UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

Carolina Patrícia Aires

CIRURGIÃ-DENTISTA

"EFEITO DA CONCENTRAÇÃO DE SACAROSE NO BIOFILME DENTAL FORMADO *IN SITU* E NO DESENVOLVIMENTO DE CÁRIE"

Dissertação apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas para a obtenção do título de Mestre em Odontologia – área de concentração em Cariologia.

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A Comissão Julgadora dos trabalhos de Defesa de Tese de MESTRADO, em sessão pública realizada em 19 de Fevereiro de 2004, considerou a candidata CAROLINA PATRÍCIA AIRES aprovada.

1. Profa. Dra. CINTHIA PEREIRA MACHADO TABCHOURY Cinthia Pereira Machado Tabchoury

2. Profa. Dra. MÁRCIA PINTO ALVES MAYER Marcia Pinto Alves Mayer

3. Prof. Dr. PEDRO LUIZ ROSALEN Pedro

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SUMÁRIO

RESUMO

Tendo em vista que a relação entre concentração de sacarose e cárie não é bem estabelecida, o objetivo do presente estudo foi avaliar a acidogenicidade e a composição bioquímica do biofilme formado *in situ* na presença de diferentes concentrações de sacarose e a sua relação com a iniciação e a progressão da cárie dental. Desta forma, foi conduzido um estudo cego e cruzado, com 3 fases experimentais de 14 dias cada. Doze voluntários adultos utilizaram dispositivos intra-orais palatinos contendo 4 blocos de esmalte dental de obtidos a partir terceiros molares humanos retidos. Os blocos dentais foram submetidos 8 x/dia aos tratamentos: água destilada e deionizada, solução de sacarose 1, 5, 10, 20 e 40%. Em cada fase experimental, 2 tratamentos foram avaliados, sendo que cada par de blocos dentais de um lado do dispositivo recebeu um tratamento diferente, definido por um sorteio: água destilada e deionizada e sacarose 1%; sacarose 10% e 20%; sacarose 5% e 40%. Os voluntários utilizaram dentifrício não-fluoretado nos períodos de "lead-in" e "washout" e durantes as fases experimentais. Ao término de cada fase, a acidogenicidade (pH inicial e 5 minutos após os tratamentos) e a composição inorgânica e de polissacarídeo insolúvel do biofilme dental formado sobre os blocos de esmalte foram analisadas; a porcentagem de perda de dureza de superfície (%PDS) e a área de perda mineral (ΔZ) foram determinadas no esmalte. Os resultados sugerem que a concentração mínima de sacarose para a formação de um biofilme dental cariogênico foi 5%, proporcionando potencial semelhante àquele observado em 10 e 20%. Em acréscimo, a concentração de sacarose 40% foi capaz de acentuar as propriedades cariogênicas do biofilme dental, aumentando a desmineralização.

ABSTRACT

Considering that the relationship between sugar concentration and caries is not clearly established, the objective of the present study was to evaluate the acidogenicity and the composition of biofilm formed *in situ* in the presence of different sucrose concentrations and its relation with the initiation and the progression of dental caries. Thus, a blind and cross-over study was conducted, with 3 experimental phases of 14 days each. Adult volunteers wore intra-oral palatal appliances containing 4 blocks of human dental enamel, which were submitted 8 x/day treatments: deionized distilled water, 1, 5, 10, 20 and 40% sucrose solution. In each experimental phase, 2 treatments were evaluated, considering that each pair of dental blocks in a side of the appliance received a different treatment, randomly defined: deionized distilled water and 1% sucrose; 10 and 20% sucrose; 5 and 40% sucrose. The volunteers used non-fluoridated dentifrice in the lead-in and washout periods and during the experimental phases. At the end of each experimental phase, the acidogenicity (baseline pH and 5 minutes after treatment), inorganic composition and insoluble polyssacharide of dental biofilm formed on the enamel blocks were analyzed; percentage of surface microhardness change (%SMC) and area of mineral loss (ΔZ) were determined on enamel. The results suggest that the threshold of sucrose concentration for the formation a cariogenic biofilm was 5%, providing a cariogenic potential as outstanding as that observed in 10 and 20%. In addition, carbohydrate concentration at 40% was able to enhance the cariogenic properties of dental biofilm, increasing enamel demineralization.

1. INTRODUÇÃO

Cárie dental é uma doença multifatorial e seu desenvolvimento está associado à presença de microrganismos cariogênicos [van Houte, 1980] e ao alto consumo de carboidratos [Bowen *et al.*, 1980]. Dentre os carboidratos, a sacarose é considerada o mais cariogênico [Newbrun, 1969] e tal característica é atribuída à alta energia de sua hidrólise, que pode ser utilizada pelas bactérias do biofilme dental para a síntese de polissacarídeos extracelulares [Hamada & Slade, 1980; Rölla *et al.*, 1985], um dos principais fatores de virulência destes microrganismos [Li & Burne, 2001].

A presença do polissacarídeo extracelular aumenta a porosidade do biofilme dental [Dibdin & Shellis, 1988], pois aumenta o espaço entre os microrganismos, facilitando a difusão da sacarose. O aumento da porosidade pode, assim, acentuar a acidogênese, prolongando o decréscimo de pH [Zero *et al.*, 1986] causado pelos ácidos provenientes do metabolismo bacteriano. Em acréscimo, a composição inorgânica do biofilme também é relevante para cárie dental, pois as reservas de flúor, cálcio e fósforo inorgânico contribuem para a supersaturação do fluido da placa em relação ao esmalte dental quando ocorrem decréscimos de pH devido à fermentação dos carboidratos [Kleinberg, 1985]. Além disso, a concentração inorgânica no biofilme apresenta uma relação inversamente proporcional ao desenvolvimento de cárie [Cury *et al.*, 1997; 2000].

Em relação ao consumo de sacarose, a freqüência pode influenciar no desenvolvimento de lesões cariosas [Newbrun, 1982], pois a alta freqüência do consumo de sacarose leva a um maior número de episódios de decréscimos de pH no biofilme dental [van Houte, 1994], que rompe a homeostase microbiana e resulta no enriquecimento de espécies acidogênicas e acidúricas, tais como estreptococos do grupo mutans e lactobacilos [Marsh, 2003]. Esses freqüentes episódios de decréscimos de pH do biofilme diminuem o tempo de remineralização pela saliva, favorecendo a desmineralização progressiva da superfície dental [Loesche, 1986]. Avaliando a composição do biofilme dental, Cury *et al.* [1997] verificaram que a maior freqüência de exposição à sacarose 20%, isto é, 8 x/dia, reduziu significativamente os níveis de flúor, cálcio, fósforo no biofilme e aumentou a

concentração de polissacarídeo insolúvel, favorecendo a formação de um biofilme cariogênico. Ainda estudando a freqüência de exposição à sacarose, Cury *et al.* [2001], em um estudo *in situ* de 28 dias, analisaram a relação entre cárie e sacarose 20% nas freqüências de 0, 2, 4 e 8 vezes ao dia. Neste estudo, a maior perda mineral do esmalte foi observada nos blocos dentais que receberam o maior número de exposições ao carboidrato. Em um estudo *in situ,* utilizando dispositivos mandibulares removíveis, Duggal *et al.* [2001] analisaram a desmineralização do esmalte com freqüências de exposição de 1, 3, 5, 7 ou 10 vezes ao dia utilizando solução de sacarose 12%, na presença ou não de dentifrício fluoretado. Os resultados mostraram que, utilizando dentifrício fluoretado, a perda mineral de esmalte foi observada na freqüência de 7 x/dia, enquanto que na ausência de dentifrício com flúor a desmineralização ocorreu na freqüência de 3 x/dia.

Embora esteja bem definido que a freqüência de consumo de açúcar influencia na composição do biofilme e no desenvolvimento de cárie dental, há evidências de que a concentração de sacarose em cada exposição possa também ser um fator relevante. Um estudo em animais inoculados com microrganismos cariogênicos verificou que concentrações de sacarose de 20, 30, 40, 50 e 56% incorporadas na dieta dos animais apresentaram uma relação direta com cárie dental [Hefti & Schmid, 1979], embora os autores não tenham analisado menores concentrações deste carboidrato. McDonald & Stookey [1977] verificaram uma relação direta entre sacarose contida em cereais e cárie em hamsters. Em acréscimo, foi observada uma relação direta entre a concentração de sacarose em soluções para bochechos e decréscimos de pH na placa dental formada *in vivo* [Imfeld, 1983]. Duggal *et al.* [2002], utilizando dispositivo mandibular removível contendo blocos de esmalte com lesão cariosa artificial, analisaram o efeito de soluções com 5, 10, 15, 20 ou 25% de sacarose na freqüência de exposição de 7 x/dia. Os autores observaram que, quando o dentifrício sem flúor foi utilizado pelos voluntários, todos os tratamentos com sacarose levaram à desmineralização dos blocos dentais. Tehrani *et al.* [1983], utilizando um modelo intra-oral de curta duração com biofilme artificial, analisaram o efeito de bochechos com baixas concentrações de sacarose sobre a desmineralização do esmalte dental por um período de 1,5 h com 4 voluntários. Através da perda mineral, avaliada pelo teste de permeabilidade ao iodo, os autores verificaram que somente dois bochechos com solução

de sacarose 1% não resultaram em perda mineral, a qual foi observada quando sacarose 5% foi utilizada. Entretanto, este estudo não analisou concentrações de sacarose maiores que 5% e, em acréscimo, nenhum dos estudos citados relacionou concentração de sacarose, composição do biofilme e desenvolvimento de cárie dental. Tendo em vista que a composição do biofilme dental também influencia, entre outros fatores, os decréscimos de pH e, conseqüentemente o desenvolvimento de cárie, torna-se necessário avaliar o efeito da concentração de sacarose em um biofilme formado na sua presença.

Desta forma, o objetivo do presente trabalho foi avaliar a acidogenicidade e a composição bioquímica do biofilme dental formado *in situ* na presença de diferentes concentrações de sacarose e sua relação com a iniciação e a progressão da cárie dental.

2. CAPÍTULO

O presente artigo foi submetido, conforme carta confirmando seu recebimento (Anexo 1), ao periódico "Caries Research".

Effect of Sucrose Concentration on Dental Biofilm Formed in situ and Enamel Demineralization

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Running Title: Effect of sucrose concentration on biofilm and caries

Key Words: sucrose, dental caries, dental biofilm, enamel, pH, concentration

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ABSTRACT

Considering that there is not a clear correlation between sugar concentration and caries, this relationship was studied in situ, evaluating dental biofilm and enamel demineralization. Adult volunteers wore intra-oral palatal appliances containing human dental enamel blocks, which were submitted 8 x/day, during 14 days, to the treatments: deionized distilled water, or sucrose solutions from 1 to 40%. The biofilm formed was analyzed with respect to acidogenicity and biochemical composition; enamel demineralization was evaluated by microhardness. The results suggest that the threshold of sucrose concentration for the formation of a cariogenic biofilm was 5%, providing to it a cariogenic potential as outstanding as that observed for 10 and 20% sucrose solutions. In addition, carbohydrate concentration of 40% was able to enhance such properties of dental biofilm, increasing enamel demineralization.

INTRODUCTION

Dental caries is a multifactorial disease and its development is associated with the presence of cariogenic microorganisms, such as mutans streptococci and lactobacilli [van Houte, 1980], and the high consumption of carbohydrates [Bowen et al., 1980]. Among the carbohydrates, sucrose is considered the most cariogenic [Newbrun, 1969] and this characteristic is mainly attributed to the high energy of its hydrolysis, which may be utilized by glucosyltransferase for synthesis of extracellular polysaccharides [Hamada & Slade, 1980; Rölla et al., 1985]. The presence of extracellular polysaccharides increases the porosity of dental biofilm [Dibdin & Shellis, 1988] and maintains the low pH [Zero et al., 1986] caused by organic acids from bacterial metabolism. Thus, a high frequency of sucrose consumption or exposure leads to repeated cycles of pH decreases in dental biofilm [van Houte, 1994], which disrupt microbial homeostasis and result in the enrichment of acidogenic and aciduric species, such as mutans streptococci and lactobacilli [Marsh, 2003]. This frequent exposure to such conditions of low pH may also diminish the time for saliva to replenish any lost tooth mineral [Loesche, 1986; Cury et al., 1997]. In addition, high frequency exposure to 20% sucrose solution can modify the biochemical characteristics of this biofilm, which then presents high concentration of insoluble polysaccharides and low levels of calcium, inorganic phosphorus and fluoride [Cury et al., 1997].

Even though the role of frequency of sugar consumption is well defined, there is evidence that sucrose concentration may also be a relevant factor. In this context, animal studies have shown that the concentration of sucrose in the diet strongly influences the incidence of smooth surface and fissure caries [McDonald & Stookey, 1977; Hefti &

Schmid, 1979], as well as there is a relationship between sucrose concentration and pH decreases in dental plaque in vivo [Imfeld, 1983]. In a short-term study, evaluating low sucrose concentrations in artificial biofilm, Tehrani et al. [1983] verified that two rinses with 1% sucrose solution did not result in mineral loss, which was observed when 5% sucrose solution was utilized. This research, however, did not evaluate sucrose concentrations higher than 5% and, in addition, none of the previous cited studies were designed to investigate relationship between sucrose concentration, dental biofilm composition and caries.

Therefore, the aim of this in situ study was to evaluate the effect of sucrose concentration on the acidogenicity and biochemical composition of dental biofilm formed in its presence and the relationship with enamel demineralization.

MATERIALS AND METHODS

Experimental design

The study involved a crossover, blind design and was performed in three experimental phases of 14 days each. Twelve healthy adult volunteers took part in this study, approved by the Research and Ethics Committee of FOP/UNICAMP (protocol no. 102/2002), and signed a written informed consent for participation. The volunteers wore acrylic intra-oral palatal appliances each containing four dental enamel blocks, which were submitted to the following treatments: distilled and deionized water (DDW), 1, 5, 10, 20 or 40% sucrose solution, 8 x/day. In each experimental phase, two treatments were evaluated, considering that each pair of dental blocks placed in a side of the appliance received a different treatment, randomly defined: DDW and 1% sucrose; 10% and 20% sucrose; 5%

and 40% sucrose. The use of two treatments (split-mouth) in the same intra-oral appliance was supported by the literature [Cury et al., 2001; Hara et al., 2003; Paes Leme et. al., 2003]. At the end of each experimental phase, the acidogenicity (baseline pH and 5 minutes after the treatments), inorganic composition and insoluble polyssacharide content of dental biofilm formed on the enamel blocks were analyzed; percentage of surface microhardness change (%SMC) and area of mineral loss (ΔZ) were determined on enamel.

Enamel block and appliance preparation

One hundred and forty-four human enamel blocks, prepared as previously described [Cury et al., 1997, 2000], with a known surface microhardness (336.5 ± 11.6) were randomized to the treatments. The volunteers wore intra-oral palatal appliances containing 4 enamel blocks, each pair of dental blocks located in one side and placed as close as possible to the posterior teeth. A 3.0-mm-deep space was created in the intra-oral appliance, leaving a 1.0-mm space for biofilm accumulation [Cury et al., 2000]. Dental biofilm was formed on the enamel blocks, which were protected from mechanical disturbance by a plastic mesh fixed to the acrylic surface. Eight times a day the volunteers removed the appliances and the treatment solutions were dripped onto the enamel blocks. After 5 min, the appliances were replaced in the mouth. A washout period of at least 7 days was allowed between the phases. During a 7-day pre-experimental period, the washout periods and the experimental phases, the volunteers brushed their natural teeth with non-fluoridated toothpaste, but drank fluoridated water (0.74 ± 0.03 mg F/L). The volunteers received instructions to wear the appliances all the time, including at night, but to remove them during meals and oral care. The test subjects received oral and written information to

refrain from using any antibacterial or fluoridated product during the pre-experimental and experimental periods. Considering that the study followed a crossover design, with the participation of the volunteers in all the steps, the subjects did not receive any advice regarding their daily diet.

Dental biofilm acidogenicity

On the 13th day of the experiment, approximately 10 hours after the last exposure to treatment solutions and before toothbrushing, pH of the overnight-starved biofilm and its variation after the respective treatment solution were determined. For this analysis, the intra-oral appliance remained in the volunteers' mouth. The pH measurements were done with a touch microelectrode (Beetrode MEPH-3L, WPI, New Haven, CT, USA) connected to a pHmeter in combination with a glass reference electrode (Orion Res Inc., Cambridge, MA, USA). The microelectrode tip was inserted between the plastic meshes that covered the dental blocks, in order to directly touch the dental biofilm formed. A reference salt bridge was established by each test subject dipping one finger in a KCl 3 M solution, into which the glass reference electrode was also inserted [Lingström et al., 2000]. The microelectrode was calibrated against standard pH buffers (pH 4.0 and 7.0) prior to and after each test as well as during tests if necessary. The pH determinations were made in duplicate for all the treatments in time 0 (baseline). After determining the baseline pH, the palatal appliance was removed and the treatment with the solutions was carried out as usual. After 1 minute the appliance was replaced in the mouth and after 4 minutes, duplicates of new pH measurements were taken (pH_{5min}) at the same places as the initial ones. The data of pH values were converted to hidrogenionic concentration (cH^+) and the

area between the cH^+ at baseline pH and pH_{5min} (cH^+ area) for each treatment was calculated [Larsen and Pearce, 1997].

Dental biofilm composition

On the 14th day of the experiment, approximately 10 hours after the last exposure to treatment solutions, dental biofilm formed on the enamel blocks was collected. Dental biofilm was dried for 24 hours in vacuum over P_2O_5 and treated as described previously [Cury et al., 1997, 2000], but with a modification in the proportion of the solutions (100 μL of HCl and 200 μL of NaOH/mg of dry weight of biofilm) for the extraction of the inorganic and polysaccharide components, respectively. Fluoride (F), calcium (Ca), inorganic phosphorus (P_i) and insoluble polysaccharide (IP) analyses were done as described by Cury et al. [1997; 2000; 2003].

Enamel analysis

At the end of each experimental phase, the enamel blocks were removed from the appliances and the surface microhardness (SMH) of all enamel blocks from all treatments was again measured. This analysis was performed according to Cury et al., [2000] and the percentage of surface microhardness change (%SMC) was calculated.

After SMH analysis, all the blocks were longitudinally sectioned through the center, one half of each block was embedded in acrylic resin and the cut surfaces were exposed and polished. The cross-sectional microhardness determination (CSMH) was performed according to Cury et al. [2000], but the indentations were made at 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180, and 200 μm from the outer enamel surface. CSMH values were

converted to mineral content (vol %) [Featherstone et al., 1983] and area of mineral loss (ΔZ) for each treatment was calculated [White & Featherstone, 1987].

Statistical Analysis

The experimental unit considered was the volunteer and the mean value for each volunteer in each treatment was submitted to statistical analysis. The assumptions of equality of variances and normal distribution of errors were checked for all the response variables tested and those that did not satisfy were transformed [Box et al., 1978]. Data of F and PI were transformed by power of –0.5. The results of Ca, P_i, final pH and ΔZ were transformed by power of –0.2 and %PDS was transformed by power 2.9. Data of cH^+ area were transformed by power of –0.1. The analysis of variance followed by Tukey test (SAS program, version 8.02, SAS Institute Incorporation, Cary: NC, 1999) was used for all the variables and the significance limit was set at 5%.

RESULTS

The results of baseline pH of dental biofilm formed in the presence of DDW, 1, 5 and 10% sucrose (table 1) did not differ statistically from each other and were higher than that found for 40% sucrose treatment ($p<0.01$), which was not statistically different from 20% sucrose treatment. With regard to the pH drop (pH_{5min}), the biofilms treated with sucrose solutions presented lower values than the negative control ($p<0.01$). The lowest pH_{5min} was found after treatment with 40% sucrose solution, although it did not differ statistically from 10 and 20% sucrose treatments. Even though dental biofilm treated with

5% sucrose solution presented a significantly higher pH_{5min} than 40%, it did not differ from 10 or 20% sucrose treatments. The hidrogenionic concentration area (cH^+ area) in dental biofilm after treatments with DDW and sucrose 1% were statistically lower than all other treatments ($p<0.01$). In addition, cH^+ area values of 5, 10 and 20% sucrose treatments did not differ from each other, but the values of groups 5 and 10% sucrose were statistically lower than that of 40% sucrose treatment ($p<0.01$).

In relation to the biochemical composition of dental biofilm (table 2), the highest F concentrations were found in DDW and 1% sucrose treatments, which did not differ statistically from each other, but both of them differed from 10, 20 and 40% sucrose groups ($p<0.01$). Moreover, F concentration in biofilm formed in the presence of 5% sucrose solution only differed statistically from 40% ($p<0.01$). Ca and P_i concentrations in DDW and 1% sucrose treatments did not differ from each other, but both of them were statistically higher than all other treatments ($p<0.01$). From 5% sucrose on, no statistically significant differences were observed for the other sucrose concentration treatments considering Ca and P_i values. IP concentration in dental biofilm formed in presence of DDW was statistically lower than all other treatments ($p<0.01$). Furthermore, IP concentrations in 5, 10 and 20% sucrose treatments did not differ from each other, but were statistically higher than 1% sucrose and lower than 40% sucrose treatment ($p<0.01$).

The analysis of dental enamel (table 3) showed that the %SMC of the dental blocks treated with DDW and sucrose 1% did not differ statistically from each other, but both of them were significantly lower than all other treatments ($p<0.01$); the same pattern was observed for ΔZ. From 5% sucrose on, no statistically significant difference was observed

for the other sucrose concentration treatments considering %SMC ($p>0.05$). In relation to ΔZ, the dental blocks treated with 5, 10 and 20% sucrose solution did not differ statistically from each other, nevertheless the group treated with 40% sucrose solution presented significantly higher mineral loss than 5 and 10% sucrose ($p<0.01$).

DISCUSSION

Data of enamel demineralization show that the treatment with 5% sucrose solution resulted in similar mineral loss when compared with 10 and 20% sucrose treatments. The enamel demineralization observed in dental blocks treated with 5% sucrose solution is in agreement with Tehrani et al. [1983], who verified that a rinse with 5% sucrose solution resulted in mineral loss when compared with 1% concentration. However, different from the present work, these authors did not compare the behavior of 5% sucrose solution with higher sucrose concentrations. In this context, sucrose concentration at 5% shows potential for demineralization similar to 10 and 20% and the findings from the study reported here suggest that a gradual increase of sucrose concentration did not clearly result in a gradual intensification of the demineralization process, which disagrees with McDonald & Stookey [1977] and Hefti & Schmid [1979]. Both studies show a direct relationship, however sucrose was present in cereal or in the diet of animals, which may explain these differences. In addition, our results are in agreement with Duggal et al. [2002], who analyzed in an in situ study the effect of solutions containing from 5 to 25% sucrose in an exposure frequency of 7 x/day and observed demineralization in all sucrose treatments. It is noteworthy that both microhardness analyses in dental enamel seemed to have different

behaviors according to the sucrose treatments. Our data of surface microhardness showed similar mineral loss from 5% sucrose solution on. However, the results were different for area of mineral loss (ΔZ) that may reflect better what happened in enamel according to the treatments from 5 to 40%.

In the present study, demineralization of dental enamel seems to be closely associated to the acidogenicity of biofilm when exposed to sucrose at different concentrations. There was a tendency to higher decreases of pH and cH^+ area when higher concentrations of carbohydrate were used (table 1), but it is also possible to observe that, in the treatment with 5% sucrose, this decrease did not differ statistically from that of the treatment with 20% sucrose. In terms of cariogenicity, it seems that there is a plateau among 5, 10 and 20% sucrose treatments, only modified by sucrose at the concentration of 40%. Considering that there is a definite ecological effect of sucrose on the oral microflora [Staat et al., 1975; Minah et al., 1985; Marsh, 2003], it can be suggested that an increase in carbohydrate intake induced specific bacterial succession, which caused an intensification of the biofilm acidogenicity after sugar exposure. Probably, this bacterial succession was kept unaltered in dental biofilm formed in the presence of 5, 10 and 20% sucrose solution and noted bacterial shifts may have occurred when sucrose concentration was increased to 40%. Although the microbiological composition was not analyzed in the present study, this complexity is not surprising in view of the heterogeneity of the biofilm microflora. These findings are supported by Bradshaw et al [1989], who showed that the low pH generated from sugar metabolism is the responsible factor for the breakdown of microbial homeostasis in dental plaque.

The biochemical composition of dental biofilm may also help explaining the different levels of caries development verified in this research. In the present study, increasing sucrose concentration from 5 to 20% did not result in higher amounts of IP, similar behavior shown by data of cH^+ area and demineralization. It should be noted that insoluble polysaccharide seemed to be closely related to the results of acidogenicity in the present study, emphasizing the cariogenic potential of biofilm and enhancing mineral loss, which is probably due to alterations in the diffusion properties and prolongation of pH decrease [Zero et al., 1986]. According to Marsh [2003], the relationship between the environment and the microbial community is not unidirectional, that is, even though the properties of the environment may dictate which microorganisms can occupy a given site, the metabolism of this community can modify the properties of their surroundings. In the present research, this fact was observed considering IP and pH decreases. Furthermore, it seems that the amount of IP in biofilms formed in the presence of sucrose from 5 to 20% were already enough for changes in the matrix structure and behavior of biofilm and consequently in the development of dental caries. In addition, the treatment with 40% sucrose showed the highest concentration of IP and significantly higher area of mineral loss than 5, 10 and 20% sucrose concentration, suggesting that higher IP content influenced pH decrease, which reflected in greater enamel demineralization.

Another point that needs to be addressed is the initial pH of the biofilm formed in the presence of 40% sucrose solution, which was significantly lower than that of the other treatments (table 1). According to earlier reports [Gibbons & Kapsimalis, 1963; Spatafora et al., 1995], this result can be attributed to acid production from endogenous carbohydrate

source. In addition, the literature reports that high sucrose concentration eventually impaired the growth of the microorganisms [Gibbons and Banghart, 1967]. This observation was not confirmed in our study, because the biofilm formed in the presence of 40% sucrose solution presented higher cH^+ area than other groups, which indicates carbohydrate fermentation.

In relation to the inorganic composition of dental biofilm in the present study (table 2), the levels of F, Ca and P_i were inversely related to the concentration of sucrose in the treatment solutions, although, from 5% sucrose on there was no significant decrease in the values of Ca and P_i as the concentration of carbohydrate increased in the treatments. Such data emphasize that sucrose concentration present during the formation of dental biofilm influenced the levels of these ions, as well as Cury et al. [1997] and Paes Leme et al. [2003] showed that frequency of sucrose consumption resulted in significant changes of Ca and P_i levels in the biofilm. Such findings of low concentration of Ca and P_i probably reflected in demineralization of dental enamel, since reservoirs of Ca and P_i in the dental biofilm are important to counteract the development of a state of subsaturation in the fluid in relation to dental enamel, when pH diminishes after sugar fermentation [Kleinberg, 1985]. Furthermore, data of the present study seem to suggest that Ca and P_i are more susceptible than F to changes promoted by sucrose in the biofilm. Some hypothesis for this low concentration of Ca and P_i have already been formulated, which includes diminished formation of minerals of Ca due to acid production from sucrose fermentation and synthesis of considerable amounts of insoluble polysaccharide resulting in less binding sites in the biofilm, since this biomolecule does not bind to Ca [Rose et al, 1993]. These interactions need to be further studied. With respect to F in dental biofilm, data from the present study

seem to suggest that Ca may not be the only element to form F reservoirs in biofilm, since the biofilm formed in the presence of 5% sucrose presented low concentrations of Ca, but showed relatively high amounts of F, implying that this element may have binding sites independent of Ca.

In summary, the findings of the present study suggest that the threshold of sucrose concentration for the formation of a biofilm with properties of acidogenicity and biochemical composition able to promote dental caries development is around 5%. In addition, carbohydrate concentration at 40% was able to enhance such properties of dental biofilm, increasing enamel demineralization.

ACKNOWLEDGEMENTS

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Table 1. pH analysis (mean ± sd) of dental biofilm according to the treatments.

Treatments	Baseline pH	pH_{5min}	cH^+ area (µmol/L x min)
DDW	7.6 ± 0.6 a (n=9)	7.5 ± 0.6 a (n=9)	0.1 ± 0.2 c (n=9)
1% sucrose	7.6 ± 0.5 a (n=12)	6.7 ± 0.5 b (n=12)	0.7 ± 1.3 c (n=12)
5% sucrose	7.5 ± 0.6 a (n=12)	5.6 ± 0.3 c (n=12)	9.1 ± 8.1 b (n=12)
10% sucrose	7.5 ± 0.3 a (n=12)	5.8 ± 0.5 cd (n=12)	8.2 ± 8.4 b (n=12)
20% sucrose	7.1 ± 0.7 ab (n=12)	5.5 ± 0.5 cd (n=12)	14.6 ± 13.5 ab (n=12)
40% sucrose	6.7 ± 0.6 b (n=11)	5.3 ± 0.4 d (n=11)	24.0 ± 16.5 a (n=11)

Treatments whose means are followed by distinct letters differ statistically ($p<0.01$).

Table 2. Biochemical composition (mean ± sd) of dental biofilm according to the treatments.

Treatments	F (μg/g)	Ca (μg/mg)	P_i (μg/mg)	IP (μg/mg)
DDW	55.1 ± 46.1 a (n=10)	39.4 ± 17.2 a (n=10)	22.0 ± 10.5 a (n=10)	28.1 ± 6.5 d (n=10)
1% sucrose	64.0 ± 82.8 a (n=12)	25.1 ± 23.1 a (n=12)	14.9 ± 12.2 a (n=12)	41.6 ± 13.5 c (n=12)
5% sucrose	35.4 ± 53.4 ab (n=11)	5.7 ± 5.3 b (n=11)	4.2 ± 3.0 b (n=11)	108.7 ± 85.9 b (n=11)
10% sucrose	11.9 ± 11.9 bc (n=12)	4.7 ± 5.1 b (n=12)	3.2 ± 2.2 b (n=11)	102.2 ± 76.0 b (n=12)
20% sucrose	12.3 ± 14.8 bc (n=12)	5.4 ± 6.1 b (n=12)	3.7 ± 2.6 b (n=12)	124.7 ± 85.4 b (n=12)
40% sucrose	5.5 ± 2.4 c (n=12)	2.6 ± 1.0 b (n=12)	2.3 ± 0.8 b (n=12)	268.1 ± 163.1 a (n=12)

Treatments whose means are followed by distinct letters differ statistically ($p<0.01$).

Table 3. Analysis (mean ± sd) of the enamel blocks according to the treatments (n=12).

Treatments	%SMC* (kg/mm^2)	ΔZ** (% vol. min x μm)
DDW	-3.9 ± 5.0 a	252.9 ± 129.3 c
1% sucrose	-10.5 ± 14.5 a	381.8 ± 224.2 c
5% sucrose	-48.8 ± 34.4 b	795.5 ± 600.0 b
10% sucrose	-43.8 ± 31.2 b	1054.3 ± 1239.8 b
20% sucrose	-58.2 ± 32.6 b	1577.4 ± 1540.8 ab
40% sucrose	-65.7 ± 40.1 b	1603.3 ± 862.3 a

Treatments whose means are followed by distinct letters differ statistically ($p<0.01$).
**SMC=surface microhardness change.*
***ΔZ= area of mineral loss.*

3. CONCLUSÃO

O presente estudo *in situ* sugere que a concentração mínima de sacarose para a formação de um biofilme com propriedades acidogênicas e composição bioquímica capazes de promover desenvolvimento de cárie é em torno de 5%. Em acréscimo, a concentração de sacarose 40% foi capaz de acentuar estas propriedades do biofilme dental formado, aumentando a desmineralização.

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* De acordo com a norma UNICAMP/FOP, baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o Medline

ANEXO 1

----- Mensagem encaminhada de Peter Shellis <r.p.shellis@bristol.ac.uk>
----- Data: Mon, 05 Jan 2004 15:38:38 +0000
De: Peter Shellis <r.p.shellis@bristol.ac.uk>
Reponder para: Peter Shellis <r.p.shellis@bristol.ac.uk>
Assunto: Receipt of Caries Research paper
Para: cinthia@fop.unicamp.br

Dear Dr Tabchoury MS
No.: 2/04 MS
Title: Effect of sucrose concentration on dental biofilm formed in situ and enamel demineralization

This is to acknowledge receipt of your manuscript Following standard practice, it will be sent to reviewers and I will let you know of my decision as to publication in due course. Should you need to contact me about the paper, please quote the MS No. given above.

Peter Shellis Division of Restorative Dentistry
Bristol University Dental School
Bristol BS1 2LY U.K.
Phone (0)117-928-4328
Fax: (0)117-928-4778

UNICAMP

COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "Efeito da concentração de sacarose na composição da placa dental e no desenvolvimento de cárie dental - estudo *in situ*", sob o protocolo nº **102/2002**, da Pesquisadora ***Carolina Patrícia Aires***, sob a responsabilidade da Profa. Dra. **Cínthia Pereira Machado Tabchoury**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP.

Piracicaba, 07 de agosto de 2002

We certify that the research project with title "Effect of sucrose concentration on the composition of dental plaque and on the development of dental caries – *in situ* study", protocol nº **102/2002**, by Researcher ***Carolina Patrícia Aires***, responsibility by Prof. Dr. **Cínthia Pereira Machado Tabchoury**, is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Research at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, August 07 2002

Prof. Dr. Pedro Luiz Rosalen
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Antonio Bento Alves de Moraes
Coordenador
CEP/FOP/UNICAMP

ANEXO 2

ANEXO 3

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título da Pesquisa

Efeito da concentração de sacarose na composição da placa dental e no desenvolvimento de cárie dental - estudo *in situ*.

Objetivo da Pesquisa

Avaliar o efeito da concentração de sacarose na composição da placa dental e no desenvolvimento de cárie dental. A composição orgânica e inorgânica da placa dental formada na presença de diferentes concentrações de sacarose será determinada e a cárie dental será avaliada pela dureza de superfície e pela dureza do esmalte seccionado longitudinalmente.

Justificativa

Considerando que a relação entre freqüência de sacarose, composição da placa e cárie tem sido estabelecida nos últimos anos a justificativa para este estudo é verificar se o efeito da concentração de sacarose na composição da placa e no desenvolvimento da cárie pode ser tão importante quanto a freqüência de exposição à esse carboidrato.

Procedimentos

O estudo será do tipo cruzado (onde todos os voluntários passam por todos os tratamentos) com 15 voluntários divididos em 5 tratamentos de 14 dias. Os tratamentos serão os seguintes: **1)** Água (controle negativo); **2)** Solução de sacarose 1%; **3)** Solução de sacarose 5%; **4)** Solução de sacarose 10%; **5)** Solução de sacarose 20% ou **6)** Solução de sacarose 40%. Os voluntários utilizarão dispositivos intra-orais palatinos contendo 4 blocos (3x3x2 mm) de esmalte humano, sobre o qual deverão gotejar as soluções descritas acima 8 vezes ao dia. Ao final de cada fase experimental, a placa será coletada e os blocos de esmalte serão removidos dos dispositivos para as análises.

Desconfortos e Riscos

Os voluntários poderão apresentar discreta halitose durante o período experimental, o que poderá ser resolvido com adequada higiene dental. O uso das soluções será apenas

como gotas sobre os blocos de esmalte presentes nos dispositivos intra-orais, não implicando em qualquer aumento de cárie dental nos voluntários. O dispositivo intra-oral pode causar um leve desconforto, que é, entretanto, semelhante ao desconforto causado por um aparelho ortodôntico móvel. Durante todo o período da pesquisa, acompanhamentos semanais serão realizados, para verificar as condições do aparelho e da sua saúde bucal. O benefício que os voluntários terão será um auxílio indireto, contribuindo para a realização deste projeto e o conhecimento que os mesmos adquirirão sobre a composição da placa dental formada na presença de diferentes concentrações de sacarose e o desenvolvimento de cárie dental.

Forma de acompanhamento e assistência

Os pesquisadores envolvidos na pesquisa estarão à disposição dos voluntários para ajuste no aparelho intra-oral a fim de minimizar qualquer desconforto.

Garantia de esclarecimento

O voluntário tem garantia de que receberá resposta ou esclarecimento de qualquer dúvida quanto aos procedimentos, riscos, benefícios e outros assuntos relacionados à pesquisa ainda que isso possa afetar a vontade do indivíduo em continuar participando. Qualquer dúvida ou problema com o dispositivo intra-oral, por favor, comunicar-nos com a maior brevidade possível.

Tel: 3412-5393 (Sala dos alunos – Cariologia) e 3433-0531 (Residência Carolina)
3412-5304 (Sala da Profª Cínthia) e 3434-4869 (Residência Profª Cínthia)

Formas de ressarcimento

Os voluntários serão ressarcidos de eventuais despesas com o transporte-alimentação para a coleta das amostras contidas nos dispositivos.

Formas de indenização

Não há danos previsíveis decorrentes desta pesquisa.

Garantia de sigilo

Os pesquisadores asseguram a sua privacidade quanto aos dados confidenciais envolvidos na pesquisa.

Liberdade para se recusar em participar da pesquisa

A decisão de fazer parte desta pesquisa é voluntária. O voluntário pode escolher se quer ou não participar, assim como poderá desistir de participar a qualquer momento.

SUA ASSINATURA INDICA QUE VOCÊ DECIDIU PARTICIPAR DA PESQUISA COMO VOLUNTÁRIO E QUE LEU E ENTENDEU TODAS AS INFORMAÇÕES ACIMA EXPLICADAS.

Nome do voluntário	Assinatura do voluntário
Nome do Representante Legal	Assinatura do Representante Legal

Documento: _____

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 DA FOP-UNICAMP.**

Endereço: Av Limeira, 901 CEP – FOP, CEP 13.414-903 Piracicaba, SP

ANEXO 4

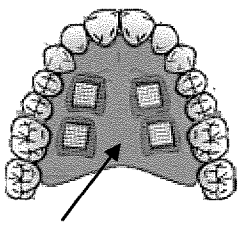
ANEXO AO TERMO DE CONSENTIMENTO

INSTRUÇÕES AOS VOLUNTÁRIOS

Antes do início de cada fase, cada voluntário receberá um dentifrício não fluoretado, frasco conta-gotas contendo a solução correspondente ao seu tratamento, estojo de aparelho ortodôntico (acomodação do dispositivo) e um dispositivo intra-oral. As instruções fornecidas a cada voluntário serão as seguintes:

INSTRUÇÕES GERAIS

- UTILIZAR APENAS O DENTIFRÍCIO E A ESCOVA DADOS PELO LABORATÓRIO.
- NÃO UTILIZAR BOCHECHOS, FIO DENTAL COM FLÚOR, CLOREXIDINA.
- NÃO UTILIZAR ALIMENTOS QUE POSSAM SER FONTE DE FLÚOR. COMO POR EXEMPLO CHÁ PRETO E CHÁ VERDE

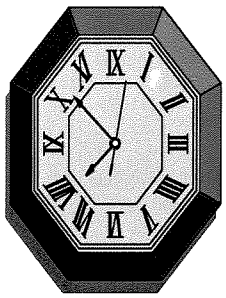


tela plástica

- DURANTE A HIGIENE ORAL O DISPOSITIVO DEVERÁ SER REMOVIDO, LAVADO COM DENTIFRÍCIO NÃO FLUORETADO E RETOMADO PARA A BOCA IMEDIATAMENTE APÓS A ESCOVAÇÃO
- VOCÊ PODE ESCOVAR TODO O APARELHO EXCETO A TELA PLÁSTICA QUE COBRE OS BLOCOS DE ESMALTE.
- CUIDADO AO ESCOVAR: NÃO DEIXE QUE JATOS DE ÁGUA DA TORNEIRA ATINJAM DIRETAMENTE A TELA PLÁSTICA QUE COBRE OS BLOCOS DENTAIS. ISSO PODE CAUSAR PERDA DA PLACA DENTAL ALI ACUMULADA.

- BEBER ÁGUA DE ABASTECIMENTO DE PIRACICABA. SE VOCÊ CONSOME APENAS ÁGUA MINERAL O LABORATÓRIO SE ENCARREGARÁ DE CEDÊ-LA TAMBÉM.

- DORMIR COM O APARELHO;
- SÓ REMOVER O APARELHO PARA FAZER REFEIÇÕES (INCLUSIVE MASCAR CHICLETE, BALA OU TOMAR MEDICAMENTOS)E HIGIENIZAÇÃO;
- QUANDO REMOVÊ-LO, COLOCAR O MESMO NO PORTA-APARELHO COM ALGODÃO ÚMIDO;



UTILIZAR 1 GOTA DA SOLUÇÃO DETERMINADA SOBRE CADA BLOCO DE ESMALTE 8 VEZES AO DIA NOS SEGUINTES HORÁRIOS:

08:00	**15:30**
09:30	**17:00**
11:30	**19:00**
14:00	**21:00**

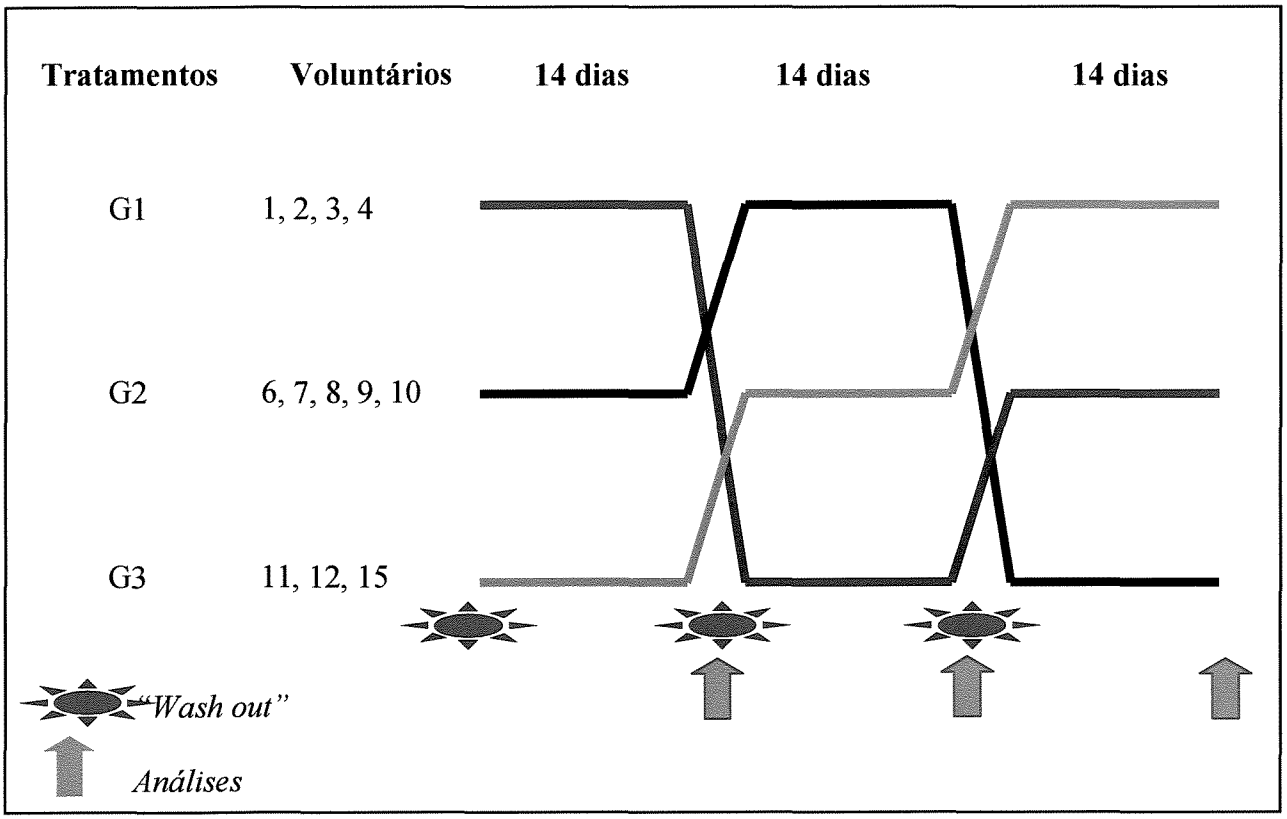
APÓS GOTEJAR, AGURADAR 5 MINUTOS ANTES DE COLOCÁR O APARELHO NOVAMENTE NA BOCA;

INTRUÇÕES PARA TÉRMINO DE CADA FASE

- AO ACORDAR, NÃO RETIRE O APARELHO DA BOCA, NÃO ESCOVE OS DENTES, NÃO TOME ÁGUA, NÃO SE ALIMENTE DE FORMA ALGUMA;
- CONTINUE USANDO O APARELHO ATÉ O MOMENTO EM QUE CHEGAR AO LABORATÓRIO E FOR SOLICITADO PARA REMOVÊ-LO;
- PREPARAMOS PARA VOCÊ UM DELICIOSO CAFÉ DA MANHÃ, QUE SERÁ SERVIDO NO LABORATÓRIO DE BIOQUÍMICA ÀS 07:00 HORAS.

ANEXO 5

Fluxograma dos tratamentos em relação aos voluntários



TRATAMENTOS

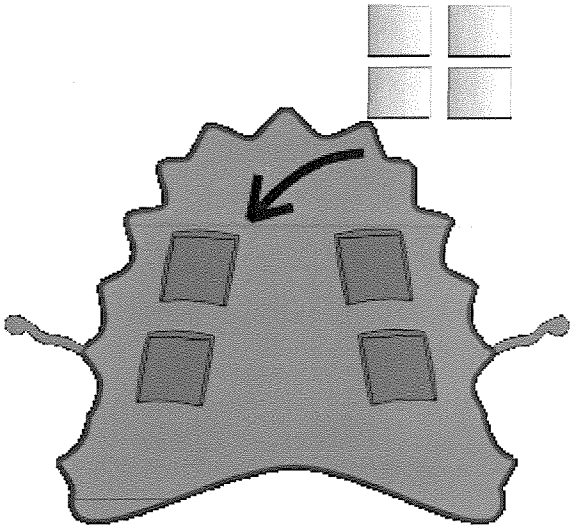
G1= Água e sacarose 1%

G2= Sacarose 10% e sacarose 20%

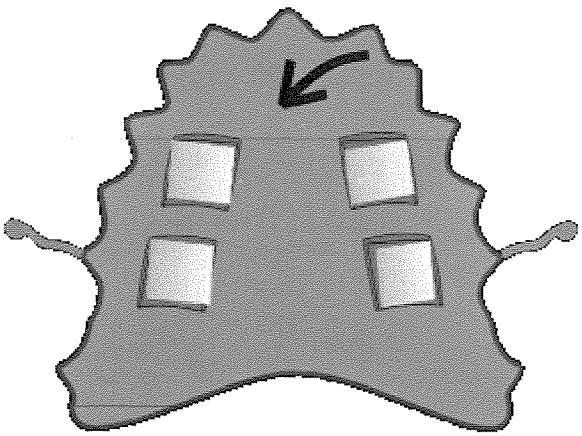
G3= Sacarose 5% e sacarose 40%

ANEXO 6

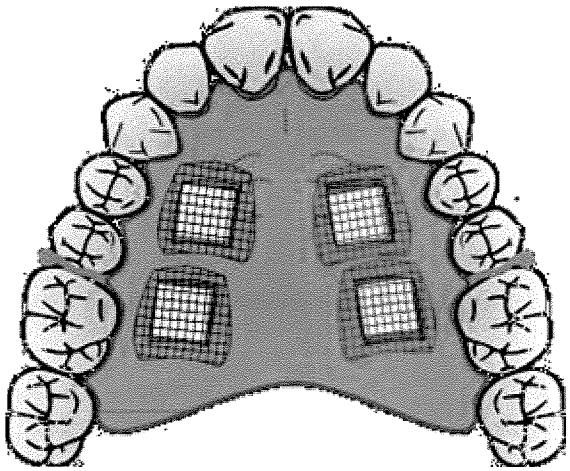
Dispositivo intra-oral palatino



A- Sítios de 3 mm de profundidade criados no dispositivo intra-oral palatino para receber blocos dentais com 2 mm de altura.



B- Blocos dentais colocados no dispositivo intra-oral palatino

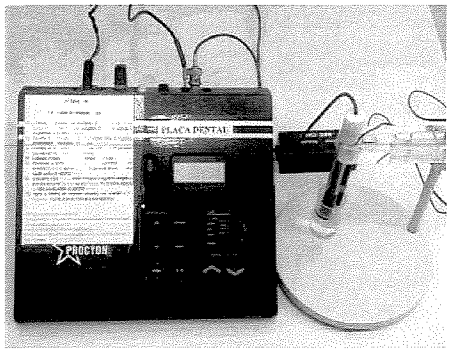


C-Telas plásticas fixadas no acrílico para minimizar distúrbios mecânicos no biofilme.

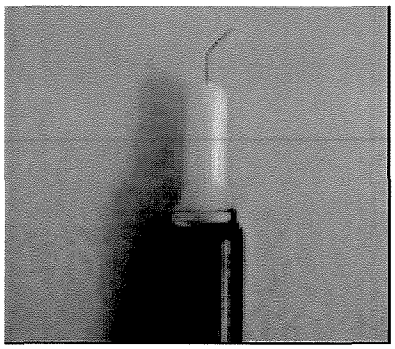
Cury *et al.*, 2000

ANEXO 7

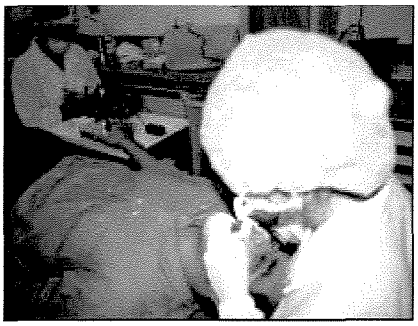
Análise da acidogenicidade do biofilme dental



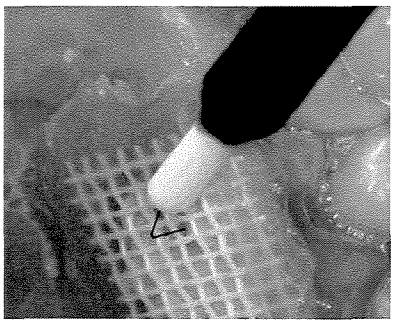
A- pHmetro acoplado ao microeletrodo Beetrode® e a um eletrodo de referência.



B- Microeletrodo Beetrode®



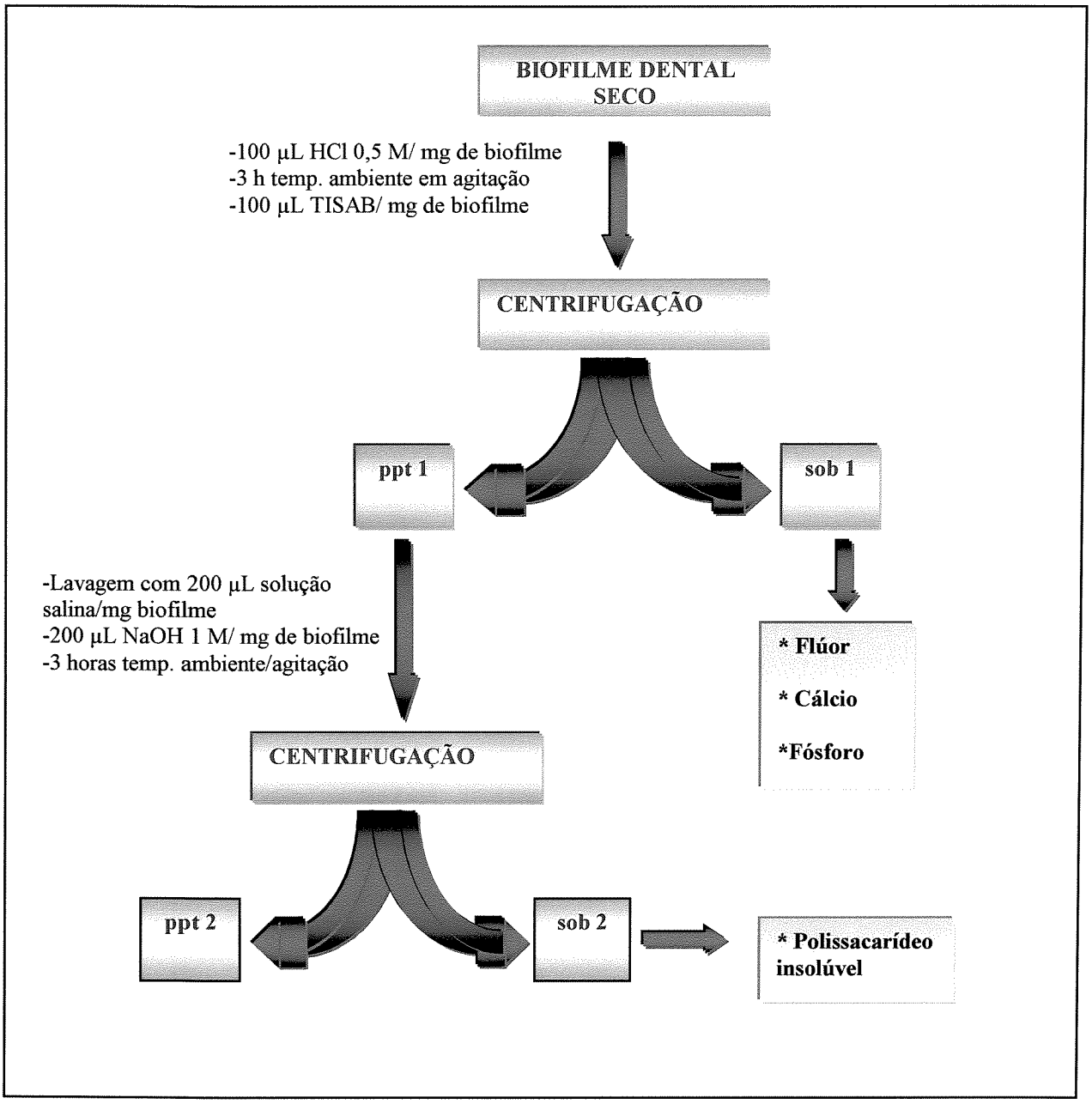
C-Posicionamento do voluntário durante a análise



D- Microeletrodo Beetrode® posicionado entre as malhas da tela plástica para mensuração do pH do biofilme dental.

ANEXO 8

Análise da composição do biofilme dental

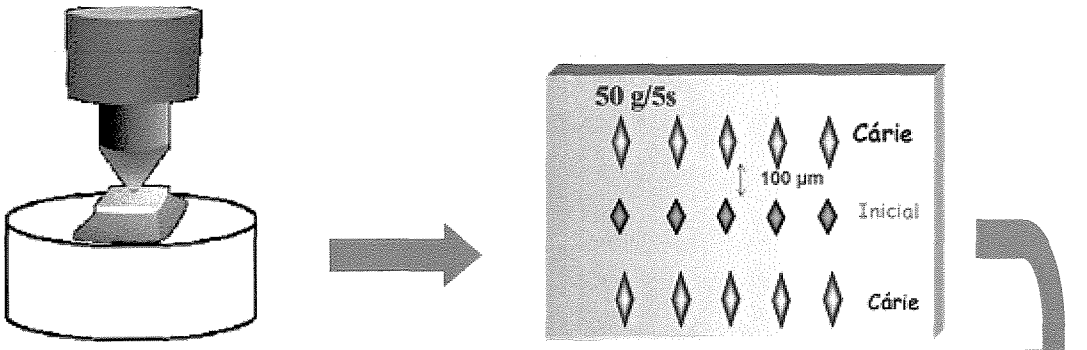


Cury *et al.* 2000 e Nobre dos Santos *et al.*, 2002 modificados.

ANEXO 9

Análises no esmalte dental

Microdureza de superfície



Análise do bloco dental no microdurômetro

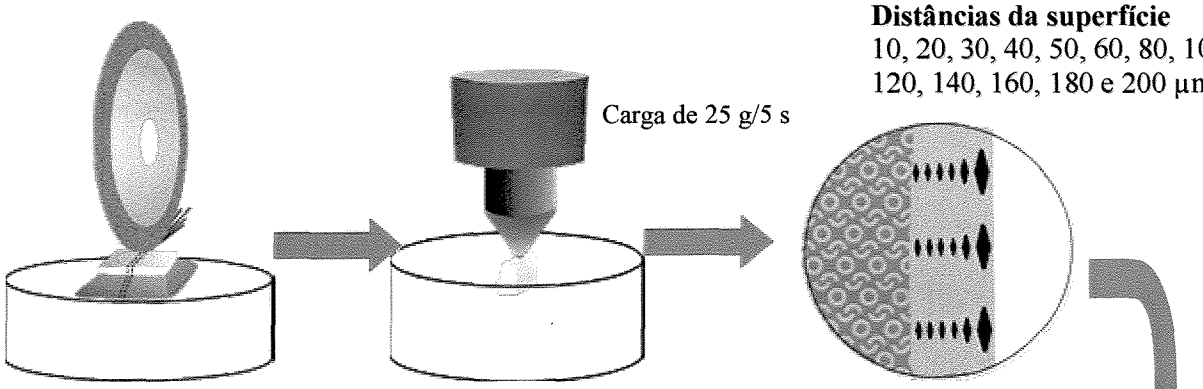
Bloco dental após a análise

% PDS = Inicial - Pós-tratamento x 100/ Inicial

Microdureza do esmalte seccionado longitudinalmente

Distâncias da superfície
10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180 e 200 µm

Carga de 25 g/5 s



Seccionamento longitudinal do esmalte dental

Esmalte dental embutido para leitura no microdurômetro

Bloco dental após análise

Delta Z =% Vol mineral x µm