Changes in caries resistance by irradiation

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Changes in caries resistance by irradiation

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먼저 묵묵히 학문과 임상에 열정적인 모습으로 많은 가르침과 참된 학자의 길을 손수 보여주시며 너무나 부족한 저를 이끌어주시고 가르침을 주신 박성호 교수님께 깊은 감사를 드립니다. 또한 부족한 제가 논문을 작성하는데 의미 있는 가르침을 주신 이찬영 교수님, 박정원 교수님, 김백일 교수님, 안형준 교수님께도 감사 드립니다.

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진심 어린 충고를 아끼지 않은 문원규 원장님과 백석기 원장님과 여러 고운미소 가족들에게도 이 지면을 통해 소중한 인연에 감사 드립니다.

힘든 실험 과정에서도 보다 의미 있는 결과를 만들기 위해 밝은 모습으로 함께 고생해주신 김도현 선생님께도 진심으로 감사 드립니다.

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Abstract

Changes in caries resistance by irradiation

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In patients undergoing radiation therapy, the likelihood of caries becomes high because of the reduced saliva secretion and changes in its composition in response to the radiation. Another cause is the possibility of radiation therapy having direct effects on the dental hard tissues, which is controversial. This study examined whether caries resistance in teeth was changed because of the alteration of the physical properties of the enamel, cementum and dentin surfaces after radiation therapy. For this, using 60 extracted teeth, this study examined whether the caries resistance of the crown and the root prior to irradiation was different from after irradiation by measuring the depth, area of caries, and the density of caries. In addition, to

investigate methods for improving the caries resistance, the teeth surface were treated with fluoride varnish, 1.1 % NaF, 5 % hydroxyapatite (HA) and chlorohexidine under a range of conditions prior to irradiation, and the change in caries resistance was examined. To examine the effective methods for treating the tooth with caries developed after irradiation, caries was induced artificially, treated with fluoride varnish, 1.1 % NaF, or 5 % hydroxyapatite (HA), and the remineralization pattern was examined.

The difference in caries with or without radiation was compared. A polarized microscopy examination showed that more caries developed after irradiation ($p<0.05$). Moreover, the caries resistance of the cementum area of the root area was enhanced when fluoride varnish (Cavity Shield, 3M/ESPE. St Paul. MN. U.S.A) was applied and subsequently irradiated ($p<0.05$). When the remineralization capacity was examined after the induction of artificial caries, remineralization was achieved successfully in the group without irradiation using fluoride varnish. On the other hand, remineralization could not be achieved in the irradiation group regardless of the treatments.

Keywords: irradiation, artificial caries, remineralization, Fluoride varnish, caries resistance, Hydroxyapatite, enamel, cementum, dentin

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I. Introduction

In patients receiving radiation therapy in the head and neck area, dental caries in the area where caries rarely forms can occur at very rapid rates. Moreover, the probability of developing secondary caries in the vicinity of the teeth that had been treated already is high, and the progress of dental caries occurs without pain (Vissink et al., 2003; Kielbassa et al., 2006). The probability of developing dental caries is higher in patients receiving radiation therapy, and several causes have been reported. First, the reduction of the saliva due to irradiation as well as the levels of IgA, pH, bicarbonate, etc. are reduced (Anderson et al., 1981; Marks et al., 1981). In patients

receiving 20-30 Gy (gray) irradiation, the effect on the salivary gland might be reversible. On the other hand, in cases whose irradiation dose is > 50 Gy, irradiation may exert fatal effects on acinar cells (Wolff et al., 1990). In addition, inflammation is frequently induced in the oral mucosa, and the risk of caries is increased due to the consequent diet changes (Vissink et al., 2003).

The level of saliva secretion is reduced in response to radiation therapy from the early phase of radiation therapy. Generally, during the first week of radiation therapy, a 50 - 60 % decrease in saliva secretion occurs, and at approximately 7 weeks after radiation therapy, the secretion volume was reported to be 20 % lower (Frazen et al., 1992).

The decrease in saliva secretion by radiation therapy in the head and neck area causes a significant decrease in the reaction of calcium ions and potassium ions within the saliva. These two ions play very important roles in the formation the calcium phosphate salts, such as calcium hydroxyapatite, which is not dissolved during the remineralization of teeth. Remineralization is a natural compensatory action for the release of inorganic substances caused by acids within plaque formed by acid forming bacteria (Backer et al., 1966). In healthy individuals, the lost inorganic substances are replenished by remineralization from the calcium and phosphate ions within the saliva and the balance is maintained.

Fluoride ions facilitate remineralization and prevent the development to dental caries (Featherstone et al., 1990; Gibbs et al., 1995). Nevertheless, remineralization is limited if calcium and potassium ions are reduced due to the decreased saliva secretion, even in the presence of fluoride.

Gortz et al. (1997) reported that irradiation can directly damage the dentinoenamel junction. Jansma et al. (1988) reported cases whose healthy dentin adjacent to the enamel was lost completely after irradiation due to these effects. In studies that examined the effect of radiation therapy on the microhardness of dental hard tissues, 60 Gy irradiation has serious adverse effects on the dentin and reduced microhardness was observed resulting in the breakdown of the enamel or the formation of gaps in the dentinoenamel junction as well as caries in the cervical area (Kielbassa et al., 1997, 2002). In studies that examined the stability of the dentinoenamel junction, one compared the shear bond strength of the dentinoenamel junction after 70 Gy irradiation and reported that the stability was decreased significantly in the irradiation group. In particular, it was reported that in the irradiated group, the fractured area was generated in the dentinoenamel junction, and the fracture pattern in the dentinoenamel junction was increased. This suggests that the physical properties of teeth can be changed directly by irradiation (Pioch et al,. 1992).

The physical properties of dental hard tissues after irradiation can be analyzed using the ultrasound transmission velocity that evaluates the physical properties of bone tissues without destroying them. The results of such analysis revealed the ultrasound transmission velocity to concur with the modulus of elasticity of hard tissues. Compared to healthy teeth, an increase in the ultrasound transmission velocity was observed in the enamel and dentin irradiated with 36 Gy or 62 Gy, which suggests a change in the modulus of elasticity of the hard tissues after irradiation. In addition, it was reported that in vitro, organic components of the interprismatic enamel was damaged severely when irradiated with 500 Gy, regardless of the water content within the tooth (Al-Nawas et al., 2000).

In addition, irradiation was reported to affect the pulp directly as well as the metabolism of odontoblasts within the pulp, which impedes the formation of the secondary dentin resulting in the rapid progression of dental caries (Grotz et al., 1997; Al-Nawas et al., 2000).

Springer et al. (2005) examined the direct effect of irradiation on the collagen of dental hard tissues. The change in dental collagen after 31.5 Gy irradiation was examined. They reported that the collagen content in dental hard tissues was small, and thus the direct effect of irradiation on dental

hard tissues could not be assessed precisely. Nevertheless, they reported that irradiation has direct effects on collagen within the pulp.

In attempts to reduce the risk of dental caries, efforts have been made to improve oral hygiene and facilitate saliva secretion by a range of methods, and topical fluoride agents containing a high concentration of fluoride and calcium have been prescribed (Wei et al., 1993). The application of fluoride varnish is the most commonly prescribed topical fluoride in western countries in the past 30 years. In addition, methods to apply fluoride using a tray are also effective (Epstein et al., 1995). Patients who cannot use the tray well enough are instructed to brush their teeth or gargle using a gel containing a high concentration of fluoride (1.1 % NaF gel)(Mcycrowitz et al., 1991). Fluoride released into the oral cavity facilitates the remineralization of teeth and plays a role in preventing the leakage of inorganic substances from teeth (Ten Cate, 1990). Such prophylactic effects of fluoride on dental caries are widely known. Recently, since fluoride is stable, the use of tooth paste or mouth washes containing a low fluoride concentration has been recommended (Navarro et al., 2001). More than 95 % of the mature enamel is comprised of inorganic substances, and enamel crystals are composed of carbonated hydroxyapatite (Manly et al., 1939). Recently, many studies on the remineralization of teeth with a

similar structure to enamel have been conducted, and amelogenin was reported to be of help in forming needle-like fluoridated hydroxyapatite (Yuwei et al., 2009).

The reason why dental caries develops abundantly in irradiated teeth may be due not only to problems associated with saliva secretion but also the physical and chemical changes in hard tissues caused by irradiation.

Using extracted teeth, this study examined whether caries resistance is altered in the enamel, cementum and dentine after irradiation. In addition, to assess methods to enhance the caries resistance of irradiated teeth, the teeth were treated with several substances known to have prophylactic effects on dental caries either prior to or after irradiation, and it was examined whether the caries resistance was altered. In addition, to examine effective remineralization methods for caries, remineralization using several substances was attempted, and the patterns were compared.

II. Materials and methods

1. Preparation of the specimens prior to irradiation

Sixty premolars without dental caries or restorations within one month after extraction were selected. The soft tissues and dental calculus attached to the tooth surface were removed by a periodontal instrument. The tooth surface was polished with abrasives not containing fluoride, cleaned with an ultrasonic cleaner for 10 minutes, washed with distilled water and dried. The teeth were sectioned buccolingually into two parts, and a total 120 samples were prepared.

2. Preparation of the specimens after irradiation

1. Classification of experimental groups

Each group used 10 samples, group NR was the non-irradiation group, group PR was irradiated with 60 Gy without treatment, and the group FV was applied a fluoride varnish (Cavity Shield, 3M/ESPE. St Paul. MN. U.S.A) on the surface and irradiated with 60Gy radiation. The HA groups were pretreated with 5 % Hydroxyapatite (Diome, Seoul, Korea) for 5, 15 or 30 minutes and then irradiated. The SF groups were pretreated with 1.1 %

sodium fluoride for 5, 15 or 30 minutes and then irradiated. The CH groups were pretreated with chlorhexidine (Hexamedine, Bukwang Pharm. Co., LTD. Seoul. Korea) for 5, 15 or 30 minutes and then irradiated (Table 1).

Table 1. Experimental Groups for demineralization (N=10)

Groups	Pretreatment solutions	Conditoin
Group NR	No radiation	Control
Group PR	Radiation	
Group FV	Fluoride varnish	
Group HA5		5 minutes
Group HA15	Hydroxy apatite	15 minutes
Group HA30		30 minutes
Group SF5		5 minutes
Group SF15	Sodium Fluoride	15 minutes
Group SF30		30 minutes
Group CH5		5 minutes
Group CH15	Chlorohexidine	15 minutes
Group CH30		30 minutes

NR, No radiation; PR, Radiation; FV, Fluoride varnish; HA, Hydroxyapatite; SF, Sodium Fluoride; CH, Chlorohexidine.

2 .The preparation of samples after irradiation

Resin blocks, 2 cm in height, 4 cm in length and 2 cm in width, were prepared using dental acryl resin. After the curing the acryl resin, the crown area and root area were removed by a low speed diamond wheel saw (Diamond Cutter RB205 Metsaw –LS, R&B co., LTD. Deajeon. Korea), and polished again to a 100-150 μm thickness using SiC paper. For each group, 10 crown samples, 10 root samples and a total 240 samples were prepared. The surface except for the window was polymerized with bonding agents (Scotchbond Multi-Purpose, 3M/ESPE. St Paul. MN. U.S.A). Nail varnish was then applied to the buccolingual area of the side of sample without injury or fissure except for an approximately 1-2 mm window(Figure 1).

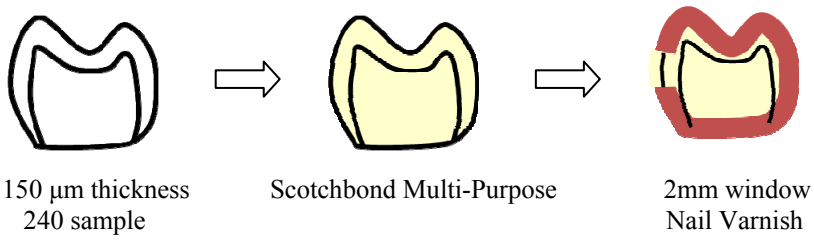

Figure 1. Schematic diagram of sample preparation.

3. The formation of artificial dental caries and observation

The tooth samples were treated with the artificial tooth caries solution (pH 4.3, lactic acid 100 mM, calcium 16 mM, Potassium phosphate 8 mM, Sodium Azide 3 mM) at 25 ^{o}C for 3 days. The samples were examined using a polarized microscope at magnification of x50 and x100. Polarizing photographs were taken using a digital camera attached to the microscope.

4. Statistical analysis between groups for depth and area in demineralization

Using the pictures taken under polarized microscope, the artificial dental caries areas were divided to 4 parts. The depth of caries was assessed by measuring the vertical length from the surface of the four areas to the end of the positive birefringence. In addition, the area of caries was measured by calculating the 0.5mm unit area (Figure 2).

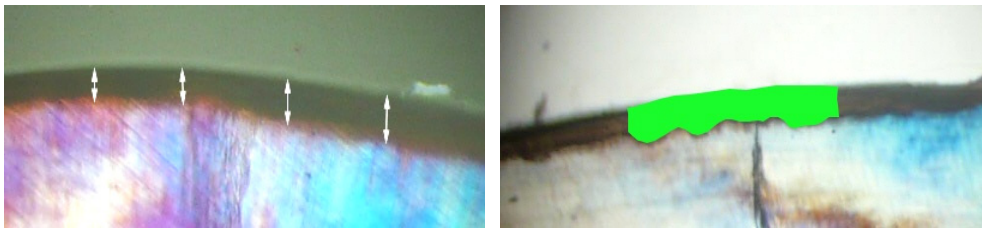

Figure 2. Measurement of the depth of caries(A) and the area of caries(B).

5. Remineralization of artificial dental caries

Among the samples with induced artificial dental caries, the NR Group without irradiation was remineralized with the Fluoride varnish (Cavity Shield, 3M/ESPE. St Paul. MN. U.S.A) at 25^{o}C. After 10 minutes, 30 minutes, or 1 day remineralization, the samples were examined at x50 and x100 magnification under a polarized microscope. Images were taken using a digital camera attached to the microscope. In addition, the FV group treated with Fluoride varnish (Cavity Shield, 3M/ESPE. St Paul. MN. U.S.A,), and the group treated with 5 % Hydroxyapatite (Group HA30) or 1.1 % Sodium fluoride (Group SF30) for 30 minutes and then irradiated were remineralized using the corresponding solution at 25^{o}C for 1 day. The samples were examined using a polarized microscope at x50 and x100 magnification. Images were taken by a digital camera attached to the microscope (Table 2).

Table 2. Experimental Groups in remineralization (N=10)

Groups	Pretreatment and remineralization solutions	Time
Group NR(10min)	No radiation / Fluoride varnish	10min
Group NR (30min)	No radiation / Fluoride varnish	30min
Group NR (1day)	No radiation / Fluoride varnish	1day
Group FV	Radiation +Fluoride varnish / Fluoride varnish	1day
Group HA30	Radiation +Hydroxyapatite / Hydroxyapatite	1day
Group SF30	Radiation + Sodium Fluoride / Sodium Fluoride	1day

NR, No radiation; PR, Radiation; FV, Fluoride varnish; HA, Hydroxyapatite; SF, Sodium Fluoride; CH, Chlorohexidine.

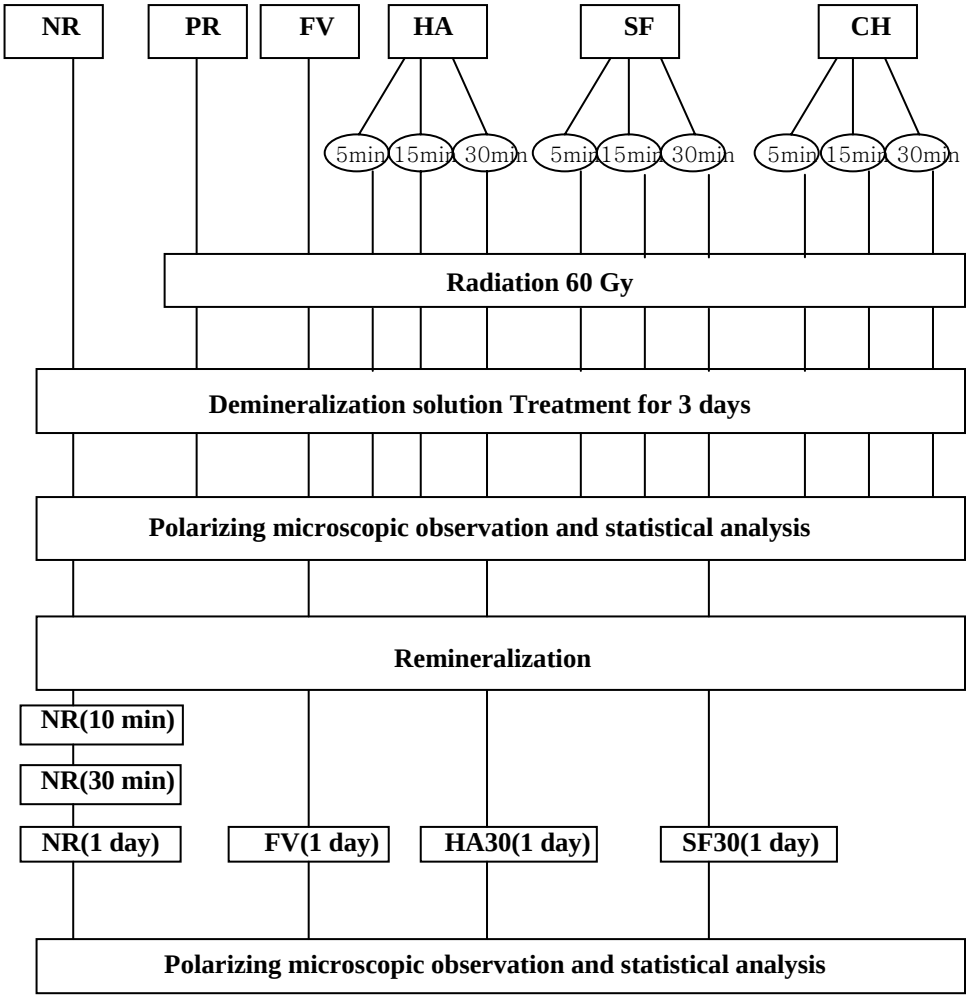

Figure 3. Flow chart of the experimental Groups in demineralization and remineralization. NR, No radiation; PR, Radiation; FV, Fluoride varnish; HA, Hydroxyapatite; SF, Sodium Fluoride; CH, Chlorohexidine.

6. Statistical analysis between groups for depth and area in remineralization

From the images taken under the polarized microscope, the artificial dental caries areas were divided to 4 parts. The depth of caries was assessed by measuring the vertical length from the surface of the four areas to the end of the positive birefringence. The area of caries was measured by calculating the 0.5mm unit area (Figure 3). In the root area, the entire caries depth to the dentin and cementum areas was measured.

For a quantitative evaluation of the amount of remineralization, the density of the samples was measured using the plot profile tool of the ImageJ analysis program (NIH, Maryland, U.S.A). The graphs were analyzed using the Spread sheet program Excel (MS Office 2007, Microsoft, U.S.A). The graph was calculated, and the area of the remineralized layer is presented as the percentage of the demineralized layer.

The difference in the depth of caries, area of caries and amount of remineralization obtained from the above results were analyzed by One-way ANOVA using SPSS 12.0. For the significant variables, post-hoc analysis was performed by a Tukey test at the significance level of 0.05. In the remineralization experiments, a Paired t-test was performed to validate the

statistical significance of the caries depth and the caries area before and after remineralization.

III. Results

1. Measurement of the depth of caries

1. The depth of caries in the crown area

The characteristics of the early caries lesions in the enamel of the surface demineralized area and subsurface demineralized area could be observed, and a difference in the caries pattern between before and after irradiation was noted (Figure 4-A,B). The mean caries depth of the non-irradiated and irradiation group was 82.41 ㎛ and 104.00 ㎛, respectively. The caries pattern after irradiation showed a significant increase ($p<0.05$, Table 3). In addition, in the irradiation group, the caries pattern was more aggressive and the caries progressed more than the non-irradiation group. In the group treated with fluoride varnish at the time of irradiation, the mean caries depth was reduced to 90.72 ㎛. Nevertheless, no significant differences were observed (Figure 4-C). In all samples treated with sodium fluoride, hydroxyapatite or chlorhexidine for 5 minutes, 15 minutes or 30 minutes, the depth of caries was decreased slightly. However, there were no significant differences compared to the irradiation group.

Table 3. Changes in the demineralized lesion depth in the enamel (N=10)

Groups	demineralized depth（㎛）
Group NR	82.41 ± 32.63[a]
Group PR	104.00 ± 27.07[b]
Group FV	90.72 ± 16.85[a,b]
Group HA5	101.64 ± 9.32[a,b]
Group HA15	97.26 ± 17.58[a,b]
Group HA30	94.15 ± 17.06[a,b]
Group SF5	99.84 ± 8.84[a,b]
Group SF15	97.66 ± 12.37[a,b]
Group SF30	94.70 ± 13.41[a,b]
Group CH5	96.81 ± 12.02[a,b]
Group CH15	97.49 ± 14.78[a,b]
Group CH30	95.80 ± 15.63[a,b]

a,b: superscript letters means a statistical difference ($p<0.05$).

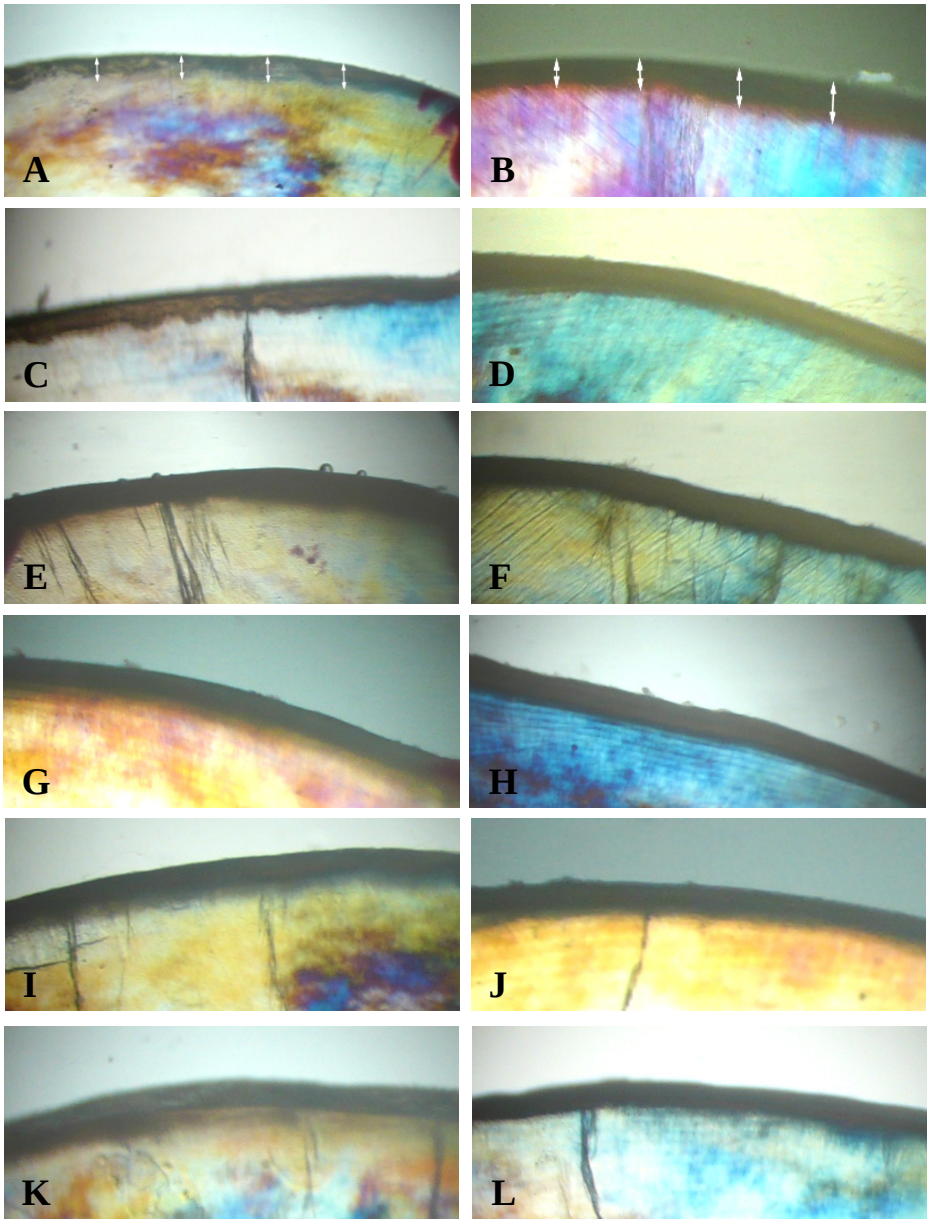

Figure 4. Polarizing microscopy images of demineralized enamel(magnification, x100). A, Group NR, B, Group PR, C, Group FV, D, Group HA5, E, Group HA15, F, Group HA30. G, Group SF5, H, Group SF15, I, Group SF30, J, Group CH5, K, Group CH15, L, Group CH30.

2. *The depth of caries in the root area*

In the root area, the depth of caries in the cementum area and the entire caries depth to the dentin area were measured. The demineralized depth measured in the cementum area of the non-irradiate group was 56.51 ㎛, and the demineralized depth of the irradiation group was 77.39 ㎛. The caries patterns after irradiation showed significant differences ($p<0.05$, Figure 5-A,B, Table 4). In the group treated with fluoride varnish at the time of irradiation, the demineralized depth was 65.99 ㎛, which was significantly different lower than that of the irradiation group ($p<0.05$). In the caries in the entire root area, only the depth of caries in the dentin excluding the cementum area was compared. No significant differences were observed between the non-irradiation group and irradiation group. The entire depth of the caries to the dentin area was measured, the caries depth of the non-irradiation and irradiation group was 126.62 ㎛ and 174.10 ㎛, respectively. The caries pattern after irradiation showed significant increases ($p<0.05$, Figure 5-A,B, Table 4). In the group treated with fluoride varnish at the time of irradiation, the caries depth was 147.76 ㎛, and the caries pattern was lower than that of the irradiation group but the difference was not significant (Figure 5-C). In the group treated with sodium fluoride,

hydroxyapatite, or chlorhexidine for 5, 15 or 30 minutes, deeper caries was detected compared to the non-irradiation group ($p<0.05$). The depth of the caries surface was slightly reduced but there was no significant difference compared to the irradiation group.

Table 4. Changes in the demineralized lesion depth in the cemnetum and dentin (N=10)

Groups	demineralized depth（μm）		
	Cementum	Dentin	Cementum+Dentin
Group NR	56.51 ± 14.39^{a}	70.11 ± 16.34^{a}	126.62 ± 10.54^{a}
Group PR	77.96 ± 10.72^{c}	96.13 ± 21.57^{a}	174.10 ± 25.08^{b}
Group FV	$65.99 \pm 11.36^{a,b}$	81.73 ± 15.30^{a}	$147.76 \pm 17.35^{a,b}$
Group HA5	$74.52 \pm 6.57^{b,c}$	89.01 ± 17.81^{a}	163.53 ± 20.74^{b}
Group HA15	$72.95 \pm 8.33^{b,c}$	89.06 ± 19.83^{a}	162.01 ± 24.47^{b}
Group HA30	$73.89 \pm 10.85^{b,c}$	95.37 ± 28.00^{a}	169.27 ± 34.16^{b}
Group SF5	$76.36 \pm 7.31^{b,c}$	89.94 ± 28.46^{a}	166.30 ± 29.86^{b}
Group SF15	$74.37 \pm 10.76^{b,c}$	86.96 ± 24.50^{a}	161.34 ± 36.85^{b}
Group SF30	$72.74 \pm 10.32^{b,c}$	88.13 ± 26.70^{a}	160.87 ± 36.78^{b}
Group CH5	$75.65 \pm 7.65^{b,c}$	92.75 ± 24.93^{a}	168.41 ± 28.34^{b}
Group CH15	$76.29 \pm 8.38^{b,c}$	88.34 ± 6.93^{a}	164.64 ± 12.52^{b}
Group CH30	$76.34 \pm 13.24^{b,c}$	86.73 ± 12.07^{a}	163.08 ± 16.80^{b}

a,b,c: Different superscript letters means statistical difference ($p<0.05$).

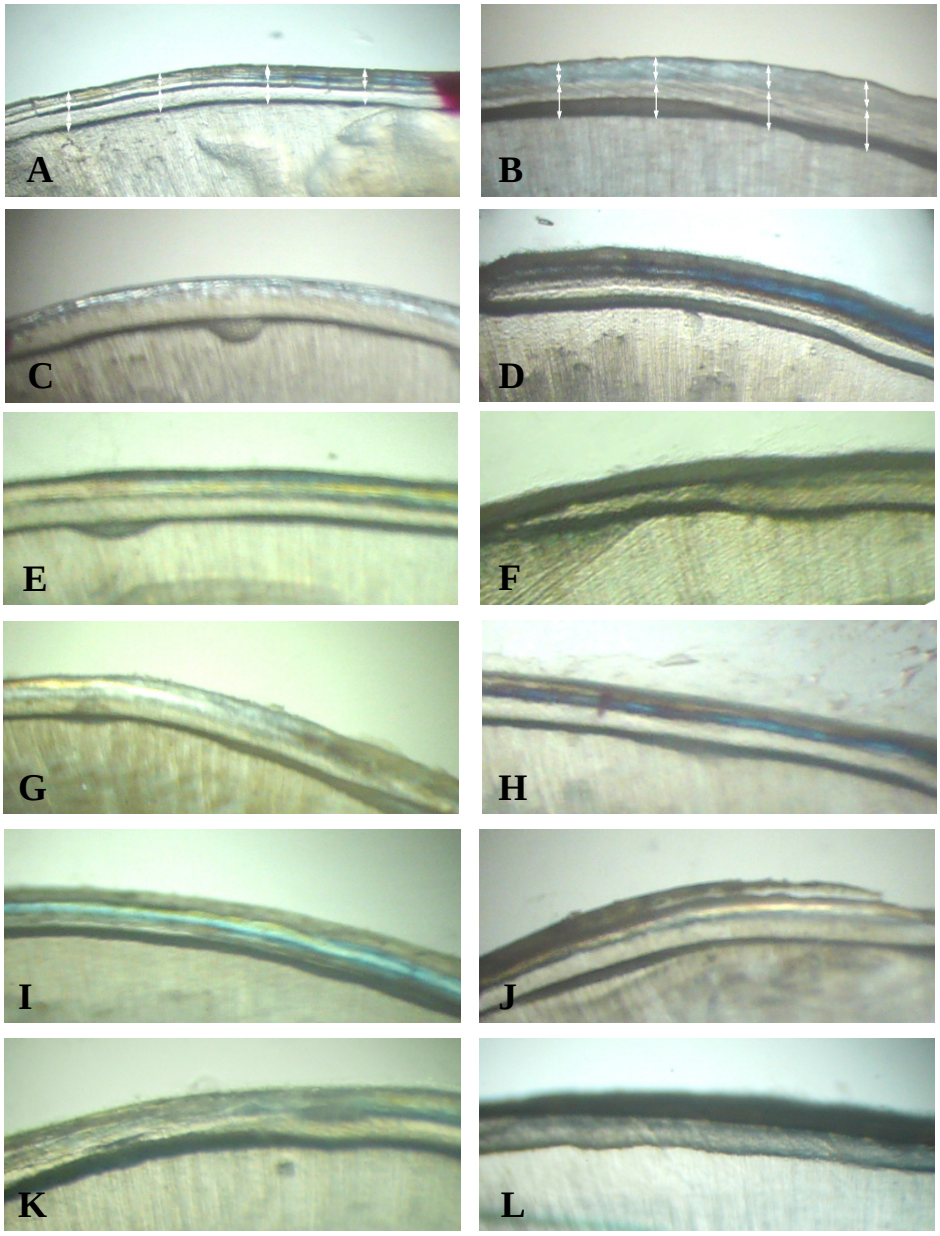

Figure 5. Polarizing microscopy images of the demineralized cementum and dentin (magnification, x100). A, Group NR, B, Group PR, C, Group FV, D, Group HA5, E, Group HA15, F, Group HA30, G, Group SF5, H, Group SF15, I, Group SF30, J, Group CH5, K, Group CH15, L, Group CH30.

2. Measurement of the area of caries

1. The area of caries in the crown area

The area per unit length was measured to evaluate the level of caries, together with the depth of the demineralized areas. The area of caries is uneven. Therefore, the entire area rather than the depth of caries was measured to evaluate the level of caries more accurately. The caries area of the non-irradiation and irradiation group was 42,227 μm^2 and 53,332 μm^2, respectively showing a significant increase ($p<0.05$, Table 5). In the group treated with fluoride varnish, the area of caries was 45,518 μm^2, but there was no significant difference compared to the untreated group. In the group treated with sodium fluoride, hydroxyapatite, or chlorhexidine for 5, 15 or 30 minutes, the area of the caries surface layer was smaller than the irradiation group but larger than the non-irradiation group but the difference was not significant.

Table 5. Changes in the demineralized lesion area in the enamel (N=10)

Groups	demineralized area (㎛ ^{2})
Group NR	42,227 ± 4393[a]
Group PR	53,332 ± 7084[b]
Group FV	45,518 ± 9720[a,b]
Group HA5	49,892 ± 4899[a,b]
Group HA15	49,216 ± 7320[a,b]
Group HA30	48,108 ± 8390[a,b]
Group SF5	49,161 ± 2686[a,b]
Group SF15	49,258 ± 8542[a,b]
Group SF30	48,078 ± 10061[a,b]
Group CH5	48,302 ± 7196[a,b]
Group CH15	49,717 ± 9142[a,b]
Group CH30	48,964 ± 5364[a,b]

a,b: Different superscript letters means statistical difference ($p<0.05$).

2. The area of caries in the root area

The caries area of the non-irradiation and irradiation group was 62,080 μm^2 and 87,706 μm^2, respectively, showing a significant increase ($p<0.05$, Table 6). In the group treated with fluoride varnish, the area of caries was 74,105 μm^2, showing a non-significant decrease. In the group treated with sodium fluoride, hydroxyapatite or chlorhexidine for 5, 15 or 30, the area of the caries surface layer was smaller than that of the irradiation group, but larger than that of the non-irradiation group but the difference was not significant.

Table 6. Changes in the demineralized lesion area in the cementum and dentin (N=10)

Groups	demineralized area (㎛ ^{2})		
	Cementum	Dentin	Cementum+Dentin
Group NR	27,745 ± 4393^{a}	34,335 ± 7301^{a}	62,080 ± 4995^{a}
Group PR	39,022 ± 3655^{b}	8,684 ± 12832^{a}	87,706 ± 13065^{b}
Group FV	32,931 ± 2850^{a,b}	41,174 ± 4930^{a}	74,105 ± 5626^{a,b}
Group HA5	38,330 ± 3877^{b}	46,914 ± 9058^{a}	85,244 ± 6543^{b}
Group HA15	35,166 ± 3163^{a,b}	44,570 ± 12205^{a}	79,737 ± 12093^{b}
Group HA30	36,873 ± 4853^{b}	43,839 ± 7829^{a}	80,712 ± 6555^{b}
Group SF5	36,100 ± 9169^{a,b}	44,753 ± 25179^{a}	80,853 ± 21567^{b}
Group SF15	36,961 ± 2686^{b}	45,152 ± 14859^{a}	82,114 ± 13178^{b}
Group SF30	36,050 ± 9259^{a,b}	44,963 ± 10607^{a}	81,014 ± 8958^{b}
Group CH5	35,590 ± 9429^{a,b}	46,000 ± 13989^{a}	81,590 ± 11067^{b}
Group CH15	37,540 ± 4662^{b}	45,925 ± 6321^{a}	83,465 ± 5257^{b}
Group CH30	38,211 ± 4854^{b}	45,850 ± 10404^{a}	84,061 ± 8618^{b}

a,b: Different superscript letters means statistical difference ($p<0.05$).

3. Measurement of the depth of caries after remineralization

1. The depth of caries after the remineralization of the crown area

The samples of the NR Group were remineralized with fluoride varnish (Cavity Shield, 3M/ESPE. St Paul. MN. U.S.A) for 10 minutes, 30 minutes or 1 day after the induction of artificial caries. The depth of caries was 82.41 ㎛, whereas the depth of caries measured after 10 and 30 minutes remineralization was 78.06 ㎛ and 70.69 ㎛, respectively, showing a decrease albeit not significant. On the other hand, the depth of caries after 1 day remineralization was 52.74 ㎛, showing a significant decrease ($p<0.05$, Figure 6).

The samples from Group FV, HA30, and SF30 were remineralized using the corresponding solution, and the depth of caries was measured. In the FV Group, in which fluoride varnish was used, the depth of caries before and after remineralization was 90.72 ㎛ and 91.13 ㎛, respectively, but the pattern of remineralization could not be observed. Similarly, in the other group there was no difference of the level of caries between before and after remineralization (Table 7).

Table 7. Changes in the demineralized lesion depth in the enamel after remineralization (N=10)

Groups	demineralized depth(㎛) before	demineralized depth(㎛) after
Group NR(10min)	82.41 ± 32.63	78.06 ± 16.50
Group NR(30min)	82.41 ± 32.63	70.69 ± 15.23
Group NR(1day)	82.41 ± 32.63	52.74 ± 9.50†
Group FV	90.72 ± 16.85	91.13 ± 11.86
Group HA30	94.15 ± 17.06	93.76 ± 16.59
Group SF30	94.70 ± 13.41	93.18 ± 11.85

Paired t-test analysis, significantly different at $p<0.05$.

2.The depth of caries after the remineralization of the root area

The depth of caries was measured in the cementum area of the root area after remineralization. After inducing artificial caries, the caries depth was 56.51 ㎛. After 10 minutes, the caries depth was reduced to 50.97 ㎛, but the difference was not significant. The caries depth measured after 30 minutes and 1 day remineralization was 42.88 ㎛ and 35.17 ㎛, respectively, showing a significant difference ($p<0.05$, Table 8). The results

of the measurement of the caries of the entire root area showed that in the caries of dentin only (excluding the cementum area) the remineralization was increased in the order of 10 minutes, 30 minutes and 1 day, and the caries depth was decreased. After 30 minutes or 1 day remineralization, the depth of caries decreased significantly ($p<0.05$). The depth of caries in the dentin area after the induction of artificial caries was 126.62 ㎛. After 10 minutes, 30 minutes and 1 day remineralization, the caries depth was reduced significantly to 110.26 ㎛, 94.01 ㎛ and 79.14 ㎛, respectively ($p<0.05$, Table 8).

The samples in Group FV, HA30, and SF30 were remineralized using the corresponding solution, and the caries depth was measured. In Group FV, the caries depth before and after remineralization was 147.76 ㎛ and 144.65 ㎛, respectively, but the remineralization patterns could not be detected (Figure 7). In the other groups, there was no difference in the level of caries between before and after remineralization (Table 8).

Table 8. Changes in the demineralized lesion depth in the cementum and dentin after remineralization (N=10)

Groups	demineralized depth(㎛) before	demineralized depth(㎛) after
Group NR (10min)		
Cementum	56.51 ± 14.39	50.97 ± 10.62
Dentin	70.11 ± 16.34	59.28 ± 15.57
Cementum+Dentin	126.62 ± 10.54	110.26 ± 15.14†
Group NR (30min)		
Cementum	56.51 ± 14.39	42.88 ± 9.68†
Dentin	70.11 ± 16.34	51.13 ± 13.21†
Cementum+Dentin	126.62 ± 10.54	94.01 ± 17.62†
Group NR(1day)		
Cementum	56.51 ± 14.39	35.17 ± 10.69†
Dentin	70.11 ± 16.34	43.97 ± 11.67†
Cementum+Dentin	126.62 ± 10.54	79.14 ± 10.99†
Group FV		
Cementum	65.99 ± 11.36	64.89 ± 11.03
Dentin	81.77 ± 15.30	79.76 ± 25.31
Cementum+Dentin	147.76 ± 17.35	144.65 ± 22.47
Group HA30		
Cementum	73.89 ± 10.85	72.04 ± 8.94
Dentin	95.37 ± 28.00	90.60 ± 10.60
Cementum+Dentin	169.27 ± 34.16	162.65 ± 20.10
Group SF30		
Cementum	72.74 ± 10.32	72.40 ± 9.68
Dentin	88.13 ± 26.70	89.02 ± 36.62
Cementum+Dentin	160.87 ± 36.78	161.42 ± 39.09

Paired t-test analysis, significantly different at $p<0.05$.

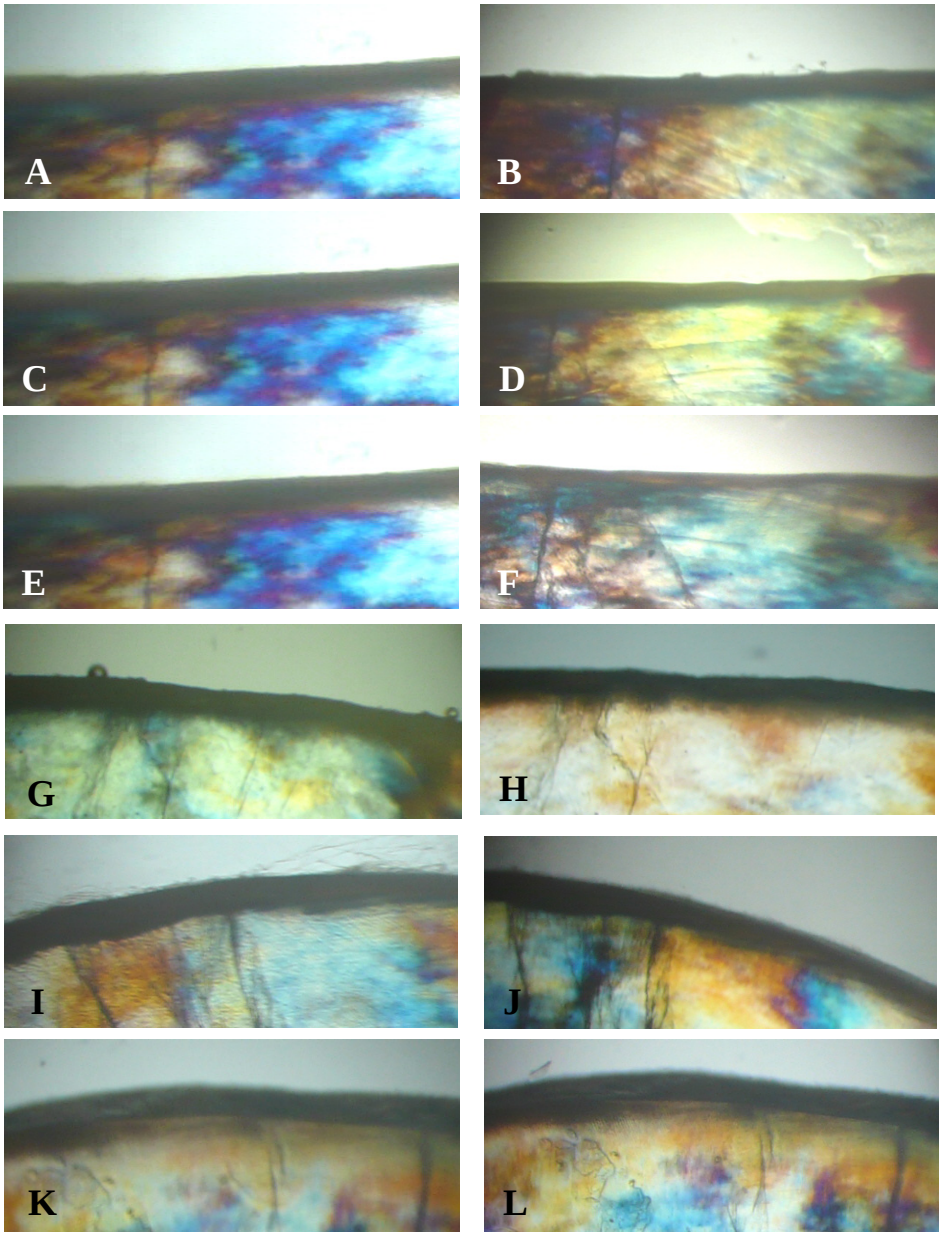

Figure 6. Polarizing microscopy images of remineralized enamel(magnification, x100). A, Group NR(before), B, Group NR(10min), C, Group NR(before), D, Group NR(30min), E, Group NR(before), F, Group NR(1 day), G, Group FV(before), H, Group FV(after), I, Group CH30(before), J, Group CH30(after), K, Group SF30(before), L, Group SF30(after).

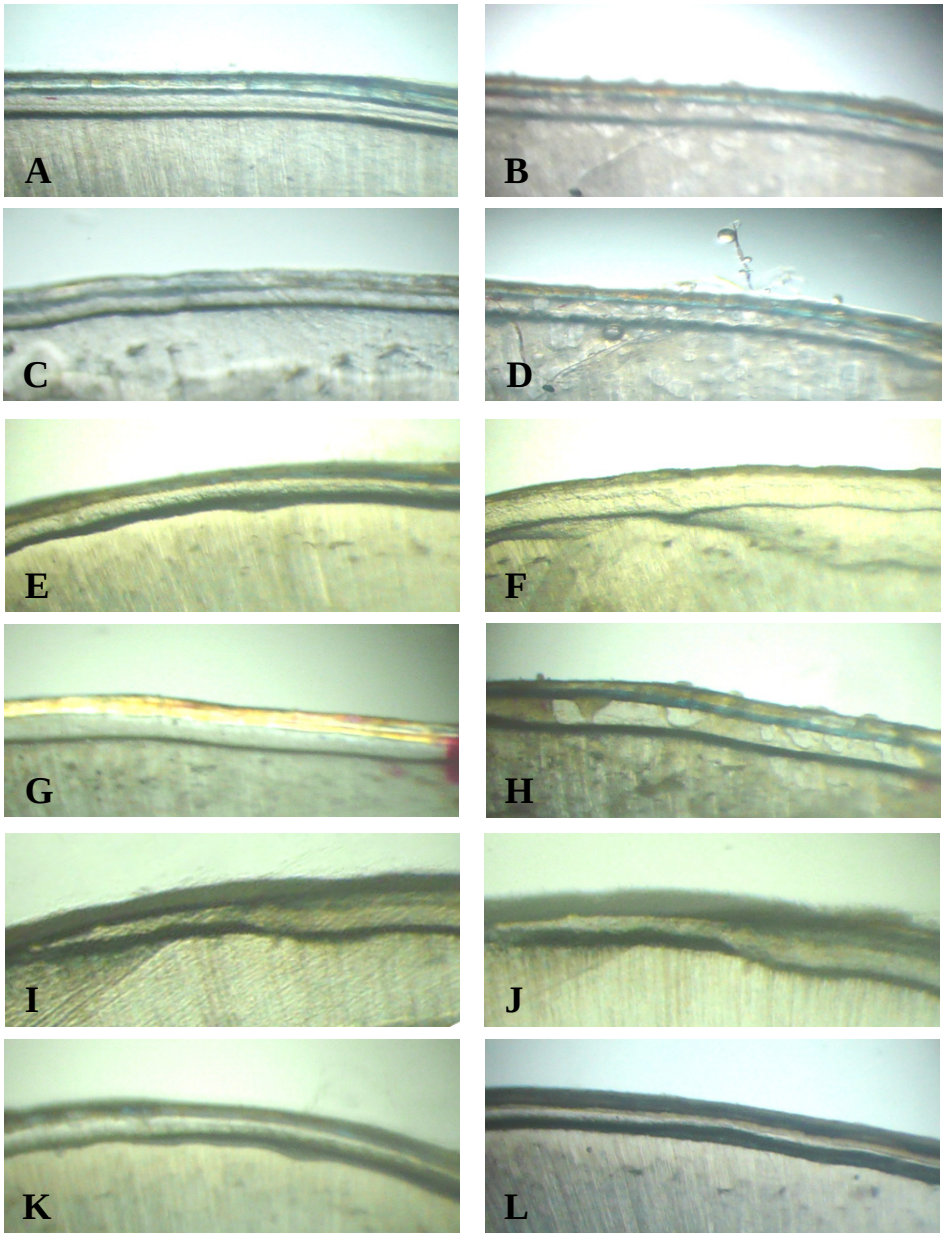

Figure 7. Polarizing microscopy images of remineralized cementum and dentin(magnification, x100). A, Group NR(before), B, Group NR(10min), C, Group NR(before), D, Group NR(30min), E, Group NR(before), F, Group NR(1 day), G, Group FV(before), H, Group FV(after), I, Group CH30(before), J, Group CH30(after), K, Group SF30(before), L, Group SF30(after).

4. Measurement of the area of caries after remineralization

1. The area of caries after the remineralization of the crown area

In the remineralized samples of the NR group using Fluoride varnish (Cavity Shield, 3M/ESPE. St Paul. MN. U.S.A) for 10 minutes, 30 minutes and 1 day, the caries area after inducing artificial caries was 42,227 μm^2. After 10 and 30 minutes remineralization, the caries area was 38,247 μm^2 and 34,515 μm^2, respectively, but the difference was not significant. On the other hand, after 1 day, there was a significant decrease in caries area to 28,632 μm^2 ($p<0.05$, Table 9).

The samples in Group FV, HA30, and SF30 were remineralized using the corresponding solution, and the caries area was measured. In the FV Group treated with fluoride varnish, the caries area before and after remineralization was 45,518 μm^2 and 45,108 μm^2, respectively, but no remineralization pattern could be observed (Figure 6). Similarly, in the other groups, there was no difference in the level of caries between before and after remineralization (Table 9).

Table 9. Changes in the demineralized lesion area in the enamel after remineralization (N=10)

Groups	demineralized area(㎛ ^{2}) before	demineralized area(㎛ ^{2}) after
Group NR(10min)	42,227 ± 3567	38,247 ± 9079
Group NR(30min)	42,227 ± 3567	34,515 ± 11758
Group NR(1day)	42,227 ± 3567	28,632 ± 8450†
Group FV	45,518 ± 9720	45,108 ± 7068
Group HA30	48,108 ± 8390	47,643 ± 7508
Group SF30	48,078 ± 10061	47,133 ± 5968

Paired t-test analysis, significantly different at $p<0.05$.

2. The area of caries after remineraliztion of the root area

The area of caries was measured in the root area after remineralization of the cementum area. The caries area after inducing artificial caries and after 10 minutes remineralization was 27,745 ㎛ ^{2} and 24,712 ㎛ ^{2}, respectively, showing no significant difference. On the other hand, the caries area measured after 30 minutes and 1 day remineralization was 21,136 ㎛ ^{2} and 18,319 ㎛ ^{2}, respectively, indicating a significant decrease ($p<0.05$, Table

10). Among the caries in the entire root area, remineralization was assessed in the caries area only in the dentin excluding the cementum area. The level of remineralization increased in the order of 10 minutes, 30 minutes, and 1 day, and the caries area decreased. The caries area decreased significantly in the cases remineralized for 30 minutes or 1 day ($p<0.05$, Table 10). In the result of the caries area of the root area to the dentin area measured after remineralization, the caries area after inducing artificial caries was 62,080 μm^2. The caries area measured after 10 minutes, 30 minutes and 1 day remineralization was reduced to 55,673 μm^2, 47,476 μm^2 and 40,227 μm^2, respectively, indicating a significant decrease ($p<0.05$).

The samples in Group FV, HA30, and SF30 were remineralized using the corresponding solution, and the caries area was measured. In the cases in the FV group, the caries area before and after remineralization was 74,105 μm^2 and 72,922 μm^2, respectively, but the remineralization patterns could not be detected (Figure 7). Similarly, in other groups, there was no significant difference in the caries level between before and after remineralization.

Table 10. Changes in demineralized lesion area in the cementum and dentin after remineralization (N=10)

Groups	demineralized area(㎛ ^{2}) before	demineralized area(㎛ ^{2}) after
Group NR (10min)		
Cementum	27,745 ± 4393	24,712 ± 5132
Dentin	34,335 ± 7301	30,961 ± 6778
Cementum+Dentin	62,080 ± 4995	55,673 ± 4830†
Group NR (30min)		
Cementum	27,745 ± 4393	21,136 ± 6477†
Dentin	34,335 ± 7301	26,340 ± 7372†
Cementum+Dentin	62,080 ± 4995	47,476 ± 6280†
Group NR(1day)		
Cementum	27,745 ± 4393	18,319 ± 5486†
Dentin	34,335 ± 7301	21,908 ± 8882†
Cementum+Dentin	62,080 ± 4995	40,227 ± 5049†
Group FV		
Cementum	32,931 ± 2850	32,094 ± 6640
Dentin	41,174 ± 4930	40,828 ± 13133
Cementum+Dentin	74,105 ± 5626	72,922 ± 10963
Group HA30		
Cementum	36,873 ± 4853	36,898 ± 7030
Dentin	43,839 ± 7829	46,066 ± 10451
Cementum+Dentin	80,712 ± 6555	82,964 ± 9052
Group SF30		
Cementum	36,050 ± 9259	36,731 ± 8889
Dentin	44,963 ± 10607	43,720 ± 13983
Cementum+Dentin	81,014 ± 8958	80,452 ± 10561

Paired t-test analysis, significantly different at $p<0.05$.

5. Quantitative evaluation of the amount of remineralization

1. Quantitative evaluation of the amount of remineralization in the crown area

In the NR Group, the mineral density was decreased with remineralization time and an increase in the amount of remineralization was observed. It After 10 minutes remineralization, remineralization occurred primarily in the surface layer. After 1 day remineralization, remineralization progressed even to the deep areas (Figure 8). Remineralization progressed in the NR group remineralized for 1 day ($p<0.05$, Figure 9).

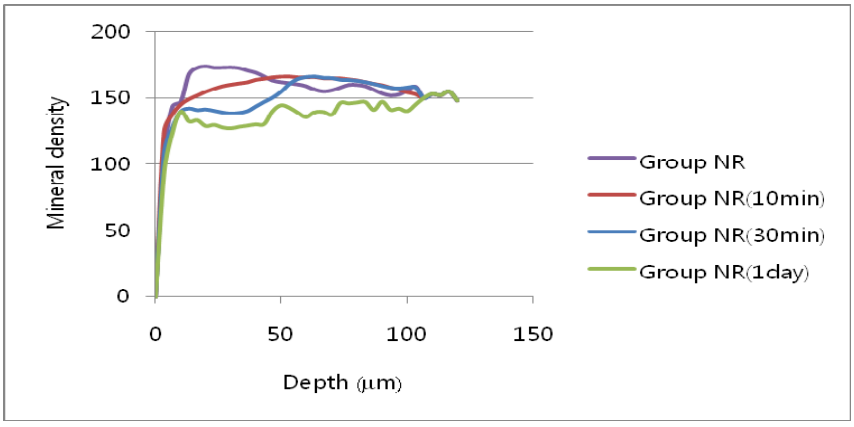

Figure 8. Comparison of the density in the enamel area before and after remineralization (Group NR).

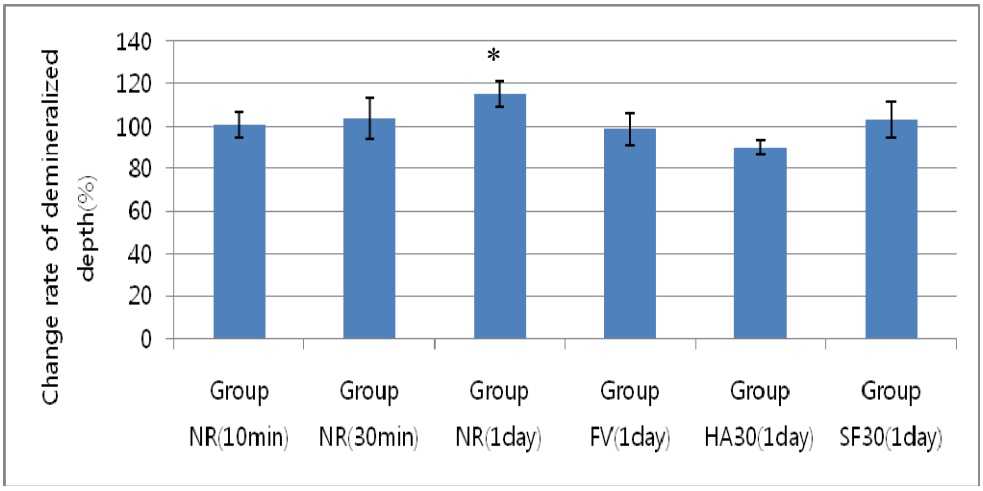

Figure 9. Change in the remineralized amount in the enamel before and after remineralization ((demineralized area before remineralization-demineralized area after remineralization/ demineralized area before remineralization) x 100 + 100(%)). *Significant at the significance level of 0.05.

2. Qualitative evaluation of the amount of remineralization in the root area

In the NR Group, the mineral density of the surface layer decreased with increasing remineralization time. However, in the deep area, the remineralization amount did not increase constantly (Figure 10). Significantly different remineralization was progressing in the NR group remineralized for 30 minutes and 1 day ($p<0.05$, Figure 11).

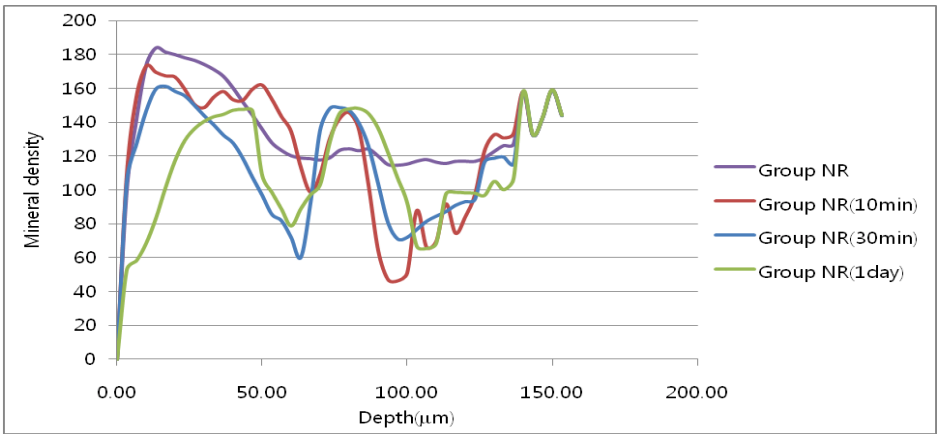

Figure 10. Comparison of the density in the dentin area before and after remineralization (Group NR).

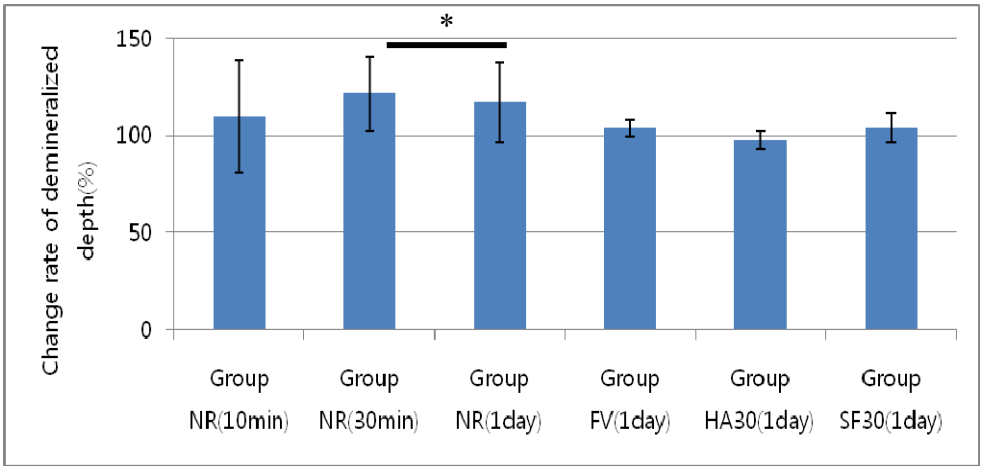

Figure 11. Change in the remineralized amount in the dentin before and after remineralization ((demineralized area before remineralization-demineralized area after remineralization/ demineralized area before remineralization) x 100 + 100(%)). *Significant at the significance level of 0.05.

IV. Discussion

In this study, the caries resistance of the enamel of the crown area and the cementum and dentin of the root of the irradiated teeth was lower than the non-irradiated teeth($p<0.05$). Polarized microscopy revealed discoloration of the demineralized area of the enamel to brown, and the brown lesion infiltrated the enamel, cementum and dentin. The brown discoloration that developed after irradiation is considered to be early caries and requires early treatment.

Silva et al.(2009) reported that compared to general caries, radiation-induced caries involves the demineralized dentin, translucent zone, dentin dead tracts, reactionary dentin, intratubular dentin deposition, etc., and the morphological changes and demineralization pattern are similar. Although the demineralization pattern caused by caries is similar, in regard to the progression of caries, caries occurs more abundantly after irradiation.

In previous studies, the morphological changes to the hard tissues of the tooth induced by irradiation could not be detected but the microdensity of the cementum was reported to decrease (Kielbassa et al., 1997, 2002), and

the fracture feature was increased in the dentoenamel junction (Pioch et al., 1992). These results reflect the change in the physical properties of the tooth, and such physical changes may be associated with the change in caries resistance. Baker et al. (1982) reported that the major component of the enamel hydroxyapatite is unaffected by irradiation, but the organic components of the interprismatic enamel are damaged by radiation injury due to the formation of radicals from the decomposition of water molecules. Because of the denaturation of organic substances, dental caries progresses rapidly in the area between inorganic substances, and dental caries progresses more rapidly than a normal tooth. Grotz et al. (1997) detected the atrophy of the odontoblastic process after irradiation in vivo by confocal laser scanning microscopy(CLSM), which could be considered to be the histomorphological proof that could explain the reason for the changes in the physical properties of the dental hard tissues after irradiation. However, in their study, after irradiation, there was no difference in the morphological changes or the demineralization pattern of caries in the enamel, cementum and dentin. Therefore, more studies will be needed to determine the correlation between the change in caries resistance and the morphological changes.

In this study, the depth of the caries lesion in the dentin was deeper than in the enamel, which is in agreement with the results of other studies (Kielbassa et al., 2006).

In the group treated with radiation (PR Group) and the untreated group (NR Group), when the depth of caries induced in the root was analyzed in the cementum and dentin separately, the non-irradiation group showed a shallower depth of caries in the cementum than the irradiation group. However, it was similar in the dentin. This is because the dentin has a higher content of organic substances than the enamel or cementum, and the concentration of the artificial caries solution in this study was high.

To examine the methods that improve the dental caries resistance prior to irradiation, fluoride varnish (Cavity Shield, 3M/ESPE. St Paul. MN. U.S.A), 1.1 % sodium fluoride, hydroxyapatite (HA) or chlorhexidine was applied for 5 minutes, 15 minutes, or 30 minutes prior to irradiation, and compared. Considering that the application of such reagents requires approximately 5 minutes, the application of these reagents once, three times and six times before irradiation and subsequently can be used to examine the effect on the improvement in caries resistance. Although fluoride varnish was effective, the other materials could not improve caries resistance against irradiation. In other words, the caries depth in the cementum was shallower than the group

treated with irradiation only (Group PR), and similar to the non-irradiation group (Group NR). Similarly, in the crown area, although not statistically significant, the caries depth was shallower than the group treated with irradiation only, and the brown caries area appeared to be lighter.

In this study, a comparison with the fluoride varnish application group showed no improvement of caries resistance in the groups applied 1.1 % sodium fluoride, 5 % hydroxyapatite (HA), or chlorhexidine. Therefore, caries resistance after radiation therapy is enhanced in patients receiving radiation therapy after applying fluoride varnish as a pretreatment for the oral cavity prior to radiation therapy. However, more study will be needed to assess other treatment methods.

In artificially induced caries, demineralized enamel over a wide area could be examined by polarized microscopy. Generally, this type of dental caries does not occur readily, and smooth surface caries is a characteristic observed in patients receiving radiation therapy (Vissink et al., 2003). Therefore, these patients require more comprehensive observation than general patients, and caries induced by radiation therapy could be managed effectively through continuous maintenance of oral hygiene, early diagnosis, prevention and treatments, which can result in improved oral health.

It was reported when remineralization progresses in the oral cavity environment, enamel crystals are surrounded partially by organic substances, which reduce the reactive surface and the diffusion rate of external ions. This can limit the rate of remineralization. The apatite in the enamel is composed of apatites with different compositions and Ca/P ratios, and the factors impeding precipitation, such as saliva proteins, plaque bacteria, etc., are present and more complex than the remineralization of artificial lesions. Therefore, the process in a real tooth takes a longer time (Ten Cate et al., 1990). Busckes et al., who examined bovine incisors, reported that 15 days was appropriate (Ten Cate 1982). Rooijand and Nancollas reported that remineralization is complete after 10-15 hours (Rooij et al., 1984). Silverstone performed experiments on the remineralization phenomenon in caries of the enamel, and reported that major changes occur within the initial 4 days, and no changes occur after 10 days (Silverstone 1977). Ten cate et al. showed that remineralization is reduced after 3 days (Ten Cate 1982).

In this study, based on the actual time used for general oral hygiene, the treatment was for 10 minutes for a single time, and assuming that it would be used for approximately 2 months, 1 day was selected as the remineralization time.

In this study, in the experiments for remineralization, the samples were not stored in artificial saliva but distilled water because in patients receiving radiation therapy, the calcium ions and potassium ions required for remineralization are decreased substantially due to the reduction of the saliva, and it was important to examine remineralization under the similar conditions. Different results may be obtained if artificial saliva or buffer solution for remineralization was used.

Generally, remineralization is carried out in the presence of fluoride, and calcium hydroxyapatite is formed by the reaction of calcium ions and potassium irons. The hydroxyapatite is then deposited in the demineralized area, and remineralization is achieved. In a previous study of remineralization, the depth of caries did not decrease, and even increased in some cases (Gwak et al., 2008). However, in this study, the depth of caries was decreased in the group NR. If fluoride varnish is used, some components of varnish rather than the effect of fluoride are deposited in the demineralized empty space, which may appear to be remineralization. The effect of fluoride varnish on remineralization requires further investigation.

In the experiments of the induction of artificial caries in the FV group, the caries resistance was enhanced significantly in the cementum of the root area ($p<0.05$). However, in the remineralization experiments,

remineralization barely occurred in both root and crown areas (Figure 28, 34). In addition, in the HA30 and SF30 groups, although remineralization was attempted, remineralization barely occurred, which is similar to the FV group (Figure 29,30,35,36). Therefore, remineralization could not be achieved in the group irradiated and induced artificial dental caries, regardless of the reagents used.

Dental caries in the cementum and dentin were increased significantly by irradiation ($p<0.05$). After irradiation, it was observed that the caries resistance was enhanced significantly in the cementum of the root area if a fluoride varnish was applied and subsequently irradiated ($p<0.05$). In addition, remineralization hardly occurred when remineralization was attempted in the group applied fluoride varnish first and subsequently irradiated. On the other hand, in the group induced artificial dental caries without irradiation, the caries area was reduced significantly when remineralization was attempted using fluoride varnish ($p<0.05$).

Overall, the caries resistance is decreased after irradiation, and protecting the tooth using fluoride varnish prior to irradiation may prevent the activation of dental caries induced by irradiation. On the other hand, once dental caries has developed after irradiation, remineralization is difficult compared to a tooth without irradiation, and more aggressive treatments may be required.

V. Conclusion

1. Under a polarized microscope, with or without irradiation of the hard tissues of the tooth, the difference in caries in the enamel, the cementum, and the dentin was compared. The results showed that more dental caries were developed after irradiation.
2. In the irradiation of dental hard tissues, the caries resistance in the cementum of the dental root was enhanced when fluoride varnish was applied first and irradiated.
3. Remineralization was observed when artificial caries was induced in dental hard tissues without radiation and remineralization was carried out using fluoride varnish.
4. Remineralization could not be achieved in the group treated with fluoride varnish, 5 % hydroxyapatite or 1.1 % sodium fluoride for 30 minutes, irradiated, and induced artificial caries, regardless of the reagents applied.
5. Clinically, in radiation therapy, the application of fluoride varnish followed by radiation therapy can enhance the dental caries resistance.

After irradiation, remineralization is difficult by any method, and more attention should be paid to the prevention of dental caries.

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ABSTRACT ((in Korean)

방사선조사 전후 치아의 우식 저항성 연구

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방사선 치료를 받는 환자에서 우식 유발 가능성이 높아지는데, 이는 방사선 조사에 따른 타액분비량의 감소와 조성의 변화와 관계 있는 것으로 사료된다. 또 다른 원인으로 방사선 조사가 치아 경조직의 물리적 성질에 직접적으로 영향을 줄 가능성이 있으며 이에 대해서는 다양한 의견이 존재한다. 이번 연구의 목적은 방사선 조사 전 후의 법랑질과 백악질 및 상아질 표면이 물리적인 성질의 변화로 인해 우식 저항성이 변화하는지 관찰하기 위함이다. 이를 위하여 발치한 치아 60 개를 대상으로 치관부와 치근부에서 방사선 조사 전 후 우식 저항성에 차이가 있는지 우식 깊이와 우식 면적을 측정하여 통계 분석하였다. 또한 우식 저항성을 증진시키기 위한 방법을 연구하기 위하여 Fluoride varnish 와 1.1% NaF 와 5% HA(Hydroxyapatite), Chlorohexidine 등을 방사선 조사 전에 처치하고 우식 저항성의 변화가 있는지를 관찰하였다.

또한 방사선 조사에 따른 우식 치아의 치료에 효과적인 방법을 알아보고자 인공적으로 우식을 유발 한 후에 Fluoride varnish, 1.1% NaF, 5% HA(Hydroxyapatite)등을 처치한 후 재광화 양상을 관찰하였다.

방사선 조사 유, 무에 따른 우식의 차이를 비교해 본 결과 편광현미경상에서 방사선 조사 후 우식이 더 많이 생기는 것을 관찰할 수 있었고($p<0.05$), 방사선을 조사할 때에 fluoride varnish(Cavity Shield, 3M/ESPE. St Paul. MN. U.S.A)를 도포한 후 조사하였을 때 치근부의 백악질부위의 우식 저항성이 증가함을 알 수 있었다($p<0.05$). 인공 우식을 유발 시킨 후의 재광화 능력을 알아본 결과 방사선 조사를 받지 않은 군에서는 fluoride varnish 를 이용하여 성공적으로 재광화되었지만 방사선 조사를 받은 군에서는 어떠한 처치로도 재광화가 일어나지 않았다.

중심되는 말 : 방사선, 인공우식, 재광화, Fluoride varnish, 우식 저항성, Hydroxyapatite, 법랑질, 백악질, 상아질