UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
– NÍVEL MESTRADO –
ÁREA DE CONCENTRAÇÃO CLÍNICA ODONTOLÓGICA
– ÊNFASE EM ODONTOPEDIATRIA –

AVALIAÇÃO *IN SITU* DA COMPOSIÇÃO DO BIOFILME E DA PROGRESSÃO DE LESÕES CARIOSAS EM ESMALTE DE DENTES DECÍDUOS E PERMANENTES HUMANOS, NA PRESENÇA E NA AUSÊNCIA DE DENTIFRÍCIO FLUORETADO.

Gisele Pedroso Moi

Porto Alegre
Dezembro, 2005.

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Linha de Pesquisa
Diagnóstico de Afecções Buco-Faciais

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Orientador: Prof. Dr. Fernando Borba de Araujo

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, Nível Mestrado, da Universidade Federal do Rio Grande do Sul como pré-requisito final para a obtenção do título de mestre em Clínica Odontológica, ênfase em Odontopediatria.

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Dedico este trabalho...

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"A odontologia é uma profissão que exige dos que a ela se dedicam o conhecimento científico de um médico, a destreza manual de um cirurgião, o senso estético de um artista e a paciência de um monge."
(Pio XII)

SUMÁRIO

RESUMO

Evidências na literatura sugerem que a velocidade de progressão de cárie pode ser influenciada por diversos fatores, entre eles as peculiaridades químicas, morfológicas e fisiológicas pertinentes aos dentes decíduos e permanentes. Estas informações são fundamentais para a correta abordagem clínica do paciente odontopediátrico, principalmente quando esta não for invasiva. Este estudo *in situ* avaliou a progressão de lesões cariosas em esmalte de dentes decíduos e permanentes, em um mesmo desenho experimental, na presença e na ausência de dentifrício fluoretado (1100 ppm NaF). Onze voluntários, em duas fases distintas, utilizaram um dispositivo palatino de acrílico contendo blocos de esmalte decíduos e permanentes. Estes blocos foram tratados com sacarose 20%, 8x/dia, por 7, 14 e 21 dias. O biofilme formado sobre os blocos dentários foi coletado para análise bioquímica e as perdas minerais dos mesmos foram acessadas através da inspeção visual (IV), microdureza transversa (ΔZ) e microscopia de luz polarizada (MLP). Os resultados foram submetidos ao teste de Tukey ($p=0,05$) e mostraram que o biofilme formado na presença de dentifrício fluoretado apresentou maiores ($p<0,05$) concentrações de Ca, P_i, F que o tratado com dentifrício placebo. A concentração de PI foi significativamente maior no biofilme tratado com dentifrício placebo. Os fatores substrato, dentifrício e tempo influenciaram de forma significativa nas perdas minerais mensuradas através da IV, ΔZ e MLP. Em todos os períodos e fases estudadas, a velocidade de progressão de cárie no esmalte do dente decíduo foi maior que no permanente ($p<0,05$).

ABSTRAC

The literature evidences suggest that carious progression rate can be influenced by various factors, among these: the chemical, morphologic and physiologic peculiar characteristics of the deciduous and permanent teeth. This information is fundamental to a correct pediatric patient approach, mainly when this approach is not invasive.

This *in situ* study evaluated the of carious lesions progression in the deciduous and permanent tooth enamel, in the same experimental design, with and without fluoride dentifrice (1100 ppm NaF), in two distinct phases, with or without fluoride dentifrice. Eleven volunteers wore a palatal appliance containing deciduous and permanent enamel blocks. These blocks were treated with sucrose 20% 8x/day for 7, 14 and 21 days. Then, the biofilm which was formed on the deciduous and permanent enamel surfaces were collected for analysis and the enamel mineral losses were assessed in the blocks through visual inspection (VI), cross-sectional microhardness (CSMH) and by polarized light microscopy (PLM). The outcomes were analyzed by Tukey test ($p<0.05$). The outcome's researches showed that the dental biofilm in the presence fluoride dentifrice had bigger Ca, P_i, and F concentration than the dental biofilm treated by placebo dentifrice. The IP concentration in the biofilm treated by placebo dentifrice was significantly greater than biofilm treated by fluoride dentifrice. The substrate, dentifrice and time factors fluoride dentifrice influenced the mineral losses measures (VI, ΔZ, PLM). The carious progression rates in deciduous enamel were faster than in permanent enamel in all periods and phases ($p< 0.05$).

1. INTRODUÇÃO GERAL

A cárie dental é uma doença infecciosa crônica multifatorial biofilme-dependente (Marsh, 1995; Thylstrup e Fejerskov, 2001). Entretanto, a atividade do biofilme é modulada por muitos fatores, como dieta, saliva, flúor, tempo, higiene oral e suscetibilidade da superfície dental (Marsh, 1995; Cury, et al, 1997; Kidd e Fejerskov, 2004). A interação entre estes fatores e o biofilme dental determina a incidência e prevalência da doença cárie (Marsh, 1995; Thylstrup e Fejerskov, 2001). Assim, o incremento das lesões cariosas e a sua progressão refletem a atividade do processo carioso (Pitts, 1983).

O surgimento de lesões cariosas é determinado pela velocidade de progressão das perdas minerais decorrentes do processo de desmineralização (Araujo et al., 2005). Seus sinais podem variar desde de perdas minerais ao nível ultraestrutural até a destruição completa do elemento dental (Thylstrup e Fejerskov, 2001).

A incidência de lesões cariosas tem sido avaliada por alguns estudos (Berman et al., 1973; Pitts, 1983). No entanto, há poucos relatos na literatura a respeito de sua progressão (Pitts, 1983). A maior parte dos estudos que determinam a velocidade de progressão de lesões cariosas são realizados em dentes permanentes (Grondahl e Hollender, 1979; Pitts, 1983). Estes mostram que sua progressão tem se apresentado como um processo lento (Silverstone, Hicks e Featherstone, 1988), sendo influenciada pela atividade cariosa do indivíduo (Ismail, 2004).

Estes achados podem ter uma relevância particular para a odontopediatria. Considerando que os dentes decíduos possuem um ciclo biológico limitado na cavidade bucal, eles podem esfoliar muito antes que a lesão cariosa evolua, acometendo a parte interna do tecido dentinário (Murray e Majid, 1978; Craig, Powell e Cooper, 1981).

Embora existam informações escassas sobre a velocidade de progressão de lesões cariosas na dentição decídua, a literatura sugere que a sua velocidade de progressão é maior do que nos dentes permanentes (Pitts, 1983).

Além de ser influenciada pela atividade cariosa do indivíduo, a velocidade de progressão de cárie é influenciada por outros fatores (Sonju-Clasen e Ruyter, 1997). Assim, variações na composição química, na morfologia e nas características fisiológicas entre dentes decíduos e permanentes (Mortimer, 1970; Sonju-Clasen e Ruyter, 1997) podem influenciar na velocidade de progressão de lesões cariosas (Pitts,1983; Schwartz et al., 1984; Peyron et al., 1992).

O esmalte dos dentes decíduos, além de apresentar menos prismas na superfície externa, possui espaços intercristalinos maiores que os permanentes (Ripa et al., 1966), apresentando assim, maior porosidade que o esmalte permanente (Shellis, 1984; Sonju-Clasen et al., 1997). Os dentes decíduos possuem um menor grau de mineralização, baixo conteúdo de fósforo, e um alto conteúdo de carbono (Sonju-Clasen et al., 1997). Além disso, o esmalte decíduo contém um maior conteúdo de carbonato que o do seu sucessor, (Sonju-Clasen et al., 1997; Sonju-Clasen e Ruyter, 1997) possuindo a metade da espessura do permanente, sendo seus prismas aproximadamente 2 μm menores do que os do esmalte do dente sucessor (Mortimer, 1970). Quando comparado ao permanente, o esmalte do dente decíduo apresenta menor conteúdo de flúor em todas as profundidades (Mellberg et al., 1970).

Por isso, muitos estudos sugerem que a velocidade de progressão de cárie no esmalte dos dentes decíduos é aproximadamente 1,5 vezes mais rápida do que no permanente (Pitts, 1983).

Embora as evidências científicas demonstrem que a cárie dentária é uma doença biofilme-dependente, ela pode ser controlada a partir da abordagem dos seus fatores etiológicos. O fluoreto presente no meio bucal é capaz de reduzir a desmineralização do esmalte provocada pelos ácidos produzidos pelo biofilme dental cariogênico (Tenuta et al., 2005). Porém, existem poucos estudos comparando a velocidade de progressão da doença cárie na dentição decídua e permanente em um mesmo desenho experimental.

Num programa de atenção odontológica à criança baseada no diagnóstico oportuno da atividade da doença, as informações sobre a velocidade de progressão das lesões cariosas, em ambas as dentições, são de grande relevância clínica, pois possibilitam uma adequada abordagem das mesmas.

Assim, os objetivos deste estudo foram realizar uma revisão da literatura sobre a velocidade de progressão de lesões cariosas em ambas dentições humanas, como também avaliar *in situ* a composição do biofilme dental e a velocidade de progressão de lesões cariosas em esmalte de dentes decíduos e permanentes humanos, na presença e na ausência de dentifrício fluoretado.

2. REFERÊNCIAS GERAIS

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3. PROPOSIÇÃO

Esta dissertação de mestrado será apresentada na forma de dois capítulos, tendo como objetivos:

Capítulo 1: Discutir, com base na literatura, a velocidade de progressão de lesões cariosas em dentes decíduos e permanentes.

Capítulo 2: Avaliar, *in situ*, a composição do biofilme e a velocidade de progressão de lesões cariosas no esmalte de dentes decíduos e permanentes humanos, na presença e na ausência de dentifrício fluoretado.

4. CAPÍTULOS

CAPÍTULO 1: A contemporary approach of the caries progression rates in pediatric dentistry: an insight review. GP Moi, AF Paes Leme, CPM Tabchoury, JA Cury; FB Araujo. De acordo com as normas do periódico "*Journal of Dentistry"*.

CAPÍTULO 2: *In situ* effect of F dentifrice on caries lesion progression in deciduous and permanent enamel and biofilm composition. Moi, AF Paes Leme, CPM Tabchoury, FB Araujo; JA Cury. De acordo com as normas do periódico "*Journal of Dental Research"*.

CAPÍTULO 1*

A contemporary approach of the caries progression rates in pediatric dentistry: an insight review

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Short Title: The caries progression in pediatric dentistry: an insight review

Key Words: Dental caries, caries progression, deciduous tooth, permanent tooth

*De acordo com as normas da revista "***Journal of Dentistr"**.

SUMMARY

The caries progression rate is influenced by several factors. Evidences in the literature suggest that peculiar characteristics of deciduous and permanent teeth can influence it. The pediatric dentistry must look for additional knowledge about the health-disease process. Information about caries progression rates is important in the planning of treatment, in determining the correct clinical approach, and in the evaluation of appropriate time intervals for dental recalls. Thus, the dental practice should promote the clinical approach that overcomes the simple decision of restoring or not the affected tooth. Therefore, it is of a great scientific relevance the accomplishment of an insight study review based on the literature that clearly approaches the caries progression rate in deciduous and permanent dentition.

INTRODUCTION

Dental caries is a chronic multi-factorial infectious disease that involves interactions among diet, saliva, biofilm or dental plaque, and a susceptible tooth surface. [1, 2]

The development of the caries lesions results from an imbalance between demineralization-remineralization of enamel.[1] This imbalance, with predominance of demineralization, is due to a biofilm accumulated over teeth, without mechanical disturbance and supplemented with fermentable sugar. [3, 4 and 5]

Then, since the biofilm is always being formed and always metabolically active, it has been suggested that the caries process might be regarded as a ubiquitous and natural phenomenon.[6] On the other hand, there are several factors other than the composition and thickness of the biofilm that will influence the magnitude of the decreases of the pH.[1, 3, 5 and 7] This process cannot be prevented, but the cariogenic biofilm formation can be controlled.[1] Therefore, the caries increment and its progression reflect the carious activity process.[8]

It is important to point out that the term "caries" can be used to refer to both the caries process and the caries lesion that are formed as a result of that process.[7] Despite of the progress in understanding the caries process, the caries "cavities" are not synonymous of carious activity process.[9] The caries "cavity" is a clinical signal that a tooth lost mineral due to the caries process. But, this clinical signal, when it considered alone, is not able to show the activity of the carious process.[7] Then, the dental caries process must overtake the "cavity" cut off and should include the etiological factors that can promote the caries progression.[7, 10 and 11]

The contemporary dental practice must look for additional knowledge about the health-disease process. The modern dental practice must promote the clinical approach that overcomes the simple decision of restoring or not the affected tooth.[9, 10 and 11]

In a model of oral health promotion, considering the control of individual's carious activity, information about carious progression rates is important in the planning of treatment, in determining the correct clinical approach (invasive or not), and in the evaluation of appropriate time intervals for dental recalls.[9, 11] Such information would have additional significance taking into consideration that incidence, prevalence and the progression rates of dental caries are different in children's dentition around the world.[12, 13] Surprisingly a few number of researches have been devoted to the study of the carious lesions progression rates, or the lack of them.[8]

The majority of the studies that determine the speed of carious lesions progression are carried through in permanent teeth.[8] They show that their progression has been presented as a slow process [14], which may be followed up if lesion's carious activity is controlled.[9] Thus, an incipient caries lesion may take an average of 3-4 years to involve the dentin tissue.[8]

In this way, it is of a great scientific relevance the accomplishment of an insight study review based on the literature that clearly approaches the caries progression in deciduous and permanent dentition.

MATERIALS & METHODS

Experimental Design

This is a systematic literature review study. It was included in this review all researches that analyzed the carious progression rate, in both human dentition, in the same experimental design. They were looked for independently on the basis of key words (dental caries, caries progression, deciduous tooth, permanent tooth). But, this assumption was found only in two electronic data bases (ncbi and bireme).

Caries Progression in Pediatric Dentistry

The caries progression rate is influenced by several factors.[16] Among these are variations in the chemical composition, morphology and physiological characteristics of deciduous and permanent teeth.[16, 18] The literature indicates that these differences can influence the caries progression speed.[8, 19 and 20]

Deciduous tooth enamel often contains prismless enamel in outer surface, with larger intercrystalline spaces than permanent tooth.[21] The enamel of deciduous tooth has more porosities than the permanent one,[17] because the interprismatic fraction and the prism-junction density are significantly greater in deciduous than in permanent.[22] Deciduous tooth presents a lower degree of mineralization, lower content of phosphorus, and a higher content of carbon dioxide.[17] Besides that, it contains significantly more type A carbonate than in permanent, and the total content (sum of type A and type B carbonates) is also significantly higher in deciduous enamel than in permanent tooth.[16, 17] Deciduous enamel is half as thick as permanent enamel, and the enamel prisms in deciduous enamel are approximately 2 μm narrower than those in permanent enamel.[18] The deciduous enamel also presents lesser fluoride at all depths than permanent one.[23]

Thereby, some studies suggest that caries progression rate in deciduous enamel is approximately 1.5 times faster than in permanent one.[8] Ando et al. (2001) showed through a diagnostic study that the lesions depth is greater in deciduous enamel than in permanent, on average, when chemically demineralized for the same period.

The first step to control and to prevent some diseases is to collect and analyze information of the prevalence/incidence and etiologic factors that are involved in them.[9] By the analyses of this information, the oral health community realized that if the biofilm

activity were controlled, the carious progression rates could be controlled too. In this meantime, some researches were done in order to get to this conclusion.

RESULTS

Looking for evidences

Although there are many studies about caries development, surprisingly, there is little research devoted to study the carious progression rate in both human dentitions.

Therefore, Pitts (1983) made a review of the literature about caries progression. He examined the researches, which have monitored caries progression in posterior approximal enamel surfaces, in deciduous or permanent teeth, by reference to the size of radiolucency which was seen on bitewing radiographs. This review indicated that the majority of approximal caries had a slow progress, and large numbers of lesions remained unchanged for long periods. He compared the results of the studies through the mathematical model of negative exponential. Then, the results of the compared studies were that the mean time, which a lesion remains radiographically confined to the permanent enamel is of the order of 3-4 year and 1-2 years in deciduous enamel, although in caries active individuals a much lower time, is reported.

With different results found between caries active and caries inactive individuals, Sonju-Clasen et al. (1997) were compared initial caries development in fluoridated and non-fluoridated deciduous and permanent enamel *in situ*. These caries lesions were analyzed by means of quantitative microradiography and confocal laser scanning microscopy. The enamel slabs were mounted in removable appliances and they were worn for 4 weeks. They found significantly larger lesions developed in deciduous than in

permanent enamel, when no topical fluorides were used. Fluoride mouthrinsing partly prevented lesion development in deciduous and completely in permanent enamel.

In order to look for appropriate time intervals for recalls of dental child patients and the right moment to choose for an invasive treatment when necessary, Vanderas et al. (2003) made a 4-year prospective study. Thereby, their research investigated the survival rate and median survival time of different stages of proximal caries in mixed dentition from bitewing radiographs taken at one year interval over a period of four years. So, they concluded that the progression of the external-half of enamel lesions of the first permanent molars is lower for the first 3 years and becomes faster thereafter; and as the time period that the teeth are exposed to increased cariogenic factors, the progression of the proximal caries is faster for all stages of the lesion in deciduous teeth.

At the same time, Issa et al. (2003) investigated the incidence of artificial sub-surface caries lesion formation in the enamel of deciduous and permanent teeth *in vitro.* So, they quantitatively compared the mineral content and the distribution of these lesions and assessed the possible influence of the fluoride upon the lesion parameters. The research showed that fluoride significantly reduced the severity of sub-surface caries lesions formed *in vitro*, but there were no differences in lesion parameters between permanent and deciduous teeth.

DISCUSSION

Dental caries is a chronic multi-factorial infection disease.[1, 2] It has been considered a biofilm-dependent oral disease. However, the activity of the biofilm is modulated by many factors such as diet, saliva, fluoride, time, oral hygiene, and a susceptible tooth surface.[1, 3, 5 and 7] The interactions among these factors and dental biofilm determine the incidence and

prevalence of dental caries.[1, 2] Therefore, the caries increment and its progression reflect the carious activity process.[8]

Dental biofilm is always formed and it is always metabolically active.[6] Although, this process cannot be prevented, the cariogenic biofilm formation can be controlled.[1] Therefore, even if dental caries is considered a biofilm-dependent oral disease, the caries incidence can be prevented and the caries progression can be followed-up if the cariogenic biofilm formation is controlled. In this way new lesions could be prevented and the existing ones could be inhibited.[15]

Evidence based dentistry must promote the clinical approach, which overcomes the simple decision of restoring or not the teeth with carious lesions. The contemporary dentistry must look for additional knowledge about the health-disease process, and the modern dental practice must be based on oral health promotion model. So, information about caries progression rates is important in the planning of dental health promotion, in determining the correct clinical approach, and in the evaluation of appropriate time intervals for dental recalls.[12, 9 and 10]

There are some studies, which have showed that dental caries progression has been presented as a slow process,[14] that is able to take some years so that an incipient caries lesion progresses and involves the dentin tissue.[8] These findings can present a particular relevance for the dental child, because deciduous teeth have a limited biological cycle in the oral mouth and can exfoliate before the carious lesions have involved the inner part of the dentin.[13, 15] Although many studies have been made about caries development, there are few researches devoted to compare the carious progression rate in deciduous and in permanent teeth.

Thus, Pitts (1983) looked for the caries progression parameters in deciduous and permanent teeth, because he knew that carious progression rate is influenced by several factors. Among these are variations in the chemical composition, morphology and physiological characteristics of deciduous and permanent teeth.[16, 18] Therefore, he made a review of the works, which have monitored caries progression in posterior approximal enamel surfaces, in deciduous or permanent teeth, by referring to the size of radiolucency seen on bitewing radiographs. Then, the results of the compared studies showed that the mean time in which a lesion remains radiographically confined in the permanent enamel is of the order of 3-4 years and 1-2 years in deciduous enamel, although in caries active individuals the carious progression rate is quicker. But, these results must be carefully used in dental practice, because they were obtained by studies' outcomes with different experimental designs.

Taking into consideration faster carious progression rate in caries active individuals, Sonju-Clasen et al. (1997) studied initial caries development in fluoridated and non-fluoridated deciduous and permanent enamel *in situ*. They found significantly larger lesions developed in deciduous enamel than in permanent when no topical fluorides were used, and showed that fluoride partly prevented lesion development in deciduous enamel and completely in permanent one. Although, this study supports the hypothesis that deciduous enamel is more susceptible to caries than permanent one, its experimental design does not permit to get to this conclusion, because the same volunteer did not participate in both phases of the study. And also, the enamel slabs were obtained from teeth with different maturation degrees. The permanent enamel was taken from newly erupted premolars enamel and the deciduous enamel from mature teeth. This may have contributed to different results of the fluorinated group. Even if this study shows that carious progression rate in

deciduous enamel is faster than permanent enamel, its results may be questioned because they used an unsuitable experimental design.

Vanderas et al. (2003) suggested through the results of their study that the progression of the external-half of enamel lesions of the first permanent molars is lower for the first 3 years and it becomes faster thereafter. But if these teeth are exposed to increased cariogenic factors, the progression of the proximal caries will be faster for all stages of the lesion. In deciduous teeth, the progression rate is fast in all stages. Maybe, these results can help to find appropriate time intervals for recalls of dental child and the right moment to choose for an invasive treatment when necessary. But, the preventive measures applied in this study do not consider the presence of patient's carious activity and the efficiency of the preventive measurements used in the study were not controlled. Thereby, it is difficult to apply this study's outcomes in dental child approach.

On the other hand, Issa et al. (2003) showed that fluoride significantly reduced the severity of sub-surfaces caries lesions formed *in vitro*. It has been reported that different preventive measures, especially fluoride, have a significant effect on retardation of the lesion progression. However, the researchers did not find any differences in lesion parameters between permanent and deciduous teeth. This may have happened because of the experimental design of this study. There were differences in enamel porosities and chemical composition due to different degrees of maturation of permanent and deciduous enamel.

In contemporary pediatric dentistry practice, it is very important to set up an attention model based on opportune diagnostic of caries activity and the establishment of the appropriate treatment, because many teeth may exfoliate before restorative treatment is required.[15] In this model, the patient must be regularly followed-up. Therefore, the results

of these previous studies will be able to give support to further studies. Thus, it is suggested the need of a study with an experimental design, which reproduces the oral mouth environment.

CONCLUSION

Although the scientific evidences show that the dental caries is a biofilm-dependent oral disease, it can be controlled by an approach of etiological caries factors. There are only few studies about caries progression both in deciduous as well as in permanent teeth, using them in the same experimental design. At the same time, the information about caries progression rates is of great clinical relevance to determine the correct caries lesion approach, considering the no-invasive treatment. According to the peculiarity of each dentition there should be further studies in which the clinical situation could be reproduced.

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CAPÍTULO 2*

***In situ* effect of F dentifrice on carious lesion progression in deciduous and permanent enamel and biofilm composition**

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Short Title: Effect of F dentifrice on deciduous and permanent caries lesions

Key Words: Dental caries, carious progression, dental biofilm, deciduous tooth, permanent tooth

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ABSTRACT

The carious progression, in deciduous enamel and permanent one are not clearly established. This *in situ* study evaluated the of carious lesions progression in the deciduous and permanent tooth enamel, in the same experimental design, with and without fluoride dentifrice (1100 ppm NaF), in two distinct phases. Eleven volunteers wore a palatal appliance containing deciduous and permanent enamel blocks. These blocks were treated with sucrose 20% 8x/day for 7, 14 and 21 days. Then, the biofilm which was formed on the deciduous and permanent enamel surfaces were collected for analysis and the enamel mineral losses were assessed in the blocks through visual inspection (VI), cross-sectional microhardness (CSMH) and by polarized light microscopy (PLM). The outcomes were analyzed by Tukey test ($p<0.05$), and showed that the dental biofilm in the presence fluoride dentifrice had bigger Ca, P_i, and F concentration than the dental biofilm treated by placebo dentifrice. The IP concentration in the biofilm treated by placebo dentifrice was significantly greater than biofilm treated by fluoride dentifrice. The substrate, dentifrice and time factors fluoride dentifrice influenced the mineral losses measures (VI, ΔZ, PLM). The carious progression rates in deciduous enamel were faster than in permanent enamel in all periods and phases.

INTRODUCTION

Caries is a multi-factorial infection disease that involves interactions among diet, saliva, biofilm or dental plaque, and a susceptible tooth surface (Marsh, 1995; Baelum and Fejerskov, 2005). The development of the caries lesions results from an imbalance between demineralization-remineralization process (Marsh, 1995). Its signals can vary from the mineral losses in the ultra-structural level until the total destruction of the tooth (Baelum and Fejerskov, 2005). Although many researches, evaluating the incidence of caries lesions, have been performed, there are few studies in literature regarding its progression (Pitts, 1983), and the ones, which are carried out, are done in permanent teeth. Their outcomes show that carious progression rate has being presented as a slow process (Pitts, 1983; Silverstone et al., 1988; Mejare et al., 1999), which may take, in average, 3-4 years for an incipient lesion to progresses and reach the inner part of the permanent dentin tissue (Pitts, 1983).

Although, the literature presents little information about the carious progression rate in deciduous teeth, studies suggest that is faster than in permanent teeth (Pitts, 1983). On the other hand, there are some differences in the chemical composition, morphology and physiological characteristics between deciduous and permanent teeth (Mortimer, 1970, Sonju-Clasen et al., 1997). Indeed, there are some evidences that low fluoride environments are able to act on the demineralization process (Bradshaw et al. 2002). So, low fluoride in oral mouth environment can reduce the carious progression rate (Duggal et al., 2001), if the mineral losses are prevented (Bradshaw et al., 2002).

Thus, the objective of this study was to evaluate the caries lesions progression in the deciduous and permanent tooth enamel in the presence or absence of fluoride in an *in situ* study.

MATERIALS & METHODS

Experimental Design

This is an *in situ* longitudinal study, double-blind that involved a split-mouth design of caries induction by plaque accumulation and sucrose use.

Eleven healthy adults' volunteers participated in the two phases of the study and they were randomly assigned to the treatment: (A) PD (placebo dentifrice, without abrasive); (B) FD (dentifrice with 1100 ppm F as NaF, without abrasive). It was performed in three different periods of biofilm accumulation (7, 14 and 21 days). The volunteers signed an informed after provided informed written consent (approved by the Research and Ethics Committee of FO/UFRGS).

The appliances and the enamel blocks from deciduous molars (10.2 oral environment year's ±4.5) and premolars (9.8 oral environment years ±3.8) were prepared according to Cury et al. (2000). The enamel blocks with the same maturation periods were randomly selected and a band of 1 mm (thickness) was coated with acid-resistant varnish to serve as the control of each tooth. In each phase, the volunteers wore acrylical palatal appliances containing six dental blocks randomly divided into groups of treatment (3 permanent blocks x 3 deciduous blocks).

During the two experimental periods and the 7 days washout periods, the volunteers brushed (3x/day) their natural teeth with dentifrice supplied by the researchers. The volunteers drank fluoridated water (0.7 mg F/L ±1.2) and received instructions as previously described (Cury et al., 2000).

To provide a cariogenic challenge, the volunteers were instructed to remove the device and drip 20% sucrose solution onto the blocks 8x/day (8:00, 9:30, 11:00, 14:00,

15:30, 17:30, 19:00, 21:00 hrs) (Cury et al., 2000). Five minutes later, the device was re-inserted into the mouth.

After 7, 14 and 21 days, the biofilm, which was formed on the deciduous and permanent enamel surfaces was collected for biochemical analysis and enamel mineral loss was assessed in the dental blocks.

Dental Biofilm Analysis

At the end of each experimental period, the dental biofilm was collected, homogenized with a spatula and dehydrated for biochemical analysis and concentrations of acid-soluble fluoride (F), calcium (Ca), inorganic phosphorus (P_i) and insoluble polysaccharide (IP) in dental plaque were determined according to previous studies (Pecharki et al., 2005).

Enamel Blocks Analyses

After plaque removal, the enamel blocks were cleaned and evaluated by visual examination for the determination of the presence or not of visible mineral loss (white spot) as described by Moi et al. (2005).

Then, all the blocks were longitudinally sectioned through the center for cross-sectional microhardness determination (CSMH) according to Cury et al. (2000). At the sound part of the enamel and the exposed area of the same tissue which was submitted to treatment, three lines of indentations at 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160, 180 and 200 μm from the outer enamel surface were made with a 25-g load for 5 sec. The average of CSMH values were converted to mineral content (%vol) according to Featherstone et al. (1983) and the area of mineral loss (ΔZ), for each treatment, was

calculated (Paes Leme et al., 2004). The microhardness tester, Future-Tech FM, coupled to software FM-ARS, was used for these analyses.

Thereafter, longitudinal sections of 100 μm (±10) were obtained from the remaining half of each block (Hara et al., 2003). They were embedded in distilled deionized water, mounted in glass-slides and the carious lesions depth was analyzed in a polarized light microscope at 100X magnification (DM LSP, Leica, Wetzlar GmbH, Germany). Digital images were taken, and the lesions depths were measured at five different sites with Image-Pro Plus software (Media Cybernetics) and the average depth was calculated.

Statistical Analyses

A factorial 3x3 was considered for the statistical analyses of plaque composition and enamel mineral changes. For the evaluation it was considered: substrate factors at 2 levels (deciduous and permanent), treatment at 2 levels (PD and FD) and time at 3 levels (7, 14 and 21 days). The volunteers were considered as statistical blocks. The non-homogeneous data were transformed (Box et al., 1978) and when the homogeneity in the change was not possible a non-parametric statistics was performed. The SAS software system (version 8.02, SAS Institute Inc., Cary, NC, USA) was used, and the significance limit was set at 5%.

RESULTS

The outcomes of F, Ca, IP, ΔZ were transformed, respectively, by 1/SQRT, log, power 0.3 and 0.2, allowing a parametric statistical analysis of the data to be performed. For P_i and visual examination a non-parametric statistical analysis was performed.

The F concentration (mean ±SD) formed in biofilm in the presence of fluoride dentifrice was statistically higher (24.3 μg/g ± 22.9) than that found in non-fluoride dentifrice (8.6μg/g ± 4.4). There are no statistical differences in time of plaque formation, but there was more F concentration in dental plaque formed on permanent teeth (19.8 μg/g ± 23.3) than in deciduous ones (13.4 μg/g ± 10.2).

With respect to Ca, a significant statistical effect of fluoride dentifrice and the time were observed. For P_i, the only factor that showed a statistical effect was fluoride dentifrice (Table 1).

For IP in dental plaque, a lower concentration in biofilm formed in the presence of fluoride dentifrice (169.5 mg/g ± 136.4) than in the one formed in the presence of placebo dentifrice (198.9 mg/g ± 108.7) was observed. The time effect was statistically significant ($p<0.05$).

For ΔZ (Figure 1.), a significant statistical effect was observed for the factors: substrate, dentifrice and time. Thus, deciduous enamel (1402.8 vol% min.x μm ± 1261.9) presented more mineral losses than permanent enamel (925.2 vol% min.x μm ± 726.3). When fluoride dentifrice was present, a lower mineral change ($p=0.01$) was found when the results were compared. Indeed, higher mineral loss was statistically found after 21 days (1638.1 vol% min.x μm ± 1167.3) compared with 7 days (800.7 vol% min.x μm ± 710.80) and 14 days (1053.9 vol% min.x μm ± 1064.9). However, there was not any statistically significant difference between 7 and 14 days ($p<0.05$).

The isolated factors substrate, dentifrice, and time significantly ($p<0.05$) influenced the mineral losses in polarizing light microscopy analysis (Figure 2.) and in clinical exam analysis, a statistic effect of substrate, time, substrate-dentifrice, substrate-time was found (Table 1).

DISCUSSION

This *in situ* model was able to simulate a carious process activity, thereby this methodology permitted high cariogenic challenges (Cury et al., 1997, 2000). Direct comparisons of this research's outcomes with those found in the literature are not always simple to make because it is the first *in situ* study with a specific experimental design of this type. It is convenient to highlight that the low concentration of F, Ca, P_i and the high concentration IP found in all periods and phases are in agreement with previous publications (Cury et al., 1997, 2000; Paes Leme et al., 2004; Ribeiro et al. 2005).

At the same time that F, Ca and Pi can be found in dental biofilm as mineral reservoirs, these reservoirs can be dissolved by the acid produced by dental plaque when sucrose is fermented by the bacteria of the dental biofilm (Cury et al., 2000). These ions can be diffused from dental plaque to saliva, but in this study, there was enough time for plaque to be saturated again by these ions through the simple law of mass action (Cury et al., 1997, 2000; Paes Leme et al., 2004). This ions diffusion can be modified by some oral environment factors such as pH changes and saturations ions changes. In this way, the fluoride dentifrice may have contributed to the increase of F, Ca and Pi concentration of this study.

There is more F concentration in dental plaque formed on permanent teeth than in deciduous ones. This can be justified through biofilm cariogenic challenges controlled by the law of mass action (Cury et al., 2000) since previous studies have showed that the enamel fluoride concentration in the permanent tooth's surface layer is always higher than that in deciduous enamel (Mellberg et al., 1970, Issa et al., 2003). The permanent enamel also has more fluoride at all depths than deciduous counter-part, possibly because the crowns of the premolars remain in their crypts in the jaws longer before eruption of

deciduous crowns. Accordingly, the premolar crowns would be exposed to cryptal tissue fluids that contain fluoridated water for a longer period than the deciduous crowns and they would thereby accumulate more fluoride by the longer and continuous topical action of those fluids during their pre-eruption maturation period (Mellberg et al., 1970).

Although the high IP concentration is in accordance with previous studies, there was a statistically significant difference ($p=0.007$) among IP in the two phases. This result contests previous evidences (Arthur et al., 2004; Paes Leme et al., 2004). Maybe, methodological differences can justify this. Although, these findings come from an *in situ* longitudinal study, there was no statistical difference in biofilm weight between in the two phases ($p = 0.59$). It can suggest that the phase did not influence the dental biofilm formation. There are some researches that provide important evidences that the fluoride (at sub-millimolar concentration) can exert inter-related (antimicrobial) actions on *S. mutans* and indirect effects by preventing the development of favorable low pH environments for cariogenic bacteria in biofilm (Hamilton, 1990; Marquis, 1990; Marsh and Bradshaw, 1990; Bradshaw et al., 2002; Marquis et al., 2003).

The experimental design of this research did not permit the use of SMH as other dental caries studies (Cury et al., 1997; Cury et al., 2000; Paes Leme et al, 2004; Aires et al., 2006). Therefore, the surface of all enamel blocks was not polished so that any alteration, in the outcomes of this study, was promoted.

Thus, the mineral changes were measured by visible mineral losses (Moi et al., 2005), polarizing light microscopy (Hara et al., 2003), and cross-sectional microhardness (Cury et al., 2000). All the results showed by these analyses presented a significant statistical effect when the factors, dentifrice and time, were observed ($p<0.05$). Then, these outcomes proved that dentifrice fluoride interfered significantly in progression of caries

throughout the time (Duggal et al., 2001; Issa et al., 2003). There is also clear evidence that the levels of fluoride in the environment may influence the carious process (Duggal et al., 2001; Bradshaw et al., 2002; Issa et al., 2003).

When carious progression was evaluated by the visual inspection and polarizing light microscopy (lesions depth), it presented a faster rate in deciduous enamel than in permanent one throughout the time ($p<0.05$), being supported by previous evidences (Pitts, 1983; Sonju-Clasen et al., 1997; Issa et al., 2003; Vanderas et al., 2003),

The clinical manifestation of the deciduous enamel carious lesions can be clinically diagnosed from 14 days ($p<0.001$), but in permanent enamel carious lesions it can only be diagnosed from 21 days ($p<0.001$). However, enamel micro-structural analyses pointed out mineral losses from 7 days in the enamel blocks. Although the enamel blocks presented mineral losses in the ultra-structural level in all periods, dental caries was not diagnosed in the first periods by visual inspection. These outcomes contribute to the evidences found in the literature, because first enamel cariogenic challenges show a sub-clinical manifestation (Thylstrup et al., 2001).

For ΔZ, a significant statistical effect was observed for the factors substrate, dentifrice and time. So, deciduous enamel presented more mineral losses than permanent enamel ($p<0.05$). When the fluoride dentifrice was present, a lower mineral change ($p=0.01$) was found. Indeed, a statistical higher mineral loss was found after 21 days ($p=0.0001$) compared with 7 days and 14 days. However, there was no significant difference between 7 and 14 days ($p<0.05$).

Some studies suggest that a carious progression rate in deciduous enamel is approximately 1.5 times faster than in permanent one (Pitts, 1983). Besides, there is also clear evidence that the levels of fluoride in the environment may influence the carious

process (Duggal et al., 2001; Bradshaw et al., 2002; Issa et al., 2003). So, after careful analyses of this study's outcomes medias (VI, ΔZ, lesion depth) it is suggested that carious progression rate in deciduous enamel is next to 1.5 times ±1.2 (p=0.02) faster than in permanent one, and it can also be observed that fluoride dentifrice reduces the carious progression rate around 1.5 times ±1.0 (p=0.08).

These findings may present a particular relevance for the pediatric dentistry practice. Therefore, a program of child attention, based on the opportune diagnostic of carious activity and in the establishment of the treatment, should focus on these clinical parameters in order to determine the correct caries lesion approach, considering the non-invasive treatment, since the patient needs to be regularly followed-up. Pondering that the deciduous teeth have a limited biological cycle in the mouth, they can exfoliate before the carious process increase. Then, the dental practice must give emphasis on the prevention of new caries lesion and on the growth inhibition of the existing ones (Craig et al., 1981).

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Table 1. Statistical Results (p-values)

Factors and interactions	**F**(μm/g)	**Ca**(mg/g)	**Pi**(mg/g)	**IP**(mg/g)	**ΔZ**(vol% min x μm)	**Polarized light micoscopy** (μm)	**Visual inpection** (1=white spot/ 0=sound teeth)
Fluoride dentifrice X Placebo dentifrice	<0.0001	0.0447	0.0011	0.0069	0.0101	0.0082	0.0500
Deciduous teeth x Permanent teeth	0.0275	ns	ns	ns	0.0052	0.0202	0.0004
Time (7, 14 and 21 days)	ns	ns	ns	0.0111	<0.0001	<0.0001	<0.001

ns = not statiscally significant (p>0.05).

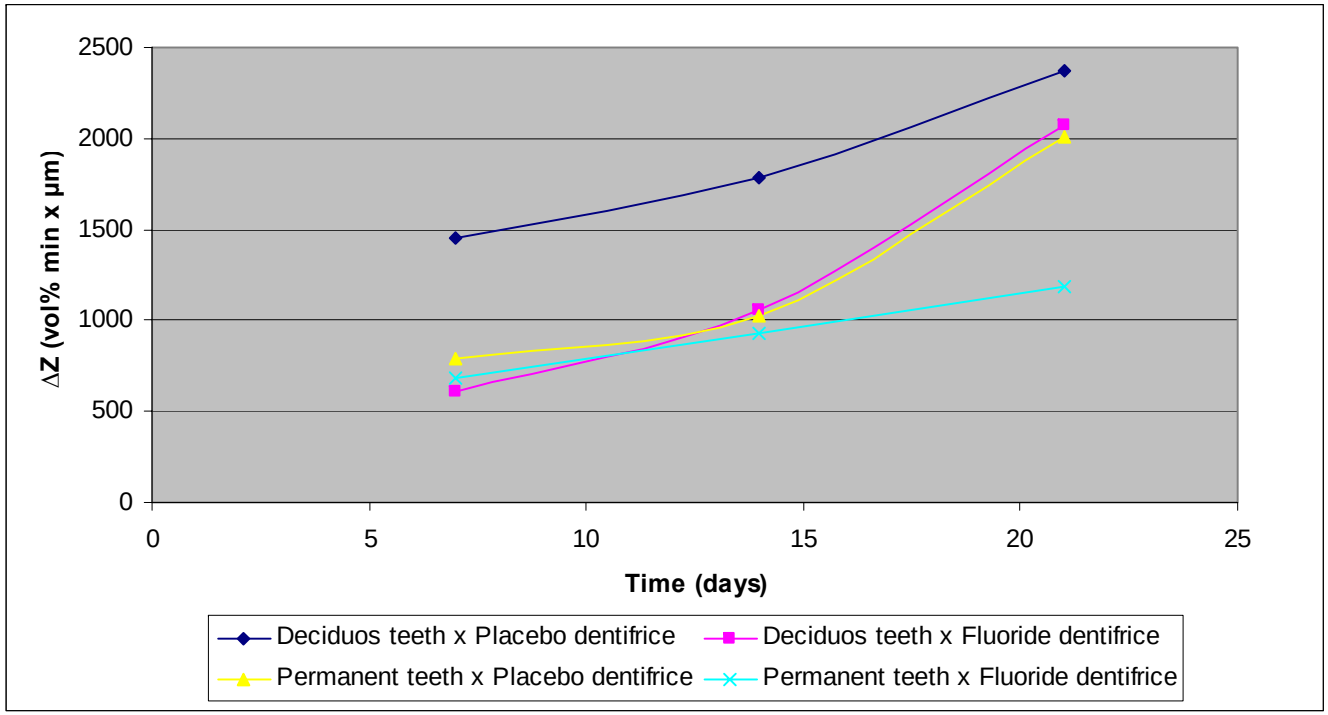

Figure 1. ΔZ (vol% min x µm) of deciduous enamel blocks and permanent ones being treated with placebo dentifrice/fluoride dentifrice for 7, 14, 21 days. A significant statistical effect was observed for the factors substrate, dentifrice and time ($p<0.05$).

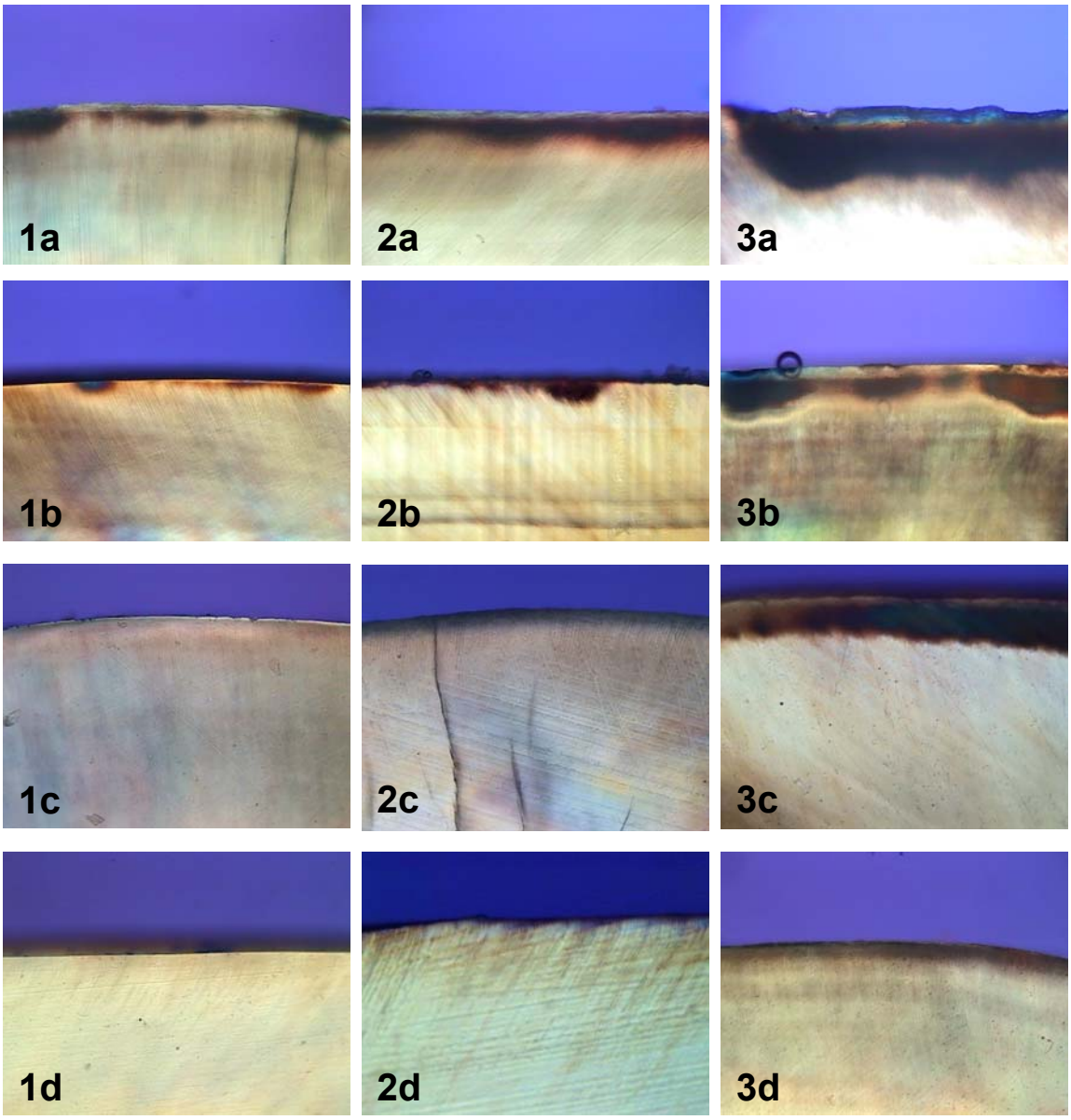

Fig 2 – Images under polarized light microscopy at 100 x magnification:

1a, deciduous enamel after 7 days being treated with placebo dentifrice. **2a**, deciduous enamel after 14 days being treated with placebo dentifrice. **3a**, deciduous enamel after 21 days being treated with placebo dentifrice.

1b, deciduous enamel after 7 days being treated with fluoride dentifrice. **2b**, deciduous enamel after 14 days being treated with fluoride dentifrice. **3b**, deciduous enamel after 21 days being treated with fluoride dentifrice.

1c, permanent enamel after 7 days being treated with placebo dentifrice. **2c**, permanent enamel after 14 days being treated with placebo dentifrice. **3c**, permanent enamel after 21 days being treated with placebo dentifrice.

1d, permanent enamel after 7 days being treated with fluoride dentifrice**. 2d**, permanent enamel after 14 days being treated with fluoride dentifrice. **3d**, permanent enamel after 21 days being treated with fluoride dentifrice.

5. CONCLUSÃO GERAL

Com base na sugestão proposta no artigo de revisão de literatura, de se realizar um ensaio clínico que pudesse contemplar a velocidade de progressão de cárie em ambas as dentições contemplando um mesmo desenho experimental, pode-se concluir que nestas condições o dentifrício fluoretado (1100 ppm NaF) pode interferir nas diferentes velocidades de progressão de lesão cariosas em esmalte dentes decíduos e permanentes.

ANEXO I**.**

Termo de Consentimento Livre e Esclarecido
Universidade Federal do Rio Grande do Sul
Faculdade de Odontologia

Este estudo será realizado no ambulatório de odontopediatria da Faculdade de Odontologia da UFRGS, em duas fases experimentais de 21 dias, em vistas a comparar *in situ* a velocidade de progressão da lesão cariosa em dentes decíduos e permanentes na presença e na ausência de flúor.

Declaro que fui informado(a) detalhadamente sobre as etapas do experimento do qual vou participar: moldagem; uso de dispositivo ortodôntico intra-oral e de creme dental com e sem flúor. Fui informado(a) de que tais procedimentos não implicarão em danos a minha saúde bucal e geral, e ainda que posso retira-me do estudo a qualquer momento se assim o desejar.

Nome:_____
Documento:_____
Assinatura:_____

Porto Alegre,_____de_____de _____.

Pesquisadores Responsáveis:
Prof. Dr. Fernando Borba de Araujo
Dra.Gisele Pedroso Moi

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ANEXO II.

Instruções aos Voluntários
Em Anexo ao Termo de Consentimento Livre e Esclarecido
Universidade Federal do Rio Grande do Sul
Faculdade de Odontologia

Antes do início de cada fase experimental, cada voluntário receberá o dentifrício a ser usado, frasco com conta-gotas contendo a solução de sacarose, estojo de aparelho ortodôntico (acomodação do dispositivo) e um dispositivo intra-oral.
As instruções fornecidas a cada voluntário serão as seguintes:

Instruções Gerais:

- O Estudo será constituído de duas fases experimentais de 21 dias.
- Durante este período não deve ser utilizado nenhum produto ou alimento rico em fluoreto (exceto o creme dental fornecido pelo pesquisador).
- O dispositivo deverá ser removido apenas para a higienização e alimentação.
- Ao remover o aparelho durante as refeições (qualquer tipo de refeição) mantenha-o envolvido em uma gaze umedecida. Isso é muito importante para não resssecar os blocos dentários e manter a placa dental (biofilme), formada sobre esses blocos, em condições adequadas.
- A limpeza do aparelho deve ser feita com escova e creme dental (fornecido pelo pesquisador), sendo que a escova não deve, em hipótese alguma, tocar as telas.
- Sua alimentação, assim como a higiene bucal, deve permanecer da mesma forma.
- Antes de gotejar a solução de sacarose sobre os blocos dentários, secar delicadamente o aparelho com gaze.
- A solução deve ser gotejada oito (8) vezes por dia sobre os blocos dentários, nos seguintes horários previamente estabelecido: 8:00, 9:30, 11:00, 14:00, 15:30, 17:30, 19:00, 21:00 horas. Espera-se 5 minutos e recoloque o aparelho em posição.

- O uso da solução de sacarose será apenas como gotas sobre os blocos dentários presentes nos dispositivos intra-orais, não implicando em qualquer aumento de risco de cárie dental aos voluntários.
- Não fazer bochechos com anti-sépticos durante o experimento.
- Os voluntários poderão apresentar discreta halitose durante o período experimental, o que poderá ser resolvido com adequada higiene da parte interna do dispositivo intra-oral.
- Caso seja necessário o uso de algum tipo de medicação durante o experimento, comunicar imediatamente o pesquisador responsável.
- O dispositivo intra-oral pode causar um leve desconforto, que é, no entanto, 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.

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