2012년 2월
석사학위 논문

불소 함유 PVA 테잎이 법랑질의 탈회에 미치는 영향에 관한 실험실 연구

조선대학교 대학원

치 의 학 과

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Effect of PVA tape supplemented with 5% NaF
on enamel demineralization *in vitro*

2012년 2월 24일

조선대학교 대학원

치의학과

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이 논문을 치의학 석사학위신청 논문으로 제출함

2011년 10월

조 선 대 학 교 대 학 원

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2011년 11월

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김 민 정
지도교수 : 치의학 박사 이 상 호
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본 연구의 목적은 자체 개발한 불소 함유 PVA 테잎 (F-PVA tape)의 법랑질 탈회 억제 효과를 평가하기 위한 것이다. 소의 전치로 법랑질 표본을 제작한 후, 네 개의 그룹으로 나누어, 아무 처치도 하지 않은 대조군과 각각 F-PVA tape, 불소바니쉬, CPP-ACFP으로 전처치를 시행한 세 개의 군을 설정한 후, 7일 간의 pH-cycling 과정을 거쳤다. pH-cycling 전, 후 법랑질의 표면 미세경도를 측정, 비교하여 미세경도 소실량을 분석하였고, 주사전자현미경을 통해 법랑질의 표면 양상을 관찰하였다. 실험 결과는 다음과 같다.

1. 법랑질 표면 미세 경도 분석

pH-cycling 전 후, 평균 미세경도 소실량은 대조군, CPP-ACFP군, 불소바니쉬군, F-PVA tape군 순으로 높게 나타났다. 대조군은 다른 군에 비해 미세경도 소실량이 유의하게 컸고($p<0.05$), CPP-ACFP군은 F-PVA tape군, 불소바니쉬군과 비교 시 유의하게 컸으며($p<0.05$), F-PVA tape군, 불소바니쉬군 사이에는 유의적인 차이가 없었다.

2. 주사전자현미경 관찰

탈회된 법랑질 표면 양상의 관찰을 통해, 재광화 제제로 전처치를 시행한 모든 군은 대조군에 비해 탈회 억제 효과가 있었음을 확인하였고, 그 효과는 F-PVA tape군, 불소바니쉬군에서 CPP-ACP군에 비해 높게 관찰되었다.

본 연구의 결과를 토대로 다음과 같은 결론을 얻었다. F-PVA tape은 법랑질의 탈회를 억제하는 효과가 있으며, 1회 적용시 함유된 불소의 양은 불소바니쉬의 1/3 정도로 낮지만, 그 효과는 현재 전문가 불소도포 제제로 사용되고 있는 불소바니쉬와 견줄 만 하다. PVA가 갖는 우수한 물리적 특성과 적용의 용이함으로 인하여 전문가 불소도포 제제로 뿐만 아니라, 가정에서 사용가능한 불소 제제로의 임상적 활용이 기대된다.

I. Introduction

The demineralization and remineralization are dynamic processes in the initiation, progression, and reversal of dental caries. Sign 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 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 improve esthetic, strength, and function[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, mouthrinses, fluoridated varnishes and gels all have helped to reduce the prevalence of dental caries[7]. To improve convenience and effectiveness of fluoride treatment with minimizing adverse effect of overdose-uptake, various forms of fluoride delivery materials have been developed. Fluoride varnish which has been used as a professional topical fluoride agent adheres to the tooth surface, so the complication from children's swallowing might decrease, but it has disadvantages like stickiness, bitter taste, and unesthetic appearance after application. To overcome those disadvantages, Authors have developed fluoride containing - polyvinyl alcohol tape (F-PVA tape) as an experimental fluoride delivery material.

The first 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 solvent. 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. Also, it has 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 PVA-degraders are Gram-negative bacteria belonging to the Pseudomonads and Sphingomonads, but Gram-positive bacteria also have PVA-degrading abilities[8,9].

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.

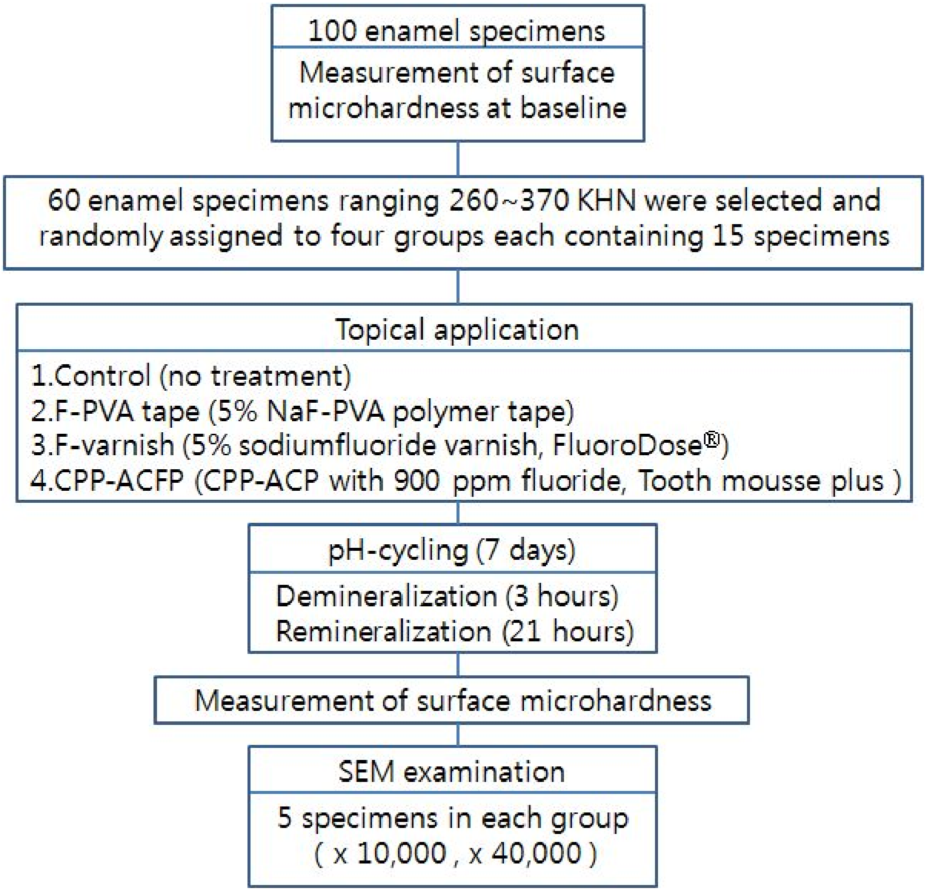


Fig. 1. Illustration of the study design.

2. Preparation of F-PVA tape

PVA (10g) and polyacrylic acid (5g) were added to 85g of distilled water, and the mixture was stirred for 2 hours at 85℃. Polyethylene glycol (3g) as a plasticizer and NaF (0.95g) were added progressively and stirred for 2 hours at 85℃. Then, the mixture was poured onto the glass plate, and made to be uniform in width (20μm) using applicator, and dried for 24 hours at 60℃(Fig. 2)[10]. The F-PVA tape is shown in the Fig. 3.

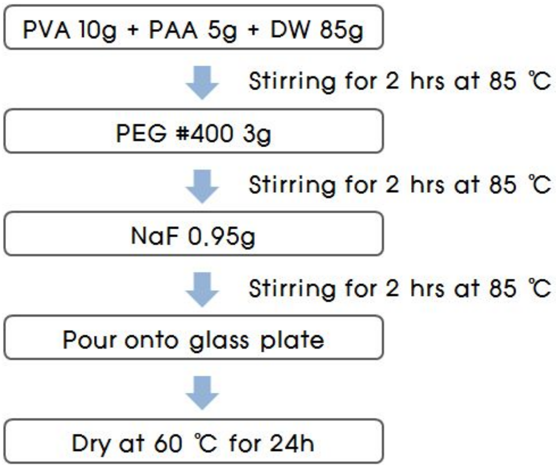


Fig. 2. The procedure for preparation of F-PVA tape.

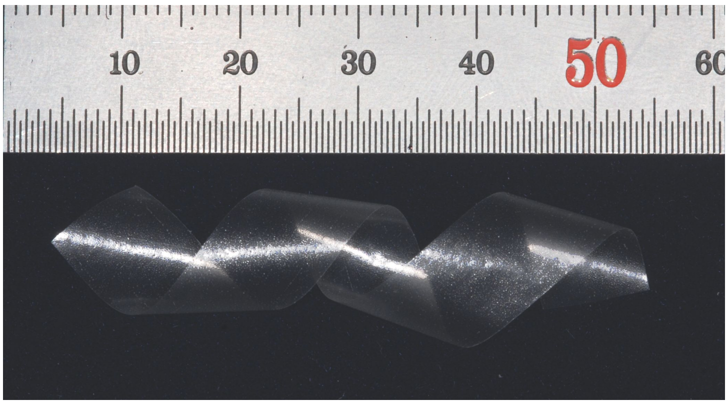


Fig. 3. The experimental F-PVA tape.

3. Specimen preparation

One hundred enamel blocks (5 mm x 5 mm) were prepared from bovine incisor teeth, 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.

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 x 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 not to 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 immersed (30mL/specimen) in the demineralizing solution [2.2mM $Ca(NO_3)_2$, 2.2mM KH_2PO_4, 50mM acetic acid, pH 4.5] at 37℃, for 3 hours. After thorough rinsing with deionized water and careful drying, the specimens were stored (15mL/specimens) in remineralizing solution [1.5mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.9mM $NaH_2PO_4 \cdot 2H_2O$, 150mM KCl, 0.1mM Tris buffer, 0.03 ppm F , pH 7.0] at 37℃, for 21 hours. The demineralizing and remineralizing solutions were changed every cycle[11-13].

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, 100g, 5s, 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 μm 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[14,15]:

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

6. 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 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.

7. Statistical analysis

All data were processed by the 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 the 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 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 relation to control group. 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. Concerning %SML, the values obtained in the F-PVA tape and F-varnish group were significantly lower than that of CPP-ACFP group.

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 ± 10.00	0.000
F-PVA tape	304.39 ± 36.03	178.52 ± 10.60	0.000
F-varnish	306.75 ± 35.90	173.57 ± 6.71	0.000
CPP-ACFP	322.63 ± 25.93	166.33 ± 5.45	0.000

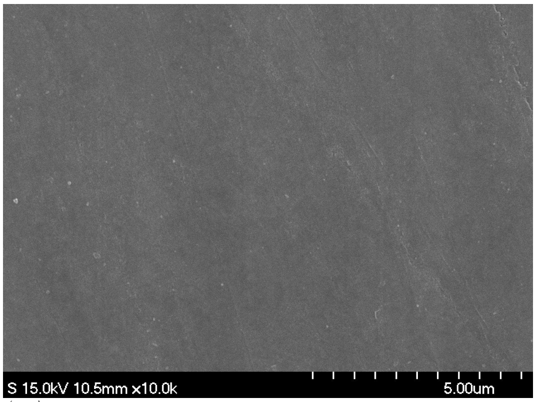
Table 2 : Analysis of surface microhardness and %SML (mean ± SD).

Group	SMH (baseline)	SMH (after pH-cycling)	%SML
Control	303.62 ± 25.34[a]	123.00 ± 10.00[a]	59.27 ± 4.25[a]
F-PVA tape	304.39 ± 36.03[a]	178.52 ± 10.60[b]	40.80 ± 5.83[b]
F-varnish	306.75 ± 35.90[a]	173.57 ± 6.71[b]	42.73 ± 6.71[b]
CPP-ACFP	322.63 ± 25.93[a]	166.33 ± 5.45[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 ($p<0.05$).

2. SEM morphological characters

The typical SEM images of the enamel surface in the different groups are shown in the Fig. 4. and Fig. 5. Sound enamel had an orderly arranged rod appearance and enamel crystals were homogeneously arranged with a clear outline. For comparison, the surfaces of the demineralized enamel were also examined. They were immersed in demineralizing solution for 21 hours as the same period other experimental groups had been immersed in it throughout the pH-cycling. Demineralized enamel had a smaller number of enamel crystals and interrod and intercrystal spaces were very prominent. In control group, interrod spaces were discernable in low magnification, and the density of enamel crystals was lower than other groups. In 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 F-varnish group. In CPP-ACFP group, the irregularity of enamel surface was more prominent than in F-PVA tape and F-varnish group, and some pothole was observed. Enamel crystals were irregularly arranged with variable widths and some were fused together and showed obvious intercrystal space.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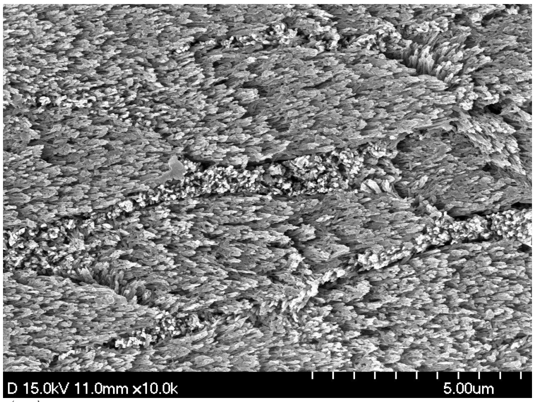
(A)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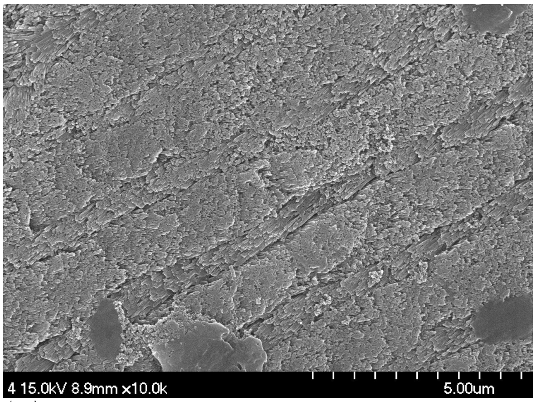
(B)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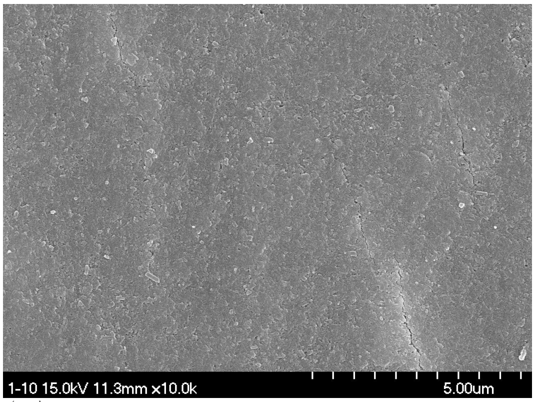
(C)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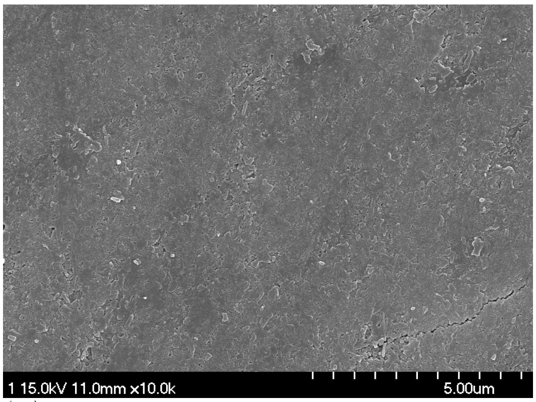
(D)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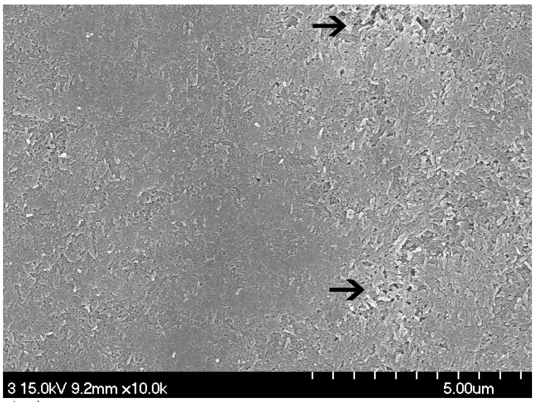
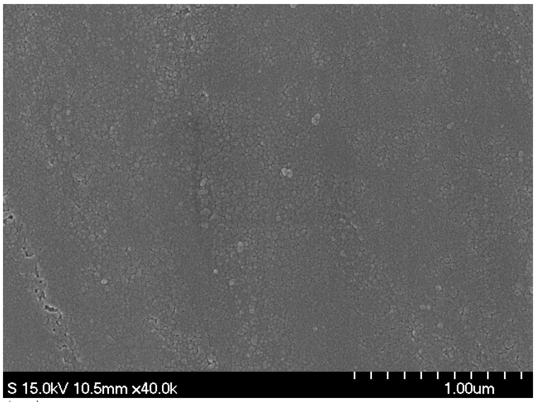
(E)


3 15.0kV 9.2mm x10.0k
5.00um

(F)

Fig. 4. The SEM images of the enamel surfaces in each group (X 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 pothole was observed (arrows).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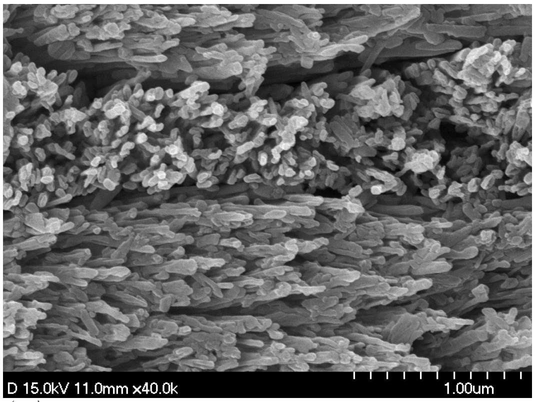
(A)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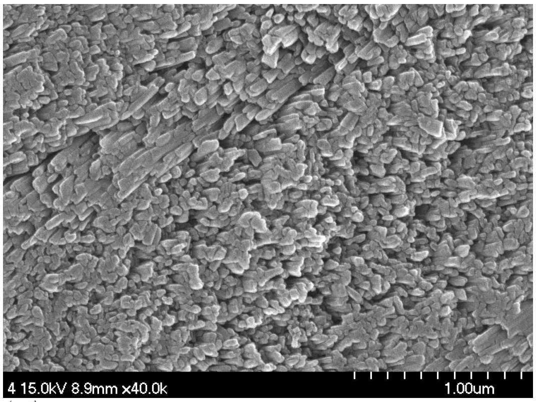
(B)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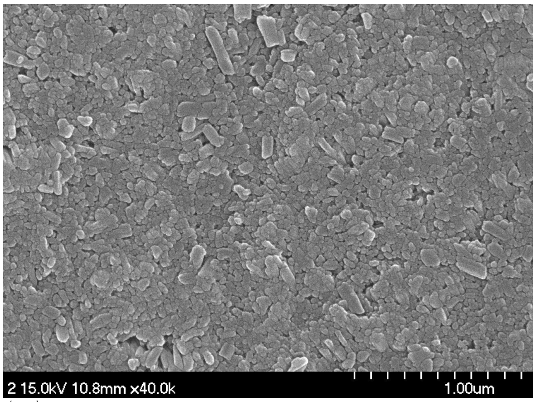
(C)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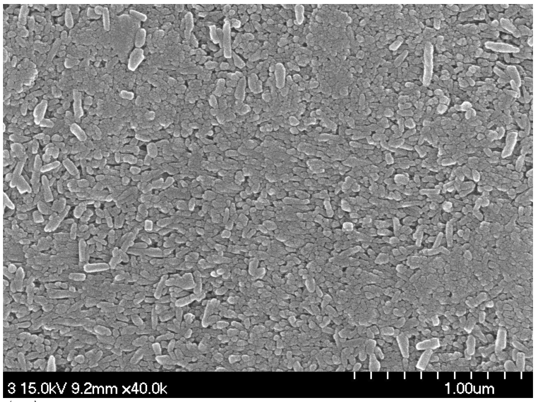
(D)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(E)

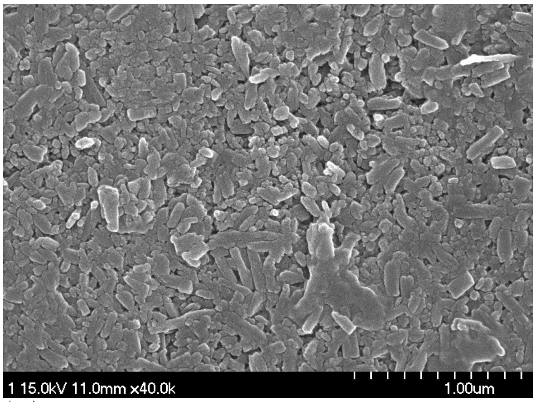
1 15.0kV 11.0mm x40.0k
1.00um
(F)

Fig. 5. The SEM images of the enamel surfaces in each group (X 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 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 space.

IV. Discussion

The mineral loss or gain in the enamel resulted 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 the initial enamel hardness, the enamel softening after demineralization, and the enamel hardening after remineralization. Both the indenters are suitable for the hardness testing of nonmetallic materials. The measurement of the Knoop long diagonal is less affected by the 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 mineral[1,16,17)].

The study design required a sufficiently flat enamel area to allow the enamel SMH measurement. Thus, 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 of obtained in the uncut enamel. Removal of the outer layer of the enamel made the enamel more susceptible to the demineralization[1)].

According to the previous study, the pH-cycling model was composed of 3-hour demineralization and 21-hour remineralization. The period for demineralization was to simulate the duration of deminsralization (low cariogenic challenge) that occurs in the oral cavity[18)]. The composition of demineralizing solution was as follow; 2.2 mM $Ca(NO_3)_2$, 2.2 mM KH_2PO_4, 50 mM acetic acid (pH 4.5)[12)], and the composition of remineralizing solution as follow; 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)[13)].

Ideally, remineralizing agents need to rapidly precipitate on partially demineralized tooth structure and transform into a more stable, less acid-soluble apatite than the hard tissue 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[19].

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[20]. 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[21]. 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 great caries reduction[22].

The experimental F-PVA tape contains 2.26% fluoride the same as in the fluoride varnish (FluoroDose®, Centrix Inc., USA). Supposing the application of the 2 tapes, sized 1cm x 12cm, in maxillary and mandibular arches respectively, the content of fluoride in 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. The tape is transparent, therefore esthetic after application, and it doesn't cause any discomfort like stickiness or bitter taste of fluoride varnish. Moreover, the tooth surface doesn't need to be dried before application of the F-PVA tape, because saliva on tooth surface can help the F-PVA tape to adhere on it. So, the application procedure of it is simple. F-PVA tape provides improved safety and acceptibility. It is possible to use it not only for the professional fluoride treatment, but also for 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, and this product has been used clinically in several published reports for successful non-invasive treatment of mild to moderate fluorotic lesion and for the reversal of early caries and for caries stabilization[23]. According to the *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 the primary teeth, a little more effective than 500 ppm NaF. On the other hand, Lata S, *et al.*[11], 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 the mixture of CPP-ACP and 900 ppm fluoride, and according to some authors, it is more effective in remineralization of enamel than CPP-ACP[24,25]. However, there has been no study on comparison of the efficacy of CPP-ACP (or CPP-ACFP) with high-concentration fluoride.

As a result of this *in vitro* study, CPP-ACFP is 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 authors speculated 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 let them for 6 hours to act on enamel surface. On the other hand, enamel specimens in CPP-ACFP group entered pH-cycling just after 5-minute application. However, according to the previous study design applying fluoride varnish for 24 hours to evaluate the effect of fluoride vanish[14,15], 6 hours isn't long relatively. We applied CPP-ACFP at 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[26]. In this study, the period of pH-cycling was 7 days, and 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 short relatively 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.

V. Conclusion

The object of this *in vitro* study is 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. 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. Concerning %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 control and CPP-ACFP group. Similar patterns were observed in F-varnish group.

F-PVA tape is effective in inhibition of enamel demineralization. Inhibition potential of enamel demineralization of F-PVA tape is comparable with that of fluoride vanish and greater than that of CPP-ACFP. In the advantage of excellent physical properties and ease of application, it can be used as the home use delivery system of fluoride. Further study should be needed to evaluate long-term effect of F-PVA tape, and clinical evaluation should be followed to overcome the limitation of *in vitro* study.

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