UNIVERSIDADE ESTADUAL DECAMPINAS
Faculdade de Odontologia de Piracicaba

CAMILA NOBRE DE FREITAS

IMPACTO DO TRATAMENTO RESTAURADOR ATRAUMÁTICO ASSOCIADO À ESTRATÉGIA EDUCATIVA EM SAÚDE BUCAL NAS CARACTERÍSTICAS FÍSICO-QUÍMICAS E MICROBIOLÓGICAS SALIVARES DE ESCOLARES: UM ESTUDO CASO-CONTROLE

IMPACT OF ATRAUMATIC RESTORATIVE TREATMENT ASSOCIATED WITH ORAL HEALTH EDUCATIONAL STRATEGY ON SALIVARY PHYSICOCHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF SCHOOLCHILDREN: A CASE-CONTROL STUDY (*)

PIRACICABA
2016

CAMILA NOBRE DE FREITAS

IMPACTO DO TRATAMENTO RESTAURADOR ATRAUMÁTICO ASSOCIADO À ESTRATÉGIA EDUCATIVA EM SAÚDE BUCAL NAS CARACTERÍSTICAS FÍSICO-QUÍMICAS E MICROBIOLÓGICAS SALIVARES DE ESCOLARES: UM ESTUDO CASO-CONTROLE

IMPACT OF ATRAUMATIC RESTORATIVE TREATMENT ASSOCIATED WITH ORAL HEALTH EDUCATIONAL STRATEGY ON SALIVARY PHYSICOCHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF SCHOOLCHILDREN: A CASE-CONTROL STUDY

Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Mestra em Odontologia, na Área de Odontopediatria.

Dissertation presented to the Piracicaba Dental School, University of Campinas in partial fulfillment of the requirements for the Master degree in Dentistry, Pediatric Dentistry area.

Orientadora: PROF[a]. DR[a]. TAÍS DE SOUZA BARBOSA

Coorientadora: PROF[a]. DR[a]. PAULA MIDORI CASTELO FERRUA

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO DEFENDIDA PELA ALUNA CAMILA NOBRE DE FREITAS, E ORIENTADA PELA PROFA. DRA. TAÍS DE SOUZA BARBOSA

PIRACICABA

2016

FICHA CATALOGRÁFICA

Agência(s) de fomento e nº(s) de processo(s): Não se aplica.

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Odontologia de Piracicaba
Marilene Girello - CRB 8/6159

F884i

Freitas, Camila Nobre de, 1990-
Impacto do tratamento restaurador atraumático associado à estratégia educativa em saúde bucal nas características físico-químicas e microbiológicas salivares de escolares : um estudo caso-controle / Camila Nobre de Freitas. – Piracicaba, SP : [s.n.], 2016.

Orientador: Taís de Souza Barbosa.
Coorientador: Paula Midori Castelo.
Dissertação (mestrado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Criança. 2. Tratamento dentário restaurador sem trauma. 3. Boca - Cuidado e higiene. 4. Microbiota. 5. Saliva. 6. Educação em saúde. I. Barbosa, Taís de Souza,1980-. II. Castelo, Paula Midori,1978-. III. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: Impact of atraumatic restorative treatment associated with oral health educational strategy on salivary physicochemical and microbiological characteristics of schoolchildren : a case-control study
Palavras-chave em inglês:
Child
Dental atraumatic restorative treatment
Mouth - Care and hygiene
Microbiota
Saliva
Health education
Área de concentração: Odontopediatria
Titulação: Mestra em Odontologia
Banca examinadora:
Taís de Souza Barbosa [Orientador]
Sandra Kalil Bussadori
Maria Beatriz Duarte Gavião
Data de defesa: 29-02-2016
Programa de Pós-Graduação: Odontologia

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Dissertação de Mestrado, em sessão pública realizada em 29 de Fevereiro de 2016, considerou a candidata CAMILA NOBRE DE FREITAS aprovada.

PROFª. DRª. TAÍS DE SOUZA BARBOSA

PROFª. DRª. SANDRA KALIL BUSSADORI

PROFª. DRª. MARIA BEATRIZ DUARTE GAVIÃO

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno.

DEDICATÓRIA

Dedico este trabalho aos meus pais, por representarem meu maior exemplo e referencial.

AGRADECIMENTOS

À Universidade Estadual de Campinas, na pessoa do seu Magnífico Reitor **Prof. Dr. José Jorge Tadeu**; à Faculdade de Odontologia de Piracicaba, na pessoa do seu diretor **Prof. Dr. Guilherme Elias Pessanha Henriques**; à coordenadora do programa de Pós-Graduação da FOP UNICAMP **Profa. Dra. Cínthia Pereira Machado Tabchoury**; à Coordenadora do programa de Pós-Graduação em Odontologia**, Profa. Dra. Juliana Trindade Clemente Napimoga**, agradeço a oportunidade de poder fazer parte como aluna de pós-graduação desta conceituada universidade.

À **Fundação de Amparo à Pesquisa do Estado de São Paulo** (FAPESP, DP, Brasil, processo nº 2012 / 04492-2; 2014 / 20464-4) e ao **Conselho Nacional de Desenvolvimento Científico e Tecnológico** (CNPq, DF, Brasil, n. 162163 / 2014-2), por conceder financiamento para a execução deste trabalho.

À minha orientadora **Profª Drª Taís de Souza Barbosa** e coorientadora **Profª Drª Paula Midori Castelo**, por toda dedicação, pelos ensinamentos, incentivos e experiência transmitidos.

Ao **Prof. Dr. Jaime Aparecido Cury**, meu orientador de iniciação científica, com quem tive meu primeiro contato com a pesquisa.

Aos professores da banca de pré-qualificação **Profª Drª Taís de Souza Barbosa, Profª Marinês Nobre dos Santos Uchôa e Prof. Dr. Fábio Luiz Mialhe**, aos professores da banca de qualificação **Profª Marinês Nobre dos Santos Uchôa, Profa. Dra. Cínthia Pereira Machado Tabchoury e Prof. Dr. Fábio Luiz Mialhe**, e da banca da defesa, **Profª Drª Taís de Souza Barbosa**, **Profª Drª Maria Beatriz Duarte Gavião e Profª Drª Sandra Kalil Bussadori** por todas as contribuições.

À **Universidade Estadual Paulista** (UNESP); à **Universidade Federal de São Paulo** (UNIFESP), por possibilitarem a realização das análises do estudo.

À **Profª Drª Marlise Inêz Klein,** pela dedicação, pelos ensinamentos, incentivos e experiência transmitidos.

Às professoras do departamento de Odontologia Infantil da FOP-UNICAMP **Profª Fernanda Miori Pascon**, **Profª Maria Beatriz Duarte Gavião**, **Profª Marinês Nobre dos Santos Uchôa e Profª Regina Puppin-Rontani** e aos funcionários **Marcelo Maistro** e **Shirley Rosana Sbravatti Moreto** pela colaboração.

Às escolas dos municípios de Charqueada, Piracicaba e Saltinho, por possibilitar a execução dessa pesquisa.

Aos voluntários desta pesquisa, que aceitaram participar do trabalho.

Agradeço a Deus, a quem deposito minha fé e me apoio em todos os momentos.

Aos meus pais e irmãos, pelo amor incondicional, apoio e compreensão.

À toda minha família, avós, tios, tias e primos, por estarem sempre ao meu lado.

Aos colegas da pós-graduação, em especial a Darlle Araújo, Lívia Pagotto, Cláudia Lobelli e Rosa Abuhadba pelos momentos de descontração, apoio e cumplicidade.

Aos colegas da UNESP, Midian Castillo, Geisiane Bueno e Gabriela Alonso, pela hospitalidade, receptividade, colaboração e apoio.

À minha amiga Nayara Faria pelo apoio em todos os momentos.

RESUMO

A cárie dentária é um problema de saúde pública no mundo, sendo necessária a implementação de medidas preventivas e tratamentos adequados. **Objetivo:** Assim, o objetivo deste estudo foi avaliar o impacto do tratamento restaurador atraumático (ART, *Atraumatic Restorative Treatment*) associado à estratégia educativa em saúde bucal nas características físico-químicas e microbiológicas salivares de escolares. **Material e Métodos:** Este estudo caso-controle foi composto por crianças de escolas públicas que apresentavam pelo menos um molar decíduo com cárie em dentina (G_{ART}), pareadas por gênero e idade com crianças livres de cárie (G_C), compreendendo 36 crianças por grupo com seis e sete anos de idade. A estratégia educativa consistiu em quatro sessões consecutivas (uma sessão/semana) sobre os fatores etiológicos da cárie, instruções de higiene bucal, escovação supervisionada e explicações sobre o ART. O ART foi realizado utilizando-se instrumentos manuais para abertura e limpeza das cavidades e restauração com ionômero de vidro de alta viscosidade. Os participantes foram avaliados em três momentos: T1–*baseline*, T2–uma semana após estratégia educativa (para G_C e G_{ART}) e T3–um mês após estratégia ou ART para G_C e G_{ART}, respectivamente. A presença de biofilme e gengivite foi avaliada nas superfícies vestibulares de incisivos superiores decíduos/permanentes. O fluxo salivar estimulado foi estimado por via mecânica de 0,3 g de *parafilm* por 5 minutos, sendo o pH salivar determinado imediatamente após a coleta. A capacidade tampão foi mensurada pela adição 1,5 ml de HCl (5 mM) em 0,5 mL de saliva estimulada e posterior aferimento do pH. Para detecção e quantificação de bactérias totais e *Streptococcus mutans*, o DNA microbiano de amostras de saliva não estimulada foi isolado e submetido a reações quantitativas de PCR (qPCR). **Resultados:** As frequências de crianças do G_C com biofilme escore zero e escore 4 aumentaram e diminuíram, respectivamente, após a estratégia educativa; a frequência de gengivite escore 2 também diminuiu significativamente. O pH salivar e capacidade tampão foram significativamente menores no G_{ART} em relação ao G_C em todos os momentos e aumentaram significativamente após as intervenções em ambos os grupos. A contagem de bactérias totais e *S. mutans* no G_C diminuiu de T1 para T2, aumentando significativamente no T3 (em relação a T2). No G_{ART}, a contagem de bactérias totais diminuiu do T1 para T2, permanecendo baixa até T3; já a contagem de *S. mutans* diminuiu de T1 para T2, embora sua porcentagem em relação às bactérias totais tenha aumentado até T3. Ainda, a porcentagem de *S. mutans* foi maior no G_{ART} nos momentos T2 e T3 em comparação ao G_C. Uma tendência de retorno aos níveis microbiológicos iniciais foi observada após as intervenções em ambos os grupos, o que enfatiza a necessidade de estratégias continuadas de

prevenção em saúde bucal. **Conclusões:** Os achados deste estudo reforçam a importância de programas preventivos em saúde bucal na melhora de parâmetros clínicos e das características físico-químicas e microbiológicas salivares tanto em crianças livres de cárie como aquelas com experiência de cárie e submetidas a intervenções.

Palavras-chave: Criança, Tratamento Dentário Restaurador Atraumático, Educação em Saúde, Microbiota, Higiene Bucal, Saliva

ABSTRACT

Dental caries is a public health problem in the world, which requires preventive measures and appropriate treatments. **Objective:** The objective of this study was to evaluate the impact of atraumatic restorative treatment (ART) associated with oral health educational strategy on salivary physicochemical and microbiological characteristics of schoolchildren. **Material and Methods:** This case-control study was composed of children from public schools with at least one decayed primary molar in dentin (G_{ART}), matched for age and gender with caries-free children (G_C), comprising 36 six- to seven-yr-old children each group with 6-7 years. The educational strategy consisted of four consecutive sessions (one session/week) about etiological factors of dental caries, oral hygiene instructions, supervised toothbrushing and explanations about the ART. ART was performed using hand instruments for opening and cleaning the cavities and restoration with high-viscosity glass ionomer. Children were evaluated at three moments: T1-baseline, T2-one week after educational strategy (for G_C and G_{ART}) and T3-one month after strategy or ART for G_C and G_{ART}, respectively. Presence of biofilm and gingivitis was assessed on buccal surfaces of primary/permanent upper incisors. Stimulated salivary flow was estimated by via mechanical on 0.3 g of *parafilm* for 5 minutes, and pH being determined immediately after salivary collection. Buffer capacity was measured by adding 1.5 ml HCl (5 mM) in 0.5 mL of stimulated saliva and subsequent pH measurement. For detection and quantification of total bacteria and *Streptococcus mutans*, microbial DNA from unstimulated saliva samples was isolated and subjected to quantitative PCR reactions (qPCR). **Results:** In G_C, the frequency of biofilm score zero and score 4 increased and decreased, respectively, after the educational strategy; gingivitis score 2 also decreased significantly. Salivary pH and buffer capacity were significantly lower in G_{ART} compared to G_C at all moments and increased significantly after interventions in both groups. In G_C, total bacteria and *S. mutans* counts decreased from T1 to T2, increasing significantly in T3 (compared to T2). In G_{ART}, total bacteria count decreased from T1 to T2, remaining low at T3, while *S. mutans* count decreased from T1 to T2. In G_{ART}, the percentage of *S. mutans*/total bacteria increased from T1 to T3, and it was higher in T2 and T3 than in G_C. A trend toward returning to baseline microbial levels was observed after interventions in both groups, which emphasizes the need for continuous oral health prevention strategies. **Conclusions:** The findings of this study reinforce the importance of preventive programs on oral health in the improvement of clinical parameters and salivary physicochemical and microbiological characteristics in both caries-free children and those with caries experience, subjected to interventions.

Key words: Child, Dental Atraumatic Restorative Treatment, Health Education, Microbiota, Oral Hygiene, Saliva

SUMÁRIO

1 INTRODUÇÃO

A cárie é uma doença dependente de biofilme-açúcar (Fejerskov, 2004), onde o acúmulo de biofilme é necessário e a exposição ao açúcar é fator determinante para a progressão da lesão de cárie em qualquer superfície dentária intacta ou restaurada. O biofilme formado na presença de sacarose tem baixas concentrações de cálcio, fosfato e flúor, íons envolvidos no processo de des-remineralização do esmalte e dentina (Paes Leme *et al.,* 2006); como a saliva por si só não é 100% eficaz para reparar todos os minerais perdidos durante o processo de desmineralização, a remoção mecânica do biofilme e restrição de açúcar se fazem necessários para o controle da doença (Cury *et al.,* 2016).

Procedimentos restauradores diminuem os locais livres para acúmulo de biofilme reduzindo significativamente as populações bacterianas (Wright *et al.*, 1992). Entretanto, o biofilme pode acumular-se também sobre a superfície da restauração, por isso a necessidade de implementação de estratégias educativas em saúde bucal em crianças em idade escolar (Vadiakas *et al.*, 2012). As estratégias educativas em saúde bucal devem consistir de instruções de saúde bucal e métodos de controle do biofilme, visando à motivação e incorporação de conhecimento, atitude e comportamento em saúde (Bhardwaj *et al.*, 2013). O controle por meio do uso do dentifrício fluoretado altera a relação dose-resposta entre açúcares e cárie, uma vez que retarda a ocorrência de cavitação para um determinado nível de açúcares (Zero, 2004) e pode reduzir a força da associação entre a frequência de ingestão de açúcares e a incidência de cárie (Duggal *et al.,* 2001;. Bernabé *et al.,* 2014).

O tratamento restaurador atraumático (ART, do original em inglês *Atraumatic Restorative Treatment*) foi desenvolvido para ser utilizado no tratamento de populações carentes ou comunidades distantes dos grandes centros (Frencken, 2009), embora também utilizado como opção de tratamento em clínicas privadas. Quando comparado aos tratamentos convencionais, o ART é considerado uma abordagem menos traumática e melhor aceita pela população infantil (Rahimtoola & van Amerongen, 2002; Schriks & van Amerongen, 2003). Quando avaliada a taxa de sobrevivência do cimento de ionômero de vidro (CIV), material frequentemente utilizado para o ART em dentes posteriores decíduos e permanentes, não se observou diferença significativa em relação às restaurações convencionais com amálgama realizadas em superfície única ou oclusal em estudos prévios (Frencken *et al.*, 2004; van't Hof *et al.*, 2006; de Amorim *et al.*, 2012).

O CIV de alta viscosidade é o material de escolha para o ART, uma vez que é biocompatível, apresenta coeficiente de expansão térmica semelhante ao dos dentes e libera

íons fluoreto (Bonifácio *et al.*, 2009; Guglielmi *et al.*, 2011). A presença de fluoreto em materiais restauradores interfere com o avanço da lesão de cárie, reduzindo a desmineralização e promovendo remineralização do esmalte e dentina (Cury & Tenuta, 2009; Mickenautsch *et al.*, 2010), uma vez que o fluoreto é o único agente terapêutico conhecido para controlar de forma eficaz a progressão da doença cárie (Fejerskov *et al.,* 2015). Estes materiais, além de liberar fluoreto por um longo tempo, podem também recarregar o íon a partir de outras fontes, tais como o fluoreto do dentifrício (Vieira *et al.,* 1999). Alguns estudos encontrados na literatura reportaram um efeito do CIV sobre a redução no número de *Streptococcus mutans* (Forss *et al.*, 1991; Forsten, 1991; Saku *et al.*, 2010); no entanto, poucos ensaios clínicos controlados foram realizados para mostrar evidências do efeito antibacteriano destes materiais, sendo necessários mais estudos para confirmar estes achados (Cury *et al.,* 2016).

Sugere-se que o desenvolvimento da cárie dentária esteja associado à presença de *S. mutans* (Baca *et al.*, 2002) e, após a irrupção dentária, estes microrganismos colonizam diferentes superfícies, incluindo lesões de cárie e fissuras. Os poucos estudos clínicos encontrados na literatura que tenham realizado programas educativos em saúde bucal (Klock and Krasse, 1978) e ART em crianças de pouca idade (Carvalho & Bezerra, 2003; Roshan *et al.*, 2010) encontraram melhora significativa nos parâmetros salivares, em termos de pH, capacidade tampão, níveis de microrganismos cariogênicos e atividade de cárie; no entanto, a contribuição dessas intervenções separadamente e a comparação do efeito do ART com um grupo controle de crianças livres de cárie não foi realizada. O diferencial deste estudo, foi a avaliação da população microbiana através da reação em cadeia da polimerase quantitativa qPCR, uma alternativa mais sensível e versátil para técnicas baseadas em cultura durante a avaliação de amostras in vivo (Klein *et al.*, 2012). Atualmente as características do biofilme dental têm recebido bastante atenção da comunidade científica, embora a avaliação salivar seja de interesse pelo seu fácil acesso e possibilidade de comparação com achados prévios (Loesche *et al.*, 1977; Klock & Krasse, 1978; Wright *et al.*, 1992; Carvalho & Bezerra, 2003; Roshan *et al.*, 2010), fornecendo um panorama das alterações fisicoquímicas e microbiológicas da saliva, que efetivamente banha todas as superfícies da boca.

Sendo assim, o objetivo deste estudo foi avaliar o impacto do ART associado à estratégia educativa em saúde bucal nas características físico-químicas e microbiológicas salivares de crianças de 6 a 7 anos em um estudo caso-controle.

2 ARTIGO

Impact of atraumatic restorative treatment associated with oral health educational strategy on salivary physicochemical and microbiological characteristics of schoolchildren: a case-control study

Camila Nobre de Freitas [a]
Karina Guedes de Souza [a]
Gabriela Caroline Alonso [b]
Marlise Inês Klein [b]
Paula Midori Castelo [c]
Taís de Souza Barbosa [a]

[a] Department of Pediatric Dentistry, Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas (UNICAMP), Brazil
[b] Department of Dental Materials and Prosthodontics, Araraquara Dental School, Universidade Estadual Paulista, UNESP, São Paulo, Brazil
[c] Department of Biological Sciences, Universidade Federal de São Paulo (UNIFESP), Campus Diadema, Brazil

Correspondence to:
Taís de Souza Barbosa, DDS, MSc, PhD
Departamento de Odontologia Infantil, Faculdade de Odontologia de Piracicaba, UNICAMP.
Av. Limeira, 901, Piracicaba, SP, Brazil. CEP13414-903
E-mail: tais_sb@fop.unicamp.br

ABSTRACT

Dental caries is still a public health concern. Therefore, the aim was to evaluate the impact of atraumatic restorative treatment (ART) associated with oral health educational strategy on salivary physicochemical and microbiological characteristics of schoolchildren (6-7y old). This case-control study included children with at least one decayed primary molar in dentin ($G_{ART;}$ n=36), matched by age and gender with caries-free children (G_C; n=36). Educational strategy consisted of 4 consecutive sessions (1/week) about etiological factors of caries, oral hygiene and supervised toothbrushing for both groups. ART was performed using hand instruments and restoration with high-viscosity glass-ionomer. Children were evaluated at: T1–baseline, T2–one week after educational strategy (for G_C and G_{ART}) and T3–one month after strategy or ART for G_C and G_{ART}, respectively. Presence of biofilm and gingivitis was assessed on buccal surfaces of upper incisors (scoring 0-4). Salivary flow rate, pH and buffer capacity were assessed in stimulated saliva. Detection and quantification of total bacteria and *S. mutans* were performed in unstimulated saliva (qPCR). It was observed that children with biofilm score zero and score 4 increased and decreased, respectively, and the frequency of gingivitis score 2 decreased after strategy in G_C. Salivary pH and buffering capacity were significantly lower in G_{ART} in all collections, increasing significantly after interventions in both groups. In G_C, total bacteria and *S. mutans* counts dropped from T1 to T2, increasing significantly in T3. In G_{ART}, total bacteria count decreased from T1 to T2, remaining low at T3; *S. mutans* count decreased from T1 to T2. The percentage of *S. mutans*/total bacteria increased from T1 to T3 in G_{ART}, and it was higher in G_{ART} compared to G_C in T2 and T3. A trend toward returning to baseline microbial levels was observed, emphasizing the need of continuous oral health strategies.

Key words: Child, Dental Atraumatic Restorative Treatment, Health Education, Microbiota, Oral Hygiene, Saliva

INTRODUCTION

In recent decades, there has been progress in the prevention of dental caries among children, adolescents and adults; however, dental caries remains a public health concern (Marcenes *et al.*, 2013; Kassebaum *et al.*, 2015), despite the widespread use of fluoridated water, fluoride toothpaste and sealant use (Narvai *et al.*, 2006; Griffin *et al.,* 2008). In Brazil, the last survey showed that tooth decay remains highly prevalent, especially in early infancy (Ministry of Health, Brazil, 2010), and similar findings were observed in other developing countries (Sutthavong *et al.*, 2010). These increased levels of dental caries are still observed especially where community and school-based preventive oral care programs are not established (Rao *et al.*, 1999). Once a strong association between tooth decay and oral hygiene was observed (Esan *et al.*, 2015), efficient dental health education should incorporate oral health instructions and methods to reduce dental biofilm, to motivate and incorporate oral health knowledge, attitude and behavior. It has been shown that even a short-term oral health educational program may be useful in improving oral hygiene and gingival health (Bhardwaj *et al.*, 2013).

Tooth decay is a multifactorial disease, biofilm-sugar dependent (Fejerskov, 2004), where the dental biofilm formation is necessary and the exposure to sugar is a determinant factor for the progression of carious lesions on any tooth surface (intact or restored). A relationship between dental caries and *Streptococcus mutans* has also been reported (Tanzer *et al.*, 2001) and, after dental eruption, these microorganisms colonize different tooth surfaces, including caries lesions and fissures (Baca *et al.*, 2002). Restorative procedures are thought to reduce biofilm accumulation sites, resulting in significant reductions in bacterial populations (Wright *et al.*, 1992). However, even when lesions or fissures are filled, biofilm can develop on the surface of these materials; therefore recent findings have emphasized the need to educate children prior to the age of 12 in oral health issues (Vadiakas *et al.*, 2012).

The atraumatic restorative treatment (ART) was developed for use in poor communities or those far from urban centers (Frencken, 2009), and it is considered a less traumatic approach and better accepted by child patients (Rahimtoola and van Amerongen, 2002; Schriks and van Amerongen, 2003). Removing decayed tissue with hand instruments and restoring the cavity by adhesive material shall conserve as much as possible tooth structure and will prevent further decay. In addition, when comparing glass ionomer cement survival rate -- a material often used in ART in primary and permanent posterior teeth -- no significant difference between conventional amalgam fillings and glass ionomer cement restoration performed in single or occlusal surface was found (Frencken *et al.*, 2004; Van't Hof *et al.*, 2006;

de Amorim et al, 2012).

Past studies reported some properties of the glass ionomer cement, such as continuing fluoride release, biocompatibility, and cariostatic potential (Benelli *et al.*, 1993; Souto and Donly, 1994), which could inhibit new caries lesions, acting in remineralization of initial lesions (Serra and Cury, 1992) and promoting mineralization of adjacent tissue (ten Cate and van Duinen, 1995); however, there is a lack of controlled clinical trials and these effects are still not based on clinical evidence (Cury *et al.*, 2016).

Few clinical studies were conducted to evaluate the effect of oral health educational programs (Klock and Krasse, 1978) and ART (Carvalho and Bezerra, 2003; Roshan *et al.*, 2010) on salivary parameters (*i.e.*, salivary pH, buffer capacity and cariogenic microorganisms' levels). In most of them, the absence of sample size calculation and a control group, and the lack of controlling for potential confounding factors make it difficult to determine comparisons. The return of the oral bacteria levels to pre-treatment levels was observed in previous studies (Loesche *et al.*, 1977; Wright *et al.*, 1992; Roshan *et al.*, 2010), which may imply that a restorative treatment alone would be a questionable modality for altering the oral flora in a clinically significant way. The differential of this study was to evaluate the microbial population via quantitative polymerase chain reaction (qPCR), a more sensitive and versatile alternative to culturing-based techniques for evaluation of in vivo samples (Klein *et al.*, 2012).

Currently, the dental biofilm characteristics and its relationship with tooth decay have received much attention from the scientific community (Fejerskov, 2004; Parisotto *et al.*, 2011), although the evaluation of salivary characteristics are of interest due to its easy access and the possibility of comparing with previous longitudinal clinical studies (Loesche *et al.*, 1977; Klock and Krasse, 1978; Wright *et al.*, 1992; Carvalho and Bezerra, 2003; Roshan *et al.*, 2010). Furthermore, it provides an overview of the physicochemical and microbiological changes in saliva caused by preventive strategies, which effectively bathes all mouth surfaces and is also related to other oral diseases beyond dental caries.

Thus, the objective of the present study was to evaluate the impact of ART associated with oral health educational strategy on salivary physicochemical and microbiological characteristics of children aged 6-7 years in a case-control design.

MATERIAL AND METHODS

Participants

A clinical longitudinal study design (ClinicalTrials.gov, CJK-2014-ART) was used with children recruited from public schools of Piracicaba, Saltinho and Charqueada, SP, Brazil. The number of population and the Human Development Index (HDI) of these cities are approximately: 364,571 habitants and HDI 0.78; 7,059 and 0.791; 16,440 and 0.736, respectively. All families have access to public water supply services, with fluoride levels of 0.7 ppm. Five hundred twenty-one forms with information and instructions about the study were distributed to six- to eight-years-old schoolchildren, and consents were obtained from 380 parents/guardians, who were informed of the procedures, possible discomforts or risks and the possible benefits of the study (approved by the Ethics Committee of Piracicaba Dental School, University of Campinas, protocol no. 136/2014).

Sample size was calculated from results of two previous studies (Klock and Krasse, 1978; Roshan *et al.*, 2010). The study done by Roshan *et al.* (2010) examined *S. mutans* counts in saliva of children submitted to atraumatic restorative treatment, while Klock and Krasse (1978) evaluated the effect of caries-preventive program on salivary pH and buffer capacity. Considering a power of the test=0.80 and alpha level=0.05, a minimum of 35 subjects in each group would be necessary to perform such evaluations. Confounding factors, such as gender and age, were considered for pairing the groups.

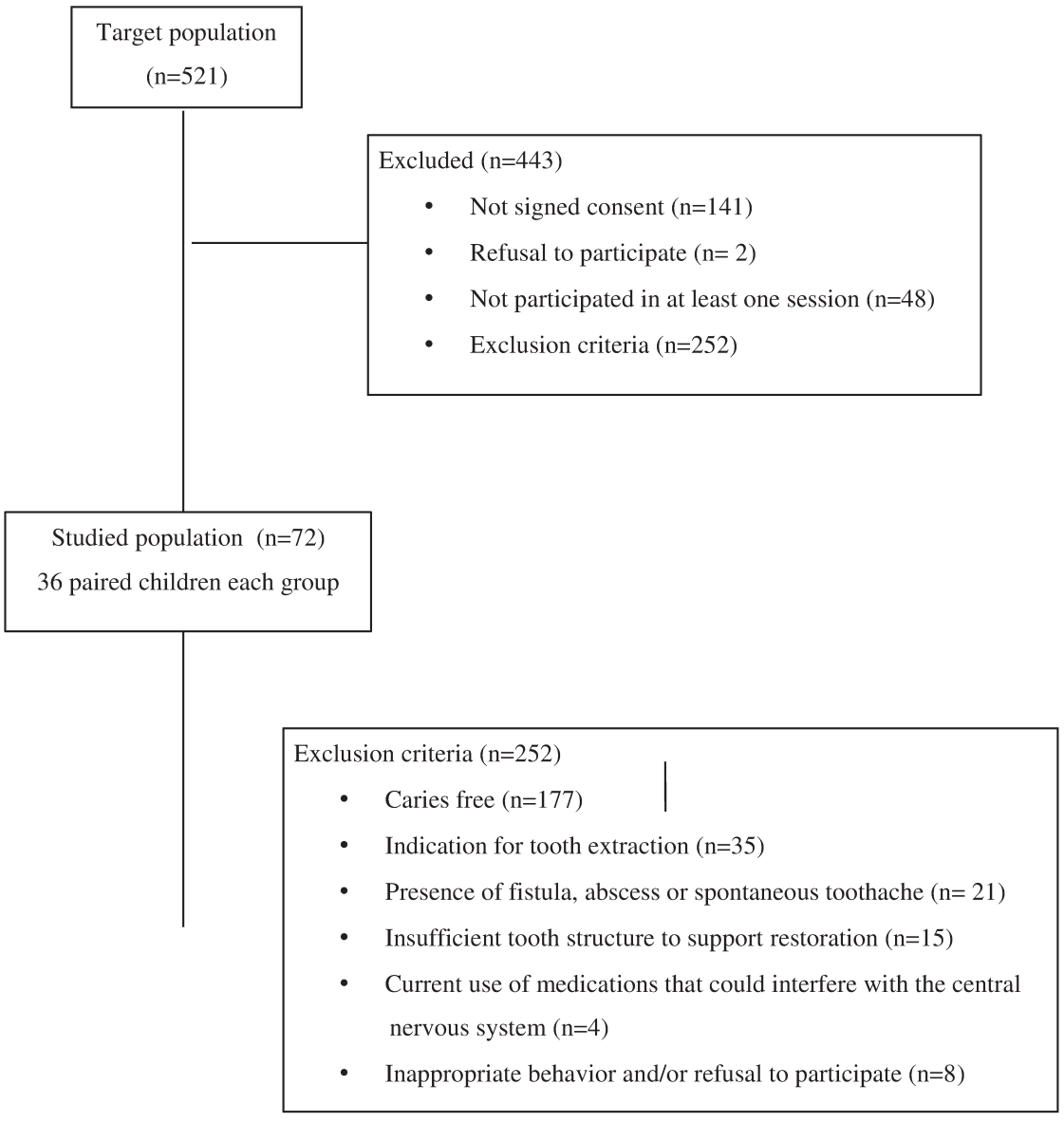
A total of 72 schoolchildren were selected after the conduction of clinical examination to verify the dental caries experience using dmft (sum of decayed, missing, and filled teeth in the primary dentition) and DMFT (sum of decayed, missing, and filled teeth in the permanent dentition) indices (WHO, 1997). All examinations took place at the schools, out of doors in daylight but not direct sunlight, by the first author previously trained using in lux method (Ministry of Health, Brazil, 2009). This calibration process consisted of the visual observation of 33 images (during 1 minute each figure) and diagnostic (presence or absence) of caries lesions with different levels of severity; then, the results were compared to the gold standard records.

Subjects were excluded in accordance with the following exclusion criteria: indication for tooth extraction, presence of fistula, abscess or spontaneous toothache, insufficient tooth structure to support restoration, presence of caries lesions in permanent teeth, chronic diseases or conditions (psychiatric diseases, tumours, traumas, diabetes, and others), current use of medications that would interfere with salivary secretion and inappropriate

behavior and/or refusal to participate. Subjects who did not participate in at least one of the sessions were also excluded. Patients in need of emergency care (toothache) and/or other dental procedures (*e.g.,* tooth extraction or endodontic treatment) were referred to the Pediatric Dentistry Clinics at Piracicaba Dental School, University of Campinas.

The study was designed to compare two paired groups. Caries-free children (DMFT/dmft=0) was allocated in the control group (G_C, n=36) and submitted to oral health educational strategy; the other group of children with at least one decayed primary molar in dentin (without painful symptoms, mobility and abscess) was submitted to oral health educational strategy and further ART (G_{ART}, n=36). See more details about sample selection in Figure 1.

Figure 1. Flow chart of sample selection.



Oral health educational strategy

Oral health educational strategy was standardized and similar for both groups and modified based on previous studies (Bhardwaj *et al.*, 2013; Angelopoulou *et al.*, 2015). Schoolchildren were submitted to four consecutive sessions (once a week, at the school, by the same researcher) of oral health educational strategy, which consisted of:

1st. session: visualization of the mouth's structures: primary and permanent teeth, tongue, palate, and other;

2nd session: explanations regarding etiological factors of dental caries (diet, microbiota and host); visualization of caries lesions using mirror;

3rd. session: oral hygiene instructions (Modified Stillman technique and flossing) using models (*i.e.*, tell-show-do technique); supervised toothbrushing;

4th. session: explanations regarding the use of ART (indications and stages).

The sessions were based on active learning methodology, interaction and reinforcement. Next, G_{ART} was submitted to ART, as described below:

Atraumatic restorative treatment

ARTs were performed by one trained pediatric dentist using protocols developed for this approach (Frencken *et al.*, 1996; 2014). Only hand instruments were used for opening and cleaning the cavities in primary molars. Cleaned cavities were restored with a high-viscosity glass-ionomer (Ketac Molar Easymix®; 3M ESPE, Sumaré, São Paulo, Brazil). ARTs were performed at school using folders and chairs, which were adapted to simulate a dental chair near to a window for natural light.

At this stage, the following exclusion criteria were considered: exfoliated and/or restored teeth after initial selection; pulp exposure during excavation (in these cases, children were submitted to emergency care and were referred to finish treatment in the Clinics of Pediatric Dentistry at Piracicaba Dental School, University of Campinas); inappropriate behavior and/or refusal to participate.

Assessment of oral hygiene

Oral hygiene was assessed by one trained researcher (CNF) considering the presence of dental biofilm and gingivitis. The presence of visible biofilm was evaluated in buccal surfaces of primary and/or permanent upper incisors, the presence of biofilm in one incisor was scored as 1, two incisors as score 2, and thereby until score 4 (Bonanato *et al.*, 2010).

The presence of gingivitis was confirmed by gingival amendment contouring and gum color in upper incisors and it was also scored from 0 to 4 (Löe, 1967; Alaluusua and Malmivirta, 1994). The evaluations were performed in three moments: T1 – baseline, T2 - one week after educational strategy, T3 - one month after the educational strategy for G_C; and T1 – baseline, T2 - one week after educational strategy, T3 - one month after ART for G_{ART}. Between T2 and T3, no other intervention was made.

Salivary collection and biochemical evaluation

Unstimulated and stimulated saliva collections were carried out in schools, at least 2 hours after the last meal and 1 hour after oral hygiene. Unstimulated saliva was collected with subjects comfortably seated, and after a few minutes of relaxation, they rinsed their mouth with distilled water. Then, they were trained to avoid swallowing saliva and asked to lean forward and spit all the saliva produced for five minutes into a cooled tube, through a glass funnel. After, stimulated saliva was collected by subjects chewing on 0.3g of an inert and tasteless material (*Parafilm*, Merifeld, USA), for approximately 70 cycles/min and spitting all the saliva produced for five minutes into another cooled tube. Salivary flow rate was estimated as the volume of saliva secreted per min (mL/min).

Salivary pH was determined immediately in stimulated saliva, using a portable pH-meter (Orion 3 Star Benchtop, Thermo Electron Corporation, USA). After calibration, the electrode was immersed in a Falcon tube containing saliva for 30 seconds for measurement.

Salivary buffer capacity was measured according to the methodology described by Kitasako *et al.* (2008). The evaluation was performed by adding 1.5 ml of 5 mM HCl to a tube containing 0.5 mL of stimulated saliva. The tube was shaken and opened to release CO_2 dissolved in the saliva and, after 5 minutes, the final pH of the solution was assessed in the way similar to determine salivary pH and taken as an expression of the buffer capacity of the salivary samples for further comparisons among collections (T1, T2 and T3) and between groups.

Salivary microbiological evaluation

For detection and quantification of microorganisms, the microbial DNA in unstimulated saliva samples was isolated and subjected to quantitative PCR reactions (qPCR). For the isolation of genomic DNA of potentially viable cells, 1 ml of saliva was centrifuged (13.000 xg/10 min/4°C) and the supernatant discarded. The precipitate was suspended in 500 µl of TE buffer (50mM Tris, 10mM EDTA, pH 8.0) and 0.75 µl of propidium monoazida (PMA: 20 mM in 20% dimethylsulfoxide; Biotium, Hayward, CA) (Nocker *et al.*, 2007). After

incubation for 5 min in the dark, under agitation, the samples were exposed to light (500W) for 3 min. After the light-induced crosslinking to extracellular DNA, including DNA from nonviable or dead cells, samples were centrifuged (5.000 xg / 5 min / 4°C) and the supernatant discarded. The precipitate was then suspended in 100 µl of TE buffer, 10.9 µl lysozyme (stock 100 mg/mL, Sigma) and 2.5 µl mutanolysin (stock 5 U/µL, Sigma). This suspension was incubated at 37°C for 30 min. After, DNA was isolated with MasterPure DNA Purification kit (Epicenter Technologies, Madison, Wis, USA) following the manufacturer's recommendations. The amount and purity of the DNA was assessed by OD260nm and the ratio OD260/280, respectively. Ten ηg (or more) of genomic DNA from each sample were mixed with 12.5 µl of 2X iQ SYBR Green Supermix (Bio-Rad Laboratories, USA) and 0.5 ul of 1 mM specific primers for *S. mutans* or total bacteria (Table 1). The reactions were loaded into 96-Well PCR Plates (MLL9601, Bio-Rad), and were carried out by a CFX96 system (Bio-Rad). The amplification cycle included an initial denaturation step (95°C / 3 min), followed by 40 cycles of denaturation (94°C / 30 sec), annealing (58°C / 30 sec), and extension (68°C / 1 min), and melt curve was analyzed. The standard curves were based on the genome size of *S. mutans* UA159 (2.01 Mb) and average of total oral bacteria known genomes (2.45 Mb) according to Dolezel *et al.* (2003). Specifically, a copy of the genome is a cell of each organism. The curve was used to transform the values of the critical threshold cycle (Ct) with respect to cell number. For *S. mutans* standard curve was constructed using genomic DNA of the lab strain *S. mutans* UA159 (ATCC 700610), while for total bacteria a DNA extracted from a saliva sample was selected arbitrarily (Klein *et al.*, 2012). To determine the number of *S. mutans* and total bacteria cells in the original sample, the numbers of cells detected in the qPCR runs were multiplied by the dilution factor from the DNA dilution step.

Bacteria quantification was performed in the three collections (T1, T2 and T3) for both groups.

Table 1. Microorganisms of interest for detection and quantification via qPCR

Microorganisms (genus and species)	**Primer sequence (forward and reverse)**	**Reference**
"All bacteria" or total bacterial load	ACTCCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	Fierer *et al.* (2005)
Streptococcus mutans	TCGCGAAAAAGATAAACAAACA GCCCCTTCACAGTTGGTTAG	Chen *et al.* (2007)

Statistics

Statistical analysis was performed using BioEstat 5.3 (Mamirauá, Belém, PA, Brazil) and SigmaPlot 13 (Systat Software Inc., San Jose, CA, USA) statistical packages with a 5% significance level. The distribution of data was evaluated using Shapiro-Wilk normality test.

Descriptive statistics consisted of means, standard deviations, medians, interquartile range, and percentages.

The frequencies of biofilm and gingivitis scores among the different collections (T1, T2 and T3) for each group were evaluated by means of Chi-square partition test. Comparisons between groups in the same moment were performed using Chi-square or Fisher's Exact tests.

Comparisons of salivary flow rate, pH, buffering capacity and bacteria counts between groups were performed using t-test/Mann-Whitney test; among collections (T1, T2 and T3) data were compared using One way ANOVA repeated measures/Friedman (Tukey post-test). Because data from bacteria quantification were not normally distributed, logarithmic transformation was applied (Ln).

RESULTS

Table 2 shows the description of the two groups (G_C and G_{ART}) evaluated according to demographic and clinical variables.

Table 2. Demographic and clinical characteristics of the studied sample

Group (n)	Gender	Age (y)	Number of decayed teeth	Baseline biofilm scores	Baseline gingivitis scores
	♀/♂	Mean (SD)	Mean (SD)	Median (25-75%)	Median (25-75%)
G_C (36)	18/18	6.50 (0.51)	0	2 (0-4)	0 (0-2)
G_{ART} (36)	18/18	6.50 (0.51)	1.78 (1.24)	0 (0-3)	0 (0-1.25)

G_C, control group; G_{ART}, atraumatic restorative treatment group.

The frequencies of biofilm and gingivitis scores according to the groups (G_C and G_{ART}) and moments (T1, T2 and T3), and the comparisons between them are shown in Table 3. The frequencies of children in G_C with biofilm score zero and score 4 increased and decreased, respectively, after educational strategy. In addition, the frequency of gingivitis score 2 in G_C decreased significantly after educational strategy. On the other hand, the frequencies of biofilm and gingivitis scores did not vary significantly between moments in G_{ART}.

Table 3. Distribution [n (%)] of biofilm and gingivitis scores according to the groups (G_C and G_{ART}) and moments (T1, T2, T3).

	Biofilm				**Gingivitis**			
G_C (n=36)	**T1**	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**
score 0	13 (36.1)	22 (61.1)	31 (86.1)	**0.010**	21 (58.3)	29 (80.6)	33 (91.7)	0.452
score 1	2 (5.6)	3 (8.3)	1 (2.8)	0.622	2 (5.6)	5 (13.9)	1 (2.8)	0.227
score 2	8 (22.2)	9 (25.0)	2 (5.6)	0.130	6 (16.7)	1 (2.8)	0 (0.0)	**0.018**
score 3	2 (5.6)	0 (0.0)	0 (0.0)	0.145	2 (5.6)	1 (2.8)	0 (0.0)	0.377
score 4	11 (30.6)	2 (5.6)	2 (5.6)	**0.011**	5 (13.9)	0 (0.0)	2 (5.6)	0.079
G_{ART} (n=36)	**T1**	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**
score 0	19 (52.8)	16 (44.4)	28 (77.8)	0.322	24 (66.7)	30 (83.3)	35 (97.2)	0.566
score 1	1 (2.8)	4 (11.1)	3 (8.3)	0.437	3 (8.3)	1 (2.8)	1 (2.8)	0.471
score 2	6 (16.7)	9 (25.0)	4 (11.1)	0.430	4 (11.1)	4 (11.1)	0 (0.0)	0.144
score 3	4 (11.1)	4 (11.1)	0 (0.0)	0.144	1 (2.8)	0 (0.0)	0 (0.0)	0.374
score 4	6 (16.7)	3 (8.3)	1 (2.8)	0.178	4 (11.1)	1 (2.8)	0 (0.0)	0.087
G_C vs. G_{ART}	**T1**	**T2**	**T3**		**T1**	**T2**	**T3**	
score 0	p=0,502[a]	p=0,556[a]	p=0,909[a]	score 0	p=0,872[a]	p=0,937[a]	p=0,766[a]	
score 1	p=1,000[b]	p=1,000[b]	p=0,614[b]	score 1	p=1,000[b]	p=0,198[b]	p=0,614[b]	
score 2	p=0,843[a]	p=1,000[b]	p=0,673[b]	score 2	p=0,521[b]	p=0,357[b]	p=0,114[b]	
score 3	p=0,673[b]	p=0,114[b]	p=1,000[b]	score 3	p=1,000[b]	p=1,000[b]	p=1,000[b]	
score 4	p*=0,410[a]	p=1,000[b]	p=1,000[b]	score 4	p=0,739[b]	p=1,000[b]	p=1,000[b]	

G_C, control group; G_{ART}, atraumatic restorative treatment group; T1, baseline; T2, one week after educational strategy; T3, one month after the educational strategy or ART.
Comparison among collections in the same group: Chi-square partition.
Comparison between groups: [a]Chi-square independence; [b]Fisher's Exact test.

The salivary characteristics and multiple comparisons are shown in Table 4. At baseline, stimulated salivary flow rate did not differ between groups, although at T2 and T3, G_{ART} showed lower salivary flow than G_C. Salivary pH and buffering capacity were significantly lower in G_{ART} in all moments (T1, T2 and T3).

Stimulated salivary flow rate did not differ after educational strategy or ART, while salivary pH and buffering capacity increased significantly after educational strategy and after ART in G_C and G_{ART}, respectively.

Table 4. Stimulated salivary flow (mL/min), pH and buffering capacity according to the groups (G_C and G_{ART}) and moments (T1, T2, T3). Means and standard deviations are shown.

	Collections			
G_C (n=36)	**T1**	**T2**	**T3**	**p-value**
Flow	1.26 (0.56)	1.23 (0.50)	1.09 (0.47)	0.300‡
pH	7.15 (0.54)[a]	7.37 (0.42)[b]	7.53 (0.37)[c]	<0.05§
Buffering capacity	6.35 (0.18)[a]	6.48 (0.22)[b]	6.53 (0.16)[b]	<0.0001‡
G_{ART} (n=36)	**T1**	**T2**	**T3**	**p-value**
Flow	1.13 (0.54)	1.13 (0.43)	0.86 (0.29)	0.062§
pH	6.65 (0.34)[a]	6.77 (0.30)[b]	7.27 (0.26)[c]	<0.0001‡
Buffering capacity	5.16 (0.47)[a]	5.33 (0.43)[b]	6.15 (0.24)[c]	<0.0001§
G_C *vs.* G_{ART}	**T1**	**T2**	**T3**	
Flow	p=0.2890†	p<0.0001*	p<0.0001†	
pH	p<0.0001†	p<0.0001*	p=0.0003*	
Buffering capacity	p<0.0001†	p<0.0001†	p<0.0001†	

G_C, control group; G_{ART}, atraumatic restorative treatment group; T1, baseline; T2, one week after educational strategy; T3, one month after the educational strategy or ART.

‡ANOVA repeated measures; § Friedman (comparison between moments in the same group).

* unpaired t test; † Mann-Whitney (comparison between groups).

Different letters in the same line means statistical significant difference between moments.

Figures 2A and 2B show the total bacteria quantification of G_C and G_{ART}, respectively, in the three collections (T1, T2 and T3). The intragroup comparison showed that total bacteria count dropped from T1 to T2, increasing significantly in T3 in G_C. In G_{ART}, total bacteria count decreased from T1 to T2 (after educational strategy), and remaining lower than T1 in T3.

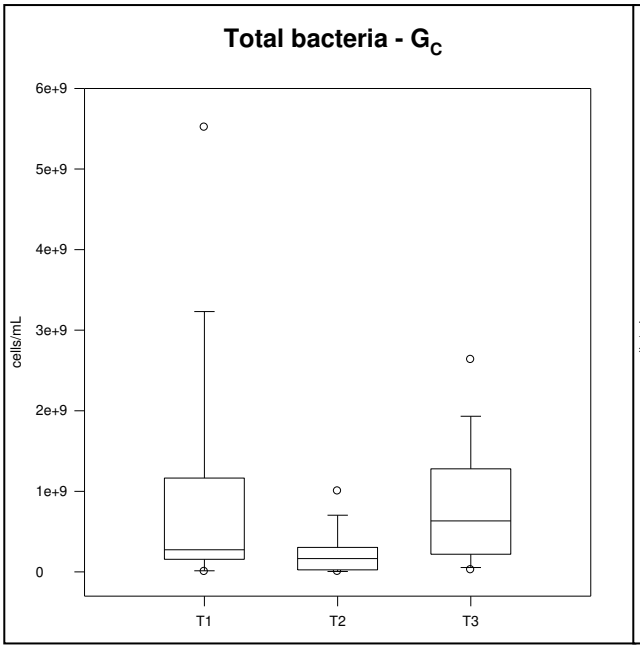


Figure 2A. Total bacteria quantification in **G_C**. Intragroup comparison was performed: T1 ≠ T2 and T2 ≠ T3 ($p<0.05$; One way Repeated Measures Analysis of Variance and Tukey post-test; logarithmic transformation was applied).
T1: baseline; T2: one week after educational strategy; T3: one month after educational strategy.

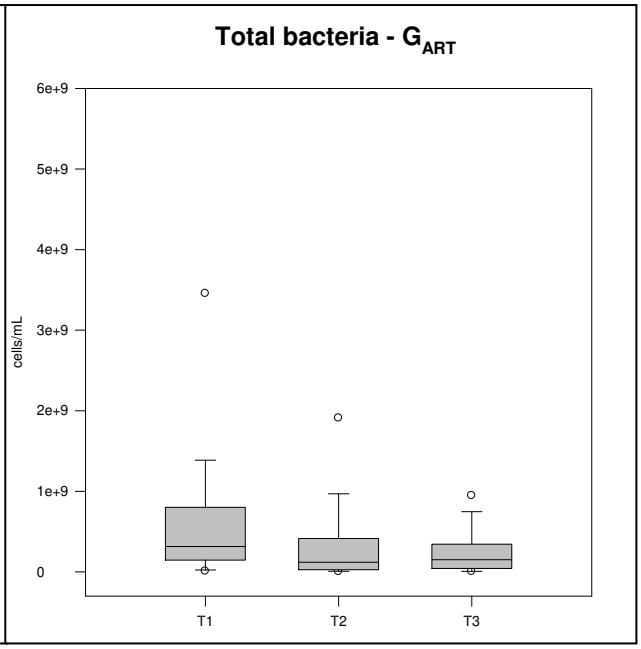


Figure 2B. Total bacteria quantification in **G_{ART}.** Intragroup comparison was performed: T1 ≠ T2 and T1 ≠ T3 ($p<0.05$; One way Repeated Measures Analysis of Variance and Tukey post-test; logarithmic transformation was applied).
T1: baseline; T2: one week after educational strategy; T3: one month after ART.

The quantification of *S. mutans* in G_C and G_{ART} are shown in Figures 3A and 2B, respectively. In G_C, *S. mutans* count dropped from T1 to T2, and increased significantly in T3 (comparing to T2), a behavior similar to that observed for total bacteria quantification. In G_{ART}, a significant decrease in *S. mutans* count was observed from T1 to T2, that is, after educational strategy; after one month of ART, *S. mutans* count was not significantly different than in T1 and T2).

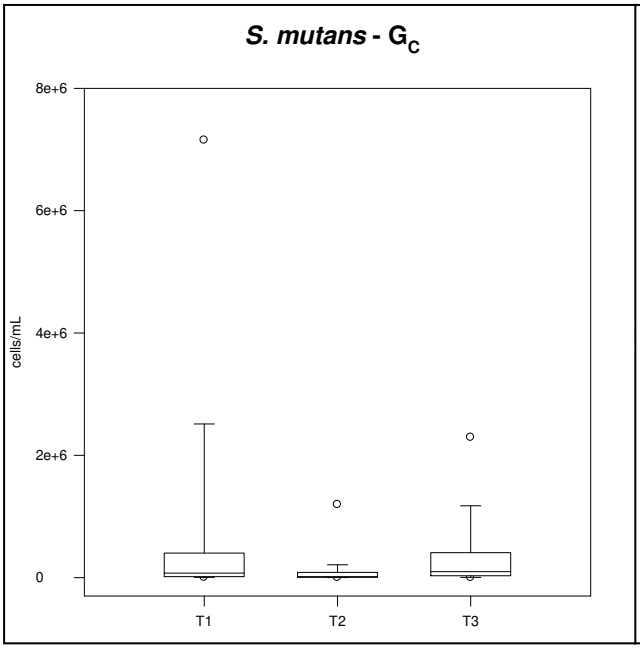


Figure 3A. *S. mutans* quantification in the **G_C**. Intragroup comparison was performed: T1 ≠ T2 and T2 ≠ T3 ($p<0.05$; One way Repeated Measures Analysis of Variance and Tukey post-test; logarithmic transformation was applied).
T1: baseline; T2: one week after educational strategy; T3: one month after educational strategy.

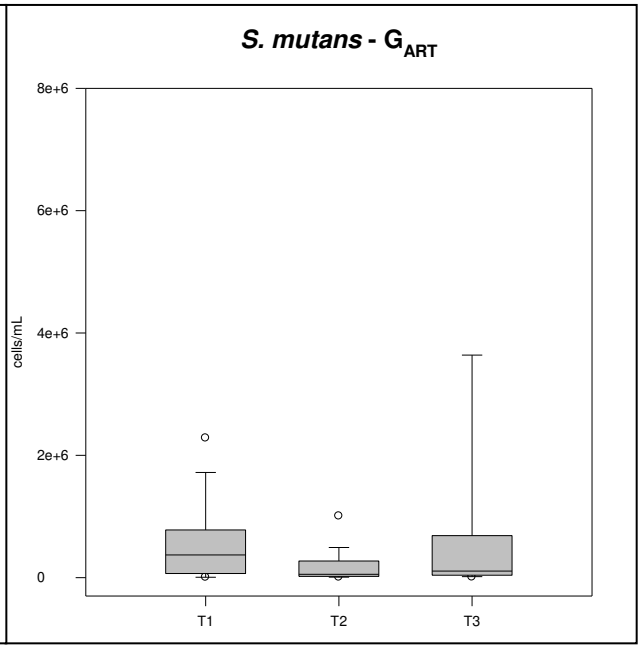


Figure 3B. *S. mutans* quantification in **G_{ART}**. Intragroup comparison was performed: T1 ≠ T2 ($p<0.05$; One way Repeated Measures Analysis of Variance and Tukey post-test; logarithmic transformation was applied).
T1: baseline; T2: one week after educational strategy; T3: one month after ART.

The intergroup comparison showed that total bacteria count was significantly higher in G_C than G_{ART} only in T3 (Mann-Whitney test; $U=222.000$; $p<0.001$); *S. mutans* count was significantly higher in G_{ART} than G_C only in T2 (t-test; $p=0.005$; power=0.77).

Table 4 shows the percentage of *S. mutans* in relation to total bacteria quantified in the three moments. The percentage increased from T1 to T3 in G_{ART}. Although *S. mutans* count of G_{ART} did not differ from G_C at baseline, the percentages of *S. mutans* in relation to total bacteria of G_{ART} were significantly higher than G_C in two of three evaluations (T2 and T3).

Table 4. Percentage of *S. mutans* within total bacteria in the three moments. The data are expressed as median (25-75%).

Percentage of *Streptococcus mutans*/Total bacteria				
	T1	**T2**	**T3**	**Friedman Repeated Measures**
G_C	0.046 (0.005-0.257)	0.021‡ (0.006-0.047)	0.020‡ (0.005-0.081)	p=0.121 Chi-square= 4.222; 2 degrees of freedom
G_{ART}	0.087* (0.020-0.228)	0.064‡ (0.024-0.214)	0.190‡* (0.069-0.492)	*p=0.035 Chi-square= 6.690; 2 degrees of freedom

G_C, control group; G_{ART}, atraumatic restorative treatment group; T1, baseline; T2, one week after educational strategy; T3, one month after the educational strategy or ART.
‡ $p<0.01$ (comparison between groups; Mann-Whitney test).

DISCUSSION

Previous studies suggest that *S. mutans* is one of the most cariogenic microorganisms because of its features and metabolic activities and, therefore, the level of these microorganisms in biofilm and saliva is of importance in determining the patient's risk of caries development (Carvalho and Bezerra, 2003; Mattos-Graner *et al.,* 2014; Hajishengallis *et al.,* 2015). Although restorative procedures with glass ionomer cement are thought to temporarily reduce biofilm accumulation sites with a bacteriostatic effect, there are very few reports of changes in cariogenic microorganisms levels after ART (Carvalho and Bezerra 2003; Roshan *et al.*, 2010). In addition, knowledge about the impact of educational preventive strategies in caries control is also limited (Klock and Krasse, 1978; Bhardwaj *et al.*, 2013). The study of Hilgert *et al.* (2015) demonstrated that supervised toothbrushing (twice a day) has a potential for arresting carious lesions progression, as they did not progress to cavitation in dentine after 3y, even in high–caries risk surfaces.

As regards biofilm and gingivitis evaluations, at baseline the frequencies of children with different scores did not differ between the two clinical groups. However, after educational

strategy, G_C showed the higher short term improvement of oral hygiene. On the other hand, the scores of the caries-activity group (G_{ART}) did not show significant changes after educational strategy and ART; once this group included more children with exfoliated incisors than G_C, the results might be underestimated. Looking at Table 3 with attention, it is possible to observe that the frequencies of biofilm and gingivitis score zero and 4 increased and decrease, respectively, but the results did not reach statistical significance. Nevertheless, this high risk group of children may have bad oral health attitudes and behaviors, such as diet and poor hygiene, which need to be modified by implementing an intensive and more frequent health program. Moreover, as follow-up was relatively short (one month), more time would be needed to verify significantly changes in clinical conditions, especially an improvement in gingival health. Current findings have shown that oral health education should be consistently repeated in order to maintain its positive results longitudinally (Angelopoulou *et al.*, 2015), allowing children to acquire sufficient knowledge and be motivated.

Saliva plays an important role in maintaining the integrity of oral tissues, and the acid buffer capacity of saliva is essential to maintain the pH in the oral environment, protecting teeth against acid produced by microorganisms (Redmo Emanuelsson and Thornqvist, 2001). Salivary flow rate, pH and buffering capacity significantly differed between caries-free (G_C) and caries-active children (G_{ART}), except of flow rate at baseline, corroborating previous studies (Gopinath and Arzreanne; Malekipour *et al.*, 2008; Singh *et al.*, 2015). Probably, the lower salivary flow rate showed by caries-active children comparing to G_C in T2 and T3 may be justified by fear or stress as an anticipation of the dental treatment, as observed by Mejía-Rubalcava *et al.* (2015). In addition, the oral health strategy implemented improved salivary pH and buffering capacity for both groups, and one month after educational strategy or ART (G_C and G_{ART}, respectively), this effect was maintained. Buffer capacity was not affected in pre-school children subjected to extensive restorative treatment under general anesthesia in a previous study (Twetman *et al.*, 1999), thus demonstrating the effect of oral health preventive programs in such parameters.

Very few studies were found in the literature that examined the changes in salivary parameters after preventive strategies. The study of Klock and Krasse (1978) observed that a caries-preventive program (prophylaxis, dietary and oral hygiene instruction, fluoride application, and sealing of pits and fissures) applied once a month during 2 years gave a significant reduction of the caries activity, while microbial conditions, saliva secretion rate, buffer capacity and saliva pH were not influenced by the preventive program. The results of the present study support the concept that oral health education significantly changed salivary

parameters considered beneficial to lower caries activity, such as salivary pH and buffering capacity, even in children with active caries lesions at baseline and one week after educational strategy.

Although *S. mutans* count of G_{ART} did not differ from G_C at baseline, the percentage of *S. mutans* in relation to total bacteria were significantly higher in two of three evaluations. And corroborating the present results, previous findings showed that *S. mutans* counts usually drop after conventional restorative procedures (Wright *et al.*, 1992), antimicrobial treatment associated to restorative procedures (Loesche *et al.*, 1977) or after ART (Carvalho and Bezerra, 2003; Roshan *et al.*, 2010); however, to our knowledge, this is the first study which compared the effect between preventive measures and ART on salivary cariogenic microorganisms' levels. In the study of Carvalho and Bezerra (2003), a significant reduction in *S. mutans* was observed, although the children who received ART were also receiving a preventive oral health program, thus confounding the results found. In the present study, one week after educational strategy and before ART, total bacteria and *S. mutans* counts significantly dropped in both groups; thus, the assumption that sealing caries lesions may be responsible solely for the decrease in bacteria counts may not be entirely correct. It is important to consider that even children who are subjected only to conventional restoration/ART may be indirectly motivated to take care of their teeth.

In G_C, a trend toward returning to baseline levels of total bacteria and *S. mutans* counts was observed one month after the educational strategy. In G_{ART}, total bacteria count remained lower than baseline one month after tooth restoration, although an increasing trend for *S. mutans* percentage in relation to total bacteria was observed, as showed by previous studies (Loesche *et al.*, 1977; Wright *et al.*, 1992). This finding may be explained by the fact that between T2 and T3, no other preventive intervention was made, and a lack of motivation may have occurred. In the study of Wright *et al.* (1992), all microbial populations monitored were predicted to return to their baseline levels within five months after restorative treatment in 50% of the participants. Other previous studies have observed that *S. mutans* continued to be at lower levels at least 6 months after restorative treatments (Twetman *et al.*, 1999; Roshan *et al.*, 2010). Removing infected carious dentine and sealing the cavities may help reduce biofilm accumulation, although continuous preventive strategies are needed to maintain low levels of *S. mutans*, as patient motivation, supervised toothbrushing, and other efforts.

Though saliva may not direct reflect the bacterial composition or metabolic activity found in biofilm of the diseased sites (Simón-Soro and Mira, 2015), it has been useful in monitoring early childhood caries risk (Nie *et al.*, 2014), in therapeutic approaches (Carvalho

and Bezerra, 2003; Roshan *et al.*, 2010), and in the development of diagnostic tools (Khanna and Walt, 2015). The early monitoring for caries risk may help guiding educational programs for caregivers and early dental referring to preventive therapies (Hajishengallis *et al.*, 2015). Although microorganisms are recognized as having a central role in dental caries, this disease result from improper interactions between oral microorganisms and host that stimulate the establishment of cariogenic biofilms on tooth surfaces, promoting demineralization of tooth tissues; environmental factors such as diet has significant impact, especially regarding its carbohydrate content (Mattos-Graner *et al.*, 2014).

The number of carious lesions, fillings and other retention sites are thought to influence microbial colonization in a negative way, and the restoration of carious teeth assists in biofilm control. While sealants or restorations are surface-located treatments, educating children in applying oral hygiene daily by educational strategies might have a further-reaching impact in preventing carious lesions (Hilgert *et al.*, 2015). Studies have observed that primary school children are influenced by parents' attitudes and behavior (Okada *et al.*, 2002); thus, it would be interesting if educational strategies could be emphasized with parents' involvement (Angelopoulou *et al.*, 2015). The present findings reinforce the concept that oral health programs may be especially relevant in the primary school level (Graham *et al.*, 2005), a better place for changing behaviors intervention, especially those target-groups in which healthy habits were not established earlier. These behaviors, once established, may persist throughout adulthood.

CONCLUSIONS

The findings of the present study emphasize the importance of continuous educational and preventive programs on oral health to improve clinical parameters and salivary physicochemical and microbiological characteristics for both caries-free children and children with caries experience.

ACKNOWLEDGMENTS

The authors thank the children, parents, directors and teachers of the public schools for participating in the study. They are grateful to the State of São Paulo Research Foundation (FAPESP, SP, Brazil, n. 2012/04492-2; 2014/20464-4) and the National Council for Scientific and Technological Development (CNPq, DF, Brazil, n. 162163/2014-2), for funding the study. Our thanks are extended to Juana Salas Rosmeri Huamani for her help on data collection; Dr

Soraya Coelho Leal and the staff of the Department of Dentistry of the Faculty of Health Sciences, University of Brasília, Brasília, Brazil, for their hospitality and contribution during the ART training phase; and Dr. Fábio Luiz Mialhe, Department of Community Dentistry of Piracicaba Dental School, University of Campinas, Piracicaba, Brazil, for his help on DMFT calibration and cooperation during the project.

AUTHORS' CONTRIBUTIONS

C.N.F. participated in conception and design of the study, acquisition of data and drafting the manuscript. K.G.S. contributed to the data collection; G.C.A. and M.I.K. were responsible for microbiological analysis. P.M.C., M.I.K. and T.S.B. participated in the conception and design of the study, data analysis, interpretation and critical revision of the manuscript. All authors have read and approved the final manuscript.

REFERENCES

Alaluusua S, Malmivirta R. Early plaque accumulation-a sign for caries risk in young children. Community Dent Oral Epidemiol. 1994 Oct;22(5 Pt 1):273-6.

Angelopoulou MV, Kavvadia K, Taoufik K, Oulis CJ. Comparative clinical study testing the effectiveness of school based oral health education using experiential learning or traditional lecturing in 10 year-old children. BMC Oral Health. 2015 Apr 28;15:51.

Baca P, Castillo AM, Bravo M, Junco P, Baca AP, Llodra JC. Mutans streptococci and lactobacilli in saliva after the application of fissure sealants. Oper Dent. 2002 Mar-Apr;27(2):107-11.

Bernabé E, Vehkalahti MM, Sheiham A, Aromaa A, Suominen AL. Sugar-sweetened beverages and dental caries in adults: a 4-year prospective study. J Dent. 2014 Aug;42(8):952-8.

Benelli EM, Serra MC, Rodrigues AL Jr, Cury JA. In situ anticariogenic potential of glass ionomer cement. Caries Res. 1993;27(4):280-4.

Bhardwaj VK, Sharma KR, Luthra RP, Jhingta P, Sharma D, Justa A. Impact of school-based oral health education program on oral health of 12 and 15 years old school children. J Educ Health Promot. 2013 Jul 31;2:33.

Bonanato K, Pordeus IA, Moura-Leite FR, Ramos-Jorge ML, Vale MP, Paiva SM. Oral disease and social class in a random sample of five-year-old preschool children in a Brazilian city. Oral Health Prev Dent. 2010;8(2):125-32.

Bonifácio CC, Kleverlaan CJ, Raggio DP, Werner A, Carvalho RC, Amerongen WE. Propriedades físico-mecânicas dos cimentos de ionômero de vidro indicados para o tratamento restaurador atraumático. Aust Dent J. 2009; 54 (3): 233-7.

Carvalho CK, Bezerra AC. Microbiological assessment of saliva from children subsequent to atraumatic restorative treatment (ART). Int J Paediatr Dent. 2003 May;13(3):186-92.

Chen Z, Saxena D, Caufield PW, Ge Y, Wang M, Li Y. Development of species-specific primers for detection of *Streptococcus mutans* in mixed bacterial samples. FEMS Microbiol Lett. 2007 Jul;272(2):154-62.

Cury JA, de Oliveira BH, Dos Santos AP, Tenuta LM. Are fluoride releasing dental materials clinically effective on caries control? Dent Mater. 2016 Jan 5.

de Amorim RG, Leal SC, Frencken JE. Survival of atraumatic restorative treatment (ART) sealants and restorations: a meta-analysis. Clin Oral Investig. 2012 Apr;16(2):429-41.

Dolezel J, Bartos J, Voglmayr H, Greilhuber J. Nuclear DNA content and genome size of trout and human. Cytometry A. 2003 Feb;51(2):127-8.

Duggal MS, Toumba KJ, Amaechi BT, Kowash MB, Higham SM. enamel demineralization in situ with various frequencies of carbohydrate consumption with and without fluoridetoothpaste. J Dent Res. 2001 Aug;80(8):1721-4.

Esan A, Folayan MO, Egbetade GO, Oyedele TA. Effect of a school-based oral health education programme on use of recommended oral self-care for reducing the risk of caries by children in Nigeria. Int J Paediatr Dent. 2015 Jul;25(4):282-90.

Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res. 2004 May-Jun;38(3):182-91.

Fierer N, Jackson JA, Vilgalys R, Jackson RB. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl Environ Microbiol. 2005 Jul;71(7):4117-20.

Frencken JE, Pilot T, Songpaisan Y, Phantumvanit P. Atraumatic restorative treatment (ART): rationale, technique, and development. J Public Health Dent. 1996;56(3 Spec No):135-40.

Frencken JE, van 't Hof MA, van Amerongen WE, Holmgren CJ. Effectiveness of single-surface ART restorations in the permanent dentition: a meta-analysis. J Dent Res. 2004 Feb;83(2):120-3.

Frencken JE. Evolution of the the ART approach: highlights and achievements. J Appl Oral Sci. 2009;17 Suppl:78-83.

Frencken JE. The state-of-the-Art of ART Sealants. Dent Update. 2014 Mar;41(2):119-20,

122-4.

Gopinath VK, Arzreanne AR. Saliva as a diagnostic tool for assessment of dental caries. Arch Orofac Sci. 2006;1:57-9.

Graham, Palmer, Stephen, George, Elliott, Eloise. Effects of a Web-Based Health Program on Fifth Grade Children's Physical Activity Knowledge, Attitudes and Behavior. Am J Health Educ. 2005 Mar-Apr; 36(2):86-93.

Griffin SO, Oong E, Kohn W, Vidakovic B, Gooch BF; CDC Dental Sealant Systematic Review Work Group, Bader J, Clarkson J, Fontana MR, Meyer DM, Rozier RG, Weintraub JA, Zero DT. The effectiveness of sealants in managing caries lesions. J Dent Res. 2008 Feb;87(2):169-74.

Guglielmi CA, Raggio DP, Takeuti ML, Camargo LB, Imparato JC. [A liberação de flúor e absorção de ionômero de vidro cimentos indicado para o tratamento restaurador atraumático]. Pesq Bras Odontoped Clin Integr. 2011; 11 (4): 561-5.

Hajishengallis E, Parsaei Y, Klein MI, Koo H. Advances in the microbial etiology and pathogenesis of early childhood caries. Mol Oral Microbiol. 2015 Dec 30.

Hilgert LA, Leal SC, Mulder J, Creugers NH, Frencken JE. Caries-preventive Effect of Supervised Toothbrushing and Sealants. J Dent Res. 2015 Sep;94(9):1218-24.

Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global Burden of Untreated Caries: A Systematic Review and Metaregression. J Dent Res. 2015 May;94(5):650-658.

Khanna P, Walt DR. Salivary diagnostics using a portable point-of-service platform: a review. Clin Ther. 2015 Mar 1;37(3):498-504.

Kitasako Y, Burrow MF, Stacey M, Huq L, Reynolds EC, Tagami J. Comparative analysis of three commercial saliva testing kits with a standard saliva buffering test. Aust Dent J. 2008 Jun;53(2):140-4.

Klein MI, Scott-Anne KM, Gregoire S, Rosalen PL, Koo H. Molecular approaches for viable bacterial population and transcriptional analyses in a rodent model of dentalcaries. Mol Oral Microbiol. 2012 Oct;27(5):350-61.

Klock B, Krasse B. Effect of caries-preventive measures in children with high numbers of S. mutans and lactobacilli. Scand J Dent Res. 1978 Jul;86(4):221-30.

Löe H. The Gingival Index, the Plaque Index and the Retention Index Systems. J Periodontol. 1967 Nov-Dec;38(6):Suppl:610-6.

Loesche WJ, Bradbury DR, Woolfolk MP. Reduction of dental decay in rampant caries

individuals following short-term kanamycin treatment. J Dent Res. 1977 Mar;56(3):254-65.

Malekipour MR, Messripour M, Shirani F. Buffering capacity of saliva in patients with active dental caries. Asian J Biochem. 2008;3:280–3.

Marcenes W, Kassebaum NJ, Bernabé E, Flaxman A, Naghavi M, Lopez A, Murray CJ. Global burden of oral conditions in 1990-2010: a systematic analysis. J Dent Res. 2013; 92(7):592-7.

Mattos-Graner RO, Klein MI, Smith DJ. Lessons Learned from Clinical Studies: Roles of Mutans Streptococci in the Pathogenesis of Dental Caries. Curr Oral Health Rep. 2014;1:70-8.

Mejía-Rubalcava C, Alanís-Tavira J, Mendieta-Zerón H, Sánchez-Pérez L. Changes induced by music therapy to physiologic parameters in patients with dental anxiety. Complement Ther Clin Pract. 2015 Nov;21(4):282-6.

Mickenautsch S, Yengopal V. desmineralização do tecido dentário duro ao lado ionômeros modificados por resina e resinas compostas: uma revisão sistemática quantitativa. J Sci Oral. 2010; 52 (3): 347-57.

Ministry of Health, Brazil. Health Attention Secretariat. Basic Attention Department. National Oral Health Coordination. SB Brasil 2010: Pesquisa Nacional de Saúde Bucal (SB Brasil 2010: National oral health research). Technical project. Brasília, 2009.

Ministry of Health, Brazil. Projeto Saúde Bucal Brasil: levantamento nacional em saúde bucal 2010 [last Access: May 21th, 2014]. Available at: http://189.28.128.100/dab/docs/geral/apresentacao _SB2010.pdf. 2011

Narvai PC, Frazão P, Roncalli AG, Antunes JL. Dental caries in Brazil: decline, polarization, inequality and social exclusion. Rev Panam Salud Publica. 2006 Jun;19(6):385-93.

Nie S, Henley WH, Miller SE, Zhang H, Mayer KM, Dennis PJ, Oblath EA, Alarie JP, Wu Y, Oppenheim FG, Little FF, Uluer AZ, Wang P, Ramsey JM, Walt DR. An automated integrated platform for rapid and sensitive multiplexed protein profiling using human saliva samples. Lab Chip. 2014 Mar 21;14(6):1087-98.

Nocker A, Sossa-Fernandez P, Burr MD, Camper AK. Use of propidium monoazide for live/dead distinction in microbial ecology. Appl Environ Microbiol. 2007 Aug;73(16):5111-7.

Okada M, Kawamura M, Kaihara Y, Matsuzaki Y, Kuwahara S, Ishidori H, Miura K. Influence of parents' oral health behaviour on oral health status of their school children: an exploratory study employing a causal modelling technique. Int J Paediatr Dent. 2002

Mar;12(2):101-8.

Parisotto TM, King WF, Duque C, Mattos-Graner RO, Steiner-Oliveira C, Nobre-Dos-Santos M, Smith DJ. Immunological and microbiologic changes during caries development in young children. Caries Res. 2011;45(4):377-85.

Rahimtoola S, van Amerongen E. Comparison of two tooth-saving preparation techniques for one-surface cavities. ASDC J Dent Child. 2002 Jan-Apr;69(1):16-26, 11.

Rao A, Sequeira SP, Peter S. Prevalence of dental caries among school children of Modibidri. J Indian Soc Pedod Prev Dent. 1999 Jun;17(2):45-8.

Redmo Emanuelsson IM, Thornqvist E. Distribution of mutans streptococci in families: a longitudinal study. Acta Odontol Scand. 2001 Apr;59(2):93-8.

Roshan NM, Shigli AL, Deshpande SD. Microbiological evaluation of salivary Streptococcus mutans from children of age 5-7 years, pre- and post-atraumatic restorative treatment. Contemp Clin Dent. 2010 Apr;1(2):94-7.

Schriks MC, van Amerongen WE. Atraumatic perspectives of ART: psychological and physiological aspects of treatment with and without rotary instruments. Community Dent Oral Epidemiol. 2003 Feb;31(1):15-20.

Serra MC, Cury JA. The in vitro effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. Quintessence Int. 1992 Feb;23(2):143-7.

Simón-Soro A, Mira A. Solving the etiology of dental caries. Trends Microbiol. 2015 Feb;23(2):76-82.

Singh S, Sharma A, Sood PB, Sood A, Zaidi I, Sinha A. Saliva as a prediction tool for dental caries: An in vivo study. J Oral Biol Craniofac Res. 2015 May-Aug;5(2):59-64.

Souto M, Donly KJ. Caries inhibition of glass ionomers. Am J Dent. 1994 Apr;7(2):1224.

Sutthavong S, Taebanpakul S, Kuruchitkosol C, Ayudhya TI, Chantveerawong T, Fuangroong S, Cae-Ngow S, Rangsin R. Oral health status, dental caries risk factors of the children of public kindergarten and schools in Phranakornsriayudhya, Thailand. J Med Assoc Thai. 2010 Nov;93 Suppl 6:S71-8.

Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ. 2001 Oct;65(10):1028-37.

ten Cate JM, van Duinen RN. Hypermineralization of dentinal lesions adjacent to glass-ionomer cement restorations. J Dent Res. 1995 Jun;74(6):1266-71.

Twetman S, Fritzon B, Jensen B, Hallberg U, Ståhl B. Pre- and post-treatment levels of salivary mutans streptococci and lactobacilli in pre-school children. Int J Paediatr Dent.

1999 Jun;9(2):93-8.

van't Hof MA, Frencken JE, van Palenstein Helderman WH, Holmgren CJ. The atraumatic restorative treatment (ART) approach for managing dental caries: a meta-analysis. Int Dent J. 2006 Dec;56(6):345-51.

Vadiakas G, Oulis CJ, Tsinidou K, Mamai-Homata E, Polychronopoulou A. Oral hygiene and periodontal status of 12 and 15-year-old Greek adolescents. A national pathfinder survey Eur Arch Paediatr Dent. 2012;13(1):11–20.

World Health Organization. Oral health surveys. Basic methods. 4th ed. Geneva: World Health Organization, 1997.

Wright JT, Cutter GR, Dasanayake AP, Stiles HM, Caufield PW. Effect of conventional dental restorative treatment on bacteria in saliva. Community Dent Oral Epidemiol. 1992 Jun;20(3):138-43.

Zero DT. Sugars - the arch criminal?. Caries Res. 2004 May-Jun;38(3):277-85.

3 CONCLUSÃO

Os achados do presente estudo reforçam a importância de programas preventivos em saúde bucal para a melhora de parâmetros clínicos e das características físico-químicas e microbiológicas salivares tanto em crianças livres de cárie como aquelas com experiência de cárie, submetidas a intervenções. Uma tendência de retorno aos níveis microbiológicos iniciais foi observada, enfatizando a necessidade de estratégias contínuas em saúde bucal.

REFERÊNCIAS

Baca P, Castillo AM, Bravo M, Junco P, Baca AP, Llodra JC. Mutans streptococci and lactobacilli in saliva after the application of fissure sealants. Oper Dent. 2002 Mar-Apr;27(2):107-11.

Bhardwaj VK, Sharma KR, Luthra RP, Jhingta P, Sharma D, Justa A. Impact of school-based oral health education program on oral health of 12 and 15 years old school childr en. J Educ Health Promot. 2013 Jul 31;2:33.

Carvalho CK, Bezerra AC. Microbiological assessment of saliva from children subsequent to atraumatic restorative treatment (ART). Int J Paediatr Dent. 2003 May;13(3):186-92.

Cury JA, Tenuta LM. Enamel remineralization: controlling the caries disease or treating early caries lesions? Braz Oral Res. 2009;23 Suppl 1:23-30.

Cury JA, de Oliveira BH, Dos Santos AP, Tenuta LM. Are fluoride releasing dental materials clinically effective on caries control? Dent Mater. 2016 Jan 5.

de Amorim RG, Leal SC, Frencken JE. Survival of atraumatic restorative treatment (ART) sealants and restorations: a meta-analysis. Clin Oral Investig. 2012 Apr;16(2):429-41.

Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res. 2004 May-Jun;38(3):182-91.

Fejerskov O, Cury JA, Tenuta LMA, Marinho V. Fluorides in caries control. In: Fejerskov O, Kidd E, editors. Dental caries: the disease and its clinical management. 3rd ed. Oxford: Wiley Blackwell; 2015. p. 245–76.

Forss H, Jokinen J, Spets-Happonen S, Seppä L, Luoma H. Fluoride and mutans streptococci in plaque grown on glass ionomer and composite. Caries Res. 1991;25(6):454-8.

Forsten L. Fluoride release and uptake by glass ionomers. Scand J Dent Res. 1991 Jun;99(3):241-5.

Frencken JE, van 't Hof MA, van Amerongen WE, Holmgren CJ. Effectiveness of single-surface ART restorations in the permanent dentition: a meta-analysis. J Dent Res. 2004 Feb;83(2):120-3.

Frencken JE. Evolution of the the ART approach: highlights and achievements. J Appl Oral Sci. 2009;17 Suppl:78-83.

Klock B, Krasse B. Effect of caries-preventive measures in children with high numbers of S. mutans and lactobacilli. Scand J Dent Res. 1978 Jul;86(4):221-30.

Loesche WJ, Bradbury DR, Woolfolk MP. Reduction of dental decay in rampant caries individuals following short-term kanamycin treatment. J Dent Res. 1977 Mar;56(3):254-

65.

Paes Leme AF, Koo H, Bellato CM, Bedi G, Cury JA. The role of sucrose in cariogenic dental biofilm formation--new insight. J Dent Res. 2006 Oct;85(10):878-87.

Rahimtoola S, van Amerongen E. Comparison of two tooth-saving preparation techniques for one-surface cavities. ASDC J Dent Child. 2002 Jan-Apr;69(1):16-26, 11.

Roshan NM, Shigli AL, Deshpande SD. Microbiological evaluation of salivary Streptococcus mutans from children of age 5-7 years, pre- and post-atraumatic restorative treatment. Contemp Clin Dent. 2010 Apr;1(2):94-7.

Saku S, Kotake H, Scougall-Vilchis RJ, Ohashi S, Hotta M, Horiuchi S, Hamada K, Asaoka K, Tanaka E, Yamamoto K. Antibacterial activity of composite resin with glass-ionomer filler particles. Dent Mater J. 2010 Mar;29(2):193-8.

Schriks MC, van Amerongen WE. Atraumatic perspectives of ART: psychological and physiological aspects of treatment with and without rotary instruments. Community Dent Oral Epidemiol. 2003 Feb;31(1):15-20.

Vadiakas G, Oulis CJ, Tsinidou K, Mamai-Homata E, Polychronopoulou A. Oral hygiene and periodontal status of 12 and 15-year-old Greek adolescents. A national pathfinder survey Eur Arch Paediatr Dent. 2012;13(1):11–20.

van't Hof MA, Frencken JE, van Palenstein Helderman WH, Holmgren CJ. The atraumatic restorative treatment (ART) approach for managing dental caries: a meta-analysis. Int Dent J. 2006 Dec;56(6):345-51.

Vieira AR, de Souza IP, Modesto A. Fluoride uptake and release by composites and glass ionomers in a high caries challenge situation. Am J Dent. 1999 Feb;12(1):14-8.

Wright JT, Cutter GR, Dasanayake AP, Stiles HM, Caufield PW. Effect of conventional dental restorative treatment on bacteria in saliva. Community Dent Oral Epidemiol. 1992 Jun;20(3):138-43.

APÊNDICES

APÊNDICE 1

Termo de Consentimento Livre e Esclarecido

As informações contidas neste documento visam convidá-lo a participar, com capacidade de livre arbítrio e sem qualquer coação.

Título do trabalho: "Impacto do tratamento restaurador atraumático associado a estratégias educativas em saúde bucal na ansiedade odontológica, na qualidade de vida relacionada à saúde bucal e na composição bioquímica e microbiológica salivar de escolares".

Responsáveis pela pesquisa: são as Cirurgiãs Dentistas Profa. Dra. Paula Midori Castelo (responsável), Profa. Dra. Taís de Souza Barbosa, Karina Guedes de Sousa (Mestranda), Juana Rosmeri Salas Huamani (Mestranda) e Camila Nobre de Freitas (mestranda).

Objetivos. Temos como objetivo avaliar o impacto do tratamento restaurador atraumático associado às abordagens educativas em saúde bucal na qualidade de vida relacionada à saúde bucal, no nível de ansiedade ao tratamento odontológico e nas características bioquímicas e microbiológicas da saliva de escolares de seis e sete anos de idade, da rede pública do município de Saltinho, SP.

Justificativa. A cárie dentária ainda acomete um número grande de crianças e isso pode ser explicado pela dificuldade de acesso aos serviços odontológicos, pelo medo e ansiedade vinculados ao tratamento. A dor, o desconforto e as infecções causados pela cárie tem um impacto negativo na qualidade de vida da criança, como: faltas escolares, problemas nutricionais, hospitalizações e medo. Estratégias em saúde bucal têm sido utilizadas mundialmente, principalmente nas escolas, para melhorar os hábitos de saúde bucal das crianças, prevenindo assim o aparecimento de doenças da boca. O tratamento restaurador atraumático remove a cárie com instrumentos manuais, sem a utilização de "motor", evitando- se ao máximo a geração de dor ou desconforto, além de ser realizado em ambiente escolar, sem que a criança necessite ir a um consultório odontológico. Assim, é importante verificar se tais métodos já descritos na literatura são realmente efetivos na prevenção da ansiedade odontológica, melhora da qualidade de vida relacionada à saúde bucal e na manutenção de condições salivares favoráveis à saúde bucal.

Procedimentos da pesquisa. Todos os procedimentos da pesquisa serão realizados pelas pesquisadoras (Cirurgiãs Dentistas): Karina Guedes de Sousa, Juana Rosmeri Salas Huamani e Camila Nobre de Freitas nas escolas da rede pública do município de Saltinho - SP, em horários que não atrapalhem as atividades universitárias, sob supervisão das Professoras Doutoras Paula Midori Castelo e Taís de Souza Barbosa.

Seleção dos voluntários: serão selecionados 225 escolares, meninas e meninos, alunos da rede pública do município de Saltinho - SP, sendo 135 escolares com cárie, sem dor espontânea e que não precisem de tratamento endodôntico (tratamento de canal) nem exodontias (extração do dente) e que receberão tratamento restaurador atraumático; e selecionados outros 90 escolares sem cárie que formarão o grupo controle.

Informações sobre a possibilidade de inclusão em grupo controle ou placebo: serão selecionados 90 escolares sem cárie que formarão o grupo controle e participarão das estratégias educativas (prevenção à doença cárie).

Exame clínico odontológico: o instrumental utilizado no exame clínico será o de uso rotineiro da clínica

odontológica (espelho bucal e sonda esterilizados e gaze); as Dentistas usarão equipamentos de proteção (gorro, máscara, avental e luvas descartáveis). Serão avaliados a experiência de cárie (número de superfícies dos dentes cariadas, perdidas e restauradas), presença de biofilme (placa dental) e gengivite (inflamação da gengiva). Escolares que necessitem tratamento odontológico mais complexo serão encaminhados para a Clínica de Odontopediatria da Faculdade de Odontologia de Piracicaba.

Avaliação da qualidade de vida e percepção da saúde bucal: os escolares serão entrevistados individualmente com um questionário de 16 itens que verificarão o impacto das doenças bucais nos sintomas bucais, limitações funcionais, bem-estar emocional e bem-estar social da criança. Haverá uma pergunta adicional que verificará se a criança percebeu alguma mudança em relação a sua saúde bucal nos últimos dias.

Estratégias Educativas: serão oferecidas orientações sobre cuidados de saúde bucal, explicando as causas das doenças que afetam a boca e como preveni-las, que serão ofertadas uma vez por semana durante quatro semanas na escola.

O "tratamento restaurador atraumático": Serão tratados com esta técnica os dentes com cárie que não apresentem dor espontânea, nem indicação para tratamento de endodôntico (canal) ou exodontia (extração); serão usados instrumentos manuais esterilizados (espelho clínico, escavador, pinça clínica – não é usado o "motor"), sendo que depois de removida a cárie, o dente limpo será restaurado com cimento ionômero de vidro. Pode ser que seja necessária a aplicação de anestesia em caso de dor e por isso a Dentista pede neste momento a você (responsável pela criança) que a informe se seu filho/a já tomou anestesia e se teve alguma reação alérgica. Os retornos e acompanhamentos necessários serão agendados pelas Dentistas e as crianças e seus responsáveis terão livre acesso a elas para qualquer dúvida ou necessidade de esclarecimentos por telefone, mensagem ou email.

Avaliação da saliva: A saliva da criança será coletada para medir cortisol (hormônio relacionado com o estresse), cálcio, fosfato e bactérias existentes na boca, utilizando-se material esterilizado, sem dor nem desconforto durante as coletas.

Métodos alternativos existentes: Não há métodos alternativos para coleta de saliva, bem como para o tratamento restaurador atraumático. Os mesmos não serão realizados caso seu filho(a) manifeste recusa.

Riscos previsíveis, benefícios e vantagens: Os exames clínicos seguirão os passos da rotina clínica, utilizando-se instrumental e material adequados e esterilizados. Os possíveis riscos que envolvem um tratamento odontológico serão sempre minimizados, como dor, desconforto e trauma. Todas as crianças serão avaliadas e receberão os mesmos procedimentos e tratamentos necessários, bem como orientações de prevenção de doenças bucais; assim, os pais/responsáveis serão informados quanto à condição de saúde bucal de seu filho/a e quais tratamentos serão necessários por meio de carta; nela, estará assegurada à criança o atendimento odontológico a ser realizado na escola ou na Faculdade de Odontologia de Piracicaba – UNICAMP em todos os casos, eletivos ou emergenciais, mesmo que a criança deixe de participar do estudo.

Forma de acompanhamento e assistência: Os pesquisadores envolvidos na pesquisa estarão à disposição para quaisquer informações desejadas, para esclarecer as dúvidas e para minimizar qualquer desconforto.

Forma de contato com os pesquisadores e com o CEP: *Qualquer dúvida ou problema, por favor, comunicar-nos com a maior brevidade possível.*

O endereço para contato com o CEP se encontra logo abaixo do campo destinado às assinaturas deste termo.

Garantia de esclarecimentos, indenização e reparação de dano: A crianças e seu responsável têm a garantia de que receberá respostas a qualquer pergunta sobre qualquer dúvida referente aos procedimentos, riscos e benefícios

empregados nesta pesquisa, em qualquer momento.

Garantia de sigilo e retirada do consentimento: Haverá sigilo e anonimato quanto aos dados confidenciais obtidos da criança e o responsável tem a liberdade de retirar seu consentimento a qualquer momento e a criança deixar de participar do estudo, sem qualquer prejuízo dos benefícios advindos da pesquisa (como por ex., as palestras educativas).

Formas de ressarcimento: Não há previsão de ressarcimento ou indenização por dano, pois a participação na pesquisa não trará riscos, nem causará despesas ao voluntário.

Entrega de cópia: Este termo de consentimento compõe-se de duas cópias idênticas, sendo uma entregue ao responsável e outra que será arquivada pelos pesquisadores.

SUA ASSINATURA EM TODAS AS PÁGINAS INDICA QUE VOCÊ PERMITIU QUE SEU FILHO/A PARTICIPE DA PESQUISA COMO VOLUNTÁRIO E QUE VOCÊ LEU E ENTENDEU TODAS AS INFORMAÇÕES ACIMAEXPLICADAS.

_____ _____

Nome da criança Nome do responsável

RG/CPF:

Telefone:

Endereço:_____

_____ _____

Assinatura do responsável Assinatura do pesquisador

ATENÇÃO: A SUA PARTICIPAÇÃO EM QUALQUER TIPO DE PESQUISA É VOLUNTÁRIA. EM CASO DE DÚVIDA QUANTO AOS SEUS DIREITOS ESCREVA PARA O COMITÊ DE ÉTICA EM PESQUISA DAFOP-UNICAMP:

Endereço: Av Limeira, 901 CEP – FOP, CEP 13.414-903 Piracicaba, SP Email: cep@fop.unicamp.br; website: http://www.fop.unicamp.br/cep/index.htm

Tel/Fax-CEP (0xx19)2106-5349

APÊNDICE 2

Termo de Assentimento

Pesquisa intitulada: "**Impacto do tratamento restaurador atraumático associado a estratégias educativas em saúde bucal na ansiedade odontológica, na qualidade de vida relacionada à saúde bucal e na composição bioquímica e microbiológica salivar de escolares**", realizada pelas pesquisadoras (Cirurgiãs Dentistas) Karina Guedes de Sousa, Juana Rosmeri Salas Huamani, Camila Nobre de Freitas, Profa. Dra. Taís de Souza Barbosa e Profa. Dra. Paula Midori Castelo (responsável). A seguir, encontra-se o termo de assentimento que deverá ser lido a cada criança, de modo que ela possa compreender e manifestar a aprovação ou desaprovação na participação desta pesquisa, por meio da concordância ou recusa em participar das fases de exame clínico, coleta de saliva e tratamento restaurador atraumático se for o caso, sendo essa recusa manifestada por choro ou negação a qualquer um dos procedimentos a que será submetida para a realização da pesquisa.

TERMO DE ASSENTIMENTO

"Olá, bom dia, sou sua Dentista, tudo bem? Posso olhar sua boca para ver quantos dentes você tem? Tenho uma luz e um espelho de dentes, que vou mostrar para você. Vou olhar seus dentes e se tiver algum bicho aí dentro, vamos tirar ele e fazer um curativo com sua ajuda. Depois faremos uma coisa bem legal!, você vai deixar cair a saliva da sua boca dentro de um copinho, tudo o que você puder. Vamos ver quanto de saliva você consegue cuspir. É bem rápido e fácil. Quando terminar, você pode voltar para sua sala, junto de seus amiguinhos." Em caso de recusa a participar da pesquisa, de alguma forma, será dito à criança: *"Tudo bem, não tem problema. Fica para uma próxima vez que eu vier aqui na escola. Você foi muito legal em conversar comigo. Muito obrigada. Vamos voltar para sua sala?"*

Nome da criança:_____

A criança demonstrou em relação ao que foi dito: ( ) aceite / ( ) recusa

Data:_____/___/____

Pesquisadoras / Cirurgiãs Dentistas:

_____	_____	_____
Karina G. de Sousa	Juana S. Huamani	Camila N. de Freitas
CPF: 008.489.424-50	CPF: 236.924.078-40	CPF: 395.086.128-94

ANEXOS

ANEXO 1

Certificado do Comitê de Ética em Pesquisa

27/02/2015 Comitê de Ética em Pesquisa - Certificado

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Impacto do tratamento restaurador atraumático associado a estratégias educativas em saúde bucal na ansiedade odontológica, na qualidade de vida relacionada à saúde bucal e na composição bioquímica e microbiológica salivar de escolares** ", protocolo nº 136/2014, dos pesquisadores Paula Midori Castelo Ferruá, Camila Nobre de Freitas, Juana Rosmeri Salas Huamani, Karina Guedes de Sousa e Taís de Souza Barbosa, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 27/02/2015.

The Ethics Committee in Research of the Piracicaba Dental School - University of Campinas, certify that the project "**Impact of atraumatic restorative treatment associated with oral health educational strategies on dental anxiety, oral health-related quality of life and salivary biochemical and microbiological composition of schoolchildren**", register number 136/2014, of Paula Midori Castelo Ferruá, Camila Nobre de Freitas, Juana Rosmeri Salas Huamani, Karina Guedes de Sousa and Taís de Souza Barbosa, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee on Feb 27, 2015.

Prof. Dr. Jacks Jorge Junior
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Felippe Bevilacqua Prado
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 2

Declaração de Treinamento

Universidade de Brasília

Brasília, 27 de Agosto de 2014

Declaração

Declaro para os devidos fins que Camila Nobre de Freitas participou de um treinamento sobre o Tratamento Restaurador Atraumático – ART entre os dias 25 e 27 de agosto do corrente ano.

O curso foi coordenado pela professora Soraya Coelho Leal e foi dividido em 02 etapas: teoria (8 horas) e prática (12 horas). As atividades práticas foram realizadas na clínica Odontológica da Universidade de Brasília e na Escola CF2 da Estrutural, Distrito Federal.

Soraya Coelho Leal
Coordenadora da disciplina de Odontopediatria
Universidade de Brasília