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AVALIAÇÃO DO POTENCIAL CARIOGÊNICO DA MALTODEXTRINA E DE SUA ASSOCIAÇÃO COM A SACAROSE

Porto Alegre, julho de 2015.

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Epidemiologia, etiopatogenia e repercussão das doenças da cavidade bucal e estruturas anexas

AVALIAÇÃO DO POTENCIAL CARIOGÊNICO DA MALTODEXTRINA

E DE SUA ASSOCIAÇÃO COM A SACAROSE

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul como parte dos requisitos obrigatórios para obteção do título de Mestre em Clínica Odontológica concentração em Cariologia/Dentística.

Orientadora: Prof.ª Dr.ª Lina Naomi Hashizume

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DEDICATÓRIA

Dedico esta dissertação de mestrado aos meus pais e minha irmã, que sempre me incentivaram e estiveram ao meu lado durante minha trajetória profissional, apoiando em todos os momentos. Ao meu namorado que esteve sempre presente. A todos meus familiares e amigos, que sempre torceram por mim.

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RESUMO

A maltodextrina é um oligossacarídeo derivado a partir da hidrólise ácida e/ou enzimática do amido de milho e está cada vez mais presente em uma variedade de alimentos tais como fórmulas infantis, bebidas esportivas e suplementos energéticos. Muitos produtos à base de maltodextrina apresentam também sacarose em sua composição. Contudo, o seu papel no desenvolvimento da cárie dentária não é claro. O objetivo deste estudo foi: realizar uma revisão da literatura sobre a cariogenicidade da maltodextrina e avaliar o potencial cariogênico e a estrutura organizacional do biofilme formado *in situ* na presença de maltodextrina e de sua associação com a sacarose. A partir das evidências encontradas na revisão de literatura, verificou-se uma escassez de estudos avaliando o potencial cariogênico da maltodextrina em esmalte. A partir do estudo *in situ*, conclui-se que a maltodextrina não apresenta potencial cariogênico em esmalte. Entretanto, a adição de sacarose à maltodextrina causa modificações na composição bioquímica e na organização estrutural do biofilme formado em sua presença, aumentando o seu potencial cariogênico em esmalte.

Palavras-Chave

Biofilme · Cárie Dentária · Maltodextrina · Sacarose

ABSTRACT

Maltodextrin is an oligosaccharide derived from the acidic and/or enzymatic hydrolysis of corn starch and is increasingly present in a variety of foods such as infant formulas, sports drinks and energy supplements. Many products containing of maltodextrin have also sucrose in its composition. However, its role in the development of dental caries is not clear. The aim of this study was: to conduct a literature review on the cariogenicity of maltodextrin and to evaluate the cariogenic potential and the organizational structure of the biofilm formed *in situ* in the presence of maltodextrin and its association with sucrose. From the evidence found in the literature review, there was a lack of studies by assessing the cariogenic potential of maltodextrin in enamel. From the *in situ* study, it was concluded that maltodextrin has no cariogenic potential on enamel. However, the addition of sucrose to maltodextrin causes changes in the biochemical composition and organizational structure of the biofilm formed, increasing its cariogenic potential in enamel.

Key-Words
Biofilm · Dental caries · Maltodextrin · Sucrose

SUMÁRIO

1. **INTRODUÇÃO**

A maltodextrina, também conhecida como polímero de glicose, é um oligossacarídeo (polímeros de cadeia longa), composto de glicose e derivado da hidrólise ácida e/ou enzimática do amido de milho e a sua posterior secagem, para a obtenção de pó que será utilizado pela indústria alimentícia. A quantidade de glicose e maltose presentes é considerada baixa em comparação a polissacarídeos de cadeias longas. Pode ser caracterizada como praticamente sem gosto e sem odor, e a sua composição irá determinar o seu potencial edulcorante. Sua doçura aumenta com o aumento da concentração de sacarídeos de baixo peso molecular, como a presença de glicose [Kearsley and Sicard, 1989; Moynihan et al., 1996; Moynihan, 1998].

A maltodextrina é utilizada pela indústria alimentícia como agente espessante em alimentos para bebês, molhos e sopas. Também pode ser utilizada como agente antiaglomerante, melhorando as propriedades de armazenamento e textura em produtos secos, como sopas instantâneas. Este oligossacarídeo não possui um papel adoçante, é utilizada para aumentar o teor calórico de fórmulas infantis, suplementos dietéticos para auxiliar no ganho de peso e em algumas bebidas esportivas. Os produtos à base de maltodextrina, principalmente as fórmulas infantis, são fornecidas às crianças em uma alta frequência e à noite, onde o fluxo salivar é reduzido, o que acaba favorecendo o desenvolvimento da cárie dentária [Moynihan et al., 1996; Levine, 1998; Grenby e Mistry, 2000; De Mazer Papa et al, 2010].

Muitos produtos à base de maltodextrina também apresentam sacarose em sua composição, ou são utilizados em associação com este carboidrato. Ainda não está claro na literatura se essa associação da maltodextrina com a sacarose apresenta algum efeito em relação à cariogenicidade desses alimentos. Verificando a escassez de estudos avaliando a associação da maltodextrina com a sacarose e seu uso cada vez maior pela indústria alimentícia, torna-se necessário uma maior investigação do seu potencial cariogênico quando consumida como única fonte de carboidrato, ou ainda, quando associado à sacarose.

OBJETIVO

2.1 Objetivo Geral

Avaliar o potencial cariogênico da maltodextrina e de sua associação com sacarose.

2.2 Objetivos Específicos

A) Avaliar as evidências existentes na literatura sobre a relação entre a maltodextrina e a cárie dentária;

B) Avaliar a composição microbiológica e bioquímica do biofilme dentário, formado *in situ* na presença da maltodextrina e da maltodextrina associada à sacarose.

C) Avaliar o efeito da maltodextrina e de sua associação com a sacarose na desmineralização do esmalte dentário;

D) Avaliar a estrutura organizacional do biofilme dentário formado *in situ*, na presença da maltodextrina e da maltodextrina associada à sacarose, através da análise com a microscopia confocal de escaneamento a laser.

2. **ARTIGO 1**

Maltodextrin and dental caries – a literature review

Abstract

Carbohydrates are largely present in our diet. Sucrose the most commonly consumed carbohydrat and presents a high cariogenic potential. Starch has low cariogenic potential but this effect may be increased if it is consumed in combination with a sucrose-rich diet due to a prolonged retention on tooth surfaces. Maltodextrin is derived from the acid hydrolysis and/or enzymatic hydrolysis of corn starch and it is increasingly present in a variety of industrialized foods such as infant formulas, sports drinks and energy supplements. Yet, its role in the development of dental caries is not clear. The objective of this study was to conduct a literature review of the association between maltodextrin and dental caries. Based on the studies included in this review it can be concluded that maltodextrin has an acidogenic potential lower than sucrose, and that the combination of maltodextrin and sucrose may have an increased cariogenic potential on dental enamel. It is suggested that more studies be conducted to verify this possible cariogenicity of maltodextrin and its association with sucrose.

Key-Words

Starch · Maltodextrin · Dental Caries · Dietary

Introduction

Maltodextrin is a starch hydrolysate oftenly used by the industry and is increasingly present in a variety of foods such as infant formulas, sports drinks and energy supplements. The maltodextrin-based products, particularly infant formulas and dietary are used as food supplement products for enteral diet, consumed orally or through tubes.[1] They are commonly used in children who have allergies or lactose intolerance [2] and given to children at high frequency and at night, where the salivary flow is reduced, which ends up favoring the development of dental caries.[3,4] Yet, its role in the development of dental caries is not clear. Therefore, the objective of this study was to conduct a literature review about the association between maltodextrin and dental caries.

Diet and Dental Caries

Although epidemiological indicators of dental caries have show important declines in the prevalence of this disease, it still appears to be present in childhood in many countries.[5,6,7] The association between dental caries and excessive consumption of sugars is affirmed by experts to the World Health Organization, on an assessment of the evidence in the literature relating diet and caries in 2003. Experts have reported an increase in caries development risk associated with frequent intake of sugars.[8]

With the changes in eating habits and the growing process of food industrialization, foods high in fiber and nutrients are being replaced by processed foods with an excess of fats and carbohydrates, thus the development of dental caries is facilitated.[9] Carbohydrate is the food group most present in our diet. They can be classified in different ways, such as: chain length (monosaccharides, disaccharides, polysaccharides) and originated from food (fruit, milk, sugar cane).[10]

Sucrose is the most commonly consumed carbohydrate and it presents a greater cariogenic potential. This feature is in part attributable to the fact that this is the only carbohydrate that can be used as a substrate for the synthesis of soluble and insoluble extracellular polysaccharides by the dental biofilm, favoring the colonization and adherence of microorganisms to dental surfaces and increasing the porosity of the dental biofilm.[11,12,13]

Dental caries is considered a multifactorial disease and its development is strongly associated with a diet high in carbohydrates. Ingestion of high quantities of fermentable carbohydrates induces the formation of a biofilm with cariogenic microorganisms.[14,15] These microorganisms, such as mutans streptococci induce the formation of acids, dramatically reducing the pH of the environment, reaching the critical pH for demineralization of enamel or dentin and promoting calcium and inorganic phosphate subsaturation. These changes lead to demineralization of dental structures due to loss of hydroxyapatite mediated by frequent pH falls.[16,17] The time required for the pH increase depends on how the carbohydrate was consumed, the time of day it occurs and the saliva action. The pH will return to normal quicker, if the sugar is ingested in liquid form, whereas in solid form it will remain on the tooth surface for a longer period before being removed by the mechanical action of brushing, for example, or by salivary action. Similarly, the pH will return to normal

quicker if the sugar is ingested soon after meals, than if ingested at night, where the salivary flow is reduced. In return above the critical value 5.5 or 6.5 (enamel and dentin respectively) saliva can replace lost minerals, occurring remineralization.[18]

Classical studies assessing the relationship of dental caries with diet show that there is increased of caries with the increased consumption of carbohydrates, and great was the influence of consistency of foods and how often foods high in sugar are consumed.[19,20,21]

GUSTAFSSON et al (1954) conducted monitoring mentally disabled adults over 5 years in order to assess the caries incremente the increase of sucrose intake, the influence of food consistency and frequency of sugar intake. They observed that there was a low incidence of caries when the diet was almost free of carbohydrates, and less caries increment with the consumption of starch derivatives carbohydrates at meals (bread and pasta).[19] It is noted in this study the absence of fluoride, which leads to more extreme results.

HARRIS (1963) we conducted a study with children from an orphanage who had lacto vegetarian diet with minimal amounts of sugar and refined flour, compared with children from another orphanage diet with no restrictions. Observed a lower prevalence of caries in the control group, an increased prevalence occurred when children leave the orphanage. They concluded that the diet had extreme influence on the development of caries as compared children with similar socioeconomic conditions.[20]

SÖDERLING et al. (1976) they conducted a study for 25 months at three groups of adults a diet with sucrose, fructose and xylitol, with the objective of evaluating caries levels when the sucrose is replaced by fructose or xylitol. The group with xylitol and fructose showed a reduction in caries levels compared to the group using sucrose.[21]

A recent systematic review aimed to assess the evidence on the association between sugar intake and dental caries, plus the effect of restricting the intake of sugars. From the 55 studies analysis, the authors concluded that there consistent evidence of moderate quality supporting a relationship between the amount of sugars consumed and dental caries development.[22]

The ratio of sucrose and dental caries is well established in the literature. Despite the development of this disease being associated with a variety of factors, the frequency and consistency of food containing sucrose have a great influence on

the development of dental caries.

Starch and dental caries

Starch is a glucose polymer, composed of two polysaccharides (amylose and amylopectin) which are found in a variety of foods as in cereals, roots and seeds. The starch molecules are inside of insoluble beads, when subjected to heating processes undergo a series of changes in a process called gelatinization. The products having an increased gelatinization are more susceptible to enzymatic breakdown, causing a partial degradation to a soluble form that can be fully dissolved by salivary and bacterial amylase, and be transformed into maltose, maltotriose, dextrins and small amounts of glucose. Salivary amylase performs the hydrolysis of glycosidic chains, resulting in smaller units which are readily metabolized by the microorganisms of the oral cavity. The extent of this hydrolysis is determined by the time that food is retained in the oral cavity. [23,24,25]

Starch has a minimal influence in dental caries if consumed in a diet with small quantities and limited frequency of sucrose. However, this effect may be magnified if starch is eaten in combination with a diet rich in sucrose due to its prolonged retention on tooth surfaces. The starch cariogenicity differs by food source, amount and frequency of consumption and its method of preparation.[17]

Although studies indicate that starchy foods do not have a cariogenic potential,[19,26] some studies have demonstrated the cariogenic role of starch consumed with or without sucrose.[25,27,28]

GUSTAFSSON et al (1954) found a low incidence of caries in the group that consumed bread and pasta associated with reduced quantities of sugars.[19] However must be considered that the study had a long duration, and modified several times over the period and the great variability among the subjects studied. Also, there was difference in the consistency of food and the frequency in which they were offered to different study groups, where the group receiving a higher frequency of carbohydrates with sticky consistency, between meals, showed a higher caries prevalence than the group who received bread and pasta less frequently.

RUGG-GUNN et al (1987) they conducted a study for two years with 405 school children with the aim of evaluate the cariogenic potential of starch and sugars diet of children, in addition, to evaluate the simultaneous effect of starch with sucrose relating the cariogenic potential of sucrose with the viscosity of the starch They found

that children who had a diet with high consumption of starch and low sugar had a lower incidence of dental caries.[26]

Pollard (1995) investigated the cariogenicity of the starch and fruit by measuring acidogenicity of biofilm and enamel demineralization. The study tested fruits and cereals (no sugar added), white bread, brown bread, rice and pasta. The results indicated that all foods were less acidogenic than sucrose (positive control). All the foods reached the maximum drop of pH and produced enamel demineralization. The autor concluded that all foods were less cariogenic than sucrose, but more than sorbitol (negative control).[27]

Llenaa and Forner (2008) conducted a study with children between 6-10 years of age with low prevalence of caries analyzing caries experience and the consumption of cariogenic foods. A questionnaire was provided to the parents to evaluate food consumption, its frequency, and the relation of diet-habits with caries experience was evaluated. Foods such as industrialized bread, sweet snacks and soft drinks showed a positive association with dental caries, since foods like cheese and walnuts showed a negative association. The authors also suggest that sugary liquid foods, foods high in semi-hydrolysate starch (whether or not combined with sugar) and sugary drinks are factors that are strongly associated with caries experience in the evaluated children, regardless of age, sex, and toothbrushing.[28]

A literature review investigated the relationship between food starch and dental caries. The authors considered a determinant for starch cariogenicity the following factors: consumption intensity (quantity and frequency), exposure of tooth surfaces, starch bioavailability, microorganisms present in the biofilm, the biofilm pH fall and salivary flow. Several experimental studies indicate that foods containing starch have a significant cariogenic potential, and studies with humans have yielded only limited information and inconclusive.[25]

Starch relationship with dental caries is not clearly established in the literature as compared with sucrose. The most recent studies showing the cariogenic potential of starch are its most experimental, and in vivo studies did not show conclusive results.

Starch hydrolysate

The starch hydrolysate can be divided into maltodextrins and glucose syrups. They are composed generally of glucose, maltose, maltotriose and glucose

polymers, whose final concentration depends on the method and degree of hydrolysis of corn starch which is chemically defined as "dextrose equivalent" (DE) of this oligosaccharide. DE is expressed as dextrose and calculated as a percentage of total dry mass. The higher the value of DE, the greater the amount of reducing sugars it will contain and the more readily the product will be metabolised by the oral bacteria. Maltodextrins are complex in nature and have a DE of less than 20, and glucose syrups have a DE higher than 20.[10]

Maltodextrin

Maltodextrin is the most used starch by food industry and it is classified as oligosaccharide (long chain polymer) and derived from acid hydrolysis and/or enzymatic hydrolysis of corn starch and subsequent drying. The amount of glucose and maltose is considered low in comparison to long chains of polysaccharides. Also called glucose polymer, maltodextrin can be characterized as virtually tasteless and odourless, and its composition will determine its potential sweetener. Its sweetness increases with increased concentration of low molecular weight saccharides, such as the presence of glucose.[3,10,29,30] Maltodextrins are used by the food industry as thickeners in baby food, sauces and soups. Additionally, it is used as anti-caking agents to improve storage properties and texture in dry products such as instant soups. Maltodextrins have a non-sweetener role. They are used to increase the caloric content of infant formulas and dietary supplements (to assist in weight gain) and in some sports beverages.[29,31,32]

Many maltodextrin-based products have sucrose in its composition and is not known if this association has a particular effect on the cariogenic potential of these foods. There is in literature a variety of studies evaluating the acidogenicity of maltodextrin on biofilm, showing that the maltodextrin is less acidogenic than sucrose.[2,30,33,34,35,36] It was located only a study in which the authors evaluated the cariogenic potential of maltodextrin in infant formulas.[4]

Moynihan et al. (1996) evaluated the acidogenicity potencial of products containing maltodextrin, glucose syrup, milk and cow's solution used as milk replacer (Calogen), compared with sucrose in 14 adult volunteers. They observed that the tested products containing maltodextrin caused a decrease in pH of the biofilm, but to a lesser extent than the pH drop caused by the sucrose.[33]

Meyerowitz et al. (1996) assessed the effects of artificial sweeteners present

in iced teas on the pH of dental biofilm. In this study, the acidogenic potential of tea without added sugar was compared. Teas with different compositions were compared: sucralose; sucralose and maltodextrin; sucralose, maltodextrin and dextrose; and finally tea containing sucrose. It was concluded that sucrose had the greatest drop in pH, either alone or combined with maltodextrin. Sucralose, maltodextrin and dextrose exhibited a significantly lower pH drop.[34]

Al-Khatib, Duggal, Toumba (2001) investigated the *in vivo* effects of three different maltodextrins (DE 5.5, DE 14.0 and DE 18.5) on the pH fall of biofilms of 10 volunteers, as well as the acidogenicity of children's drinks containing maltodextrin compared with sucrose and sorbitol solution. It was concluded that, although being less acidogenic than sucrose, maltodextrin led to a substantial drop in the pH of the biofilm and may have an enamel demineralization potential.[30]

Raju et al. (2012) evaluated the effect of infant milk formula composed of different sugars and their effects on dental plaque pH. Six formulas were tested, three containing maltodextrin associated with sucrose, two containing lactose and one containing only sucrose, in ten children 7-10 years. The results showed that all formulas reduced the biofilm pH to lower values than those obtained after washing with water. The formulations containing sucrose in combination with other carbohydrates such as maltodextrin showed a greater decrease in the pH, than formulas containing lactose.[2]

Bhat and Dubey (2003) conducted a study with 75 Indian children caries free. They measured the pH of the biofilm after rinsing with regular infant formulas (for children without dietary restrictions, containing lactose), soy formula (for children with specific dietary restrictions, containing maltodextrin), bovine milk, water and sucrose solution. The sucrose solution showed the biggest drop of pH, being more acidogenic. Among the tested formulas, soy formula containing maltodextrin showed the biggest drop in pH, presenting itself as more acidogenic, similar to sucrose.[35]

Dashper et al. (2012) selected four soy beverages, three formulas containing maltodextrin and two containing bovine milk, and determined the acidogenic potential of each. The authors evaluated *in vitro* the acid production by mutans streptococci, the acid buffering capacity and the calcium and inorganic phosphate concentrations of the beverages. The rate of acid production by mutans streptococci in the milk-based beverage was 5 to 6 times lower than the pH of 6.5, in the production of soy based beverages, and 3 to 5 fold lower than pH 5.5, showing a lower buffer capacity

in the soy beverage. Calcium levels in the soluble soy-based beverages were lower than in milk; even the entire contents are similar. From beverages evaluated in the study, authors concluded that soy-based beverages have a higher acidogenic potential than bovine milk beverages.[36]

De Mazer Papa et al. (2010) conducted an *in situ* study with 11 volunteers using palatal appliances with deciduous dental enamel blocks, with the objective of assessing the effects of milk and soy-based infant formula associated or not with sucrose on enamel demineralization and its effect on the biofilm composition. Both infant formulas, special for babies, contained a small percentage of maltodextrin (milk-based formula contains 3.2% of maltodextrin and 4.06% of lactose, and soy-based formula contains 7.31% of maltodextrin). After the analysis of mineral loss, biochemical and microbiological biofilm, the authors concluded that milk and soy-based infant formula present potential to induce demineralization in deciduous enamel, which was increased when sweetened with sucrose.[4]

Most studies were performed with isolated maltodextrin or comparing different maltodextrin's DE, and a reduced number of studies evaluated its association with other sugars. Moreover the studies have different evaluation parameters, which difficult the results comparison. The studies, mostly, were limited to *in vivo* acidogenicity evaluation of maltodextrin in biofilm, and showed that maltodextrin has an acidogenic potential but lower than sucrose. A study evaluating the combination of maltodextrin with sucrose showed that its cariogenic potential increases when it is associated with sucrose.[2] However, the study was developed from deciduous dental enamel, which presents different mineral structure of permanent enamel, that has lower levels of calcium and phosphorus, smaller thickness and a higher density enamel prisms compared with the permanent enamel,[37] so the results can not be generalized para esmalte permanente.

All the studies found, in this literature review, were conducted with enamel, it was not found studies evaluating the acidogenicity or cariogenicity maltodextrin on dentin. It is suggested that more studies must be conducted to verify the cariogenic potential of maltodextrin alone and its association with sucrose and other carbohydrates in permanent enamel and dentin.

Conclusion

Based on the studies included in this review it can be concluded that

maltodextrin has an acidogenic potential lower than sucrose, and that the maltodextrin and combination of maltodextrin and sucrose have a cariogenic potential on deciduous dental enamel.

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3. **ARTIGO 2**

Cariogenic potential of maltodextrin and its association with sucrose on enamel: study *in situ*

Abstract

Maltodextrin which has been widely used in the food industry is obtained by hydrolysis of corn starch. Many products containing starch derivatives have sucrose in its composition. The aim of this study was to evaluate the effect of maltodextrin and its association with sucrose on *in situ* demineralization of enamel and in the microbiological and biochemical composition dental biofilm formed on its presence. This *in situ* study was also cross-over, split-mouth and doubleblind, where 19 volunteers wore intraoral appliances containing bovine enamel blocks during two periods of 14 days. Volunteers were instructed to drop 8 times per day the following solutions on the enamel blocks: deionized distilled water (DDW), maltodextrin, maltodextrin+sucrose or sucrose. At the end of each experimental phase biofilm were assessed by microbiological (mutans streptococci and lactobacilli counts) and biochemical (calcium, inorganic phosphate, fluoride and insoluble extracellular polysaccharides (IEPS) concentrations) and analisis enamel blocks had their surface enamel microhardnes also assessed. Microbiological analysis showed no difference among the treatments. Biofilms formed in the presence of sucrose and maltodextrin+sucrose displayed a lower inorganic composition, a higher demineralization, and a higher concentration of IEPS than the maltodextrin alone. Isolated maltodextrin showed similar results to the DDW. It is concluded that the isolated maltodextrin has no cariogenic potential in enamel, and the addition of maltodextrin to sucrose not increases its cariogenicity.

Key-Words

Maltodextrin · Dental Biofilm · Dental Caries · Sucrose

Introduction

Maltodextrin, also called as glucose polymer, is an oligosaccharide derivative of acid hydrolysis and/or enzymatic corn starch and subsequent drying. It is composed generally of glucose, maltose, maltotriose and glucose polymers, and its final concentration varies, depending on the method and degree of hydrolysis which is chemically defined as "dextrose equivalent" (DE) of this oligosaccharide. The

higher the value of DE, the greater the amount of reducing sugars present in the maltodextrin and the faster the product will be metabolised by oral bacteria [Kearsley and Sicard, 1989; Moynihan et al., 1996].

Maltodextrins are used by the food industry as thickeners in baby food, sauces and soups as well as anti-caking agents in order to improve the storage properties and the texture of dry products, such as instant soups. Maltodextrins do not have the function of a sweetener; they are used in infant formulas to increase the amount of calories, in dietary supplements to assist in weight gain and in some sports drinks [Moynihan, et al., 1996; Levine, 1998; Grenby and Mistry, 2000].

Many maltodextrin-based products have sucrose in their composition. Among the carbohydrates, sucrose is considered the most cariogenic one and this feature is partly attributable to the fact that this carbohydrate is used as a substrate for the synthesis of extracellular polysaccharides (EPS) in dental biofilm [Bowen, 2002; Paes Leme, et al., 2006]. *In vivo* studies report that the cariogenic potential of starch consumption associated with sucrose is greater than the cariogenic potential of these carbohydrates consumed by alone [Bowen et al., 1980; Firestone, Shmid, Muhlemann, 1982; Thurnheer et al., 2008].

It is not clear whether the combination of maltodextrin with sucrose exerts any particular effect on the cariogenic potential of these foods. There are a variety of studies in the literature which evaluate the acidogenicity of maltodextrin isolated in biofilm, showing that the maltodextrin has a cariogenic potential, however, lower than that of sucrose, despite of both showing significant decreases in pH [Meyerowitz, Syrrakou, Raubertas, 1996; Moynihan, et al., 1996; Al-Khatib, Duggal, Toumba, 2001; Bhat SS and Dubey A, 2003; Dashper SG et al., Raju et al., 2012]. Only one study evaluated infant formulas, containing different percentages of maltodextrin, associated or not with sucrose, in relation to its cariogenic potential and effects on biofilm formed *in situ*. The authors concluded that infant formulas are cariogenic for primary enamel and that the cariogenicity is increased when associated with sucrose [de Mazer Papa et al., 2010]. Considering the scarcity of studies evaluating the association of maltodextrin with sucrose, it is necessary further investigation of its cariogenic potential when consumed as a sole source of carbohydrate, or when associated with sucrose.

Taking into account that the maltodextrin has been widely and often used in the food industry with or without other sugars, the aim of this study was to evaluate

the effect of maltodextrin and its association with sucrose on *in situ* demineralization of enamel and in the microbiological and biochemical composition of dental biofilm formed in its presence.

Material and Methods

Experimental Design

This study was *in situ*, randomized, double-blind, split-mouth and crossover. There were two experimental phases with duration of 14 days each, with nineteen volunteers, with average age of 24.75 ± 3.3 years (mean ± SD). It was approved by the Ethics and Research Committee of the Dental School of the Federal University of Rio Grande do Sul (Protocol 666.924). Informed consent was obtained from each participant prior to the beginning of the study and only volunteers with good oral health conditions and favorable systemic and availability to attend to the research site on request were included. In addition, the volunteers could not be smokers, could not present xerostomy, should not use any type of orthodontic appliance or have used any antibiotics for at least two months prior to baseline.

Enamel Blocks and Palatal Appliance Preparation

Enamel blocks were obtained from bovine incisors. All teeth which presented fractures on the external enamel surface, as well as cracks and hypocalcification were excluded from the samples. Before the preparation of blocks, the teeth were subjected to a removal of tissue debris and stored on a formaldehyde solution pH 7.0 and 2% with phosphate buffer during 30 days for disinfection [Cury, Rebello, Del Bel Cury, 1997]. The blocks were prepared with dimensions of 3x3x2mm [Cury, Rebello, Del Bel Cury, 1997], obtaining a total of 148 blocks with a defined initial surface microhardness (340.9 ± 51.1 kg/mm²), wich were randomly divided into four different groups according to each treatment.

For each volunteer an intraoral palatal appliance was made from acrylic resin containing four blocks, two blocks on the right side and two on the left [Cury et al., 2003]. The cavities for the blocks were created in the appliance leaving 1.0 mm for biofilm formation and covered with a plastic screen to protect from the mechanical friction [Hara et al., 2003]. Each side was identified with a color correspondent to the test solution that would be dropped (a red side and a white side) [Cury et al., 2001; Paes Leme et al., 2004; Pecharki et al., 2005; Ribeiro et al., 2005].

Treatments

The test solutions were prepared and provided to the volunteers, in milky white bottles, with only white or red color identification. Solution were prepared as follow: in the following treatments: (1) deionized distilled water (DDW); (2) 10% sucrose solution; (3) 10% maltodextrin solution (DE 13-17, Sigma-Aldrich, St. Louis, MO, EUA); (4) 10% maltodextrin solution and 10% sucrose solution. In the experimental period, two treatments were evaluated in each volunteer, considering that two enamel blocks were built in each side of the appliance and received one different treatment: DDW (control) and sucrose solution or maltodextrin+sucrose solution and maltodextrin solution. The use of the two treatments on the same oral appliance (split-mouth) is supported by previous studies for the absence of cross effect [Cury et al., 2001; Hara et al., 2003; Paes Leme et al., 2004, Pecharki et al., 2005; Ribeiro et al., 2005].

The solutions were prepared and provided to the volunteers every three days during the experimental period. During the 14 days, 8 times a day, at predetermined times (8:00, 9:30, 11:00, 13:30, 15:00, 16:30, 18:00 and 21:00) the volunteers were instructed to remove the appliance from the mouth, drip one drop of the assigned solution on the enamel block according to the treatment protocol, dry the excess with gauze and wait 5 minutes before putting the appliance in the mouth. The volunteers were instructed to use the appliance at all times, removing it only for the intake of food and drinks and to realize oral hygiene. No restriction were made in diet and for the entire period the volunteers used fluoridated water (0.8 ppmF) and non-fluoridated toothpaste, which was provided by the researchers. A seven-day washout period was performed previously to the beginning of each experimental phase. Upon completion, the appliances were collected for analysis of the composition of biofilm and caries development.

The biofilm formed was collected and microbiological (total aerobic microorganisms, mutans streptococci and lactobacilli counts) and biochemical (concentrations of calcium (Ca), inorganic phosphate (Pi), fluoride (F) and insoluble extracellular polysaccharides [IEPS]) analyses were conducted. After collecting the biofilm, the enamel blocks were evaluated for microhardness after the treatment.

Colection of Dental Biofilm

The samples of the formed biofilm on two of the enamel blocks of each treatment were collected on the 14th day of each experimental phase and about 12 hours after the last exposure to the treatments. The screen over the blocks was removed, the biofilm was collected, with sterile curettes, and immediately transferred to pre-weighed microcentrifuge tubes, sterile and identified with the volunteer number and the corresponding treatment. An aliquot of biofilm was collected for microbiological analysis and the remaining biofilm was reserved for subsequent biochemical analysis.

Microbiological Analysis

From the biofilm collected for the microbiological determination, the weight of 1mg wet biofilm was suspended in 1 mL of sterile saline (0.9% NaCl) and sonicated [Aires et al., 2008]. The suspension was serially diluted in sterile 0.9% NaCl solution. Two drops of each dilution were plated in the following culture media: mitis salivarius agar plus 0.2 units bacitracin/ml (MSB) (HI Media - Ghatkopar West, Mumbai, India) for growth of mutans streptococci; Rogosa SL agar (HI Media - Ghatkopar West, Mumbai, India) for growth of lactobacilli; Brain Heart Infusion (BHI) agar (Kasvi - Atuba, Curitiba, Brazil) plus sheep blood, vitamin K and hemin for growth of total microorganisms. The media: BHI agar and MSB agar were incubated for 48 hours at 37°C in microaerophilic; and Rogosa SL agar incubated for 72 hours at 37°C in microaerophilic. After this period, the number of colony forming units in each culture media was counted with the aid of a stereomicroscope (Olympus SZ51 - Shinjuku-ku, Tokyo, Japan). The results were expressed in a number of colony forming units per milliliter (CFU / mL).

Biochemical Analysis of Biofilm

Dental biofilm was dehydrated in a vacuum desiccator over phosphorus pentoxide (P_2O_5) and the dry weight was obtained (±0.01 mg, Sartorius BP 210D, Sartorius, Goettingen, Germany). For biochemical analysis, 0.5 M hydrochloric acid (HCl) was added to the microtube (0.1 mL HCl/mg dry weight dental biofilm). After 3h at room temperature under constant agitation, the same volume of TISAB II pH 5.0 (containing 20 g NaOH/L) was added as a buffer. The samples were centrifuged for 10 min at 14000 rpm (Eppendorf 5410, Eppendorf AG, Hamburg, Germany) and the supernatant retained for determination of F, Pi and Ca. F was determined by ion-

selective electrode specific for fluoride (Orion 9609, Thermo Fisher Scientific Inc., Waltham, MA) connected to an ion analyzer [Cury et al., 1997] and Ca and Pi were determined colorimetrically using reagents Arsenazo III and molybdate, respectively. [Vogel et al., 1983; Fiske and Subarrow, 1925]. To the precipitate, 1.0 M NaOH (0.2 mL/mg dry weight plaque) was added. After 3h at room temperature under constant agitation, the samples were centrifuged [Pecharki et al., 2005] and the concentration of IEPS, in the supernatant, was determined using the phenol-sulfuric method [Dubois et al., 1956].

Microhardness Analysis

The determination of initial enamel surface microhardness aimed to select the enamel blocks for the study and enables the calculation of the percentage loss of surface microhardness after treatments. From the center of the enamel block 1000 µm below the upper limit and 1500 µm right from the left edge an indentation reference was performed using a static load of 100g for 10 seconds. The indentations were made with the long axis of the diamond parallel to the external enamel surface, with a Knoop diamond static weight of 50g, applied for 10 seconds on a microhardness tester HMV-2T (Shimadzu, Kyoto, Japan). Five indentations were carried out in sequence, separated by a distance of 100 µm [Cury et al., 2000].

It was measured final surface microhardness of the enamel blocks, after the end of each experimental phase, there were five indentations with 50g load for 10 seconds. From the mean value of the indentations was calculated the average post-experiment microhardness. Then, the mean value of the 4 blocks for each volunteer in each phase and the percentage of surface microhardness loss (%SML) were calculated [Aires et al., 2002].

Statistical Analysis

The data obtained after laboratory analysis of biochemical, microbiological composition of biofilm, and of surface microhardness of enamel blocks, were tabulated and then submitted to the SPSS (Statistical Package for Social Sciences - IBM, Armonk, NY, USA) version 21.0 for Windows. Statistical analysis using the Shapiro-Wilks normality test detected heterogeneous variances in some variables. For the statistical analysis of the percentage of mutans streptococci and the analysis of lactobacilli among total microorganisms was used the nonparametric Friedman test

(p<0.05). In relation to the statistical analysis of biochemical data and surface microhardness, the data that showed normal distribution did not undergo any changes (%SML, Pi and IEPS), on the contrary, the data without normal distribution was transformed into log10 (x) (Ca, F and biomass). After the test with Bonferroni correction (p <0.05), all data underwent a statistical analysis through the Generalized Estimating Equations (GEE).

Results

Table 1 presents the results of the statistical analysis of the percentage of mutans streptococci on the total number of microorganisms (%MS/TM) and lactobacilli on the total number of microorganisms (%LB/TM). The %MS/TM showed no difference among treatments (p>0.05). The same occurred with the analysis of %LB/TM, with no difference among treatments (p>0.05).

Table 2 displays the results of the statistical analysis of biochemical variables. In relation to the F concentration in the biofilm, maltodextrin, sucrose and maltodextrin+sucrose showed lower concentration than the DDW (p<0.05) but they were not different among each other. The analysis of the Ca concentration in the biofilm resulted in lower values (p<0.05) in the presence of sucrose, compared to the treatments with maltodextrin, maltodextrin+sucrose and DDW, the last one presenting a lower concentration of Ca than the other treatments. The analysis of the concentration of Pi in the biofilm demonstrated lower values (p<0.05) by the treatment with maltodextrin+sucrose, the remaining treatments had similar values (p>0.05).

The concentration of IEPS presented higher values by the treatment with sucrose and maltodextrin+sucrose, compared to the treatment with maltodextrin (p<0.05), that had a similar value with DDW.

The %SML displayed lower values (p<0.05) by the treatments with DDW and maltodextrin in comparison with sucrose and maltodextrin+sucrose, those presenting a greater %SML.

The analysis of the biomass of the biofilm demonstrated higher values (p<0.05) by the treatment with sucrose compared to the treatments with the maltodextrin and the maltodextrin+sucrose, which values are similar to DDW (p>0.05).

Discussion

Maltodextrin has been widely used in the food industry, usually associated with other sugars; however, its use associated with sucrose received insufficient investigation in the literature. Therefore, the aim of this study was to evaluate the cariogenic potential of isolated maltodextrin and also associated with sucrose *in situ*.

The microbiological analysis of %MS/TM and %LB/TM after treatment showed no difference (p>0.05) among the tested treatments. Although not found to have statistically significant difference in microbiological analysis, the groups with treatment with sucrose and maltodextrin+sucrose showed median values in %MS/TM higher than the rest, as well as verified with the %LB/TM where median values was greater in the groups which received treatment with maltodextrin and maltodextrin+sucrose. It is suggested that the number of microorganisms does not necessarily express their virulence in the biofilm. Authors conducted a study with the aim of assessing the levels of mutans streptococci and the development of caries in children, and concluded that the incidence of caries is much more related to the mutans streptococci´s ability to synthesize EPS than to the proportions of those bacteria present in the biofilm. Furthermore The authors suggest that the diet composition influences the virulence characteristics of the mutans streptococci, which in the presence of starch and sucrose results in an increase of virulence (cariogenic) of the biofilm [Mattos-Graner et al., 2000].

These microorganisms such as mutans streptococci cause the formation of acids, reducing the pH, reaching the critical value for the demineralization of enamel or dentin, promoting the subsaturation of calcium and phosphorus which leads to the demineralization of the dental structures due to the loss of hydroxyapatite ions [Bezerra and Toledo, 1999; Fejerskov and Kidd, 2011]. The determination of the Ca, Pi and F concentrations is an additional factor in order to evaluate the cariogenic potential of dental biofilms. When biofilm is formed in the presence of sucrose these ions are found in low concentrations [Cury et al., 2003; Paes Leme et al., 2006]. This fact is confirmed in previous studies that show a low concentration of Ca, Pi and F in the biofilm formed in situ in the presence of sucrose [Cury, Rebelo, Del Bel Cury, 1997; Cury et al., 2000].

In the present study the analysis of the F concentration in the biofilm resulted in lower values by the treatments with maltodextrin, sucrose and maltodextrin+sucrose in comparison to the control. The treatment realized with

sucrose had a lower concentration of Ca in the biofilm compared to the rest of the performed treatments. A similar result was confirmed *in situ* study, with declines in F and Ca concentrations in the presence of sucrose when comparing infant formulas containing maltodextrin associated or not to sucrose [De Mazer Papa et al., 2010]. The concentration of Pi in the biofilm presented lower values by the treatment with maltodextrin+sucrose compared to the rest of the treatments. A similar result to the one cited above was found in a previous study where Pi concentration was lower by the treatment with a starch+sucrose than by the treatment with sucrose and starch isolated [Ribeiro et al., 2005]. The concentration of Ca, Pi and F ions can be verified in several studies *in situ*, showing a decrease in the concentration of these ions when the frequency of carbohydrates consumption is increased [Cury, Rebello, Del Bel Cury, 1997; Vale et al. 2007; Tenuta et al. 2006; Aires et al., 2006]. Ingestion of high quantities of fermentable carbohydrates induces the formation of a biofilm with cariogenic microorganisms [Van Houte, 1989; Bowen et al., 1980].

Mostly using sucrose, the EPS are synthesized by microorganisms present in the tooth surface. These are mostly insoluble and promote the selective adhesion [Schilling and Bowen, 1992; Vacca-Smith et al., 1996] and the accumulation of a large number of cariogenic microorganisms on the tooth surfaces [Rolla, 1989; Mattos-Graner et al., 2000; Nobre dos Santos et al, 2002]. The increased production of extracellular polysaccharides promotes an increased porosity of the biofilm, allowing greater substrate diffusion into the enamel surface and with this a higher acid production by the microorganisms [Zero et al., 1992; Dibdin and Shellis, 1988]. The analysis of the IEPS concentration in the biofilm presented a lower value by the treatment with maltodextrin, similar to DDW, and higher concentration by the treatment with sucrose and maltodextrin+sucrose; showing that the production of IEPS is increased in the presence of sucrose. A similar result in a study that evaluated infant formula with the addition of sucrose [De Mazer Papa et al., 2010] and *in situ* studies assessing the biofilm with the addition of sucrose [Aires et al., 2006; Ribeiro et al., 2005]. In the present study it was not verified difference between treatments with sucrose and maltodextrin+sucrose in this experimental model of 14 days, but we can see a numerical increase in the concentration of IEPS with the combination of these carbohydrates. The result agrees with previous study in which the authors found no statistical difference in the concentration of IEPS in the biofilm formed *in situ* in the treatments with starch and starch+sucrose, using similar

experimental design to this study [Ribeiro et al., 2008]. Another previous study conducted with the aim to assess the starch and sucrose (alone or associated) for the formation and EPS composition, gene expression and acidogenicity of mutans streptococci on the *in vitro* biofilm. The authors concluded that the combination of starch with sucrose has great effects on the composition and structure of the EPS matrix in addition to the effects on gene expression of mutans streptococci, which may increase the cariogenic potential of the biofilm [Duarte et al., 2008].

These studies have report an increased in cariogenic potential of sucrose with the combination of starch, however they were conducted with deciduous dental enamel, which differs from the present study. The deciduous dental enamel has a different mineral structure of permanent dental enamel, with lower levels of calcium and phosphorus, low thickness and a high density of enamel prisms [de Menezes et al., 2010].

The biomass of the biofilm was higher in the treatment with sucrose ($p < 0.05$) compared to the treatment with maltodextrin, maltodextrin+sucrose and DDW. This result is in agreement with the findings of a previous study, where the value of the biomass present in the biofilm receiving treatment with sucrose and sucrose associated to starch was higher than the one found in biofilms which received treatment only with starch or water [Ribeiro et al., 2005].

Considering the analysis of %SML were confirmed higher values by the treatment with sucrose and maltodextrin+sucrose, compared to the treatment with maltodextrin. It can be compared to the results found in a previous study with higher %SML values by the treatment with infant formulas with the addition of sucrose in comparison with the formulas without the addition of sucrose [De Mazer Papa et al., 2010]. Other study reported similar results where the highest value of %SML was observed in the association of starch with sucrose, suggesting that a small amount of starch added to food which contains sucrose results in an increase of the cariogenic potential of this food [Ribeiro et al., 2005]. The higher %SML in the enamel occurs in the presence of fermentable carbohydrates because when metabolized by the microorganisms in the oral cavity, they synthesize soluble and insoluble EPS, increasing the viscosity of the biofilm and thus favoring the colonization and adherence of microorganisms. With growing colonization increases the production of acid, which reduces the pH and changes the concentration of Ca, Pi and F in the biofilm. With the decline of the concentration of these ions, there is a mineral loss

and a demineralization of the tooth surface [Dibdin and Shellis, 1988; Cury, Rebello, Del Bel Cury, 1997; Bowen, 2002].

In the present study it was not observed cariogenicity of the maltodextrin isolated on enamel, however, *in vivo* studies demonstrate that the maltodextrin has a potential acidogenic that can cause pH to drop in the biofilm formed on the enamel [Meyerowitz, Syrrakou, Raubertas, 1996; Moynihan, et al., 1996; Al-Khatib, Duggal, Toumba, 2001; Bhat and Dubey, 2003; Dashper et al., Raju et al., 2012].

Although previous studies [Ribeiro et al., 2005; Duarte et al., 2008] show that the addition of starch to sucrose increases its cariogenic potential, however this study has not obtained the same results with the combination of maltodextrin+sucrose. The combination of these carbohydrates showed the same potential cariogenic of sucrose isolated. A possible reason for this difference between the results of these studies would be difference in the molecular structure of carbohydrates used that reflect the degree of viscosity of the solution. The physico-chemical properties of maltodextrins as their sweetness and viscosity varies according to the extent of starch hydrolysis, determined by the DE value, which increases over time. The viscosity decreases with time at which the starch is subjected to hydrolysis [Moore et al., 2005; Soto et al., 2012]. With the increase in DE, is the increase in its water solubility, fluidity, osmolarity, sweetness and digestibility in infant formulas. But, the decrease of the DE and the consequent increase in molecular size, result in starch maltodextrins characteristics due to its viscosity-increasing [Chronakis, 1998].

The results may be relevant to the dentin, since it has a critical pH higher than enamel. However, they were not found studies that assessed the cariogenic potential of maltodextrin in dentin. *In situ* studies evaluating starch and its association with sucrose showed a cariogenic potential in dentin [Lingstrom et al., 1994; Aires et al.,2008]. Therefore, as the maltodextrin is a hydrolisate starch, it may present a cariogenic potential higher than the starch, which is a polysaccharide with a larger size and higher molecular weight. The DE is also a measure which characterizes the extent of hydrolysis of the starch and also indicates an average molecular weight. With increasing the degree of hydrolysis, the average molecular weight decreases and increases DE [Alexander, 1992]. Since maltodextrin, low DE and presents smaller molecules, than starch, it could be more easily metabolized by the acidogenic microorganisms of the biofilm [Bhat and Dubey, 2003].

Conclusion

Based on the results of this study, it is concluded that the maltodextrin has no cariogenic potential on the enamel, and the addition of maltodextrin do not increase the cariogenic potential of sucrose.

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Table 1. Median and percentiles and median values (25 and 75) for percentages of mutans streptococci, lactobacilli in relation to total number of microorganisms.

Variables	DDW	Maltodextrin	Sucrose	Maltodextrin+Sucrose
		Treatments		
%MS/TM	0.00001	0.0048	0.0235	0.0237
	(0.00-0.0025)	(0.00001-0.0555)	(0.000-0.0880)	(0.00-0.0693)
	(n=19)[A]	(n=18) [A]	(n=19) [A]	(n=18) [A]
%LB/TM	0.0006	0.1460	0.0750	0.1740
	(0.00-0.0228)	(0.0002-0.5550)	(0.0029-0.2280)	(0.0469-0.6879)
	(n=19) [A]	(n=18) [A]	(n=19) [A]	(n=18) [A]

*MS= mutans streptococci; TM= total microorganisms; LB= lactobacilli

**Distinct capital letters show statistically significant differences, Friedman test ($p < 0.05$)

Table 2. Biochemical composition of the dental biofilm according to the treatments.

Variables	*Treatments (Mean+SD)*			
	DDW	**Maltodextrin**	**Sucrose**	**Maltodextrin+Sucrose**
F (µg/mg)	95.92 ± 154.10 (n=13)^A	52.74 ± 96.73 (n=17)^B	26.16 ± 17.89 (n=18)^B	22.75 ± 18.83 (n=15) ^{B}
Ca (µg/mg)	1.31 ± 2.06 (n=13)^A	0.48 ± 0.78 (n=17)^A	0.22 ± 0.19 (n=18) ^{B}	0.26 ± 0.26 (n=16)^A
Pi (µg/mg)	8.44 ± 7.14 (n=13)^A	3.85 ± 3.64 (n=17)^A	3.55 ± 1.63 (n=18)^A	2.96 ± 1.76 (n=16)^B
IEPS (µg/mg)	53.24 ± 28.88 (n=14)^A	65.04 ± 24.95 (n=16)^{AB}	115.23 ± 93.47 (n=19) ^{BC}	149.43 ± 89.84 (n=17)^C
%SML	18.77 ± 10.86 (n=19)^A	31.86 ± 20.44 (n=18)^A	52.87 ± 24.98 (n=19)^B	55.27 ± 25.62 (n=18)^B
Biomass (mg dry weight)	1.18 ± 1.08 (n=19)^A	1.45 ± 1.04 (n=18)^A	2.38 ± 2.63 (n=19)^B	2.02 ± 1.23 (n=18)^A

*F=Fluoride; Ca=Calcium; Pi= Inorganic phosphate; IEPS= Insoluble extracellular polysaccharide; %SML= Percentage of surface microhardness loss

***Distinct capital letters show statistically significant differences; transformed data for $\log_{10}(x)$ (Ca, F and biomass); folllowed by Generalized Estimating Equations test and Bonferroni test (p<0.05)

4. **ARTIGO 3**

Structural organization of dental biofilm formed *in situ* in the presence of maltodextrin or under this association with sucrose

Abstract

Maltodextrin is a corn starch hydrolysate used in various foods, such as infant formulas, dietary supplements and sports drinks. Many marketed products containing maltodextrin are associated with the sucrose. Among the carbohydrates in the diet, sucrose is considered the most cariogenic one since it is, used as a substrate for the synthesis of extracellular polysaccharides (EPS) by the biofilm. It is not clear in the literature the maltodextrin cariogenicity and its association with sucrose in foods. This study aimed to evaluate the organizational structure of the biofilm formed *in situ* in the presence of maltodextrin and its association with sucrose, by confocal laser scanning microscopy (CLSM). This *in situ* study was also randomized, split-mouth and doubleblind, where 6 volunteers wore intraoral appliances containing bovine enamel blocks during two periods of 14 days. Volunteers were instructed to drop 8 times per day the following solutions on the enamel blocks: maltodextrin and maltodextrin+sucrose, or sucrose and deionized distilled water (DDW). Biofilms were stained with Syto-9 and Calcofluor in order to analyze live cells and EPS, respectively. From the biofilm formed on the blocks, were obtained images and then were analyzed the percentage of EPS and biofilm thickness. The treatment with maltodextrin showed similar results with DDW ($p>0.05$). The treatment with maltodextrin+sucrose and with sucrose showed a higher percentage of EPS and thickness, compared with treatment with maltodextrin ($p<0.05$). From the images obtained by CLSM, it is concluded that the maltodextrin used alone has similar results to DDW. Maltodextrin do not cause structural changes in the biofilm organization. However, when the maltodextrin is combined with sucrose, there is an increase of the biofilm cariogenic potential with changes in its structural organization.

Key-Words

Maltodextrin · Dental Biofilm · Dental Caries · Confocal Laser Scanning Microscopy

Introduction

The maltodextrin, also known as a glucose polymer, is obtained by acid hydrolysis and/or enzymatic hydrolysis of corn starch and subsequent drying. It consists of glucose, maltose, maltotriose and glucose polymers, and its final concentration varies depending on the method and degree of hydrolysis, chemically defined as "dextrose equivalent" (DE) of this oligosaccharide. The higher the value of DE, greater the amount of reducing sugars present, and the product will be more readily metabolised by oral bacteria [Kearsley and Sicard, 1989; Moynihan et al, 1996; Moynihan et al., 1998].

Maltodextrin is used as a thickening agent and anti-caking in baby food, sauces and soups, improving storage properties and texture of dry products. This oligosaccharide is not used as a sweetener, but to increase the caloric content of infant formulas, dietary supplements and sports drinks [Moynihan, et al., 1996; Levine, 1998; Grenby & Mistry, 2000].

Many commercialized products containing maltodextrin are associated with sucrose. Among the carbohydrates in the diet, the sucrose is considered the most cariogenic and this feature is in part attributable to the fact that only carbohydrat to be used for the synthesis of extracellular polysaccharides (EPS) in biofilm [Bowen, 2002; Paes Leme, et al., 2006]. *In vivo* studies have reported that cariogenic potential of starch consumption, associated with sucrose, is greater than the cariogenic potential of these carbohydrates consumed alone [Bowen et al., 1980; Firestone, Shmid, Muhlemann, 1982; Thurnheer et al., 2008].

It is unclear, if the maltodextrin in combination with sucrose has a particular effect on the cariogenic potential of these foods. There is in the literature a variety of studies evaluating the acidogenicity maltodextrin isolated in biofilm, showing that the maltodextrin has a acidogenic potential, but lower than that sucrose, despite, showing significant decreases in pH [Meyerowitz, Syrrakou, Raubertas, 1996; Moynihan, et al., 1996; Al-Khatib, Duggal, Toumba, 2001; Raju et al., 2012]. It was found only one study evaluating the combination of maltodextrin with sucrose, from infant formulas containing different percentages of maltodextrin, associated or not with sucrose, compared to the cariogenic potential and their effects on biofilm formed *in situ*. The authors concluded that the formulas have a deciduous enamel demineralization potential when associated with sucrose [De Mazer Papa et al., 2010].

Traditionally, electron microscopy is used to study the composition and structure of the biofilm, due to its high resolution [Listgarten, 1976; Theilade et al., 1976]. However, the dehydration, fixing and incorporation of the samples is necessary, which can cause a distortion in the structure and may change the relationship of one component relative to another. Another microscopy technique that can be used to study biofilm is a confocal laser scanning microscopy (CLSM), with which makes possible to study the three dimensional structure of the biofilm. With this technique biofilms can be viewed in their natural state without the need for dehydration, fixing or incorporation. Furthermore, it is possible through its optical sectioning properties make several thin sections [Shotton, 1989; Costerton et al., 1995; Wood et al., 2000]. Biofilm architecture is an important factor in the biological modulation and microbial physiology. The behavior of microorganisms will depend on the thickness and density of the formed biofilm in addition to the proportion of cells and EPS [Wood et al., 2000].

The present study aimed to evaluate the organizational structure of the biofilm formed *in situ* in the presence of maltodextrin and maltodextrin associated with sucrose in order to be better understand the cariogenic potential of maltodextrin or the association between maltodextrin+sucrose

Material and Methods

Experimental Design

This study was *in situ*, randomized, doubleblind and split-mouth. The experimental phase was 14 days, with six volunteers, aged between 21 and 27 years. It was approved by the Ethics and Research Committee of the Dental School of the Federal University of Rio Grande do Sul (Protocol 666.924). Informed consent was obtained from each participant prior to the beginning of the study and only volunteers with oral health conditions and favorable systemic and availability to attend to the research site on request were included. In addition, the volunteers could not be smokers, could not present xerostomy, should not use any type of orthodontic appliance or have used any antibiotics for at least two months prior to baseline.

Enamel Blocks and Palatal Appliance Preparation

Enamel blocks were obtained from bovine incisors. All teeth which presented fractures on the external enamel surface, as well as cracks and hypocalcification

were excluded from the samples. Before the preparation of blocks, the teeth were subjected to a removal of tissue debris and stored on a formaldehyde solution pH 7.0 and 2% with phosphate buffer during 30 days for sterilization [Cury, Rebello, Del Bel Cury, 1997]. The blocks were prepared with dimensions of 3x3x2mm [Cury, Rebello, Del Bel Cury, 1997] and they were randomly divided into four different groups according to each treatment.

For each volunteer it was made one intraoral palatal appliance from acrylic resin containing two blocks, one block on the right side and one on the left [Cury et al., 2003]. The cavities for the blocks were created in the appliance leaving 1.0mm for biofilm formation and covered with a plastic screen to protect from the mechanical friction [Hara et al., 2003]. Each side was identified with a color according to the solution that would be dropped (a red side and a white side) [Cury et al., 2001; Paes Leme et al., 2004; Pecharki et al., 2005; Ribeiro et al., 2005].

Treatments

The solutions were prepared and distributed to the volunteers, in milky white bottles, with only white or red color identification, in the following treatments: (1) deionized distilled water (DDW); (2) 10% sucrose solution; (3) 10% maltodextrin solution; (4) 10% maltodextrin solution and 10% sucrose solution. In the experimental period, two treatments were evaluated in each volunteer, considering that one enamel blocks were built in each side of the appliance and received one different treatment: DDW (control) and sucrose solution or maltodextrin+sucrose solution and maltodextrin solution. The use of the two treatments on the same oral appliance (split-mouth) is supported by previous studies for the absence of cross effect [Cury et al., 2001; Hara et al., 2003; Paes Leme et al., 2004, Pecharki et al., 2005; Ribeiro et al., 2005].

The solutions were prepared and changed every three days during the experimental period. During the 14 days, 8 times a day, at predetermined times (8:00, 9:30, 11:00, 13:30, 15:00, 16:30, 18:00 and 21:00) the volunteers were instructed to remove the appliance from the mouth, drip one drop of each solution of each enamel block in accordance with the treatment protocol, dry the excess with gauze and wait 5 minutes before putting the appliance in the mouth. The volunteers were instructed to use the appliance at all times, removing it only for the intake of food and drinks and to realize oral hygiene. No restriction were made in diet and for

the entire period the volunteers used fluoridated water (0.8 ppmF) and non-fluoridated toothpaste, provided by the researchers. A seven-day washout period was performed previously to the beginning of the experimental phase. Upon completion, each volunteer appeared in the biochemistry and oral microbiology laboratory and the blocks were collected for analysis of the biofilm.

Confocal Laser Scanning Microscopy and Images Analysis

One enamel block of each treatment was aseptically transferred to 24-well plates. The solution biofilm was fixed with 4% paraformaldehyde for 60 minutes. Then, the biofilms were immersed in the lysozyme solution for 2 minutes to allow the permeabilization of Gram-positive bacteria. Further, biofilms were stained by immersion in 0.3% Syto-9 (Invitrogen, Carlsbad, CA, USA) for 30 minutes at room temperature. The carbohydrates of the extracellular matrix were stained by immersion of biofilms Calcofluor (Sigma-Aldrich, Poole, Dorset, UK) for 60 minutes [Thurnheer et al., 2003]. Then biofilms were immersed in Mowiol (Sigma-Aldrich, Poole, Dorset, UK) and maintained on the surface of strip (LabTek, Paludi, Pieve D'alpago, Italy).

Biofilms were analyzed using an inverted confocal microscope FV-1000-PME (Olympus, Shinjuku, Tokyo, Japan), with UV laser (Coherent Inc., Santa Clara, California, USA), He-Ne (Uniphase Vertriebs GmbH, Eching, Munich, Germany) and Air (Coherent Inc., Santa Clara, California, USA) and a confocal laser operated by computer. The filters were set to 488nm for detection of Syto-9 and the detection Calcofluor, the filters were set to 430nm. Confocal images were obtained using objective 40x (an opening 1.25) and 100x (an opening 1.4) of 3 distinct regions of each biofilm [Thurnheer et al., 2003]. Vertical sections of approximately 0.44 mm thick were obtained for each field. The images were exported to Image J software (National Institutes of Health, Montgomery, MD) in which was performed the calculation of the area occupied by the green and blue dyes (Syto-9 and Calcofluor, respectively) in each sample. Then an average was calculated from the areas occupied by Syto-9 (representing the biofilm bacteria) and areas occupied by Calcofluor (which is the extracellular matrix of the biofilm - percentage of EPS) to each sample. At the end of each enamel block analysis, each treatment resulted in three pictures of the biofilm. It also determined the thickness of the biofilm in each image and an average was calculated of three cuts performed.

Statistical Analysis

The data obtained after analysis of CLSM were tabulated and submitted to the SPSS (Statistical Package for Social Sciences - IBM, Armonk, NY, USA) version 21.0 for Windows. The one-way ANOVA followed by LSD test was used for comparisons between different groups ($p < 0.05$).

Results

Three images of each enamel block in each treatment were obtained by CLSM. The %EPS and the thickness of the biofilm formed in each image was analyzed (Table 1). After the observation, representative images of each treatment were selected. They are shown in Figure 1 in transverse cross-sections and Figure 2 in vertical cross-sections. The green area represents cells stained from Syto-9, and blue area is the carbohydrate (EPS) present in the extracellular matrix, stained with Calcofluor.

The percentage of EPS analysis showed lower values in the treatments with DDW and maltodextrin compared to the treatments of sucrose and maltodextrin+sucrose ($p < 0.05$), images 1a and 1b, where there is a predominance of cells compared to the EPS, represented by the area stained blue. The analysis of the biofilm thickness showed the highest values with treatments with sucrose and maltodextrin+sucrose ($p < 0.05$), images 2c and 2d, with a greater thickness of the biofilm formed from these treatments. Treatment with maltodextrin showed no difference ($p > 0.05$) when compared to DDW in both variables.

Discussion

The food industry is increasingly using maltodextrin in their products, and often it is associated with sucrose, however there are few studies evaluating its role in the development of dental caries. Therefore, the aim of this study was to evaluate the organizational structure of the biofilm formed *in situ* in the presence of maltodextrin and maltodextrin associated with sucrose by CLSM.

The biofilms formed in this study exhibited considerable heterogeneity in the distribution of cells, extracellular matrix. These results were also reported by previous studies, in which samples of biofilms containing channels extending throughout the thickness of the biofilm, providing direct communication between the oral

environment and the tooth surface [Wood et al., 2000]. Extracellular matrix is the largest part of the biofilm and is comprised of water and aqueous products; the dry part corresponds to a mixture of EPS, protein, salts and cellular material [Socransky, Haffajee, 2002]. Soluble and insoluble EPS are produced by microorganisms of the oral cavity from sucrose; the only carbohydrate that can be used for this synthesis. Their presence promotes colonization and adherence of this microorganisms [Rolla, Scheie, Ciardi, 1985; Dibdin and Shellis, 1988; Bowen, 2002].

The analysis of the %EPS in biofilm showed a higher %EPS, with treatments with sucrose and maltodextrin+sucrose ($p<0.05$) compared to treatment with maltodextrin, which showed a lower %EPS in relation to cells. These results demonstrate greater production of the EPS by microorganisms in the treatments with sucrose agreeing with those found in a previous study where the authors also observed an increased production of EPS in the treatment with sucrose compared to other groups [Ribeiro et al., 2005; Aires et al., 2006; de Mazer Papa et al., 2010].

Biofilms formed in the treatments with sucrose and maltodextrin+sucrose showed thicker ($p<0.05$) than the biofilm formed with maltodextrin alone or DDW. This finding agrees with the analysis of EPS percentage in biofilms formed on different treatments. We suggest that with increased production of EPS, consequently there is an increase in the thickness of the biofilm, as shown *in vitro* study, where formed biofilms from sucrose were denser and thicker than the biofilms formed in the absence of this carbohydrate [Singleton et al., 1997]. The increase in thickness, caused by the increased production of EPS, is also a consequence of increasing the porosity of the biofilm. A more porous biofilm allows greater diffusion of substrates across the biofilm, promoting increased metabolism and acid production by the microorganisms present [Zero et al., 1992; Dibdin and Shellis, 1988].

Conclusion

Maltodextrin alone do not cause structural changes in the biofilm organization. However, when the maltodextrin is combined with sucrose, there are changes on structural organization of biofilm which could be related to an increased cariogenic potential.

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Table 1. Mean and standard deviation of the percentage values of extracellular polysaccharides and the thickness of the biofilm on the different treatments.

| Variables	Treatments Mean+SD			
	DDW	Maltodextrin	Sucrose	Maltodextrin+Sucrose
%EPS	25.83 ± 4.17^A	23.06 ± 3.61^A	45.63 ± 12.98^B	41.85 ± 10.13^B
	(n=12)	(n=3)	(n=12)	(n=3)
Thickness	56.92 ± 18.60^A	42.5 ± 2.92^A	84.7 ± 15.40^B	106.8 ± 14.40^B
(µm)	(n=9)	(n=3)	(n=9)	(n=3)

*EPS= extracellular polysaccharide

**Distinct capital letters show statistically significant differences, ANOVA followed by LSD test ($p<0.05$)

Figure 1. Representative images of each treatment of CLSM transverse cross-sections view. The green area represents cells stained from Syto-9, and blue area is the carbohydrate (extracellular polysaccharides) present in the extracellular matrix, stained with Calcofluor. A-Treatment with DDW; B-Treatment with maltodextrin; C-Treatment with sucrose; D-Treatment with maltodextrin+sucrose. Images were obtained by using an X40 objective.
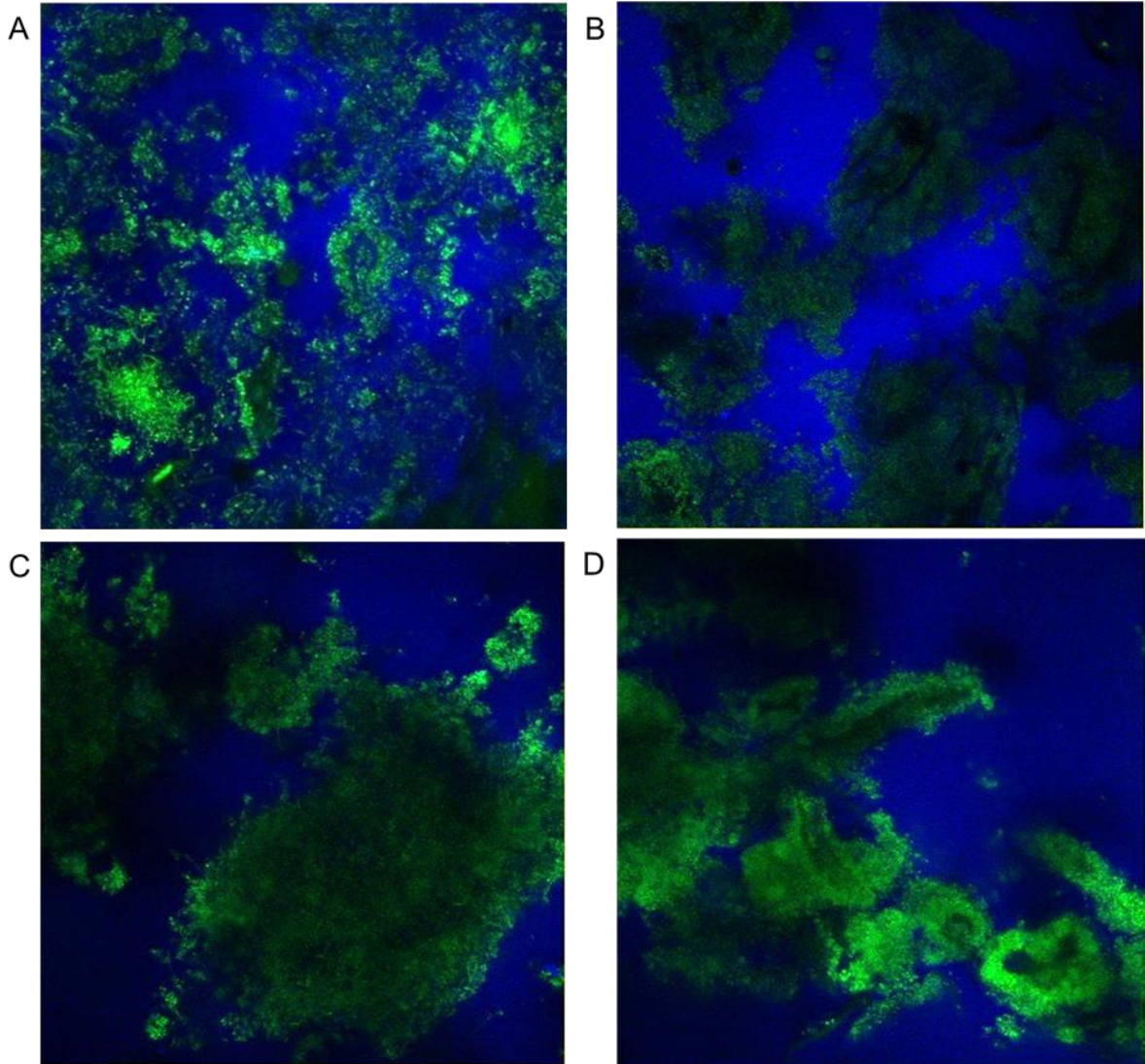
Figure 2. Representative images of each treatment of CLSM vertical cross-sections view. The green area represents cells stained from Syto-9, and blue area is the carbohydrate (extracellular polysaccharides) present in the extracellular matrix, stained with Calcofluor. A-Treatment with DDW; B-Treatment with maltodextrin; C-Treatment with sucrose; D-Treatment with maltodextrin+sucrose. Images were obtained by using an X40 objective.
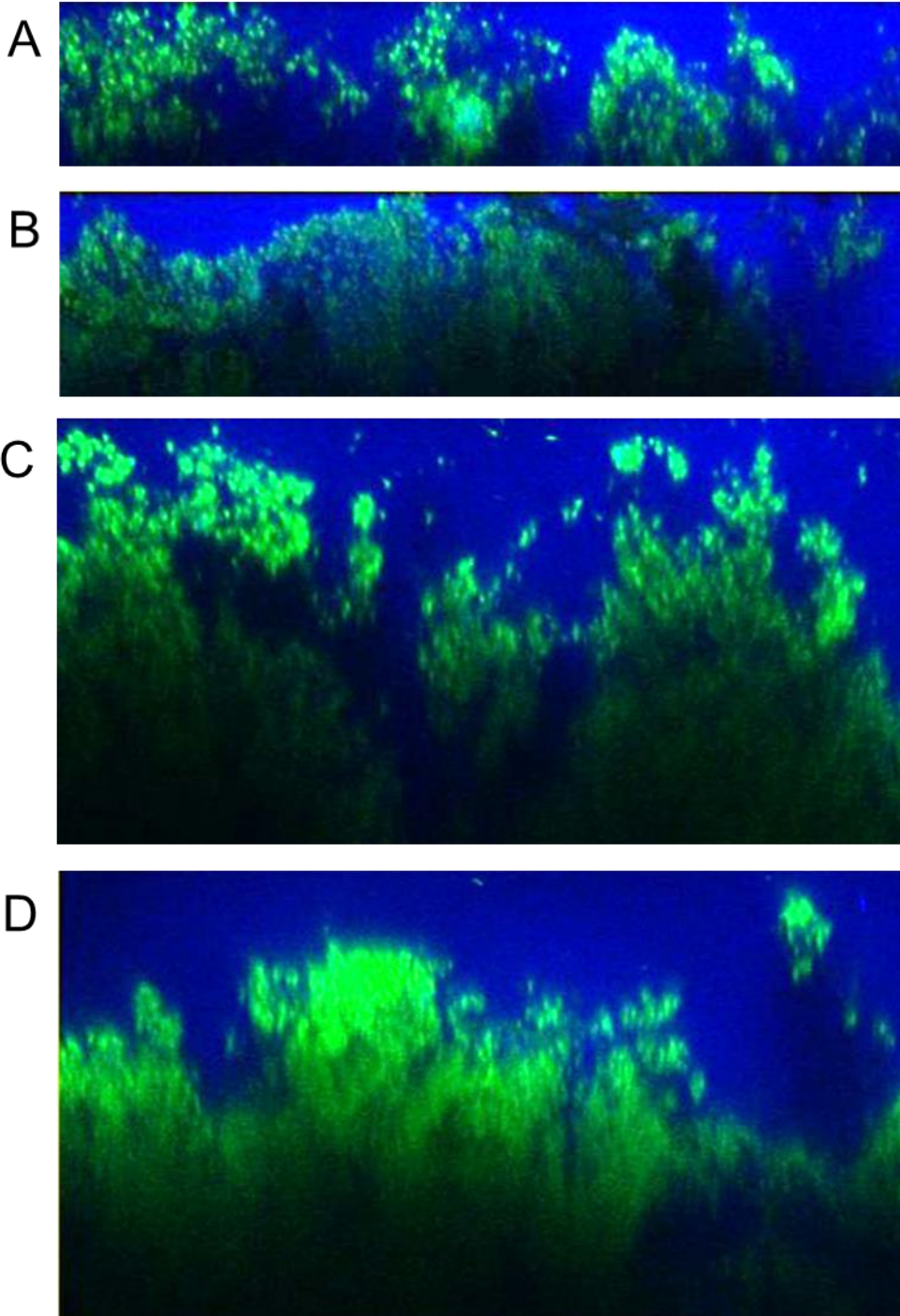
5. **CONSIDERAÇÕES FINAIS**

- A partir da revisão de literatura realizada, verificou-se uma escassez de estudos que avaliassem o potencial cariogênico da maltodextrina em esmalte dentário, assim como a sua associação com a sacarose.
- É possível concluir, a partir dos resultados dos estudos realizados nesta dissertação de mestrado, que a maltodextrina isolada não apresenta potencial cariogênico em esmalte dentário. Entretanto a associação da maltodextrina à sacarose causa alterações na composição bioquímica e na estrutura organizacional do biofilme formado, aumentando o seu potencial cariogênico.
- Portanto recomenda-se cautela no consumo frequente de produtos alimentícios contendo esta associação (maltodextrina+sacarose) por serem potencialmente cariogênicos em esmalte dentário.
- Sugere-se que mais estudos sejam conduzidos para avaliar também o potencial cariogênico da maltodextrina e associações com outros carboidratos em dentina.

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TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Caro participante,

Estamos realizando um estudo para avaliar o potencial cariogênico de dois açúcares (maltodextrina e sacarose) sobre a placa dental, de título: "Avaliação do potencial cariogênico da maltodextrina e da sua associação com a sacarose: estudo in situ". O estudo está sendo realizado pelos professores Drª. Lina Naomi Hashizume e Dr. Rodrigo Alex Arthur e a aluna Gabriela Rezende da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul.

Você deverá utilizar um aparelho removível, semelhante a um aparelho ortodôntico, durante todo o dia, por 28 dias, dividido em duas fases de 14 dias. Este aparelho contém blocos de esmalte dental que foram cortados a partir de dente de boi, limpo e desinfetado em laboratório. Este aparelho deverá ser retirado somente para higiene e alimentação. Você deverá gotejar uma solução com cada um esses açúcares sobre os blocos de esmalte 8 vezes ao dia. A placa dental formada sobre os blocos, durante cada período será analisada, para isso, serão necessárias 5 (cinco) consultas, uma para remoção de placa profissional de toda a boca e quatro para distribuir as soluções testadas. Ao final de cada período de 14 dias, você deverá comparecer ao Laboratório de Bioquímica e Microbiologia Bucal (LABIM) para que seja realizada a coleta da placa dental formada sobre os blocos do aparelho. Nós usaremos a placa dental formada para análise laboratorial e também usaremos os blocos de esmalte para determinarmos a quantidade de cárie dental desenvolvida durante o período de uso do aparelho.

Os possíveis desconfortos associados à sua participação neste estudo somente são aqueles decorrentes da utilização do aparelho (leve mau hálito e sensibilidade na língua, nos primeiros dias de uso do aparelho). Além disso, há o desconforto associado ao seu deslocamento até o LABIM para coleta do aparelho e da placa dental. Adicionalmente toda e qualquer ocorrência durante o experimento estará sendo avaliada. O benefício associado à sua participação nessa pesquisa será um auxílio indireto, contribuindo para a realização desse projeto e para a ciência como um todo.

Será assegurada a liberdade de recusar-se a participar ou retirar-se da pesquisa a qualquer momento. Assim como o direito ao sigilo de todas as informações coletadas, não sendo permitido acesso por outra pessoa que não o próprio participante ou pesquisadores envolvidos com o projeto de pesquisa. Todas as informações coletadas e a identidade dos indivíduos ficarão sob o poder restrito dos pesquisadores. Os pesquisadores se comprometem em manter a confidencialidade dos dados de identificação pessoal dos participantes e os resultados serão divulgados de maneira agrupada, sem a identificação dos indivíduos que participaram do estudo.

Toda e qualquer dúvida no decorrer do estudo poderá ser esclarecida pelos envolvidos nesta pesquisa. O pesquisador responsável por este projeto é a professora Drª. Lina Naomi Hashizume. Contato pode ser feito pelo telefone (51) 3308-5193, e no endereço do Departamento de Odontologia Preventiva e Social da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2492, Porto Alegre. Possíveis problemas, também, podem ser reportados diretamente ao Comitê de Ética em Pesquisa da UFRGS 3308.3738 de segunda a sexta das 8 às 17 horas.

Eu, _____, declaro que fui informado dos objetivos e procedimentos que serão realizados nesta pesquisa, bem como sei dos meus direitos e dos deveres dos pesquisadores. Declaro, ainda, que recebi uma cópia deste Termo.

Porto Alegre, _____ de _____ de 2014.
Assinatura do participante: _____
Pesquisador Responsável: Dra. Lina Naomi Hashizume
Assinatura do pesquisador responsável: _____