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Frequency of intake and amount of fluoride in milk for remineralisation of artificial caries on enamel and dentine: *Ex vivo/in situ* study

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ABSTRACT

Objectives: This study analysed the effect of frequency of intake and amount of fluoride in milk on the remineralisation of artificial enamel and dentine caries lesions *ex vivo/in situ*.
Materials and methods: Pre-demineralised bovine enamel and dentine slabs were randomly allocated into 5 groups and fixed in removable appliances used by subjects for 7 days in each phase. Each treatment comprised milk containing 2.5 ppm fluoride daily (T1), or every other day (T2), 5.0 ppm F daily (T3), or every other day (T4) or no treatment (T5).
Results: Enamel alterations were quantified by surface hardness recovery (%SHR) and transversal microradiography (TMR), and in dentine by TMR only. Data were analysed by ANOVA and Tukey's test ($p < 0.05$). For enamel, the highest %SHR was found for T1 and T3 compared to control, without significant differences between them. All groups showed positive values of $\Delta\Delta Z$ – T1 (247.3 ± 198.5); T2 (110.9 ± 303.2); T3 (226.0 ± 299.2); T5 (5.0 ± 288.0), except T4 (-274.5 ± 407.3). For dentine, the only group that presented remineralisation was T2 (350.0 ± 657.5).
Conclusions: Fluoridated milk daily seems to have higher remineralising effect on enamel than its use every other day. Dentine, does not seem to benefit from daily use of fluoridated milk.

1. Introduction

Fluoride is considered the main responsible for the dramatic decrease in caries incidence and prevalence observed worldwide over the last decades (Bratthall, Hansel-Petersson, & Sundberg, 1996). It can be administered by community, self-applied and professional methods, which are often used in association (Pessan, Toumba, & Buzalaf, 2011). Water fluoridation has been regarded as the main primary preventive and public health measure for caries control, as it reaches most of the population, including socially deprived groups. The benefits of water fluoridation, however, are unavailable to a large proportion of the world's population, mainly due to political, geographical and technical reasons (Sampaio & Levy, 2011). In order to overcome this problem, other methods of community fluoridation have been suggested. As milk is an important part of children's diet, fluoridated milk has been used in school-based preventive programmes for many decades, in different parts of the world. One of the main advantages of this method is that it allows the delivery of a precise amount of fluoride under controlled conditions (Banoczy, Rugg-Gunn, & Woodward, 2013).

The effectiveness of fluoridated milk in caries control has been assessed by *in vitro*, *in situ* and clinical studies, which show a

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positive effect of its regular consumption on caries prevention (Banoczy et al., 2013). However, while the evidence of water fluoridation in caries control has been firmly established over the last decades (McDonagh et al., 2000), systematic reviews have concluded that the number of studies with good quality that evaluated the effects of fluoridated milk in preventing caries is insufficient. Overall, the included studies suggested that fluoridated milk was beneficial to school children, to prevent caries in the permanent (Yeung et al., 2005) or primary (Cagetti, Campus, Milia, & Lingstrom, 2013) dentitions. High-quality studies, nonetheless, are still necessary in order that unequivocal evidence can be established. In addition, many aspects involving the milk fluoridation programmes still need to be addressed.

There are distinct ongoing school based milk fluoridation programmes worldwide in countries like Chile, Thailand, UK, Russia, Bulgaria and Republic of Macedonia, involving over a million children. These fluoridated milk schemes comprise a single drink of cow's milk at school during a morning break. However, the amount of fluoride delivered through milk in these programmes ranges between 0.5 and 0.85 mg per day (Banoczy et al., 2013). Also the frequency of fluoridated milk consumption varies in the different milk fluoridation schemes. In the UK, the amount of fluoride delivered from milk is 0.5 mg per day on school days (200 days/year), while in Chile quantities ranging between 0.25 and 0.75 mg per day (depending on the age of the children) are delivered 365 days each year (Banoczy et al., 2013).

The impact of these differences in the amount of fluoride delivered and frequencies of intake on the caries preventive potential of milk fluoridation schemes is not completely known. The influence of two different fluoride concentrations in milk (2.5 and 5.0 ppm, corresponding to 0.5 and 1.0 mg fluoride delivered in 200 mL of milk, respectively) on the prevention of demineralisation of sound enamel was recently evaluated *in situ*. Both fluoride concentrations significantly protected enamel from demineralisation when compared with non-fluoridated milk, but did not significantly differ from each other (Malinowski, Duggal, Strafford, & Toumba, 2012a). A recent *in vitro* study evaluated the effect of different fluoride concentrations in milk (2.5, 5.0 or 10.0 ppm) and also of distinct frequencies of use (once daily, twice daily or on alternate days) for remineralising initial enamel carious lesions. The best remineralising effect was observed for 2.5 ppm fluoride milk used twice daily (Ongtenco et al., 2014). Regarding dentine, an *in vitro* study analysed the remineralising effect of different solutions (sodium chloride, artificial saliva, milk, milk + 2.5 ppm fluoride, milk + 10 ppm fluoride and artificial saliva + 10 ppm fluoride). The results showed a positive effect of the fluoridated milk, but the group treated with sodium chloride presented a better effect (Arnold, Heidt, Kuntz, & Naumova, 2014). However, only one *in situ* study evaluated the influence of different fluoride concentrations in milk on enamel remineralisation, but the quantities of fluoride used added in milk (1.5 or 3.0 mg) were higher than those typically employed in milk fluoridation schemes and the authors evaluated only surface rehardening (Lippert, Martinez-Mier, & Zero, 2014).

In addition, to date no studies evaluated the effect of distinct fluoride concentrations in milk on dentine remineralisation *in situ* neither the effect of frequencies of use of milk on the remineralisation of dentine and enamel caries *in situ*. Thus, the present study evaluated if there was any additional benefit of increasing the amount of fluoride in milk from 2.5 ppm to 5 ppm per day on *ex vivo/in situ* enamel and root dentine remineralisation. The effect of different frequencies of drinking fluoridated milk (every day or every other day) was also investigated. It was hypothesized that higher fluoride concentration and frequency of milk intake would lead to enhanced remineralisation of enamel and dentine.

2. Materials and methods

2.1. Ethical aspects and subjects

The study followed a double-blind, randomised, crossover protocol, comprising 5 phases of 7 days each, with an interval of 7 days among them. Twenty-three young adult subjects (2 male, 21 female) took part in the study, after approval by the IRB of Bauru Dental School, University of São Paulo, Brazil (No.179/2011). Sample size was based on an *in situ* study recently conducted with similar research protocol (Malinowski et al., 2012a). According with that study, a sample size of 12 volunteers would be needed, in order to find a significant difference between the negative control (T5 – no treatment) and milk containing 5.0 ppm of fluoride, considering an α value of 0.05 and 80% of power. Considering possible dropouts, our study began with 25 volunteers, with 5 volunteers in each phase. The inclusion criteria were: stimulated salivary flow > 1 mL/min, unstimulated salivary flow > 0.25 mL/min; salivary pH > 6, good oral conditions (presenting full permanent dentition, no open cavities or deficient restorations, absence of gingivitis and periodontal disease). Other factors taken into consideration were related to the overall health of the volunteer. Pregnant women, patients with systemic diseases and using chronic medication were not eligible to participate. Subjects signed an informed consent document prior to the beginning of the study.

2.2. Specimen preparation

Bovine lower incisors were freshly extracted and stored in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature. Teeth were submitted to careful visual inspection in order to detect stains and cracks. If these were detected, the teeth were excluded. Selected teeth were carefully cleaned from gingival tissue using a periodontal curette (Duflex, Duflex do Brasil, Brazil) before being cut. Initially the roots were separated from the crowns using a diamond disk (Diaflex-F, Wilcos do Brasil, Petrópolis, Brazil) by sectioning the cervical portion of the tooth. The crown was used for obtaining enamel slabs and, from the root, dentine slabs were prepared. Then the crowns or roots were fixed in acrylic plaques ($40 \times 40 \times 5$ mm) that were placed in the ISOMET Low Speed Saw cutting machine (Buehler Ltda., Lake Bluff, IL, USA). Two diamond disks (XL 12205, $102 \times 0.3 \times 12.7$ mm, Extec Corp., Enfield, CT, USA), which were separated by a 4-mm diameter spacer (7 cm diameter, 4 mm thickness and central role of 1.3 cm), were used to cut the slabs (300 rpm, under refrigeration). One enamel and one dentine slabs (4×4 mm) were obtained from the flattest portion of each crown or root, respectively, through double sections in the longitudinal and transversal directions. The surface of the slabs was ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA) and polished with felt paper wet by diamond spray (1 μm; Buehler), resulting in removal of about 100 μm depth of the enamel, what was controlled with a micrometer. After polishing, the specimens were cleaned in an ultrasonic device with deionised water for 10 min.

Baseline surface hardness (BSH) determination was performed on all the enamel slabs ($n = 230$) for selection purposes (five indentations; Knoop diamond, 25 g, 10 s; HMV-2; Shimadzu Corporation, Tokyo, Japan) (Mean KHN 335.3 ± 2.4). Dentine slabs ($n = 230$) were not submitted to surface hardness analysis since previous studies have revealed that this type of analysis is not accurate for this substrate, considering that hardness is not evenly distributed even in sound dentine (Moron et al., 2013). After that, one third of the (enamel/dentine) surface was covered with nail varnish to create a sound control area. The slabs were subjected to