Effect of fluoride on remineralization of artificial enamel caries in pH 4.3 and pH 7.0 remineralizing solution

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Effect of fluoride on remineralization of artificial enamel caries in pH 4.3 and pH 7.0 remineralizing solution

Directed by Professor Chan-Young Lee

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This certifies that the Doctoral Dissertation of
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또한, 부족한 논문을 완성하는데, 조언을 아끼지 않으셨던, 이승일 선생님, 정일영 선생님, 서정택 선생님, 박정원 선생님께도 감사드립니다.

보존과라는 학문을 가르쳐주시고, 지금의 치과의사가 되게 해 주신 이승종 선생님, 노병덕 선생님, 박성호 선생님, 김의성 선생님, 신수정 선생님, 신유석 선생님께도 감사드립니다.

힘든 실험과정에도 늦게까지 남아서 결과를 정리해 주었던 신한얼 선생님과 실험과정과 논문 작성을 가르쳐 주었던 여러 보존과 선후배님께 감사드립니다.

진료나절을 실험으로 비워도 묵묵히 참고 지켜봐 주었던 김동진 원장님께도 감사드립니다.

저를 낳아주시고 지금까지도 믿어주시는 어머님과 하늘나라에 계시지만 같이 기뻐해 주실 아버님께 감사드립니다. 가까이 계시면서 삶의 의미를 일깨워 주시는 장인어른과 장모님께도 감사드립니다.

많은 주말들을 실험과 논문으로 함께 하지 못했지만, 한번도 불평하지 않았고 아빠를 반겨주었던 시현이 채현이와 논문을 만드는데 함께 노력하고 고민했던 사랑하는 아내 김덕주에게 사랑과 감사의 마음을 전합니다.

2010 년 6 월

송상채

<TABLE OF CONTENTS>

<LIST OF FIGURES>

<LIST OF TABLES>

Abstract

Effect of fluoride on remineralization of artificial enamel caries in pH 4.3 and pH 7.0 remineralizing solution

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In many epidemiologic studies, the caries incidence becomes low in drinking water with fluoride and initial enamel caries has been disappeared by fluoride, and crystallographically fluoridated hydroxyapatite has much lower solubility than normal hydroxyapatite due to fluoride crystal's stability and its growth mechanism.

With this characteristic of fluoride, there have been many studies for remineralization of caries lesion; Margolis et al. explained the precipitation of fluoridated hydoxyapatite for remineralization using the degree of saturation physiologically.

Although the remineralization occurs much more with increasing degree of saturation, it doesn’t occur to the inner portion completely. Therefore, there have been attempts of the remineralzation to the inner portion by lowering the degree of saturation on the surface zone in order to resolve this kind of problem.

In many cases, there have been many applications to remineralize the caries with fluoride yet clear theoretical explanations or evidences are not enough.

The objective of this experiment was to figure out how fluoride affects the remineralization of artificial enamel caries lesion with the comparison of the phase of the remineralization to the artificial enamel caries in terms of different fluoride concentrations in the remineralizing solutions with pH 4.3 and pH 7.0.

The following experimental results were obtained after the calculation of the degree of density of the thin sections whose enamel was demineralized in the demineralizing solution and remineralization was observed under the polarizing microscope for eight days at maximum.

1. The more fluoride concentration increased, the more the remineralization occurred in pH4.3 remineralizing solution. (group 1,2,4, $p<0.05$)

2. The entire rate of remineralization increased in the pH 7.0 remineralizing solution. however; there was no significant difference in fluoride concentration.
3. As fluoride concentration increased in pH 4.3 remineralizing solution, the rate of remineralization both on the surface zone and subsurface zone increased.
4. As fluoride concentration increased in pH 7.0 remineralizing solution, the rate of remineralization on the surface zone exceeds that of on the subsurface zone.

In conclusion, fluoride affected the remineralization of the enamel artificial caries more in pH 4.3 than in pH 7.0.

Keywords: formation of artificial caries, remineralization, Fluoride, Hydroxyapatite, Fluoridated Hydroxyapatite

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I. Introduction

In many epidemiologic studies, the caries incidence becomes low in drinking water having fluoride (Backer dirks. 1974; Forrest 1965), and clinically it has been observed that the initial enamel caries is disappeared by fluoride (Edgar et al. 1978). Crystallographically, the small fluoride ions replace the hydroxyl group in hydroxyapatite to become more dense and stable structure (Kay et al. 1964; Young and Elliott 1966). Then, fluoride increases the driving force for the formation of apatite crystal and the growth of apatite is accelerated (Moreno et al. 1974).

Due to fluoride crystal’s stability and its growth mechanism, fluoridated hydroxyapatite is discovered to have much lower solubility than normal hydroxyapatite. Also, fluoride forms the calcium fluoride-like deposits on the surface zone of the enamel, and the deposit roles a reservoir releasing fluoride in low pH (ten Cate 1997).

With this nature of fluoride, many studies have tried to demonstrate the remineralization of the caries lesion; if the remineralizing solution has fluoride, the precipitation of hydroxyapatite becomes increasing, and the rate of remineralization also escalates (ten Cate and Arends 1977). Moreover, the sound enamel is demineralized in demineralizing solution with fluoride, the demineralized ion reacts with fluoride ion and leads to the formation of fluoridated hydroxyapatite with low solubility and further demineralization does not occur (Margolis and Moreno 1990).

Margolis et al. proposed the degree of saturation by calculating the activity of fluoridated hydroxyapatite ion and the solubility of the product in order to explain the precipitation of the fluoridated hydroxyapatite for remineralization (Margolis and Moreno 1990). The degree of saturation is informed to affect the phase of demineralization (Lee 1992) and a study shows as the higher the degree of the saturation becomes, the more the remineralization occurs (Park et al. 2000). Thus, the demineralization of the enamel and the phase of remineralization can result differently by the surrounding ions such as Ca^{2+}, P^{+}, F^{-} in terms of the concept of degree of saturation as well as that of the pH.

Even though the degree of saturation is higher, the remineralization does not affect the inner portion completely. It blocks the spread of ions due to both bigger size of the precipitation on the surface zone and relatively narrower diffusion channel for the crystals in the process of the remineralization from the high degree of the saturation (Aoba et al. 1978; Silverstone et al. 1981).

In order to for the minerals to penetrate easily through the enamel surface zone, there have been attempts remineralizing to inner portion by lowering the degree of the saturation on the surface zone.

There have been studies that remineralization occurred easily after acid-etching on the caries lesion of the enamel (Flaitz and Hicks 1994) and the remineralization is attempted in the acidic solution with fluoride in order to occur the demineralization of the enamel surface and the precipitation of the fluoridated hydroxyapatite easily (Fox et al. 1983).

Remineralization occurred at the inner portion with lower pH; however, it also led to the demineralization at the same time. Thus, over 25 ppm fluoride concentration is needed to have only the remineralization without the demineralization in the pH 4.8 acidic solution (Yamazaki et al. 2007).

The remineralization occurs a lot in the lower fluoride concentration in pH 6.8 whereas there is no significant difference of the fluoride concentration in pH 5.5 for there is the difference between the OCP (octacalcium phosphate in pH 6.8) and DCPD (dicalcium phosphate dehydrate in pH 5.5), the precursors of the fluoridated hydroxyapatie (Lammers et al. 1992).

Despite many case studies indicated remineralization of caries with fluoride, concrete evidence or explanations are still insufficient.

In this experiment, in order to figure out how fluoride affects the remineralization of artificial enamel caries lesion, the phase of the remineralization has been compared to the artificial enamel caries in terms of different fluoride concentrations in the remineralizing solutions with pH 4.3 and pH 7.0.

II. Materials and methods

1. Preparation of thin section

Non-carious sound human third molars extracted within one month were chosen and its attached soft tissues on the tooth surface and calculus have been removed by the perio curette. The surface of tooth was cleaned by polishing material which does not contain fluoride and washed for 10 minutes with distilled water and dried.

The coronal portion was exposed and the root portion was embedded into the dental acrylic resin, put into the cylinder-like tube, 1cm height x 1cm length made of 10ml syringe. After acrylic resin hardened, it was sectioned into 0.2 ~0.3 mm thickness, parallel to tooth longitudinal axis by using Minitom (Struers, Ballerup, Denmark) and ground into 0.15mm thickness by using #800 sandpaper.

After grinding and washing for 10 minutes by ultrasonic washing machine of molar section, each upper and lower surface were covered by Scotchbond Multi-purpose (3M, St. Paul, MN, U.S.A.) and cured by UV light under the nitrogen gas. Then, the nail varnish was applied to both sides of the sections except 1mm ~ 2mm windows with no defects or cracks on the mesio-distal sides. Eighty sections were prepared and each ten sections were distributed into eight groups.

2. Preparation of experimental solution

2.1 Preparation of stock solution

Using 30% of lactic acid solution (Sigma Co., St. Louis., MO, U.S.A.), 1M concentration of the lactic adic solution was prepared and titrated for stock solution. 1M calcium was prepared by using calcium chloride powder (Sigma Co., St. Louis., MO, U.S.A.) and 1M phosphate was prepared by using potassium phostphate powder. Concentrations of each ion were analyzed by 790 Personal Ion chromatography (Metrohm, Herisau, Switzerland) and prepared as the stock solutions.

2.2 Preparation of demineralizing solution

The calculated rates from the stock solutions were taken and then, the concentrations of each lactic acid, calcium and phosphate were prepared for demineralizing solution. 3.08mM of sodium azide was added into the solutions and 8N potassium hydroxide standard solution was used to adjust the solutions pH level as 4.3 under the pH meter. (Orion Research Inc., Jacksonville, FL, U.S.A.) Concentrations of each ion were analyzed quantitatively by using 790 Personal Ion Chromatography (Metrohm, Herisau, Switzerland) and used to make the demineralizing solution for the experimental samples (Table 1).

Composition	Concentration
Lactic acid (mM)	100
Calcium (mM)	15.5
Phosphate (mM)	8.5
Sodium azide (mM)	3.08
pH	4.3

Table 1 Initial composition of demineralizing solution

2.3 Preparation of remineralizing solution

The calculated rates from the stock solutions were taken and the concentrations of each lactic acid, calcium and phosphate were prepared to demineralizing solution. 3.08mM of sodium azide was added into the solutions and 8N potassium hydroxide standard solution was used to adjust the solutions pH level 4.3 and 7.0 under the pH meter (Orion Research Inc., Jacksonville, FL, U.S.A.).

Concentrations of each ion were analyzed quantitatively by using 790 Personal Ion Chromatography (Metrohm, Herisau, Switzerland), and the concentration of fluoride was analyzed by 9609BNWP and 960900 fluoride combination electrode (Thermo scientific, Waltham, MA, U.S.A.) and used for remineralizing solution for thin sections (Table 2).

Group	1	2	3	4
Lactic acid (mM)	10	10	10	10
Calcium (mM)	9.3	9.2	9.0	9.0
Phosphate (mM)	31.6	30	30.8	29
Sodium azide (mM)	3.08	3.08	3.08	3.08
pH	4.3	4.3	4.3	4.3
Fluoride (ppm)	0	1	2	4

Group	5	6	7	8
Lactic acid (mM)	0	0	0	0
Calcium (mM)	0.11	0.13	0.11	0.11
Phosphate (mM)	0.31	0.31	0.31	0.31
Sodium azide (mM)	3.08	3.08	3.08	3.08
pH	7.0	7.0	7.0	7.0
Fluoride (ppm)	0	1	2	4

Table 2 Initial composition of remineralizing solution

3. The formation and observation of artificial dental caries

The thin sections were submerged in 30 ml of demineralizing solution in a 50ml plastic basket and reacted for 24 hours in warm water bath. Then the thin sections saturated in distilled water were examined at x40 and x100 magnification under the polarizing microscope I221 (Dongwon, Seoul, Korea) and their pictures were taken to confirm the formation of artificial caries. The samples were prepared for the experiment (Fig. 1).

4. Remineralization and observation of artificial enamel caries

The thin sections were submerged in 30 ml of remineralizing solution in a 50ml plastic bottle and reacted for eight days in warm water bath with the solutions replaced at a two-day interval. 0.15 mm thickness of thin sections were saturated in distilled water and observed under the polarizing microscope I221 at x40 and x100 magnification. Then, the samples were observed at intervals about one, two, four, six, and eight days and their pictures were taken (Fig. 1).

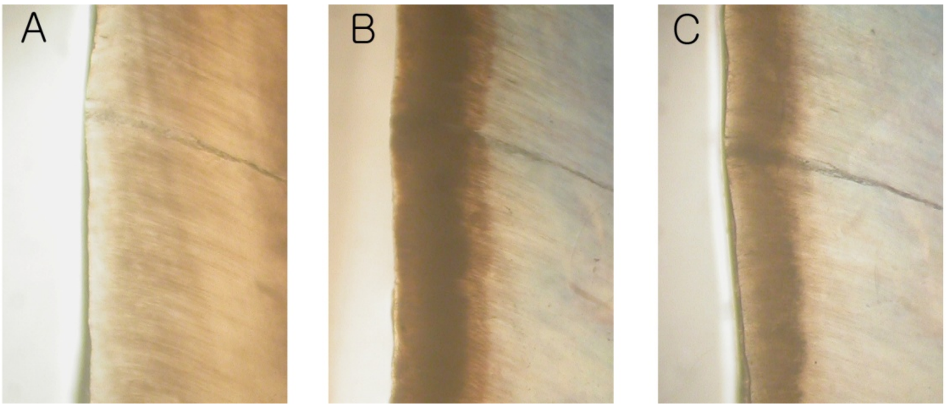


Fig. 1 Polarizing microscopic images of thin sections: (A) sound enamel (B) decalcified enamel (C) remineralized enamel

5. Analysis of the experimental materials

The thin sections were observed and pictured at x100 magnification under the polarizing microscope with mounted digital camera. After demineralization and remineralization of the experimental samples occurred, their corresponding areas were aligned and edited by Photoshop program (Adobe, San Jose, CA, U.S.A.) in order to determine the changes. With the fixation of the changes into gray scale, the density was evaluated by Image J program (National Institutes of Health, Bethesda, U.S.A.), and their pictures and graphs were compared to distinguish both the surface and subsurface zones (Fig. 2).

Then, the measured density was processed by Excel (Microsoft, Redmond, WA, U.S.A.) and the percentages of the demineralized area and that of the remineralized area were compared to measure the demineralization area (Fig. 3).

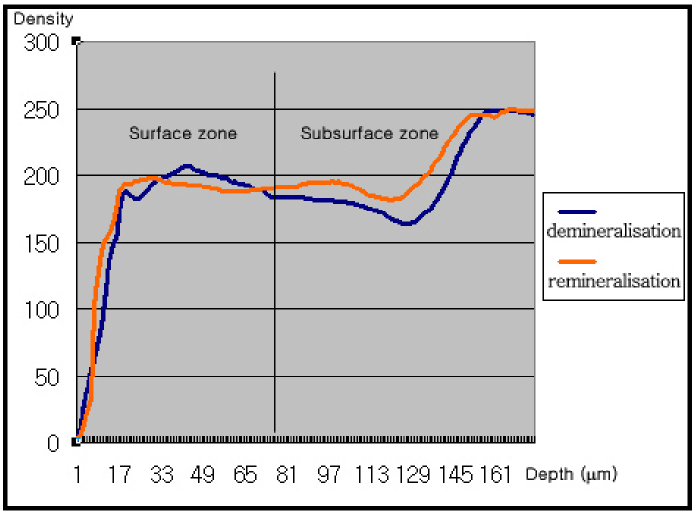


Fig. 2 Classification of surface zone and subsurface zone

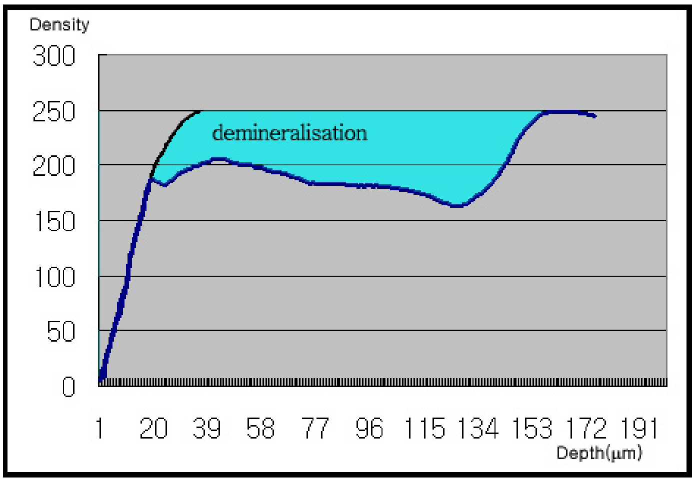


Fig. 3 Measuring method of demineralization area

6. Statistical analysis

The differences with respect to fluoride concentration were measured in pH 4.3 and pH 7.0 remineralizing solutions. Oneway ANOVA was carried into effect and its significant variables were tested by a Tukey. Also, the differences of the average rate of remineralization between pH 4.3 and pH 7.0 remineralizing solutions were assessed by t-test.

The differences of the width of the surface zone between before and after the remineralization were assessed in length from the graph and their differences of the lengths were calculated into percentage and examined by oneway ANOVA.

The differences of the remineralization of each surface and subsurface zone were calculated with the widths measured from the remineralized surface zone and calculated the percentages of the difference and then analyzed by oneway ANOVA.

III. Results

1. Phase of polarizing microscopic picture and density graph

1.1 Phase of remineralization of fluoride concentration in pH 4.3 remineralizing solutions (Fig. 4)

a. Group 1 (pH 4.3, Fluoride 0 ppm)

As the remineralization was in progress on the surface zone, the demineralization occurred simultaneously as well as the progression of demineralization in the inner portion. Moreover, there were some cases showing that the entire rate of remineralization was low or the demineralization exceeded the remineralization.

b. Group 2 (pH 4.3, Fluoride 1 ppm)

Although the remineralization occurred more on the surface zone, the formation of remineralization was witnessed uniformly on both surface and subsurface zones and the progressive demineralization in the inner portion was also observed.

c. Group 3 (pH 4.3, Fluoride 2 ppm)

The surface zone was clearly displayed in the pictures taken by the polarizing microscope and the even distribution of the remineralization of the surface zone and subsurface zone was progressive and also the demineralization was progressive in the inner portion.

d. Group 4 (pH 4.3, Fluoride 4 ppm)

The surface zone was even clearer in the pictures under the polarizing microscope, and there was even distribution of the remineralization and the demineralization was progressive in the inner portion.

1.2 The phase of remineralization of fluoride concentration in pH 7.0 remineralizing solutions (Fig. 5)

a. Group 5 (pH 7.0, Fluoride 0 ppm)

Even distribution of the remineralization on both the surface and subsurface zones was distinctive; there was demineralization neither on the surface zone nor its inner portion.

b. Group 6 (pH 7.0, Fluoride 1 ppm)

The surface zone was clearly noticed on the polarized microscopic pictures, and there was the remineralization of the surface and subsurface zones but demineralization did not occur.

c. Group 7 (pH 7.0, Fluoride 2 ppm)

The thickness of the surface zone was broader and clearer on the polarized microscopic pictures; there was no demineralization but the remineralization occurred on both surface and subsurface zones.

d. Group 8 (pH 7.0, Fluoride 4 ppm)

The thickness of the surface zone was much broader and more apparent in the polarized microscopic pictures; and there was the significant remineralization on both surface and subsurface zones however, the demineralization was not detected.

2. Statistical analysis of the graphs using computer program

2.1 Comparison of the rate of the remineralization depending on fluoride concentration in pH 4.3 remineralizing solutions

There was gradually increasing phase of the entire rate of remineralization depending on fluoride concentration in the remineralizing solutions, whereas the demineralization was also in progress (group 1, 2, 4, $p<0.05$) (Fig. 6).

2.2 Comparison of the rate of remineralization in pH 7.0 remineralizing solution depending on fluoride concentration

There was the entire rate of the remineralization in the remineralizing solution increased whereas; there was no significant difference in the rate of remineralization depending on the changes in fluoride concentration (Fig. 7).

2.3 Comparison of the average rate of the remineralization in both pH 4.3 and pH 7.0 remineralizing solutions

The average of the remineralization rate in the remineralizing solution was observed higher in pH 7.0 without any significant difference.

2.4 Comparison of the remineralization rate of the surface zone and subsurface zone in pH 4.3 remineralizing solutions

Either the demineralization was observed or the rate of the remineralization on the surface zone was significantly low without fluoride in pH 4.3 but the remineralization was detected on the subsurface zone However, fluoride concentration was high, the rate of remineralization increased both on surface and subsurface zones ($p<0.05$)(Fig. 9).

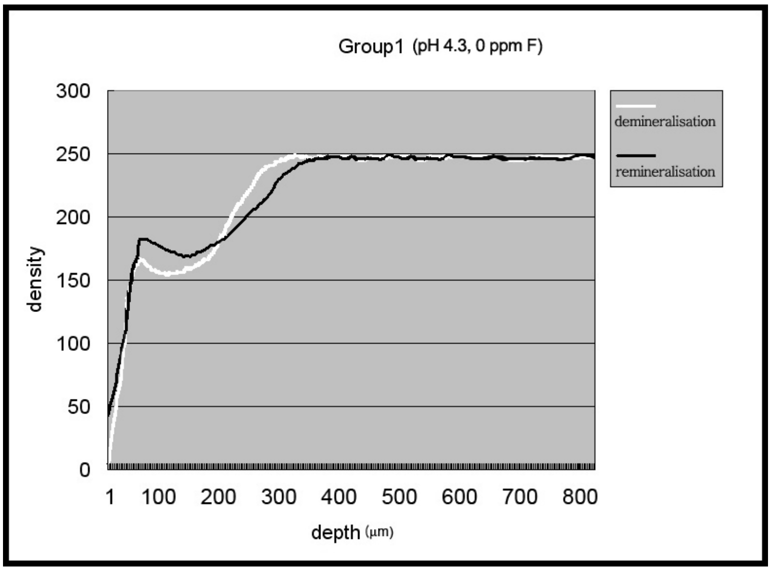
2.5 Comparison of the remineralization rate of surface and subsurface zones in the pH 7.0 remineralizing solutions

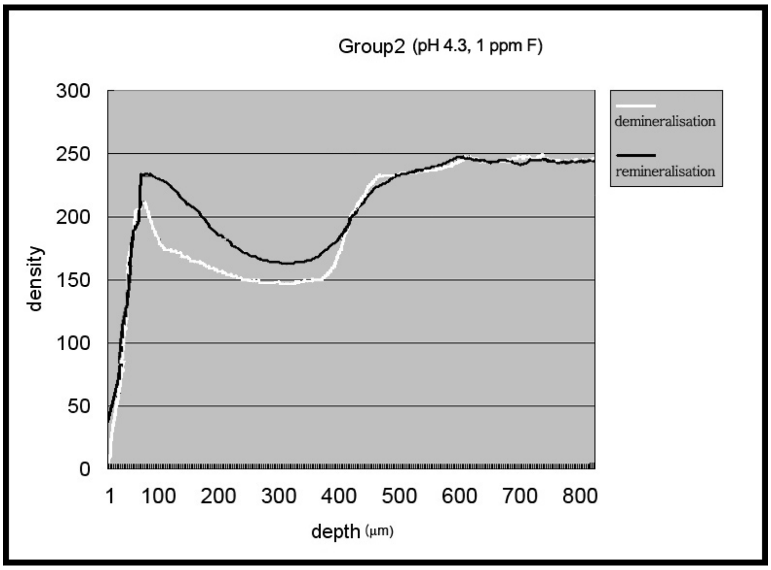
As fluoride concentration in the pH 7.0 remineralizing solution was high, the rate of remineralization on the surface zone was elevated without any significant difference between the groups. However, the statistical difference was observed between the surface and subsurface zones in 4 ppm fluoride concentration ($p<0.05$) (Fig. 10).

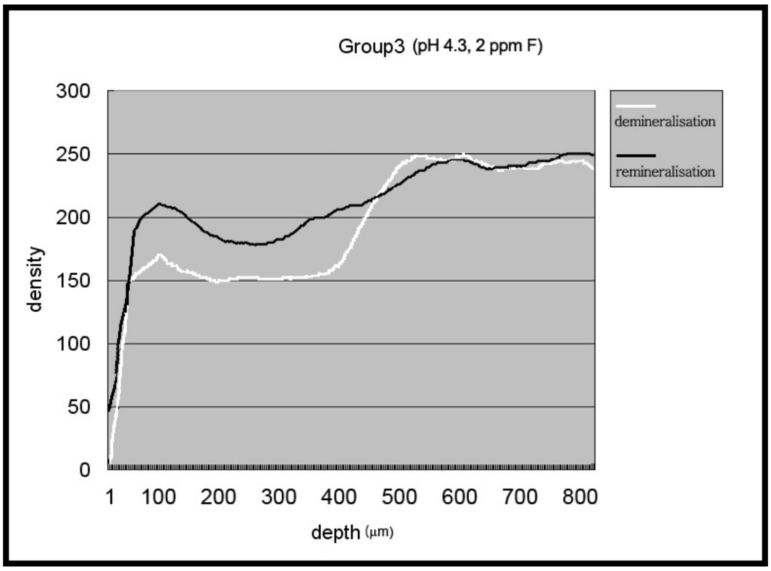
2.6 Changes of the width on the surface zone before and after the remineralization

Although there were small changes of width on the surface zone before and after the remineralization without fluoride in pH4.3 remineralizing solutions, there was no change in the width of the surface zone depending on fluoride concentration.

As fluoride concentration in pH 7.0 remineralizing solution increased, the phase showed the width increasing without any significant difference (Fig. 11).


Group1 (pH 4.3, 0 ppm F)
density
depth (μm)
demineralisation
remineralisation
300
250
200
150
100
50
0
1
100
200
300
400
500
600
700
800


Group2 (pH 4.3, 1 ppm F)
density
depth (μm)
demineralisation
remineralisation
300
250
200
150
100
50
0
1
100
200
300
400
500
600
700
800



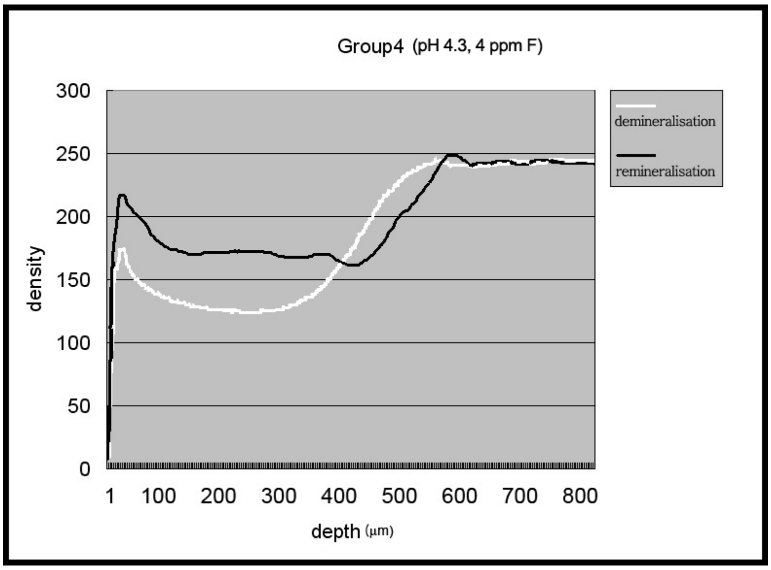
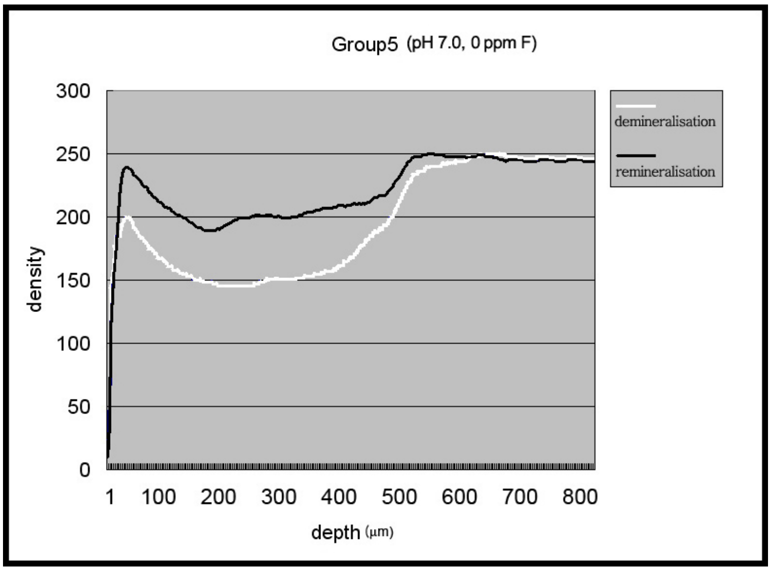
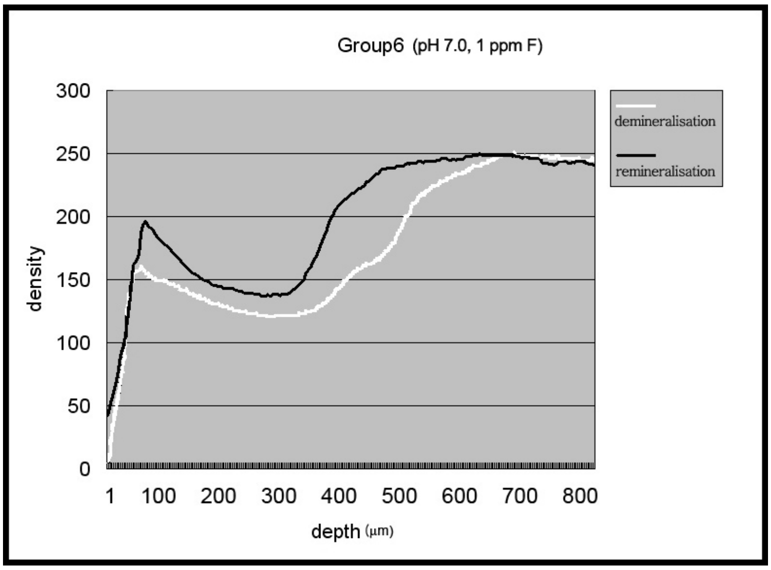
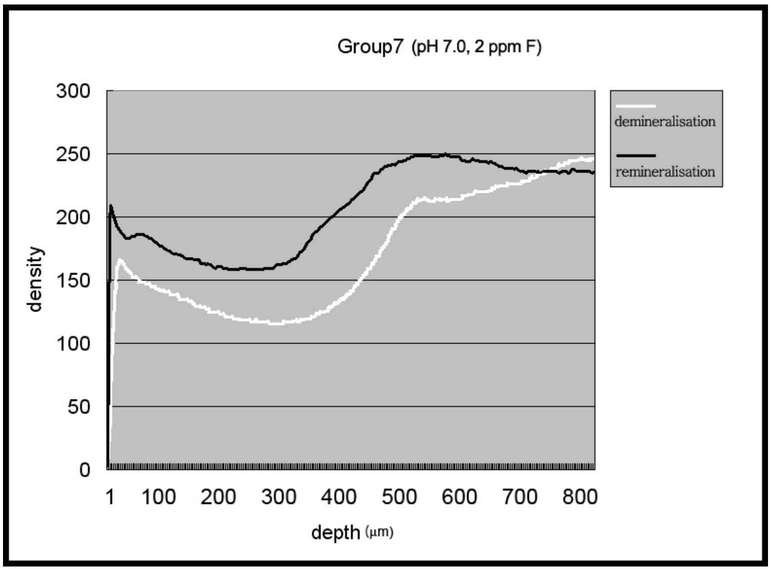


Fig. 4 Comparison of density before and after remineralization in pH 4.3 group


Group5 (pH 7.0, 0 ppm F)
density
depth (µm)
demineralisation
remineralisation
300
250
200
150
100
50
0
1
100
200
300
400
500
600
700
800


Group6 (pH 7.0, 1 ppm F)
density
depth (µm)
demineralisation
remineralisation
300
250
200
150
100
50
0
1
100
200
300
400
500
600
700
800



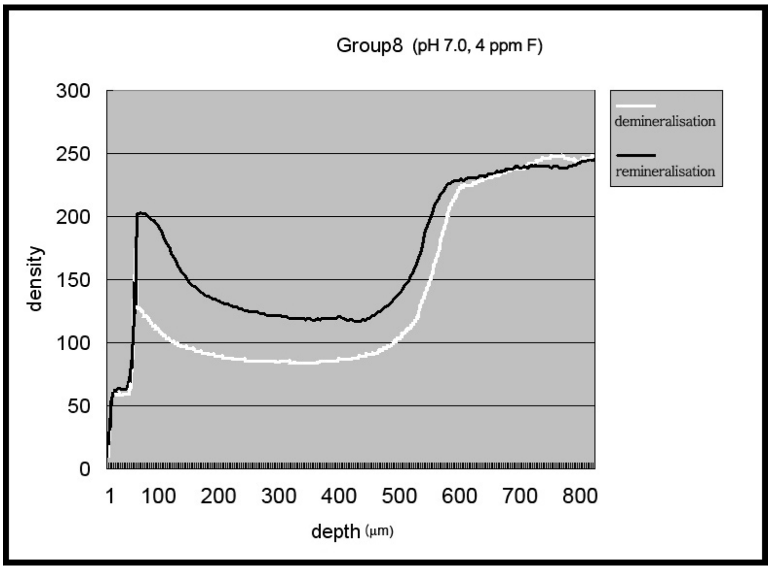


Fig. 5 Comparison of density before and after remineralization in pH 7.0 group

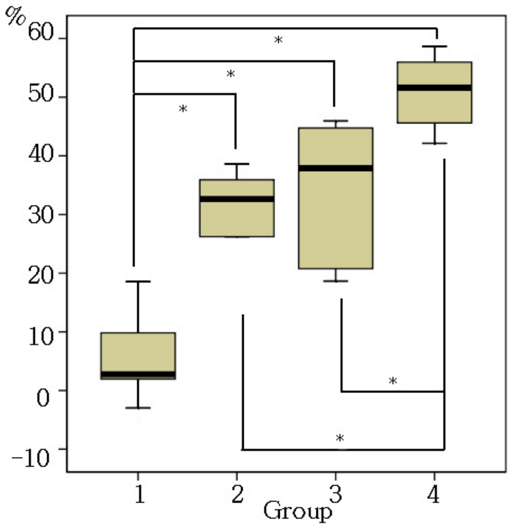


Fig. 6 Volume percentage of remineralization between group 1,2,3 and 4 (pH 4.3)

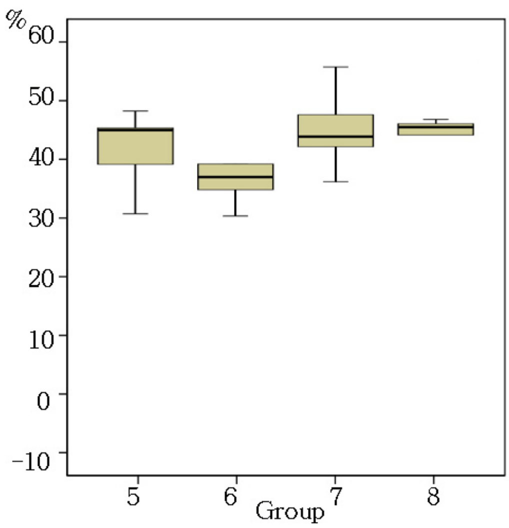


Fig. 7 Volume percentage of remineralization between group 5,6,7 and 8 (pH 7.0)

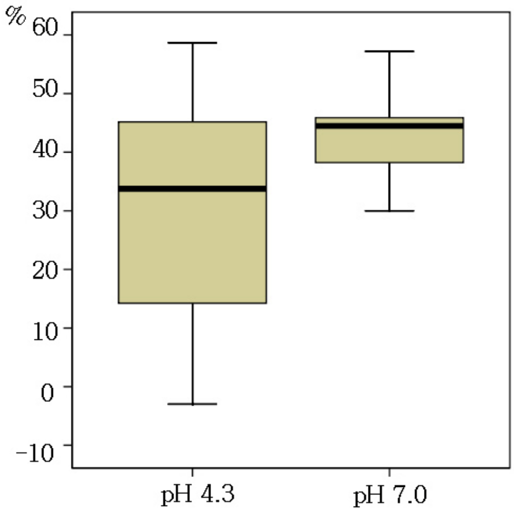


Fig. 8 Volume percentage of remineralization between pH 4.3 group and pH 7.0 group

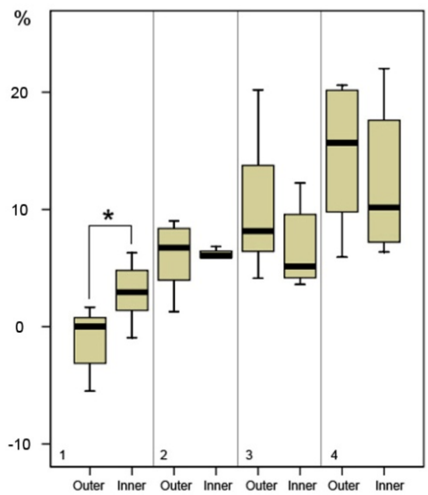


Fig. 9 Volume percentage of remineralization between surface zone and subsurface zone in pH 4.3 group

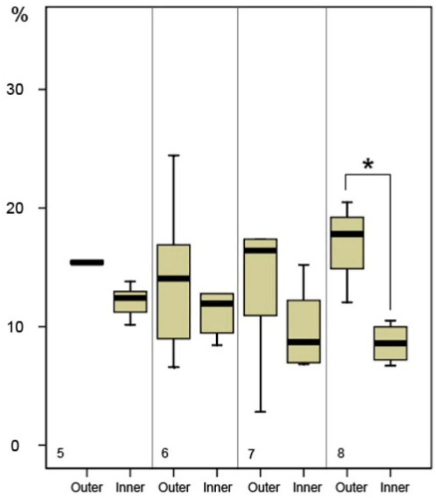


Fig. 10 Volume percentage of remineralization between surface zone and subsurface zone in pH 7.0 group

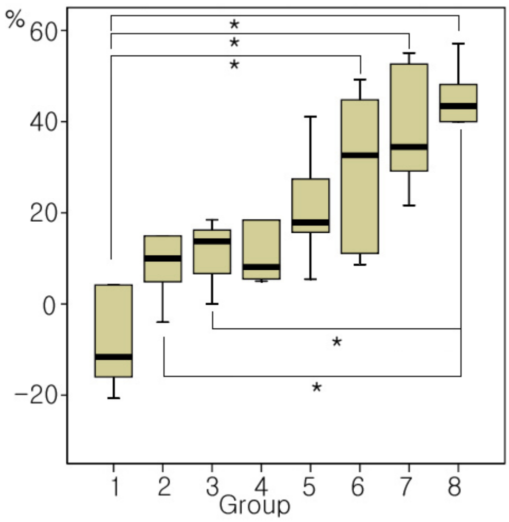


Fig. 11 Change rate of surface zone width between group 1,2,3,4,5,6,7 and 8

IV. Discussion

There have been a lot of experimental trials of recovery to the normal condition of teeth through the remineralization as well as the prevention of the caries lesion using fluoride.

When the sound enamel is placed in pH 4.3 lactic acid, there is no demineralization even in low fluoride concentration due to the low solubility of fluoridated hydroxyapatite moreover, it does not lead to the loss of the entire mineral since the rate of precipitation exceeds that of mineral loss (Margolis et al, 1986).

However, with various factors in the remineralization of caries enamel, many studies have tried to find the concentration of the surrounding ions and proper pH to lead the remineralization to the inner portion of the caries enamel.

The objective of this experiment was to identify fluoride participation in the remineralization through the phase of the remineralization in caries lesion with the different concentration of fluoride in both pH 4.3 and pH 7.0 remineralizing solutions.

The experimental methods for the artificial enamel caries and the remineralization can be divided into two: one using the acidified gelatin gel used by Silverstone (Silverstone, 1968) and another using acid buffer system suggested by Moreno. (Moreno et al. 1974) The acid buffer system was used in this experiment to form caries lesion fast and separate the affecting factor from the remineralization precisely.

In order to obtain the tooth without exposure to fluoride, the extracted third molar was used. Teeth with caries were discarded after microscopic examination. After the teeth were submerged in demineralizing solution for 24 hours, those with uniformly spread demineralization were chosen to progress the experiment.

Even though the thin sections were not exposed to the fluoride in the remineralizatnion, some had to be discarded and examined under the polarizing microscope; the discarded included the groups of non-remineralization, deeply caries lesion and the ones without bondings.

Under the polarizing microscope, the surface zone was not observed distinctively in some samples however, the surface zone was more visible as the remineralization was progressive at the same time.

The images of the experimental samples might have been observed differently due to several factors including the degree of the polarization, the intensity of light and the focal point after the observation under the polarizing microscope. Using Photoshop program, the pictures of thin sections were aligned, and the artificial caries lesion parts were chosen and their density were observed. Due to the difference in brightness, the density could have resulted differently with the density of the sound enamel setting at 250 in the graph.

As the rate of the remineralization differed from the depth of the demineralization and remineralization in each group, the rate of the demineralization and the remineralization in percentage from the sound enamel area was calculated.

The phase of the remineralization was defined through the calculation of remineralization rate of surface zone and subsurface zone and it was to see if the remineralization was evenly infiltrated to the inner portion.

The previous study showed there are the correlation between pH and remineralization; the demineralization in 2 ppm fluoride concentration is observed in low pH (lower than pH 4.0), both remineralization and demineralization occur simultaneously in between pH 4.3 and pH 5.0 and the only remineralization occurs in pH 5.5 above (Kim et al. 2007; Kim et al. 1997).

The various results are concluded in the study of the remineralization depending on the fluoride concentration (Han et al. 1996).

Lynch et al. suggested as fluoride concentrations in pH 4.8, 5.0, and 5.2 increases 1, 2, 6 ppm respectively as the rate of the remineralization increases (Lynch et al. 2006), and more remineralization significantly increases in pH 5.5 with 0.03 ppm fluoride concentration between pH 5.5 and pH 6.8, and it shows there is no relationship between the remineralization and pH in low fluoride concentration, 0.03 ~ 1.0 ppm (Lammers et al. 1992).

Yamazaki et al. indicated that the demineralization in the inner portion is progressed at the outside between 2.1 ppm and 10.1 ppm of the fluoride concentration in pH 4.8, and 25 ppm fluoride concentrations is needed for the remineralization without any demineralization (Yamazaki et al. 2007).

In this experiment, as fluoride concentration in pH 4.3 remineralizing solution increased, the remineralization occurred more as well as the demineralization was observed simultaneously. This result was identical to the result from the study by Yamazaki et al. and referred as to see the saturation of the remineralizing solution to the inner portion since the enamel artificial caries lesion occurred.

The result of remineralization observed in pH 7.0 remineralizing solution was also identical to the experiment performed by Kwak at al. that the depth of the demineralization in over the pH 5.5 solution does not increase (Kwak et al. 2008).

Without fluoride in the pH 4.3 remineralizing solution, the rate of remineralization on the surface zone was decreasing or the demineralization was seen on the surface zone. As fluoride concentration increased, the remineralization increased significantly. While the rate of the remineralization in pH 7.0 remineralizing solution increased, there was no significant difference in fluoride concentration.

In conclusion, the remineralization was more affected by fluoride in pH 4.3 than pH 7.0.

In terms of the difference of the remineralization rate on the surface zone and subsurface zone, as fluoride concentration in pH 4.3 remineralizing solution increased, the rate of remineralization on both the surface zone and subsurface zone increased. However, as fluoride concentration in the pH 7.0 remineralizing solution was elevated, the rate of remineralization on the surface zone exceeded that of it on the subsurface zone.

Yamazaki et al. concluded that the remineralization occurs to deeper inner portion of enamel in the acidic solution when observed in two conditions with different acidity: one in pH 4.8 with 20 ppm F and another in pH 7.0 with 10 ppm F. The remineralization is observed to be favor in acidic solution for more remineralization occurs in acidic solution with respect to the lesion types (Yamazaki et al. 2008).

The result from this experiment indicated the average of the remineralization rate in the remineralizing solution was observed higher in pH 7.0 however; the result was identical to the one drawn from the study of Yamazaki et al. that the more remineralization in pH 4.3 was shown as fluoride concentration increased.

Therefore, following hypotheses about the occurrence of the remineralization to the inner portion can be drawn from the result that fluoride has affected the remineralization more in pH 4.3 remineralizing solution than in pH 7.0.

First, it can be inferred that the demineralization on the surface zone might have led to the continuance of the diffusion channel which eventually leads to the remineralization of the inner portion.

Flaitz showed that the remineralization in neutral remineralizing solution occurs much more after the acid etching of the surface zone of the enamel artificial caries (Flaitz et al. 1994).

Second, the remineralzing solution could penetrate the inner portion due to the smaller size of hydroxyapatite crystal in pH 4.3 than in pH 7.0.

Ishikawa suggested that the size of hydroxyapatite from the hydrolysis of DCPA (dicalcium phosphate anhydrous) in pH6.5 becomes significantly smaller than that in pH7.0 (Ishikawa et al. 1993).

Jeon et al. proved that the crystallines are arranged more densely in pH 5.0 and pH 6.0 than in pH 4.3 under the observation of scanning electromicroscope and it blocks the diffusion of the remineralizing solution to the inner portion (Jeon et al. 2007).

Finally, it is assumed that there might be the difference of the formation and the growth of the fluoridated hydroxyapatite; the remineralization could not occur since the amount of fluoride was small because of the expense of fluoride ions on the surface zone in pH 7.0.

The formation mechanism of fluoridated hydroxyapatite could be made by three methods: the precipitation of fluoridated hydroxyapatite, the exchange of hydroxyl ion to fluoride and the recrystallization of hydroxyapatite to fluoridated hydroxyapatite (Barone J.P. et al. 1978). Among those three methods, the exchange of hydroxyl ion to fluoride occurs frequently in pH 7.0 for it is the easiest and fastest way to use up fluoride mainly on the surface zone.

This could indicate that less fluoride concentration on the surface zone was used since the precipitation and recrystallization from the hydrolysis of precursor led to the slow formation and the growth of the crystals in pH 4.3, whereas the more expense of fluoride on the surface zone was occurred as the hydroxyapatite was exchanged to fluoridated hydroxyapatite in pH 7.0.

Lammers et al. suggested the precursor of hydroxyapatite in pH 5.5 is DCPD(dicalcium phosphate dehydrate) and that of it in pH 6.8 is OCP(octacalcium phosphate) which alters into hydroxyapatite in low fluoride concentration. It also indicated that fluoridated hydroxyapatite can be made from without OCP(octacalcium phosphate) as fluoride concentration increases (Lammers et al. 1992).

The remineralization rate occurred less than the experiment by Lynch et al. with the depth of the lesion at 300 ~ 500 µm however, the depth of the demineralization was 40 ~ 50 µm in this experiment.

This experiment could be designed differently in order to prove more remineralization occurring in pH 4.3 than in pH 7.0, using higher fluoride concentration where the demineralization does not occur or the shallow depth of demineralization.

Thus, it needs to analyze the component from the crystals since the the remineralization phases were shown differently both in pH 4.3 and pH 7.0 remineralizing solutions to figure out either the hydroxyapatite precursor or hydroxyapatite crystal growth phase resulted from the experiment by Lammers et al. was affected.

V. Conclusion

In order to figure out the effect of fluoride of the enamel artificial caries on the remineralization, the phase of remineralization was observed and compared by using different fluoride concentration in pH 4.3 and pH 7.0 remineralizing solutions and the following results were drawn from the experiment.

1. The more fluoride concentration increased, the more the remineralization occurred in pH 4.3 remineralizing solution. (group 1,2,4, $p<0.05$)
2. There was entire rate of remineralization increasing in the remineralizing solution however; there was no significant difference in fluoride concentration.
3. As fluoride concentration increased in pH 4.3 remineralizing solution, the rate of remineralization both on the surface zone and subsurface zone increased.
4. As fluoride concentration increased in pH 7.0 remineralizing solution, the rate of remineralization on the surface zone exceeded that of on the subsurface zone.

In conclusion, fluoride affected the remineralization of the enamel artificial caries more in pH 4.3 than in pH 7.0.

VI. References

김민경, 금기연, 이찬영. 법랑질 인공우식의 재광하에 미치는 pH 의 영향에 관한 연구. 대한치과보존학회지 22(1): 193-208, 1997

김성철, 노병덕, 정일영, 이찬영. 재광화 완충용액의 pH 변화가 상아질 재광화에 미치는 영향. 대한치과보존학회지 32(2):151-161, 2007

곽영준, 김의성, 박성호, 공형규, 이윤, 이찬영. pH5.5 에서 재광화 용액의 포화도 변화가 인공 탈회된 법랑질의 재광화에 미치는 영향. 대한치과보존학회지 33(5):481-492, 2008

박정원, 허복, 이찬영. 유기산 완충 용액의 포화도가 법랑질 및 상아질의 재광화에 미치는 영향과 산화 인회석의 AFM 관찰. 대한치과보존학회지 25:459-473. 2001

이찬영. 산 완충 용액을 이용한 인공 치아 우식 형성. 연세치대논문집 7:34-41, 1992

한원섭, 금기연, 이찬영. 인공 치아 우식의 재광화에 미치는 불소의 영향. 대한치과보존학회지 21(1): 161-173, 1996

전영의, 정일영, 노병덕, 이찬영. 유산 완충 용액의 pH 및 포화도 변화에 따른 법랑질 내 수산화인회석 결정 형태의 변화. 대한치과보존학회지 32(6):498-513, 2007

Aoba T, Okazaki M, Takahashi J, Moriwaki Y. X-ray diffraction study on remineralization using synthetic hydroxyapatite pellets. Caries Res 12:220-230. 1978

Backer dirks, O. The benefits of water fluoridation, Caries Res (Suppl) 8:2-15, 1974

Barone J.P., Nancollas G.H. The growth of calcium phosphates on hydroxyapatite crystals. The effect of fluoride and phosphate. J Dent Res 57(5-6):735-742, 1978

Edgar, W.M., Rugg-Gunn, A.J., Jenkins, G.N. Geddes, D.A. Photographic and direct visual recording of experimental caries-like changes in human dental enamel. Arch Oral Biol 23,667-673, 1978

Flaitz C.M., Hicks M.J. Role of the acid-etch technique in remineralization of caries-like lesion of enamel: a polarized light and scanning electron microscopic study. ASDC J Dent Child 61:32-38 1994

Forrest. J.R. Caries incidence and enamel defects in areas with different levels of fluoride in drinking water. Br Dent J 100:195-200, 1965

Fox J.L., Iyer B.V., Higuchi W.I. Hefferren J.J. Solution acitivity product (K_{FAP}) and simultaneous demineralization-remineralization in bovine tooth enamel and hydroxyapatite pellets. J Pharm Sci 72:1252-1255. 1983

Ishikawa K., Eanes E.D. The hydrolysis of anhydrous dicalcium phosphate into hydroxyapatite. J Dent Res 72(2):474-480. 1993

Kay M.I., Young, R.A., Posner, A.S. The crystal structure of hydroxyapatite. Nature (Lond) 204:1050-1052

Lammers P.C., Borggreven J.M.P.M., Driessens F.C.M. Influence of fluoride and pH in vitro remineralization of bovine enamel. Caries Res 26:8-13. 1992

Lynch R.J.M., Mony U., ten Cate J.M. The effect of fluoride at plaque fluid concentrations on enamel de- and remineralisation at low pH. Caries Res 40:533-539. 2006

Moreno E.C., Zahradnik R.T. Chemistry of enamel subsurface zone zone demineralization in vitro. J Dent Res 53:226-235, 1974.

Moreno E.C., Kresak, M, Zahradnik RT. Fluoridated hydroxyapatite solubility and caries formation. Nature 247:64-65. 1974

Margolis H.C., Moreno E.C., Murphy B.J. Effect of low levels of fluoride in solution on enamel demineralization in vitro. J Dent Res 65(1):23-29, 1986

Margolis H.C., Moreno, E.C. Physicochemical perspectives on the cariostatic mechanisms of systemic and topical fluoride. J Dent Res 69, 606-613, 1990

Silverstone L.M. The surface zone zone in caries and in caries-like lesions produced in vitro. Brit Dent J 20;125(4): 145-157, 1968

Silverstone L.M. Remineralization phenomena. Caries Res 11(Suppl 1) : 59-84, 1977

Silverstone L.M., Wefel J.S., Zimmerman B.F., Clarkson B.H., Featherstone MJ Remineralization of nature and artificial lesion s in human dental enamel in vitro. Effect of calcium concentration of the calcifying fluid. Caries Res 15:138-157, 1981

ten Cate J.M. In vitro studies on the effects of fluoride on de- and remineralization. J Dent Res 69 (special issue):614-619, 19901

ten Cate J.M. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. Eur J Oral Sci 1997;105:461–5.

ten Cate J.M.., Arends J. Remineralization of artificial enamel lesions in vitro. Caries Res 11:277-286, 1977

Young, R.A., Elliott, J.C. Atomic-scale bases for several properties of apatites. Arch Oral Biol 11:699-707, 1966

Yamazaki H. Litman A, Margolis H.C Effect of fluoride on artificial caries lesion progression and repair in human enamel : Regulation of mineral deposition and dissolution under in vivo-like condition. Arch Oral Bio 52:110-120, 2007

Yamazaki, H., Margolis, H.C. Enhanced enamel remineralization under acidic condition in vitro. J Dent Res 87(6):569-574, 2008

ABSTRACT (in Korean)

pH 4.3 과 7.0 재광화 완충용액에서 법랑질 인공우식의

재광화에 있어 불소의 영향

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치의학과

여러 역학적 연구에서 식수에 불소가 들어있는 경우 충치 이환율을 낮추고 초기 법랑질 우식이 불소에 의해 사라지는 것을 관찰하였다. 결정학적으로 불소 결정의 안정성과 성장기전으로 인해 불화 수산화인회석은 일반 수산화인회석 보다 낮은 용해도를 가진다.

이런 불소의 성질을 이용하여 우식 병소를 재광화 하려는 연구가 있어왔는데, Marolis 등은 재광화에 사용되는 불화 수산화인회석의 침착을 포화도를 이용하여 생리학적으로 설명하였다.

포화도가 높을수록 재광화가 많이 일어나지만, 재광화가 심부까지 완전하게 일어나지는 않는데, 이런 문제를 해결하기 위해서 표면 부위의 포화도를 낮추어 심부까지 재광화가 이루어지게 하려는 시도가 있어왔다.

치아 우식의 재광화를 위해서 불소가 여러가지 조건으로 활용되었지만, 명확한 이론적인 설명이나 근거가 아직 부족한 상태이다.

이번 실험에서는 불소가 인공 우식 치아의 재광화에 미치는 영향을 알기위해 pH 4.3 과 pH 7.0 재광화 완충용액에서 불소의 농도를 다르게 하여 법랑질 인공 우식에 나타나는 재광화 양상을 비교 관찰하여 다소의 지견을 얻었기에 이를 보고하는 바이다.

이 연구에서는 탈회 완충용액을 이용하여 법랑질을 탈회시키고, 그 시편을 각각 재광화 완충용액에서 최대 8 일간 편광현미경으로 관찰한 후 흑화도를 구하여 분석한 후 다음과 같은 결과를 얻었다.

1. pH 4.3 재광화 완충용액에서는 불소의 농도가 증가할수록 재광화양이 더 증가했다. (group 1,2,4, $p<0.05$)
2. pH 7.0 재광화 완충용액에서는 전체적인 재광화양은 증가하지만 불소 농도에 따른 차이는 없었다.
3. pH 4.3 재광화 완충용액에서는 불소의 농도가 증가함에 따라 표면층과 표면하층 모두 재광화양이 증가했다.
4. pH 7.0 재광화 완충용액에서는 불소의 농도가 증가함에 따라 표면층이 표면하층보다 재광화양이 증가했다. (group 8,$p<0.05$)

결론적으로 불소는 pH 7.0 보다 pH 4.3 에서 법랑질 인공 우식치아의 재광화에 더 영향을 주었다.

핵심되는 말: 인공우식 형성, 재광화, 불소, 수산화인회석, 불화 수산화인회석