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ORIGINAL ARTICLE

In vivo effects of fluoride on enamel permeability

Stefano Chersoni · Angelica Bertacci · David H. Pashley · Franklin R. Tay · Lucio Montebugnoli · Carlo Prati

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Abstract This in vivo study evaluated the effects of topical fluoride application on enamel by repeated scanning electron microscopy analysis of replicas. Baseline fluid droplets were employed as qualitative indication of enamel permeability. CaF_2-like globules were detected in vivo after fluoride application and were not found after professional brushing, ultrasound action, or chemical extraction. Absence of water permeability of enamel was demonstrated even after removal of CaF_2-like globules. Droplets reappeared within 1 h in sodium fluoride-treated teeth, but they did not reappear even after 1 week following topical enamel treatment with acidulated phosphate fluoride. Teeth treated with an acidulate fluoride-free solution showed lack of CaF_2-like globules and no droplets for at least 1 week as detected in acidulate phosphate fluoride-treated teeth. The caries-preventing action of fluoride may be due to its ability to decrease permeability and diffusion pathways. CaF_2-like globules seem to be indirectly involved in enamel protection over time maintaining an impermeable barrier, and phosphoric acid seemed to play an unexpected fluoride-independent preventive role.

Keywords NaF · APF · Enamel permeability · CaF_2-like globules · Phosphoric acid

S. Chersoni · A. Bertacci (✉) · L. Montebugnoli · C. Prati
Endodontics Unit, Department of Oral Sciences,
Alma Mater Studiorum-University of Bologna,
via San Vitale 59,
40125 Bologna, Italy
e-mail: angelica.bertacci2@unibo.it

D. H. Pashley · F. R. Tay
Department of Oral Biology, School of Dentistry,
Medical College of Georgia,
Augusta, GA, USA

Introduction

It has been demonstrated that most of the cariostatic effect of topical fluoride cannot be attributed to the incorporation of fluoride in the hydroxyapatite crystal lattice [1]. CaF_2 globular complexes, also called phosphate-contaminated calcium fluoride [2], are a major reaction product following topical treatment of dental hard tissues coating dental enamel [3–6]. Previous studies reported that calcium fluoride-like deposits are affected by pH [7], by the type of fluoride applied, and by the length of application [3, 4]. It has been suggested that CaF_2 acts as a pH-controlled reservoir of fluoride due to high solubility and to the release of a large amount of fluoride during dissolution [8]. The effect of these CaF_2 globules in fluoride-preventing action is still debated. Nevertheless, few simulated in vivo studies have been carried out to evaluate the retention of CaF_2 globules and the effect of fluoride treatment on human permanent enamel [9, 10], but the detection of CaF_2-like globules in vivo is still a challenge. Fluoride–tooth interactions following topical fluoride application in mature permanent teeth involves enamel surfaces, oral fluids, and fluids flowing through dental hard tissues [11]. Calcium fluoride might not only form a reservoir of fluoride but also may offer a more effective diffusion barrier than fluoridated apatite at the tooth surface [12]. No correlation between fluoride treatment and enamel permeability has been found, although it has been hypothesized by one study [13]. Recently, enamel permeability in vivo has been evaluated by replica technique [14].

The aim of this study was to evaluate in vivo changes in enamel structure produced by topical fluoride application to mature permanent teeth by scanning electron microscopy (SEM) inspection of sequential replicas made in vivo. The first tested null hypothesis was that topical fluoride

application in vivo does not produce CaF_2-like globular aggregates. The second test null hypothesis was that fluoride treatment did not affect enamel permeability in vivo.

Materials and methods

Eighty permanent sound upper central incisors from 40 subjects aged 25–42 years were selected after obtaining their informed consent. All the subjects did not use any fluoridated product at least during the previous week and in the week following the treatments. All subjects enrolled in the study gave their informed consent to the procedure, which was noninvasive and risk free.

Patients were randomly assigned to four groups (n=10). Two different fluoride treatments and a phosphoric acid-based solution fluoride-free were compared to control group: 10 mL of Oral-B Fluorinse® (Procter & Gamble, Cincinnati, OH, USA), 0.2% sodium fluoride (1,000 ppm F^-) at neutral pH, was maintained in the mouth for 2 min (NaF group); Fluorine Gel® (Dental Medical, Conegliano, Italy), an acidulated phosphate fluoride (APF) 0.33% NaF (1,300 ppm F) and phosphoric acid at pH 3, applied with a cotton-tip applicator for 2 min and then water-rinsed (APF group); a phosphoric acid-based solution (0.01%) fluoride-free at pH 3 applied with a cotton-tip applicator for 2 min and then water-rinsed (AP group); and not fluoridated mineral water was used as a control treatment (San Benedetto, Scorzè, Venezia, Italy).

Replicas were sequentially obtained in the right central incisors in order to study permeability variation. Before taking each impression, each tooth was professionally brushed with a prophylactic brush (Prophy minicups, Westpoint-Perident, Firenze, Italy) mounted on a rotary micromotor handpiece (4,000 rpm) for 30 s with a pressure adequate to bend brush bristles and finally air-dried for 10 s.

The impressions of the buccal surface of central incisors were made using polyvinylsiloxane (Affinis light body; Coltene, Altstätten, Switzerland). The material was allowed to set for 5 min. Then the impression was removed from the enamel surface and later cast in polyether impression material (Permadyne Garant; 3 M ESPE, St. Paul, MN, USA). After separation, the casts were gold-sputtered and inspected by scanning electron microscope (Model 5400, JEOL; Tokyo, Japan) accordingly to our previous study [14].

Baseline impressions of buccal enamel of central incisors of each subject were made. Immediately after each treatment, left incisors were washed and air-dried but not professionally brushed in order to avoid any removal from enamel surface before taking the impressions; then, the patients were dismissed and asked to return in 1 h. During this time, the patients could only drink water. When they returned, the right incisors were washed and air-dried but not professionally brushed before taking the impressions 1 h after treatment. Immediately after, same incisors were professionally brushed (rotary prophylaxis brush) and air-dried before getting impression. Additional sequential impressions were carried out 1 h later and after 1 week. Each patient at the end of the study, after the last impression, received a prophylaxis APF application.

Additional controls consisted of incisors from different subjects that separately received topical treatment as NaF group with Oral-B Fluorinse®. Two of these received 30 s of water-spray sonication (Castellini, Castel Maggiore, Bologna, Italy) with ultrasonic tips (EMS, Geneva, Switzerland) placed parallel to enamel surface with no pressure before taking an impression. Two-incisor enamel surfaces were adjusted to be parallel to the floor, and a 2-μL droplet of 1 M potassium hydroxide (KOH) was placed on the fluoride-treated surface to extract the surface globules [15]. After 5 min, the drop of KOH was carefully removed by absorbent paper, and the surface rinsed with water and air-dried was impressed. Four central incisors were brushed with an electric toothbrush with pressure control system (Oral B triumph, Oral B, Procter & Gamble, Cincinnati, OH, USA) and followed by taking impressions.

Evaluation and statistical analysis

For each sample, except replicas of unbrushed treated teeth that were not included in the statistical analysis, a representative area of 100 μm^2 was examined at ×2,000 by two operators, randomly examining, in a double-blind manner, and the mean value of the number of droplets observed was recorded.

One-way analysis showed that standardized skewness and kurtosis values were within the range expected for data from a normal distribution. After fitting a general linear model, ANOVA for repeated measures with split-plot design was performed to evaluate differences between treatments, time-related variations in the number of droplets, and the interaction between treatments and time-related variations in the number of droplets. Bonferroni's t test was applied as a multiple comparison t test for significant values.

Results

The results estimating a general linear model relating the number of droplets to three predictive values (treatment, time, and the interaction between treatment and time) showed a statistically significant relationship ($p<0.01$) with

all three predictive variables (F=371.8 concerning treatments, F=284.3 concerning time, and F=74.9 concerning the interaction between treatment and time), the R-squared statistic indicating that the model as fitted explains 95.13% of the variability in the number of droplets.

Statistical analyses of the permeability results are summarized in Table 1.

No significance between-treatment difference was found at baseline. All baseline replicas of incisors showed the presence of microscopic droplets on enamel surfaces (Fig. 1a, b) that represent outward fluid flow from enamel during the setting time of the material, arranged accordingly with a previous study [14]. Immediately (left incisors) or 1 h after (right incisors) either containing fluoride treatment, the replicas (unbrushed) revealed extensive deposition of globular aggregates of approximately 1 μ or less covering almost the entire surface (Fig. 1c, d). These globules although similar to fluid droplets were completely removable by rotary prophylaxis brush as demonstrated for both experimental groups that did not show any globular aggregates or droplets (Fig. 2a, b), while phosphoric acid treatment did not involve such globules deposition.

Professional brush fails to completely remove globules in the approximal area where only few single bristles of the brush reach this convex zone (Fig. 2d). Moreover, APF group is characterized by areas of slightly, inhomogeneous honeycomb morphology (Fig. 2b). Higher magnification of some of these areas displayed an uneven surface with pits and microroughness (Fig. 2c).

NaF-treated surfaces showed increase (p=0.01) in number of fluid droplets 1 h after CaF_2-like globules removal by professional brushing (Fig. 3a). On the contrary, APF group, after 1 h, showed absence of droplets (Fig. 3b). One week later, APF treatment droplets were still absent (Fig. 3c), and only two samples showed few localized areas covered with droplets as displayed (Fig. 3d).

Replicas obtained from AP group teeth showed the absence of droplets without any time-dependent variation compared to APF group also in absence of any globules deposition.

Table 1 Effects of treatments on enamel permeability

	Baseline	Treatments	1h later	1week later
NaF	39.3±7.4 a	1.7±0.7 b	36.7±5.1 a	38.3±6.6 a
APF	38.0±6.7 a	1.1±0.3 b	1.4±0.5 b	1.2±0.4 b
AP	37.6±6.4 a	1.2±0.4 b	1.2±0.4 b	1.4±0.5 b
Control	37.8±7.8 a	35.9±5.0 a	38.6±4.4 a	36.4±5.8 a

Values are means (±SD) of droplets/100 μ^2 in the four treatment groups at baseline, after treatments, 1 h later, and 1 week later. Significant differences between means are expressed by different letters

Control group did not show any significant statistical differences.

Ultrasound action on teeth that had been previously treated with topical fluoride application is able to remove CaF_2 aggregates on enamel surface that is free of water droplets (Fig. 4a).

Application of the strong base KOH removed the CaF_2 globules, while the surrounding area remained covered with such globules (Figs. 4b, c). Teeth brushed with the electric toothbrush after NaF fluoride treatment still showed CaF_2 globules (Fig. 4d).

Thus, topical fluoride treatment produced deposition of globular aggregates that were easily removed by rotary prophylaxis brushing, ultrasonic action, or chemical extraction with KOH. In the presence of CaF_2 globules, the enamel permeability remained low 1 h after treatment, but after removal of CaF_2 globules by brushing, the fluid permeability returns to pretreatment levels within 1 h in neutral NaF-treated samples, while permeability remained low until 1 week, for acid treatment irrespective of the presence of fluoride.

Discussion

The major effect of topical fluoride treatment is the formation of CaF_2-like globules on the enamel surface or in decalcified enamel lesions [16–18]. This globular surface material is often combined with phosphates or proteins and is regarded as being relatively insoluble [19, 20] but is reported to be lost from enamel surface over time in a period ranging from days to weeks, as result of daily brushing and mastication [4, 21]. Therefore, some researchers have argued that these deposits provide not more than a limited protective capability [22]. The present study confirmed that these surface deposits are removed mechanically and are unlikely to be responsible of the long-term effect. It has been suggested that CaF_2 is formed not only on surfaces but also to some extent into the underlying enamel [22, 23], but TEM examination fails to confirm this [8, 24]. We speculate that topical fluoride treatments reduce enamel permeability by creating submicron calcium fluoride precipitates along the imbrication lines between enamel prisms where there is more water and protein [25]. Using acidic fluoride-containing gel at a pH of 3, which is below the critical pH of enamel, the acidic gel creates higher local calcium concentrations in “enamel water” than normally would occur that would tend to precipitate more submicron calcium fluoride or less soluble calcium form. This results in a prolonged reduction in enamel fluid permeability. Fluoride reduces permeability, and fluid movement in this way not only can enhance precipitation and remineralization but also can prevent deeper ions

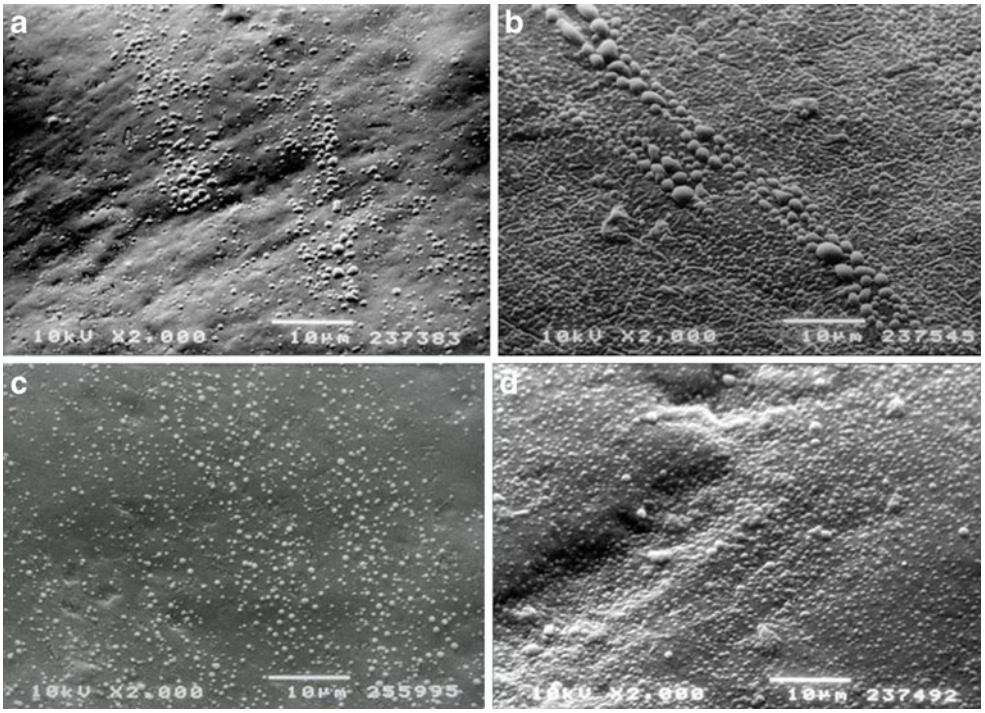

Fig. 1 SEM replicas at baseline and after fluoride treatments for each group in the same subject. **a** Baseline of NaF group showed many fluid droplets; **b** baseline of APF group showed a similar morphology. **c** NaF-treated enamel displayed a deposition of CaF_2-like globular aggregates; **d** the same globular aggregates are present after APF treatment

fluoride diffusion and explain fluoride enamel outer deposition [12]. On the other hand, a recent study showed that the underlying enamel surface showed a 22% increase in the fluoride content after removal of surface CaF_2 using KOH compared with control enamel [22]. KOH-soluble fluoride (unbonded fluoride) significantly inhibited caries lesion development [16], while the increase in KOH-insoluble fluoride (firmly bounded or apatitically incorporated fluoride) had apparently no clinical significance and that even pure fluorapatite has been shown to have a limited cariostatic potential effect in intra-oral models [1]. Professional cleaning with a rotating prophylaxis brush is

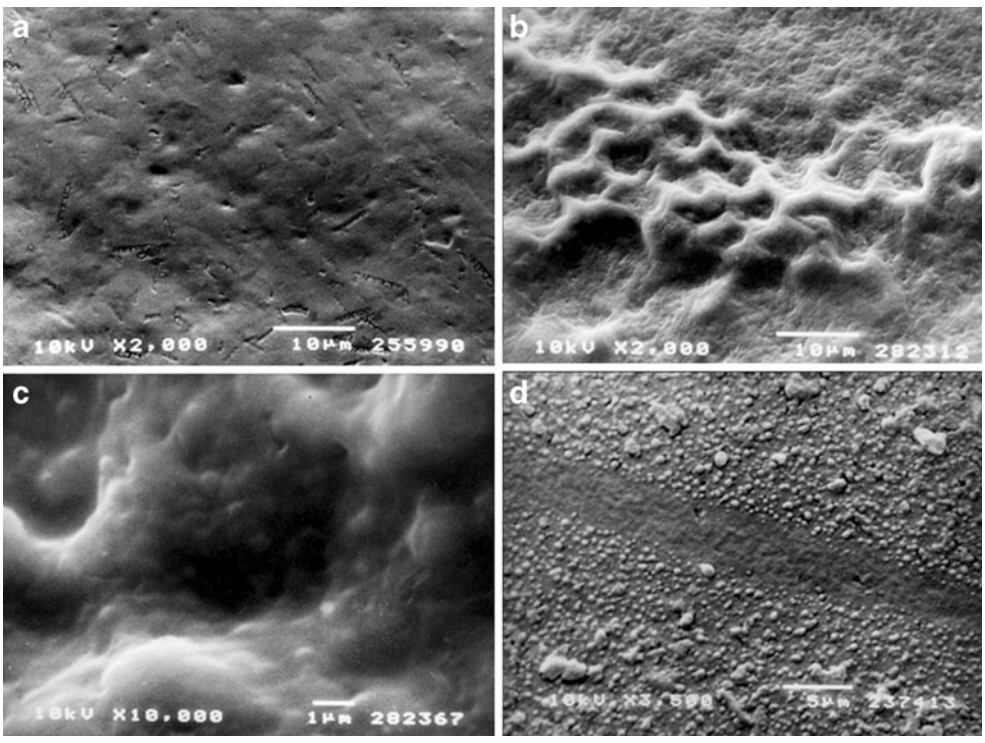

Fig. 2 SEM replicas of fluoride treated after CaF_2-like globules removal by brushing **a** NaF group showing no CaF_2-like aggregates and no water droplets observable; **b** APF group displaying the typical honeycomb appearance of etched enamel surface. No CaF_2-like aggregates and no droplets observable. **c** Higher magnification of this area revealed an uneven surface with pits and micro-roughness. **d** SEM photomicrograph of approximal enamel showing the detail of a stripped area where CaF_2 globules have been removed by a single bristle of the brush in a NaF-treated sample

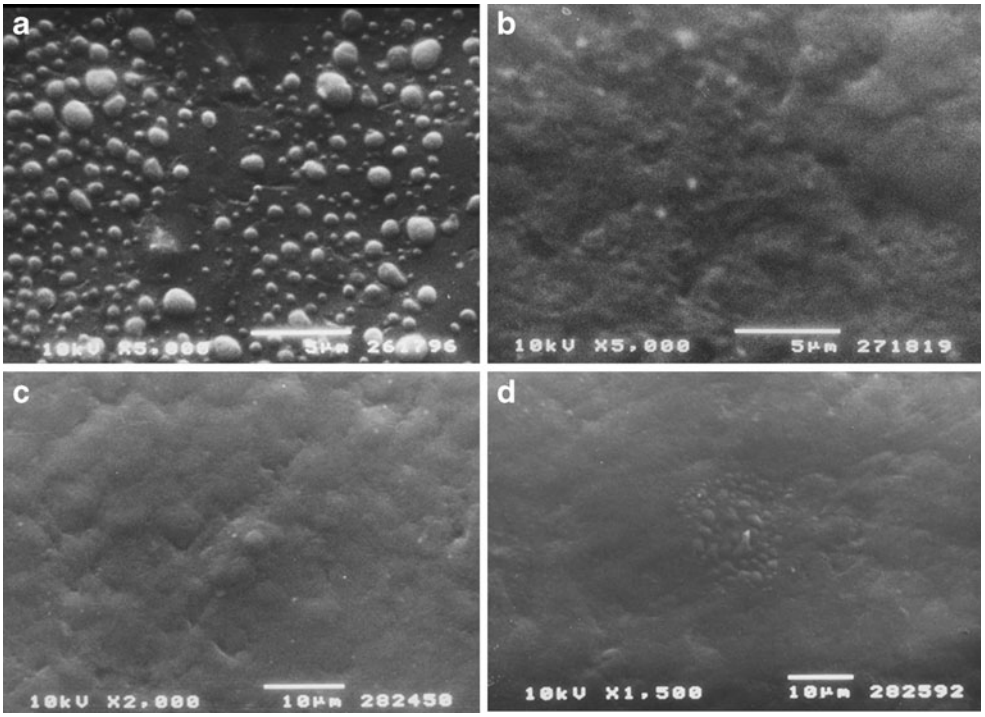

Fig. 3 SEM replicas of fluoride-treated enamel 1 h or 1 week after CaF_2-like globules removal: **a** NaF sample showing many fluid droplets 1 h after; **b** APF replicas showing no droplets after 1 h. **c** After 1 week, APF group still showed absence of fluid droplets. **d** Representative image of two samples that showed few localized areas covered with contiguous bounded droplets after 1 week

unlikely to remove apatitically bound fluoride, and so, the present study suggested that this one did not affect permeability supporting the paramount relevance of permeability in caries.

Present study demonstrated that fluoride-containing globules could be removed in vivo by a sonic scaler, by a rotary brush, and by KOH and that daily toothbrushing did not significantly affect this protective layer according to Tenuta et al. [26]. We speculate that although surface CaF_2 deposits are mechanically removable, their anticaries activity could be ascribed either to transient decreases in enamel permeability or subsurface uptake of calcium fluoride. The fact that enamel exposed to fluoride and professionally brushed had neither CaF_2-like globules nor

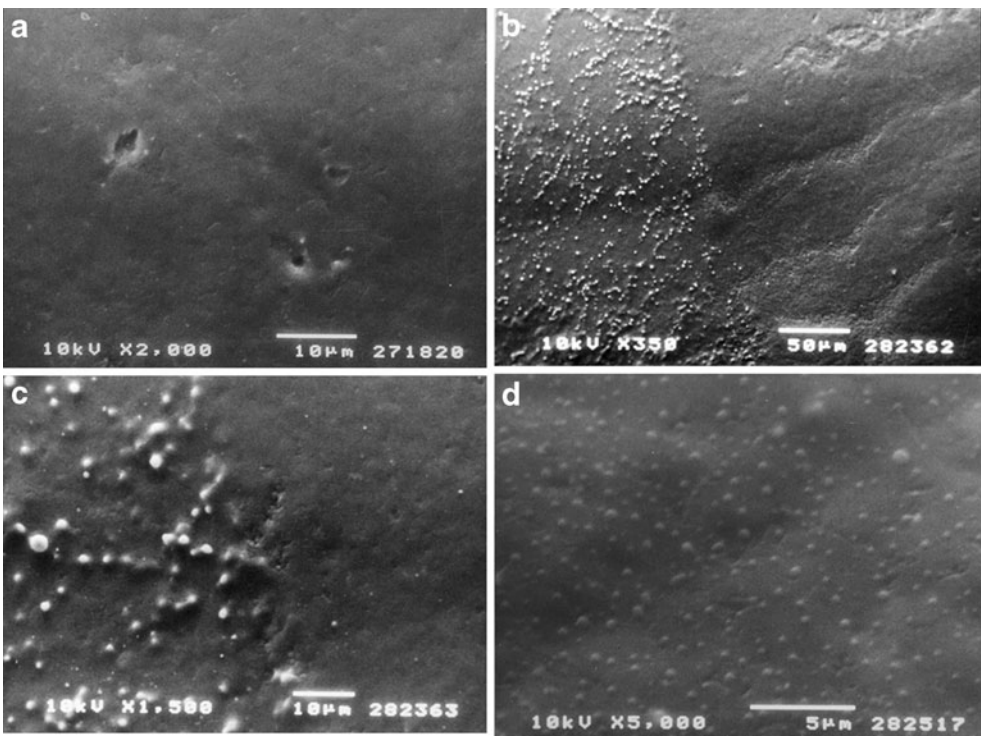

Fig. 4 SEM replicas of additional controls obtained from NaF-treated enamel. **a** After sonication of NaF-treated enamel, no CaF_2-like globules or fluid droplets were detected. **b**, **c** Low and higher power micrographs after KOH treatment; note the presence of globules of CaF_2 deposits on the left side and their absence on the right side due to the droplet of KOH that extracted the globules. **d** Partial removal of CaF_2-like globules after brushing with an electric tooth brush

fluid droplets meant that fluoride treatment of enamel temporarily reduced enamel permeability even in the absence of surface CaF_2-like globules.

It is likely that fluoride treatments produce relatively insoluble [2] precipitates of fluoride in subsurface interprismatic porosities or other products as demonstrated by Gerth et al. [27] that blocked outward fluid movement but that solubilize within 1 h. On the other hand, acidulated treatment reduced enamel permeability at least for 1 week. The low pH may solubilized calcium and phosphate and created more complex relatively insoluble salts. Thus, calcium fluoride deposition may be the outward manifestation of relatively insoluble subsurface blockage of enamel surface that may block enamel pores [11] and other diffusion pathways [3, 25].

The effectiveness of a topical fluoride agent may be related to the changes that fluoride produces in enamel permeability, and these changes could be related to the pH of the fluoride solution [28], with a more prolonged effect in permeability reduction demonstrated for acidic APF gel in this study. This finding could explain why acidic fluoride solutions are known to be more effective in caries prevention [29, 30] and in reducing early enamel erosion in comparison to neutral fluoride formulations [31]. Lack of control studies about the preventive role of phosphoric acid alone confounds the role of fluoride in APF formulations and does not allow to establish the effective role of fluoride. On the best of our knowledge, this is the first study that evaluated the influence of phosphoric acid alone on enamel permeability in absence of fluoride, recognizing the effect of the acid component of the APF gel. The results obtained in this study showed the absence of enamel permeability persisting for at least 1 week. Moreover, in vivo 30-s application of 37% phosphoric acid to enamel prevented droplet formation for at least 6 months in orthodontic patients (unpublished results). The existence of a precipitation of calcium phosphate crystals at low pH could explain the long-lasting effect of phosphoric acid etching on enamel permeability. Obviously, this hypothesis required further clinical and laboratory tests to be confirmed.

That being so phosphoric acid etching in enamel adhesive procedures could play an unexpected preventive role. This suggests, for example, that the respective effect of acid and resin during enamel fissure sealing should be further evaluated.

Otherwise, phosphoric acid-containing fluoride products are thought to be clinically more effective in caries prevention [32], but the true mechanism is still unclear. As the pathogenesis of caries and erosion involves acid demineralization, a possible bias on the real effect of phosphoric acid might exist, and it could be hypothesized that different acids could set different effects on enamel.

The induced decrease of enamel permeability could have clinical relevance. It has been speculated that specific occlusion of the pathways of outward fluid flow may increase caries resistance considering the negative correlation between posteruptive enamel maturation and caries incidence [14]. We speculate that calcium fluoride can simulate a reversible posteruptive enamel maturation effect that promotes remineralization solely by occluding diffusion pathways and indirectly enabling mineral precipitation.

The cariostatic effect of fluoride could be achieved by blocking demineralizing episodes, creating transient reductions in enamel permeability, rather than the reinforcing of the enamel surface, in as much as the deposited layer is potentially lost in a short time [22].

Superficial calcium fluoride-like globules seem to be indirectly involved in enamel protection over time since their removal did not immediately restore enamel permeability to outward fluid flow. The presence of these globular deposits of CaF_2 may protect enamel and prolong the presence of subsurface salts by delaying their solubilization.

In conclusion, when enamel surfaces were treated with NaF mouth rinse and with acidulated fluoride gel, the CaF_2 globules deposited on the surface blocked outward fluid movement through enamel, and this result requires rejection of both the tested null hypotheses: Fluoride treatment in vivo allowed the deposition of CaF_2-like globules and affected at least temporary reduced enamel permeability. The application of phosphoric acid produced a more lasting effect.

Results support a fluoride role in enamel permeability reduction although did not exclude additional mechanisms. If the caries preventive action of fluoride mainly depends on decreasing enamel permeability, new substances with longer effects could be tested. Further studies will be necessary to confirm this hypothesis. The proposed mechanism of the caries-preventing action of fluoride supports the evidence that the primary effect of fluoride is posteruptive [33, 34].

Conflict of interest The authors declare that they have no conflict of interest.

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