available at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/arob

Effect of calcium pre-rinse and fluoride dentifrice on remineralisation of artificially demineralised enamel and on the composition of the dental biofilm formed *in situ*

Ana Carolina Magalhães [a], *Tatiana de Almeida Furlani* [b], *Flávia de Moraes Italiani* [b], *Flávia Godoy Iano* [b], *Alberto Carlos Botazzo Delbem* [a], *Marília Afonso Rabelo Buzalaf* [b,*]

[a] *Department of Pediatric Dentistry, Faculty of Dentistry of Araçatuba, UNESP – São Paulo State University, Araçatuba, SP, Rua José Bonifácio, 1193 Araçatuba-SP 16015-050, Brazil*

[b] *Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, Bauru, SP, Al. Octávio Pinheiro Brisolla, 9-75 Bauru-SP 17012-901, Brazil*

ARTICLE INFO

Article history:
Accepted 4 June 2007

Keywords:
Enamel
Dental biofilm
Demineralisation
Calcium
Fluoride dentifrice

ABSTRACT

Objective: This *in situ* blind crossover study investigated the effect of calcium (Ca) rinse prior to the use fluoride (F) dentifrice on remineralisation of artificially demineralised enamel and on the composition of biofilm. *Design*: During four phases of 14 days, 10 volunteers wore appliances containing two artificially demineralised bovine enamel blocks. Three times a day, they rinsed with 10 mL of Ca (150 mM) or placebo rinse (1 min). A slurry (1:3, w/v) of F (1030 ppm) or placebo dentifrice was dripped onto the blocks. During 1 min, the volunteers brushed their teeth with the respective dentifrice. The appliance was replaced into the mouth and the volunteers rinsed with water. The biofilm formed on the blocks was analysed for F and Ca. Enamel alterations were evaluated by the percentage of surface microhardness change (%SMHC), cross-sectional microhardness (% mineral volume) and alkali-soluble F analysis. Data were analysed by ANOVA ($p < 0.05$). *Results*: The use of the Ca pre-rinse before the F dentifrice produced a six- and four-fold increase in biofilm F and Ca concentrations, respectively. For enamel, the remineralisation was significantly improved by the Ca pre-rinse when compared to the other treatments. There was a significantly higher concentration of alkali-soluble F in enamel when the F dentifrice was used, but the Ca pre-rinse did not have any significant additive effect. *Conclusions*: According to our protocol, the Ca pre-rinse significantly increased biofilm F concentration and, regardless the use of F dentifrice, significantly enhanced the remineralisation of artificially demineralised enamel.

1. Introduction

The cariostatic effect of fluoride (F) dentifrices has been recognised for a long time, and, in conjunction with improved daily oral hygiene, F dentifrice is regarded as the major factor responsible for the dramatic caries reduction in children and young adults in most industrialised countries during the last decades.[1]

F may be deposited on the enamel by formation of a CaF_2-like reservoir. During a cariogenic challenge, F released from this reservoir may diffuse into the enamel promoting reformation of apatite.[2] In addition, demineralised enamel

* *Corresponding author*. Tel.: +55 14 32358246; fax: +55 14 32343164.
E-mail address: mbuzalaf@fob.usp.br (M.A.R. Buzalaf).

0003–9969/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.archoralbio.2007.06.006

acquires larger amounts of fluoride than does sound enamel.[3,4] The dental biofilm is an important reservoir of this compound. Clinical studies have demonstrated an inverse relationship between the F concentrations in the dental biofilm and the prevalence of dental caries.[5–7] It now appears certain that the uptake and retention of F by dental biofilm is mainly dependent on biofilm calcium (Ca) concentrations.[1,8–13]

Following this rationale, attempts to increase the cariostatic effectiveness of F have focused on methods to increase Ca concentrations in dental biofilm. It was reported that a 150 mmol/L Ca-lactate pre-rinse significantly increased salivary fluoride concentrations after the use of a 228-ppm F rinse.[14] Significant increases in F concentrations in biofilm fluid[15] and in whole biofilm[16] were also reported when the Ca-lactate pre-rinse was used.

In the studies mentioned above, the vehicle for use of F was a rinse. However, F dentifrice is by far the most frequently used topical fluoride agent.[17] Despite some authors have reported a significant increase in salivary F levels after the use of F dentifrice preceded by a calcium pre-rinse,[18] others have not[13] or have identified an increase only in the short time.[19]

This study was designed in an attempt to conciliate these different findings. For this purpose, an *in situ* model using artificially demineralised enamel was employed, in order that besides the evaluation of biofilm F and Ca concentrations, the associated enamel alterations could also be assessed.

2. Materials and methods

2.1. Experimental design

This study was approved by the Research and Ethics Committee of the Bauru School of Dentistry, University of São Paulo (Proc. no. 318/2003). Ten volunteers[20,21] with good oral and general health (five male and five female, mean age 24 years, mean DMFT 7.4, PHP index between 0 and 1) took part in a blind crossover protocol conducted in four phases of 14 days, after signing an informed consent. The following treatments were used: Ca solution rinse and F dentifrice (Ca–F), Ca solution rinse and placebo dentifrice (Ca), placebo solution rinse and F dentifrice (F) and placebo solution rinse and placebo dentifrice (P). For this purpose, the volunteers wore appliances containing two artificially demineralised enamel blocks. Three times/day, they rinsed with 10 mL of Ca (150 mM) or placebo rinse (1 min). A slurry (1:3, w/v) of F (1030 ppm) or placebo dentifrice was dripped onto the blocks. During 1 min, the volunteers brushed their teeth with the respective dentifrice. The volunteers replaced the appliance in the mouth and rinsed with water. The response variables evaluated were the percentage of surface microhardness change (%SMHC), cross-sectional microhardness (% mineral volume) and alkali-soluble F analysis for enamel alterations, as well as the Ca and F concentrations present in the biofilm formed on the blocks.

2.2. Enamel blocks and palatal appliance preparation

Enamel blocks (4 mm × 4 mm × 2.5 mm) were prepared from incisor bovine teeth, freshly extracted, sterilised by storage in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature. The enamel surface of the blocks 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. This was controlled with a micrometer. The surface microhardness determination was performed by five indentations (Knoop diamond, 25 g, 10 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan).

Eighty blocks were obtained and subjected to artificial demineralisation by immersion in 32 mL of 50 mM buffer acetate solution [1.28 mM $Ca(NO_3)_2{\cdot}4H_2O$, 0.74 mM $NaH_2PO_4{\cdot}2H_2O$, 0.03 ppm F, pH 5.0, 37 °C], during 16 h.[22] After that, the microhardness was again evaluated and the percentage of surface microhardness change was calculated [%SMHC demin = 100(SMH demin − SMH sound)/SMH sound]. Blocks with mean %SMHC around 65–90 were selected and randomly allocated for the intra-oral phases. In order to evaluate the profile of the lesion formed, 10 blocks were analysed for cross-sectional microhardness (Fig. 1).

One cavity of 5 mm × 5 mm × 3 mm was made on the left and right sides of the acrylic palatal appliances and in each of them one block of enamel was fixed with wax. For this, a 4-mm-deep space was created in the acrylic appliance, leaving a 1.5-mm space for plaque accumulation.[23] For the formation of dental biofilm on the enamel block, it was protected from mechanical disturbance by a plastic mesh fixed in the acrylic surface. The blocks were replaced after each phase.

2.3. Treatment

Three times a day, after the meals, the volunteers rinsed with 10 mL of a 150 mM calcium lactate (Sigma–Aldrich, Atlanta, Georgia, USA) or placebo (deionised water) solution, during 1 min. In sequence, the solution was expectorated and a slurry (1:3, w/v) of fluoride (Crest®, NaF, 1030 ppm) or placebo dentifrice (Crest®, Proctor & Gamble, Cincinnati, Ohio, United States) was dripped onto the enamel blocks (3 drops/block). During 1 min, the volunteers brushed their teeth with a pea-size amount (~0.3 g) of the respective dentifrice. The Crest® placebo was identical to Crest® except that it contained no detectable fluoride. The abrasive system in the products is hydrated silica and they contain no detectable calcium. After the time elapsed, the volunteers replaced the appliance in the mouth and rinsed their mouth with 5 mL of drinking water (0.7 ppm F). All solutions and dentifrices used were placed in separated vials which did not allow their identification by the volunteers, in order to conform with the blind protocol of the study.

A 7-day washout period was allowed before the beginning of the study and between the phases to eliminate possible residual effect from the previous treatment. During the experimental period, the volunteers received instructions to wear the appliance all the time, including at night, but to remove it during meals (1 h × 3 meals/day), when it was involved in a gauze wet with deionised water. The volunteers received oral and written information to refrain from using any antibacterial or fluoridated product.