ARTICLE IN PRESS

Journal of Dentistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Full Length Article

In situ effect of the combination of fluoridated toothpaste and fluoridated gel containing sodium trimetaphosphate on enamel demineralization

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ARTICLE INFO

Keywords:
Caries
Biofilm
Fluoride
Demineralization

ABSTRACT

Objective: This *in* situ study evaluated the effect of the association of low-F (4500 μg F/g) gel containing TMP and FT (1100 μg F/g) on enamel demineralization.

Methods: This crossover and double-blind study consisted of five phases of seven days each. Volunteers (n = 12) wore palatal appliances containing four enamel blocks. The cariogenic challenge was performed with 30% sucrose solution (six times/day). Treatments were: placebo toothpaste (PT, no fluoride/TMP); 1100 μg F/g toothpaste (FT); FT + 4500 μg F/g + 5%TMP gel (FT + TMP gel); FT + 9000 μg F/g gel (FT + 9000 gel) and FT + 12,300 μg F/g (FT + Acid gel). After topical application of treatments for one min, two blocks were removed for analysis of loosely bound fluoride (CaF_2), calcium (Ca), phosphorus (P) and firmly bound fluoride (FA) formed in enamel. After the seven-day experimental periods, the percentage of surface hardness loss (%SH), integrated subsurface hardness loss (ΔKHN), CaF_2, Ca, P and FA retained were determined. Moreover, the biofilms formed on the blocks were analyzed for F, Ca, P and insoluble extracellular polysaccharide (EPS) concentrations.

Results: FT + TMP gel promoted the lowest%SH and ΔKHN ($p < 0.001$). The highest concentration of CaF_2 formed was observed for the FT + Acid gel ($p < 0.001$), followed by FT + 9000 gel > FT + TMP gel > FT > PT. CaF_2 retained on the blocks was reduced across all groups ($p < 0.001$). Similar values were observed for the Ca/P/F and EPS in enamel and biofilm for all fluoride groups.

Conclusion: The association of FT + TMP gel significantly reduced enamel demineralization *in situ*. *Clinical Significance:* The association of treatments may be an alternative for patients with high caries risk.

1. Introduction

Dental caries is considered a multifactorial disease that requires the complex interaction of several factors to develop clinical manifestation [1]. Fluoride (F) has been the main agent used for dental caries prevention worldwide. The use of F toothpaste (FT) is considered the primary reason for the reduction in caries prevalence observed over the last decades [2,3].

Topical application of F (TAF) is often used in preventive programs as well as in patients at high risk of developing dental caries as an adjunct measure for the reduction of lesions [4]. When a product with high F concentration is applied on the tooth surface, there is deposition of calcium fluoride (CaF_2), which is covered by calcium and phosphate ions, and saliva proteins that delay the solubility of the compound [5]. Thus, it functions as a source of F, thereby interfering with the dynamics of the de-remineralization processes [6]. However, the cost of products with high F concentration can be high, especially fluoridated varnishes that are the first choice for infants with high caries risk [7–9]. This implies restricted access in terms of public health, especially in developing countries where fluoride gels are most commonly employed. Notwithstanding, the risk of ingestion and adverse events (mainly nausea and vomiting) can be present when fluoride gel is applied in children younger than six years, overcoming the potential benefits of its application [9–11].

One of the possible strategies that could be utilized to reduce the risk of acute toxicity is the reduction of F composition along with supplementation by calcium and/or phosphate salts. Among the phosphate salts with anticariogenic activity, sodium trimetaphosphate (TMP) appears to be the most effective [12]. The addition of TMP to experimental gels with lower F content (4500 μg F/g) was shown to

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https://doi.org/10.1016/j.jdent.2017.10.013
Received 10 May 2017; Received in revised form 24 October 2017; Accepted 28 October 2017
0300-5712/

reduce demineralization and promote remineralization of tooth enamel similarly to conventional gels (9000 μg F/g and 12,300 μg F/g − Acid gel), as demonstrated in *in vitro* [13] and *in situ* [14] studies.

In situations of high caries risk, the combination of two topical methods, TAF and fluoride toothpaste (FT), has been suggested [15]. A randomized controlled clinical trial demonstrated that TAF with Acid gel associated with supervised brushing with FT showed there to be the similar capacity of remineralizing initial carious lesions when compared with FT alone [16]. On the other hand, an *in situ* study [17] that evaluated the remineralization of enamel lesions after daily applications of high concentration gel (12,300 μg F/g, Acid gel) associated with FT (1450 μg F/g) indicated there was an increase in mineral volume for FT associated with acid gel compared to FT, concluding that there was an association of increased F incorporation into lesions for the FT + Acid gel group when compared to just FT. With this, there is no consensus on the additional effect of the association of F methods for dental caries control [18].

Thus, the aim of this *in situ* study was to assess the effect of the association of low-F (4500 μg F/g) gel containing TMP and FT (1100 μg F/g) on enamel demineralization. The null hypothesis was that the association of treatments with low-F/TMP gel and FT would promote a similar reduction in demineralization when compared to the association of 9000 μg F/g gel and FT treatments.

2. Material and methods

2.1. Experimental design

This study was approved by the Institutional Review Board of São Paulo State University (Unesp), School of Dentistry, Araçatuba, Brazil (Protocol: 50723315.1.0000.5420), and all participants read and signed an informed consent form prior to study onset. This crossover double-blind study was conducted in five phases of seven days each. The sample size of volunteers was based on a previous study [19], considering the primary outcome from surface and cross-sectional hardness analysis, in terms of the mean difference between groups (30 and 1300, respectively), standard deviation (20 and 900, respectively), an α-error of 5% and a β-error of 20%. Volunteers (n = 12) aged 20 to 30 years who were in good general and oral health [20] wore palatal appliances initially containing four bovine enamel blocks (Fig. 1A) selected by initial surface hardness (SHi). The cariogenic challenge was performed with 30% sucrose (six times/day). Treatments were: no fluoride/TMP toothpaste − Placebo (PT); 1100 μg F/g toothpaste (FT); FT and 4500 μg F/g gel containing 5%TMP (FT + TMP gel); FT and 9000 μg F/g gel (FT + 9000 gel) and FT and 12,300 μg F/g (FT + Acid gel). After topical application of treatments for one min, two blocks were removed for analysis of loosely bound (CaF_2) and firmly bound (FA) fluoride formed in enamel; and calcium (Ca) and phosphorus (P) on the same blocks. After the seven-day experimental period, the percentage of surface hardness loss (%SH), integrated subsurface hardness loss (ΔKHN), CaF_2 and FA retained as well as Ca and P in enamel were determined. Moreover, biofilms were analyzed for F, Ca, P and insoluble extracellular polysaccharide (EPS) concentrations. Data were assess using one-way and two-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test ($p < 0.001$).

2.2. Gel/toothpaste formulation and fluoride/pH assessment

An experimental gel (i.e., 100 g) with neutral pH was prepared in the laboratory with the following ingredients: 8.0 g of carboxymethyl cellulose (Synth, Diadema, São Paulo, Brazil), 0.1 g sodium saccharin (Vetec, Duque de Caxias, Rio de Janeiro, Brazil), 28.0 g glycerol (Sigma-Aldrich Co., St. Louis, MO, USA) and 0.5 g of peppermint oil (Synth, Diadema, São Paulo, Brazil) adjusted with deionized water to 100 g. F (NaF, Merck, Darmstadt, Germany) was added to the gel at concentrations of 4500 (1.0 g of NaF) or 9000 μg F/g (2.0 g of NaF). Subsequently, TMP (Sigma-Aldrich Co., St. Louis, MO, USA) was added at a 5% concentration (5.0 g) to the gel with F concentrations of 4500 μg F/g. A commercial acid gel was used as positive control (12,300 μg F/g, Acid gel, pH = 4.5, DFL Indústria e Comércio S.A., Rio de Janeiro, RJ, Brazil). In addition, toothpastes were produced with the following constituents: 0.5 g of titanium dioxide (Sigma-Aldrich Co., St. Louis, MO, USA), 1.7 g of carboxymethyl cellulose (Sigma-Aldrich Co., St. Louis, MO, USA), 0.08 g of methyl *p*-hydroxybenzoate sodium (Sigma-Aldrich Co., St. Louis, MO, USA), 0.1 g of saccharin (Vetec, Duque de Caxias, Rio de Janeiro, Brazil), 0.5 g of peppermint oil (Synth, Diadema, São Paulo, Brazil), 26.6 g of glycerol (Sigma-Aldrich Co., St. Louis, MO, USA), 10 g of abrasive silica (Tixosil 73®, Rhodia, São Paulo, Brazil) and 1.7 g of sodium lauryl sulfate (Sigma-Aldrich Co., St. Louis, MO, USA) adjusted with deionized water to 100 g. NaF (Merck, Darmstadt, Germany) was added to the F toothpaste to reach a concentration of 1100 μg F/g. A toothpaste without F (Placebo) was also prepared.

The F concentrations in the gels and toothpastes were determined with a F ion-specific electrode (9609 BN; Orion Research Inc., Beverly, MA, USA) attached to an ion analyzer (Orion 720 A+; Orion Research

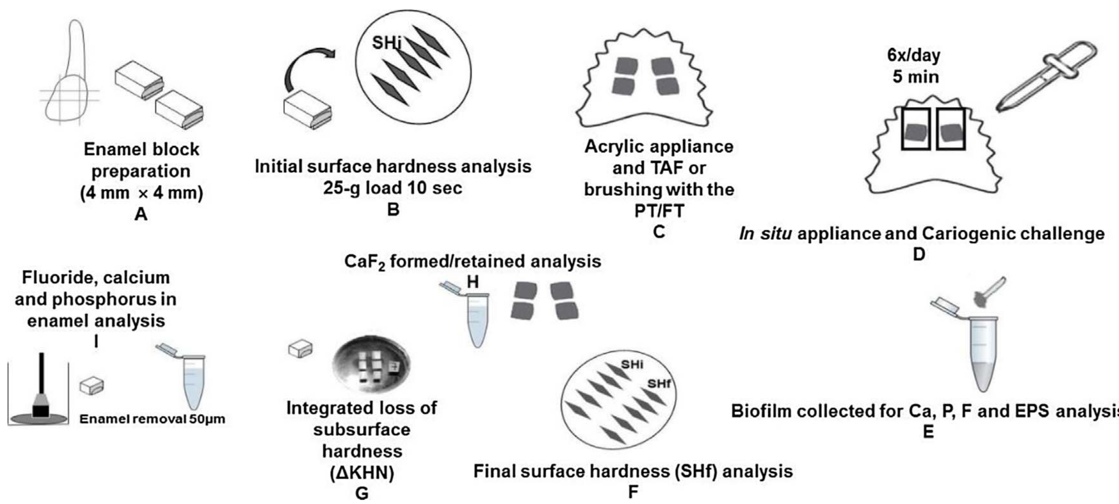

Fig. 1. A Enamel block preparation. B Initial surface hardness analysis. C Acrylic appliance and TAF or brushing with the PT/FT. D *In situ* appliance and Cariogenic challenge. E Biofilm collected for Ca, P, F and EPS analysis. F Final surface hardness analysis. G Integrated loss of subsurface hardness (ΔKHN). H CaF_2 formed/retained analysis. I Fluoride, calcium and phosphorus in enamel analysis.