MAXIMILIANO SÉRGIO CENCI

AVALIAÇÃO DA ASSOCIAÇÃO ENTRE INFILTRAÇÃO MARGINAL E CÁRIE ADJACENTE A RESTAURAÇÕES DENTÁRIAS

Tese de Doutorado apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas para obtenção do Título de Doutor em Odontologia – Área de Cariologia

Orientador: Prof. Dr. Jaime Aparecido Cury

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“Do you have...” asked the first man.

“...an answer for you? Yes I have.” said *Deep Thought*

The two men shivered with expectancy. Their waiting has not been in vain.

“There really is one?” breathed the second man.

“There really is one” confirmed *Deep Thought*.

“To everything, to the great question of life, the universe and everything?”

“Yes”

Both of the men have been trained for this moment. Their lives have been a preparation for it. They have been selected at birth as those who would witness the answer. But even so they found themselves gasping and squirming like excited children.

“And you’re ready to give it to us?” asked him.

“I am”

They both licked their dry lips.

“Though I don’t think… that you’re going to like it”

“Doesn’t matter! We must know it! Now!”

“Now?” inquired *Deep Thought*.

“Yes, now!”

“All right”, said the computer and settled into silence again.

The two men fidgeted. The tension was unbearable.

“You’re really not going to like it” observed *Deep Thought*.

“Tell us!”

“All right” said *Deep Thought*. “The answer to the great question…”

“Yes?”

“…of life, the universe and everything” said *Deep Thought*

“Yes?”

“Is…”

“Yes?”

“42” said *Deep Thought*, with infinite majesty and calm.

…

"I think the problem is that the question was too broadly based..."

"Forty two?! Is that all you've got to show for seven and a half million years' work?"

"I checked it very thoroughly," said the computer, "and that quite definitely is the answer. I think the problem, to be quite honest with you, is that you've never actually known what the question is…"

Douglas N. Adams, 1978, *The Hitchhiker’s Guide to the Galaxy*

RESUMO

Cárie secundária é considerada a principal causa para a substituição de restaurações, e tem sido atribuída à microinfiltração ao longo das interfaces restauradoras. No entanto, além de não haver clara associação entre microinfiltração e essas lesões, essa associação poderia ser irrelevante se fluoreto (F) estivesse presente nessas interfaces. Portanto, este estudo *in situ* objetivou avaliar o efeito de microinfiltração em cárie adjacente a restaurações em presença de F, tanto individualmente fornecido por material restaurador ou dentifrício fluoretado como associado a partir dessas fontes. O estudo foi realizado em 4 fases de 14 dias cada. Os fatores em avaliação foram material restaurador em 2 níveis: RC – resina composta, e CIV – cimento de ionômero de vidro modificado por resina; condição de infiltração marginal em 2 níveis: L^- -sem indução de infiltração, e L^+ - com indução de infiltração; e tratamento com F em 2 níveis: DNF – dentifrício não fluoretado (placebo), e DF – dentifrício fluoretado. Da associação destes fatores foram obtidos subgrupos, os quais foram aleatoriamente designados aos voluntários (n=14). Durante todas as fases experimentais, foi permitido o acúmulo de biofilme sobre os blocos restaurados sendo esses submetido a um alto desafio cariogênico pela exposição a uma solução de sacarose 20%, 10x/dia, o que foi feito extra-oralmente. Os voluntários usaram três vezes por dia os dentifrícios DNF ou DF, dependendo da fase experimental. Não foram encontradas diferenças entre restaurações L^+ e L^-, considerando todas as variáveis estudadas ($p>0.05$). Maior desmineralização em esmalte e em dentina foi observada adjacente a restaurações de RC do que adjacente a restaurações de CIV quando DNF foi usado ($p<0.05$). A concentração de F foi maior no fluido do biofilme exposto ao dentifrício F ou formado sobre restaurações de CIV ($p<0.05$). Conclui-se que a microinfiltração não teve influência no desenvolvimento de cárie secundária em presença ou não de F fornecido por dentifrício ou material restaurador, e que a presença de F no biofilme fornecido por CIV ou DF é importante para inibir desmineralização adjacente às restaurações.

Palavras-chave: Cárie secundária (recorrente), flúor, microinfiltração, fendas marginais, biofilme dental.

ABSTRACT

Secondary caries is considered the main cause for restorations replacement, which has been attributed to microleakage throughout restorations interface. However, besides the fact that there is no clear association between microleakage and these lesions, this association could not be relevant if fluoride (F) is present at the restoration interface. Thus, this study aimed to evaluate the effect of microleakage on caries around enamel-dentine restoration under F presence, either individually from dental materials or dentifrice, or in combination. For this an *in situ* study involving a double-blinded, crossover design was carried out in 4 phases of 14 days each. The factors under evaluation were restorative materials at 2 levels: CR - composite resin, and GI - resin-modified glass ionomer cement; marginal leakage status at 2 levels: L^- - without leakage induction, and L^+ - with leakage induction; and fluoride treatment at 2 levels: NF – non-fluoride dentifrice (placebo), and FD - fluoride dentifrice. Therefore, experimental subsets were obtained from the association of these factors, which were randomly assigned to the volunteers (n = 14). Through all the experimental phases, biofilm accumulation was allowed on the restored slabs and a high cariogenic challenge was provided by exposing the biofilms to a 20% sucrose solution 10x/ day extra-orally. The volunteers used NF or FD 3x/day, depending on the experimental phase. No differences were found between L^+ or L^- restorations for all the variables studied ($p>0.05$). Higher demineralisation in both enamel and dentine around RC restorations than around GI restorations was observed under NF dentifrice use ($p<0.05$). F concentration was higher in the fluid of biofilm exposed to FD or formed onto GI restoration ($p<0.05$). It can be concluded from the results that microleakage has no influence on secondary caries in the presence or absence of F from dentifrices or restorative materials, and that the presence of F in the biofilm provided by GI and /or FD is important to inhibit demineralisation adjacent to restorations.

Key Words: Secondary (recurrent) caries, fluoride, microleakage, marginal gap, biofilm

SUMÁRIO

1 INTRODUÇÃO GERAL

A substituição de restaurações antigas consideradas defeituosas é responsável não só por dois terços de todas as restaurações realizadas por cirurgiões-dentistas como consome a maior parte do tempo de trabalho desses profissionais (Kidd, 1989, Özer, 1997, Mjör 2005). Lesões de cárie secundária são freqüentemente apontadas como o fator isolado mais importante e de maior prevalência relacionado ao diagnóstico de falha restauradora, sobretudo em estudos transversais, e muitas restaurações são indicadas para substituição com intenção de prevenir essas lesões (Mjör, 1997, Özer & Thylstrup, 1995; Rezwani-Kaminski *et al*., 2002).

Cárie secundária tem sido associada com o processo de microinfiltração de bactérias e carboidratos fermentáveis, e/ou de ácidos orgânicos entre a parede da cavidade e o material restaurador (Kidd *et al*., 1994; Fontana & González-Cabezas, 2000). Essa hipotética associação foi elaborada a partir da observação histológica de lesões de cárie de origem natural ou artificial adjacente a restaurações, onde linhas de desmineralização foram identificas ao longo das paredes cavitárias (Hals & Nernaes, 1971; Kidd, 1976; Kidd, 2001). Essas linhas de desmineralização, as chamadas "lesões de parede", seriam decorrentes da presença de microinfiltração (Kidd *et al*., 1992; Mjör & Toffenetti, 2000; Kidd, 2001) (Figura).

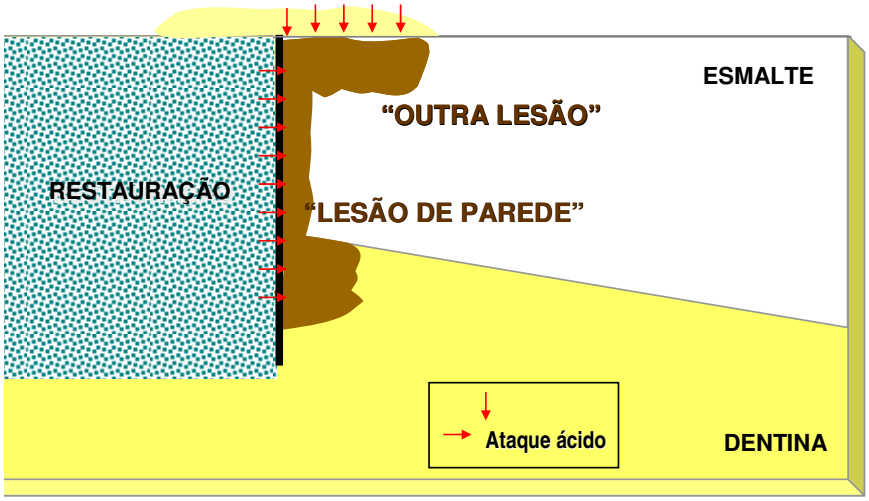

Figura - Aspecto histopatológico das lesões de cárie secundária, adaptado de Hals & Nernaes (1971).

Embora seja razoável esperar que a microinfiltração tenha um papel relevante no desenvolvimento de lesões de cárie adjacentes às restaurações, uma vez que ela representaria um caminho de difusão ao longo da interface dente-restauração, alguns questionamentos têm sido levantados contestando essa associação (Söderholm *et al.,* 1989; Anusavice, 1995; Özer & Thylstrup, 1995; Mjör, 1998; Mjör & Toffenetti, 2000; Mjör 2005). Enquanto alguns estudos sugerem clara associação entre cárie e defeitos marginais (Hodges *et al.*, 1995) e microinfiltração (Hals & Nernaes, 1971; Gilmour *et al.*, 1993; 1997; Grossman & Matejka, 1999), outros estudos refutam essa relação, alegando que apenas fendas marginais maiores do que 250 ou 400 µm poderiam permitir acúmulo de biofilme e conduzir à desmineralização ativa ao longo da interface dente-restauração (Söderholm *et al.*, 1989; Kidd *et al.*, 1994; Özer, 1997; Mjör, 2005). No entanto, a maioria dos estudos publicados fundamenta suas conclusões no exame de lesões de cárie em dentes extraídos caracterizando uma avaliação transversal, sendo necessários estudos avaliando a associação entre microinfiltração e cárie de forma prospectiva e em condições semelhantes às encontradas *in vivo* para contribuir com o seu completo entendimento.

Existe ainda divergência de definição quanto a esse tipo de lesão de cárie, tanto do ponto de vista histopatológico quanto clínico (Özer & Thylstrup, 1995; Mjör & Toffenetti, 2000; Thomas *et al.*, 2007). Os termos cárie secundária e cárie recorrente têm sido utilizados como sinônimos, e se referem a lesões que se desenvolvem adjacentemente a restaurações. A presença de cárie decorrente de tecido cariado deixado (propositadamente - principalmente junto a parede pulpar para evitar dano pulpar - ou não) nas cavidades após tratamento restaurador é definida como cárie residual ou remanescente, e pode estar presente nas margens das cavidades (Özer & Thylstrup, 1995). Uma vez que não é possível clinicamente diferenciar lesões adjacentes às restaurações não removidas durante preparo cavitário anterior ou “novas lesões” se desenvolvendo em contato com o material restaurador (cárie secundária), carie residual pode ser confundida com cárie secundária e vice-versa (Özer & Thylstrup, 1995).

Recentemente, a terminologia “lesões de cárie que se desenvolvem

adjacente às restaurações" foi proposta em substituição aos termos cárie secundária e cárie recorrente (Kidd & Fejerskov, 2004; Thomas *et al.*, 2007). Isso com base no entendimento de que cárie secundária é na verdade cárie primária ao longo das margens de restaurações (Kidd, 2001; Mjör 2005), sendo que essas lesões são mais prevalentes em região de acúmulo de biofilme, como as margens cervicais das restaurações (Özer,1997; Mjör & Toffenetti, 2000; Kidd, 2001). Com base neste entendimento, fatores relacionados às características do biofilme acumulado sobre a superfície do dente e restauração seriam mais relevantes para o desenvolvimento destas lesões, explicando sua iniciação e progressão. Dentre estes fatores, a presença de fluoreto (F) no biofilme, fornecido regularmente por dentifrício fluoretado ou liberado por material restaurador poderia modular o desenvolvimento das lesões, e interferir na relevância da presença de infiltração ou fendas marginais para o processo.

Desta forma, é bem conhecido que a presença de F no meio reduz cárie dental, reduzindo a desmineralização e ativando a remineralização de esmalte e dentina (Cury, 2001). Assim, estudos realizados *in vitro* e *in situ* têm demonstrado diminuição da progressão de lesões de cárie adjacentes às restaurações com materiais que liberam F (Benelli *et al.*, 1993; Dionysopoulos *et al.*, 1998; Kotsanos, 2001; Shinkai *et al*, 2001, Hara *et al.*, 2006). Esse efeito tem sido atribuído à liberação de íons-flúor por estes materiais e seu efeito físico-químico na manutenção da estrutura mineral dos dentes (Kotsanos, 2001; Hara *et al.*, 2002), embora algum efeito antimicrobiano do F per si ou potencializado por outras substâncias liberadas possa ocorrer (Haycibara *et al.*, 2003).

Portanto, embora possa haver associação entre microinfiltração e cárie, a presença de fluoreto no meio poderia anular essa relação. Assim, este estudo foi realizado com o objetivo de avaliar *in situ* o efeito de microinfiltração na desmineralização de esmalte e dentina adjacente a restaurações na presença F individualmente fornecido por material restaurador ou dentifrício, ou associado a partir dessas duas fontes.

2 CAPÍTULO*

Effect of microleakage and fluoride on enamel-dentine demineralisation around restorations

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Key Words: Secondary (recurrent) caries, fluoride, microleakage, marginal gap, demineralisation, biofilm

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Abstract

There is no consensus about an association between microleakage and secondary caries and even if there was, the presence of fluoride (F) at the restorations' interface could conceal it. Thus, a randomized, double-blinded, crossover study was carried out to evaluate *in situ* the effect of microleakage on caries around enamel-dentine restorations under F presence from dental materials or dentifrice, either alone or in combination. In 4 phases of 14 days each, 14 volunteers wore palatal devices containing dental slabs restored with RC – resin composite (Z250), or GI - resin-modified glass ionomer cement (Vitremer). Restorations were made without leakage (L^-), following the recommended adhesive procedures, or with leakage (L^+), in absence of adhesive procedures. Biofilm was left to accumulate on the restored slabs, which were exposed extra-orally to a 20% sucrose solution 10x/day. The volunteers used a non-F (NF) or a F (F) dentifrice 3x/day, depending on the experimental phase. Biofilm was collected for analysis and dental slabs were evaluated regarding mineral loss by transversal microradiography. No differences were found between L^+ or L^- restorations ($p>0.05$). Higher demineralisation in both enamel and dentine around RC restorations was observed under NF dentifrice ($p<0.05$). F concentration was higher in the fluid of biofilm exposed to F dentifrice or formed onto GI restoration ($p<0.05$). These results suggest that while microleakage does not affect caries development, GI or F dentifrice may maintain increased F levels in the biofilm, decreasing caries formation and progression.

Introduction

Secondary caries is considered the main cause for replacement of restorations [Mjör, 2005]. It has been associated to microleakage of bacteria and fermentable substrate, and/or diffusion of organic acids produced by the biofilm formed on the tooth/restoration surface throughout the restoration/cavity wall interface [Mjör and Toffentti, 2000; Mjör, 2005]. However, this association has been questioned based on clinical findings.

Some studies have shown a clear relationship between marginal defects and secondary caries [Hodges et al., 1995], including a bulk of available evidence from in vitro studies on the caries association with microleakage [Hals and Nernaes, 1971; Kidd, 1976; Grossman and Matejka, 1999]. However, most clinical studies have not confirmed that leakage or even marginal gaps lower than 250 or 400 µm could lead to active demineralisation beneath restorations [Kidd et al., 1994; Özer, 1997; Mjör, 2005].

In fact, secondary caries might equally well be considered as primary caries at the margins of existing fillings [Kidd and Fejerskov, 2004]. These lesions occur mainly in areas of dental biofilm stagnation, such as the cervical margins of restorations [Özer, 1997; Mjör and Toffenetti, 2000; Mjör 2005]. Therefore, the microbiological and biochemical composition of the biofilm accumulated on the tooth/restoration surface could better explain the development of these lesions than the presence of microleakage itself. Furthermore, the presence of fluoride (F) either from dentifrice use [Hara et al., 2006] or released from a dental material [Benelli et al., 1993; ten Cate and van Duinen, 1995] may significantly interfere with the initiation and progression of these lesions. However, F has been quantified in the whole biofilm rather than in biofilm fluid, the place where it exerts its effect on caries control [Tenuta et al., 2006].

Thus, this study aimed to evaluate in situ the effect of microleakage on caries around enamel-dentine restorations under the presence of F, either independently from a F-releasing dental material or F dentifrice, or by their combination. Also, the composition of the biofilm formed on the restoration was evaluated.

Materials and Methods

Panelists and Ethical Aspects

The study was approved by the local Research and Ethics Committee (Process 030/2004). Fourteen volunteers (aged 18 to 31 years; 7 male and 7 female) who fulfilled inclusion criteria (normal salivary flow rate, good general and oral health, ability to comply with the experimental protocol, not having used antibiotics during the 2 months prior to the study, not using fixed or removable orthodontic devices) were invited to take part in this study and those who agreed to participate signed an informed written consent. phase

Experimental Design

This in situ study involved a double-blinded, crossover design for caries induction by biofilm accumulation and sucrose exposure, carried out in 4 phases of 14 days each. The blinding procedures involved the codification of the treatments and materials used during the experiment, in such a way where both the volunteers and the researches could not identify them during the experimental and analyses stages. The factors under evaluation were marginal leakage status at 2 levels: L^- - without leakage induction, and L^+ - with leakage induction (a preliminary study was carried out to establish these conditions); restorative materials at 2 levels: RC = resin composite, and GI = resin-modified glass ionomer cement; and F treatment at 2 levels: NF = non-F dentifrice (placebo), and FD = F dentifrice (1100 µg F/g as NaF, silica-based – Tandy, Colgate Palmolive), as a factorial 2 X 2 X 2 design. The null hypotheses tested were that there would not be any effect of (1) presence of induced microleakage, (2) type of restorative material or (3) dentifrice use on the response variables assessed. Therefore, the 8 experimental subsets obtained from the association of these factors were assigned to the volunteers. At each experimental phase, volunteers used a palatal device loaded with four slabs restored with one material, with both marginal conditions: L^- (2 slabs) and L^+ (2 slabs) and used one of the dentifrices. Volunteers that had been assigned to one subset of restorative materials/dentifrice at one phase were randomized into different experimental subsets, characterizing a crossover design. Volunteers (n=14) were considered

experimental units for all variables under study. Biofilms were allowed to accumulate on the restored slabs and a high cariogenic challenge was provided by exposing the biofilms to a 20% sucrose solution 10 times/ day extra-orally. After each phase the biofilm formed was collected for microbiological and F concentration in the fluid and whole biofilm analyses. Ca and P_i were also analyzed in the fluid. Inorganic analyses of the biofilm were performed on fasting or after a cariogenic challenge. Mineral loss (ΔZ: vol % min.µm) was assessed by TMR (tranversal microradiography) in enamel and dentine adjacent to the restorations at various distances from the tooth-restoration interface (Figure 1).

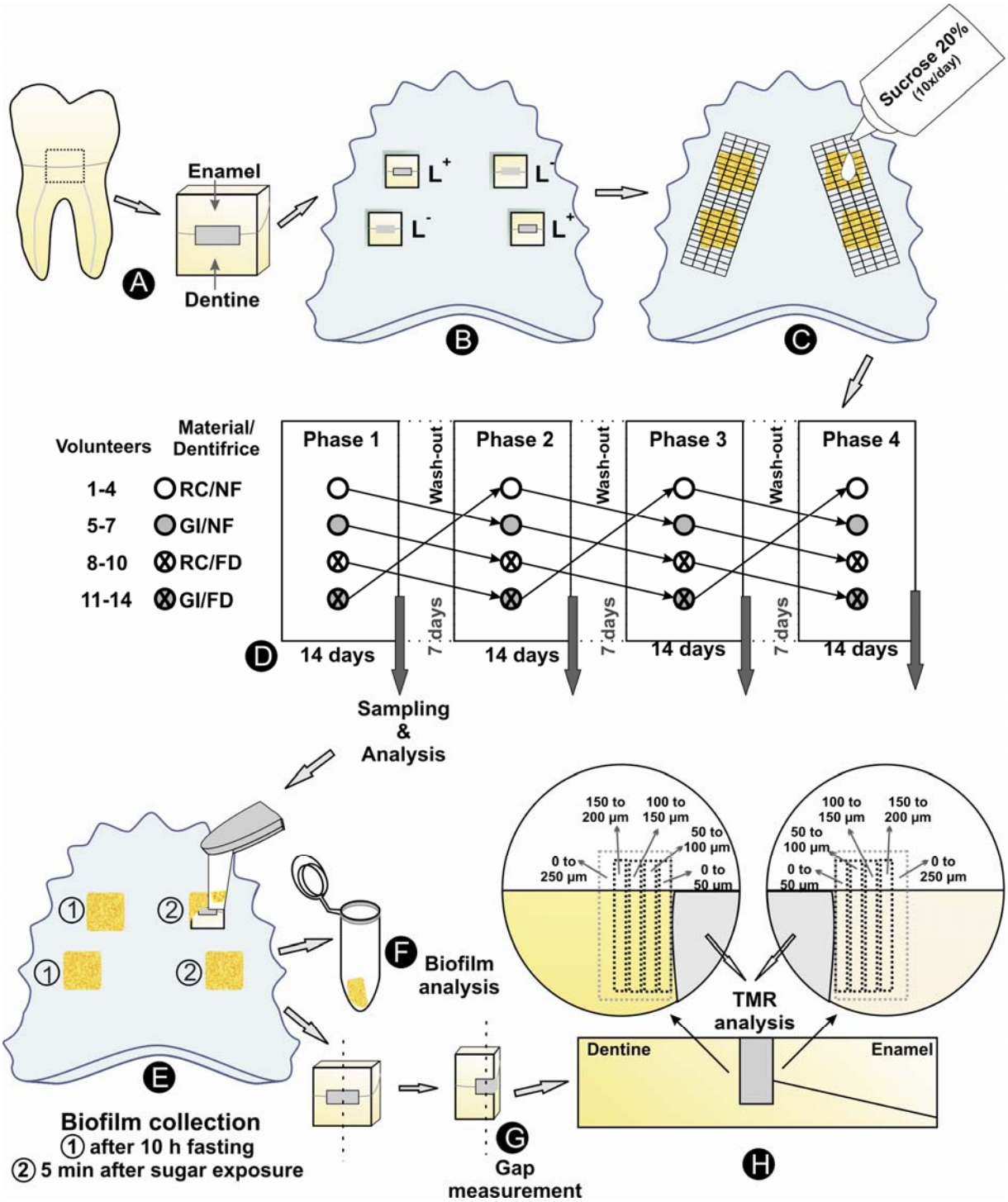

Figure 1. Illustration of the experimental design.

Preparation of Enamel-dentine Slabs

Enamel-dentine slabs (4 x 4 x 2 mm^3) were prepared from the cervical region of non-erupted human third molars (2 mm above and 2 mm below the cemento-enamel junction) previously stored in 10% formaldehyde solution, pH 7.0, for at least one week [Hara et al., 2003]. Box-shaped standardized cavities (2.5 x 1.0 x 1.5 mm) were prepared at the centre of each slab, with carbide burs (# 255, KG Sorensen, Barueri SP, Brazil, replaced after 5 preparations) at high-speed rotation and under water/air spray cooling. The cavities' occlusal margin was located in enamel, while the gingival margin was located in dentin (Figure 1A). The specimens were kept moist throughout all the steps. Before restorations placement, all cavities and slabs surfaces were cleaned with rotating brushes and non-F dentifrice and washed with distilled water.

Restorative Procedures

Resin composite restorations (RC)

Without leakage induction (L^-): adhesive procedures were carried out following the manufacturer's instructions with the SingleBond adhesive system (3M ESPE, St. Paul, MN, USA) and resin composite (Z250, 3M ESPE, shade A3) was placed in three layers of ~0.5 mm, each one polymerized for 20 seconds with a photocuring unit (RadII, SDI, Bayswater, Victoria, Australia). Final restorations were additionally polymerized for 40 s. Finishing and polishing were carried out 24 h later with aluminum oxide discs (SofLex System, 3M ESPE), according to the manufacturer's instructions.

With leakage induction (L^+): the slabs were restored as described above, but no acid etching or adhesive system was used. This procedure was previously shown to allow significantly higher microleakage (Table 1).

Resin-modified glass ionomer restorations (GI)

Without leakage induction (L^-): cavities were treated following the manufacturer's recommendations. After primer polymerization, the glass ionomer (Vitremer, 3M ESPE) was placed in a single bulk with a syringe (Centrix Inc., Shelton,

CT, USA) and polymerized for 40 s. After 24 h, restorations were finished and polished with aluminum oxide discs (SofLex System).

With leakage induction (L^+): in a pilot study, several approaches (water, saliva, and other contaminants) were tested in order to decrease the material's adhesion and to increase microleakage. Among the tested substances, distilled water (kept in excess in the cavity walls during restorations' placement) promoted the highest microleakage (data not shown) and was chosen for the experiment. Therefore, each cavity was covered with distilled water with a thin brush. Water accumulation was not allowed in the cavity floor. Next, the glass ionomer cement was placed in the cavities, polymerized, and finished and polished after 24 h as described.

After polishing, all the restored slabs were stored for up to 24 h in a moist environment, at 37 °C, until the preparation of the palatal devices.

Preparation of the Palatal Devices

A custom-made acrylic resin intraoral palatal device, containing two lateral cavities on each side measuring 5 x 5 x 3 mm, in which two slabs were placed, was made for each volunteer (Figure 1B). Plastic meshes were fixed leaving a 1 mm space for accumulation of dental biofilm on the slabs [Hara et al, 2003 for details].

Clinical Phase

Before and throughout each experimental phase, volunteers followed a 1-week lead-in period using the phase-designed dentifrice (non-F or F dentifrice).

Cariogenic challenge to the restored specimens was provided by dripping a 20% sucrose solution onto all slabs, 10 times/day (Figure 1C). Volunteers were instructed to remove the devices from the oral cavity, to remove the excess of saliva with gauze, and drip one drop of the solution onto each slab at 8.00, 9.30, 11.00, 12.30, 14.00, 15.30, 17.00, 19.00, 20.00 and 21.00 h. Reinsertion of the device was carried out 5 min after dripping the solution to allow sucrose diffusion through the biofilm. The volunteers were instructed to wear the devices all the time. During meal times, devices were kept moist in plastic boxes. Volunteers brushed their teeth and the device with the assigned dentifrice 3 times/day, after the main mealtimes. The

device was cleaned by brushing with the dentifrice, except for the area containing the slabs, where only the slurry from the dentifrice in use was gently spread. Volunteers lived in an optimally fluoridated city (0.7 mg F/L, for the region), and drank and consumed foods prepared with this water. No restriction was made with regard to the volunteers' diet. After each experimental phase, a one week wash-out period was allowed before the commencement of the new phase (Figure 1D).

Microbiological and biochemical analysis of the biofilm

The biofilm formed on the restored slabs was collected on the 14th day of each experimental phase, approximately 10 h after the last exposure to sucrose solution and dentifrice. For microbiological analyses, an aliquot (± 1 mg) was sampled with a plastic spatula after biofilm homogenization. The remaining biofilm was used for the biochemical analysis (Figure 1E and F).

Microbial analysis: biofilm was weighed to ± 10 µg (Analytical Plus AP 250D, Ohaus Corp., Florham Park, N.J., USA) in sterile microcentrifuge tubes, suspended in reduced transport fluid (RTF) [Syed and Loesche, 1972] (1 mL/mg biofilm, wet weight) and sonicated (Sonifier Vibra Cell, Sonics and Materials, Danbury, Conn., USA) at 40 W, 5% amplitude, 6 pulses of 9.9 s each [Bowen et al., 1988]. The suspensions were serially diluted in RTF and three drops of 20 µL were inoculated on blood agar (for enumeration of total bacteria), mitis salivarius agar (MSA) (total streptococci), mitis salivarius-bacitracin agar (MSB) [Gold et al., 1973] (mutans streptococci) and Rogosa SL agar (lactobacillus). The plates were incubated at 37 °C, in atmosphere of 10% CO_2 (MSB, MSA and Rogosa), or in anaerobiosis (blood agar) for 24–96 h. The colony-forming units (CFU) were counted using a stereomicroscope, and the results expressed in CFUs per milligram of dental biofilm. Different colony morphologies were identified by Gram staining and morphology, and biochemical tests of sugars fermentation were used to identify mutans streptococci.

Biochemical analysis of biofilm fluid and whole biofilm: samples were collected with a plastic spatula and immediately placed inside an oil-filled centrifuge tube [Vogel et al., 1997]. The biofilm was collected from one slab after 10 h fasting and from the other slab from each group 5 min after dripping a 20% sucrose solution

[Tenuta et al., 2006] (Figure1E). After determination of the sample weight (± 10 µg), the tube was centrifuged for 10 min (21,000 *g*) at 4°C to separate the fluid from the biofilm solids. The fluid was recovered with oil-filled capillary micropipettes and deposited on the surface of an oil-covered inverted F electrode (Orion, 94-09) with the use of a microscope and micro-reference electrode, held in a micromanipulator, to close the circuit [Vogel et al., 1997]. Biofilm fluid samples were diluted with TISAB III (1:10) on the surface of the F electrode by means of micropipettes [Vogel et al., 1997].

For analyses of total Ca and P_i in the biofilm fluid, quartz nanoliter volume pipettes [Vogel et al., 1990] were used to deposit standardized volumes of the samples or standards into Ca or P_i sensitive colorimetric reagents [Vogel et al., 1983]. The absorbance of the mixtures was read with the use of a 50-µL micro-cuvette (Hellma, 105.202, Müllheim, Germany) in a Beckman DU-65 spectrophotometer.

After fluid extraction, the tip of the centrifuge tube was cut, and the remaining biofilm was centrifuged into microcentrifuge tubes containing 0.5 M HCl (0.1 mL/10 mg of biofilm, wet weight) for extraction of acid-soluble whole biofilm F [Cury et al., 1997; Tenuta et al. 2006]. The samples were agitated at 30 rpm for 3 h at room temperature, centrifuged, and the supernatant was collected. The supernatant was then neutralized with TISAB II containing 0.5 M NaOH (1:1) and kept frozen until analyses. F in this acid extract was measured by a F-specific electrode (Orion, 96-09) coupled to a ion analyser (Orion, 720 A+).

Gap area evaluation

At the end of each experimental phase, the enamel/dentine slabs were removed from the intraoral devices and longitudinally sectioned through the middle of the restoration (Isomet 1000, Buehler Iltd. Lake Bluff, IL USA) (Figure 1G). In one hemi-section, the total area of the gaps at the restorations' interfaces was measured under a magnification of 400 X with a measuring microscope (Microhardness tester, Future-Tech FM, Future Tech Corp., Tokyo, Japan). Considering that the gap along the interface does not have any regular geometric shape, the measurement was performed in rectangular sections, as described by Loguércio et al. [2004]. The area

of each section was calculated (in μm^2) based on its width and length, and a sum of the areas from these sections up to a 200 µm depth along the restorations' interface was presented as the total gap area adjacent to restorations.

Enamel and Dentine Demineralisation Analyses

Mineral loss was quantified by tranversal microradiography (TMR) in all enamel and dentine lesions. For each sectioned slab, one slice (500 µm thickness) was cut through the middle region of the restoration using a diamond wire saw (Well, LeLocle, Switzerland). These sections were lapped to a thickness of 200 ± 10 µm (for dentine analysis) and subsequently to a final thickness of 100 ± 10 µm (for enamel analysis) using a precision lapping machine (Logitech, Glasgow, UK) making sure that sections were plano-parallel. Sections were mounted on a polyacrylate holder, together with an aluminum stepwedge, for calibration purposes. A photographic glass plate was mounted on the holder and radiographed using an X-ray generator (Philips, Eindhoven, The Netherlands). After developing the glass plate, an image of each lesion typically capturing a 500 µm area centred optically, was taken using a microscope-video camera-PC setup with dedicated software (TMR 2006 v 3.0.0.2, Inspektor Research Systems, Amsterdam, The Netherlands). Therefore, the integrated mineral loss (ΔZ; volume % mineral.µm) was obtained from 50 µm wide and 300 µm deep slices, in four distances from the restorations' margins: 0 to 50 µm; 50 to 100 µm; 100 to 150 µm; and 150 to 200 µm. A ΔZ value was also calculated for the total area, from 0 to 250 µm (Figure 1H).

Microleakage evaluation

Additional dental slabs were restored as described (n=8), sealed with nail varnish and then immersed in a 0.5% basic fuchsin solution for 24 h. Specimens were removed from the dye, cleaned, and sectioned through the middle of the restoration, in an occluso-gingival direction. Dye leakage was assessed with a video camera connected to a stereomicroscope (Leica MZ6, Wetzlar, Germany), and the images were analyzed with a dedicated software (Image Pro Plus 4.1 for Windows). Leakage along the restorations' interfaces was measured in µm (20x magnification).

Statistical analysis

Microleakage and gap area data were analyzed by the Mann-Whitney rank sum test. Data from the in situ experiment were analyzed using ANOVA. A randomized block design was used for the statistical analyses, considering the volunteers as statistical blocks, and type of dentifrice, type of restorative material and restorations' marginal condition as factors. The assumptions of equality of variances and normal distribution of errors were checked for each variable, and in case of assumptions violation, data were transformed. Enamel and dentine demineralisation were analyzed individually, at each distance from restoration. Different distances were compared by Repeated Measures ANOVA for each experimental subset fixed. Comparisons between the conditions of fasting and post-sugar challenge (biochemical analyses) were performed for each experimental subset independently, using paired tests (t test or Wilcoxon signed rank test). SAS System 9.1 software (SAS Institute Inc.) was used for ANOVA, and SigmaStat Version 3.1 (Systat Software, Inc.) was used for all paired tests and Repeated Measures ANOVA. The significance level was set at 5%.

Results

Procedures used to create non-adhering restorations were effective to induce higher leakage and a wider gap area around the restorations ($p < 0.05$), either for RC or GI, in both enamel and dentine margins (Table 1).

Table 1. Mean ± SD (median) microleakage (in µm, n= 8) and marginal gap area (in µm^2; n=14) in enamel and dentine margins.

Material	Marginal Status	Enamel		Dentine	
		Microleakage	Gap area	Microleakage	Gap area
Composte resin	Without leakage L^-	2.6 ± 7.3 (0.0)	79.1 ± 74.4 (59.8)	91.7 ± 86.4 (93.3)	276.1 ± 229.3 (184.9)
	With leakage L^+	449.7 ± 227.9 (482.1)	774.1 ± 332.7 (746.6)	732.4 ± 303.3 (729.6)	1452.4 ± 840.2 (1392.8)
Resin-modified glass ionomer	Without leakage L^-	244.2± 187.1 (276.0)	129.1 ± 128.1 (62.4)	85.0 ± 80.2 (0.0)	533.3 ± 371.0 (443.3)
	With leakage L^+	520.7± 215.1 (529.3)	1350.9 ± 999.8 (1263.3)	542.9 ± 428.6 (280.7)	2092.6 ± 1102.8 (1843.3)

L^- restorations showed lower microleakage and gap area adjacent to restorations than L^+ restorations ($p<0.05$, Mann-Whitney rank sum test).

Table 2 shows the results of the ANOVA for all variables studied. No significant effect of marginal condition was found for any of the variables under study. The effects of dentifrice and restorative material were significant for most of the variables, and their interaction was also significant for some.

For the mineral loss in the total area adjacent to restorations (0 to 250 µm), both in enamel and dentine margins, a statistically significant effect for the interaction Dentifrice*Material was found (Table 2). Demineralisation was significantly higher under NF dentifrice use on slabs restored with RC ($p < 0.05$), while under F dentifrice use demineralisation around RC and GI restorations was not statistically different ($p> 0.05$), neither for enamel nor for dentine.

Table 2. Analysis of Variance results (P-values)

Variables	Factors and interactions						
	Dentifrice	**Material**	**Marginal Condition**	**Dentifrice * Material**	**Dentifrice * Marginal Condition**	**Material * Marginal Condition**	**Dentifrice * Material * Marginal Condition**
Enamel ΔZ **0-250µm**	0.0384	0.0007	NS	0.0180	NS	NS	NS
Enamel ΔZ **0-50µm**	NS	0.0019	NS	NS	NS	NS	NS
Enamel ΔZ **50-100µm**	NS	0.0026	NS	0.0158	NS	NS	NS
Enamel ΔZ **100-150µm**	NS	0.0003	NS	0.0147	NS	NS	NS
Enamel ΔZ **150-200µm**	NS	0.0077	NS	NS	NS	NS	NS
Dentine ΔZ **0-250µm**	0.0012	0.0007	NS	0.0101	NS	NS	NS
Dentine ΔZ **0-50µm**	<.0001	<.0001	NS	0.0054	NS	NS	NS
Dentine ΔZ **50-100µm**	0.0004	<.0001	NS	0.0097	NS	NS	NS
Dentine ΔZ **100-150µm**	0.0002	<.0001	NS	0.0025	NS	NS	NS
Dentine ΔZ **150-200µm**	0.0001	<.0001	NS	0.0035	NS	0.0113	NS
TM	NS	NS	NS	NS	NS	NS	NS
TS	NS	NS	NS	NS	NS	NS	NS
SM	NS	NS	NS	NS	NS	NS	NS
LB	0.0371	NS	NS	NS	NS	NS	NS
F, Biofilm Fluid Fasting	<.0001	<.0001	NS	NS	NS	NS	NS
Ca, Biofilm Fluid Fasting	NS	0.0288	NS	NS	NS	NS	NS
Pi, Biofilm Fluid Fasting	NS	NS	NS	<.0001	NS	NS	NS
F, Biofilm Fluid Post-challenge	<.0001	<.0001	NS	NS	NS	NS	NS
Ca, Biofilm Fluid Post-challenge	NS	0.0405	NS	NS	NS	NS	NS
Pi, Biofilm Fluid Post-challenge	NS	NS	NS	NS	NS	NS	NS
F, Whole Biofilm Fasting	0.0003	0.0121	NS	NS	NS	NS	NS
F, Whole Biofilm Post-Challenge	<.0001	0.0004	NS	NS	NS	NS	NS

NS = not statistically significant (p > 0.05),TM = Total micro-organisms; TS = Total streptococci, SM = Mutans streptococci; LB = Lactobacilli.

Regarding the mineral loss in enamel at the various distances from the restorations' margins (Table 3), ANOVA showed a statistically significant effect for the factor Material in all distances, while for the distances 50 to 100 µm and 100 to 150 µm, the interaction Dentifrice*Material was also significant (Table 2). Therefore, when demineralisation was evaluated at the first 50 µm of enamel adjacent to the restoration, a higher demineralisation was observed around RC restorations when compared to GI restorations, regardless of dentifrice type ($p < 0.05$) (Table 3). The same trend was observed for the 150-200 µm area. However, for the other enamel areas, mineral loss was significantly higher around RC under NF dentifrice use, while under FD use no significant difference was found between the materials.

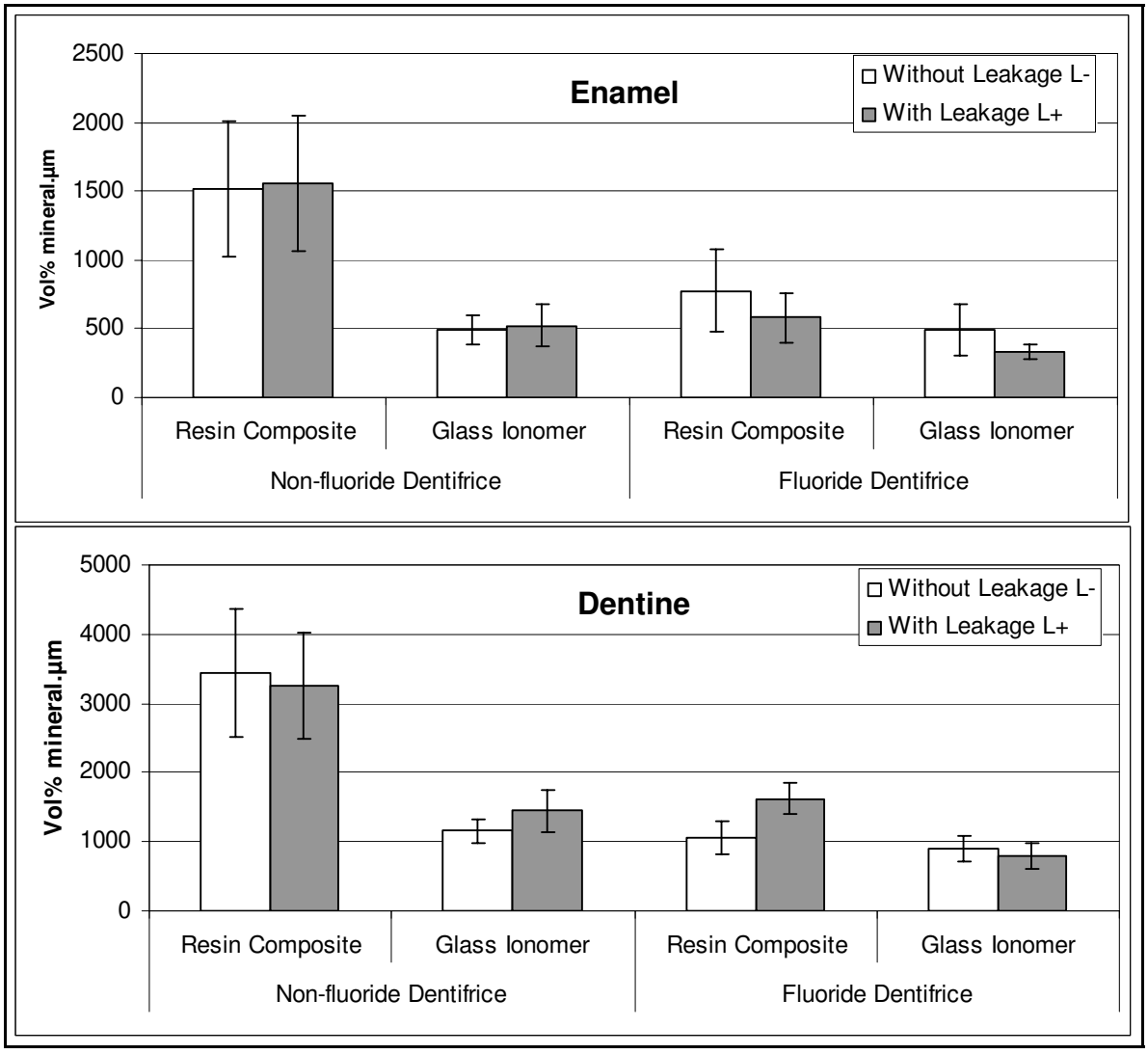

Figure 2. Mean ± SE (n = 14) enamel and dentine mineral loss (total area adjacent o restorations – 0 to 250 µm, vol% mineral*µm) according to treatment groups. Restorations with or without induced microleakage were not statistically different ($p > 0.05$). Under NF use, mineral loss was significantly higher in enamel and dentine around RC restorations ($p < 0.05$), but when FD was used, no significant differences were found between RC and GI ($p>0.05$).

Considering dentine margins, a statistically significant effect for Dentifrice*Material was found for all distances from the restoration (Table 2), with a significantly higher mineral loss around RC under NF dentifrice, although no

significant differences between the materials were observed when the F dentifrice was used (Table 3).

Table 3. Mineral loss (vol% mineral.µm), considering distances from restoration's margins.

Margin location (substratum)	Dentifrice	Restorative material	Restoration marginal status	Distance from restoration's margin (µm)			
				0 to 50	50 to 100	100 to 150	150 to 200
Enamel	Non-fluoride	Resin composite	Without leakage L^-	1748.8 ± 2128.6	1789.6 ± 2258.3	1600.8 ± 2043.9	1449.2 ± 1902.1
			With leakage L^+	1516.5 ± 1824.9	1468.8 ± 1841.7	1516.5 ± 1768.3	1371.5 ± 1717.2
		Resin-modified glass ionomer	Without leakage L^-	533.1 ± 499.3	558.5 ± 540.9	556.9 ± 496.7	618.1 ± 483.2
			With leakage L^+	540.7 ± 656.2	572.1 ± 545.0	485.0 ± 501.6	565.4 ± 626.6
	Fluoride	Resin composite	Without leakage L^-	1002.1 ± 1225.8	867.1 ± 1199.8	895.0 ± 1206.8	775.4 ± 1093.3
			With leakage L^+	686.4 ± 904.7	659.6 ± 822.2	634.3 ± 715.1	656.1 ± 756.6
		Resin-modified glass ionomer	Without leakage L^-	569.1 ± 683.8	674.5 ± 798.6	487.3 ± 588.9	432.7 ± 549.5
			With leakage L^+	345.8 ± 221.9	345.0 ± 237.9	382.9 ± 270.6	390.8 ± 263.1
Dentine	Non-fluoride	Resin composite	Without leakage L^-	3510.0 ± 3934.5	3480.4 ± 3482.1	3481.7 ± 3652.8	3310.0 ± 3434.9*
			With leakage L^+	3967.1 ± 3373.7	3477.3 ± 3315.7	3540.8 ± 3003.7	3267.1 ± 2592.1*
		Resin-modified glass ionomer	Without leakage L^-	915.8 ± 351.8	996.5 ± 560.4	974.2 ± 594.0	1136.2 ± 631.7*
			With leakage L^+	1091.9 ± 838.6	1314.2 ± 1103.7	1420.4 ± 1288.6	1464.6 ± 1236.5*
	Fluoride	Resin composite	Without leakage L^-	1038.3 ± 921.3	1024.2 ± 889.4	1002.9 ± 834.5	988.3 ± 838.5
			With leakage L^+	1405.4 ± 846.0	1625.0 ± 883.3	1605.4 ± 849.7	1604.3 ± 822.9
		Resin-modified glass ionomer	Without leakage L^-	724.2 ± 597.7	795.5 ± 575.3	870.9 ± 681.7	945.0 ± 633.3*
			With leakage L^+	504.1 ± 545.3	802.5 ± 742.6*	838.8 ± 839.4*	769.2 ± 811.5*

Values are Mean ± SD.* means a statistically significant difference in comparison to the 50 µm distance within each group ($p < 0.05$). Data from L^+ and L^- restorations are presented separately even without statistically significant differences in order to illustrate the lack of relationship between microleakage and caries adjacent to restorations.

The comparison of mineral loss at the various distances from the restoration margin within each subgroup (material/dentifrice combinations) showed differences only for dentine margins (Table 3), where a significantly higher

demineralisation was observed closer to RC than 150 to 200 µm away, when the NF dentifrice was used ($p < 0.05$) (Table 3). However, under FD use the difference was not significant ($p>0.05$). On the contrary, lower demineralisation in dentine was found closer to GI restorations for both dentifrices ($p < 0.05$, Table 3).

Differences in CFU counts [data not shown] were not found for the studied microorganisms ($p > 0.05$), except for lactobacilli, which counts under FD use were lower when compared with NF (Table 2, $p = 0.037$).

Table 4. Biochemical analysis of the biofilms, according to the experimental conditions

Biofilm's condition	Dentifrice	Restorative material	Biofilm fluid			Whole biofilm (wet weight basis)
			F (µM)	Ca (mM)	P_i (mM)	F (nmol/g)
Fasting 10 h	**Non-fluoride**	**Resin composite**	1.5 ± 0.6	2.1 ± 1.3	9.6 ± 3.9	399.2 ± 772.4
		Resin-modified glass ionomer	4.5 ± 2.3	1.7 ± 1.2	9.3 ± 4.4	664.6 ± 703.8
	Fluoride	**Resin composite**	2.7 ± 2.3	1.9 ± 1.5	9.4 ± 4.7	656.9 ± 681.2
		Resin-modified glass ionomer	7.6 ± 6.1	1.8 ± 1.1	10.9 ± 5.4	939.3 ± 811.5
5 min after sugar challenge	**Non-fluoride**	**Resin composite**	2.5 ± 2.1	4.5 ± 2.2*	7.9 ± 4.4*	241.8 ± 288.3
		Resin-modified glass ionomer	5.0 ± 2.7	3.8 ± 1.3*	7.6 ± 4.1*	400.5 ± 411.6
	Fluoride	**Resin composite**	6.3 ± 5.1*	5.3 ± 3.2*	7.8 ± 4.5*	535.8 ± 631.5
		Resin-modified glass ionomer	10.0 ± 5.6	3.8 ± 1.4*	7.4 ± 3.9*	1227.8 ± 1274.9

Values are Mean ± SD.* means significant difference ($p < 0.05$) from the fasting value, for comparisons made under the same experimental condition.

In the analysis of inorganic ions, the effects of dentifrice and material were significant for F in the fluid and whole biofilm (Table 2), with higher concentrations found on GI restorations when compared to RC or by FD use when compared to NF dentifrice (Table 4). Ca concentrations were lower in the fluid of biofilm formed on slabs restored with GI than on slabs restored with RC ($p = 0.029$), and no significant effect was found for P_i. After the sugar challenge, Ca concentration in the fluid increased and P_i significantly decreased for most groups ($p < 0.05$) (Table 4), while F concentrations did not change ($p > 0.05$), except in the fluid of biofilm on slabs

restored with RC under FD use, where the F concentration increased after sugar challenge ($p<0.05$).

In summary, the first null hypothesis formulated was not rejected since no differences were observed due to higher or lower microleakage at restorations' interfaces. The second and the third ones were rejected, since differences were found among restorative materials and dentifrices in the response variables studied.

Discussion

Understanding secondary caries is an important clinical issue, since most replacements of restorations are made because of secondary caries. This study was designed to explore the effects of marginal leakage on the initiation and progression of caries adjacent to enamel-dentine restorations in controlled in situ conditions under fluoride presence. The in situ model used was shown to be adequate to study enamel caries [Paes Leme et al., 2004], dentine caries [Hara et al., 2003] and secondary caries [Benelli et al., 1993; Hara et al., 2006]. In addition, the methods used to create marginal leakage on resin composite (RC) and glass ionomer (GI) restorations were effective to increase in 2 to 200 times the leakage and in 4 to 10 times the total gap area adjacent to restorations (Table 1).

The findings showed that the presence of microleakage around the restorations did not significantly change enamel or dentine demineralisation, even at the first 50 µm around the restorations (Figure 2; Table 3). Therefore, the results give support to a lack of association between microleakage and caries adjacent to restorations [Kidd et al., 1994; Özer and Thylstrup, 1995; Özer, 1997; Mjör, 2005], even for this highly cariogenic model, with 10x/day exposure to sucrose during 14 days of biofilm accumulation. Furthermore, the findings of the present study were obtained simulating a more realistic clinical condition, when small interfacial gap and high microleakage are produced due to the absence of ideally bonded restorations. In contrast, most of the clinical studies showing a lack of association between secondary caries and marginal defects have evaluated extracted teeth and considered the effects of marginal staining, macroscopic gaps or visual marginal defects on this association [Mjör, 2005, for review]. The disagreement of the present findings and in vitro data showing association between microleakage and gap presence with secondary caries [Hals and Nernaes, 1971; Kidd, 1976; Grossman and Matejka, 1999; Totiam et al 2007] is probably explained by the type of cariogenic model used [Grossman and Matejka, 1999]. Indeed, most of the in vitro models are more aggressive than in situ or in vivo conditions for secondary caries study [Özer

and Thylstrup, 1995], and also do not consider the presence of biofilm on the tooth-restoration surface.

For the development of caries lesions adjacent to restorations, the results showed evidence that the effect of the biofilm formed on the restorations is more important than the effect of microleakage itself. It is interesting to notice that in absence of FD a statistically higher ($p < 0.05$) demineralisation in dentine (not in enamel) was observed close to RC restoration (0 to 50 µm) than 200 µm from it (Table 3). Since this effect is independent of microleakage, it should be attributed to other factors. A possible explanation could be that bacterial colonization is facilitated closer to the restorations, due to differences in surface roughness or surface free energy among the dentine, enamel and the material and presence of shallow retentive sites, which affect bacterial colonization [Quirynen, 1994; Marsh and Nyvad, 2005]. Also, root surfaces are more heavily colonized and the biofilm develops faster than on enamel surfaces, [Nyvad and Fejerskov, 1987]. Thus, in the present in situ model, it is possible that biofilm accumulation started around the restoration margins, causing higher mineral loss in this area when RC was used. However, adjacent to GI restorations, higher demineralisation was found in dentine far from the cavity wall than close to it (Table 3). This lower demineralisation closer to GI restorations could be explained by a local high F concentration in the biofilm present in this area, as previously observed [Benelli et al, 1993; Hara et al., 2006]. In fact, a higher F concentration was found in the biofilm formed on GI than on RC when the NF was used (Table 3) but it was not possible to collect biofilm samples at different distances from the restoration for this specific evaluation.

Indeed, the effects of F, provided by either GI or FD, on the inhibition of dentine-enamel demineralisation is evident (Figure 2), since a higher demineralisation was observed around RC compared with GI when NF was used. However, in the presence of FD the demineralisation adjacent to both materials was similar. These findings are in agreement with the anticaries effect of F-releasing restorative materials or FD in enamel or dentine [Dijkman and Arends, 1992; Benelli et al., 1993; ten Cate and van Duinen, 1995; Hara et al., 2003; Paes Leme et al., 2004; Hara et al., 2006], and are supported by the analysis of F in the biofilms. Biofilms formed on

GI restorations or under FD use presented higher total F concentrations (Table 4), which agrees with previous studies evaluating F concentration in the whole biofilm formed on GI-restored slabs [Benelli et al., 1993] and under FD use [Paes Leme et al., 2004; Hara et al., 2006]. Additionally, this is the first study showing that either GI or FD are able to increase F in the biofilm fluid, where the degree of saturation with respect to tooth minerals determines their precipitation or dissolution. Moreover, this is the first time that the increase in F concentration in the fluid is demonstrated even 10 h after toothbrushing. Thus, F reservoirs formed in whole biofilm by FD use [Paes Leme et al., 2004] and the continuous release of F by GI may maintain a higher F concentration in the fluid. On the other hand, a synergistic effect of F released from GI combined with FD use was not found since the highest F concentration in the fluid of biofilm formed on GI restorations under FD use did not result in a significantly lower demineralisation in this group when compared to the GI used with NF or the RC used with FD, which finding is in agreement with Hara et al [2006].

With regard to the mechanism of F affecting microbial composition of the biofilms, the concentrations of F found in the fluid of the biofilms were lower than 10 ppm (526.3 µM), the minimum necessary to inhibit bacteria acidogenesis [Bradshaw et al., 2002]. This suggests that F effect of GI or FD may be mainly physicochemical on de/remineralisation and in fact the effect on mutans streptococci and lactobacilli abundance in the biofilms formed was marginal.

In addition to F, the biofilm fluid was also analyzed for Ca and P_i concentrations, either on fasting or 5 min after a sugar challenge. The significant lower Ca concentration in the fluid of biofilm formed on GI restorations could suggest that the higher availability of F in the fluid of these groups would affect Ca concentration, giving support to a complex equilibrium between tooth/restoration, biofilm mineral reservoirs and the fluid [Tenuta et al., 2006]. This could have been further complicated in the present study due to the presence of both enamel and dentine surfaces in the restored slabs, with different solubility and consequently different saturation levels for mineral dissolution or precipitation at a given mineral ion concentration and pH in the fluid [Lynch and ten Cate, 2006]. After the sugar challenge, the significant increase in Ca in the fluid in all groups, and in F in the group

restored with RC under FD use, suggests a pH-mediated release of these ions from biofilm reservoirs or enamel/dentine dissolution [Gao et al., 2001; Tenuta et al., 2006], which is not always observed for F due to its concomitant precipitation as fluorapatite or uptake by biofilm bacteria during the acidification of the fluid [Tenuta et al., 2006]. The utilization of P_i by bacteria during acid production could have accounted for its significant decrease after the sugar challenge [Tenuta et al., 2006].

One limitation of this study was the impossibility of studying the characteristics of biofilms formed within the interfacial spaces adjacent to restorations, due to the difficulties of sampling procedures for such micro-spaces. Future studies should also focus on the biofilm formed inside these gaps, in order to bring further information about ecological features and biochemical aspects on secondary caries progression.

In summary, microleakage does not seem to influence secondary caries while the presence of F in the biofilm provided either by GI or FD may be important to reduce demineralisation adjacent to restorations.

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3 CONSIDERAÇÕES GERAIS

A prevalência e a incidência de cárie dentária vêm diminuindo nas últimas décadas no Brasil e no mundo devido a fatores como o uso disseminado de dentifrício fluoretado (Cury et al., 2004). No entanto, as substituições de restaurações devido ao diagnóstico de cárie adjacente as mesmas (cárie secundária) têm se mantido constantes (Özer & Thylstrup, 1995; Thomas, 2007), e cárie secundária continua sendo a principal causa de falha de restaurações apontada na literatura (Mjör, 2005; Bernardo *et al.*, 2007). Essa aparente contradição induz ao raciocínio de que é necessário melhor entendimento da cárie secundária, e dos fatores possivelmente relacionados com sua iniciação e desenvolvimento. Essa tese foi desenvolvida com o objetivo de estudar *in situ* o papel da microinfiltração e da presença de fendas marginais no desenvolvimento das lesões de cárie, e da presença de fluoreto (F) liberado por material restaurador ou fornecido por dentifrício. Ainda, foram estudadas as características do biofilme formado sobre os blocos dentais restaurados, com o objetivo de melhor entender este processo.

Aspectos metodológicos

Embora os aspectos histopatológicos das lesões de cárie secundária tenham sido estabelecidos a partir de estudos *in vitro* de indução de cárie adjacente a restaurações, o valor deste tipo de ensaio tem sido questionado (Özer & Thylstrup, 1995). A principal crítica aos estudos *in vitro* consiste na dificuldade de extrapolação dos dados para situações *in vivo* (Mjör & Toffenetti, 2000) devido principalmente à falta de condições naturais de formação de película sobre as superfícies e acúmulo de biofilme sob influência dos fatores salivares e mecânicos presentes na cavidade bucal (Mjör & Toffenetti, 2000; Thomas, 2007).

Por outro lado, estudos sobre cárie secundária *in vivo* apresentam outros tipos de limitação, tais como: (1) não seria possível por motivos éticos fixar fatores ou induzir condições para, por exemplo, permitir acúmulo de biofilme sob condições controladas a fim de estudar prospectivamente o desenvolvimento de lesões de cárie; (2) não podem ser utilizados métodos precisos para avaliação quantitativa da

perda mineral (como microdureza ou microrradiografia) por serem destrutivos; (3) a alta variabilidade entre indivíduos requereria a inclusão de muitos sujeitos em um ensaio clínico, aumentando consideravelmente seu custo e dificuldade de execução; (4) no caso de estudos clínicos retrospectivos, haveria dificuldades para diferenciação entre as "novas" lesões de cárie secundária e as "velhas" lesões de cárie remanescentes, dificultando a interpretação dos resultados (Zero, 1995; Thomas, 2007).

Assim sendo, para desenvolvimento desta tese optou-se pelo modelo de estudo *in situ* o qual, além de permitir o estudo da relação entre cárie adjacente a restaurações e microinfiltração de forma controlada e prospectiva, permitiu também a inclusão de diversas variáveis de resposta. Adicionalmente, o modelo ainda apresenta as seguintes vantagens de acordo com Zero (1995): (1) estudos *in situ* podem ser conduzidos intra-oralmente, permitindo a inclusão de fatores salivares, e acúmulo natural de biofilme; (2) estes estudos facilitam o controle das variáveis experimentais em relação aos ensaios clínicos; (3) estes modelos facilitam a integração de diversas técnicas analíticas, aumentando a sensibilidade e a validade científica da metodologia; (4) o curto período de duração envolvido normalmente evita a implicação de problemas éticos relacionados a estudos com humanos; (5) o custo dos estudos *in situ* é geralmente muito mais baixo do que os dos ensaios clínicos. Ainda, estudos prévios demonstraram que o modelo utilizado é adequado para estudar cárie em esmalte, dentina, ou mesmo adjacente a restaurações. (Benelli *et al.*, 1993; Hara *et al.*, 2003; Paes Leme *et al.*, 2004; Hara *et al.*, 2006, entre outros).

Cárie adjacente a restaurações, microinfiltração, defeitos marginais e sua relação com o biofilme dental

Muitos artigos têm sido publicadas estudando microinfiltração relacionada a restaurações, tratando-se em sua maioria de estudos *in vitro* com a utilização de corantes, pressão por ar, entre outros métodos (Mjör & Toffenetti, 2000). No entanto, a aplicabilidade clínica desses ensaios tem sido questionada, assim como o papel clínico da microinfiltração *per se*, uma vez que essa propriedade poderia não estar

associada com propriedades adesivas dos materiais restauradores (Cenci *et al.*, 2005) e ainda poderia não estar associada ao desenvolvimento de cárie secundária (Mjör, 2005).

Cárie dental é uma doença localizada causada pelo acúmulo local de biofilme não perturbado mecanicamente, e pela exposição a carboidratos fermentáveis. De forma semelhante, cárie secundária é também um fenômeno localizado, intimamente associado com o acúmulo de biofilme e com sua evolução para uma condição cariogênica (Özer & Thylstrup, 1995). Como conseqüência, a presença de microinfiltração ou de fendas marginais de dimensões reduzidas nas interfaces restauradoras só seria fator predisponente ao desenvolvimento de cárie secundária se permitisse o acúmulo de biofilme e o aporte de substratos fermentáveis (Mjör, 2005). Há evidência de que os micro-espaços característicos de fissuras oclusais não permitem desenvolvimento de biofilmes com potencial cariogênico (Carvalho *et al.*, 1989, 1992; Özer & Thylstrup, 1995). De forma semelhante, é razoável esperar que os micro-espaços responsáveis pela microinfiltração também não permitam maturação desses biofilmes. Os resultados apresentados nesta tese corroboram essa hipótese, e indicam que o acúmulo de biofilme sobre restauração e dente, e sobretudo suas características, é que são os determinantes para o desenvolvimento de cárie.

Adicionalmente e de forma semelhante ao que é observado para lesões de cárie primária, foi mostrado no presente estudo que a presença de F no meio, fornecido por dentifrício ou liberado por material restaurador desempenham papel evidente na inibição das lesões. Foi possível observar esse efeito pela avaliação da concentração de F no biofilme total, e, de forma inédita, no fluido do bioflme formado sobre os blocos dentais restaurados, cujo grau de saturação em relação aos minerais do dente determina sua precipitação ou dissolução, ou em outras palavras, o potencial para desmineralização ou remineralização.

Um aspecto importante é que os resultados aqui apresentados mostrando falta de associação ente infiltração e cárie foram obtidos de forma controlada e prospectiva, simulando as condições naturais para desenvolvimento de cárie, onde restaurações com maior ou menor infiltração marginal, e com fendas marginais de

área maior ou menor foram submetidas ao mesmo desafio cariogênico para permitir inferências sobre o papel da microinfiltração no desenvolvimento de cárie, em presença ou ausência de F.

Implicações clínicas

Os resultados desta tese corroboram com a premissa de que a iniciação e progressão de cárie adjacente a restaurações é resultado do acúmulo local de biofilme, sobretudo na superfície das restaurações e estrutura dental. Assim, essas lesões de cárie podem ser prevenidas por medidas como uso de F e controle do acúmulo do biofilme (Mjör & Toffetti, 2000).

Os dados deste trabalho também suportam que a presença de defeitos marginais, manchamento e outras características associadas com microinfiltração não são indicativas da necessidade de substituição de restaurações, exceto por possíveis razões estéticas.

Outro dado relevante foi que na ausência de DF a utilização de materiais liberadores de F parece contribuir para inibição da desmineralização adjacente a restaurações. No entanto, estudos devem ser conduzidos avaliando se o potencial anticariogênico destes materiais se mantém constante após envelhecimento devido ao longo tempo de permanência na cavidade bucal.

Com base nestas afirmações, estudantes de odontologia e cirurgiões dentistas devem ser instruídos e treinados a evitar a substituição de restaurações em função da provável presença de microinfiltração ou de defeitos nas margens das restaurações, e instruídos a realizar diagnóstico de cárie secundária de forma semelhante ao de cárie primária, uma vez que os dois tipos de lesão se tratam do mesmo processo, apenas em localizações distintas.

Perspectivas futuras para o estudo de cárie secundária

Os aspectos histopatológicos relacionados à cárie secundária vêm sendo estudados há várias décadas, e um significativo aporte de informações e mudanças de conceitos foram introduzidas no final do século passado e na presente década (Mjör & Toffenetti, 2000; e Mjör 2005, para revisão). No entanto, ainda alguns

aspectos precisam ser melhor compreendidos, o que justifica a necessidade de realização de estudos nos seguintes campos: (1) características ecológicas e bioquímicas de biofilmes formados nas fendas marginais entre material restaurador e paredes cavitárias; (2) relação entre fendas marginais de diferentes dimensões e cárie secundária, em estudos prospectivos e de base clínica; e (3) potencial de inibição de cárie de materiais restauradores liberadores de F ou de substâncias antimicrobianas, levando-se em consideração o envelhecimento desses materiais após longos períodos de permanência *in loco* na cavidade bucal.

Adicionalmente, considerando-se a alta prevalência de substituição de restaurações atribuídas à presença de cárie secundária, e as dificuldades por parte dos dentistas de diagnosticar cárie adjacente a restaurações e sua relação com presença de defeitos marginais, manchamento, ou mesmo microinfiltração, verifica-se também a necessidade de melhor desenvolvimento para os seguintes aspectos: (1) métodos e ferramentas para diagnóstico de cárie adjacente a restaurações; e (2) programas de treinamento e desenvolvimento de material didático sobre cárie secundária para cursos de formação e reciclagem de cirurgiões dentistas.

Por fim, mesmo considerando-se que há muito ainda para ser trabalhado e entendido sobre cárie secundária, os resultados apresentados nesta tese trazem importante contribuição para o entendimento de aspectos relacionados à iniciação e progressão das lesões de cárie adjacentes a restaurações, reforçando a idéia de que cárie dental é uma doença causada pela quebra no equilíbrio fisiológico entre os minerais dos dentes e o fluido do biofilme dental frente a pressões ecológicas que interrompem a homeostasia, como uma condição de exposição a substratos fermentáveis e/ou baixo pH (Fejerskov, 2004; Marsh, 2006). Assim, fatores relacionados às características dos biofilmes formados na região da interface dente-restauração estão mais diretamente relacionados ao desenvolvimento de cárie secundária do que a presença de microinfiltração ou de micro-espaços entre a parede cavitária e material restaurador.

4 CONCLUSÃO GERAL

Os resultados deste estudo sugerem que a simples presença de infiltração marginal não é relevante para o desenvolvimento de lesões de cárie de esmalte e/ou dentina adjacente a restaurações, as quais podem ser inibidas por fluoreto fornecido seja por material restaurador ou dentifrício.

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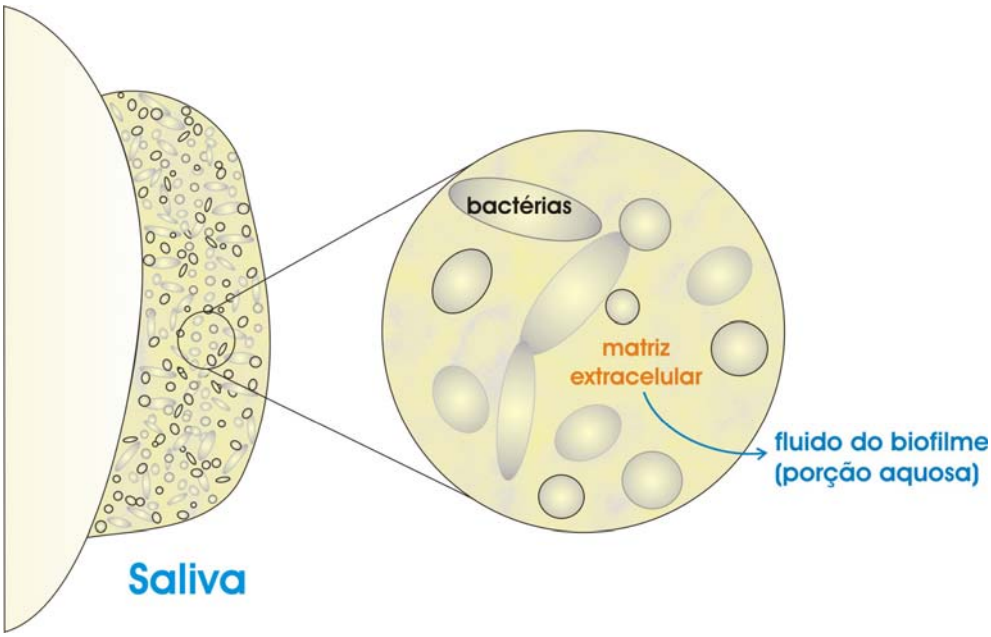
APÊNDICE 1 – Ilustração dos procedimentos para extração e análise do fluido do biofilme



Esquema ilustrando a presença do fluido, a porção aquosa constituinte do biofilme, a qual é responsável pelas trocas iônicas entre bioflime dental, minerais dos dentes e saliva, governando a ocorrência de desmineralização ou remineralização (cortesia de Tenuta, LMA).

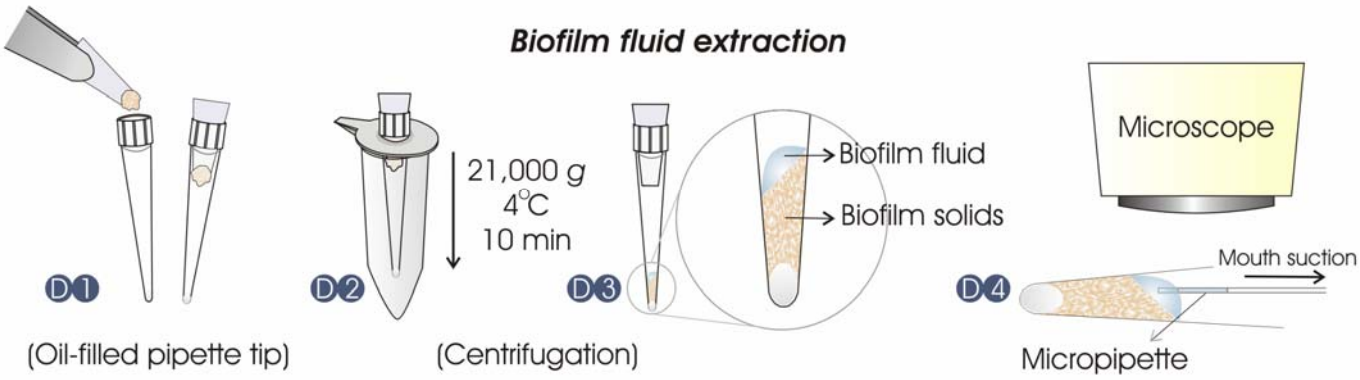


Ilustração dos procedimentos de coleta e extração do fluido do biofilme (cortesia de Tenuta, LMA).

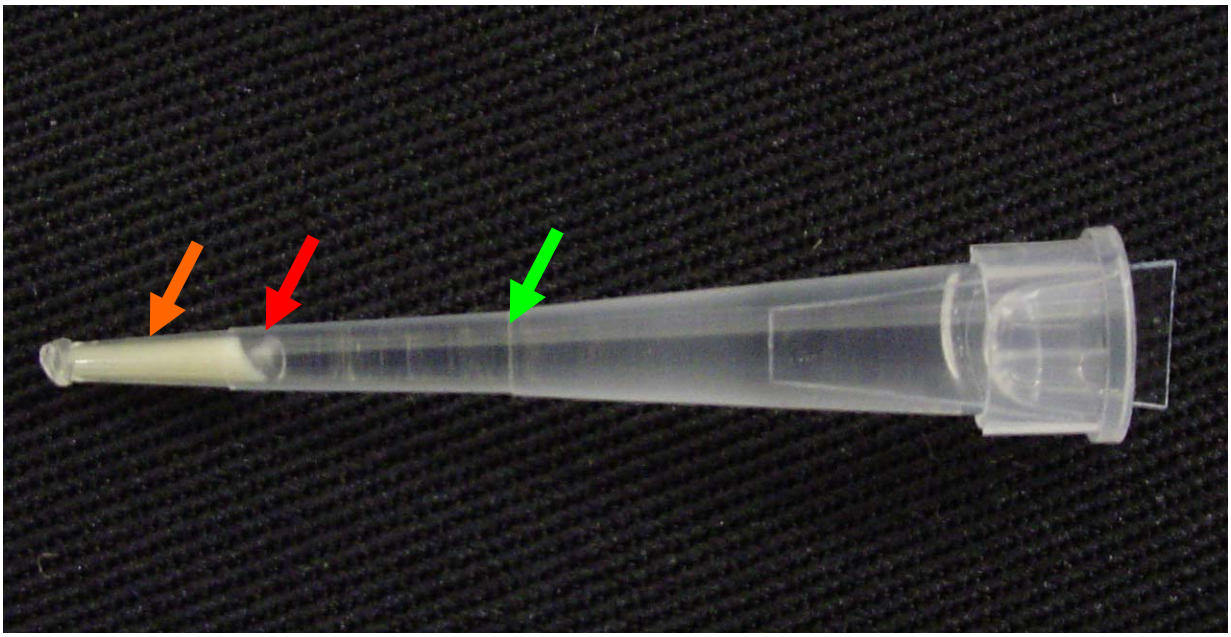


Foto mostrando o fluido do biofilme (seta vermelha) separado por centrifugação do estroma do biofilme (seta laranja), além do óleo mineral (seta verde) preenchendo a ponteira de pipeta (cortesia de Tenuta, LMA. Concentração e cinética de cálcio, fósforo inorgânico e fluoreto no fluido do biofilme dental formado na presença de sacarose (tese) Piracicaba: Faculdade de Odontologia de Piracicaba – UNICAMP; 2005.)

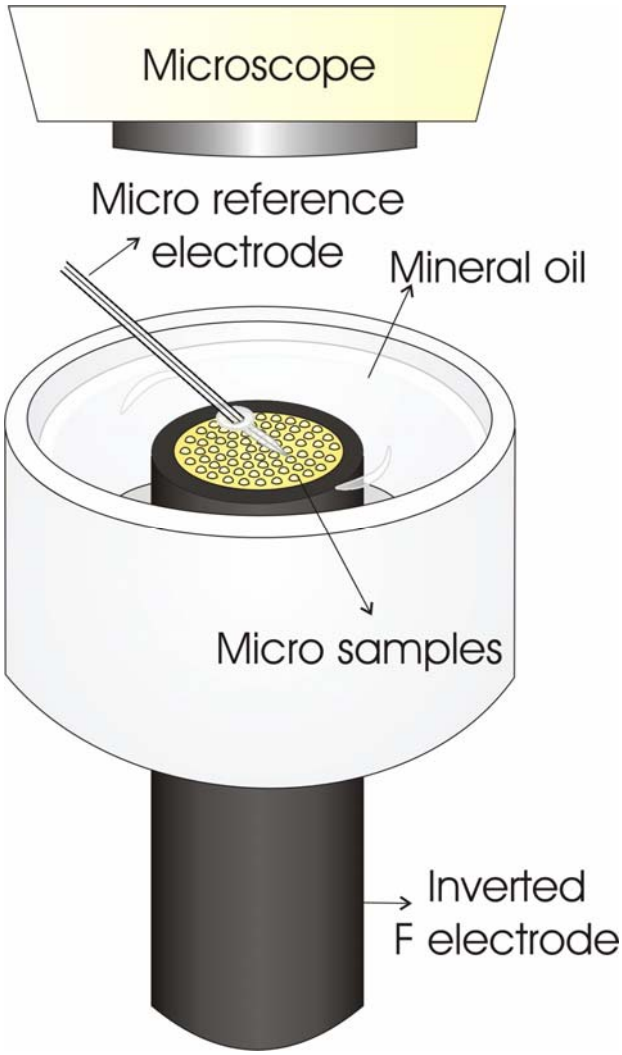


Ilustração do aparelho utilizado para microanálise de fluoreto.

As amostras eram aplicadas na superfície do cristal sensível a fluoreto no eletrodo invertido íon-específico, diluídas com TISAB III (10:1), sob óleo mineral. Com o auxílio de um microscópio, o microeletrodo de referência era posicionado em contato com as amostras, fechando o circuito e permitindo a determinação da concetranção de fluoreto através de um potenciômetro (cortesia de Tenuta, LMA. Concentração e cinética de cálcio, fósforo inorgânico e fluoreto no fluido do biofilme dental formado na presença de sacarose (tese) Piracicaba: Faculdade de Odontologia de Piracicaba – UNICAMP; 2005.)

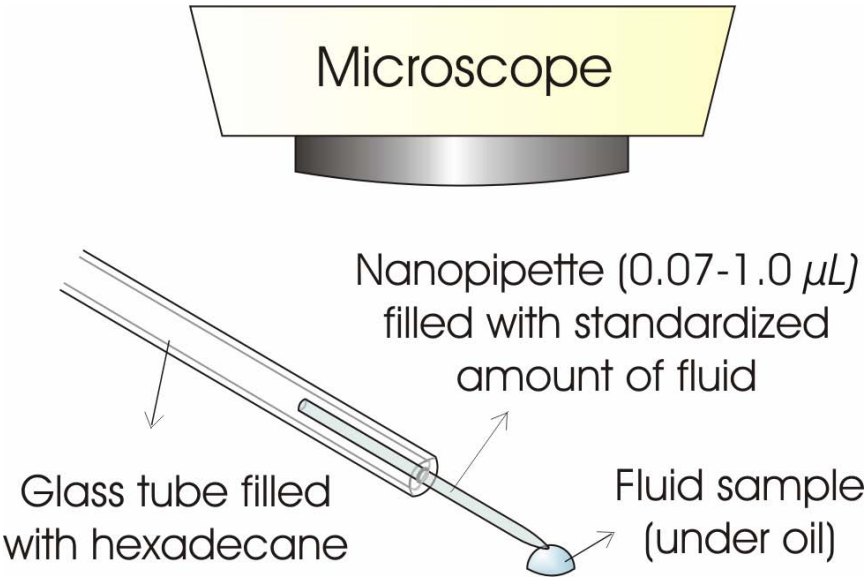
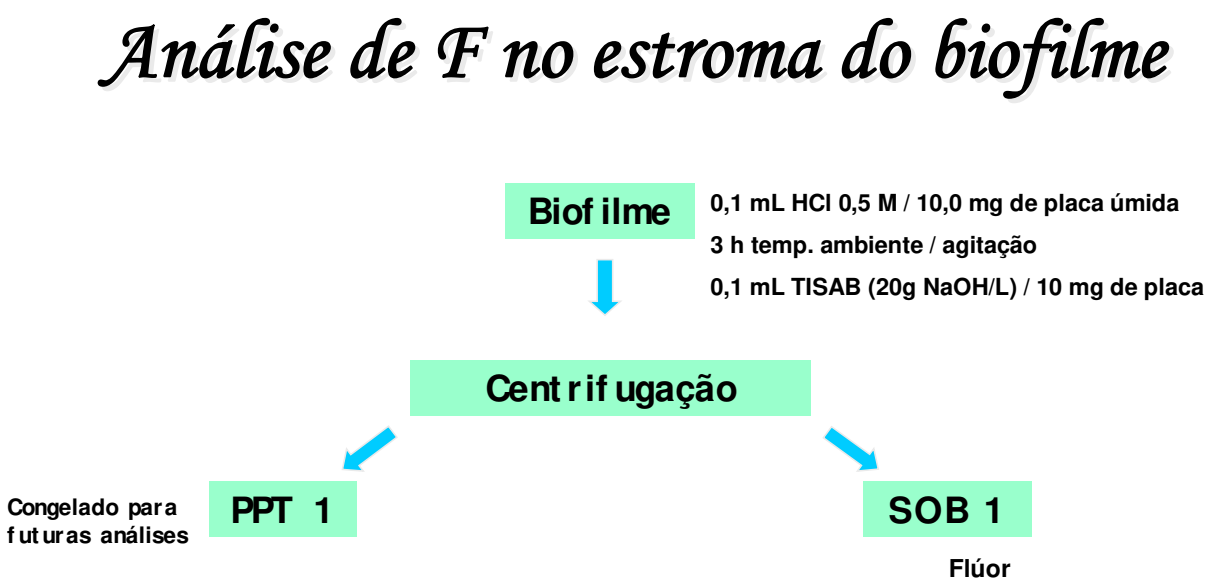


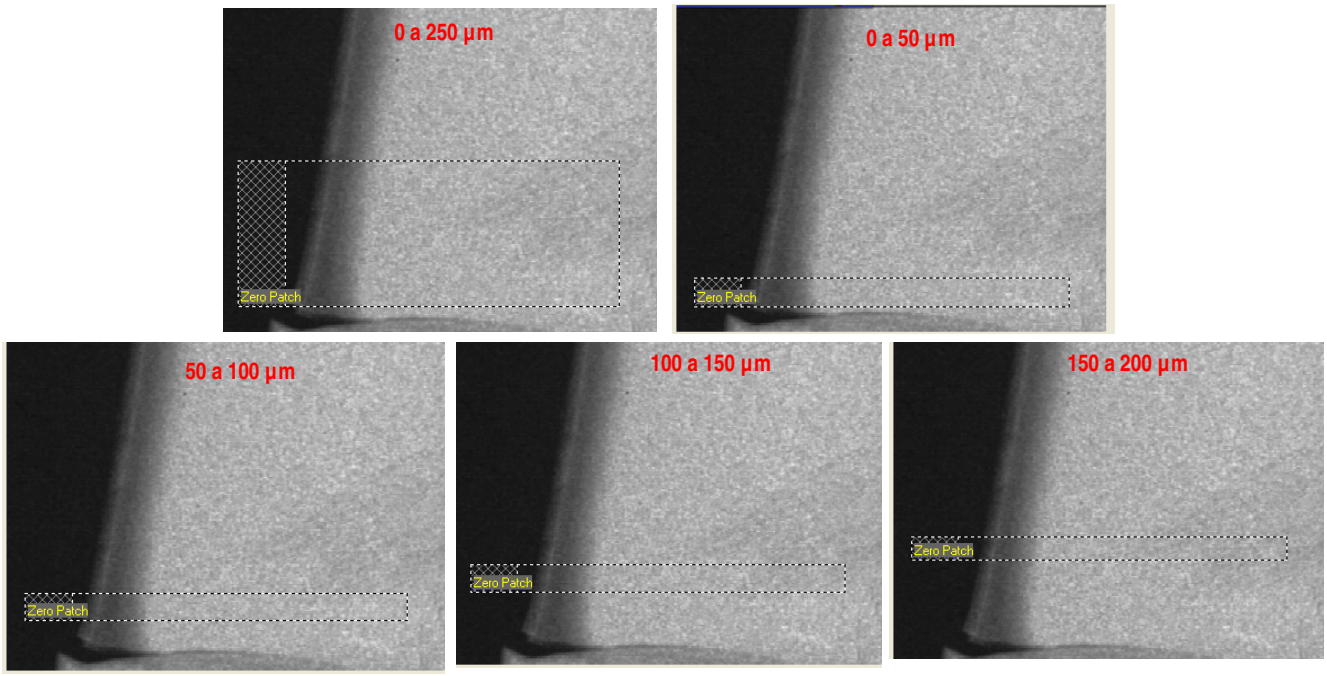
Ilustração da coleta de fluido para transferência de volumes padronizados para reações colorimétricas e determinação das concentrações de cálcio e fósforo inorgânico. (cortesia de Tenuta, LMA. Concentração e cinética de cálcio, fósforo inorgânico e fluoreto no fluido do biofilme dental formado na presença de sacarose (tese) Piracicaba: Faculdade de Odontologia de Piracicaba – UNICAMP; 2005.)

APÊNDICE 2 – Fluxograma representando o tratamento do estroma do biofilme após a retirada do fluido para extração e dosagem de fluoreto



Cury *et al.,* 1997; 2000; Tenuta *al.,* 2006.

APÊNDICE 3 – Ilustração da avaliação da perda mineral por microrradiografia de secção tranversal, realizada nas diferentes distâncias das bordas das restaurações



APÊNDICE 4 – Tabela completa com os valores de P obtidos com as análises de variância para as variáveis de resposta do estudo *in situ*.

Variables	Factors and interactions						
	Dentifrice	**Material**	**Marginal Condition**	**Dentifrice * Material**	**Dentifrice * Marginal Condition**	**Material * Marginal Condition**	**Dentifrice * Material * Marginal Condition**
Enamel ΔZ **0-250µm**	0.0384	0.0007	0.6672	0.0180	0.8842	0.8092	0.9472
Enamel ΔZ **50µm**	0.1122	0.0019	0.2388	0.0867	0.4940	0.6944	0.8950
Enamel ΔZ**100µm**	0.0920	0.0026	0.4384	0.0158	0.9724	0.9131	0.4518
Enamel ΔZ **150µm**	0.1157	0.0003	0.5092	0.0147	0.8051	0.7906	0.9635
Enamel ΔZ **200µm**	0.1250	0.0077	0.8692	0.0517	0.6908	0.8650	0.8572
Dentine ΔZ **0-250µm**	0.0012	0.0007	0.3624	0.0101	0.9570	0.0682	0.1476
Dentine ΔZ **50µm**	<.0001	<.0001	0.3408	0.0054	0.8198	0.0502	0.3996
Dentine ΔZ**100µm**	0.0004	<.0001	0.0973	0.0097	0.6313	0.2111	0.2638
Dentine ΔZ **150µm**	0.0002	<.0001	0.0521	0.0025	0.9286	0.1689	0.2557
Dentine ΔZ **200µm**	0.0001	<.0001	0.1843	0.0035	0.8821	0.0113	0.0718
TM, CFU	0.6960	0.7652	0.4082	0.1873	0.8540	0.2418	0.6747
TS, CFU	0.2665	0.7296	0.6444	0.3686	0.7393	0.2193	0.6257
SM, CFU	0.9951	0.8132	0.9786	0.0275	0.7149	0.1691	0.2886
% SM/ TM	0.9149	0.6067	0.5296	0.0360	0.7629	0.1576	0.3150
% SM / TS	0.5610	0.8150	0.8860	0.0293	0.8628	0.0806	0.4400
LB, CFU	0.0371	0.5659	0.2934	0.6328	0.9249	0.7857	0.6691
% LB/ TM	0.0539	0.3414	0.3441	0.4126	0.7576	0.5444	0.5443
Ca, Biofilm Fluid Fasting	0.2284	0.0288	0.4527	0.1275	0.7102	0.8095	0.7834
Pi, Biofilm Fluid Fasting	0.4427	0.9262	0.9650	<.0001	0.5998	0.6114	0.6931
F, Biofilm Fluid Fasting	<.0001	<.0001	0.7588	0.2576	0.6307	0.5069	0.6984
Ca, Biofilm Fluid Post-challenge	0.6915	0.0405	0.3041	0.1913	0.5621	0.3795	0.3396
Pi, Biofilm Fluid Post-challenge	0.8723	0.9558	0.3414	0.1624	0.9818	0.6392	0.9081
F, Biofilm Fluid Post-challenge	<.0001	<.0001	0.6601	0.3847	0.4658	0.9339	0.8805
F, Whole Biofilm Fasting	0.0003	0.0121	0.4119	0.8995	0.7435	0.9423	0.9591
F, Whole Biofilm Post-Challenge	<.0001	0.0004	0.5482	0.8346	0.3779	0.3830	0.5469

CFU = Colony forming units / mg biofim TM = Total micro-organisms; TS = Total streptococci, SM = Mutans streptococci; LB = Lactobacilli

APÊNDICE 5 – Tabela com os resultados da análise microbiológica dos biofilmes, cujos dados não foram apresentados no texto da tese.

Table. Microbiological analysis of the biofilms, according to the experimental conditions

Dentifrice	Material	Marginal Status	S. mutans (CFU/mg x 10^3)	Lactobacilli (CFU/mg x 10^6)	Total streptococci (CFU/mg x 10^7)	Total micro-organisms (CFU/mg x 10^7)	% S. mutans/ Total streptococci	% S. mutans/ Total micro-organisms	% Lactobacilli/ Total micro-organisms
Non-fluoride	**Composite resin**	**Without leakage L^-**	6.79 ± 18.45	2.68 ± 4.56	1.10 ± 0.61	3.80 ± 2.04	0.11 ± 0.31	0.012 ± 0.031	9.81 ± 13.08
		With leakage L^+	3.49 ± 10.55	3.13 ± 4.64	1.05 ± 0.70	3.60 ± 2.22	0.05 ± 0.13	0.009 ± 0.018	13.13 ± 19.29
	Glass ionomer	**Without leakage L^-**	0.68 ± 0.99	4.70 ± 7.24	1.36 ± 0.87	4.55 ± 3.34	0.01 ± 0.02	0.003 ± 0.006	13.30 ± 20.30
		With leakage L^+	1.36 ± 3.26	3.97 ± 5.82	1.75 ± 1.11	5.43 ± 2.28	0.01 ± 0.02	0.002 ± 0.004	10.38 ± 15.31
Fluoride	**Composite resin**	**Without leakage L^-**	2.54 ± 7.46	1.90 ± 2.15	1.79 ± 1.42	4.45 ± 3.22	0.01 ± 0.03	0.005 ± 0.015	6.91 ± 10.55
		With leakage L^+	1.58 ± 3.59	1.78 ± 2.05	1.21 ± 0.56	3.57 ± 1.59	0.01 ± 0.03	0.004 ± 0.006	6.52 ± 9.07
	Glass ionomer	**Without leakage L^-**	1.47 ± 2.14	2.15 ± 5.01	1.35 ± 0.62	4.26 ± 2.37	0.01 ± 0.01	0.003 ± 0.003	7.55 ± 18.03
		With leakage L^+	0.81 ± 1.23	2.62 ± 4.15	1.81 ± 1.50	4.71 ± 2.04	0.01 ± 0.01	0.001 ± 0.002	6.34 ± 9.67

APÊNDICE 6 – Tabela com os resultados da análise bioquímica dos biofilmes, apresentando os dados de acordo com todos os fatores em estudo e combinações de fatores.

Table. Biochemical analysis of the biofilms, according to the experimental conditions

Biofilm's condition	Dentifrice	Restorative material	Restoration marginal status	Biofilm fluid			Whole biofilm (w/w basis)
				Ca (mM)	P_i (mM)	F (µM)	F (µmol/g)
Fasting 10 h	**Non-fluoride**	**Resin composite**	**Without leakage L⁻**	2.1 ± 1.6	9.4 ± 4.1	1.5 ± 0.64	0.41 ± 0.85
			With leakage L⁺	2.1 ± 1.4	10.0 ± 4.0	1.5 ± 0.55	0.38 ± 0.72
		Glass ionomer	**Without leakage L⁻**	1.8 ± 1.4	8.8 ± 4.0	4.7 ± 3.6	0.84 ± 1.6
			With leakage L⁺	1.6 ± 1.3	8.7 ± 3.9	4.3 ± 2.3	0.49 ± 0.80
	Fluoride	**Resin composite**	**Without leakage L⁻**	1.9 ± 1.6	9.3 ± 4.9	2.8 ± 3.0	0.77 ± 1.1
			With leakage L⁺	1.8 ± 1.4	8.5 ± 2.9	2.7 ± 1.8	0.57 ± 0.69
		Glass ionomer	**Without leakage L⁻**	2.1 ± 1.7	10.4 ± 5.6	8.1 ± 6.0	0.94 ± 0.86
			With leakage L⁺	1.7 ± 0.9	11.3 ± 5.7	7.6 ± 7.2	0.95 ± 0.85
5 min after sugar challenge	**Non-fluoride**	**Resin composite**	**Without leakage L⁻**	4.2 ± 2.0*	7.7 ± 4.4*	2.3 ± 2.2	0.16 ± 0.22*
			With leakage L⁺	4.7 ± 2.8*	6.9 ± 2.7*	2.6 ± 2.1	0.31 ± 0.37
		Glass ionomer	**Without leakage L⁻**	3.5 ± 0.97*	6.7 ± 3.4*	5.0 ± 4.1	0.33 ± 0.29
			With leakage L⁺	4.1 ± 1.8*	7.2 ± 3.5*	5.0 ± 2.3	0.50 ± 0.64
	Fluoride	**Resin composite**	**Without leakage L⁻**	5.8 ± 4.2*	6.1 ± 2.8*	6.9 ± 6.8*	0.59 ± 0.64
			With leakage L⁺	4.6 ± 2.4*	7.1 ± 4.5	5.9 ± 4.6*	0.55 ± 0.70
		Glass ionomer	**Without leakage L⁻**	3.7 ± 2.2	8.2 ± 4.7*	10.3 ± 6.0	1.5 ± 1.4
			With leakage L⁺	3.7 ± 1.1*	7.8 ± 3.7*	9.5 ± 5.8	1.1 ± 1.3

Values are Mean ± SD. * means significant difference ($p < 0.05$) from the fasting value, for comparisons made under the same experimental condition

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FACULDADE DE ODONTOLOGIA DE PIRACICABA

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Certificamos que o Projeto de pesquisa "Avaliação da correlação entre microinfiltração e cárie dental adjacente a restaurações dentárias", protocolo CEP nº **030/2004**, dos Pesquisadores ***Maximiliano Sérgio Cenci*** e ***Jaime Aparecido Cury***, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde - MS e foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Odontologia - UNICAMP.

We certify that the research project "Evaluation of the relationship between microleakage and dental caries closed to restorations", register number **030/2004**, of ***Maximiliano Sérgio Cenci*** and ***Jaime Aparecido Cury***, is in agreement with the recommendations of 196/96 Resolution of the National Health Committee - Brazilian Health Department and was approved by the Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas - UNICAMP.

Piracicaba - SP, Brasil, April 29 2004

Profa. Dra. Cinthia Pereira Machado Tabchoury
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MAXIMILIANO SÉRGIO CENCI
RG: 1074408632-SSTC
Autor(a)

JAIME APARECIDO CURY
RG: 4.329.838
Orientador(a)

ANEXO 4 – Súmula Curricular

Maximiliano Sérgio Cenci nasceu em 4 de janeiro de 1978, em Caxias do Sul, Rio Grande do Sul. Completou o ensino fundamental e médio em escolas públicas estaduais na mesma cidade. No ano de 1997 ingressou na Faculdade de Odontologia da Universidade Federal de Pelotas (FO-UFPel), tendo sido graduado Cirurgião Dentista em 2001. No mesmo ano foi contratado como Professor Substituto junto ao Departamento de Odontologia Restauradora da FO-UFPel, tendo atuado nas disciplinas de Dentística Restauradora I e II. No ano seguinte (2002), ingressou no Programa de Pós-Graduação em Odontologia, área de Dentística da FO-UFPel, sob orientação do Prof. Dr. Flávio F. Demarco. Durante o período foi bolsista CAPES e obteve o título de Mestre em Odontologia em janeiro de 2004. Ainda em 2004 iniciou seu doutoramento na Faculdade de Odontologia de Piracicaba, UNICAMP, no Programa de Pós-Graduação em Odontologia, área de Cariologia, sob orientação do Prof. Dr. Jaime A. Cury. Foi bolsista FAPESP, e bolsista CAPES durante Estágio de Doutorado no Exterior, o qual foi desenvolvido no Academic Centre for Dentistry Amsterdam (ACTA), sob supervisão do Prof. Dr. Jacob M. “Bob” ten Cate.

Publicações:

1. **Cenci MS**, Venturini D, Pereira-Cenci T, Piva E, Demarco FF. The effect of polishing techniques and time on surface characteristics and sealing ability of resin composite restorations after one-year storage. Oper Dent 2008; 33(2):165-72.
2. **Cenci MS**, Pereira-Cenci T, da Rosa Rodolpho PA, Del Bel Cury AA, Demarco FF. Clinical assessment of posterior fiber-reinforced fixed partial dentures in an up to 96-month follow-up. Pract Proced Aesthet Dent. 2008. *in press*
3. Souza EM, Bretas RT, **Cenci MS**, Maia-Filho EM, Bonetti-Filho I. Periapical radiographs overestimate root canal wall thickness during post space preparation. Int Endod J 2008. *in press*
4. Piovesan EM, Demarco FF, **Cenci MS**, Pereira-Cenci T. Survival rates of endodontically treated teeth restored with fiber-reinforced custom posts and cores: a 97-month study. Int J Prosthodont. 2007;20(6):633-9.
5. Pereira-Cenci T, Pereira LJ, **Cenci MS**, Bonachela WC, Del Bel Cury AA. Maximal bite force and its association with temporomandibular disorders. Braz Dent J. 2007;18(1):65-8.

6. Pereira-Cenci T, Cury AA, **Cenci MS**, Rodrigues-Garcia RC. In vitro Candida colonization on acrylic resins and denture liners: influence of surface free energy, roughness, saliva, and adhering bacteria. Int J Prosthodont. 2007;20(3):308-10.
7. **Cenci MS,** Demarco FF, Pereira CL, Lund RG, de Carvalho RM. One-year comparison of metallic and translucent matrices in Class II composite resin restorations. Am J Dent. 2007;20(1):41-5.
8. Demarco FF, **Cenci MS**, Lima FG, Donassollo TA, André DA, Leida FL. Class II composite restorations with metallic and translucent matrices: 2-year follow-up findings. J Dent. 2007;35(3):231-7.
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10. Venturini D, **Cenci MS**, Demarco FF, Camacho GB, Powers JM. Effect of polishing techniques and time on surface roughness, hardness and microleakage of resin composite restorations. Oper Dent. 2006;31(1):11-7.
11. da Rosa Rodolpho PA, **Cenci MS**, Donassollo TA, Loguércio AD, Demarco FF. A clinical evaluation of posterior composite restorations: 17-year findings. J Dent. 2006;34(7):427-35.
12. **Cenci M**, Demarco F, de Carvalho R. Class II composite resin restorations with two polymerization techniques: relationship between microtensile bond strength and marginal leakage. J Dent. 2005;33(7):603-10.
13. Pereira CL, **Cenci MS**, Demarco FF. Sealing ability of MTA, Super EBA, Vitremer and amalgam as root-end filling materials. Braz Oral Res. 2004;18(4):317-21.
14. **Cenci MS**, Piva E, Potrich F, Formolo E, Demarco FF, Powers JM. Microleakage in bonded amalgam restorations using different adhesive materials. Braz Dent J. 2004;15(1):13-8.
15. Pereira CL, Demarco FF, **Cenci MS**, Osinaga PW, Piovesan EM. Flexural strength of composites: influences of polyethylene fiber reinforcement and type of composite. Clin Oral Investig. 2003;7(2):116-9.