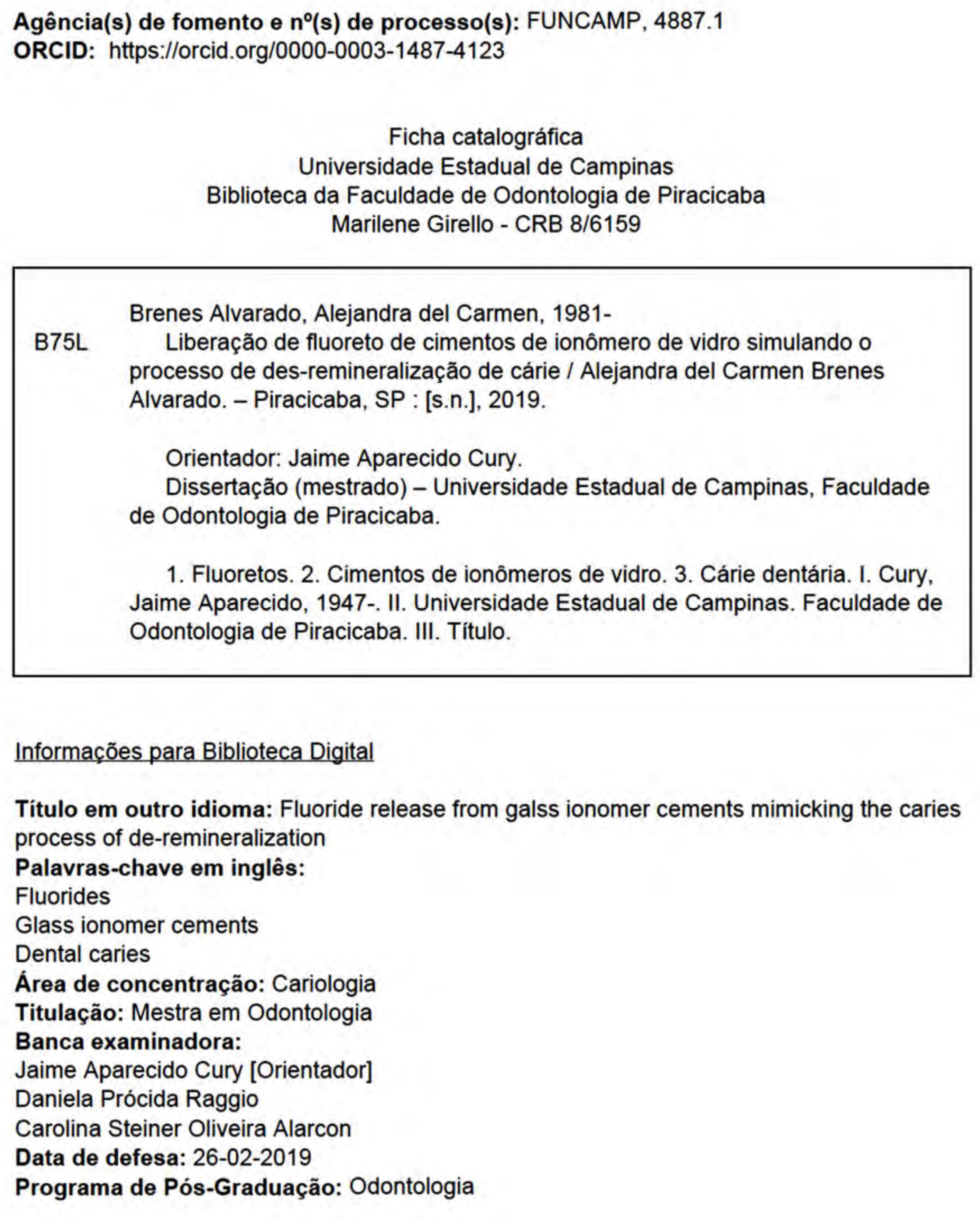

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RESUMO

Interferindo localmente com o processo físico-químico de desenvolvimento de lesões de cárie, fluoreto age eficazmente no controle da cárie dentária. Nesse sentido, por sua capacidade de liberar fluoreto constantemente no meio ambiente bucal, os cimentos de ionômero de vidro (CIV) disponibilizam fluoreto no lugar certo (fluido do biofilme), no momento certo (antes da ingestão de açúcar) e na concentração mínima necessária para interferir com o processo de cárie que ocorre ao redor das restaurações. Vários meios de imersão têm sido utilizados para estimar a liberação de fluoreto e o potencial anticárie de CIV e o mais adequado é o uso de soluções desmineralizante (Des-) e remineralizante (Re-) porque simulam a dinâmica do processo de cárie. Diferentes tipos de CIV estão disponíveis no mercado e o potencial deles liberarem fluoreto tem sido avaliado em água ou saliva, meios que não simulam o processo de cárie. O objetivo desse estudo foi avaliar a liberação de fluoreto de 15 CIV, simulando o desenvolvimento do processo de cárie. Seis corpos de prova cilíndricos (161,8 mm^2) foram preparados de acordo com as recomendações dos fabricantes. Os corpos de prova foram polidos e submetidos diariamente a ciclagens por 6 e 18 h em volumes de 1 ml de soluções Des- (tampão acetato 50 mM, pH 5,0 contendo 1,12 mM de Ca e 0,73 mM de Pi) e Re- (tampão Tris 0,1 M, pH 7,0 contendo 1,5 mM de Ca, 0,9 mM de P_i e 150 mM de KCl), respectivamente. As soluções foram trocadas diariamente durante 12 dias, nas quais a concentração de fluoreto foi determinada com eletrodo específico pela técnica direta. Os resultados foram expressos em concentração de fluoreto diária liberada nas soluções Des- e Re- (µg F/ml), quantidade de fluoreto liberada diariamente em soluções Des+Re por área de corpo de prova (µg F/cm^2/dia) e liberação acumulativa durante os 12 dias (µg F/cm^2). A diferença de liberação diária nas soluções Des- e Re- para cada material foi avaliada descritivamente. A liberação diária nas soluções Des+Re e a acumulada foram analisadas por ANOVA, considerando respectivamente os fatores material e tempo ou só material. Para ambos os casos, as diferenças entre os CIV foram comparadas pelo teste de Tukey ($p \leq 0,05$). Todos os CIV mostraram uma alta liberação inicial de fluoreto nos primeiros dias, seguido por um declínio gradual e posterior estabilização. Três padrões distintos de liberação foram observados nas soluções Des- e Re-: Maior liberação de fluoreto na solução Des- do que na Re- durante todo o período (A); Liberação inicial maior na solução Des- (B) e liberação similar em ambas das soluções durante todo o período (C). Os CIV diferiram estatisticamente na liberação diária e cumulativa de fluoreto ($p<0,05$). O Maxxion R liberou a maior quantidade diária e acumulativa de fluoreto. A menor quantidade acumulativa de fluoreto foi liberada por Resiglass R. Os CIV avaliados mostraram padrões qualitativos e quantitativos distintos de liberação de fluoreto em condições simulando o processo de cárie, o que deve refletir nas suas propriedades anti-cárie.

Palavras chave: Fluoreto, Cimento de ionômero de vidro, Cárie dentária, Ciclagem de pH

ABSTRACT

Interfering locally with the physical-chemical process of caries lesions development, fluoride is very effective to control caries. For its property to release fluoride constantly to the oral environment, glass ionomer cement (GIC) is able to maintain fluoride available at the right place (biofilm fluid), right time (before sugar intake) and in the minimum concentration required to interfere with the caries process adjacent to restorations. Several media of immersion have been used to estimate the fluoride releasing from GIC, but pH-cycling in demineralizing (De-) and remineralizing (Re-) solutions is the most appropriated because it simulates the caries process dynamics that occurs in vivo. Currently, there are several GIC in the market but their fluoride releasing potential has been studied in water or saliva, that do no replicate the caries process. The purpose of this study was to evaluate the fluoride release from 15 restorative GIC simulating the caries development process. Six cylindrical samples (161.8 mm^2) were prepared according to the manufacturer's instructions. The specimens were exposed daily to pH-cycling for 6 and 18 hours, respectively in volumes of 1 ml of De- (acetate buffer 50 mM, pH 5,0 containing 1,12 mM of Ca and 0,73 mM of P_i) and Re- (Tris buffer 0,1 M, pH 7,0 containing 1,5 mM of Ca, 0,9 mM of P_i and 150 mM of KCl) solutions. Fresh solutions were used everyday for 12 days, and their fluoride concentration was determined with ion specific electrode. Daily fluoride concentration in De- and Re- solutions were recorded in µg F/ml (ppm F). De- and Re- values were summed and presented in µg F/cm^2/day of specimen area. Also, the cumulative amount of fluoride released by each GIC during the 12 days was calculated (µg F/cm^2). Differences in the daily fluoride release of each material in the De- and Re- solutions were evaluated descriptively. Daily and cumulative release in De+Re were analyzed by ANOVA, respectively by two-way (time x material) and one-way (material). Differences between GIC were compared by Tukey test ($p \leq 0,05$). Three distinct patterns were observed for fluoride release in De- and Re- solutions: Greater fluoride release in De- than Re- solution during the period (A); Initial higher releasing on the De- solution (B) and similar releasing in both solutions during the whole period (C). All GIC showed early burst of fluoride release on the first days followed by a gradual decline. The GIC differed statistically ($p<0.05$) either on daily and cumulative fluoride release. Maxxion R released the greater daily and cumulative amount of fluoride during all the period. Resiglass R released the lowest cumulative amount of fluoride. The GICs evaluated showed distinct qualitative and quantitative patterns of fluoride releasing in conditions simulating the caries process that may reflects in their anti-caries potential.

Keywords: fluorides, glass ionomer cements, dental caries, pH cycling

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1 INTRODUÇÃO

Apesar dos esforços no controle da cárie dentária, 2,5 bilhões de pessoas apresentam lesões de cárie não tratadas (Kassebaum et al., 2017). A taxa de progressão da doença aumenta com a exposição aos fatores de risco (Tonetti et al., 2017), principalmente alto consumo de açúcar e inadequada exposição ao fluoreto (Petersen e Lennon, 2004). Quando os fatores etiológicos da doença não são controlados, a estrutura dental pode ser destruída e se a cavidade não for accessível à higienização (Kidd et al., 2015), faz-se necessário colocar uma restauração para recuperar a forma e função do dente e assim permitir um controle mais eficaz de biofilme (Ricketts et al., 2013).

Dentre as opções de materiais restauradores, os cimentos ionoméricos (CI) têm um papel importante na Odontologia Restauradora. Segundo à composição e aditivos, existem materiais ionoméricos convencionais (CIV) ou modificados por resina (CIVMR). Os CI surgiram na década dos anos 70 e sua composição está definida pela presença de silicatos de vidro que reagem com ácidos polialquenóicos, através de uma reação ácido-base (Wiegand et al., 2007). Posteriormente foram introduzidos os CIVMR, desenvolvidos para superar as baixas propriedades mecânicas iniciais típicas dos CIV, assim como melhorar a parte estética das restaurações (Wiegand et al., 2007). Os CIVMR são fabricados com partículas de vidro de menor tamanho, e adicionados com foto iniciador e componentes de metacrilato para promover uma polimerização inicial foto ativada complementada com a reação ácido-base (Wiegand et al., 2007).

A reação ácido-base que promove o endurecimento inicial dos CIV e complementa a fotoativada dos CIVMR, ocorre em duas etapas (Sidhu e Nicholson, 2016). Inicialmente os prótons do poliácido reagem com sítios básicos da superfície do vidro provocando a mobilização de íons (Ca^{++}, Al^{+++}, F^{-}, etc) do vidro para a solução de poliácidos. Sais insolúveis de poliacrilato são gerados levando a um endurecendo imediato do material em 2-6 minutos (Sidhu e Nicholson, 2016) com a formação de um cimento. Partículas de vidro permanecem sem serem atacadas pelo ácido e conferem carga ao cimento. Em seguida, os íons de alumínio mobilizados interagem com as moléculas de poliácidos para formar ligações cruzadas, ao tempo que o cimento incorpora toda a água, sendo esse segundo passo mais lento e tomando aproximadamente 24 horas (Zainuddin et al., 2009). Posteriormente ocorre o processo de maturação, que se caracteriza por mudanças nas propriedades clínicas do cimento, aumentando a resistência à compressão, declínio na dureza, diminuição da opacidade com o tempo que aumenta a translucidez e intercambio iônico com a superfície dental (Nicholson, 2018). Ao contrário dos demais íons, a quantidade de fluoreto mobilizado do vidro pelo ataque ácido e passando a fazer parte do cimento é menor (Crisp e Wilson, 1974). A forma química que o fluoreto está presente no cimento formado, seguramente afetará o quanto será liberado na boca pelas restaurações.

Biocompatibilidade, adesão química ao esmalte e dentina e liberação de fluoreto são algumas das características que favorecem o uso dos CI (Sidhu e Nicholson, 2016). Entre essas propriedades, a liberação de fluoreto torna ambos os tipos de material, uma opção desejável para ambas as dentições, decídua e permanente, principalmente para aqueles pacientes com alto risco de cárie (de Araujo et al., 1996).

A liberação de fluoreto por CIV ou CIVMR está determinada por fatores como a composição do material, a relação pó/liquido usada no preparo do cimento, a reatividade do pó e líquido e a solubilidade da partícula de vidro (Shiozawa et al., 2014). Em acréscimo, por sua capacidade de liberar fluoreto por um longo período de tempo, os CI podem ser considerados um importante fonte de fluoreto na cavidade bucal para o controle da cárie dentária tanto do esmalte como da dentina e agindo pelo mesmo mecanismo de ação que outros meios convencionais de uso de fluoreto (Cury et al., 2016).

A ação do fluoreto no processo de desenvolvimento de lesões de cárie está firmemente estabelecida. Por meio de um mecanismo físico-químico, o fluoreto disponível na cavidade bucal, interfere com o processo de des- e remineralização que ocorre no biofilme dental diariamente quando da exposição à açucares da dieta. Durante a queda de pH e a desmineralização, o fluoreto disponível no biofilme é capaz de reduzir a perda mineral, enquanto que, durante a remineralização, é possível aumentar a recuperação mineral, resultando em uma redução da progressão da lesão de cárie (Cury e Tenuta, 2008). Portanto, o fluoreto liberado por CI pode contribuir para controlar a recorrência de lesões de cárie no substrato dentário adjacente a restaurações (Tenuta e Cury, 2010) ou na remineralização de lesões iniciais de cárie de estruturas dentárias adjacentes as restaurações com CI (Tedesco et al., 2016). Por sua propriedade de liberação constante de fluoreto, os CI conseguem manter o fluoreto disponível constantemente no lugar certo (o biofilme), em concentração mínima necessária e no momento adequado (antes que o açúcar seja ingerido) (Cury et al., 2016).

O efeito do fluoreto liberado por CI na redução da desmineralização ao redor de restaurações tem sido mostrado *in vitro* (Serra e Cury, 1992) e *in situ* no esmalte (Tenuta et al., 2005; Benelli et al., 1993) e dentina (Hara et al., 2006). Resultados de eficácia de redução de cárie para a dentição decídua (Dias et al., 2018; Raggio et al., 2016) e dentição permanente (Ruengrungsom et al., 2018; Yengopal e Mickenautsch, 2011) tem sido descritas por revisões sistemáticas, porém os ensaios clínicos realizados são ainda insuficientes e tem alto risco de vieses (Cury et al., 2016).

Uma vez que o potencial anti-cárie do CI se baseia na capacidade de liberação de fluoreto, essa propriedade deve ser criteriosamente avaliada. Essa capacidade tem sido avaliada *in vitro* usando diferentes meios de armazenamento, como ácido acético diluído(Shahid et al., 2014), solução de ácido lático (Naoum et al., 2011), NaCl 133 mmol/l tamponado com diferentes pH (Moreau e Xu, 2010), água (Basso et al., 2011; Attar, 2003), saliva artificial (Rao et al., 2015; Gururaj et al., 2013) e soluções desmineralizantes (Des-) e remineralizantes (Re-) de ciclagens de pH (Carvalho e Cury, 1999; Hayacibara et al., 2004; Nigam et al., 2009). Entre esses meios, os mais utilizados são água deionizada e saliva artificial, mas estes meios são inapropriados para estimar o potencial anti-cárie de materiais liberadores de fluoreto porque eles não simulam o processo físico-químico de desenvolvimento de lesões de cárie que ocorre *in vivo* ao redor das restaurações dos CI (Cury et al., 2016).

Em acréscimo, quando diferentes CI são comparados em termos das suas propriedades de liberarem fluoreto em água deionizada, saliva artificial ou soluções Des-Re, a ordem de classificação dos materiais em termos de concentração de fluoreto liberado muda dependendo do meio que é utilizado (Carvalho e Cury, 1999; Hayacibara et al., 2004; Nigam et al., 2009). Considerando esse viés comparativo na escolha do meio usado para avaliar a liberação de fluoreto e o fato que soluções Des-Re simulam as ciclagens de pH que ocorrem durante o desenvolvimento de lesões de cárie ao redor das restaurações, essas soluções devem ser preferentemente utilizadas para estimar o potencial anti-cárie de diferentes CI que outros meios de imersão (Cury e Tenuta, 2009). Meios desvinculados do mecanismo de ação anticárie do fluoreto podem ser usados para avaliar outras propriedades dos materiais ionoméricos, como por exemplo erosão (ISO 9917-1:2007).

Atualmente, há vários CIV ou CIVMR no mercado, e considerando que resultados do potencial efeito anti-cárie de diferentes CI depende de como é avaliada a liberação de fluoreto, o objetivo do presente estudo foi determinar *in vitro* a concentração de fluoreto liberada por 15 cimentos de ionômero de vidro restauradores submetidos a condições simulando o processo físico-químico de desenvolvimento de lesões de cárie.

2 ARTIGO

Fluoride release evaluation from glass ionomer cements mimicking the caries process of de-remineralization

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Short Title: Fluoride release from GIC mimicking caries development

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Abstract

The ability of glass ionomer cements (GICs) to release fluoride should be evaluated by simulating the process of caries lesion development to estimate the anti-caries potential of these materials. We determined the amount of fluoride released from 15 commercial GICs under a pH-cycling regime that simulated the caries process of demineralization and remineralization. Six discs of each GIC were immersed for 6 and 18 h each day in demineralising (De-) and remineralising (Re-) solutions, respectively. The solutions were changed daily over 12 days, during which the fluoride concentration was determined using ISE. The results were expressed in: (1) the daily fluoride concentration in De- and Re- solutions (µg F/ml), (2) the amount of fluoride released daily in De+Re solutions per area of specimens (µg F/cm^2/day) and (3) by the cumulative release over the 12-day period (µg F/cm^2). All GIC showed an early burst of fluoride release during the first days, followed by a gradual decline; however, three distinct patterns were observed. Specifically, (A) greater fluoride release in De- compared to Re- solution during the study period; (B) initial higher release in the De- solution; and (C) similar release in both solutions over the whole period. The GICs differed statistically ($p < 0.05$) with respect to daily and cumulative fluoride release. Maxxion R and Resiglass R presented the highest and lowest ability to release fluoride, respectively. The GICs evaluated showed distinct qualitative and quantitative patterns of fluoride release under conditions simulating the caries process that might reflect their anti-caries properties.

Introduction

Despite efforts to control dental caries, 2.5 billion people have untreated caries lesions [Kassebaum et al., 2017]. The rate of disease progression increases with exposure to risk factors [Tonetti et al., 2017], which mainly include high sugar consumption and inadequate use of fluoride [Petersen and Lennon, 2004]. Further progression of lesions leads to deeper substrate dissolution and, when progression is not controlled, a cavitation occurs [Fejerskov, 1997]. When a cavitated lesion cannot be cleaned by toothbrushing [Kidd et al., 2015], operative dentistry is needed to restore tooth form and function to allow effective biofilm control [Ricketts et al., 2013].

Glass-ionomer cements (GICs) and resin modified glass ionomer cements (RMGICs) have an important role in dentistry. For GICs, an acid-base reaction occurs between a polyacid and the basic silicate glass particle surface, mobilizing various ions (including calcium, aluminium and fluoride) to the cement matrix [Wiegand et al., 2007]. The reaction continues with ionic crosslinks between these ions (e.g. Ca, Al, F) and the polyacid molecules, completing the immediate hardening within the first minutes, with a slower process continuing over the first 24h [Sidhu and Nicholson, 2016], along with a maturation process extending over days or months [Nicholson, 2018]. In RMGIC, the typical acid-base reaction of GICs is complemented with an earlier photochemical polymerization.

GICs and RMGICs exhibit properties that facilitate their use in dentistry, including biocompatibility, which involves chemical bonding to both enamel and dentin, and fluoride release [Sidhu and Nicholson, 2016]. Out of these properties, the release of fluoride facilitates their use on deciduous and permanent dentitions [Dhar et al., 2015], especially in patients with a high risk of caries (de Araujo et al., 1996). In primary molars, GICs tend to be used for conventional restoration and atraumatic restorative treatment (ART) [American Academy of Pediatric Dentistry, 2016], including dentinal occlusal and occlusoproximal caries lesions [Casagrande et al., 2013]. GIC restoration exhibits longevity and survival rates to other restorative materials that are commonly used in primary molars, such as amalgam [Mickenautsch and Yengopal, 2011] and composites [Yengopal and Mickenautsch, 2011]. However, for the permanent teeth of children, there is insufficient evidence supporting the use of GIC for long-term restoration, with single surface ART restoration being recommended [de Amorim et al., 2018].

Fluoride release from GICs and RMGICs is determined by different factors, including the composition, powder/liquid ratio used when preparing cement, the reactivity of the powder and liquid and the solubility of the glass particles [Shiozawa et al., 2014]. Because of the ability of GICs and RMGICs to release fluoride over long timeframes and the contemporary concepts of how fluoride works on caries control, they provide an important source of fluoride in the oral cavity to control dental caries on both enamel and dentin [Cury et al., 2016]. When fluoride is maintained constantly in the right place (the biofilm), is available at the right time (before sugar consumption), and is available in sufficient concentrations (low levels), it can reduce the demineralisation and enhance the remineralisation of the tooth structure adjacent to the site of restoration [Cury et al., 2016]. In fact, fluoride concentration found in biofilm [whole and fluid] formed on dental specimens restored with GIC has been found to reduce enamel and dentine demineralisation compared with composites *in situ* [Benelli et al., 1993; Hara et al., 2006; Cenci et al., 2008].

Fluoride found in biofilm derives from GIC restoration, and is released daily during the period of de- and remineralisation that biofilm-restoration is subjected to the caries process [Cury et al., 2016]. Thus, every time sugar is ingested, the pH falls in biofilm fluid, fluoride is released and the amount of mineral dissolved is reduced because Ca and Pi are lost as hydroxyapatite and return to the tooth as fluorapatite (reduction of demineralization). When the ingestion of sugar ceases and the pH rises again, fluoride released from GIC that is present in biofilm fluid enhances the natural phenomenon dental of remineralisation [Cury et al., 2016].

Therefore, the *in vitro* evaluation of fluoride release from GIC should mimic the caries process that occurs in the oral cavity and the mechanism of how GIC works on caries control [Cury et al., 2016]. At present, several GICs are available in the market; however, their anticaries potential in terms of their ability to release fluoride has only been evaluated in certain storage media, such as diluted acetic acid [Shahid et al., 2014], lactic acid [Naoum et al., 2011], NaCl 133 mmol/l buffered with different pH levels [Moreau and Xu, 2010], water [Basso et al., 2011; Attar, 2003] and artificial saliva [Rao et al., 2015; Gururaj et al., 2013]. Only one study evaluated the potential of GICs using pH-cycling solutions (de- and remineralising) by simulating the caries process [Nigam et al., 2009]. The use of appropriate media in laboratory evaluations of fluoride release from dental materials have been recommended [Cury et al., 1993; Carvalho and Cury, 1999; Hayacibara et al., 2004] because *in vitro* models should simulate what happens in the oral cavity and because the amount of fluoride released from different GICs changes with the media used.

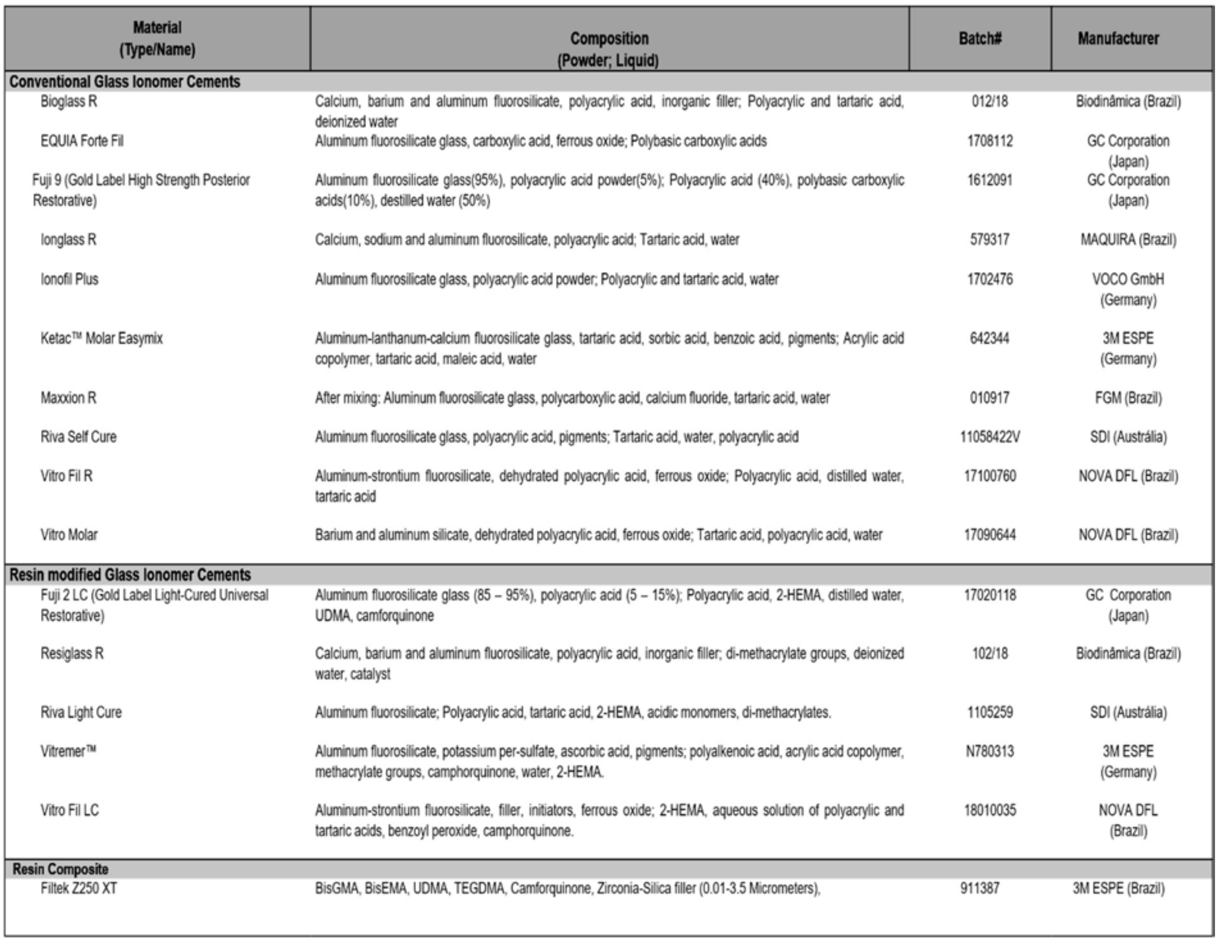
At present, the anticaries potential of different GICs *in vitro* has led to contrasting results, depending on the media used. Thus, here we determined the amount of fluoride released from 15 GICs under conditions modelling the caries process.

Materials and Methods

Experimental design

Fluoride release from 15 restorative glass-ionomer cements (nine conventional and six resin-modified) found in a Brazilian market (Table1) were evaluated in demineralising (De-) and remineralising (Re-) solutions. This approach simulated the caries process (pH-cycling regimen, please see 1st paragraph of the discussion), following the recommendation of Carvalho and Cury [1999]. Disc-shape specimens (n = 6) were immersed daily in De- and Re- solutions for 6 and 18 h, respectively. These solutions were changed daily over a 12-day period. Fluoride concentrations in the De- and Re- solutions were analysed with an ion specific electrode (ISE). Fluoride concentrations in the De- and Re- solutions for each material were compared descriptively. The amount (µg) of fluoride released daily in the De- and Re- solutions was summed. The materials were statistically compared using µg F/cm^2 per day based on (1) the 7th day, when release had stabilised, and (2) the cumulative release over the 12-day period. The materials were coded with capital letters (From A to P) in a blind analysis with ISE.

Table 1. Restorative materials used and specifications



Material (Type/Name)	Composition (Powder; Liquid)	Batch#	Manufacturer
Conventional Glass Ionomer Cements			
Bioglass R	Calcium, barium and aluminum fluorosilicate, polyacrylic acid, inorganic filler; Polyacrylic and tartaric acid, deionized water	012/18	Biodinâmica (Brazil)
EQUIA Forte Fil	Aluminum fluorosilicate glass, carboxylic acid, ferrous oxide; Polybasic carboxylic acids	1708112	GC Corporation (Japan)
Fuji 9 (Gold Label High Strength Posterior Restorative)	Aluminum fluorosilicate glass(95%), polyacrylic acid powder(5%); Polyacrylic acid (40%), polybasic carboxylic acids(10%), destilled water (50%)	1612091	GC Corporation (Japan)
Ionglass R	Calcium, sodium and aluminum fluorosilicate, polyacrylic acid; Tartaric acid, water	579317	MAQUIRA (Brazil)
Ionofil Plus	Aluminum fluorosilicate glass, polyacrylic acid powder; Polyacrylic and tartaric acid, water	1702476	VOCO GmbH (Germany)
Ketac™ Molar Easymix	Aluminum-lanthanum-calcium fluorosilicate glass, tartaric acid, sorbic acid, benzoic acid, pigments; Acrylic acid copolymer, tartaric acid, maleic acid, water	642344	3M ESPE (Germany)
Maxxion R	After mixing: Aluminum fluorosilicate glass, polycarboxylic acid, calcium fluoride, tartaric acid, water	010917	FGM (Brazil)
Riva Self Cure	Aluminum fluorosilicate glass, polyacrylic acid, pigments; Tartaric acid, water, polyacrylic acid	11058422V	SDI (Austrália)
Vitro Fil R	Aluminum-strontium fluorosilicate, dehydrated polyacrylic acid, ferrous oxide; Polyacrylic acid, distilled water, tartaric acid	17100760	NOVA DFL (Brazil)
Vitro Molar	Barium and aluminum silicate, dehydrated polyacrylic acid, ferrous oxide; Tartaric acid, polyacrylic acid, water	17090644	NOVA DFL (Brazil)
Resin modified Glass Ionomer Cements			
Fuji 2 LC (Gold Label Light-Cured Universal Restorative)	Aluminum fluorosilicate glass (85 – 95%), polyacrylic acid (5 – 15%); Polyacrylic acid, 2-HEMA, distilled water, UDMA, camforquinone	17020118	GC Corporation (Japan)
Resiglass R	Calcium, barium and aluminum fluorosilicate, polyacrylic acid, inorganic filler; di-methacrylate groups, deionized water, catalyst	102/18	Biodinâmica (Brazil)
Riva Light Cure	Aluminum fluorosilicate; Polyacrylic acid, tartaric acid, 2-HEMA, acidic monomers, di-methacrylates.	1105259	SDI (Austrália)
Vitremer™	Aluminum fluorosilicate, potassium per-sulfate, ascorbic acid, pigments; polyalkenoic acid, acrylic acid copolymer, methacrylate groups, camphorquinone, water, 2-HEMA.	N780313	3M ESPE (Germany)
Vitro Fil LC	Aluminum-strontium fluorosilicate, filler, initiators, ferrous oxide; 2-HEMA, aqueous solution of polyacrylic and tartaric acids, benzoyl peroxide, camphorquinone.	18010035	NOVA DFL (Brazil)
Resin Composite			
Filtek Z250 XT	BisGMA, BisEMA, UDMA, TEGDMA, Camforquinone, Zirconia-Silica filler (0.01-3.5 Micrometers),	911387	3M ESPE (Brazil)

Preparation of specimens

Six cylindrical specimens (7.8 mm in diameter and 2.2 mm in thickness) were prepared at room temperature (23 ± 2 °C) and (50 ± 20 %) relative humidity, according to ISO #9917-1 and # 9917-2 recommendations and the manufacturer's instructions. Materials were mixed and placed in Teflon moulds using a Centrix® system (Nova DFL, Rio de Janeiro, Brazil) Conventional glass ionomers were allowed to set under pressure between a polyester strip and glass plates for 15 min. Resin modified glass ionomers and composite resin were placed on a glass plate between polyester strips and were light activated on both upper and lower surfaces. One multipeak light curing unit (Valo Cordless, Ultradent Products Inc, South Jordan, UT, USA), with a 9.4 mm active diameter and 1477 mW/cm^2 irradiance, was used for all light curing procedures. The light curing unit was positioned 1 mm above the surface strip and was activated for 20 or 40 s according to manufacturer's specification. No protection was applied on the surface of the specimens. After hardening, specimens were stored at 37 °C and 100% relative humidity for 24 h [Carvalho & Cury, 1999; Hayacibara et al., 2004], and excess was removed with a scalpel blade and coarse/medium Opti-Disc ™ contouring discs (Kavo, Brazil) and low-speed handpiece.

pH cycling regimen

The specimens were separately immersed in polyethylene tubes containing 1.0 mL demineralizing solution (Acetate buffer 50 mM, pH 5.0, containing Ca 1.12 mM and P_i 0.73 mM). This solution was 50% undersaturated related to human enamel/dentine (Cury JA, personal information). After 6 h, a 0.1 ml volume of TISAB III was added to the tubes. The specimens were then removed, washed with purified water, dried with lab absorbent tissues, and transferred to another tube containing 1.0 mL Re- solution (Tris buffer 0.1 M, pH 7.0, containing Ca 1.5 mM, P_i 0.9 mM and KCl 150 mM) [Serra and Cury, 1992]. After 18 h of immersion in Re- solution, the same procedure as described for De- solution was repeated. The tubes were maintained at 37 °C under agitation (90 rpm) over the 12-day study period. After each daily cycle, fresh De- and Re- solutions were used. TISAB III (Orion 940911, Thermo Scientific, Cambridge, USA), instead TISAB II, was used to buffer the De- and Re- solutions to avoid the interference of aluminium released by the materials in the analysis with ISE by the direct technique [Hayacibara et al., 2004].

Fluoride determination

Fluoride concentration in De- and Re- solutions was recorded daily using ISE (Thermo Scientific Orion 96-09, Orion Research Incorporated, Cambridge, USA) coupled to ion-analyser Star A214 (Thermo Scientific Orion). The electrode was calibrated with standards of fluoride solutions, ranging from 0.059 to 90.91 µg F/mL, which were prepared from NaF 99.99% (Sigma-Aldrich, St Louis, MO, USA) and mixed with TISAB III (10:1, v/v). The accuracy of the analysis was checked with a standard fluoride solution (Orion 940907, Thermo Scientific) and the average coefficient of variation from triplicates was 2.4%. Fluoride concentration was determined by performing a linear regression of the logarithm of fluoride concentrations of the standards with the respective mV values ($r^2 = 0.9999$) using an Excel spreadsheet (Microsoft). The results obtained from the De- and Re- solutions were expressed in µg F/ml. The amount of fluoride released by the materials per day in De+Re solutions, the amount of fluoride on day 7 after the stabilisation of all materials, and the cumulative amount of the 12-day period was expressed in µg F/cm^2 of the exposed surface area of the specimens.

Statistical Analyses

The daily fluoride concentrations in the De- and Re- solutions were analysed descriptively. Data of daily F release in De+Re solutions was adjusted to a Log-normal distribution, three outliers were removed, and the remaining data were statistically analysed by repeated measures ANOVA, followed by a Tukey test [$p \leq 0.05$] for differences between materials. Data on the cumulative difference of F released among F materials over the 12-day period were log-transformed, and one outlier was removed to homogenize the variance and normally distribute the measurements. These data were then, analysed by ANOVA, followed by a Tukey test [$p \leq 0.05$]. SAS software 8.01 [SAS Institute Inc., Cary, NC, USA] was used for the analysis, and significance was set at 5%.

Results

Restorative materials evaluated

According to the manufacturers (Table 1) the GIC EQUIA Forte Fil, Fuji 9, Ionofil Plus, Riva Self Cure and Maxxion R and the RMGIC Fuji 2 LC, Riva Light Cure and Vitremer are aluminium fluorsilicate materials. Vitro Molar was modified with barium and Bioglass R and Resiglass R with calcium and barium. Ionglass R contains calcium and sodium, while Ketac Molar Easymix was modified with lanthanum. Vitro Fil R and Vitro Fil LC were modified with strontium. Vitro Molar, Vitro Fil R, Vitro Fil LC and EQUIA Forte Fil contain ferrous oxide, while Maxxion R contains calcium fluoride.

Qualitative evaluation of fluoride release in demineralising (De-) and remineralising (Re-) solutions

The general pattern of daily fluoride release by GIC in De- (Fig.1a) and Re- (Figure 1b) solutions over the 12-day period involved an early burst of fluoride release on the first days, followed by a gradual decline until stabilisation throughout the remaining period. Figure 1 shows that, in general, more fluoride was released by De- solution compared to Re- solution.

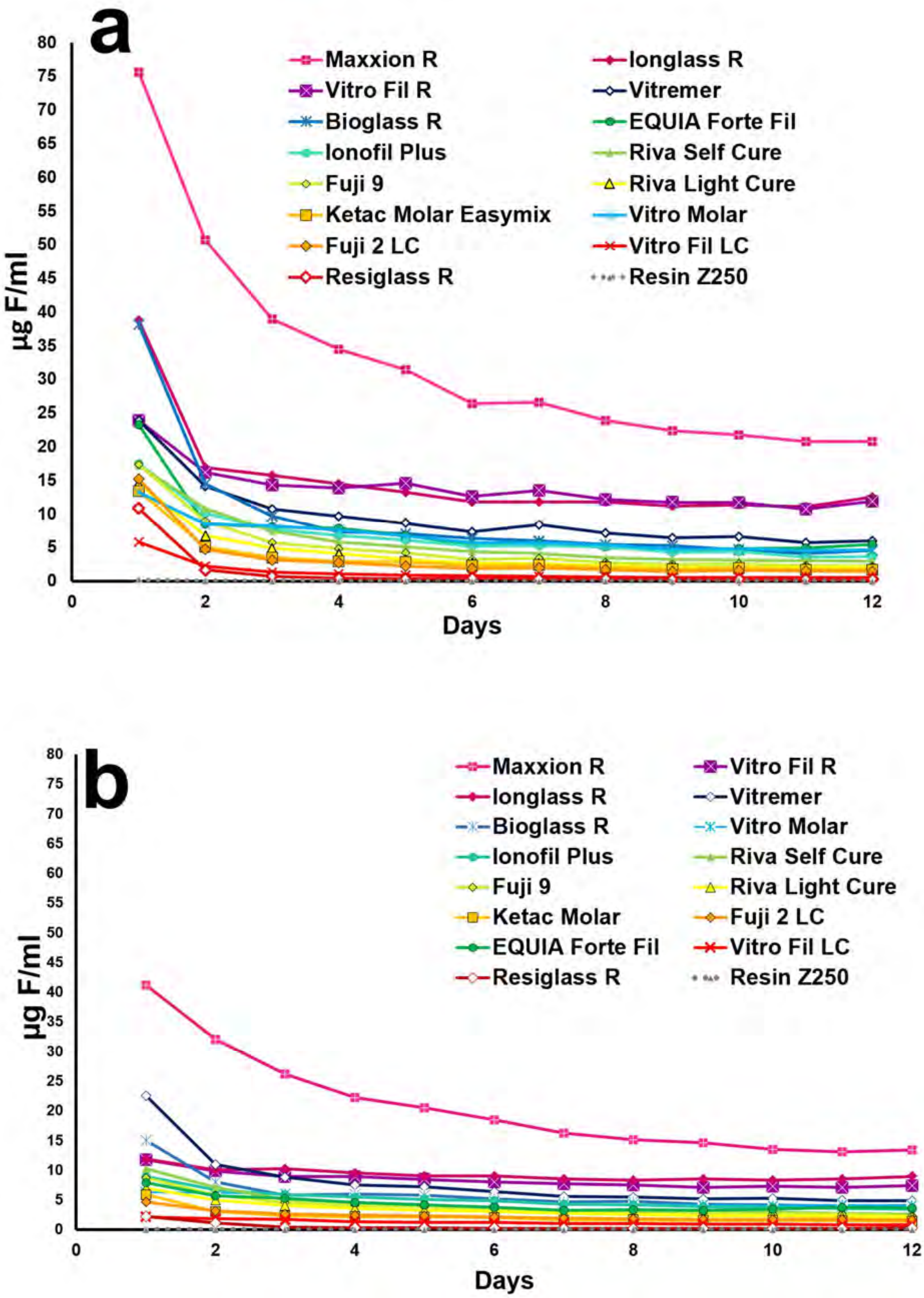


Figure 1. Mean (n = 6) daily fluoride concentration (µg F/ml) in demineralising (a) and remineralising (b) solutions by GICs and the control (Resin 250) over the 12-day period of the pH-cycling regime to which the specimens were subjected

However, when the pattern of F release in De- and Re- solutions for each GIC was compared, we detected three distinct patterns (Fig 2A, 2B and 2C).

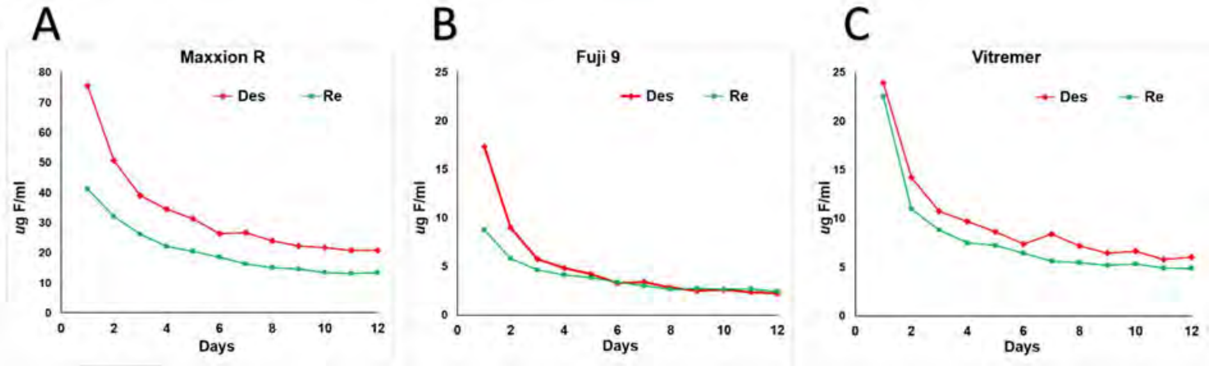


Figure 2. Representative patterns (A, B and C) of fluoride release in De- and Re- solutions (µg F/ml) by GICs over the 12 days of pH-cycling

Three GICs (Maxxion R, Vitro Fil R and Ionglass R) exhibited pattern A of fluoride release, with more fluoride being released in the De- solution compared to the Re- over the 12 days. The second pattern (Figure 2B) was exhibited by Bioglass R, EQUIA Forte Fil, Fuji 2 LC, Fuji 9, Ionofil Plus, Ketac Molar Easymix, Resiglass R, Riva Light Cure, Riva Self Cure, Vitro Fil LC and Vitro Molar materials. In this scenario, there was an initial higher release of fluoride in the De- solution compared to the Re- solution, but this difference was not maintained. In comparison, less fluoride was initially released in the De- solution compared to the Re- solution for Vitro Fil LC and Vitro Molar for this scenario. The third pattern (Figure 2C) was exhibited by the RMGIC Vitremer, which showed a very similar release of fluoride in De- and Re- solutions over the 12-day period.

Quantitative evaluation of F release

Daily release in De-+Re- solutions

The amount of released daily in De- and Re- solutions was summed. Figure 3 shows that all GICs exhibited a burst fluoride release in the first days, followed by a decline and stabilisation.

ANOVA showed statistical significance for material, time and interactions ($p < 0.0001$). The materials differed regarding the time period between the burst of fluoride release and stabilization. All GICs and RMGICs differed from the control group (Please, see supplemental material).

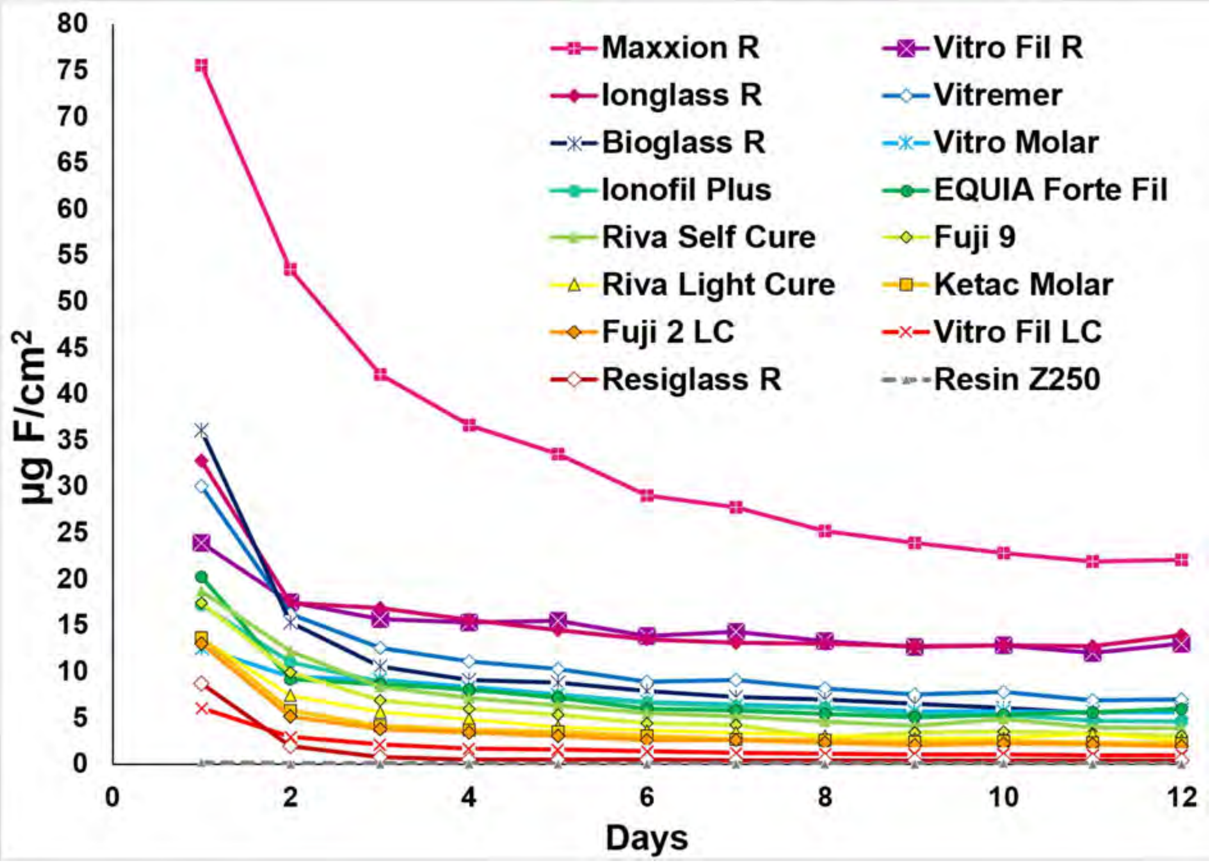


Figure 3. Daily fluoride released (mean; n = 6) (µg F/cm^2) in demineralising+ remineralising solutions by GICs and the control (Resin 250) during the 12 days of pH-cycling

Certain GICs (Ionglass R, Ionofil Plus, Vitro Fil R and Vitro Molar) exhibited a very fast burst of fluoride release, and stabilised after the 2nd day of pH-cycling. Other materials (Bioglass R, Fuji 2 LC, Ketac Molar Easymix, Maxxion R, Riva Light Cure and Vitro Fil LC) stabilised after the 3rd day. Other cements (EQUIA Forte Fil, Fuji 9, Resiglass R and Vitremer) stabilised after the 4th day, while Riva Self Cure stabilised after the 5th day.

When the materials were compared on each day, Maxxion R exhibited the greatest fluoride release over all periods, while Resiglass R exhibited the lowest fluoride release. The statistical difference among the materials on each day is shown in the supplemental material.

After the 6th day of evaluation, the burst effect of fluoride release ceased for all materials, after which the materials released a constant amount of fluoride daily. Figure 4 shows the statistical difference among GCIs on day 7 of evaluation.

On day 7 of pH-cycling, Maxxion R released the highest amount of fluoride [$p < 0.05$]. In decreasing order of fluoride release, there was no difference ($p > 0.05$) in the amount of F released by Vitro Fil R, Ionglass R and Vitremer, nor among Vitremer, Bioglass R, Vitro Molar, Ionofil Plus, Riva Self Cure and Fuji 9. Also, Riva Self Cure, Fuji 9, Riva Light Cure, Ketac Molar Easymix and Fuji 2 LC showed no difference. EQUIA Forte Fil and Vitro Fil R released an equal amount of F. Resiglass R released the lowest amount of F. All GICs differed from Z250 resin ($p < 0.05$).

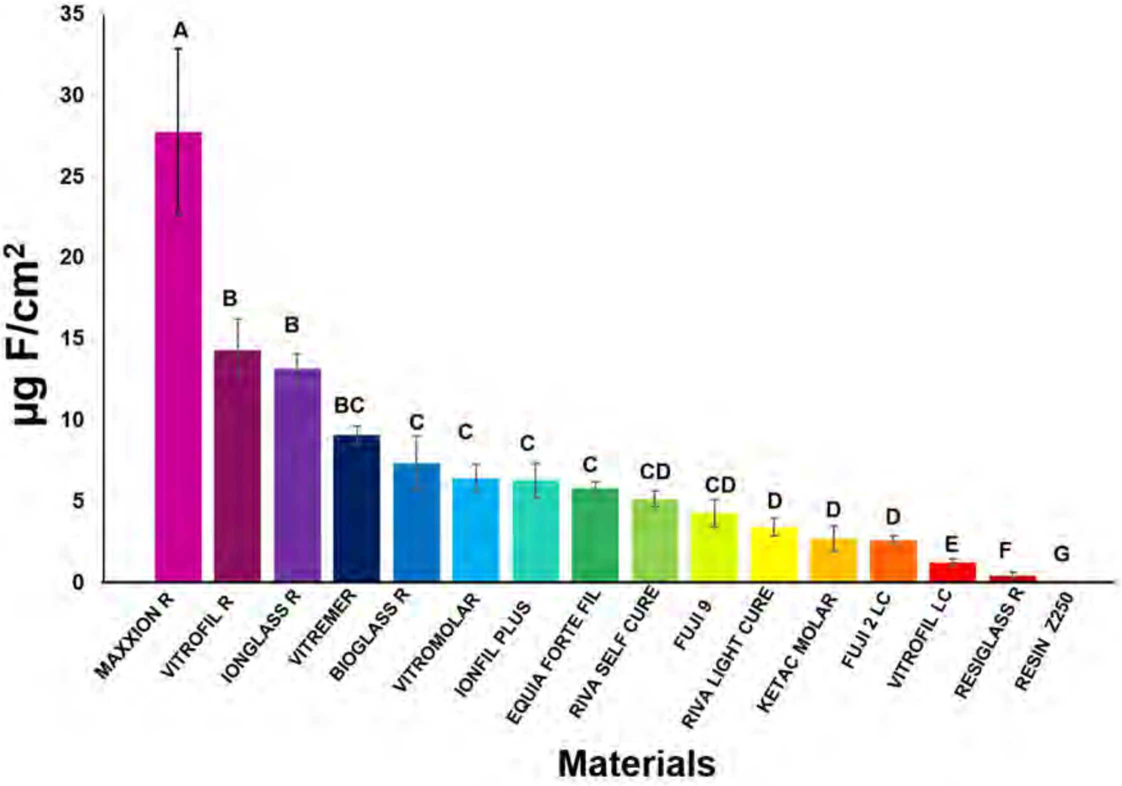


Figure 4. Mean (SD; n = 6) of fluoride release (µg F/cm^2) by the glass ionomer cements and the control (Resin Z250) in De-+Re- solutions on day 7 of the pH-cycling regime. Capital letters show differences among the materials ($p < 0.05$)

Cumulative fluoride release over the 12 days

Figure 5 shows the cumulative amount (µg F/cm^2) of fluoride release in De-+Re- solutions over the 12 days. ANOVA showed a statistically significant difference among materials. All GICs differed ($p < 0.0001$) from the negative control (Resin Composite Z250) with respect to fluoride release. Among the GICs, Maxxion R released the most fluoride, while Resiglass R released the least fluoride. The other GICs released intermediate amounts of fluoride between Maxxion R and Resiglass R (Figure 5).

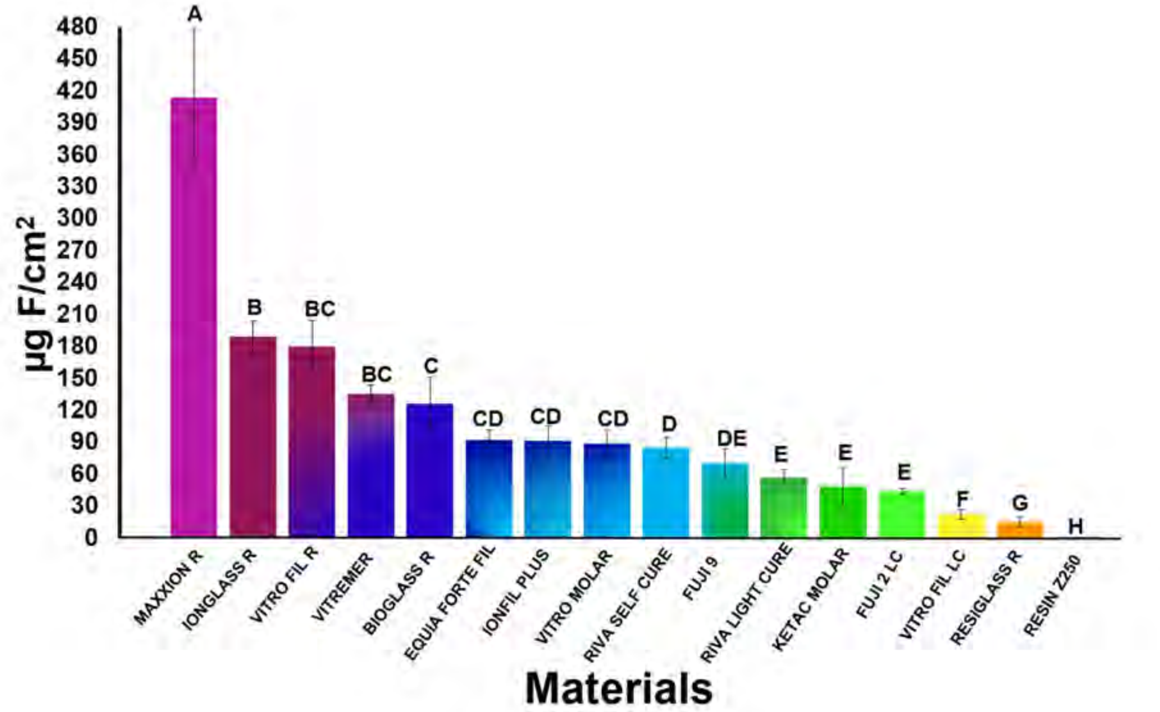


Figure 5. Mean (SD; n = 6) cumulative fluoride release (µg F/cm^2) by the glass ionomer cements and the control (Resin 250) over the 12 days of pH-cycling in De-+Re-solutions. Capital letters show differences between the materials ($p < 0.05$)

Discussion

To have some validity, models should simulate real-life. Thus, the property of glass ionomer cements (GICs) on fluoride release and their anticaries potential should be evaluated under conditions that simulate the physicochemical process of caries process development [Cury et al., 2016]. Through mimicking the caries process of demineralisation and remineralisation that teeth restored with GIC restorations are subjected daily in the mouth, we evaluated the fluoride release ability of nine conventional GICs (GIC) and six resin modified GICs (RMGIC). The model that was used simulated a high cariogenic challenge, and was originally developed to test the effect of fluoride on enamel demineralisation around fixed orthodontics appliances [Featherstone et al., 1986]. Furthermore, we used this pH-cycling model to evaluate the effect of GIC on enamel demineralisation during restoration *in vitro* [Serra and Cury, 1992]. The positive *in vitro* results showing reduced demineralisation compared to the composite were confirmed in vivo using an *in situ* model [Benelli et al., 1993].

Our findings clearly showed qualitative (Figure 1, 2) and quantitative (Figure 3–5) differences in fluoride release among the evaluated GICs. However, these differences might not be due to any common or specific component reported by the manufacturers described in Table 1. For example, when the nine conventional GICs were compared with six RMGIC based on the qualitative results of fluoride release, nothing was present that differentiated the type of material. Thus, Maxxion R, Vitro Fil R and Ionglass R were the only GICs that presented pattern A of fluoride release in De- and Re- solutions (Figs 1-2), while Vitremer was the only RMGIC that presented pattern C of fluoride release. Most of the evaluated GICs presented pattern B of fluoride release, irrespective of modification with resin. The same was true when comparing GICs and RMGICs from the same manufacturer; thus, resin and aluminium fluorosilicate doped with other ions (table 1) do not confer specific patterns in terms of qualitative fluoride release from RMGICs or GICs, respectively.

When evaluating the quantitative data of fluoride release and composition of GICs, we did not identify any distinct or common elements. No differentiation between conventional GICs (doped or not with ions) and RMGICs was detected for the early burst of fluoride release [Kuhn and Wilson, 1985] and the time taken to stabilise and to sustain release. The early burst of fluoride release among all GICs tested ranged from 48 to 120 h (Figure 3 and supplemental material), irrespective of the composition of the material (Table 1). The early burst is explained by a superficial rinse that causes an initial higher dissolution of the complexes of aluminium or phosphate with fluoride formed during the acid-base reaction [Shahid et al., 2014]. This process is mainly regulated by unreacted glass particles inside the cement [Crisp and Wilson, 1974]. After higher release during the first day, sustained release during the remaining period might be the result of diffusion through pores, fissures and bulk diffusion [Kuhn and Wilson, 1985; Gandolfi et al., 2006].

This study also ranked fluoride concentration on the 7th day of evaluation and based on the cumulative 12-day period. However, no association between the composition of the materials and their ability to release more or less fluoride was detected. Conventional aluminium fluorosilicate GICs did tend to release more fluoride compared to RMGICs (Figure 4, 5). Thus, the GIC Maxxion R and the RMGIC Resiglass R released the greatest and lowest amount of fluoride, respectively. However, while there is one RMGIC (Vitremer) among the four first materials on ability to fluoride release, among the four last, there is one conventional GIC (Ketac Molar Easymix) (Figure 4,5).

Although we could not explain the qualitative and quantitative patterns of fluoride release based on the composition of the evaluated materials, the results might indicate their anti-caries potential. Fluoride interferes with the physicochemical process of caries development, reducing the demineralisation of enamel or dentine and enhancing remineralisation during restoration [Cury et al., 2016]. Therefore, the maintenance of constant low fluoride concentrations in the oral cavity are more important than high concentrations for short periods. Thus, the burst effect of fluoride release found for all materials might be less relevant in terms of efficacy on caries control compared to the sustained releasing shown for all materials after day 6 (Figure 3 and supplemental material). However, the early burst of fluoride release might have some clinical relevance for repairing caries lesions on the surface of teeth close to GIC restoration. This possibility is remote even for the material Maxxion R, which had the highest ability to release fluoride during the first 3 days of the burst effect. During the 3 first days, this material released a mean of 55 µg F/cm^2. Thus, restoration of 1 x 1 cm surface area would release 55 µg F in the mouth, which could be relevant in terms of remineralisation, if it was not diluted very fast by salivary clearance [Hallgren et al., 1990]. Indeed, the biofilm formed around the restoration is the only site where fluoride released in the oral cavity by GICs is retained and shows anticaries effect [Cenci et al., 2008].

Thus, experimental data show that GICs are able to interfere with caries development in the enamel or dentine adjacent to restoration sites by releasing fluoride, which reduces demineralisation and enhances remineralisation [Cury et al., 2016]; however, the key mechanisms have not been identified. Our findings showed three patterns of fluoride release in De- and Re- solutions (Figs 1-2). If reducing demineralisation is more relevant, the GICs that showed pattern A of fluoride release would be more effective at controlling caries. If fluoride is as effective at reducing demineralisation as it is at enhancing remineralisation, pattern C (as exhibited by Vitremer) would be highly effective at controlling caries. For the anti-caries effect of toothpaste, the effect of fluoride on enamel and dentine remineralisation might be more relevant than reducing demineralisation [Kusano et al., 2011]. Thus, further studies are required to test this phenomenon for GICs materials.

Caries is a chronic disease, with caries lesions progressing each time sugar is ingested [Cury et al., 2016]; thus, fluoride must be constantly available in biofilm to interfere with the caries process. Thus, the anti-caries potential of the tested GICs is reflected by their ability to sustain fluoride release, as shown in the quantitative results for daily (Figure 4) and cumulative (Figure 5) fluoride release. From the 6th day of evaluation, all materials presented sustained fluoride release, differing statistically on day 7 (Figure 4). Maxxion R released the most fluoride (27.7 µg F/cm^2), while Resiglass R released the least (0.41 µg F/cm^2). Only a very low physicochemical fluoride concentration is needed to interfere with caries [Cury et al., 2016] and could be reached in biofilm fluid by all materials [Cenci et al., 2008]; however, the 10-fold difference in fluoride release between Maxxion R and Resiglass R might be relevant in mitigating caries, but it should be evaluated by further study. On the other hand, the ability of Maxxion R to release the highest amount of fluoride could provoke the erosive dissolution of restoration [Bueno et al., 2019]. Restoration survival might be balanced with the anti-caries property of the material, considering the cost-effectiveness of this intervention.

The GICs evaluated in this study showed distinct qualitative and quantitative patterns of fluoride release under conditions simulating the process of caries development and might reflect their anti-caries properties. Among the tested materials, those that exhibited greater fluoride release in demineralising solution could be used to counterbalance high-risk caries activity. Fluoride released from GICs might be effective at controlling caries control; however, clinical evidence is required [Cury et al., 2016]. In instances with high caries risk or when fluoride toothpaste is not available [Cenci et al., 2008], GICs represent a good option for fluoride supply. Because the GICs available in the market might not have the same potential to control caries, other properties, besides fluoride release, could lead to different GICs being best for different clinical applications. Finally, the relevance of the distinct qualitative and quantitative patterns of fluoride release by the studied GICs studied should be further evaluated by validated models regarding dose-responses between the effect of fluoride release and enamel-dentine demineralisation and/or remineralisation.

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“The authors have no conflicts of interest to declare.”

Author Contributions

JAC conceived and designed the experiments; ABA performed the experiment; ABA and JAC analysed the data; ABA and JAC wrote the paper.

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3- Conclusão

Tendo em vista que:

a) Os CIVs avaliados apresentaram três padrões distintos de liberação de fluoreto: Maior liberação de fluoreto na solução Des- do que na Re- durante todo o período (A); Liberação inicial maior na solução Des- (B) e liberação similar em ambas das soluções durante todo o período (C). O padrão B foi o mais frequentemente notado.

b) Eles diferiram estatisticamente no tempo (dias) para apresentarem uma liberação sustentável de fluoreto

c) Eles também diferiram estatisticamente quanto ao grau de liberação diária e cumulativa de fluoreto

d) E que eles foram avaliados em condições simulando o processo físico-químico de desenvolvimento de lesões de cárie

Nossos resultados permitem concluir que os CIV avaliados mostraram padrões qualitativos e quantitativos distintos de liberação de fluoreto que deve refletir nos seus potenciais de controlar cárie dentária.

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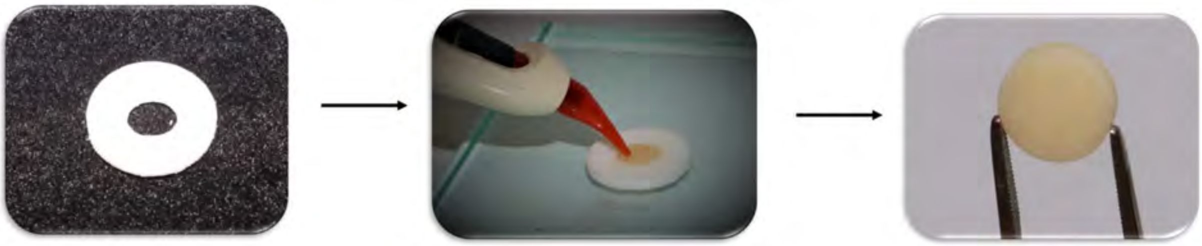
APÊNDICES

Apêndice 1

Ilustração de Material e métodos

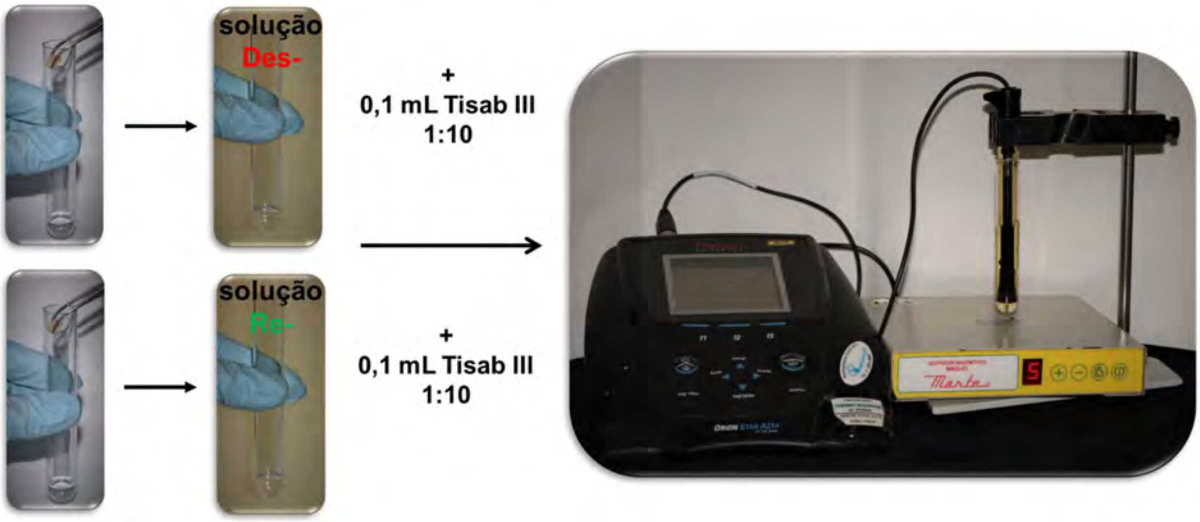
1a. Cimentos de ionômero de vidro convencionais utilizados.

1b. Cimentos de ionômero de vidro modificados por resina utilizados.



1c. Elaboração de corpos de prova

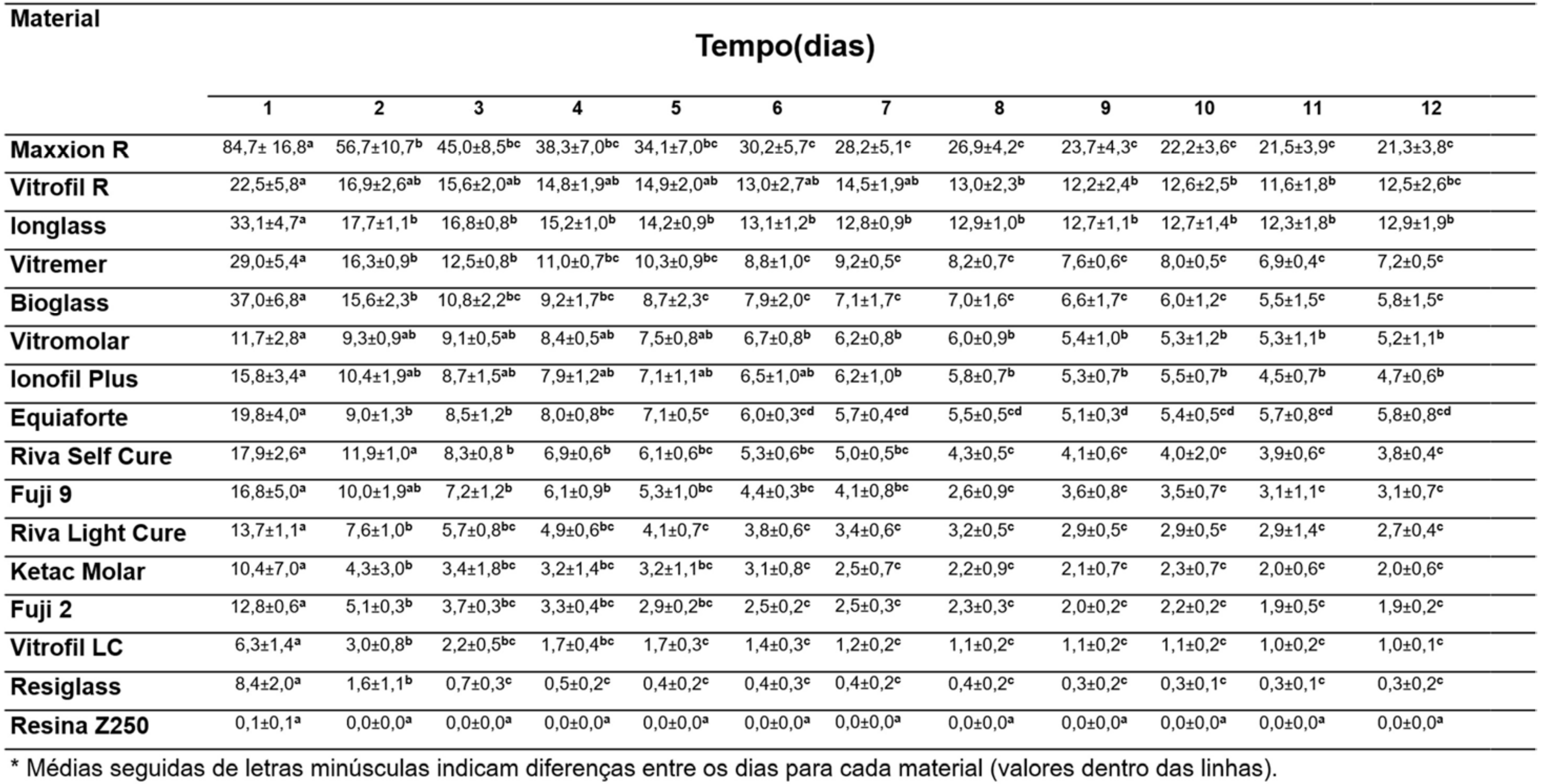
1d. Ciclagem de pH



1e . Análises de concentração de fluoreto nas soluções Des- e Re- com Eletrodo íon específico

Apêndice 2

Tabela 2. Média ± DP (n=6) da liberação diária de fluoreto (µg F/cm^{2}) dos materiais restauradores avaliados em relação ao tempo de imersão (dias) nas soluções Des+Reminerlizantes.



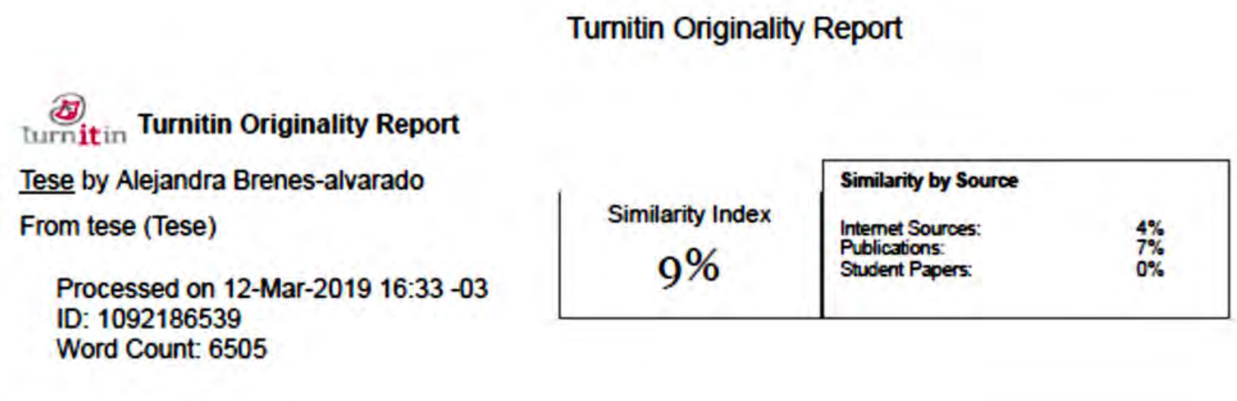
Material	Tempo(dias)											
	1	2	3	4	5	6	7	8	9	10	11	12
Maxxion R	84,7± 16,8^{a}	56,7±10,7^{b}	45,0±8,5^{bc}	38,3±7,0^{bc}	34,1±7,0^{bc}	30,2±5,7^{c}	28,2±5,1^{c}	26,9±4,2^{c}	23,7±4,3^{c}	22,2±3,6^{c}	21,5±3,9^{c}	21,3±3,8^{c}
Vitrofil R	22,5±5,8^{a}	16,9±2,6^{ab}	15,6±2,0^{ab}	14,8±1,9^{ab}	14,9±2,0^{ab}	13,0±2,7^{ab}	14,5±1,9^{ab}	13,0±2,3^{b}	12,2±2,4^{b}	12,6±2,5^{b}	11,6±1,8^{b}	12,5±2,6^{bc}
Ionglass	33,1±4,7^{a}	17,7±1,1^{b}	16,8±0,8^{b}	15,2±1,0^{b}	14,2±0,9^{b}	13,1±1,2^{b}	12,8±0,9^{b}	12,9±1,0^{b}	12,7±1,1^{b}	12,7±1,4^{b}	12,3±1,8^{b}	12,9±1,9^{b}
Vitremer	29,0±5,4^{a}	16,3±0,9^{b}	12,5±0,8^{b}	11,0±0,7^{bc}	10,3±0,9^{bc}	8,8±1,0^{c}	9,2±0,5^{c}	8,2±0,7^{c}	7,6±0,6^{c}	8,0±0,5^{c}	6,9±0,4^{c}	7,2±0,5^{c}
Bioglass	37,0±6,8^{a}	15,6±2,3^{b}	10,8±2,2^{bc}	9,2±1,7^{bc}	8,7±2,3^{c}	7,9±2,0^{c}	7,1±1,7^{c}	7,0±1,6^{c}	6,6±1,7^{c}	6,0±1,2^{c}	5,5±1,5^{c}	5,8±1,5^{c}
Vitromolar	11,7±2,8^{a}	9,3±0,9^{ab}	9,1±0,5^{ab}	8,4±0,5^{ab}	7,5±0,8^{ab}	6,7±0,8^{b}	6,2±0,8^{b}	6,0±0,9^{b}	5,4±1,0^{b}	5,3±1,2^{b}	5,3±1,1^{b}	5,2±1,1^{b}
Ionofil Plus	15,8±3,4^{a}	10,4±1,9^{ab}	8,7±1,5^{ab}	7,9±1,2^{ab}	7,1±1,1^{ab}	6,5±1,0^{ab}	6,2±1,0^{b}	5,8±0,7^{b}	5,3±0,7^{b}	5,5±0,7^{b}	4,5±0,7^{b}	4,7±0,6^{b}
Equiaforte	19,8±4,0^{a}	9,0±1,3^{b}	8,5±1,2^{b}	8,0±0,8^{bc}	7,1±0,5^{c}	6,0±0,3^{cd}	5,7±0,4^{cd}	5,5±0,5^{cd}	5,1±0,3^{d}	5,4±0,5^{cd}	5,7±0,8^{cd}	5,8±0,8^{cd}
Riva Self Cure	17,9±2,6^{a}	11,9±1,0^{a}	8,3±0,8 ^{b}	6,9±0,6^{b}	6,1±0,6^{bc}	5,3±0,6^{bc}	5,0±0,5^{bc}	4,3±0,5^{c}	4,1±0,6^{c}	4,0±2,0^{c}	3,9±0,6^{c}	3,8±0,4^{c}
Fuji 9	16,8±5,0^{a}	10,0±1,9^{ab}	7,2±1,2^{b}	6,1±0,9^{b}	5,3±1,0^{bc}	4,4±0,3^{bc}	4,1±0,8^{bc}	2,6±0,9^{c}	3,6±0,8^{c}	3,5±0,7^{c}	3,1±1,1^{c}	3,1±0,7^{c}
Riva Light Cure	13,7±1,1^{a}	7,6±1,0^{b}	5,7±0,8^{bc}	4,9±0,6^{bc}	4,1±0,7^{c}	3,8±0,6^{c}	3,4±0,6^{c}	3,2±0,5^{c}	2,9±0,5^{c}	2,9±0,5^{c}	2,9±1,4^{c}	2,7±0,4^{c}
Ketac Molar	10,4±7,0^{a}	4,3±3,0^{b}	3,4±1,8^{bc}	3,2±1,4^{bc}	3,2±1,1^{bc}	3,1±0,8^{c}	2,5±0,7^{c}	2,2±0,9^{c}	2,1±0,7^{c}	2,3±0,7^{c}	2,0±0,6^{c}	2,0±0,6^{c}
Fuji 2	12,8±0,6^{a}	5,1±0,3^{b}	3,7±0,3^{bc}	3,3±0,4^{bc}	2,9±0,2^{bc}	2,5±0,2^{c}	2,5±0,3^{c}	2,3±0,3^{c}	2,0±0,2^{c}	2,2±0,2^{c}	1,9±0,5^{c}	1,9±0,2^{c}
Vitrofil LC	6,3±1,4^{a}	3,0±0,8^{b}	2,2±0,5^{bc}	1,7±0,4^{bc}	1,7±0,3^{c}	1,4±0,3^{c}	1,2±0,2^{c}	1,1±0,2^{c}	1,1±0,2^{c}	1,1±0,2^{c}	1,0±0,2^{c}	1,0±0,1^{c}
Resiglass	8,4±2,0^{a}	1,6±1,1^{b}	0,7±0,3^{c}	0,5±0,2^{c}	0,4±0,2^{c}	0,4±0,3^{c}	0,4±0,2^{c}	0,4±0,2^{c}	0,3±0,2^{c}	0,3±0,1^{c}	0,3±0,1^{c}	0,3±0,2^{c}
Resina Z250	0,1±0,1^{a}	0,0±0,0^{a}	0,0±0,0^{a}	0,0±0,0^{a}	0,0±0,0^{a}	0,0±0,0^{a}	0,0±0,0^{a}	0,0±0,0^{a}	0,0±0,0^{a}	0,0±0,0^{a}	0,0±0,0^{a}	0,0±0,0^{a}

* Médias seguidas de letras minúsculas indicam diferenças entre os dias para cada material (valores dentro das linhas).

ANEXOS

Anexo 1

Relatório de verificação de originalidade e prevenção de plágio



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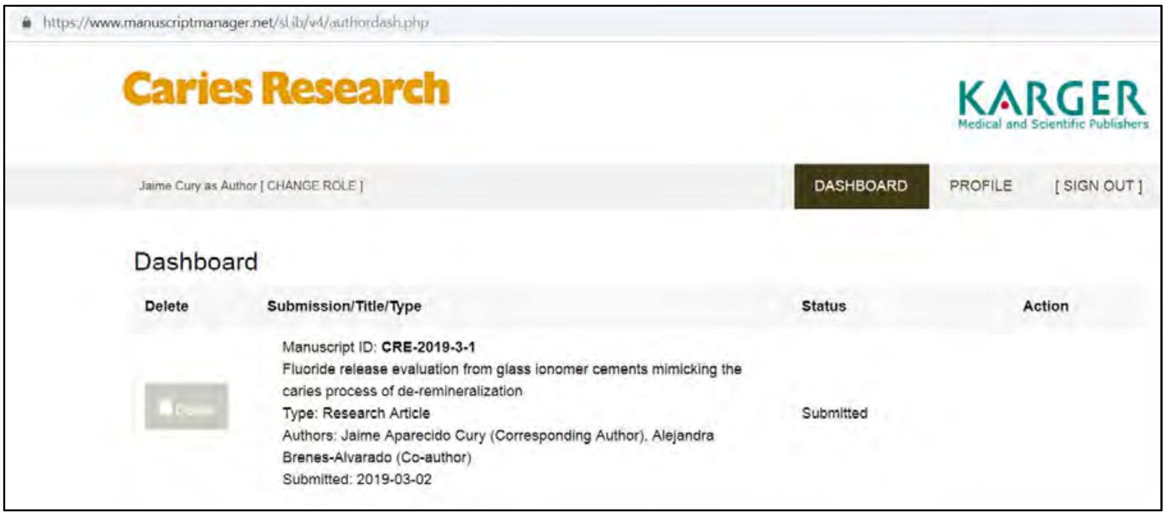

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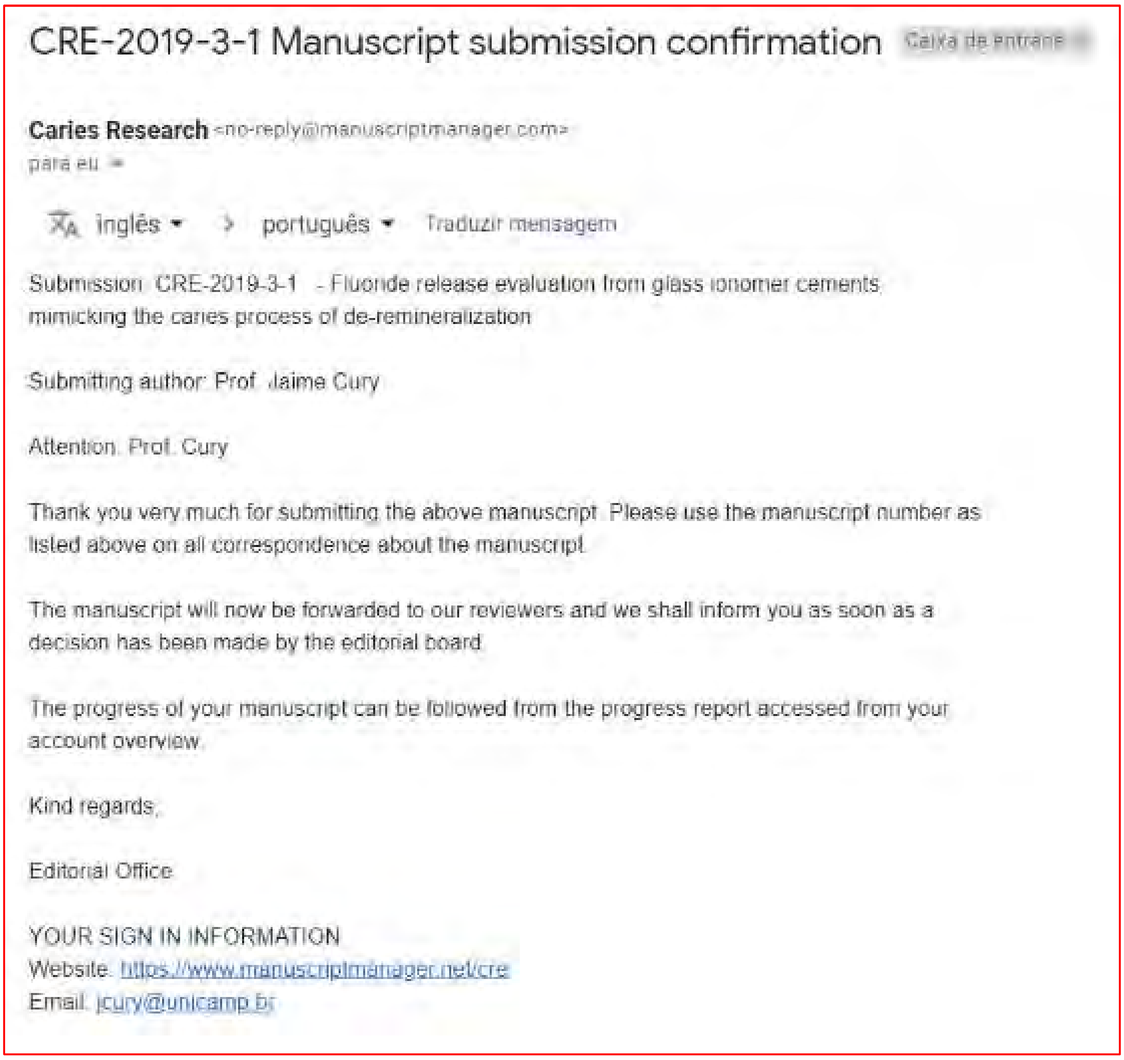

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