Remineralisation effect of SDF+KI solution on artificial caries

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Remineralisation effect of SDF+KI solution on artificial caries

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A Dissertation Thesis
Submitted to the Department of Dentistry
and the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
Master's degree in Dentistry

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February 2022

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February 2022

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Abstract

Remineralization effect and discoloration of SDF+KI solution on artificial caries

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Fluoride varnishes have become effective for preventing caries and remineralising early caries. The most commonly used fluoride agent today (38% SDF) contains 44,800 ppm, the highest of any fluoride compounds used in dentistry. Aim of this study is to see effect of silver diamine fluoride (SDF) + potassium iodide (KI) solution on the remineralisation on artificial caries. Sixty bovine incisors without cracks or white spots were demineralized and subjected to each of the following treatments: no treatment, 38% SDF+KI, 38% SDF, 5% sodium fluoride varnish (NaF) application. Acid-resistant nail varnish was applied to the surface of each specimen to create two exposed windows with a size of 3.5 mm × 3.5 mm. The specimens were immersed in demineralizing solution and stored at 37˚C in an incubator for 120 hours (n=15 each group) for manufacturing early dental caries with varying lesion depths. Microhardness of enamel surfaces was measured with Vickers Hardness Number (VHN). Evaluation of discoloration was measured with digital single-lens reflex (DSLR) camera image and Delta E. Measurement of lesion depth and histological evaluation was done by Polarized Light Microscope. Scanning Electron Microscope (SEM) images were investigated to compare morphological changes on the surfaces.

VHN values of the all demineralized enamel specimens in remineralisation groups were significantly increased after remineralisation agents were applied. Based on the VHN results, SDF+KI and SDF were more effective than NaF. DSLR camera image and Delta E measure showed that the SDF groups recorded the largest color change values in the demineralized enamel group compared to SDF+KI, NaF, and control groups. SDF group showed the most darkness among the groups. PLM showed all the demineralized enamel specimens were remineralised after remineralisation procedure. SDF groups showed black staining on PLM and DSLR camera image.

Remineralisation of incipient lesion area, measured by enamel surface hardness and PLM calibration increase, of SDF+KI and SDF were greater than NaF application. But it is important to obtain prior consent for the aesthetic results of only SDF treatment. Treatments of teeth with KI solution after SDF treatment significantly reduced the discoloration caused by SDF.

Key words: SDF+KI, Remineralisation, caries prevention, discoloration.

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I. Introduction

Dental caries is the most frequent oral disease affecting almost every country on the earth (Marthaler, 2004). Modern dentistry, unlike the past, focuses on preventing onset and spread of dental caries rather than restoring or treating them (Health and Committee, 2016). It has been reported that arresting dentin caries in primary teeth is possible without restorative intervention (Mäkinen, et al., 1995). Applying fluoride varnishes on the outer surface of the teeth is considered a non-invasive method for caries control and prevention (Yu, et al., 2018). Fluoride varnishes have become effective for preventing caries and remineralising early caries (Lo, et al., 2001). Sodium fluoride (NaF) varnish at 5% contains 22,600 ppm applied topical agent in caries management (Newbrun, 2001). The most commonly used fluoride agent today (38% SDF) contains 44,800 ppm, the highest of any fluoride compounds used in dentistry (Zhao, et al., 2018). Clinical studies have shown that applying silver diamine fluoride (SDF) at 38% can effectively treat and prevent early childhood caries (Liu, et al., 2012; Llodra, et al., 2005; Yee, et al., 2009). SDF seems to be efficient in controlling caries in vitro and in vivo cavities, according to early research (Gotjamanos and Orton, 1998; Klein, et al., 1999; McDonald and Sheiham, 1994; Yamaga, 1972). Clinical experiments have also shown that SDF is effective in reducing caries in both primary and permanent teeth (Almeida,

1994; Llodra, et al., 2005; Lo, et al., 2001; Nishino, et al., 1969; Wong, et al., 2005). It halts the progression of caries lesions and changes teeth color into black (Nguyen, et al., 2017). It is important to obtain prior consent for the aesthetic results of SDF treatment (Patel, et al., 2018).

Supplementing SDF with potassium iodide (KI) was proposed as a response to the problem of discoloration (Abdullah, et al., 2020). If KI can prevent the staining associated with SDF without reducing its effectiveness in preventing caries, it would be a win-win situation (Li, et al., 2016). Riva star (SDI Limited, Bayswater, Australia) products have capsules of two colors. Silver capsules containing 38% silver diamine fluoride (SDF) and green capsules containing potassium iodide (KI) saturated solutions claimed to alleviate black staining of arrested caries lesions (Knight and McIntyre, 2006). The silver ions in the SDF solution combine with the KI iodide ions to generate silver iodide, which reduces SDF discoloration (Seifo, et al., 2020).

Aim of this study is to evaluate the remineralising effect of 38% silver diamine fluoride+potassium iodide (SDF+KI) on demineralized enamel and compare it with the currently used 38% silver diamine fluoride (SDF) and 5% sodium fluoride varnish (NaF) application on artificial caries.

II. Materials and Methods

1. Specimen preparation

Healthy bovine incisors without caries, discoloration, or structural defects were used as specimens (n=60). The bovine teeth were kept in a freezer before being used. Dried specimens were then immersed in deionized water for 30 minutes. Using a low speed handpiece (Lasungmedice, Incheon, South Korea) with a diamond disc (NTI-Kahla, Kahla, Germany), all specimens were sectioned (10x5x5mm) from the labial surface of incisors (Figure 1). After the specimen was buried in an acrylic resin (Ortho-Jet, Lang Dental Mfg,Co., Inc., USA). The surface was polished with abrasive disc paper (SiC Sand paper, R&B Inc., Daejeon, South Korea) gradually 600, 800, 1000,

1200 grit. The schematic of the experimental procedures in this study is shown in Figure 2.

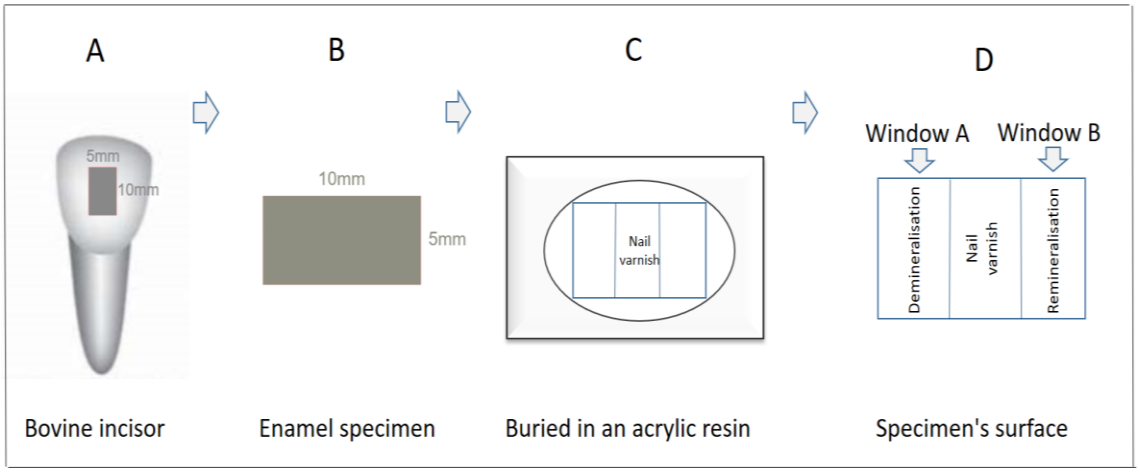

Figure 1. Preparation of a bovine enamel specimen. A. Specimen section B. Enamel specimen C. Buried in an acrylic resin D. Specimen surface covered with nail varnish is the control and the exposed specimen surface is the demineralized part (treated experimental)

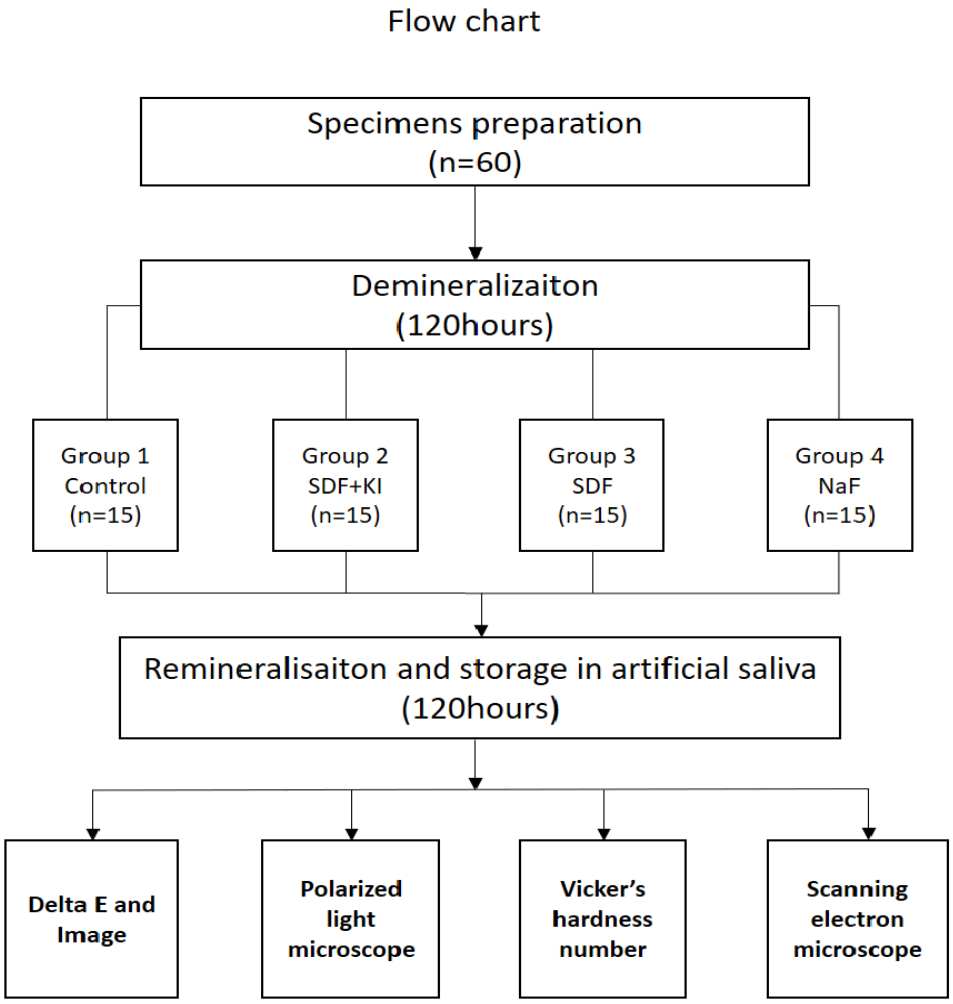

Figure 2. Flowchart of the demineralization and remineralisation on enamel.

2. Artificial carious lesion creation

For a reference area, acid-resistant nail varnish (Mix-nails, Mix & Match, Incheon, South Korea) was applied to the middle of each specimen surface before generating artificial caries lesions. Acid-resistant nail varnish was applied to the surface of each specimen to produce two exposed windows with a size of 3.5 mm × 3.5 mm. The specimens were immersed in 40ml of demineralized gel at stored at 37°C in an incubator for 120 hours for manufacturing to early dental caries with varying lesion depths (White, 1987). The compositions of the demineralizing gel were 2% carbopol (Carbopol* ETD 2050 polymer, Noveon Inc., Wickliffe, OH, USA) and 0.1 M lactic acid gel indicating pH of 4.8 and hydroxyapatite (calcium phosphate tribasic, Sigma, St. Louis, MO, USA) making up 50% of the gel.

3. Treatment protocol

Each of SDF+KI, SDF, NaF varnish were applied to each specimen's remineralisation area (Figure 1D, window B) using a commercial product and following the manufacturer's instructions.

3.1 SDF+KI

After air drying the demineralized surfaces, one drop of SDF was applied with a micro-brush for one minute. After applying SDF, a saturated KI solution was applied until creamy-white precipitates turned to be clear, and then the area was rinsed with copious volumes of distilled water for thirty seconds.

3.2 SDF

The 38% SDF application protocol was carried out according to the manufacturer's instructions.

After air drying the demineralized surfaces, one drop of SDF were added to a mixing well and applied with a micro-brush to the enamel surfaces for one minute. After two minutes, the specimens were rinsed with distilled water for thirty seconds.

3.3 NaF

5% fluoride varnish appliance was performed following the manufacturer's instructions. Specimens were air-dried and NaF was applied on the demineralized surfaces using a micro-brush for twenty seconds.

After 24 hours acetone was used to wipe off the materials that were applied previously and the materials were reapplied. The procedure above was repeated five times during one hundred twenty (120) hours (once in a day for 5 days). After the procedure, specimens stored in artificial saliva (gastric mucin 3.43μM, NaCl 6.52 mM, $CaCl_2$ $2H_2O$ 1.45 mM, KH_2PO_4 5.42 mM in 1000ml distilled water/pH=7). Table 1 shows materials used in this research.

Table 1. Materials used in this study

Material	Brand name	Composition
SDF+KI	Riva star (SDI Limited, Bayswater, Australia)	38% silver diamine fluoride+potassium iodide
SDF	Saforide (Toyo Seiyaku Kasei Co., LTD, Osaka, Japan)	38% silver diamine fluoride
NaF	3M Espe Clinpro (3M, ST.Paul, MN, USA)	5% sodium fluoride

4. Assessment of enamel demineralization and remineralisation

4.1 Colorimetric analysis

Tooth sample obtained by a general DSLR camera (model 550D, Canon, Tokyo, Japan) at the following setting: shutter speed of 1/45 s, aperture value of 13.0, and ISO speed of 1600, it is assumed that the sample is observed with the eye. Tooth color measured by Delta E (http://colormine.org/delta-e-calculator). Delta E (ΔE) is defined as the difference between two

colors in an L* a* b* color space.

4.2 Polarized light microscope (PLM)

After finishing fluoride treatment, specimens were cut perpendicular to the treated surfaces. A microblock of 300 μm section was cut (Tech-Cut 4, Rancho Dominguez, CA, USA) and these were abraded 800, 1000 and 1200 grit disc paper (SiC Sand Paper, R&B Inc., Daejeon, South Korea) until obtain the 100 μm thickness. Glass slides were used to mount the specimens on it. The slabs were submerged in deionized water, and images were acquired using a polarized light microscope (PLM, CX31-P, Olympus, Tokyo, Japan) at magnifications of 100x and 400x. PLM micrographs revealed the histological characteristics of lesion depth.

4.3 Surface Microhardness measurement (Vicker's hardness number-VHN)

In the micro durometer, 400x objective lens was used to expose the exposed area of each sample's working surface. The Vicker's microhardness was calculated using the formula, Vertical Diagonal (VD)+Horizontal Diagonal (HD)=Total Diagonal (TD), where VD is the measure of the vertical diagonal and HD is the horizontal diagonal measurement. Vicker's surface hardness tester (MMT-X, Matsuzawa, Akita, Japan) value at 200g load was used to determine the hardness in 3 areas (demineralized, sound, remineralised) of each specimen for 15 seconds.

4.4 Scanning Electron Microscope analysis (SEM)

A field emission SEM with 15kV was used to examine the surface analysis. After drying with a freeze dryer, use an ion coater (E-1010, Hitachi, Japan) to coat platinum with a thickness of 100 nm and observe with SEM (S-3000N, Hitachi, Japan). The images were obtained under SEM with x8000 magnification.

5. Statistical Analysis

The mean of VHN measurement, Delta E, PLM calibration measurements (lesion depth) of

each group were calculated. To compare the demineralization and remineralisation effects of treatment materials used in each group, repeated measured ANOVA and one-way ANOVA were conducted. All statistical analyses were carried out using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY) with 0.05 considered as significant.

2. Results

1. Comparison of color change

The SDF group recorded the largest color change values in the demineralized enamel group compared to SDF+KI, NaF and control groups (Figure 4). Delta E values in the SDF group showed significant difference in Figure 5.

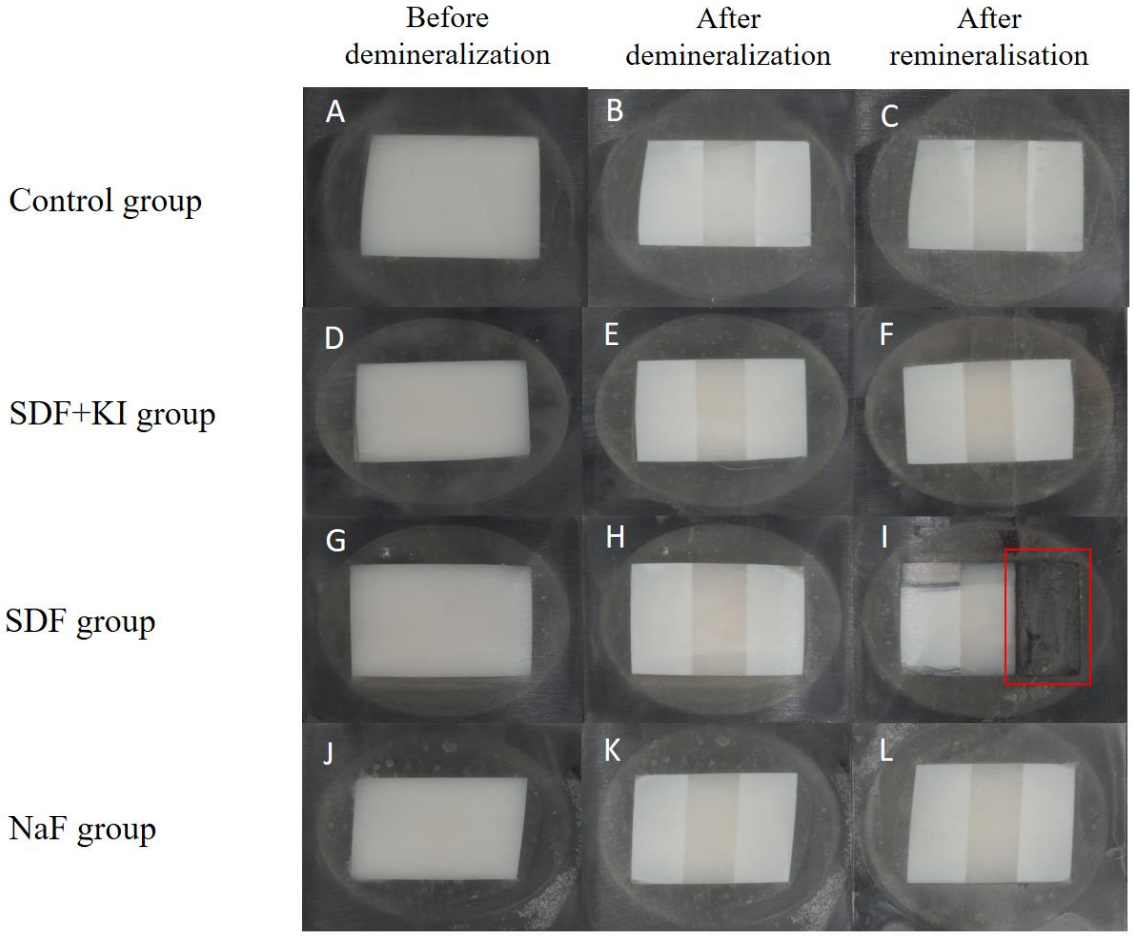

Figure 4. Representative Quantitative Light-induced Fluorescence white light image. A, D, G, J Before

demineralization, B, E, H, K after demineralization and C, F, I, L after remineralisation.

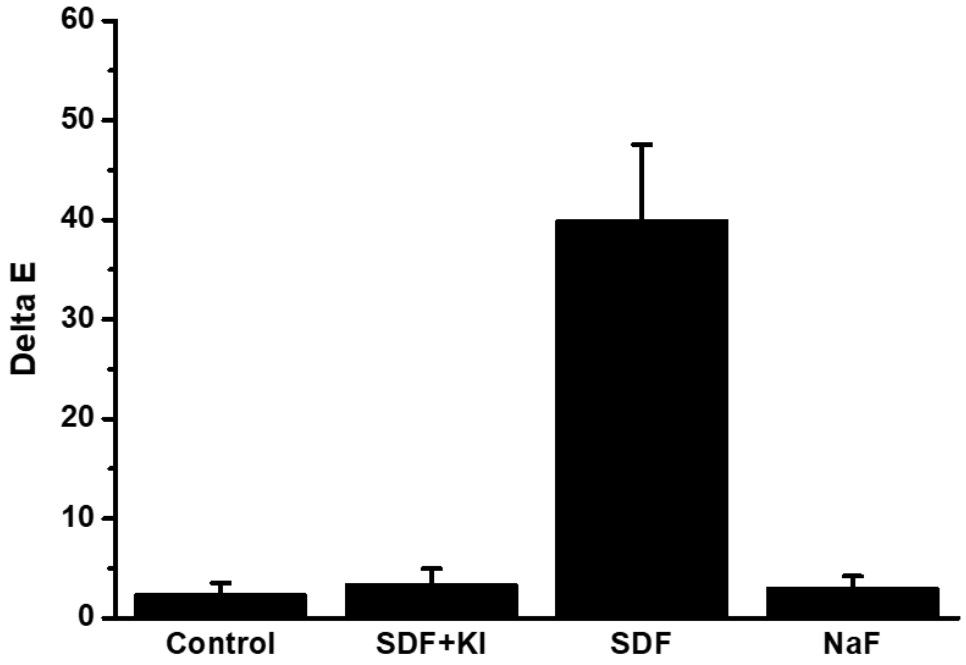

Figure 5. Delta E measurement

2. Comparison of lesion depth by Polarized Light Microscope (PLM)

Histological features in PLM micrographs of lesions demonstrated that shallow lesions had less mineral loss and a higher recovery than deep lesions. PLM showed all demineralized enamel specimens were remineralised after remineralisation procedure. Figure 6 and 7 polarized light microscope at magnification of 100x and 400x. In a figure 6 and 7 (A and C) highlighted section on figure 6 and 7 (B and D).

Even though control group was in artificial saliva PLM did not show any remineralisation. Remineralising effect of NaF group is lower than SDF+KI groups. The shade of SDF group became too dark and it was impossible to take a measurement with PLM. Lesion depths significantly reduced after fluoride treatment, as shown in Figure 8, although surface layers of deeper lesions were visibly remineralised.

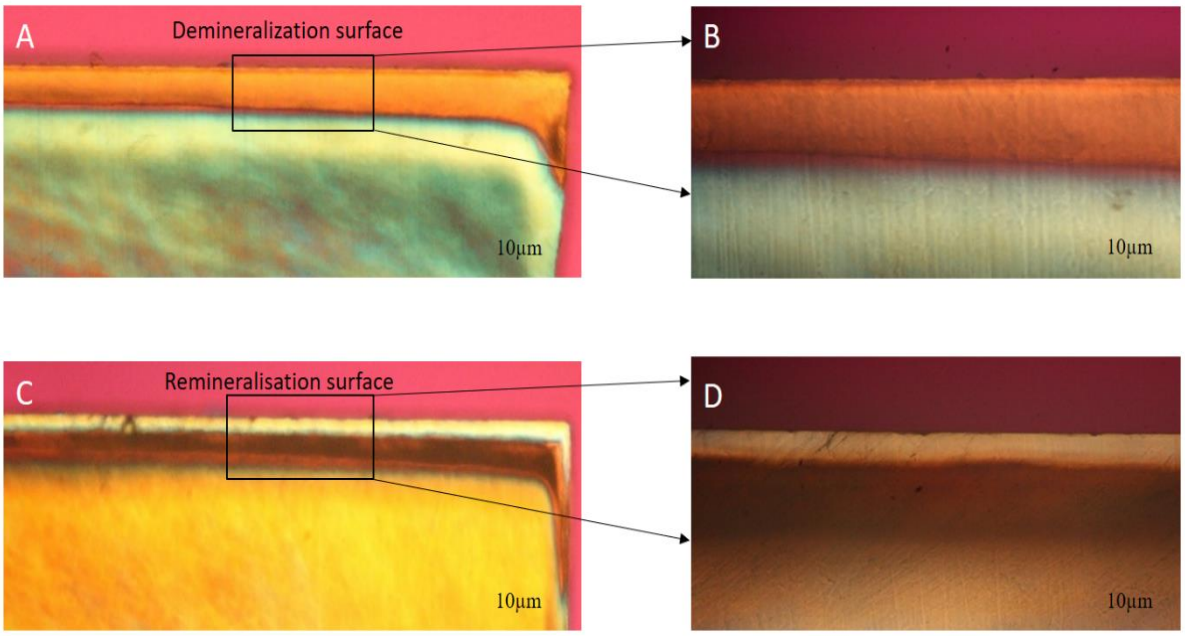

Figure 6. Representative polarized light micrographs after remineralisation (A) control group (x100) (B) control group (x400) (C) SDF+KI group (x100) (D) SDF+KI group (x400)

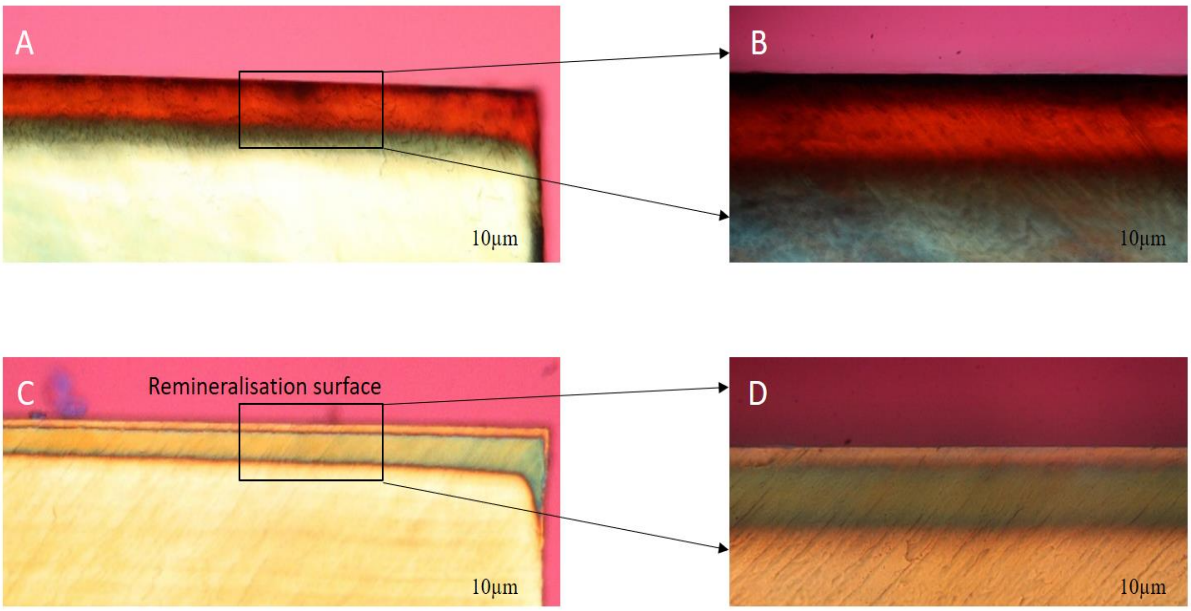

Figure 7. Representative polarized light micrographs after remineralisation (A) SDF group (x100) (B) SDF group (x400) (C) NaF group (x100) (D) NaF group (x400)

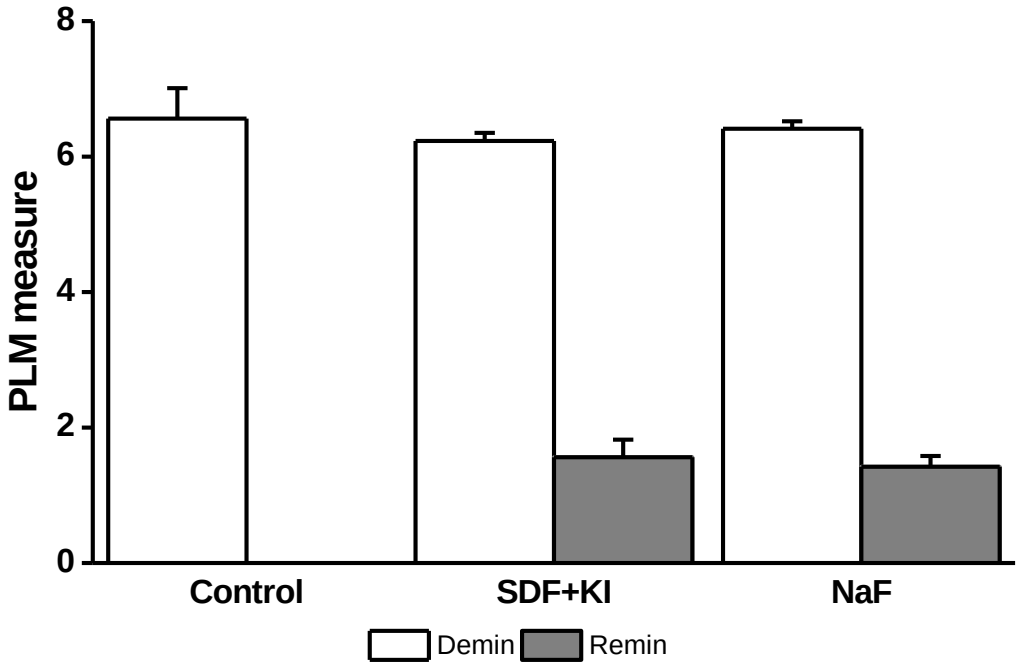

Figure 8. PLM demineralization and remineralisation measurement

3. Comparison of Surface hardness by Vickers Hardness Number (VHN)

Figure 9 shows the mean values of the VHN. In the control group, there was no statistically significant difference between the post demineralization and post remineralisation VHN values. VHN values of the all demineralized enamel specimens in remineralisation groups were significantly increased after remineralisation agents were applied. Based on the VHN results, SDF+KI and SDF were more effective than NaF. Remineralisation of the area of SDF+KI and SDF groups, measured by enamel surface hardness was significantly greater than that of NaF group. The average was calculated at 6% differently from before, and the graph is as follows Figure 10. Among them, it was confirmed that there was a statistically significant difference ($p<0.05$) in the SDF+KI and SDF groups when compared with the control group.

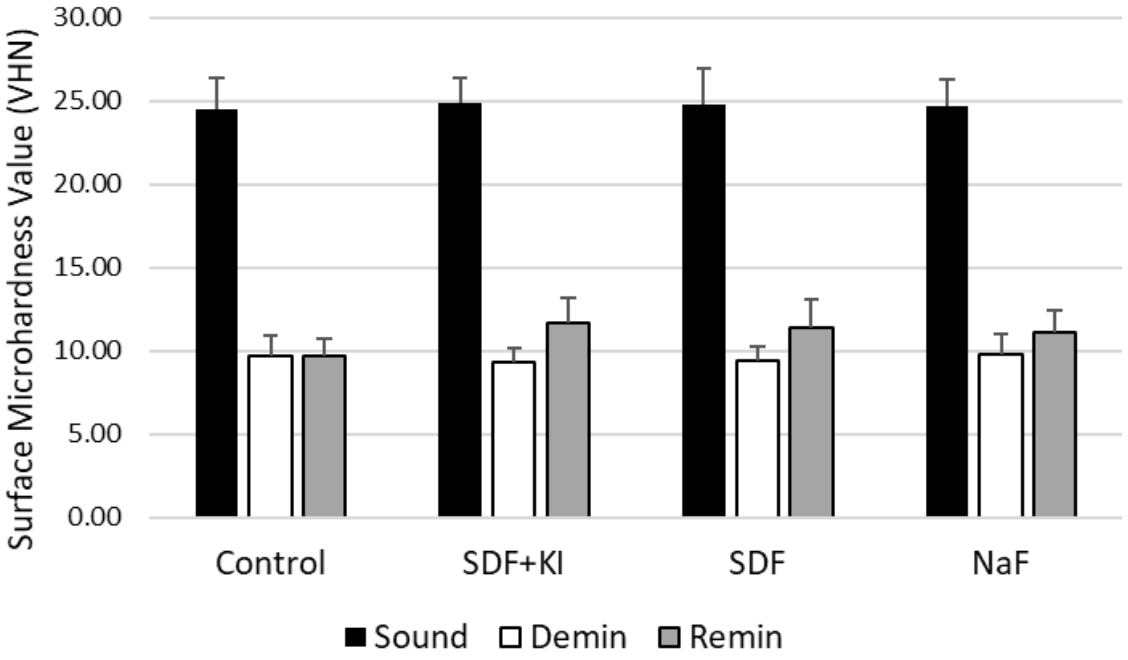

Figure 9. Surface hardness change of each group

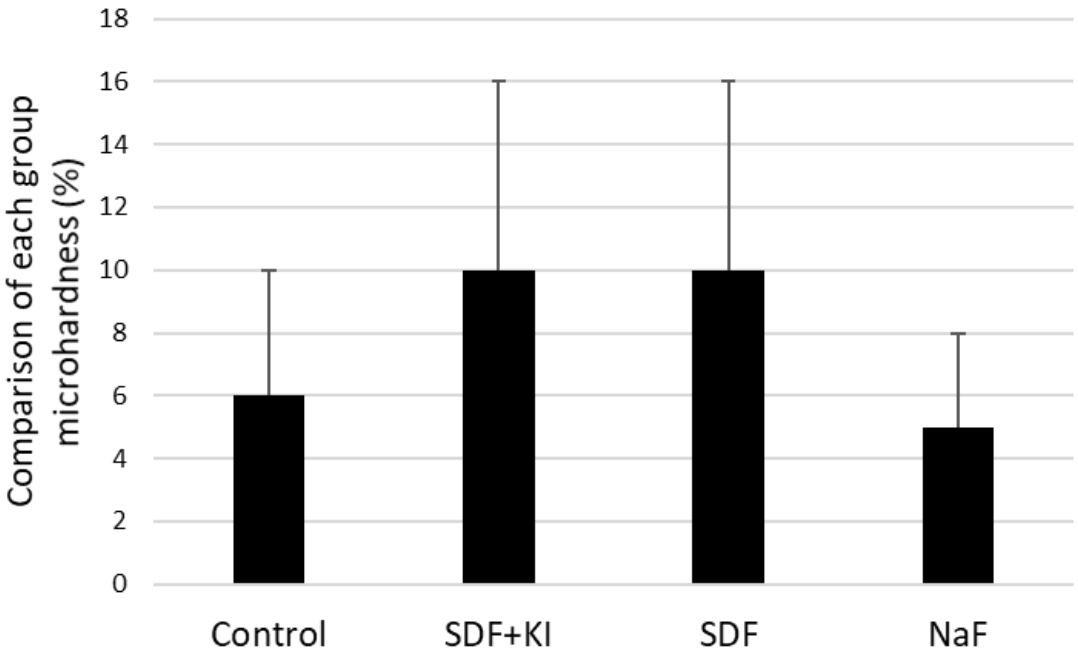

Figure 10. Comparison of each group microhardness percentage (%)

4. Scanning Electron Microscope analysis (SEM)

The process of demineralization and remineralisation was qualitatively characterized by observing

the specific morphological and structural features of the enamel surface via inspection of SEM images of the enamel surfaces. Each group is represented by images that are representative of that group Figure 11. However, after 120 hours remineralisation different enamel surface forms were observed in the four groups. The enamel surface of the control group shows more porosities than the other groups (Figure 11A). The surface was relatively smooth in the SDF+KI group (Figure 11B). The interprismatic areas were found to be partially occluded, with exposed interprismatic patches visible on the surface SDF (Figure 11C). In the NaF group, the interprismatic gaps were found to be sealed by fluoride varnish application (Figure 11D).

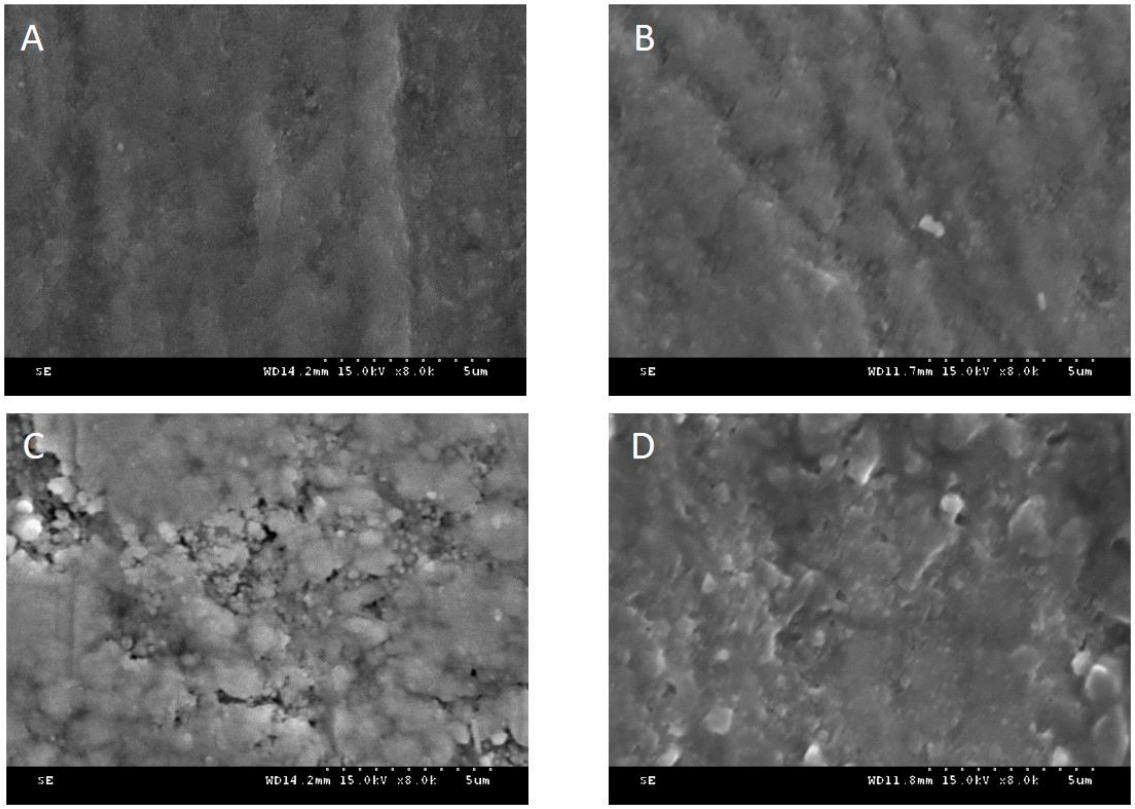

Figure 11. Representative scanning electron micrographs of the enamel surface morphology (After remineralisation) (A) control group (B) SDF+KI group (C) SDF group (D) NaF group

3. Discussion

The present study evaluated the potential effects of using 38% SDF+KI, 38% SDF, 5% NaF varnish in conjunction with artificial enamel caries lesions. This study proved that the application of 38% SDF+KI has a similar remineralising effect to that of 38% SDF on enamel caries. And both of them are more effective than NaF on promoting remineralisation. From a pharmacokinetics perspective, SDF has been shown to be a generally safe topical treatment (Vasquez, et al., 2012). But, caries lesions are blackened by silver diamine fluoride. That most significant disadvantage is the aesthetic concern caused by tooth surface discoloration (Horst, et al., 2016). Expect for the black staining of arrested caries lesions, recent clinical trials on SDF usage have shown no significant adverse effects (Nelson, et al., 2016). Colorimeters have been used in the past to assess changes in the CIE (i.e., L*a*b* parameters) system in dental shade studies (de l'Eclairage, 1978; Paul, et al., 2002).

Bovine teeth are used in this study, these teeth are easy to handle because of their size and availability. Many aspects of bovine and human teeth, such as radio-density (Fonseca, et al., 2008) enamel thickness and dentin surface hardness, have been found to be similar in research (Donassollo, et al., 2007). As compared to human teeth, bovine teeth have a more homogeneous mineral composition (Ghisi, et al., 2015).

In color evaluation experiments, this is viewed as an advantage (Sayed, et al., 2019). According to the results of this study, the QLF image and the Delta E value measurement, the SDF group has more discoloration than other groups. The use of KI after SDF has been suggested to reduce the discoloration possibility. Because silver iodide can be produced through reaction of KI with free silver ions. This silver iodide shows white color (Knight and McIntyre, 2006). Moreover, there was no color (Delta E measurement) difference between SDF+KI and NaF treatments.

The use of a saturated KI solution immediately after SDF application was suggested as a strategy

to resolve the discoloration problem related by SDF (Sayed, et al., 2020). The Vickers method was used in this investigation. Because the resulting pyramid-shaped indent is easy to measure and detect visually and digitally (Heravi, et al., 2018). The mean difference in Vickers hardness number value results, SDF+KI and SDF was more remineralisation effective than NaF. The layer with increased mineral content extends above the enamel surface, as shown by Polarized Light Microscope images (Manesh, et al., 2009). The SDF+KI group had the greatest depth of remineralisation to Polarized Light Microscope's observations. It is important to note that the effects of SDF+KI and SDF are very similar, NaF has lower in vitro remineralisation effect than SDF+KI and SDF group. It should be noted that SDF+KI is an improvable agent and additional investigation is needed to recommend it as an alternative to routine fluoride application treatments.

4. Conclusions

No significant differences were evident in the remineralisation potential SDF and KI solution after using SDF. Remineralisation of incipient lesion area of SDF+KI and SDF, measured by enamel surface hardness and polarized light microscope calibration increase, was significantly greater than NaF application. But it is important to obtain prior consent for the aesthetic results of only SDF treatment. Treatments of teeth with KI solution after SDF treatment significantly reduced the discoloration caused by SDF. In conclusion, within the limitations of the study, the lesions were not natural caries lesions, they were created artificially. Validity of the study can be improved by direct caries treatment (in vivo study).

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국문요약

인공 우식에 SDF+KI를 재광화 효과

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지도교수: 송제선

이 연구의 목적은 SDF+KI 용액이 인공우식의 재광화에 미치는 영향을 평가하는 것이다. 60개의 금이나 백점이 없는 우치를 준비하여 다음과 같은 군으로 분류하여 각각 적용하여 처리하였다. 처리 안한 군 (대조군); 38% silver diamine fluoride+potassium iodide; 38% silver diamine fluoride; 5% sodium fluoride varnish. 항산 nail varnish을 각 시편 표면에 적용하여 3.5 mm × 3.5 mm 사이즈의 2개의 창을 만들었다. 모든 시편을 탈회 용액에 넣어 37도에 120시간을 담궈 우식과 다양한 병소를 (lesion) 만들었다. 법랑질의 microhardness을 Vickers Hardness Number (VHN) 측정하였다. DSLR camera image로 변색을 측정하고, lesion 깊이는 white light image and Delta E로 측정되었다. 그리고 Polarized Light Microscope로 조직변화를 측정하였다. Scanning Electron Microscope (SEM) 사진으로 형태변화를 측정하였다. 모든 탈회된 법랑질의 VHN 값은 재광화 이후로 유의하게 증가하였다. VHN 결과에 따

르면, SDF+KI 과 SDF 는 NaF 보다 더 효과적이있다. DSLR image와 Delta E 값은 SDF 군의 변색 정도가 SDF+KI, NaF, 그리고 대조군과 비교하였을 때 가장 컸다고 보였다. SDF 군은 다른 군과 비교하였을 때 어두은 색상을 보였다. PLM 값이 탈회된 모든 법랑질 재광화됐다는 것을 입증하였다. SDF 군은 검은 PLM, DSLR image에서 검은 staining을 보였다.

SDF+KI, SDF의 재광화 값은 NaF 보다 더 유의하게 컸습니다. 항우식 치료로 SDF만 치료했을 때 심미적은 문제를 잘 알아 두는 것이 중요하다. SDF 치료후에 KI 처리하는 것이 변색을 유의하게 줄어주였다.

핵심되는 말: SDF+KI, 재광화, 충치 예방.