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IN VITRO EVALUATION OF EXPERIMENTAL FLUORIDE TAPE IN INHIBITION OF ENAMEL DEMINERALIZATION

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Abstract

The aim of this *in vitro* study was to evaluate the effectiveness of experimental 2.26% fluoride-polyvinyl alcohol (F-PVA) tape in inhibition of enamel demineralization using enamel surface microhardness (SMH) analysis and scanning electron microscopy (SEM) examination.

Enamel specimens (n=60) randomly assigned to four groups: control group, F-PVA tape group, fluoride varinish (F-varnish) group, casein phosphopeptide-amorphous calcium phosphate (CPP-ACFP) group. After topical application, pH-cycling was processed. Then, SMH was measured and the percentage loss of surface microhardness (%SML) was calculated. For the SEM examination, five sample specimens in each group were treated and the morphologic character was evaluated.

After pH-cycling, the SMH values of the enamel specimens of F-PVA tape and F-varnish group were significantly higher than that of CPP-ACFP group, there was no significant difference between F-PVA tape and F-varnish group. With SEM examination, enamel surfaces in the F-PVA tape group and F-varnish group showed numerous spherical and ovoid crystals formed on the enamel surface were also observed. The density of crystals was higher than that of both control group and CPP-ACFP group.

F-PVA tape is effective in inhibition of enamel demineralization. Also, F-PVA tape's inhibition of enamel demineralization is comparable to that of F-vanish and greater than that of CPP-ACFP.

Key words : Fluoride tape, PVA tape, Enamel demineralization

Ⅰ. Introduction

Demineralization and remineralization are dynamic processes in the initiation, progression, and reversal of dental caries. Signs of the caries process cover a continuum from the first molecular changes in the apatite crystals of the tooth, to a visible white-spot lesion, through to dentin involvement and eventual cavitation. Progression through these stages requires a continual imbalance between pathologic and protective factors that results in the dissolution of apatite crystals and the net loss of calcium, phosphate, and other ions from the tooth. Therefore, regulation of the demineralization-remineralization balance is a key to prevention of dental caries. A goal of modern dentistry is to manage non-cavitated carious lesions non-invasively through remineralization in an attempt to prevent disease progression and thereby improve esthetic, strength, and function of tooth[1-3].

Fluoride use has been credited with playing a major role in the reduction of dental caries in the pediatric population. In fact, the proportion of people entering adulthood without caries has increased dramatically[4-6]. The widespread use of fluoride in dentifrices, mouthrins-

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es, fluoridated varnishes and gels all have helped to reduce the prevalence of dental caries[7,8]. To improve convenience and effectiveness of fluoride treatment while minimizing the adverse effect of overdose-uptake, various forms of fluoride delivery materials have been developed. Fluoride varnish has been used as a professional topical fluoride agent and adheres to the tooth surface, which potentially prevents complications from children's swallowing but it has major disadvantages such as stickiness, bitter taste, and unesthetic appearance after application. To overcome those disadvantages, we have developed a fluoride-polyvinyl alcohol tape (F-PVA tape) as an experimental fluoride delivery material.

The discovery of PVA dates back to 1924, when a PVA solution was obtained by safonifying poly (vinyl ester) with caustic soda solution. PVA has excellent physical properties, such as viscosity, film forming, emulsifying, dispersing powder, adhesive strength, tensile strength, and flexibility. PVA is also resistant not only to water, but also to oil, grease, and solvents. Based on these versatile properties, PVA has been widely used, especially in fabric and paper sizing, fiber coating, adhesives, emulsion polymerization, films for packing and farming, and the production of polyvinyl butyral. PVA has also been used in manufacturing medical materials such as hydrogel to mimic soft tissue, drug delivery system, biosensor, bioreactor, antitumor agent, hemostatics. PVA is non-toxic to organisms and has biodegradibility. Most of the PVA-degraders are Gram-negative bacteria belonging to the genus Pseudomonads and Sphingomonads, but Gram-positive bacteria with PVA-degrading abilities have also been reported[9-11].

This *in vitro* study was designed to evaluate the effectiveness of experimental F-PVA tape in inhibition of enamel demineralization, through enamel surface microhardness (SMH) analysis and scanning electron microscopy (SEM) examination.

Ⅱ. Materials and methods

1. Study design

The study design is shown in Fig. 1.

2. Preparation of F-PVA tape

PVA (10 g) and polyacrylic acid (5 g) were added to 85 g of distilled water and the mixture was stirred for 2

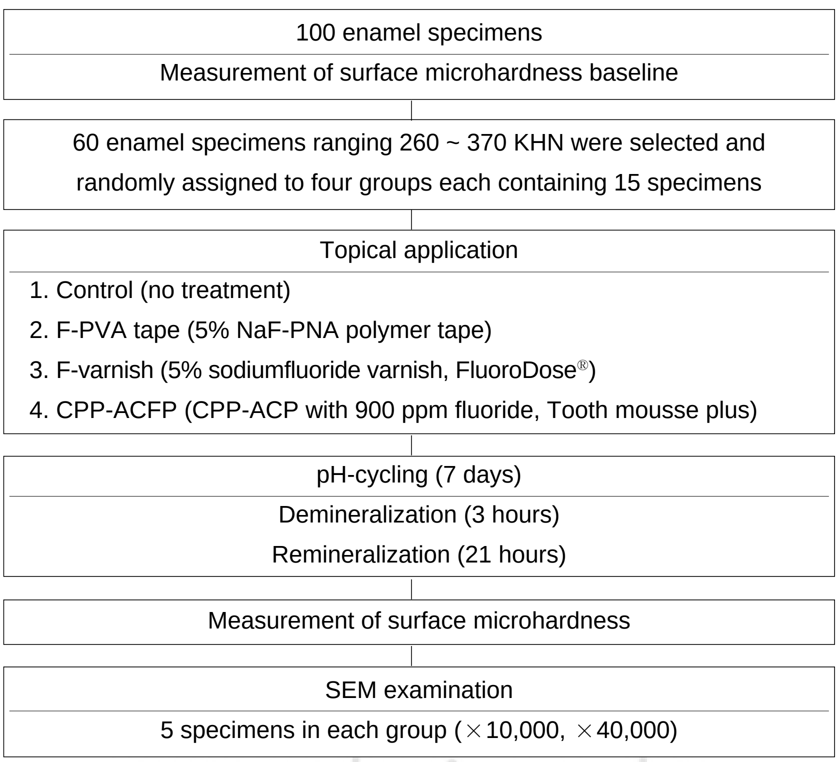

Fig. 1. Illustration of the study design.

hours at 85℃. Polyethylene glycol (3 g), as a plasticizer, and NaF (0.95 g) were added progressively and stirred for 2 hours at 85℃. Then, the mixture was poured onto a glass plate, and spread to a uniform in width (20 ㎛) using an applicator, and then dried for 24 hours at 60℃ (Fig. 2)[12]. The F-PVA tape is shown in the Fig. 3.

3. Specimen preparation

One hundred enamel blocks (5 mm × 5 mm) were prepared from bovine incisor teeth, which were freshly extracted. The teeth were cut using diamond cutting disks (Komet, USA). Custom made plastic cylindrical molds were prepared and self cured acrylic resin (Dentsply International Inc., USA) was poured on it; then each enamel block was embedded in, on top partially set, and allowed to set. Specimens were then polished flat progressively with 400, 800, 2400 and 4000 grit silicon carbide sandpapers (R&B Inc., Korea) on a rotating polishing machine (METPOL-1, R&B Inc., Korea) and stored in distilled and deionized water.

Enamel specimens (n=60) with microhardness ranging from 260 to 370 Knoop hardness number (KHN) were pooled and randomly assigned to four groups, each containing fifteen specimens.

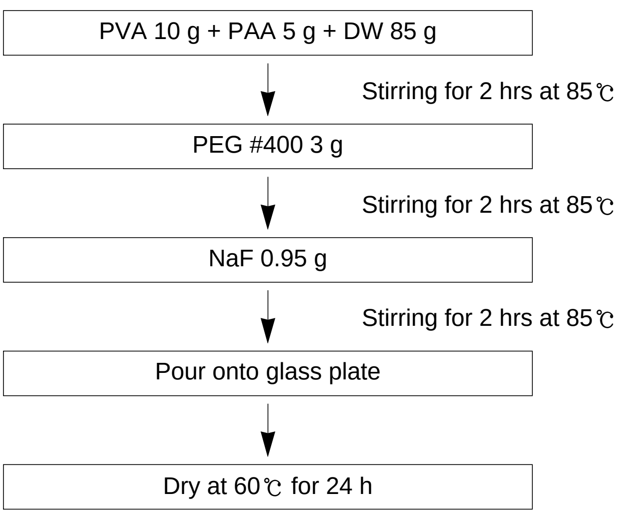

Fig. 2. The procedure for preparation of F-PVA tape.

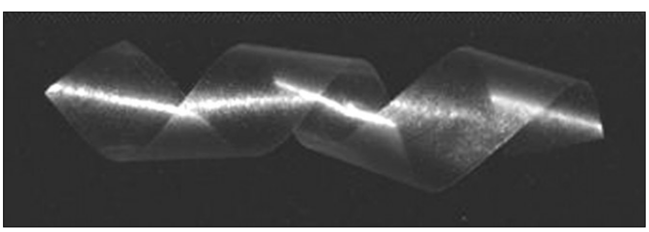

Fig. 3. The experimental F-PVA tape.

4. Topical application of agents

The following four experimental groups were used in this study:

(1) No treatment (control group). No treatment was given to the enamel surface, and the teeth were kept in the distilled and deionized water for 6 hours.

(2) 2.26% F-PVA tape (F-PVA tape group, developed material experimentally). A piece of tape, sized 5 mm × 5 mm, was put onto each enamel specimen, and soaked with distilled and deionized water using microbrush for fluoride ions to dissolve from it and act at the enamel surface for 6 hours. Then it was delicately removed from the enamel surface using cotton tips immersed in deionized water, without rubbing.

(3) 2.26% fluoride varnish (F-varnish group, FluoroDose®, Centrix Inc., USA). A thin layer of fluoride varnish was applied using a microbrush, left to act at the enamel surface for 6 hours, and then delicately removed from the enamel surface using cotton tips immersed in deionized water, without rubbing. No chemical substances were used for the removal of the varnish in order to not alter the enamel surface.

(4) Casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP group, GC Tooth mousse plus™, Recaldent, GC Corp., Japan). A thin layer of CPP-ACFP creme was applied using a microbrush, left undisturbed for 5 minutes and then washed with deionized water. Specimens in this group underwent the same application process twice a day (8:00 am, 8:00 pm) as recommended in the product manual for 7 days of pH-cycling.

5. pH-cycling

A pH-cycling regimen included alternative demineralization (3 hours) and remineralization (21 hours) for 7 days. Using separate containers, the specimens were immersed (30 mL/specimen) in the demineralizing solution [2.2 mM $Ca(NO_3)_2$, 2.2 mM KH_2PO_4, 50 mM acetic acid, pH 4.5] at 37℃, for 3 hours. After thorough rinsing with deionized water and careful drying, the specimens were stored (15 mL/specimens) in remineralizing solution [1.5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.9 mM $NaH_2PO_4 \cdot 2H_2O$, 150 mM KCl, 0.1 mM Tris buffer, 0.03 ppm F, pH 7.0] at 37℃, for 21 hours. The demineralizing and remineralizing solutions were changed every cycle[13-15].

6. Assessment of surface microhardness (SMH)

Enamel demineralization was measured as surface softening with a Knoop microhardness diamond in different regions of the specimens (Knoop diamond, 100 g, 5 s, HMV 2; Shimadzu Corporation, Tokyo, Japan) at baseline and after pH-cycling. All readings were performed by the same examiner using the same calibrated machine. In each reading, four indentations, at least 100 ㎛ apart, were made and their average was taken to represent the specimen's hardness value. Additionally, the percentage loss of surface microhardness was calculated using the following calculation[16,17]:

$$\%SML = 100\,(KHN_{(baseline)} - KHN_{(after\ pH\text{-}cycling)})/KHN_{(baseline)}$$

7. SEM examination

For the SEM examination, five sample specimens in each group were treated. Air-dried sample specimens were sputtered with platinum, resulting in platinum coating. Then, the shapes of the enamel surface were evaluated with SEM (S4700, Hitachi, Japan). For comparison of the morphologic character of the enamel surface, sound and demineralized enamel were also examined. For the latter, five enamel specimens were immersed in demineralizing solution for 21 hours as the same period other experimental groups had been immersed in it throughout the pH-cycling.

8. Statistical analysis

All data were processed by SPSS 17.0 software. The SMH values were compared at the different time intervals in each group with the paired samples *t*-test at a significance level of 0.05. The effects of the F-PVA tape, F-varnish, and CPP-ACFP on the changes in the enamel SMH were analyzed using one-way ANOVA and Student-Newman-Keuls post hoc test at a significance level of 0.05.

Ⅲ. Results

1. Surface microhardness

The average SMH values of the surface enamel in each group measured at the different time intervals during the treatment are shown in Table 1. The pH-cycling process for 7 days significantly reduced SMH in each group. The SMH values at the baseline were not significantly different among the experimental groups. Table 2 shows the SMH values and %SML among the groups. After pH-cycling, enamel specimens treated with remineralizing agents (F-PVA tape, F-varnish, and CPP-ACFP) presented significantly higher SMH and lower %SML values in comparison to the control group. The SMH values of the enamel specimens of the F-PVA tape and the F-varnish group were significantly higher than that of the CPP-ACFP group. Also, there was no significant difference between F-PVA tape group and F-varnish group. Regarding %SML, the values obtained in the F-PVA tape and F-varnish group were significantly lower than that of CPP-ACFP group.

2. SEM morphological characters

The typical SEM images of the enamel surface in the different groups are shown in Fig. 4 and Fig. 5. Sound enamel has an orderly arranged rod appearance and enamel crystals are homogeneously arranged with a

Table 1. Surface microhardness at baseline and after pH-cycling in each group (mean±SD)

Group	SMH (baseline)	SMH (after pH-cycling)	*p*-value
Control	303.62±25.34	123.00±20.00	0.000
F-PVA tape	304.39±36.03	178.52±20.60	0.000
F-varnish	306.75±35.90	173.57±16.71	0.000
CPP-ACFP	322.63±25.93	166.33±15.45	0.000

Table 2. Analysis of surface microhardness and %SML (mean±SD)

Group	SMH (baseline)	SMH (after pH-cycling)	%SML
Control	303.60±25.34[a]	123.00±20.00[a]	59.27±4.25[a]
F-PVA tape	304.39±36.03[a]	178.52±20.60[b]	40.80±5.83[b]
F-varnish	306.75±35.90[a]	173.57±16.71[b]	42.73±6.71[b]
CPP-ACFP	322.63±25.93[a]	166.33±15.45[c]	48.13±8.97[c]

[a,b,c] Different letters in each column indicate significant differences among groups by Student-Newman-Keuls post hoc test ($p<0.05$).

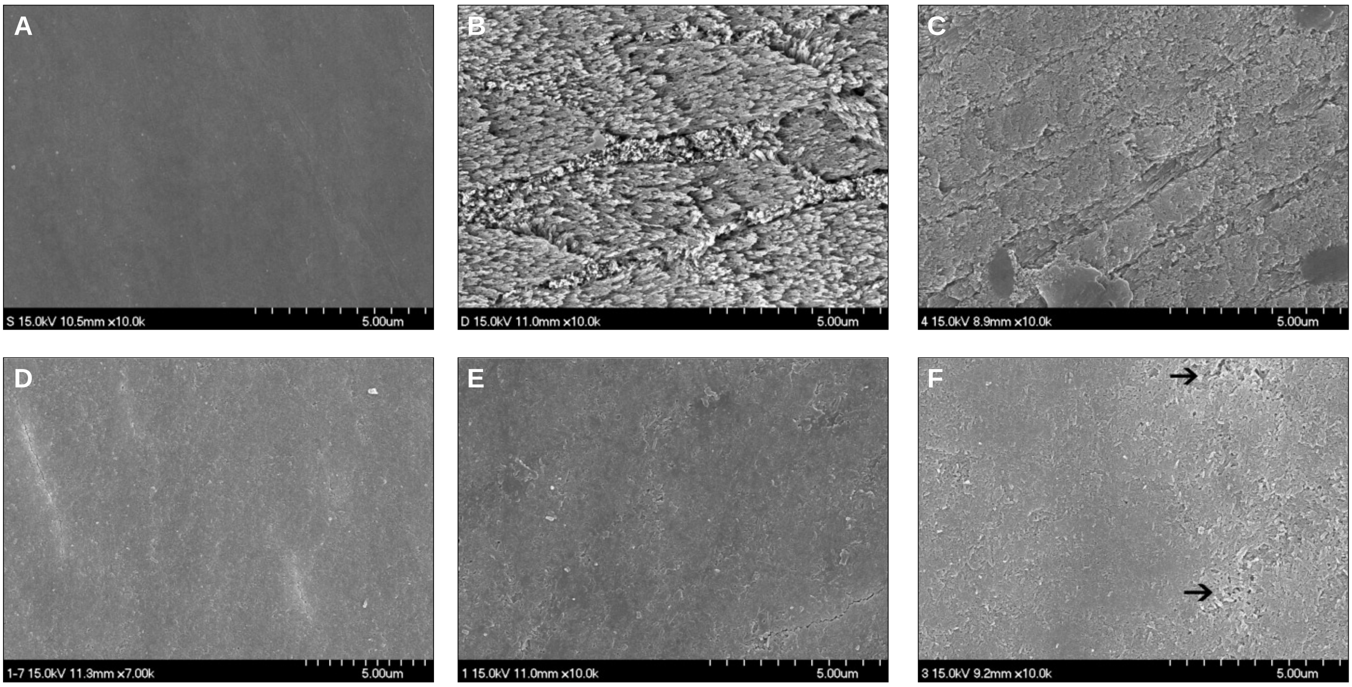

Fig. 4. The SEM images of the enamel surfaces in each group (× 10,000).
(A) Sound enamel : enamel rods are orderly arranged, (B) After demineralization : interrod and intercrystal spaces are prominent, (C) Control : interrod spaces are discernable, (D) F-PVA tape : enamel surface shows mild irregularity, (E) F-varnish : enamel surface shows mild irregularity and it is similar with that of F-PVA tape, (F) CPP-ACFP : the irregularity of enamel surface was more prominent than in F-PVA tape and F-varnish group, and some pot-holes were observed (arrows).

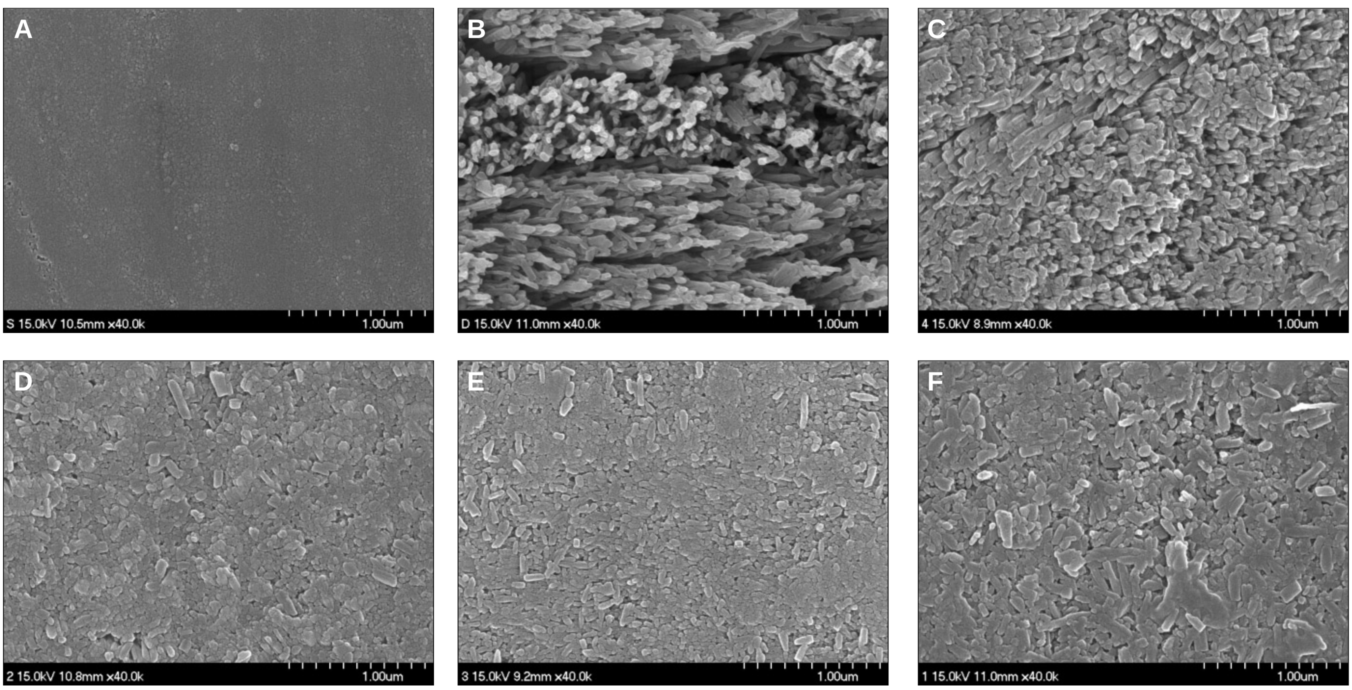

Fig. 5. The SEM images of the enamel surfaces in each group (× 40,000).
(A) Sound enamel : enamel crystals were homogeneously arranged with a clear outline, (B) After demineralization : demineralized enamel had a smaller number of enamel crystals and interrod and intercrystal spaces were very prominent, (C) Control : interrod spaces were discernable in low magnification, and the density of enamel crystals was lower than in other groups, (D) F-PVA tape : numerous spherical and ovoid crystals formed on the enamel surface were observed, and the density of crystals was higher than that of control group (E) F-varnish : numerous spherical and ovoid crystals formed on the enamel surface were observed, and it is similar with that of F-PVA tape, (F) CPP-ACFP : Enamel crystals were irregularly arranged with variable widths and some were fused together and showed obvious intercrystal spaces.

clear outline. For comparison, the surfaces of the demineralized enamel were also examined. They were immersed in demineralizing solution for 21 hours, which is the same period other experimental groups had been immersed in demineralizing solution throughout the pH-cycling. Demineralized enamel has a smaller number of enamel crystals and the interrod and intercrystal spaces are very prominent. In the control group, the interrod spaces were discernable in low magnification and the density of enamel crystals was lower than in other groups. In the F-PVA tape group, enamel surface showed mild irregularity in general and numerous spherical and ovoid crystals formed on the enamel surface were observed. The density of crystals was higher than that of demineralized specimens. Similar patterns were observed in the F-varnish group. In the CPP-ACFP group, the irregularity of the enamel surface was more prominent than in F-PVA tape and F-varnish group and some potholes were observed. Enamel crystals were irregularly arranged with variable widths and some were fused together and had obvious intercrystal spaces.

Ⅳ. Discussion

The mineral loss or gain in the enamel as a result of demineralization or remineralization can be measured as changes in the enamel surface microhardness. The indentation hardness test with either the Knoop indenter or the Vicker indenter has been used for measurements of initial enamel hardness, enamel softening after demineralization, and enamel hardening after remineralization. Both these indenters are suitable for hardness testing of nonmetallic materials. The measurement of the Knoop long diagonal is less affected by elastic recovery than the short diagonal or equal diagonals of the 136° diamond pyramid of the Vicker indenter. The Knoop hardness number has been correlated with the volume percentage of the enamel minera[11,18,19].

The study design required a sufficiently flat enamel area to allow enamel SMH measurement. Therefore, the area subjected to the pH-cycling was not the original surface of enamel. Moreover, a decrease in enamel SMH caused by pH-cycling in the polished enamel could be different from that obtained with the uncut enamel. Removal of the outer layer of the enamel made the enamel more susceptible to the demineralization[1].

Based on results from the previous study by ten Cate, the pH-cycling model was composed of 3-hour demineralization and 21-hour remineralization. The demineralization period was designed to simulate the duration of demineralization (low cariogenic challenge) that occurs in the oral cavity[20]. The composition of demineralizing solution was as follows: 2.2 mM $Ca(NO_3)_2$, 2.2 mM KH_2PO_4, 50 mM acetic acid (pH 4.5)[14]. The composition of the remineralizing solution was as follows: 1.5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.9 mM $NaH_2PO_4 \cdot 2H_2O$, 150 mM KCl, 0.1 mM Tris buffer, 0.03 ppm F (pH 7.0)[15].

Ideally, remineralizing agents need to rapidly precipitate on the partially demineralized tooth structure and transform into a more stable, less acid-soluble apatite than the hard tissue being replaced. They would need to do this in the presence of saliva and before the next acid challenge comes in contact with the newly precipitated mineral. If the mineral phase that is formed is soluble in saliva or under acid conditions, it will be rapidly lost. On the other hand, mineral that is taken up by the enamel may serve as a reservoir that could be released into fluid phase surrounding the enamel crystals during a caries attack and serve as a substrate for subsequent remineralization[21].

One of the important and well-documented methods for reducing dental caries is fluoride. Fluoride is known to reduce caries in three ways: inhibiting bacterial metabolism of fermentable carbohydrates, enhancing remineralization by the incorporation of available fluoride into the tooth structure during acid attacks, and reducing the tooth's solubility during subsequent acid attack[22]. The widespread use of fluoride in dentifrices, mouthrinses, fluoridated varnishes and gels all have helped to reduce the prevalence of dental caries[7]. Although various modes of delivery for professionally applied fluoride exist, one form has emerged that combines caries prevention efficacy with safety and versatility. The American Dental Association Council on Scientific Affairs recommends fluoride varnish as the only professionally applied fluoride for moderate to high risk patients of all age groups. In addition to demonstrating effectiveness equivalent to fluoride gels, fluoride varnish provides improved safety and acceptability[23]. There is ample clinical evidence that biannual application of fluoride varnish decrease dental caries. Repeated applications within a shorter period have been shown to result in a great reduction of caries[24].

The experimental F-PVA tape contains 2.26% fluoride similar to the amount in fluoride varnish (FluoroDose®, Centrix Inc., USA). Supposing application of 2 tapes, sized 1 cm × 12 cm, in maxillary and mandibular arches respectively, the content of fluoride administered from the tape is 2.35 mg and it is about one third of that of FluoroDose® (6.79 mg). As mentioned in the results, the inhibition potential of enamel demineralization of the experimental F-PVA tape is comparable with commercially used fluoride varnish.

Previously, we conducted an experiment to evaluate the fluoride-releasing efficacy of the F-PVA tape as compared with fluoride varnish (CavityShieldTM, 3M ESPE OMNI Preventive care INC., Bentonville, USA). We measured the concentration of fluoride in saliva samples of subjects, and found fluoride-releasing efficacy of F-PVA tape and it's comparable with that of fluoride varnish. Thus, we speculate that the effect of F-PVA tape is not limited to the buccal surface to which it adheres, and its effect extents to the proximal and occlusal surfaces[12,26].

The tape is transparent and therefore esthetic after application. It does not cause any discomforts associated with fluoride varnish such as stickiness or bitter taste. Moreover, the tooth surface does not need to be dried before application of the F-PVA tape since saliva on the tooth surface can help the F-PVA tape to adhere to the tooth. Although F-PVA tape does not have adhesive property itself, when it is moistened with saliva it can adhere to the tooth surface. So, the application procedure is simple. F-PVA tape provides improved safety and acceptability. It is possible to use it not only in professional fluoride treatment, but also in home-use.

Casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP) was used as another remineralizing agent. For every 2 fluoride ions, 10 calcium ions and 6 phosphate ions are required to form one unit cell of fluorapatite [$Ca_{10}(PO_4)_6F_2$]. Hence, on topical application of fluoride ions, the availability of calcium and phosphate ions can be the limiting factor for net enamel remineralization to occur. Tooth Mousse™ contains 10%w/w CPP-ACP nanocomplex. This product has been used clinically in several published reports for successful non-invasive treatment of mild to moderate fluorotic lesions and for the reversal of early caries and also for caries stabilization[27]. According to an *in vitro* study by Zhang Q, *et al.*[1], the casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) creme is effective in remineralizing early enamel lesion of primary teeth, a little more effective than 500 ppm NaF. On the other hand, Lata S, *et al.*[13], concluded that CPP-ACP creme is effective, but to a lesser extent than fluoride (Fluorprotector, Ivoclar Vivadent, containing 1000 ppm NaF) in remineralizing early enamel caries at surface level. CPP-ACFP is a mixture of CPP-ACP and 900 ppm fluoride, and according to some authors, it is more effective in remineralization of enamel than CPP-ACP[27,28]. However, there has been no study comparing the efficacy of CPP-ACP (or CPP-ACFP) with high-concentration fluoride.

As a result of this *in vitro* study, CPP-ACFP was observed to be less effective than experimental F-PVA tape and fluoride varnish in inhibition of enamel demineralization. Calcium and phosphate ions were contained in the demineralizing and remineralizing solutions used in pH-cycling, so we speculate that fluoride ion was the critical factor in the inhibition of enamel demineralization in the calcium- and phosphate-rich circumstance. Different application methods of each remineralizing agent might affect the results. In F-PVA tape and F-varnish group, after application of the agent, authors left them for 6 hours to act on the enamel surface. On the other hand, enamel specimens in CPP-ACFP group entered pH-cycling after just a 5-minute application. However, according to a previous study design applying fluoride varnish for 24 hours to evaluate the effect of fluoride vanish[16,17], 6 hours isn't long relatively. We applied CPP-ACFP on the enamel specimens twice a day during pH-cycling to simulate the normally recommended daily oral prophylaxis. And we should consider that fluoride varnish is recommended at least twice a year and CPP-ACP (or CPP-ACFP) is recommended twice a day[29]. In this study, the period of pH-cycling was 7 days, and thus the authors could evaluate short-term effect of F-PVA tape and other remineralizing agents. Considering that CPP-ACFP contains low-level fluoride and is recommended twice a day, 7 days might be relatively short for CPP-ACFP to act sufficiently. So, supposing that this study goes for long period, the result of CPP-ACFP group may be better than that of this study, so the effect of different application methods may be inconsiderable. Further study is needed to evaluate long-term effects of F-PVA tape along with clinical evaluation to overcome the limitations of *in vitro* study.

Ⅴ. Conclusion

The aim of this in vitro study was to evaluate the effectiveness of experimental 2.26% fluoride-polyvinyl alcohol (F-PVA) tape in inhibition of enamel demineralization using enamel surface microhardness (SMH) analysis and scanning electron microscopy (SEM) examination.

The SMH values of the enamel specimens of the F-PVA tape and the F-varnish group were significantly higher than that of the CPP-ACFP group. Also, there was no significant difference between F-PVA tape group and F-varnish group. Regarding %SML, the values obtained in the F-PVA tape and F-varnish group were significantly lower than that of CPP-ACFP group.

In SEM examination, enamel surface in F-PVA tape group showed mild irregularity in general and numerous spherical and ovoid crystals formed on the enamel surface were observed. The density of crystals was higher than that of demineralized specimens. Similar patterns were observed in the F-varnish group.

F-PVA tape is effective in inhibition of enamel demineralization. The inhibition potential of enamel demineralization of F-PVA tape is comparable with that of fluoride varnish and greater than that of CPP-ACFP. With the significant advantages of excellent physical properties, ease of application, and improved safety, F-PVA tape can be used not only for professional treatment, but also in a home-use delivery system.

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국문초록

불소 테이프의 법랑질 탈회 억제 효과에 관한 실험적 평가

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본 연구의 목적은 법랑질 표면 미세경도의 측정과 주사전자현미경 관찰을 통해 본 교실에서 개발한 2.26%의 불소를 함유한 폴리비닐알코올 테잎 (F-PVA tape)의 법랑질 탈회 억제 효과를 평가하기 위한 것이다.

60개의 법랑질 표본을 각 군당 15개 씩, 임의로 대조군, F-PVA tape 군, 불소바니쉬 군, CPP-ACFP 군으로 나누고, 각각의 재광화 제재를 도포한 후 pH-cycling 과정 거쳤다. 표본의 표면 미세경도를 측정하고 표면 미세경도 소실량을 계산하였다. 주사전자현미경 관찰을 위해, 각 군에서 5개의 표본을 추출하고 형태학적 특징을 분석하였고 다음과 같은 결론을 얻었다.

1. pH-cycling 후, F-PVA tape 군과 불소바니쉬 군의 법랑질 표면 미세경도 값은 CPP-ACFP 군과 비교 시 유의하게 높았으며, F-PVA tape 군과 불소바니쉬 군 사이에는 유의한 차이가 없었다.
2. 주사전자현미경 관찰 시, F-PVA tape 군과 불소바니쉬 군에서는 법랑질 표면에 형성된 수많은 구형 및 난원형의 결정 입자들이 관찰되었으며, 결정 입자의 밀도는 대조군 및 CPP-ACFP 군에서 보다 높았다.

F-PVA tape은 법랑질의 탈회를 억제하는데 효과적이며, 그 효과는 불소바니쉬와 견줄만하고, CPP-ACFP 보다 우수하다.

주요어: 불소 테이프, PVA tape, 법랑질 탈회