UNIVERSIDADE ESTADUAL PAULISTA
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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ODONTOLÓGICA

Kevin Bruce Hall

Influência da quantidade de dentifrício e concentração de fluoreto na retenção intrabucal de fluoreto em crianças

Araçatuba - SP

2015

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Influência da quantidade de dentifrício e concentração de fluoreto na retenção intrabucal de fluoreto em crianças

Dissertação apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Julio de Mesquita Filho" – UNESP, como parte dos requisitos para a obtenção do título de Mestre em Ciência Odontológica – Área Saúde Bucal da Criança.

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Coorientador: Prof. Dr. Alberto Carlos Botazzo Delbem

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Dedicatória

Dedico este trabalho

A **Deus**,

Por ter me guiado desde o inicio por este caminho que escolhi. Nos momentos mais difíceis, foi a Sua ajuda que busquei e sempre a encontrei. A Sua força me incentivou a lutar, todos os dias, quando me levantava para enfrentar mais um dia.

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“Pois metade de mim é partida e a outra metade é saudade.”

Oswaldo Montenegro.

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Agradecimentos

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Epígrafe

"É que tem mais chão nos meus olhos

do que cansaço nas minhas pernas.

Mais esperança nos meus passos

do que tristeza nos meus ombros.

Mais estrada no meu coração

do que medo na minha cabeça."

(Cora Coralina)

Resumo

HALL, K.B. **Influência da quantidade de dentifrício e concentração de fluoreto na retenção intrabucal de fluoreto em crianças.** 2015. Dissertação (Mestrado em Ciência Odontológica, área de Saúde Bucal da Criança) – Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2015.

RESUMO

O presente estudo teve como objetivo avaliar as concentrações de fluoreto (F) na saliva após a escovação com dentifrícios contendo diferentes concentrações de F (0, 550 e 1100 ppm F, pH neutro) em diferentes quantidades (0,1, 0,3 e 0,5 g). Os voluntários (*n* = 24, 8-10 anos de idade) foram aleatoriamente divididos em 9 grupos experimentais, de acordo com as possíveis combinações de dentifrícios e quantidades. Estes foram instruídos a escovar seus dentes 3 vezes ao dia durante uma semana com um dentifrício placebo (período de "wash out"). No sétimo dia, foi coletada uma amostra de saliva estimulada (baseline) previamente à escovação, seguindo um protocolo duplo-cego e cruzado. Em seguida, as crianças escovaram os dentes por um minuto com a respectiva combinação de dentifrício e quantidade, sendo as amostras de saliva coletadas 5, 15, 30, 60 e 120 minutos após. O protocolo foi repetido por 8 semanas adicionais, contemplando o protocolo cruzado do estudo. As amostras foram centrifugadas e os sobrenadantes, congelados para posterior análise de F, utilizando um eletrodo íon-seletivo (Orion 9409) e um eletrodo de referência (Orion 9002) após tamponamento com TISAB III. A área sob a curva (AUC) para o clearance do fluoreto na saliva foi calculada usando os pontos individuais (regra trapezoidal). Os dados de fluxo salivar, concentração de F na saliva nos diferentes tempos de coleta, bem como de AUC foram submetidos a ANOVA a dois critérios, de medidas repetidas, seguida pelo teste de Student-Newman-Keuls ($p<0.05$). Foi observado um pico nas concentrações de F na saliva 5 min após a escovação, as quais decresceram exponencialmente nas coletas seguintes. Uma relação dose-resposta evidente foi observada entre a concentração e quantidade de F nos dentifrícios e a AUC da concentração de F na saliva ($p<0,001$). A escovação com 0,3 e 0,5 g do dentifrício com concentração reduzida de F (550 ppm F) levou a valores de AUC significativamente mais altos quando comparados aos obtidos após o uso de 0,1 g do dentifrício convencional (1100 ppm F). Pode-se concluir que a escovação com um dentifrício de baixa concentração de F aplicando a técnica transversal promove uma maior concentração de F na saliva

em comparação a um dentifrício convencional usando a quantidade do tamanho de uma ervilha.

Palavras-chave: Dentifrícios – Saliva – Fluoretos

Abstract

HALL, K.B. **Influence of the amount of dentifrice and fluoride concentrations on intraoral fluoride retention in children.** 2015. Dissertação (Mestrado em Ciência Odontológica, área de Saúde Bucal da Criança) – Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2015.

ABSTRACT

This aim of this study was to evaluate fluoride (F) concentrations in saliva of children after brushing with dentifrices containing different F concentrations (0, 550 and 1100 ppm F) in different amount (0.1, 0.3 and 0.5 g). Volunteers (*n*=24, 8-10 years old) were randomly assigned into 9 experimental groups, according to the possible combinations of F concentrations in the dentifrices and amount used, following a double-blind, crossover protocol. They were instructed to brush their teeth with a placebo dentifrice, 3 times/day, during one week (wash out period). On the 7th day, stimulated saliva was collected prior to toothbrushing (baseline) with the assigned combination of dentifrice type and amount. Following, saliva samples were collected at 5, 15, 30, 60 and 120 min after toothbrushing and the protocol was then repeated for 8 additional weeks, in order to follow the crossover protocol. Saliva samples were then centrifuged and the supernatants were frozen until F analysis, which was carried out using ion-selective (Orion 9409) and reference (Orion 9002) electrodes after buffering with TISAB III. The area under the curve (AUC) for salivary fluoride clearance was calculated using the individual points (trapezoidal rule). Data of salivary flow rate, salivary F concentrations at each collection point, as well as of AUC were submitted to two-way, repeated measures ANOVA, followed by the Student-Newman-Keuls test ($p<0.05$). A marked peak in salivary fluoride concentrations was seen 5 min after brushing, decreasing exponentially afterwards. A clear dose-response relationship was seen between fluoride concentration and amount of dentifrice used and the mean AUC of salivary F concentrations ($p<0.001$). Toothbrushing with 0.3 and 0.5 g of the low-fluoride toothpaste (550 ppm F) led to significantly higher AUC values when compared with the conventional toothpaste (1100 ppm F) using 0.1 g of the product. It can be concluded that brushing with a low-fluoride toothpaste applied using the transversal technique delivers more fluoride to saliva in comparison with a conventional toothpaste in a pea-size amount.

Kevin Bruce Hall

Key words: Dentifrice – Saliva – Fluorine

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Lista de Abreviaturas e Símbolos

ANOVA = Análise de variância

AUC = Área sob a curva

CAAE = Certificado de apresentação para apreciação ética

USA = Estados Unidos da América

FOA = Faculdade de Odontologia de Araçatuba

F = Fluoreto

NaF = Fluoreto de sódio

g = Grama

h = Hora (s)

Log = Logaritmo

µg F/g = Micrograma de fluoreto por grama

µg F/mL = Micrograma de fluoreto por mililitro

µL = Microlitro

µM = Micromolar

mg F/g = Miligrama de fluoreto por grama

mL = Mililitro

mL/minuto = Mililitro por minuto

mV = Milivolt / milivoltagem

n = Número de repetições

ppm F = Parte por milhão de fluoreto

pH = Potencial hidrogeniônico

p = Probabilidade

rpm = Rotações por minuto

SD = Standard Deviation

SE = Standard Error

SP = São Paulo

TISAB = Tampão de ajuste da força iônica total

UNESP = Universidade Estadual Paulista

α = Alfa

~ = Aproximadamente

% = Porcentagem

Sumário

Sumário

Influence of the amount of dentifrice and fluoride concentrations on intraoral fluoride retention in children

Hall KB, Delbem ACB, Nagata ME, Hosida TY, Moraes FRN, Pessan JP

Araçatuba Dental School, Univ. Estadual Paulista (UNESP), Araçatuba, SP, Brasil.

Short tittle: Intraoral F retention after dentifrice use

Key-words: Dentifrice. Saliva. Fluorine.

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*De acordo com as instruções aos autores do periódico Caries Research (Anexo A).

Abstract

This study evaluated fluoride (F) concentrations in saliva of children after brushing with dentifrices containing different F concentrations (0, 550 and 1100 ppm F) in different quantities (0.1, 0.3 and 0.5 g). Volunteers (*n*=24, 8-10 years old) were randomly assigned into 9 experimental groups, according to the possible combinations of dentifrice type and amount, following a double-blind, crossover protocol. They were instructed to brush their teeth with a placebo dentifrice during one week (wash out). On the 7th day, stimulated saliva was collected prior to toothbrushing (baseline) and at 5, 15, 30, 60 and 120 min after toothbrushing. Saliva samples were then centrifuged and the supernatants used for F analysis after buffering with TISAB III. The area under the curve (AUC) for salivary fluoride clearance was calculated using the individual points (trapezoidal rule). Data of salivary flow rate, F concentrations at each collection point and AUC were submitted to two-way, repeated-measures ANOVA, followed by the Student-Newman-Keuls test ($p<0.05$). A marked peak in salivary F concentrations was seen 5 min after brushing, decreasing exponentially afterwards. A clear dose-response relationship was seen between F concentration and amount of dentifrice and the mean AUC of salivary F concentrations ($p<0.001$). Toothbrushing with 0.3 and 0.5 g of the low-fluoride toothpaste led to significantly higher AUC than the conventional toothpaste using 0.1 g. It can be concluded that brushing with a low-fluoride toothpaste applied using the transversal technique delivers more fluoride in saliva in comparison with a conventional toothpaste in a pea-size amount.

1. Introduction

Considering the multifactorial etiology of dental caries, brushing with a fluoride dentifrice can be regarded as the best method of fluoride use, as it combines mechanical removal or disorganization of the biofilm with the therapeutic effects of fluoride on the processes of de- and remineralization [Pessan *et al.*, 2006, 2011]. Due to concerns about the association between the early use of fluoridated dentifrices and the development of dental fluorosis [Wong *et al.*, 2010], some alternatives have been proposed in order to minimize the fluoride uptake by these sources, which include use of dentifrices with reduced concentration of F.

The scientific evidence for recommending these dentifrices are, however, still inconclusive or even contradictory [Walsh *et al.*, 2010; Santos *et al.*, 2013], prompting some professionals to recommend the use of dentifrices with conventional fluoride concentrations (1000-1100 mg F/g), but in small quantities [Villena, 2000]. It is noteworthy, however, that no scientific evidence is available to attest the long-term efficacy of such recommendations. In fact, short-term studies show the opposite: the amount of dentifrice applied on the toothbrush and brushing time were shown to have a direct impact on the resulting salivary fluoride levels *in vivo* [Den Besten and Ko, 1996; Zero *et al.*, 2010], as well as on enamel fluoride uptake and on the remineralization of incipient caries lesions *in situ* [Zero *et al.*, 2010].

While the influence of the amount of dentifrice used during toothbrushing on intraoral parameters has been firmly established in the above-mentioned studies [Den Besten and Ko, 1996; Zero *et al.*, 2010], no study has assessed the impact of fluoride concentration in the dentifrice as a function of the amount used during toothbrushing on the resulting clinical efficacy against dental caries. Considering that the clinical efficacy of fluoridated dentifrices is directly related to intraoral fluoride retention [Duckworth *et al.*, 1992], it would be interesting to assess the impact of using different quantities of fluoridated dentifrice in salivary fluoride levels of children using a standard fluoridated dentifrice (1100 mg F/g) and a low-fluoride toothpaste (550 mg F/g). Given the uncertainties surrounding the clinical efficacy of low fluoride toothpaastes [Walsh *et al.*, 2010], this study could bring useful information on the effects of these formulations on the fluoride concentrations in saliva. Based on dose-response considerations, it is possible that the use of a

standard amount of a low-fluoride dentifrice, such as that obtained by the transversal technique, could raise salivary fluoride concentration at levels similar to those obtained by using a conventional formulation applied in lower quantities. This could facilitate the application of the dentifrice by children and caregivers.

Thus, the aim of this study was to evaluate salivary fluoride concentrations in children after brushing with dentifrice containing different fluoride concentrations (0, 550 and 1100 ppm F, neutral pH) in different quantities (0.1, 0.3 and 0, 5 g). The null hypothesis of the study was that the amount of dentifrice used during brushing and the fluoride concentration in the product does not influence fluoride uptake by saliva.

2. Material and Method

Children aged 8-10 years old, living in Araçatuba – SP (Brazil), participated in this study after approval by the ethics committee of Araçatuba Dental School – UNESP (CAAE 22130113.0.0000.5420) and legal guardians of all participants signed an informed consent form (ANEXO B). Sample size was calculated based on the study by Pessan *et al.* [2008], according to which 19 subjects would be required to detect significant differences in salivary fluoride concentrations of children using Placebo and 1100 ppm F dentifrices (mean difference = 6.26 µmol/L, standard deviation = 4.8), considering a power of 80% (α=0.05). Given the possibility of dropouts due to the crossover protocol, and attempting to have an equal number of subjects under each experimental condition, 27 subjects were initially enrolled (*n*=3/experimental condition).

As inclusion criteria, only volunteers who exclusively consumed water from the public supply both for drinking and in food preparation could participate [Pessan *et al.*, 2006]. Individuals who were on medication that could interfere with the formation of dental plaque and with salivary flow rate could not take part in the study. There was a first meeting with participants, in which they were fully informed about the objectives and the methodology to be used in the study, as well as the need to properly follow the protocol. There were no restrictions on the inclusion of volunteers with a history of tooth caries, which was determined by clinical examination using the criteria of the World Health Organization [WHO, 1997].

Formulation and determination of F in the experimental Dentifrices

The experimental dentifrices were produced in the laboratory of Pediatric Dentistry from Araçatuba Dental School, using the same basic formulation with the following components: titanium dioxide, carboxymethyl cellulose, methyl p-hydroxybenzoate, sodium saccharine, oil peppermint, glycerin, silica abrasive, sodium lauryl sulfate and water. The dentifrices were packaged (ANEXO C) in identical tubes and coded by a researcher not involved in the experimental stage. A formulation without F (Placebo) was used, and sodium fluoride (NaF, Merck®) was added to the experimental dentifrices in order to reach concentrations of 550 and 1100 mg F/g. Total (TF) and ionic fluoride (IF) were assessed (ANEXO D) according to Delbem, *et al.* [2009], using an F ion-specific electrode (Orion 9609 BN; Orion Research Inc, Beverly, MA, USA) coupled to an ion analyzer (Orion 720 A^+; Orion Research Inc, Beverly, MA, USA), previously calibrated with five standard solutions (0.125, 0.25, 0.5, 1.0, and 2.0 µg F/mL).

Experimental Design

The factors of study were the amount of dentifrice applied on the toothbrush (0.1, 0.3 and 0.5 g) and fluoride concentration in the products (0, 550 and 1100 ppm F), resulting in 9 possible combinations of treatments. The amount of dentifrice to be placed on the toothbrush were standardized in a pilot study using Bitufo toothbrushes (ANEXO E) as follows: (1) Full bristles: the dentifrice was applied to the entire length of the brush head; (2) Transversal technique: the dentifrice was applied perpendicular to the long axis of the brush; and (3) Pea-size: the dentifrice was applied at one end of the brush head, as pea-size lump. However, to avoid variations related to the operator and the size of the orifice of the dentifrice tube, a portable scale (Weighing Scale / Lb. Precision Balance 0.01-g; Italy; BEL Engineering s.r.l) was used for weighing the exact amount of dentifrice during the experimental phases (ANEXO F).

Children were instructed to brush their teeth with a placebo dentifrice (without F) at home (ANEXO G), 3 times a day, without restrictions on the amount of dentifrice to be applied to the brush, brushing technique and rinsing habits after brushing. The study was conducted on the premises of a Public School from

Araçatuba (ANEXO H), beginning at 7:00 am. Prior to brushing, salivary flow was stimulated by chewing on a rubber band. All saliva formed during 2 minutes was collected in pre-weighed plastic containers, so that the correct volume of saliva could be obtained by weighing the containers after sample collection (considering the density equal to 1.0), making it possible to calculate salivary flow (mL/min). This sample was used as the baseline value. Children then brushed their teeth for 1 minute, as usually done at home, involving all faces of all teeth, in order to produce a more uniform dilution of the product in the mouth. They were then instructed to expectorate the dentifrice in a disposable container and rinse their mouths with 10 ml of deionized water for 5 seconds. From that point, five saliva samples were collected as described above, at 5, 15, 30, 60 and 120 minutes after brushing, totaling 6 samples per child, according to the protocol described by Zero *et al.* [2010]. At the end of each trial week, the same procedure was performed for each combination of amount and dentifrice.

Fluoride Analysis

Saliva samples were transferred (ANEXO I) from the collection vials to microcentrifuge tubes and centrifuged at 12,000 rpm for 5 minutes, in order to separate desquamated epithelial cells, food particles and bacteria from saliva itself [Vogel *et al.*, 2000]. Then, 200 uL of the supernatant were transferred to a new microcentrifuge tube, to which 20 uL of TISAB III (Orion) was added. This buffered solution was dispensed to the membrane of an ion-selective electrode (Orion 9409), and a reference electrode (Orion Reference Electrode Double Junction 9002) was used to close the circuit. The electrodes were calibrated with standard solutions of known concentrations of fluoride, also buffered with TISAB III. The values obtained in millivolt (mV) were converted into µM of F using a Microsoft Excel spreadsheet, using standard curve data to obtain the analysis parameters (linearity, slope and coefficient of variation). All readings (standards and samples) were done in triplicate (ANEXO J).

Statistical Analysis

Statistical analysis was performed on the software SigmaPlot version 12.0, at a significance level of 5%. The area under the curve (AUC) for salivary fluoride clearance was calculated using the individual points (trapezoidal rule). Data of salivary flow rate (original) and AUC (Log_{10} transformed) passed normality (Shapiro-Wilk) and homogeneity tests (Bartlett), while data of salivary fluoride concentrations at each individual collection points did not pass normality and homogeneity tests even after transformation. All data were submitted to two-way, repeated measures ANOVA, followed by the Student-Newman-Keuls test. Pearson's correlation coefficient was used to verify the relationship between salivary flow rate and AUC for salivary fluoride clearance.

3. Results

Twenty-four subjects completed the 9 experimental phases of the study. Figure 1 shows a marked peak in salivary fluoride concentrations 5 min after brushing, which was dependent on the fluoride concentrations in the products and on the amount of dentifrices used during toothbrushing. Salivary levels then decreased exponentially in two distinct phases (5-15 and 15-30 min), returning to baseline levels afterwards. No visible differences among the groups regarding baseline (pre-brushing) values were observed.

Table 1 presents mean salivary fluoride concentrations at each individual collection point, according to the possible combinations of fluoride concentration and amount of dentifrices used. Significant differences were observed among dentifrices ($F=21.6$, $p<0.001$), times of sample collection ($F=39.7$, $p<0.001$), as well as for the interaction between the variables ($F=21.4$, $p<0.001$). Overall, significant increases in salivary F concentrations were only seen for samples collected 5 min after brushing, except for 0.5 g of the 1100 ppm F toothpaste, which led to significant increases at 5 and 15 min after brushing.

Figure 2 shows the mean areas under the curve (AUC) of salivary fluoride clearance for the different combinations of fluoride concentrations in the dentifrices and amount of product used during toothbrushing. A clear dose-response relationship was seen among the dentifrices ($F=208.8$, $p<0.001$), amount of dentifrice used ($F=62.6$, $p<0.001$), as well as for the interaction between the two

variables (F=15.9, p<0.001). For the placebo dentifrice, no significant differences were seen among the different quantities used during toothbrushing. For the fluoridated dentifrices, however, significant differences were seen between the dentifrices within each amount of product used. Toothbrushing with 0.3 and 0.5 g of the low-fluoride toothpaste (550 ppm F) led to significantly higher AUC values when compared with the conventional toothpaste (1100 ppm F) using 0.1 g of the product.

Mean salivary flow rate was 1.2 mL/min throughout the entire experimental phase, without significant differences among the dentifrices (F=0.97, p=0.39), amount of dentifrice used (F=0.12, p=0.89), nor the interaction between the two variables (F=0.42, p=0.79), as shown in Table 2. An inverse moderate relationship was seen between salivary flow rates and AUC. Pearson's coefficient correlation was -0.31 (p<0.001) when considering the 9 possible combinations of dentifrices and amount, and -0.40 (p<0.001) when excluding the Placebo values.

4. Discussion

The results of a comprehensive Cochrane review revealed that while the evidence of toothpastes containing 1000 ppm F or above in caries prevention is unquestionable, no consistent evidence is available to attest the clinical efficacy of toothpastes containing 500-550 ppm F [Walsh *et al.*, 2010]. This uncertainty has led some authorities to recommend the use of low quantities of a conventional toothpaste, under the assumption that this measure would minimize fluoride intake during toothbrushing without compromising the anticaries effect of the formulation [Davies *et al.*, 2003; Wright *et al.*, 2014]. While lowering the amount of dentifrice has indeed a direct impact on the reduction of fluoride intake [de Almeida *et al.*, 2007; Kobayashi *et al.*, 2011], no scientific evidence is available to attest the effects of such recommendation on caries prevention, nor in intraoral variables likely to affect clinical efficacy. The present study showed that salivary fluoride levels are dependent on the fluoride concentration in the toothpaste, as well as on the amount of toothpaste loaded on the toothbrush, therefore leading to the rejection of the study's null hypothesis.

Baseline salivary fluoride concentrations did not significantly differ among the groups, indicating that any differences in salivary F levels after toothbrushing would result from the treatment combinations (amount of dentifrice and F concentration). Marked increases on salivary fluoride levels were observed 5 min after brushing for

both fluoridated toothpastes, which exponentially decreased for all groups afterwards, reaching levels not significantly different from baseline values 15 minutes after brushing. The only exception was for treatment combination of 0.5 g of 1100 ppm F toothpaste, which led to levels significantly higher than baseline also 15 minutes after brushing. This more pronounced effect indicates that a higher treatment intensity (fluoride concentration x amount used) could potentially lead to a higher protection against dental caries. Although this strategy could not be recommended to children under 7 years of age due to the risk of dental fluorosis, it could be a viable alternative to adults in order to maximize the preventive and therapeutic effects of fluoride [Buzalaf *et al.*, 2011]

The cumulative effect of the treatment combinations on the resulting salivary fluoride concentrations can be observed from the AUC data. Interestingly, the use of 0.3 or 0.5 g of the low-fluoride toothpaste led to higher AUC values when compared to 0.1 g of the conventional toothpaste, indicating that brushing with a low-fluoride toothpaste applied using the transversal technique delivers more fluoride to the oral environment in comparison with a conventional toothpaste in a pea-size amount. The implications of these findings to the clinical practice need to be addressed in details. First of all, there are wide variations in the amount of dentifrice prescribed to children by professionals as a “low amount”, which include descriptions as child’s little fingernail, small amount, smear, pea grain, rice grain, bean grain and transversal technique [dos Santos *et al.*, 2011]. Moreover, there seems to be no consensus regarding the quantity of toothpaste that would correspond to the application using the transversal technique or a pea-size amount, as the same quantity (0.25 g) has been referred as both pea-size [Creeth, 2013] and resulting from the “transversal technique” [Moraes *et al.*, 2007]. In addition to the discrepancies among professionals on the amount of toothpaste to be used by children, such recommendations are subject of interpretations by parents and caregivers, and therefore are likely to vary widely among them. Based on the present data and in previous studies assessing this subject [Den Besten and Ko, 1996; Zero *et al.*, 2010], these variations probably have a great impact on intraoral fluoride uptake and on enamel mineral composition, consequently affecting clinical efficacy, what emphasizes the need of more studies addressing this topic. In this sense, it is possible that the use of the transversal technique would be easier to be learned and standardized by parents and caregivers, as it is possibly less prone to

wide variations when compared to more vague descriptions; this, however, is only a hypothesis as this variable was not assessed in the present study. If this assumption is true, the use of this technique would be helpful at home and especially at schools and kindergartens, in which several children are assisted at the same time.

The above-mentioned considerations, however, should be considered with caution, given the scarcity of well-designed studies assessing the real benefits of low-fluoride toothpastes in caries prevention [Walsh *et al.*, 2010]. In addition, it has been argued that low-fluoride toothpastes may not be a viable option for all children, especially those from families from low socioeconomic status, as some low-fluoride formulations labeled as children's toothpastes (with special packaging and flavoring agents) are usually more expensive than ordinary products [Martins *et al.*, 2011]. Although this is a valid point, it only refers to one of several aspects that should be taken into account when recommending a toothpaste to a child and therefore should not be used as a reason not to recommend such products. In this sense, while more consistent evidence is not available, the recommendation of low-fluoride toothpastes could also consider financial aspects, but should mainly be based on risks and benefits [Wong *et al.*, 2010]. Since the clinical performance of low-fluoride toothpastes has been shown to be dependent on the caries activity status [Lima *et al.*, 2008], it seems justifiable that low-fluoride toothpastes could be recommended for children under the age of 3 years at low caries risk, especially for those living in areas with fluoridated drinking water [Pessan *et al.*, 2011].

Another interesting finding was that the differences between the toothpastes within the same amount of product were not proportional to the intensity of treatment (amount of dentifrice x fluoride concentration). Considering the 2-fold difference between the fluoridated toothpastes, it would be expected that the increment in AUC values after brushing with a 1100 ppm F toothpastes would, therefore, be around 100% of that obtained for the 550 ppm F dentifrice. Such increments, however, were lower than expected (~ 56, 76 and 91%, respectively for 0.1, 0.3 and 0.5 g), showing a clear tendency of increase with higher amount of dentifrice applied on the toothbrush. Despite a similar trend has also been shown for biofilm fluoride concentrations when comparing conventional and low-fluoride toothpastes [Pessan *et al.*, 2010; 2013; 2014], the reasons for such pattern are not evident. Nonetheless, considering that brushing time, amount of dentifrice and

rinsing procedures (amount of water and time to rinse the mouth) were carefully standardized throughout the study, one factor that might have influenced on the results is fluoride intake during toothbrushing, which has been shown to be influenced by the amount of toothpaste used during toothbrushing [Kobayashi *et al.*, 2011]. However, given the complex interplay of variables in intraoral fluoride uptake, the reasons for the trend observed in the present work should be further examined in future investigations. In this sense, although saliva is a good indicator of intraoral fluoride retention [Pessan *et al.*, 2015] and can be considered as an indirect indicator of clinical performance [Duckworth et al, 1992], the impact of dentifrice amount and fluoride concentration on the resulting fluoride levels in the dental biofilm, biofilm fluid, as well as on other relevant intraoral parameters could provide useful data for the determination of the optimum combination for children.

The present study brings new information on the effects of low-fluoride toothpastes on intraoral fluoride retention, suggesting that the use of low amount of a conventional toothpaste may not be as effective as the use of a low-fluoride formulation applied using the transversal technique. Therefore, the current recommendation of low quantities of a conventional toothpaste should be re-evaluated, as it is not based on sound scientific evidence considering intraoral fluoride retention or any other relevant parameter that could impact on clinical efficacy. As the controversies surrounding low-fluoride toothpastes have not yet been fully addressed, this issue should be constantly discussed and evaluated by authorities, in order to determine the best possible therapy to patients. In this sense, while more consistent evidence is not available on the recommendations of toothpastes to children, professionals, parents and caregivers should have the right to choose the treatment that better suits the children's need, based on risks, benefits, costs and personal preference.

5. Acknowledgements

The authors thank to the volunteers for taking part in the study. The study was conceived and designed by ACBD and JPP. The experiments were undertaken by KBH, MEN, TYH, FRNM and JPP. Data was analyzed and the manuscript was written by KBH, MEN, TYH, FRNM, ACBD and JPP. This study was supported by CNPq, which played no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

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Figure captions

Figure 1 – Time-course salivary fluoride concentrations in children after brushing with dentifrices containing 0 (Placebo), 550 and 1100 ppm F, using 0.1, 0.3 and 0.5 g of dentifrice during toothbrushing (Placebo values for the 3 different amount were averaged). *n*=24

Figure 2 – Mean AUC fluoride concentrations in saliva collected over 120 minutes after brushing with dentifrices containing 0 (Placebo), 550 and 1100 ppm F, using 0.1, 0.3 and 0.5 g of dentifrice during toothbrushing. Different letters indicate significant differences among the groups (Student-Newman-Keuls' test after logarithmic transformation, $p<0.05$, *n*=24). Bars indicate SE

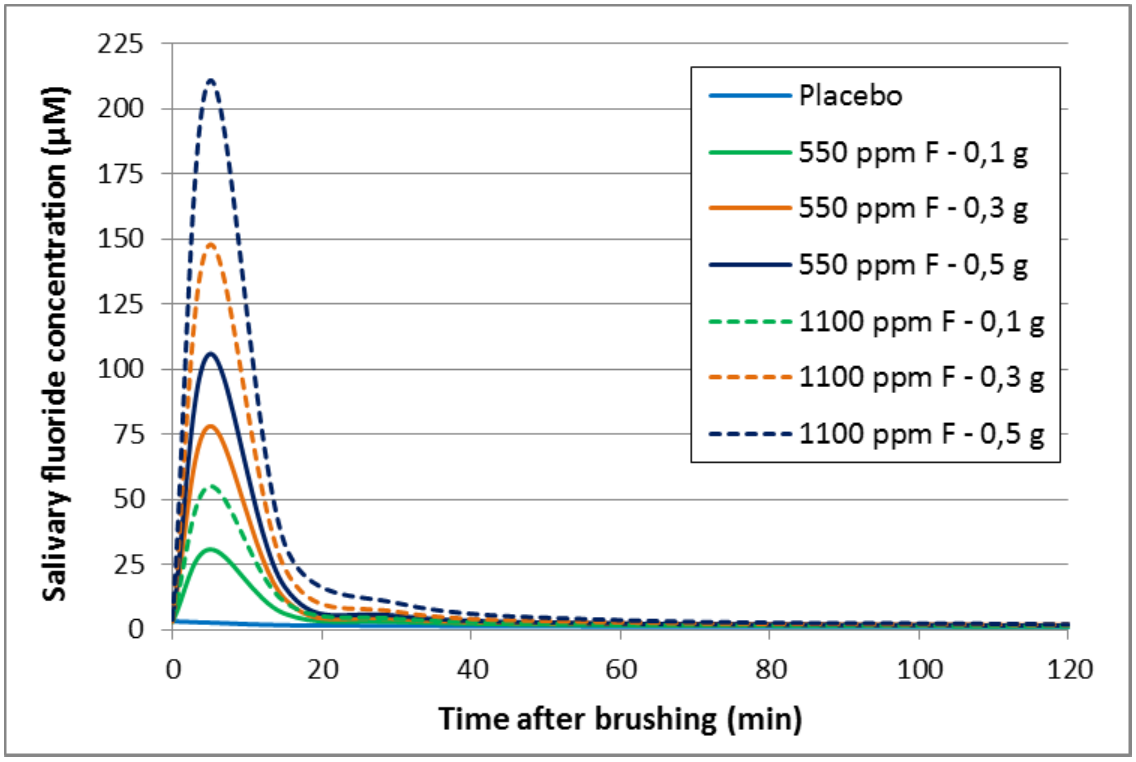
Salivary fluoride concentration (µM)
225
200
175
150
125
100
75
50
25
0
0
20
40
60
80
100
120
Time after brushing (min)
Placebo
550 ppm F - 0,1 g
550 ppm F - 0,3 g
550 ppm F - 0,5 g
1100 ppm F - 0,1 g
1100 ppm F - 0,3 g
1100 ppm F - 0,5 g

Figure 1

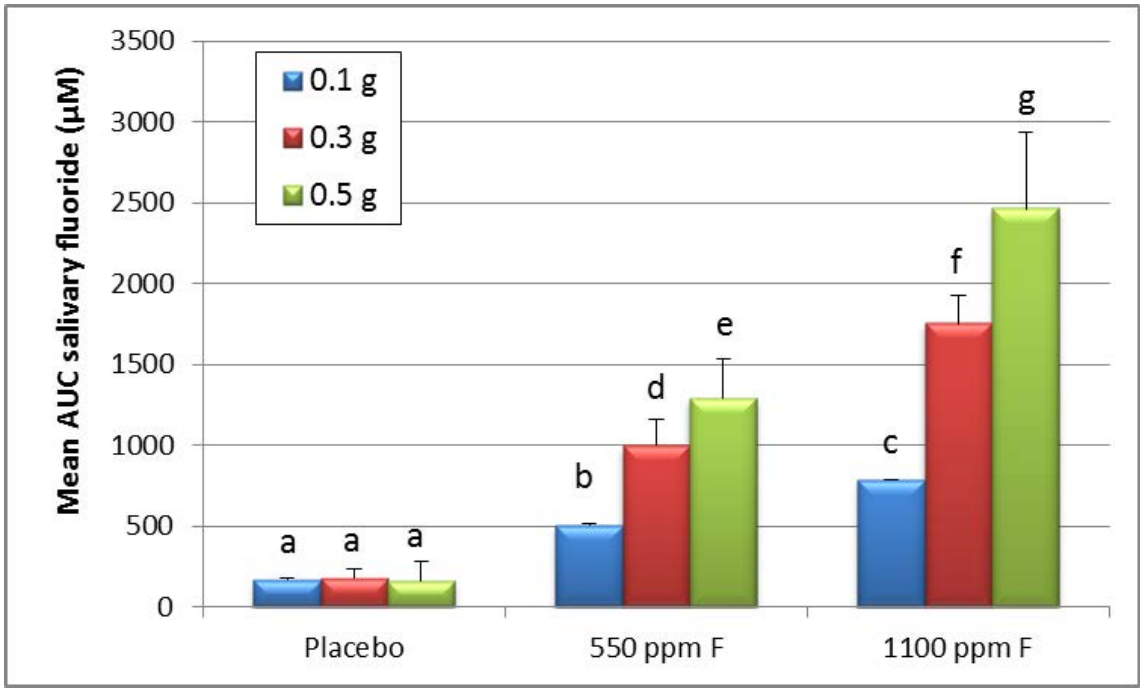
Mean AUC salivary fluoride (µM)
3500
3000
2500
2000
1500
1000
500
0
0.1 g
0.3 g
0.5 g
a
a
a
b
d
e
c
f
g
Placebo
550 ppm F
1100 ppm F

Figure 2

Table captions

Table 1 – Mean (SD) salivary fluoride concentrations at each individual collection point, according to the possible combinations of fluoride concentration and amount of dentifrices used

Table 2 – Mean salivary flow rate (mL/min) for each combination of dentifrice and amount used during toothbrushing

Table 1 – Mean (SD) salivary fluoride concentrations at each individual collection point, according to the possible combinations of fluoride concentration and amount of dentifrices used

Dentifrice	Amount (g)	Time after brushing					
		0	5	15	30	60	120
Placebo	0,1	3.0[a]	2.0[a]	1.6[a]	1.4[a]	1.3[a]	1.1[a]
		(1.3)	(0.7)	(0.6)	(0.5)	(0.6)	(0.3)
	0,3	3.2[a]	2.7[a]	1.8[a]	1.5[a]	1.3[a]	1.2[a]
		(1.7)	(2.2)	(0.9)	(0.7)	(0.4)	(0.5)
	0,5	3.0[a]	2.0[a]	1.5[a]	1.5[a]	1.2[a]	1.1[a]
		(1.6)	(0.6)	(0.6)	(0.4)	(0.2)	(0.3)
550 ppm F	0,1	3.0[a]	30.9[b]	6.2[a]	3.0[a]	1.8[a]	1.3[a]
		(1.7)	(25.6)	(4.4)	(1.5)	(0.6)	(0.3)
	0,3	3.8[a]	78.1[b]	12.0[a]	4.2[a]	2.4[a]	1.8[a]
		(2.0)	(77.5)	(11.3)	(2.2)	(1.3)	(0.9)
	0,5	3.5[a]	105.9[b]	16.6[a]	5.4[a]	2.4[a]	1.6[a]
		(2.5)	(82.7)	(12.5)	(3.3)	(1.0)	(0.5)
1100 ppm F	0,1	3.7[a]	55.1[b]	10.8[a]	4.2[a]	2.0[a]	1.5[a]
		(2.5)	(48.6)	(10.3)	(3.6)	(0.7)	(1.0)
	0,3	3.1[a]	147.8[b]	23.8[a]	6.9[a]	2.8[a]	1.9[a]
		(1.8)	(114.7)	(21.4)	(4.5)	(1.1)	(0.6)
	0,5	3.7[a]	210.9[b]	33.2[c]	10.3[a]	3.7[a]	2.1[a]
		(2.8)	(210.1)	(36.7)	(12.8)	(2.6)	(0.9)

Different superscript letters indicate significant differences among times of sample collection within each row (Student-Newman-Keuls' test, $p<0.05$, $n=24$).

Table 2 – Mean salivary flow rate (mL/min) for each combination of dentifrice and amount used during toothbrushing

Dentifrice	**Placebo**			**550 ppm F**			**1100 ppm F**		
Amount (g)	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5
Mean	1.2	1.2	1.2	1.2	1.2	1.1	1.2	1.2	1.1
SD	(0.4)	(0.5)	(0.5)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)	(0.4)

No significant difference was observed among the conditions tested (Two-way, repeated measures ANOVA, $p>0.05$, $n=24$).

Anexos

ANEXO A – INSTRUÇÕES AOS AUTORES

Caries Research

Aims and Scope

'Caries Research' is an international journal, the aim of which is to promote research in dental caries and related fields through publication of original research and critical evaluation of research findings. The journal will publish papers on the aetiology, pathogenesis, prevention and clinical control or management of dental caries. Papers on health outcomes related to dental caries are also of interest, as are papers on other disorders of dental hard tissues, such as dental erosion. Aspects of caries beyond the stage where the pulp ceases to be vital are outside the scope of the journal. The journal reviews papers dealing with natural products and other bacterial inhibitors against specific criteria, details of which are available from the Editor.

Submission

Manuscripts written in English should be submitted online:

Online Manuscript Submission

Should you experience problems with your submission, please contact:

Prof. David Beighton
(Editor-in-Chef, Caries Research)
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The Henry Wellcome Laboratories for Microbiology and Salivary Research
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During the online submission you will be asked to list complete mailing addresses, including e-mail addresses of three potential reviewers for your manuscript.

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Original papers or Short Communications are reports of original work (including systematic reviews and meta-analyses). Both have the structure outlined below but for Short Communications the abstract should be less than 100 words and the manuscript should not exceed 3 printed pages, equivalent to about 9 manuscript pages (including tables, illustrations and references).

Reviews can have a freer format but should nevertheless commence with a Title page, an Abstract and an Introduction defining the scope.

Current topics are concise articles that present critical discussion of a topic of current interest, or a fresh look at a problem, and should aim to stimulate discussion.

Letters to the Editor, commenting on recent papers in the journal, are published occasionally, together with a response from the authors of the paper concerned.

Preparation of Manuscripts

Text should be one-and-a-half-spaced, with wide margins. All pages and all lines must be numbered, starting from the title page. A conventional font, such as Times New Roman or Arial, should be used, with a font size of 11 or 12. Avoid using italics except for Linnaean names of organisms and names of genes.
Manuscripts should be prepared as a text file plus separate files for illustrations. The text file should contain the following sequence of sections: Title page; Declaration of interests; Abstract; Introduction; Materials and Methods; Results; Discussion; Acknowledgements; References; Legends; Tables. Each section should start on a new page, except for the body of the paper (Introduction to Acknowledgements), which should be continuous. Lines in the manuscript must be numbered consecutively from the title page until the last page. Submissions which do not conform to these simple guidelines will be returned to the author.

Title page: The first page of each manuscript should show, in order:

- the title, which should be informative but concise;
- the authors' names and initials, without degrees or professional status, followed by their institutes;
- a short title, maximum length 60 characters and spaces, for use as a running head;
- a list of 3-10 key words;
- the name of the corresponding author and full contact details (postal address, telephone and fax numbers, and e-mail address).

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The possible existence of a conflict of interest does not preclude consideration of a manuscript for publication, but the Editor might consider it appropriate to publish the disclosed information along with the paper.

Abstract: The abstract should summarise the contents of the paper in a single paragraph of no more than 250 words (to ensure that the abstract is published in full by on-line services such as PubMed). No attempt should be made to give numerical results in detail. References are not allowed in the abstract.

Introduction: This section should provide a concise summary of the background to the relevant field of research, introduce the specific problem addressed by

the study and state the hypotheses to be tested.

Materials and Methods (or Subjects and Methods): All relevant attributes of the material (e.g. tissue, patients or population sample) forming the subject of the research should be provided. Experimental, analytical and statistical methods should be described concisely but in enough detail that others can repeat the work. The name and brief address of the manufacturer or supplier of major equipment should be given.

Statistical methods should be described with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, findings should be quantified and appropriate measures of error or uncertainty (such as confidence intervals) given. Sole reliance on statistical hypothesis testing, such as the use of P values, should be avoided. Details about eligibility criteria for subjects, randomization and the number of observations should be included. The computer software and the statistical methods used should be specified. See Altman et al.: Statistical guidelines for contributors to medical journals [Br Med J 1983;286:1489–93] for further information.

Manuscripts reporting studies on human subjects should include evidence that the research was ethically conducted in accordance with the Declaration of Helsinki (World Medical Association).In particular, there must be a statement in Materials and Methods that the consent of an appropriate ethical committee was obtained prior to the start of the study, and that subjects were volunteers who had given informed, written consent.

Information detailing the power and sample size calculations must be included in the manuscript.

Randomized clinical trials should be reported according to the standardised protocol of the CONSORT Statement. The CONSORT checklist must be submitted together with papers reporting clinical trials.

Randomized clinical trials must be registered at clinicaltrials.gov or similar national authority and the trial number included in the manuscript.

Trials beginning after 1 July 2012 must be registered before recruitment of the first patient. Caries Research will accept 'retrospective registration' of trials that began before 1 July 2012 (retrospective meaning registration occurs after patient enrolment begins). When submitting a paper on a clinical trial, the trial registration number should be stated at the end of the abstract in the following format: Trial registration: [name of the trial registry, the registry URL and the trial registration number].

In studies on laboratory animals, the experimental procedures should conform to the principles laid down in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and/or the National Research Council Guide for the Care and Use of Laboratory Animals.

Unless the purpose of a paper is to compare specific systems or products,

commercial names of clinical and scientific equipment or techniques should only be cited, as appropriate, in the 'Materials and Methods' or 'Acknowledgements' sections. Elsewhere in the manuscript generic terms should be used.

In any manuscript involving microradiography, the following information must be included: the radiation source and filters used and the kV used (this determines the wavelength of radiation and hence the validity of using Angmar's equation).

Manuscripts on experimental enamel caries should show that the lesions retain a relatively well-preserved surface layer, i.e. are not surfacesoftened lesions. Proof of surface integrity can be provided either as illustrations in the paper or as supplementary material for the reviewers. Transverse microradiography, polarized light microscopy of a section immersed in water or backscattered scanning electron microscopy of a polished cross-section can be used to provide the necessary proof. To allow the nature of experimental changes to be assessed, microradiographs or micrographs should be provided to show part of the experimental lesion and the adjacent control (e.g. figure 2 of Zaura et al.: Caries Res 2007;41:489–492). Again, these images can be provided as part of the paper or as supplementary material for review purposes.

Results: Results should be presented without interpretation. The same data should not be presented in both tables and figures. The text should not repeat numerical data provided in tables or figures but should indicate the most important results and describe relevant trends and patterns.

Discussion: This section has the functions of describing any limitations of material or methods, of interpreting the data and of drawing inferences about the contribution of the study to the wider field of research. There should be no repetition of preceding sections, e.g. reiteration of results or the aim of the research. The discussion should end with a few sentences summarising the conclusions of the study. However, there should not be a separate 'Conclusions' section.

Acknowledgements: Acknowledge the contribution of colleagues (for technical assistance, statistical advice, critical comment etc.) and provide the position(s) of author(s) employed by commercial firms. This section should describe the source(s) of funding that have supported the work inlcuding relevant grant numbers. Please also include this sentence: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript." If this statement is not correct, you must describe the role of any sponsors or funders, and amend the sentence as needed. Additionally, the roles of all authors must be described (For example: Conceived and designed the experiments: AA, BB. Performed the clinical examination: AA, CC. Performed the experiments: DD, FF. Analyzed the data: BB, FF. Wrote the paper: AA, CC, FF, EE).

Legends: The table headings should be listed first, followed by the legends for the illustrations.

Tables: Tables should be numbered in Arabic numerals. Each table should be placed on a separate page. Tables should not be constructed using tabs but by

utilising the table facilities of the word-processing software.

Illustrations:

- Illustrations should be numbered in Arabic numerals in the sequence of citation. Figure numbers must be clearly indicated on the figures themselves, outside the image area.
- Black and white half-tone illustrations must have a final resolution of 300 dpi after scaling, line drawings one of 800-1200 dpi.
- Figures with a screen background should not be submitted.
- When possible, group several illustrations in one block for reproduction (max. size 180 x 223 mm).

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References

Reference to other publications should give due acknowledgement to previous work; provide the reader with accurate and up-to-date guidance on the field of research under discussion; and provide evidence to support lines of argument. Authors should select references carefully to fulfil these aims without attempting to be comprehensive.

Cited work should already be published or officially accepted for publication. Material submitted for publication but not yet accepted should be cited as 'unpublished results', while unpublished observations communicated to the authors by another should be cited as 'personal communication', with credit in both cases being given to the source of the information. Neither unpublished nor personally communicated material should be included in the list of references. Abstracts more than 2 years old and theses should not be cited without a good reason, which should be explained in the covering letter accompanying the paper.

References should be cited by naming the author(s) and year. Where references are cited in parenthesis, both names and date are enclosed in square brackets. Where the author is the subject or object of the sentence, only the year is enclosed in brackets.

One author: [Frostell, 1984] or Frostell [1984].

Two authors: [Dawes and ten Cate, 1990] or Dawes and ten Cate [1990].

More than two authors: [Trahan et al., 1985] or Trahan et al. [1985].

Several references cited in parenthesis should be in date order and separated by semi-colons: [Frostell, 1984; Trahan et al., 1985; Dawes and ten Cate, 1990].

Material published on the World Wide Web should be cited like a reference to a print publication, and the URL included in the reference list (not in the text), together with the year when it was accessed.

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Examples

(a) Papers published in periodicals: Lussi A, Longbottom C, Gygax M, Braig F: Influence of professional cleaning and drying of occlusal surfaces on laser fluorescence in vivo. Caries Res 2005;39:284-286.

(b) Papers published only with DOI numbers: Theoharides TC, Boucher W, Spear K: Serum interleukin-6 reflects disease severity and osteoporosis in mastocytosis patients. Int Arch Allergy Immunol DOI: 10.1159/000063858.

(c) Monographs: Matthews DE, Farewell VT: Using and Understanding Medical Statistics. Basel, Karger, 1985.

(d) Edited BOOKS: DuBois RN: Cyclooxygenase-2 and colorectal cancer; in Dannenberg AJ, DuBois RN (eds): COX-2. Prog Exp Tum Res. Basel, Karger, 2003, vol 37, pp 124-137.

(e) Patents: Diggens AA, Ross JW: Determining ionic species electrochemically. UK Patent Application GB 2 064 131 A, 1980.

(f) World Wide Web: Chaplin M: Water structure and

behavior. www.lsbu.ac.uk/water, 2004.

Supplementary Material

Supplementary material is restricted to additional information which is directly pertinent to the contentand conclusion of the paper. Please note that all supplementary files will undergo editorial review and should be submitted together with the original manuscript. The editors reserve the right to reject or limit the scope and length of supplementary material. Supplementary material must meet production quality standards for web publication without the need for any modification or editing. In general, supplementary files should not exceed 10 MB in size. Acceptable file formats are word or pdf, excel spreadsheets (only if the data cannot be converted properly to a pdf file), video files (.mov, .avi, .mpeg), and audio files (.wav), either free standing or incorporated into html or ppt files in each case to illustrate the sound. Accepted supplementary material will be published as submitted and no proofs will be provided to the authors.

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ANEXO B

CERTIFICADO DA APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA

FACULDADE DE ODONTOLOGIA - CÂMPUS DE ARAÇATUBA - JÚLIO DE

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Influência da quantidade de dentifrício e concentracão de fluoreto na retenção intrabucal de fluoreto em crianças

Pesquisador: Kevin Bruce Hall

Área Temática:

Versão: 2

CAAE: 22130113.0.0000.5420

Instituição Proponente: Faculdade de Odontologia do Campus de Araçatuba - UNESP

Patrocinador Principal: Financiamento Próprio

DADOS DA NOTIFICAÇÃO

Tipo de Notificação: Outros

Detalhe: Submissão de emenda ao projeto

Justificativa: Optou-se por realizar alterações no protocolo do estudo, de forma a contornar os

Data do Envio: 08/08/2014

Situação da Notificação: Parecer Consubstanciado Emitido

DADOS DO PARECER

Número do Parecer: 864.762

Data da Relatoria: 17/10/2014

Apresentação da Notificação:

A emenda ao projeto foi muito bem elaborada, de forma clara e objetiva.

Objetivo da Notificação:

O objetivo de realizar alterações no protocolo deste estudo, foi para contornar os problemas inerentes a cooperação dos voluntários em casa (número de escovações por dia), bem como dificuldades relacionadas à colocação dos dentifrícios sobre a escova e diluição do mesmo durante a escovação. Ressaltamos, entretanto, que as alterações sugeridas não implicarão em mudanças quanto ao objetivo principal do estudo (avaliar a retenção intrabucal de fluoreto após a escovação com dentifrícios contendo 0, 550 e 1100 ppm F, em 3 quantidades distintas, totalizando 9 possíveis combinações). As alterações propostas têm por objetivo contornar os problemas

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observados no início do estudo (descritos em detalhe nesta emenda) sem, no entanto, comprometer o objetivo principal do projeto. Considerando-se que não serão mais obtidas amostras de biofilme, acreditamos ser necessário a inclusão de mais tempos de coleta para a saliva, visto que esta análise detalhada torna possível uma comparação mais precisa entre as combinações (quantidade e concentração de fluoreto), bem como possibilita o cálculo da área sob a curva, o que tem grande relevância clínica. Assim, embora não iremos mais analisar as amostras de biofilme, acreditamos que um maior número de observações para as amostras de saliva possibilitará a interpretação mais adequada dos resultados, além de seguir protocolos bem estabelecidos na literatura científica.

Avaliação dos Riscos e Benefícios:

Não houve alterações quanto aos riscos e benefícios.

Comentários e Considerações sobre a Notificação:

Analisando-se as alterações propostas, observa-se que estas não vão alterar o objetivo principal do estudo (avaliar a retenção intrabucal de fluoreto após a escovação com dentifrícios contendo 0, 550 e 1100 ppm F, em 3 quantidades distintas, totalizando 9 possíveis combinações)

Considerações sobre os Termos de apresentação obrigatória:

Os termos de apresentação obrigatória estão adequados.

Recomendações:

Nenhuma

Conclusões ou Pendências e Lista de Inadequações:

Mediante ao exposto sou favorável à APROVAÇÃO desta Emenda ao projeto inicial.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Não havendo pendências, o CEP propõe a aprovação da emenda do projeto de pesquisa salientando que, os prazos para o envio de relatórios deverão ser mantidos.

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Continuação do Parecer: 864.762

ARACATUBA, 10 de Novembro de 2014

Assinado por:
Ana Claudia de Melo Stevanato Nakamune
(Coordenador)

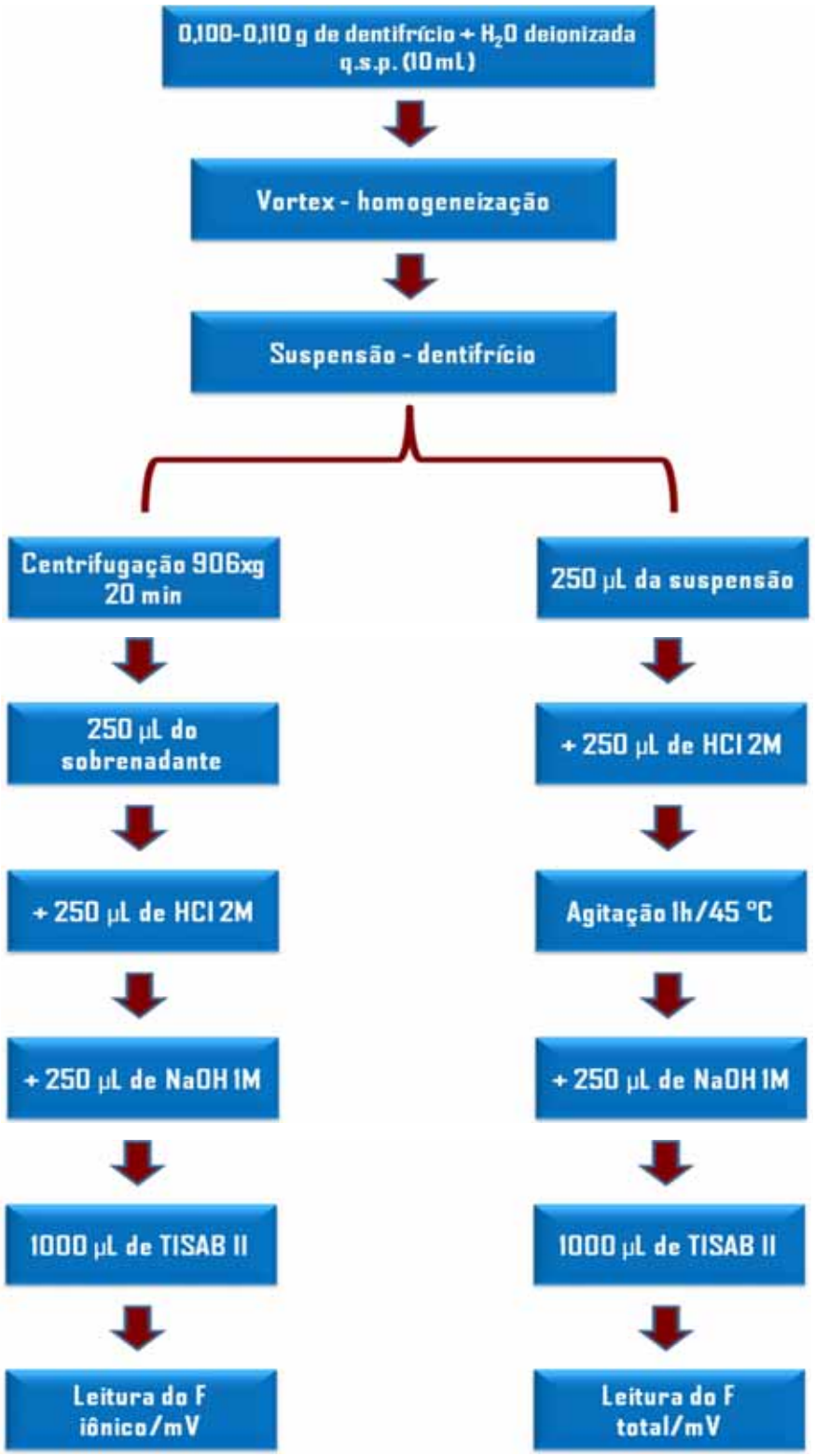
ANEXO C

DENTIFRÍCIOS EXPERIMENTAIS

1. Dentifrícios experimentais: Placebo (sem F), 550 µg F/g e 1100 µg F/g

ANEXO D

ESQUEMA REPRESENTATIVO DA DOSAGEM DO F NOS DENTIFRÍCIOS



Delbem et al., 2009

ANEXO E

QUANTIDADE DE DENTIFRÍCIO A SER APLICADO NA ESCOVA

Instrução de aplicação do dentifrício	**Quantidade de dentifrício**	**Foto demonstrativa**	
Cerdas cheias	0,5 g		
Transversal ao longo eixo da escova	0,3 g		
Tamanho de uma ervilha	0,1 g		

ANEXO F

KIT DOS VOLUNTÁRIOS

2. Escova e dentifrício placebo (sem flúor)

ANEXO G

PESAGEM DO DENTIFRÍCIO

3. Balança Portátil / Lb. Precision Balance 0.01-g; Italy; BEL Engineering s.r.l

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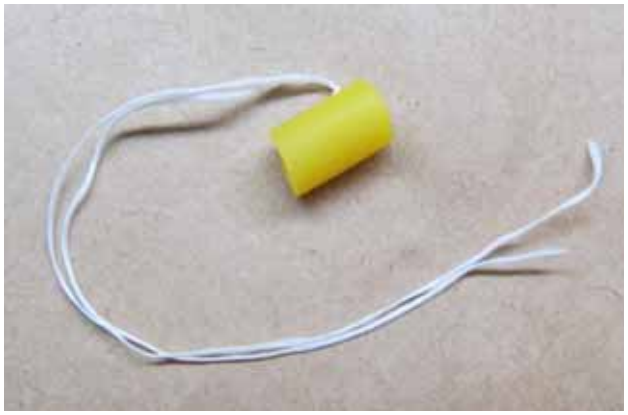
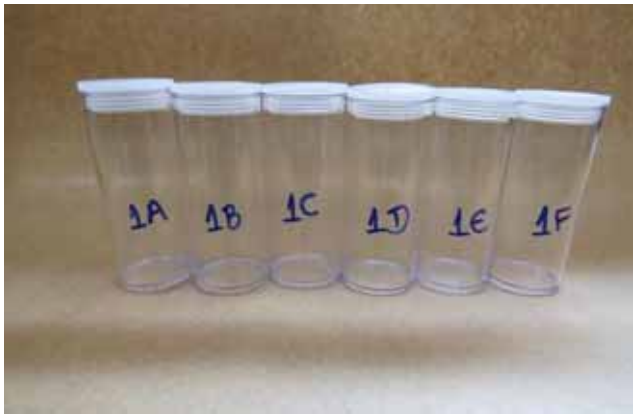
ANEXO H

COLETA DE SALIVA

4. Escola Municipal Cristiano Olsen (Araçatuba – SP)



5. Banda de borracha para estimular a salivação

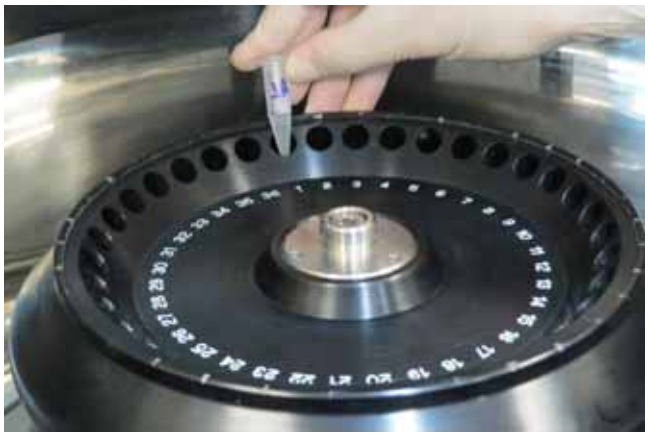


6. Tubos J40 previamente pesados

ANEXO I

PROCESSAMENTO DAS AMOSTRAS E ANÁLISE DE FLUORETO

7. Transferência da amostra

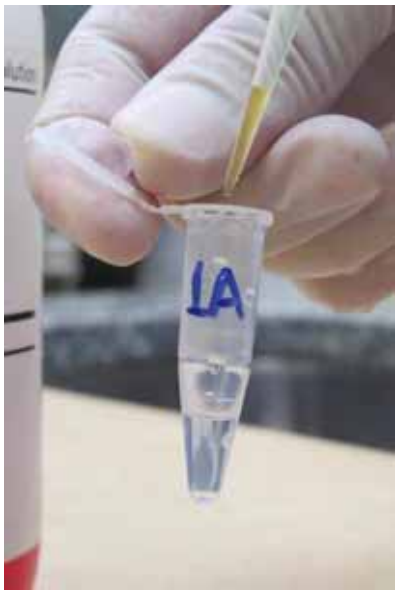


8. Centrifugação da amostra

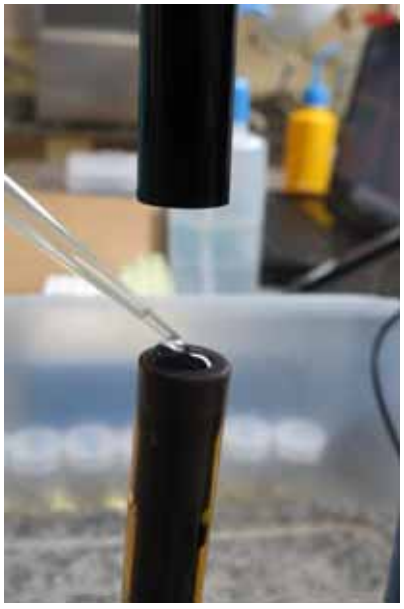
9. *Transferência de 200* uL do sobrenadante

ANEXO I

PROCESSAMENTO DAS AMOSTRAS E ANÁLISE DE FLUORETO



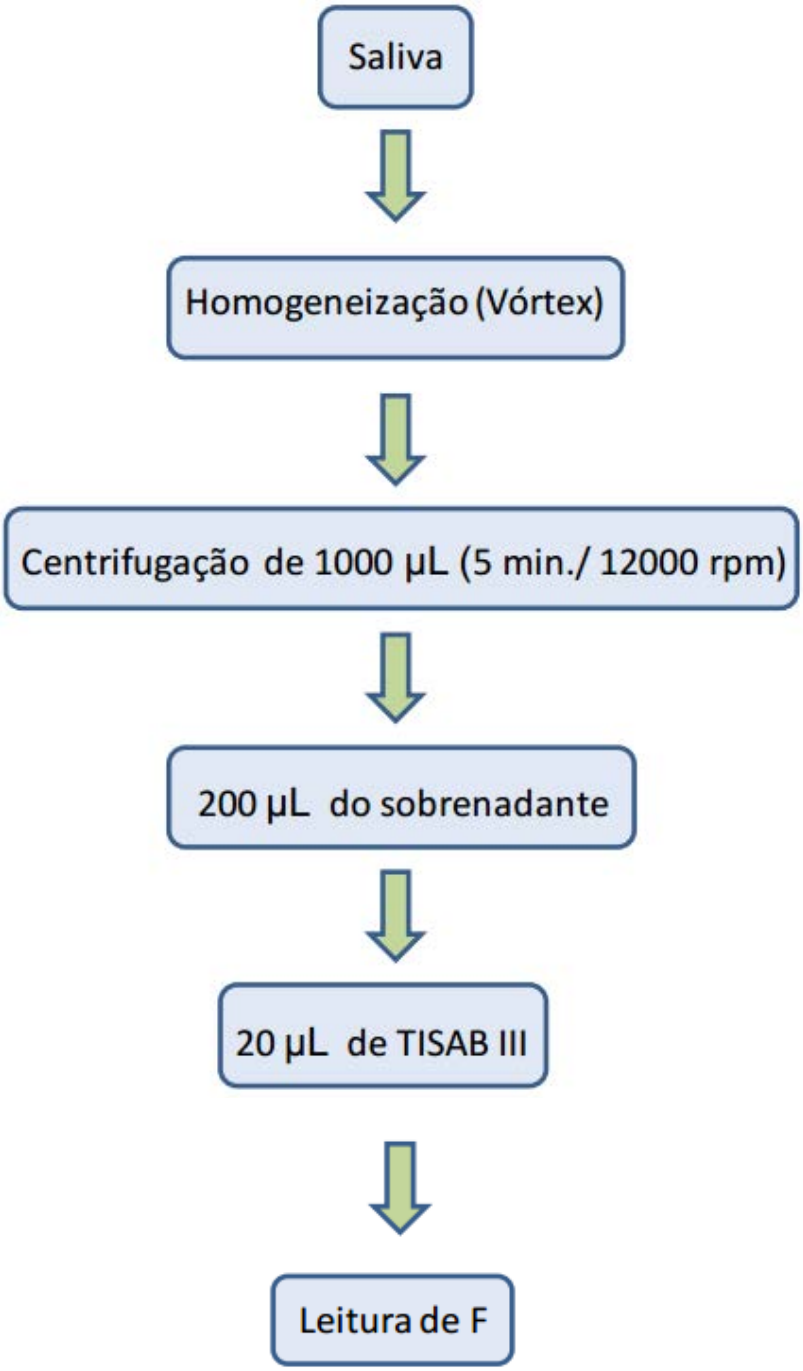
10. *Adição de 20* uL de TISAB III ao sobrenadante



11. Amostra no eletrodo

ANEXO J

ESQUEMA REPRESENTATIVO DO PROCESSAMENTO DAS AMOSTRAS DE SALIVA



Vogel *et al.*, 2000