Tufts University | School of Dental Medicine

Effect of Silver Diamine Fluoride and Potassium Iodide Treatment on Microhardness

A Thesis

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by

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ABSTRACT

Aim: This In vitro study was conducted to investigate the effect of silver diamine fluoride (SDF) and potassium iodide (KI) treatment on microhardness levels, and to evaluate the effect of the subsequent application of KI on the degree of discoloration.

Materials and Methods: Forty-five teeth were randomized into three groups of fifteen teeth. Advantage Arrest™ (AA) SDF, RIVA STAR™ (RS) step 1 only SDF, and (RS) 2 steps (SDF/KI) were tested. Vickers Hardness Number (VHN) and numerical color analysis were obtained before (phase 1) and after demineralization (phase 2), and one week after the second application of the arresting medicament (phase 3). Hardness values were normalized by dividing the VHN at phase 3 by the VHN at phase 2. The change in lightness (L) value was quantified as the difference in L value between phase 2 and phase 3 (phase 3 – phase 2). The ΔE value quantifying the overall color difference between phase 2 and phase 3 was also calculated.

Results: For the normalized VHN, means ± SD were as follows: 1.84 ± 0.30 for SDF (AA), 1.80 ± 0.19 for SDF (RS), and 1.44 ± 0.13 for SDF/KI. The comparison of materials was significant ($p < 0.001$), with significance between SDF (AA) and SDF/KI ($p < 0.001$) and SDF (RS) and SDF/KI ($p < 0.001$), but not SDF (AA) and SDF (RS) ($p = 0.905$). All treatment groups were significant ($p < 0.001$) when comparing the VHN for the three different phases of treatment, with phase 1 exhibiting the highest mean and phase 2 exhibiting the lowest mean for each treatment group. For the change in lightness (L) value,

means ± SD were as follows: -36.04 ± 7.25 for SDF (AA), -32.28 ± 6.55 for SDF (RS), and 3.29 ± 10.11 for SDF/KI. The comparison of materials was significant ($p < 0.001$), with significance between SDF (AA) and SDF/KI ($p < 0.001$) and SDF (RS) and SDF/KI ($p < 0.001$), but not SDF (AA) and SDF (RS) ($p = 0.420$). In the SDF (AA) and SDF (RS) groups, phase 1 exhibited the highest mean L value and phase 3 exhibited the lowest. For each of these two groups, the difference was significant ($p < 0.001$) when comparing the readings for the three phases of treatment; all post-hoc tests were significant as well. In the SDF/KI group, phase 1 exhibited the highest mean L value and phase 2 exhibited the lowest. The difference in L value was significant ($p < 0.001$) when comparing the readings for the three phases of treatment, and the post-hoc tests were significant between phase 1 and both phase 2 and 3 ($p < 0.001$, $p = 0.002$, respectively), but not between phase 2 and 3 ($p = 0.041$) when using the Bonferroni correction. For the ΔE value quantifying the color difference between phase 2 and phase 3, means ± SD were as follows: 37.37 ± 6.60 for SDF (AA), 34.37 ± 5.68 for SDF (RS), and 18.98 ± 6.02 for SDF/KI. The comparison of materials was significant ($p < 0.001$), with significance between SDF (AA) and SDF/KI ($p < 0.001$) and SDF (RS) and SDF/KI ($p < 0.001$), but not SDF (AA) and SDF (RS) ($p = 0.380$).

Conclusion: Application of SDF or SDF/KI increases the microhardness of the demineralized dentin. However, KI application significantly reduced the hardness of the SDF when compared to the unaccompanied SDF. Regarding color changes, SDF/KI showed less changes than SDF when compared to the baseline and was better esthetically than SDF.

DEDICATION

This work is dedicated to my parents, Ahmed and Fatimah, the source of my power to pursue my education since I began this post-doctoral enlightening journey in kindergarten. Thank you for always being there. I am forever grateful. May God always protect you and keep you as a source of blessing in my life. I love you both from the bottom of my heart.

To my older brother, Yaser, thank you for believing in me, and for your support, care and patience. You have always put my serenity as a priority. I feel blessed to have you as a brother and a best friend. And to my younger brothers, Abdulrahman, Abdullah and Faisal, who have always encouraged me and gave me continuous love, I miss you all.

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Last but not least, I dedicate this to my beloved wife and closest friend, Reyouf, for her kindness, devotion, and endless support. Her selflessness and sacrifice will always be remembered.

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Dental Caries in Pediatric Dentistry:

Dental caries is a pathological process of localized destruction and demineralization of enamel and dentin by microorganisms. [1] As it progresses, the acid produced on dietary fermentable carbohydrates by bacterial action diffuses into the tooth and dissolves the mineral carbonated hydroxyapatite resulting in demineralization. [2] Dental caries is considered the most prevalent disease worldwide. [3] Research has shown that the prevalence of dental caries for permanent teeth has decreased since the 1960s, but for primary teeth in young children, the prevalence of caries has risen from 24% to 28% between 1988 and 2004. [4]

Among young children the most severe form of dental caries, known as early childhood caries (ECC), is defined as "the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child under the age of six. In children younger than three years of age, any sign of smooth-surface caries is indicative of severe early childhood caries (S-ECC). From ages three through five, one or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing, or filled score of greater than or equal to four (age 3), greater than or equal to five (age 4), or greater than or equal to six (age 5) surfaces also constitutes S-ECC". [5] These carious lesions may advance towards odontogenic infections if they remain untreated. [6] Children with untreated severely carious teeth are affected in all aspects of their lives; their overall health, weight, education, and quality of life are all adversely impacted owing to pain and distress associated with caries progression. [7,8,9]

Young patients with extensive dental caries are usually treated in the operating room (OR) under general anesthesia (GA) in a hospital setting. Unfortunately, many variables, including accessibility of services, insurance, lengthy waiting lists, illness, no reachable anesthesiologist, poor climate, etc., may cause cancelations or delays affecting the use of OR facilities; therefore, dental rehabilitation is sometimes not given in a prompt manner. [10] Due to delayed treatment, further progression of dental caries could potentially lead to tooth loss.

Silver Diamine Fluoride (SDF):

Many recent studies have been directed toward caries prevention and arresting disease progression by using Silver Diamine Fluoride (SDF), fluoride, sealants, and arginine. [11] SDF $[Ag(NH_3)_2F]$ is a solution that has been used to arrest dental caries; it was first approved to be used therapeutically in Japan in the 1960s. [12] It was also used in countries like Argentina, Australia, Brazil and China to treat dental caries. [13] SDF has been used since 1969 to arrest carious lesions on primary teeth for children, [12] prevention of pit and fissure caries of erupting permanent molars, [14] and root caries in aged people. [15] SDF was also used to sterilize infected root canals and tooth hypersensitivity treatment. [16]

The 38% SDF was granted Breakthrough Designation Therapy by the Food and Drug Administration in 2016 for arresting and preventing tooth caries in children and adults. The code “Interim caries arresting medication application” was approved by the Code on Dental Procedures and Nomenclature (CDT) Code Maintenance Commission for 2016. [17]

The exact mechanism of action for SDF is not well understood, but it has been suggested that SDF's chemical components contribute as follows to its arresting effects: silver salts stimulate dentine sclerosis or calcification, silver nitrate acts to kill bacteria, and fluoride aids in remineralization and prevention. [18] Studies were done on SDF to test its effect on cariogenic bacteria, the mineral content of enamel and dentin, and the organic content of dentin. When it comes to cariogenic bacteria, SDF is bactericidal, it inhibits adherence and growth of *Streptococcus mutans* on the carious enamel surface; silver particles were found on the dentin surface following SDF treatment with lower colony forming unit (CFU) counts of bacteria and more dead bacteria. [19,20] Enamel blocks treated with SDF had less lesion depth and increased microhardness; also, SDF treated enamel blocks took up more calcium from the re-mineralizing solution and released less calcium in the demineralizing solution. [21,22] SDF treated dentin surfaces had less lesion depth and mineral loss and increased microhardness. When human dentin was subjected to demineralization, SDF treated dentin had less calcium and phosphorous loss and more fluoride uptake.

As a result of SDF treatment, collagens were protected and not exposed. In the presence of SDF, collagen was less degraded by matrix metalloproteinases (MMPs) than by silver nitrate ($AgNO_3$) or sodium fluoride (NaF). [23-26] The higher the concentration of SDF, the lower the degradation of collagen: when using 38% SDF, collagen was less degraded when compared to using either 30% SDF or 12% SDF. [27] A 38% SDF is more effective in arresting caries than 12% SDF; the 12% SDF has a similar effect to the control where no treatment was done to the primary teeth. [28,29]

Re-application of SDF bi-annually showed to decrease caries activity more than a single application; [30] however, in some situations where more than one application is not feasible, an option called Silver Modified Atraumatic Restorative Treatment (SMART) can be used, where SDF is applied on the carious lesion and then immediately restored or sealed with conventional glass ionomer cement (GIC) on the same appointment. This option is especially helpful when the patient is not likely to present for immediate further dental treatment, for example, patients with behavioral difficulties, very young patients, medically compromised patients and long waiting lists for hospital dentistry. In these situations, the SMART technique is considered a better option than doing nothing at all. [31]

In a single SDF application, its effectiveness ranges from 47% to 90%, depending on the size of the cavity and tooth location. [30,32] Posterior teeth have a lower rate of arrest than anterior teeth. [33] It seems that saliva also plays a major role in the caries arrest mechanism by SDF, where silver chloride was found to be the main precipitate in SDF treated dentin; when comparing rates of arrest in geriatric patients with pediatric patients, the elderly have a lower arrest rate [34] since they tend to have low salivary flow and less functioning saliva, hence a higher caries rate than that in the pediatric population where a higher rate of caries arrest was observed on the surfaces mostly covered by saliva (buccal and lingual smooth surfaces, and anterior teeth). [30]

Studies have discovered that resistance to biofilm formation and further caries formation was noted when applying SDF on artificial carious lesions, likely due to residual ionic silver. [24,35] When comparing SDF treated demineralized to SDF treated non-demineralized dentin, more silver and fluoride is deposited in demineralized dentin, and hence more resistant to caries bacteria than treated sound dentin. [36]

Previous clinical trials on SDF revealed that black stains on the caries lesions that were arrested were common and could cause an aesthetic concern. [37] These stains were the most frequently reported barrier to the usage of SDF among parents. Pediatric program directors expressed concerns regarding the staining and poor parental acceptance of aesthetics following its application. [38]

Potassium Iodide (KI) - RIVA STAR™ (RS):

It has been proposed recently that by applying a saturated potassium iodide (KI) solution directly after SDF has been applied, staining of the dentin carious lesion may be diminished while the ability of SDF to arrest caries is not affected. [39] The proposed explanation is that the KI solution's iodide ions will react to the SDF solution's excess silver ions to form a silver iodide precipitate. [40]

Some recent studies have focused on the use of the SDF/KI. Studies were done on SDF/KI to test its effect on *Streptococcus mutans* growth, secondary caries prevention and root caries arrest. SDF/KI was effective in reducing the numbers of *Streptococcus mutans* in dentinal tubules infected with this organism. [41] Furthermore, topical treatment of SDF/KI on dentine reduced in vitro caries development and inhibited surface biofilm formation. [36] Treatment with SDF/KI inhibited secondary caries development in GIC fillings but was not as effective as treatment with SDF alone. [42] SDF/KI solution application has also been effective in arresting root caries among elderly residents. [43] Moreover, in terms of dentine hypersensitivity, SDF/KI showed better results when compared to oxalic acid-based preparations. [44]

Riva Star (RS) is similar to Advantage Arrest (AA) 38% SDF. RS is a two-component device made up of RS Step 1, containing SDF, followed by RS Step 2, containing aqueous KI, which forms an immediate reaction precipitate of silver iodide when it reacts with SDF, thereby reducing tooth sensitivity by physically occluding the open dentinal tubules. Riva Star was granted an approval by the Food and Drug Administration in 2018 for treatment of dentinal hypersensitivity.

It would be beneficial if KI could hinder the staining formation of SDF without diminishing its physical properties, as it has been shown to be effective in the prevention and arrest of dental caries. Nevertheless, there is no evidence from clinical trials to support this claim.

Artificial Carious Lesions:

Dentin caries is considered more complicated than enamel caries. [45] It involves two stages: first, dissolution of biominerals by organic acids, and second, subsequent degradation of dentin matrix by proteases. Dentin has more organic component and water composition than enamel, where 50% of dentin is made of water and only 3% in enamel. Creating an artificial carious lesion that is similar to natural lesion using organic acids has been attempted since the 1970s. [45-48]

In our study, an artificial dentin caries was created in a standardized lab setting using an acetic acid buffer as the demineralizing solution where it had been commonly used in many previous studies. [45-48] Artificial root dentin lesions created with acetate buffer were found to have comparable cross-sectional microhardness, and lesion profile to natural lesions under qualitative polarized microscopy. [49]

Hardness Testing:

Enamel and dentin hardness have been determined through a variety of methods including abrasion, pendulum, scratching, and indentation techniques. [50] Since it has been shown that the hardness of enamel and dentin has significant local variations, it has been preferred to use microindentation methods. [51]

Measuring hardness has been proven to be a practical method for assessing the mineral content of teeth. [52] Several caries or arrested caries studies have revealed that alterations in dentine's microhardness are directly related to its mineral concentration. [53,54] The deterioration of carious dentine's mechanical properties, namely hardness, was positively correlated with a decrease in its mineral content. [52] Dentine strength and stiffness were obtained from its inorganic component, [55] and the reduction of carious dentine's mechanical properties was due to mineral loss. [56]

Arrested dentinal caries are caries that do not show any further progressive tendency. Such dentin is usually inactive microbiologically and approximates sound dentin's hardness. [57] Arrested caries become hypermineralized and have a high fluoride content due to continuous exposure to oral cavity fluids.

Topical fluoride agents such as 5% NaF varnish and 38% SDF solution were used to arrest caries. Following topical application of SDF solution or NaF varnish, clinical trials reported encouraging results and treatment was considered successful in those studies if carious lesions were hard on probing. [58-61] Clinical diagnosis of arrested dentinal caries, however, mostly are contingent on the subjective evaluation of the examiner. Therefore, laboratory studies were conducted to evaluate the remineralization and hardening of dentinal lesions during reversal of caries.

AIM AND HYPOTHESIS

Research Aims:

The aims of this in-vitro study were to investigate the effect of SDF/KI treatment on the microhardness levels, and to evaluate the effect of the subsequent application of potassium iodide (KI) on the degree of discoloration.

Research Hypotheses:

Our hypotheses were as follows:

1- For microhardness, SDF treated groups have greater microhardness levels when compared to the SDF/KI treated group.

2- For color change, potassium iodide application influences the color changes after the application of SDF, and results in less color changes from baseline when compared to SDF alone.

Significance:

The results of this study could help the dentist to understand the effect of SDF/KI on microhardness levels when applied on carious primary teeth.

MATERIALS AND METHODS

This in vitro study was performed in the Gavel Laboratory, Tufts University School of Dental Medicine, Boston, MA, USA. Forty-five extracted or exfoliated primary molar teeth were collected according to human subjects' regulations in the pediatric dentistry department, Tufts University School of Dental Medicine, MA, USA. All teeth were collected with no link to the patient sources. Jars with saline were kept in the pediatric dentistry department for extracted/exfoliated teeth to be collected during the day. An investigator picked them up on a regular basis. Only sound or minimally carious extracted or exfoliated primary molars (where we could get 2 X 3 mm of sound dentin) were included in the study; any teeth preserved in sodium hypochloride (NaOCl) or any chemical preservatives other than saline were excluded.

A total of 45 teeth were randomly divided into three groups of 15 teeth for each material being tested (Table 1). Each tooth was sectioned to expose the desired dentin surface. Randomization was conducted using the "sample" function of software R version 3.4.2.

Group 1: Advantage Arrest™ Silver Diamine Fluoride 38% *(*Bee Brand Medical Dental Company Ltd., Osaka, Japan)

Group 2: RIVA STAR™ step 1 only: Silver Diamine Fluoride 35-40% (SDI Limited, Victoria, Australia)

Group 3: RIVA STAR™ 2 steps: Silver Diamine Fluoride 35-40% and Potassium Iodide (SDI Limited, Victoria, Australia).

1-Sample Collection:

Collected teeth were cleaned from the residual tissues attached to the tooth surface by wiping them with a piece of gauze and then were stored in normal saline at 4°Celsius until preparation.

2-Sample preparation and manipulation:

After randomizing the 45 samples into three groups (Figures 1 and 2), each sample was prepared in the following manner: a large cylindrical plastic mold was placed at the top of the table with the cap at the bottom of the mold to hold the acrylic resin material, which was poured into the mold until desired coverage was achieved. The tooth was pushed into the resin with the occlusal surface facing down until it reached the mold's lid (Figure 3), the mounted samples were removed from the mold as soon as the resin sat, each tooth was split with a slow-speed saw with water-cooled diamond blade IsoMet 1000 (Buehler, Lake Bluff, IL, USA) (Figures 4 and 5). Then each sample was trimmed with a dental trimmer and polished with 600-grit silicon carbide paper under water to expose the desired dentin surface, which was viewed under a stereomicroscope (OLYMPUS, SZX16) to confirm it (Figures 6 and 7). The test specimens' working surfaces were covered with acid-resistant nail varnish (red color) except for a small 2 mm by 3 mm window on the working dentin surface, which was left uncovered to subject it to the demineralizing solution (Figure 8). The Vickers hardness test was performed before and after creating the artificial carious lesion on the test groups to confirm the demineralization process, and after the arresting material was applied to assess the changes.

3- Demineralizing Solution Preparation:

Artificial residual carious lesions (chemical model) were created in the unprotected area of the test groups by storing each specimen in a 60 ml glass container with 40 ml of 0.05M acetate buffer (Figure 11); the pH was adjusted to pH 5.0. First the 0.05 M acetate buffer pH 5.0 was made using acetic acid glacial (Figure 10) and sodium acetate anhydrous containing 1.28 mmol/L of Ca, 0.74 mmol/L of inorganic phosphorous and 0.03 μg F/ml, was prepared from $Ca(NO_3)_2.4H2O$, $NaH_2PO_4.2H_2O$, and NaF, respectively, where desired pH was achieved using sodium hydroxide NaOH (Figure 9). [62] Test specimens were placed in the buffer, which was changed daily for two weeks at 37°C incubator (Figure 12) for the desired demineralization to be achieved which is more than 200-μm [49] because on arrested dentinal lesion on primary teeth, it was found that 38% SDF would create a highly mineralized surface zone (about 150 μm) rich in calcium and phosphate. [26] After the test specimens had been in the artificial caries buffer for two weeks they were immediately stored in deionizing water with thymol to prevent bacterial growth.

4- Test for Hardness:

The specimen surface was characterized with hardness test and microscopic observations, where hardness was defined as the resistance to indentation. An indenter (Buehler, Lake Bluff, IL, USA, Wilson VH1202) (Figure 14) was used to perform the microhardness analysis on the surface of the dentin structure. Three indentations were performed under a load of 50 g for 15 s for each specimen. [63] The Vickers Microhardness value was obtained using the following formula: Vickers Hardness Number (VHN) = (1854.4 3 W)/ d^2 (where VHN is the Vickers hardness number expressed in kg/mm^2, W is the weight in g, and d is the length of the diagonal in l mm) for hardness. For the test groups, we assessed hardness on three different occasions: before demineralization (phase 1), after two weeks of demineralization (phase 2), and one week after the second application of the test material (phase 3). Hardness values obtained were recalculated in order to be normalized by dividing the VHN of treated dentin by the VHN of demineralized dentin (phase 3 / phase 2).

5- Silver Diamine Fluoride Application:

Advantage Arrest™ Silver Diamine Fluoride 38%:

Demineralized dentin was wiped with gauze before the application of SDF. Silver diamine fluoride 38% (Bee Brand Medical Dental Company Ltd., Osaka, Japan) (Figure 15) was applied twice as follows: after two-weeks demineralization, teeth were dried with a gentle flow of compressed air, SDF was dispensed in a plastic dappen dish, micro brush was bent (Figure 16); then SDF was applied directly on the exposed demineralized dentin surface for one minute, dried with a gentle flow of compressed air for at least one minute, and the excess SDF was removed with a cotton pellet. A second application of SDF was made after one week. A study by Duangthip et al. showed that weekly application of SDF for three weeks, then annually was the most frequent monitored application in a clinical trial that showed increased effectiveness compared to a single annual application [64] but with a limit of 1 drop (25 μL) per 10 kg per treatment visit, with weekly intervals at most. [17]

RIVA STAR™ Silver Diamine Fluoride 35-40% (SDI Limited, Victoria, Australia)

Step 1: Demineralized dentin was wiped with gauze before the application of SDF. Silver diamine fluoride 35-40% (SDI Limited, Victoria, Australia) (Figure 17) was applied twice as follows: after two-weeks demineralization, teeth were dried with a gentle flow of compressed air, then foil on the silver capsule was pierced using the silver brush. In a circular motion, foil was pushed to the edge of the opening, (Figure 18); then SDF was applied directly on the exposed demineralized dentin surface for one minute, dried with a gentle flow of compressed air for at least one minute, and the excess SDF was removed with a cotton pellet. A second application was made after one week.

Step 2: Immediately using the green brush, foil on the green capsule was pierced and pushed to the edge of the opening in a circular motion. A generous amount of solution from the green capsule was applied to treatment site for one minute (Figure 19), dried with a gentle flow of compressed air for at least one minute, and the excess KI was removed with a cotton pellet. A second application of SDF/KI was made after one week. VHN was recorded after the second application to measure its effect on dentin hardness for all test groups.

6- Spectrophotometer:

According to the Commission International del'Eclairage L* a* b* color system, each color was explained in a three-dimensional fashion. L* axis depicted lightness/ brightness or color ranging from black (0) to white (100), a* axis characterized red (+a*) to green (-a*) and yellow (+b*) to blue (-b*) is denoted by the b* axis. A spectrophotometer, Crystaleye® (Olympus, Shinjuku, Tokyo, Japan) (Figure 20) was used to capture the sample images and perform color analysis of the surface of the dentin structure.

The instrument was calibrated with the manufacturer’s instruction before examination. The L*, a* and b* values were measured three times for each sample at each time interval and the average values were documented. The difference of color between baseline and the desired time point was calculated based on the mathematical equation: [65]

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

7- Scanning electron microscope (SEM):

Following application of the arresting solutions, a scanning electron microscope (Amary 3300 FE SEM with x10,000 magnification) was used to provide images of crystals formed on specimen surfaces using a focused beam of high-energy electrons rather than light.

For qualitative SEM evaluation, a specimen was selected from each group. Before examination, specimens were completely dried, and sputter coated with gold. To differentiate the silver wires from dentin, a 20 keV operating voltage was selected.

8- Energy dispersive X-ray spectroscopy (EDS):

Analysis of the elements of the treated surfaces coupled with the SEM was conducted. To detect carbon (C), oxygen (O), phosphorous (P), calcium (Ca), silver (Ag) and iodine (I) ion levels through energy dispersive X-ray spectroscopy (EDS), the elemental point analysis was performed for the surfaces of the specimens. As part of the total weight of the sample where the reading was taken, readings were conveyed as a relative percentage weight of the recognized element.

9- Sample Size Calculation:

A sample size calculation was conducted using the statistical software nQuery Advisor (Version 7.0). Based on the effect size obtained in a pilot study, with n=3 per group, a sample size of n=15 per group was adequate to obtain a power greater than 99% alongside a Type I error rate of $\alpha = 5\%$.

10- Statistical Analysis:

Data were analyzed using descriptive statistics: means, standard deviations, medians, and inter-quartile ranges. Normality of the data was assessed with the Kolmogorov-Smirnov test. Levene's test was used to assess the assumption of equal variance.

For dentin hardness, Welch's F test was used to compare the VHNs after normalization between the different test groups due to violation of the homogeneity of variances assumption. Post-hoc tests were performed via the Games-Howell test. Repeated measures ANOVA was used to compare the VHNs for the three test groups before demineralization (phase 1), two weeks after demineralization (phase 2), and one week after the second application of the related material (phase 3). The Bonferroni correction was used in post-hoc tests.

For lightness value (L*), one-way ANOVA was conducted to compare the differences in L* value between phase 2 and phase 3 among the different test groups. Post-hoc tests were performed via Tukey's HSD. For the SDF (AA) and SDF (RS) groups, repeated measures ANOVA was used to compare the L* values before demineralization (phase 1), two weeks after demineralization (phase 2), and one week after the second application of the related material (phase 3). The Bonferroni correction was used in post-hoc tests. For the SDF/KI group, the assumption of normality was violated; hence, Friedman's test was conducted to compare the L* values of the SDF/KI group in the different phases of the experiment. The Wilcoxon signed-rank test with Bonferroni correction was used as post-hoc tests.

For changes between phase 2 and phase 3 in regard to color value (ΔE), one-way ANOVA was used to compare the ΔE between the different test groups. Post-hoc tests were performed via Tukey's HSD.

P-values less than 0.05 were considered statistically significant, except in the case of post-hoc tests in which the Bonferroni correction was used (for which $\alpha = 0.05/3 \approx 0.0167$). The statistical software SPSS (version 25) was used for the analysis.

RESULTS

1. Vickers Hardness Number (VHN):

1. Comparison Between the Materials (Table 2):

In this section, VHNs were normalized by dividing the VHN of the treated sample by the VHN of the demineralized sample.

For the SDF (AA) group and SDF (RS) group, the means (± standard deviations) were higher (1.84 ± 0.30, 1.80 ± 0.19, respectively) when compared to the SDF/KI group (1.44 ± 0.13). The comparison of materials using Welch's F test was statistically significant ($p < 0.001$); in post-hoc testing (Games-Howell), there was a statistically significant difference between the SDF/KI group and both the SDF (AA) group and the SDF (RS) group ($p < 0.001$). However, the difference between the SDF (AA) and SDF (RS) groups was not statistically significant ($p = 0.905$).

2. Comparison Between the Test Groups at the Different Phases of the Experiment (Table 3):

For all treatment groups, the highest mean (± standard deviation) was recorded for phase 1, followed by phase 3 and then phase 2. In the SDF (AA) group, results were 54.01 ± 4.90, 14.00 ± 1.82, and 25.42 ± 2.62, respectively. In the SDF (RS) group, results were 54.75 ± 4.12, 14.07 ± 1.54, and 25.16 ±1.70, respectively. In the SDF/KI group, results were 58.32 ± 6.65, 14.68 ± 1.42, and 21.10 ± 2.14, respectively. For each treatment group, repeated-measures ANOVA determined that the mean VHN value was statistically significant when comparing the readings for the three different phases ($p < 0.001$). Post-hoc tests using the Bonferroni correction revealed statistically significant differences between all phases for each group ($p < 0.001$).

3. Lightness Value (L*):

1. Comparison Between the Materials (Table 4):

Changes in lightness values between phases 2 and 3 were measured in this section, noting that the negative values correspond to a decline from the baseline or phase 2.

For the SDF (AA) group and SDF (RS) group, the means (± standard deviations) were lower (-36.04 ± 7.25, -32.28 ± 6.55, respectively) when compared to the SDF/KI group (3.29 ± 10.11). The comparison of the materials using one-way ANOVA was statistically significant ($p < 0.001$); in post-hoc testing (Tukey HSD), there was a statistically significant difference between the SDF/KI group and both the SDF (AA) group and the SDF (RS) group ($p < 0.001$). However, the difference between the SDF (AA) and SDF (RS) groups was not statistically significant ($p = 0.420$).

2. Comparison Between the Test Groups at the Different Phases of the Experiment (Table 5):

For the SDF (AA) and SDF (RS) groups, the highest means (± standard deviations) of L* value were recorded for phase 1, followed by phase 2 and then phase 3. In the SDF (AA) group, results were 77.92 ± 7.26, 66.20 ± 5.11, 30.16 ± 4.66, respectively, and in the SDF (RS) group, results were 77.75 ± 5.84, 66.15 ± 4.18, 33.87 ± 6.10, respectively. In the SDF/KI group, the highest mean (± standard deviation) was recorded for phase 1, followed by phase 3 and then phase 2 (77.59 ± 7.25 for phase 1, 63.28 ± 3.43 for phase 2, and 66.57 ± 9.28 for phase 3). In the SDF (AA) and SDF (RS) groups, repeated-measures ANOVA determined that the difference in mean L* value was statistically significant when comparing the readings for the three phases ($p < 0.001$). Post-hoc tests using the Bonferroni correction revealed statistically significant differences between all phases for each of the two groups ($p < 0.001$). In the SDF/KI group, Friedman's test was used to determine that the difference in L* value was statistically significant when comparing the readings for the three phases ($p < 0.001$). Post-hoc tests (Wilcoxon signed-rank test with Bonferroni correction) revealed a statistically significant difference between phase 1 and both phase 2 and phase 3 ($p < 0.001$, $p = 0.002$, respectively). However, the difference between phase 2 and phase 3 was not statistically significant ($p = 0.041$) when using the Bonferroni correction.

3. Changes in Color Value (ΔE) Between Phase 2 and Phase 3 (Table 6):

For the SDF (AA) group and SDF (RS) group, the means (± standard deviations) were higher (37.37 ± 6.60, 34.37 ± 5.68, respectively) than the SDF/KI group (18.98 ± 6.02). The comparison of materials regarding changes in color value (ΔE) using one-way ANOVA was statistically significant ($p < 0.001$); in post-hoc testing (Tukey HSD), there was a statistically significant difference between the SDF/KI group and both the SDF (AA) group and the SDF (RS) group ($p < 0.001$). However, the difference between the SDF (AA) and SDF (RS) groups was not statistically significant ($p = 0.380$).

4. SEM Observation:

The SEM image showed small separate cubic shaped crystals for the SDF samples but with greater surface dispersion and associated occlusion of the dentinal tubule (Figure 33). The SDF/KI sample showed sparse irregular clustered crystal formation over the surface and only partial dentinal tubular occlusion (Figure 35). The images were taken at x2,000 magnification at the end of the experiment.

5. EDS Elemental Analysis:

The EDS point analysis for crystals formed in both SDF samples showed a high silver peak (Figure 34) with a weight percentage of 67% (Table 7), verifying that the crystals were silver, while in the SDF/KI sample the analysis showed the highest silver peak trailed by iodine (Figure 36), verifying the existence of silver iodide with a weight percentage of 35% and 39.75% respectively (Table 8).

DISCUSSION

In this study, an in vitro model was used to compare the microhardness levels of different dental treatments applied to artificial carious lesions in demineralized human dentin. Based on this study's results, the null hypothesis was rejected, as differences were observed among the hardness capacities of each dental treatment. Furthermore, this study is the first to evaluate the efficacy of applying KI immediately after SDF application in terms of microhardness levels.

We created artificial carious lesions on primary dentin of primary molars on the test groups, using acetate buffer for two weeks. In order to confirm the demineralization process, VHN was assessed for our test samples before (phase 1) and after (phase 2) demineralization to confirm the weakening of dentin structure due to loss of calcium and phosphate ions. When comparing the VHN for hardness, we found that the mean VHN for phase 1 was significantly higher than phase 2, which indicated weakened dentin structure.

We also measured the VHN after applying the dental treatment on the test groups to confirm the calcifying effect of SDF on demineralized dentin. VHN had significantly increased after the two applications of SDF (Phase 3) when compared to phase 2. Studies have shown that the microhardness for the outermost dentinal surface of carious lesion that had been arrested would increase after applying surface treatment containing fluoride, [61] since SDF has the ability to remineralize artificial dentin caries-like lesions under net-de-mineralizing conditions. [66]

Hardness testing is an indirect method to track changes in the mineral content of dentin and several studies have been published on dentin microhardness. [53,57,67,68] In these studies, dentinal microhardness typically ranged from 25 to 65 (245 to 638 MPa) in VHN. However, the results of microhardness from various studies cannot be directly compared. First, the preparation method of the sample may differ. Some researchers proposed that 100% ethanol be used to dehydrate dentin specimens to minimize shrinkage due to drying. [53,67] Others proposed that samples of dentin should be examined directly without dehydration of ethanol. [57,68,69] Second, the load used in the microhardness test varied from 1.5 gf (9.8 average 10−3 N) [68] to 500 gf (4.9 N) [69] between the studies. The load used in this study was 50 gf (49×10^{-3} N), and the VHN values reported were consistent with those in a previous study done by Soekanto et al. in which the mean VHN of sound dentin was 64.82 ± 8.15 and demineralized dentin was 10.32 ± 2.27. [63] Likewise, the entire range of VHN in this study varied from 11 to 68 (104 to 667 MPa) and these values were similar to the values reported of 10 to 62 (98 to 608 MPa) for primary teeth dentin. [61]

In this study, we elected the demineralized dentin phase as our baseline when calculating the changes in color value ΔE and lightness value ΔL in order to isolate the effect of the treatment and to eliminate factors that were not accounted for before collecting the teeth. Similarly, baseline VHN was used for normalizing the treated VHN by the equation:

$$VHN_{treated} / VHN_{demineralized}$$

By using this method, we adjusted the final values measured on common scales to a different one. In other words, this normalization equation enables the comparison of the corresponding normalized values from different datasets to eliminate the effects of certain gross influences such as hypo or hyperplasia, hypo or hypermineralization, fluorosis and sclerotic dentin.

The microhardness results of the present study showed that applying KI to the SDF will decrease the microhardness significantly when compared to not applying it to the treated surface, despite increasing the hardness of the demineralized dentin. The possible reason is that the application of KI solution might diminish the amount of silver ions, as most of the free silver ions have already reacted with the iodine salt. The reaction between KI and SDF is displayed as:

$$Ag(NH_3)_2F + KI \rightarrow AgI + KF + 2NH_3$$

One of the most significant concerns for patients is the esthetic appearance of the tooth. Our assessment was to analyze color changes using more accurate and reproducible instrument-based measurements rather than assessing color differences subjectively by the naked eye. [65] As mentioned, in the SDF solution, silver ions can darken the structure of the tooth. It is proposed that the KI solution can react with SDF to produce a bright yellow dense compound (silver iodide), [43] which can reduce the black staining of excess free silver ions. [24] In this study, the results of the quantitative measurements of color differences support the difference between teeth treated with SDF and teeth treated with SDF plus KI. The mean color change for each SDF treated group was higher than the mean color change of the SDF plus KI group.

As stated, a significant warning for its use may be the clinical observation that SDF causes dark staining of carious enamel and dentin. Of paramount importance is the finding that under any circumstances, based on only esthetics, some parents found the dark color unacceptable. Within this study, we focused on reporting the lightness value (L*) after applying SDF and SDF/KI. Our results showed that SDF/KI group is more white or lighter in the L* axis. In fact, there was not any significant difference when comparing this group to its baseline. On the contrary, both SDF groups were black or in other words, darker in the L* axis.

To our knowledge, this research is the first laboratory study to investigate the efficacy of SDF/KI treatment regarding microhardness and can be considered as a reference for future studies, which we believe is a key strength of the present study. Nevertheless, it should be noted that the focus of this research is on an in-vitro model that differs from the more complicated clinical scenarios as we used artificial caries that are different from the natural carious lesions. Thus, the outcomes cannot be extrapolated straight to the in-vivo situations and caution is recommended when interpreting the results. Further studies are needed for a clearer evaluation of these dental treatments' performance in clinical services.

CONCLUSION

Within the limitations of this in vitro study it can be concluded that the application of SDF and SDF/KI increases the microhardness of the demineralized dentin. Unaccompanied SDF application is significantly higher than SDF/KI application with regards to microhardness; however, it has the disadvantage of displaying black staining and having inferior aesthetics.

Based on this study, we recommend applying unaided SDF on carious primary enamel or dentin especially on the posterior teeth.

APPENDIX A

Table 1: Surface Application Materials used in the study and application protocol:

Material	Application
Advantage Arrest™ 38% Silver Diamine Fluoride (Bee Brand Medical Dental Company Ltd., Osaka, Japan)	Teeth were dried with air, SDF was dispensed in a plastic dappen dish, then SDF was applied directly on the exposed demineralized dentin surface for one minute, dried with a gentle flow of compressed air for at least one minute, and the excess SDF was removed with a cotton pellet.
RIVA STAR™ Silver Diamine Fluoride 35-40% (SDI Limited, Victoria, Australia)	Teeth were dried with air, then foil on the silver capsule was pierced using the silver brush. In circular motion, foil was pushed to the edge of the opening, then SDF was applied directly on the exposed demineralized dentin surface for one minute, dried with a gentle flow of compressed air for at least one minute (Step 1). Step 2: Immediately using the green brush, foil on the green capsule was pierced and pushed to the edge of the opening in a circular motion. A generous amount of solution from the green capsule was applied to treatment site for one minute, dried with a gentle flow of compressed air for at least one minute, and the excess KI was removed with a cotton pellet.

Table 2: Results of the Comparison Between Test Groups' Vickers Hardness Number (VHN) After Normalization:

	SDF (**Advantage Arrest™**)	SDF capsule only (**RIVA STAR™**)	SDF/KI (**RIVA STAR™**)
Mean	1.84	1.80	1.44
Median	1.88	1.86	1.41
SD	0.30	0.19	0.13
IQR	(1.64, 1.98)	(1.68, 1.92)	(1.38, 1.52)
*p**	<0.001		

* P-value was obtained from Welch's F test. Post-hoc tests (Games-Howell) were used to determine the significantly different groups as follows:

- SDF (AA) vs. SDF (RS): $p = 0.905$
- SDF (AA) vs. SDF/KI: $p < 0.001$
- SDF (RS) vs. SDF/KI: $p < 0.001$

Table 3: Results of the Test Groups' Vickers Hardness Number at the Different Phases of the Experiment:

		SDF (**Advantage Arrest™**)	SDF capsule only (**RIVA STAR™**)	SDF/KI (**RIVA STAR™**)
Phase 1 (Sound Dentin)	Mean	54.01	54.75	58.32
	Median	51.70	53.97	59.47
	SD	4.90	4.12	6.65
	IQR	(50.35, 58.20)	(52.18, 56.75)	(52.90, 61.90)
Phase 2 (Demineralized Dentin)	Mean	14.00	14.07	14.68
	Median	14.30	14.00	14.27
	SD	1.82	1.54	1.42
	IQR	(12.48, 14.88)	(12.67, 15.32)	(13.85, 15.03)
Phase 3 (Treated Dentin)	Mean	25.42	25.16	21.10
	Median	25.77	25.17	21.17
	SD	2.62	1.70	2.14
	IQR	(23.08, 27.63)	(24.07, 26.52)	(19.55, 21.92)
	*p**	<0.001	<0.001	<0.001

* p-value was obtained using repeated measures ANOVA. Post-hoc tests were conducted using the Bonferroni correction. For each group, differences between all phases were significant ($p < 0.001$).

Table 4: Results of the Comparison Between Test Groups' Changes in Value of Lightness (∆L):

	SDF (**Advantage Arrest™**)	SDF capsule only (**RIVA STAR™**)	SDF/KI (**RIVA STAR™**)
Mean*	-36.04	-32.28	3.29
Median*	-34.49	-34.87	3.95
SD	7.25	6.55	10.11
IQR*	(-43.57, -29.29)	(-36.72, -28.00)	(1.43, 8.35)
*p***	<0.001		

* Negative values correspond to a decrease in the value of lightness from the baseline.
** p-value was obtained from one-way ANOVA. Post-hoc tests (Tukey HSD) were used to determine the significantly different groups as follows:

- SDF (AA) vs. SDF (RS): $p = 0.420$
- SDF (AA) vs. SDF/KI: $p < 0.001$
- SDF (RS) vs. SDF/KI: $p < 0.001$

Table 5: Results of the Test Groups' Value of Lightness (L*) at the Different Phases of the Experiment:

		SDF (**Advantage Arrest™**)	SDF capsule only (**RIVA STAR™**)	SDF/KI (**RIVA STAR™**)
Phase 1 (Sound Dentin)	Mean	77.92	77.75	77.59
	Median	80.52	80.99	80.59
	SD	7.26	5.84	7.25
	IQR	(74.91, 82.97)	(74.27, 81.47)	(72.41, 83.21)
Phase 2 (Demineralized Dentin)	Mean	66.20	66.15	63.28
	Median	65.13	65.85	64.44
	SD	5.11	4.18	3.43
	IQR	(63.88, 68.95)	(63.18, 68.53)	(62.06, 65.70)
Phase 3 (Treated Dentin)	Mean	30.16	33.87	66.57
	Median	30.78	34.38	67.13
	SD	4.66	6.10	9.28
	IQR	(25.91, 33.18)	(30.25, 38.58)	(63.85, 69.78)
	*p**	<0.001	<0.001	<0.001

- For SDF (AA) and SDF (RS) groups: p-value was obtained using repeated measures ANOVA. Post-hoc tests were conducted using Bonferroni correction. For each group, differences between all phases were significant ($p < 0.001$)
- For SDF/KI Group: p-value was obtained using Friedman's test. Post-hoc tests (Wilcoxon signed-rank test with Bonferroni correction) was used to determine the significantly different phases as follows:
  - Phase 1 vs. Phase 2: $p < 0.001$
  - Phase 1 vs. Phase 3: $p = 0.002$
  - Phase 2 vs. Phase 3: $p = 0.041$
    - The difference between Phase 2 and Phase 3 would have been significant at the 0.05 significance level but was not significant when using the Bonferroni correction ($p < 0.0167$).

Table 6: Results of the Comparison Between Test Groups' Changes in Color Value (ΔE):

	SDF (**Advantage Arrest™**)	SDF capsule only (**RIVA STAR™**)	SDF/KI (**RIVA STAR™**)
Mean	37.37	34.37	18.98
Median	36.65	36.85	18.25
SD	6.60	5.68	6.02
IQR	(31.63, 43.94)	(30.54, 37.71)	(14.48, 22.16)
*p**	<0.001		

* p-value was obtained from one-way ANOVA. Post-hoc tests (Tukey HSD) were used to determine the significantly different groups as follows:

- SDF (AA) vs. SDF (RS): $p = 0.380$
- SDF (AA) vs. SDF/KI: $p < 0.001$
- SDF (RS) vs. SDF/KI: $p < 0.001$

Table 7: Energy dispersive X-ray spectroscopy (EDS) analysis for SDF sample:

Element	Concentration	Units
C	27.003	Wt.%
O	2.068	Wt.%
P	0.652	Wt.%
Cl	2.576	Wt.%
Ca	0.687	Wt.%
Ag	67.014	Wt.%
Total	100	Wt.%

Table 8: Energy dispersive X-ray spectroscopy (EDS) analysis for SDF/KI sample:

Element	Concentration	Units
C	23.386	Wt.%
O	1.812	Wt.%
Ag	35.061	Wt.%
I	39.741	Wt.%
Total	100	Wt.%

APPENDIX B

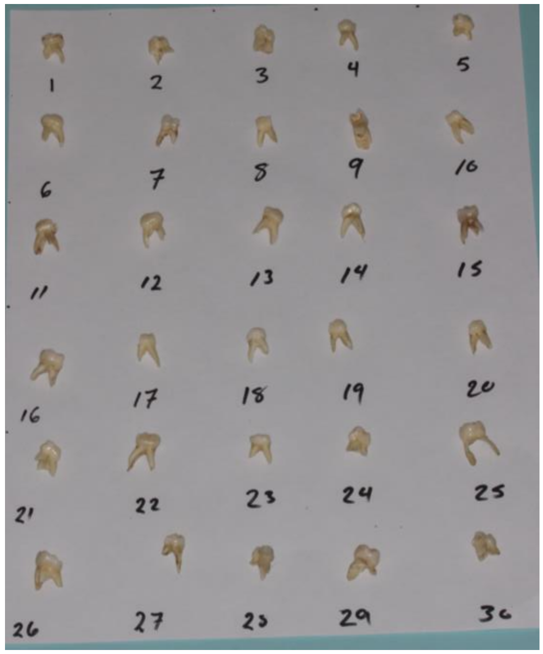


Figure 1: The collected sample of the first 30 primary molars.

Figure 2: Sample randomization using software R version 3.4.2: First 15 numbers go into group 1 SDF (AA): 1, 3, 5, 6, 10, 14, 19, 21, 24, 26, 31, 32, 36, 42 and 44; next 15 go into group 2 SDF (RS): 2, 4, 6, 12, 13, 15, 18, 22, 28, 30, 33, 40, 41, 43 and 45; last 15 go into group 3 SDF/KI: 8, 9, 11, 16, 17, 20, 23, 25, 27, 29, 34, 35, 37, 38 and 39.

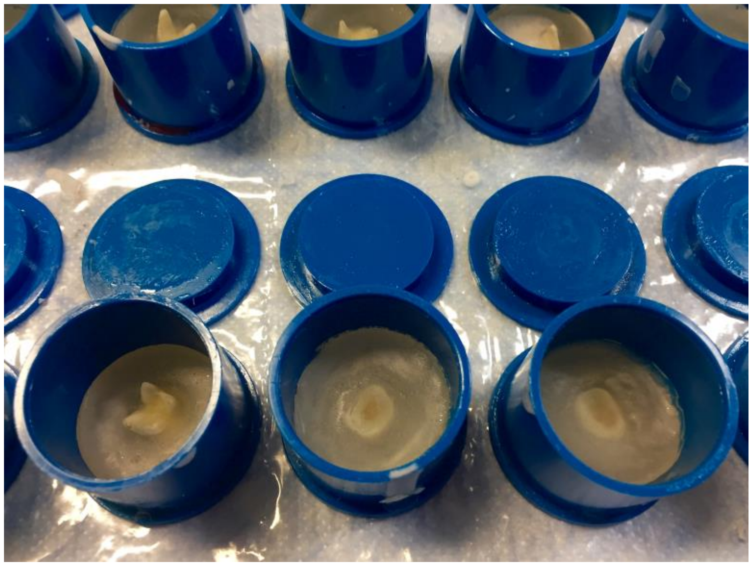
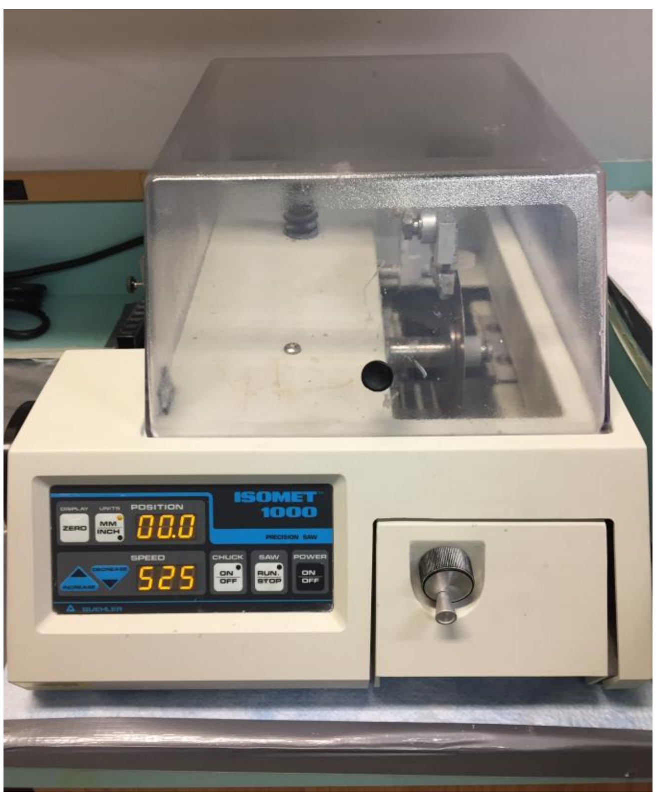
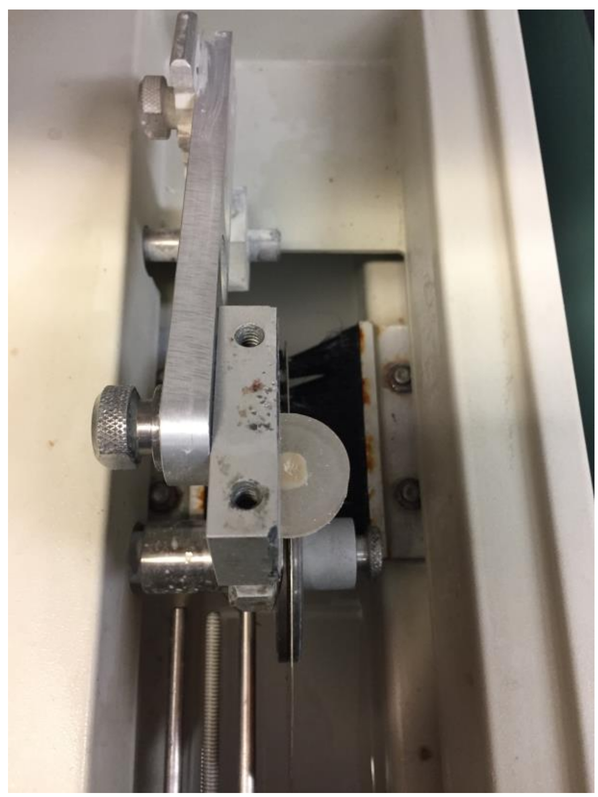


Figure 3: Mounted samples in acrylic.





Figures: 4,5: IsoMet 1000 saw (Buehler, Lake Bluff, IL, USA) used to split samples.

Figure 6: Stereomicroscope (OLYMPUS, SZX16).

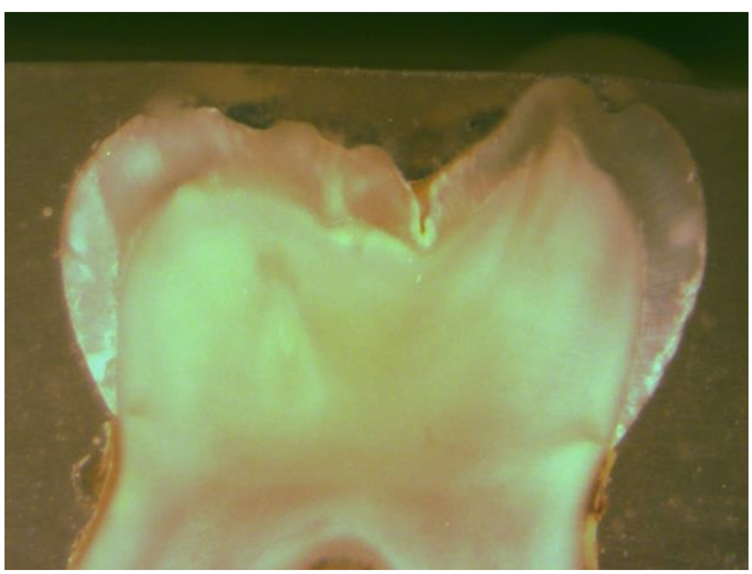


Figure 7: Each tooth sectioned was inspected under the Stereomicroscope.

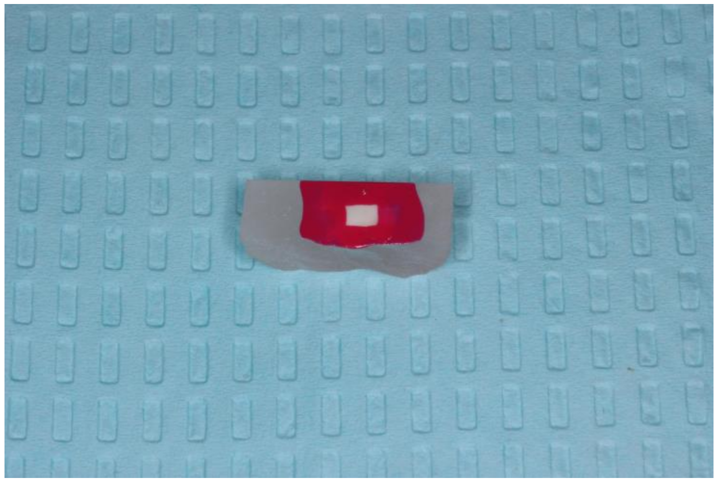
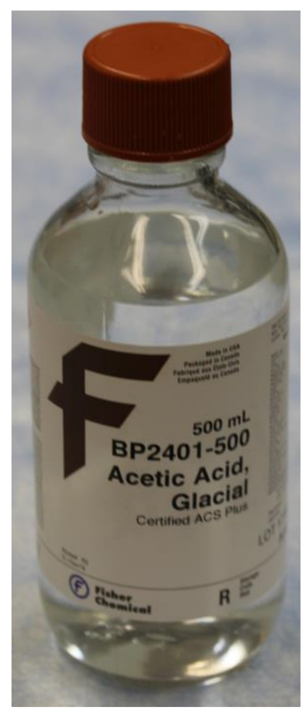


Figure 8: 2X3 dentin window on test samples.



Figures 9,10: Acetate buffer preparation using acetic acid glacial.

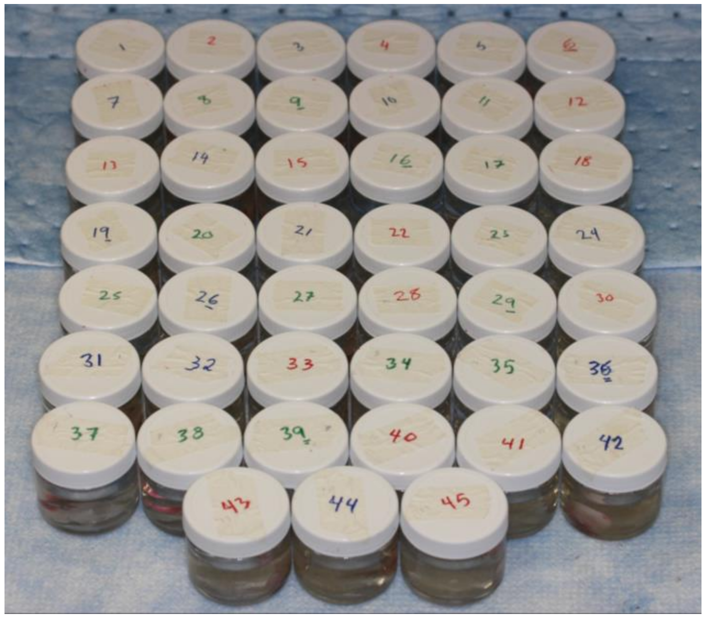


Figure 11: Samples were placed into respective 60 ml jars before applying 40 ml of demineralization solution.

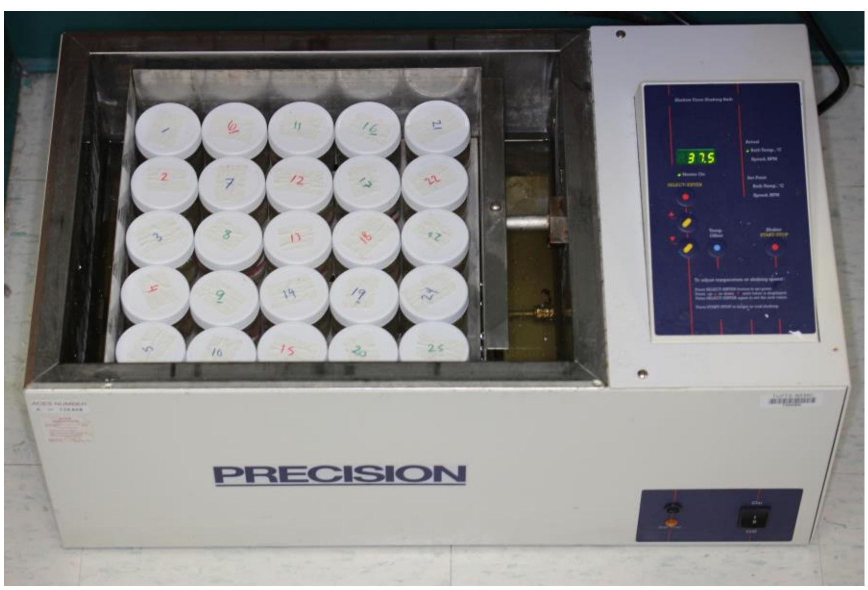


Figure 12: Incubator for test samples at 37°C for demineralization.

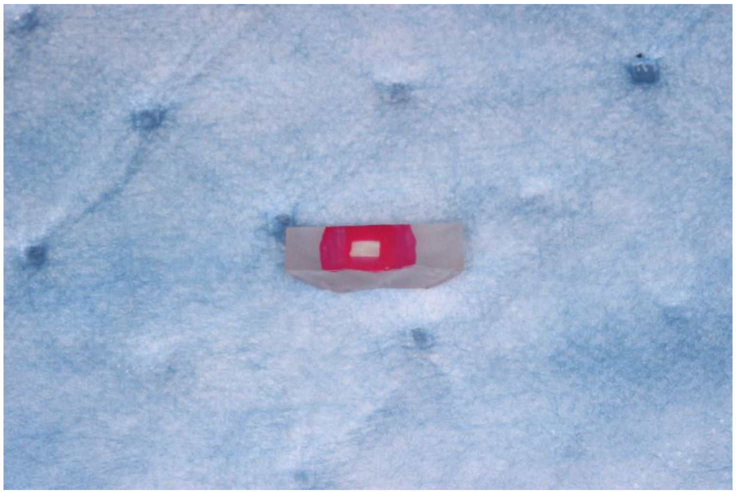


Figure 13: Test sample after 2-week demineralization.

Figure 14: Hardness assessment using (Buehler, Lake Bluff, IL, USA, Wilson VH1202).

Used to measure dentin hardness (VHN) in three different timings: Phase 1: before demineralization, Phase 2: after demineralization, Phase 3: one week after 2^{nd} SDF application.

Figure 15: 38% Silver Diamine Fluoride (Bee Brand Medical Dental Company Ltd., Osaka, Japan).

Figure 16: SDF (AA) application.

Figure 17: Silver diamine fluoride 35-40% (SDI Limited, Victoria, Australia).

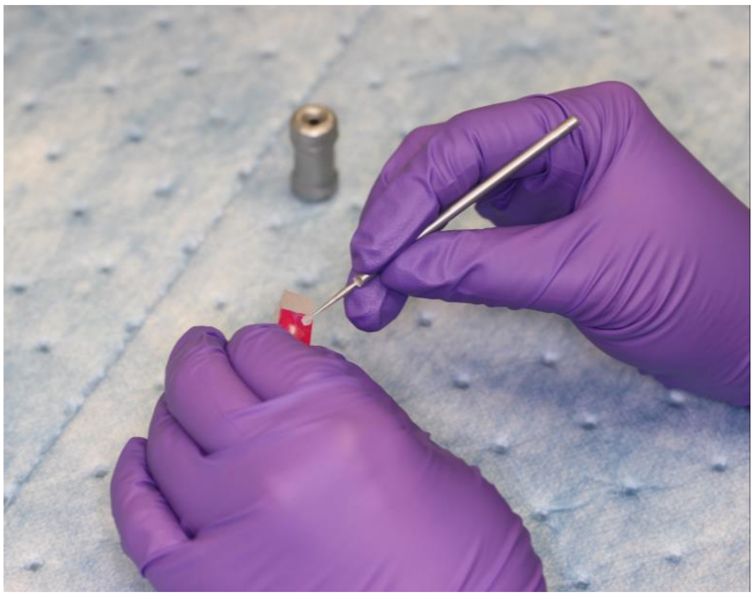


Figure 18: SDF (RS) application (step 1).

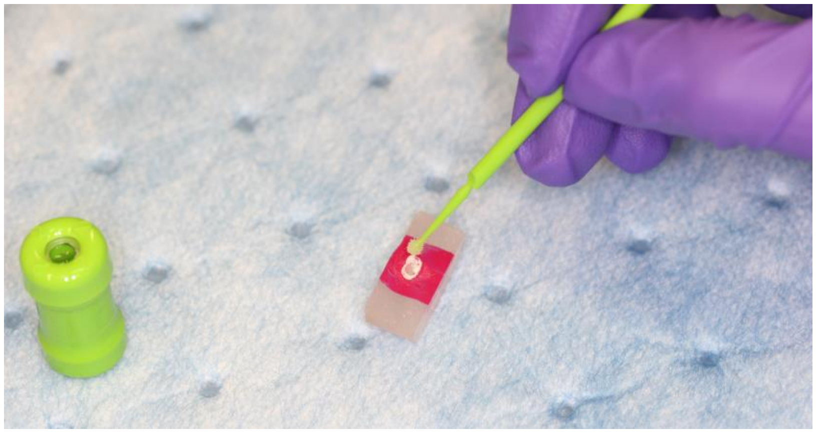
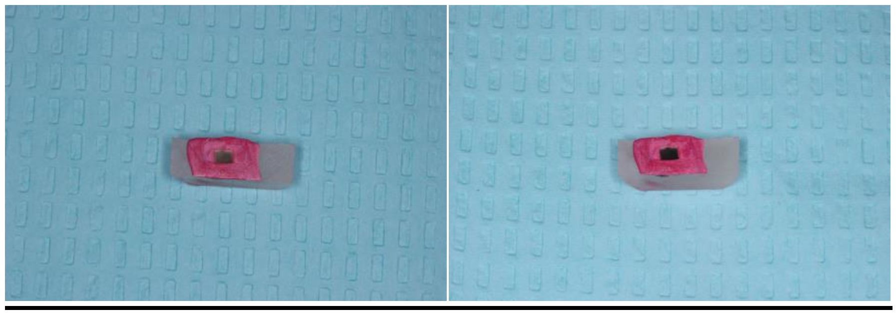
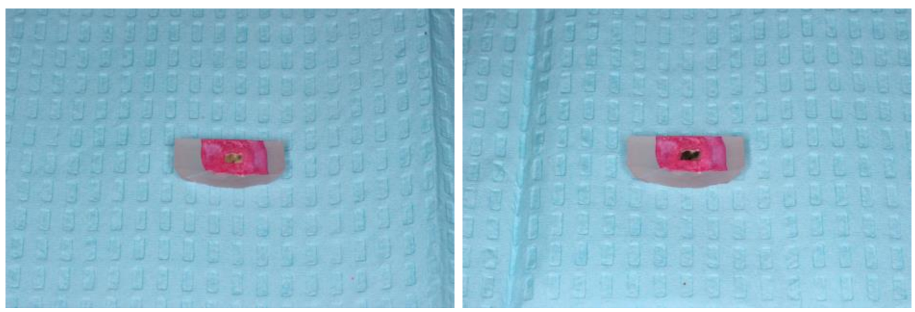


Figure 19: Potassium iodide (KI) application (RS step 2). Notice white precipitate when applied.

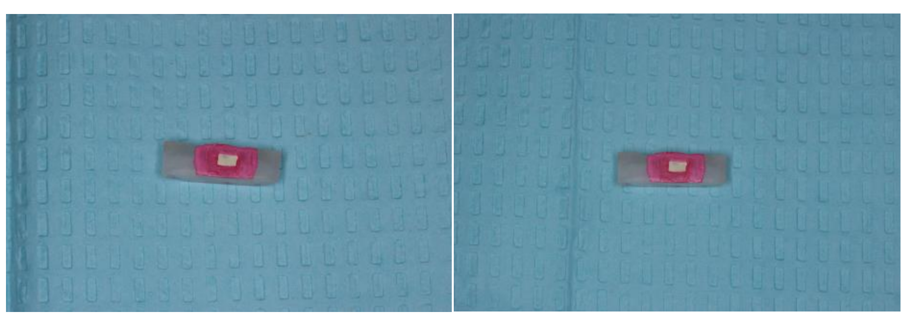
Figure 20: Color assessment using a spectrophotometer, Crystaleye® (Olympus, Shinjuku, Tokyo, Japan). Used to measure dentin hardness (VHN) in three different timings: Phase 1: before demineralization, Phase 2: after demineralization, Phase 3: one week after 2nd SDF application.



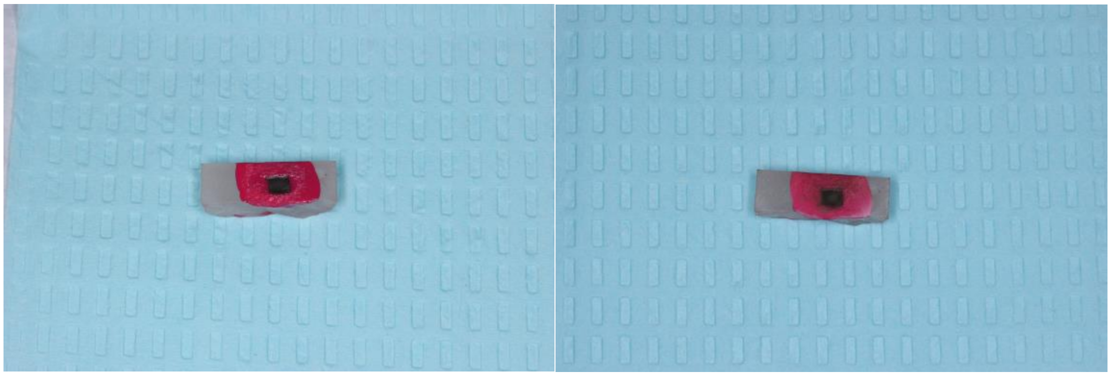
Figures 21, 22: SDF (AA) sample 5 minutes since application, 1 hour after.



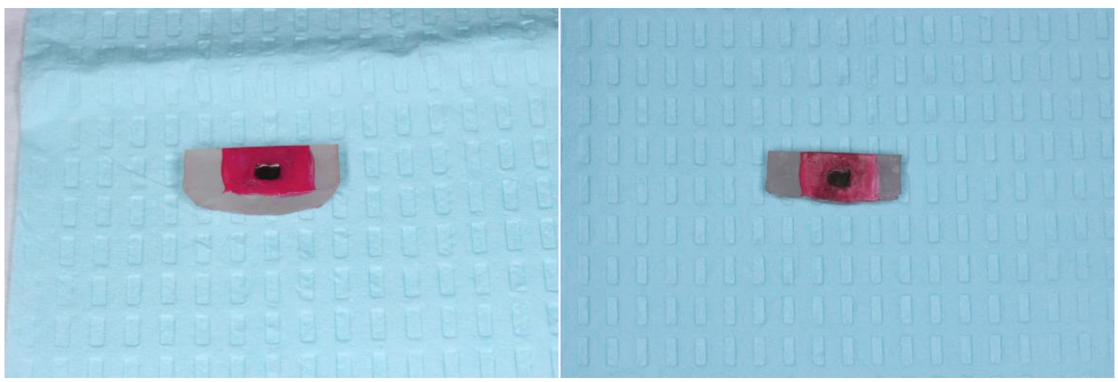
Figures 23, 24: SDF (RS) sample 5 minutes since application, 1 hour after.



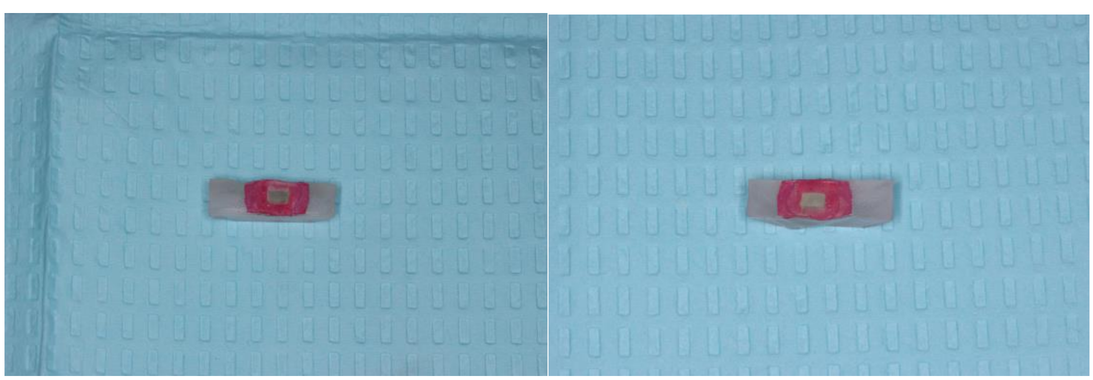
Figures 25, 26: SDF/KI sample 5 minutes since application, 1 hour after.



Figures 27, 28: SDF (AA) sample 1 week after 1st application, 1 week after 2nd application.



Figures 29, 30: SDF (RS) sample 1 week after 1st application, 1 week after 2nd application.



Figures 31, 32: SDF/KI sample 1 week after 1st application, 1 week after 2nd application.

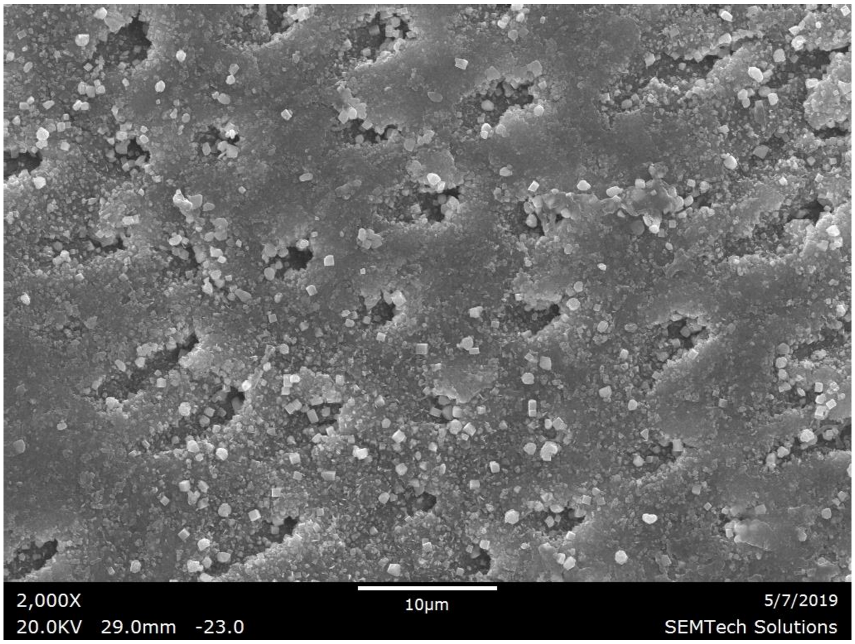


Figure 33: SEM image for the surface of the SDF sample at x2000 magnification.

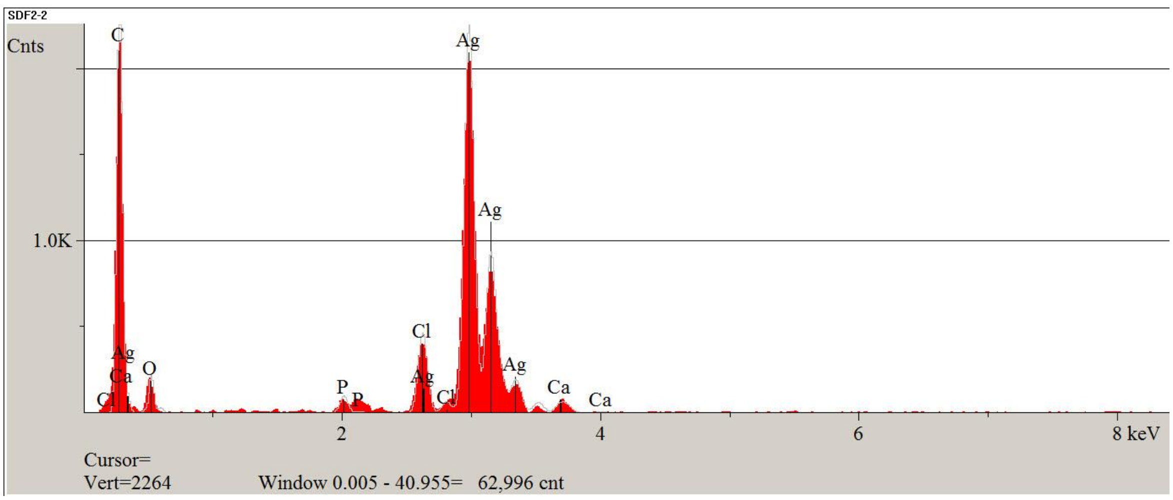


Figure 34: Energy dispersive X-ray spectroscopy (EDS) analysis for SDF sample.

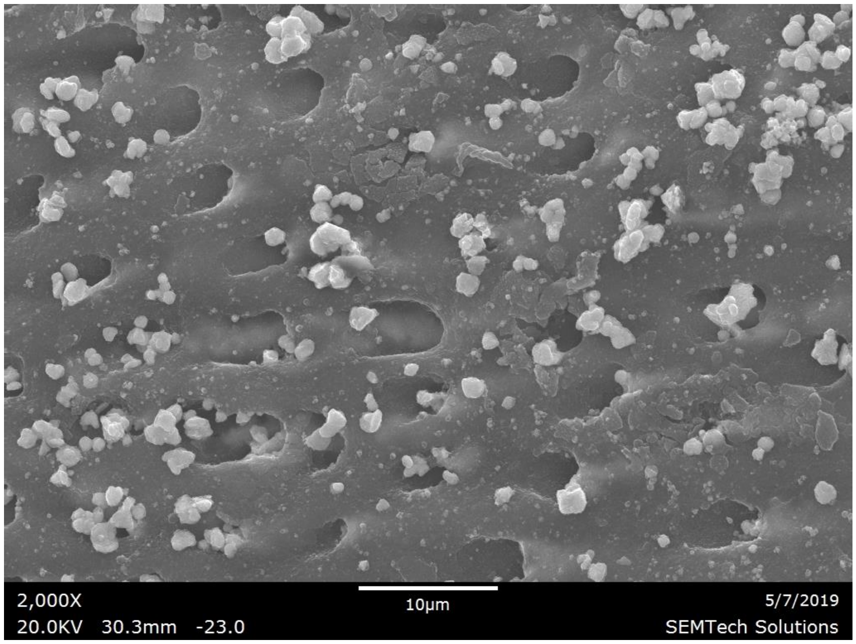


Figure 35: SEM image for the surface of the SDF/KI sample at x2000 magnification.

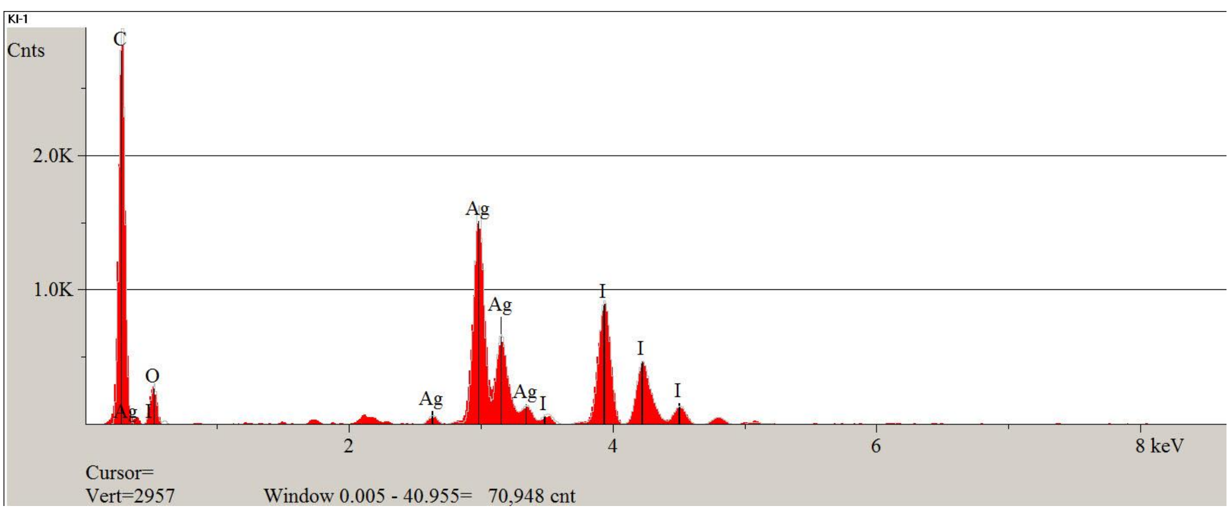


Figure 36: Energy dispersive X-ray spectroscopy (EDS) analysis for SDF/KI sample.

BIBLIOGRAPHY

1) Reynolds, E.C., 2008. Calcium phosphate-based remineralization systems: scientific evidence? Australian dental journal, 53(3), pp.268-273.

2) Vieira, A.P.G.F., Hanocock, R., Eggertsson, H., Everett, E.T. and Grynpas, M.D., 2005. Tooth Quality in Dental Fluorosis. Calcified tissue international, 76(1), pp.17-25.

3) Ozdemir D. Dental Caries: The Most Common Disease Worldwide and Preventive Strategies. Int J of Bio 2013:5. 10.5539/ijb.v5n4p55.

4) Quock RL, Barros JA, Yang SW, Patel SA. Effect of silver diamine fluoride on microtensile bond strength to dentin. Oper Dent 2012;37(6):610-6.

5) Drury TF, Horowitz AM, Ismail AI, et al. Diagnosing and reporting early childhood caries for research purposes. A report of a workshop sponsored by the National Institute of Dental and Craniofacial Research, the Health Resources and Services Administration, and the Health Care Financing Administration. J Public Health Dent 1999;59(3):192-7.

6) Grund K, Goddon I, Schuler IM, Lehmann T, Heinrich-Weltzien R. Clinical consequences of untreated dental caries in German 5- and 8-year-olds. BMC Oral Health 2015;15(1):140.

7) Heinrich-Weltzien R, Monse B, Benzian H, Heinrich J, Kromeyer-Hauschild K. Association of dental caries and weight status in 6- to 7-year-old Filipino children. Clin Oral Investig 2013;17(6):1515-23.

8) Leal SC, Bronkhorst EM, Fan M, Frencken JE. Untreated cavitated dentine lesions: impact on children's quality of life. Caries Res 2012;46(2):102-6.

9) Pourat N, Nicholson G. Unaffordable dental care is linked to frequent school absences. Policy Brief UCLA Cent Health Policy Res 2009(PB2009-10):1-6.

10) Goodwin M, Sanders C, Pretty IA. A study of the provision of hospital based dental general anaesthetic services for children in the northwest of England: part 1--a comparison of service delivery between six hospitals. BMC Oral Health 2015;15:50.

11) Sharma G, Puranik MP, K RS. Approaches to Arresting Dental Caries: An

Update. J Clin Diagn Res 2015;9(5):ZE08-11.

12) Yamaga R, Yokomizo I. Arrestment of caries of deciduous teeth with diamine silver fluoride. Dent Outlook 1969 33: 1007–1013.

13) Chu CH, Lo EC. Promoting caries arrest in children with silver diamine fluoride: a review. Oral Health Prev Dent 2008;6(4):315-21.

14) Nishino M, Ono S, Kita Y, Tsuchitani Y. Caries prevention in pits and fissures with diammine silver fluoride solution and fissure sealant. Sealing properties of pits and fissures and adhesive characteristics to enamel. J Osaka Univ Dent Sch 1974;14:1-7.

15) Tan HP, Lo EC, Dyson JE, Luo Y, Corbet EF. A randomized trial on root caries prevention in elders. J Dent Res 2010;89(10):1086-90.

16) Mei ML, Lo EC, Chu CH. Clinical Use of Silver Diamine Fluoride in Dental Treatment. Compend Contin Educ Dent 2016;37(2):93-8; quiz100.

17) Horst JA, Ellenikiotis H, Milgrom PL. UCSF Protocol for Caries Arrest Using Silver Diamine Fluoride: Rationale, Indications and Consent. J Calif Dent Assoc 2016;44(1):16-28.

18) Rosenblatt A, Stamford TC, Niederman R. Silver diamine fluoride: a caries "silver-fluoride bullet". J Dent Res 2009;88(2):116-25.

19) Tsutsumi N. Studies on topical application of Ag(NH3)2F for the control of interproximal caries in human primary molars 2. effect of Ag(NH3)2F on Streptococcus mutans on interproximal plaque. Jpn J Paediatr Dent 1981 19: 523–536.

20) Mei ML, Li QL, Chu CH, Lo EC, Samaranayake LP. Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries. Ann Clin Microbiol Antimicrob 2013;12:4.

21) Li YJ. [Effect of a silver ammonia fluoride solution on the prevention and inhibition of caries]. Zhonghua Kou Qiang Ke Za Zhi 1984;19(2):97-100.

22) Wu L, Yang F. Comparison of the effects of three fluoride-containing agents on the demineralization of deciduous teeth in vitro. J Modern Stomatol 2002 16: 216–218.

23) Yao N, Tang R. Study of the effect of Chinese nutgall water extract on

experimental root caries remineralization. Chin J Conserv Dent 2006 16: 285–287.

24) Knight GM, McIntyre JM, Craig GG, et al. Inability to form a biofilm of Streptococcus mutans on silver fluoride- and potassium iodide-treated demineralized dentin. Quintessence Int 2009;40(2):155-61.

25) Chu CH, Mei L, Seneviratne CJ, Lo EC. Effects of silver diamine fluoride on dentine carious lesions induced by Streptococcus mutans and Actinomyces naeslundii biofilms. Int J Paediatr Dent 2012;22(1):2-10.

26) Mei ML, Ito L, Cao Y, et al. An ex vivo study of arrested primary teeth caries with silver diamine fluoride therapy. J Dent 2014;42(4):395-402.

27) Mei ML, Li QL, Chu CH, Yiu CK, Lo EC. The inhibitory effects of silver diamine fluoride at different concentrations on matrix metalloproteinases. Dent Mater 2012;28(8):903-8.

28) Yee R, Holmgren C, Mulder J, et al. Efficacy of silver diamine fluoride for Arresting Caries Treatment. J Dent Res 2009;88(7):644-7.

29) Houpt M. Topical application of silver diamine fluoride may arrest dental caries. J Evid Based Dent Pract 2011;11(1):54-5.

30) Zhi QH, Lo EC, Lin HC. Randomized clinical trial on effectiveness of silver diamine fluoride and glass ionomer in arresting dentine caries in preschool children. J Dent 2012;40(11):962-7.

31) Wang AS, Botelho MG, Tsoi JH, Matinlinna JP. Effects of silver diammine fluoride on microtensile bond strength of GIC to dentine. International Journal of Adhesion & Adhesives 2016:70: 196–203.

32) Crystal YO, Niederman R. Silver Diamine Fluoride Treatment Considerations in Children's Caries Management. Pediatr Dent 2016;38(7):466-71.

33) Fung MHT, Duangthip D, Wong MCM, Lo ECM, Chu CH. Arresting Dentine Caries with Different Concentration and Periodicity of Silver Diamine Fluoride. JDR Clin Trans Res 2016;1(2):143-52.

34) Zhang W, McGrath C, Lo EC, Li JY. Silver diamine fluoride and education to prevent and arrest root caries among community-dwelling elders. Caries Res 2013;47(4):284-90.

35) Knight GM, McIntyre JM, Craig GG, et al. An in vitro model to measure the effect of a silver fluoride and potassium iodide treatment on the permeability of demineralized dentine to Streptococcus mutans. Aust Dent J 2005;50(4):242-5.

36) Knight GM, McIntyre JM, Craig GG, et al. Differences between normal and demineralized dentine pretreated with silver fluoride and potassium iodide after an in vitro challenge by Streptococcus mutans. Aust Dent J 2007;52(1):16-21.

37) Crystal YO, Janal MN, Hamilton DS, Niederman R. Parental perceptions and acceptance of silver diamine fluoride staining. J Am Dent Assoc 2017;148(7):510-8.

38) Nelson, T., Scott, J. M., Crystal, Y. O., Berg, J. H., & Milgrom, P. (2016). Silver diamine fluoride in pediatric dentistry training programs: survey of graduate program directors. Pediatric dentistry, 38(3), 212-217.

39) Knight, G. M., Mclntyre, J. M., Craig, G. G., & Mulyani. (2006). Ion uptake into demineralized dentine from glass ionomer cement following pretreatment with silver fluoride and potassium iodide. Australian dental journal, 51 (3), 237-241.

40) Knight, G. M., & McIntyre, J. M. (2006). The effect of silver fluoride and potassium iodide on the bond strength of auto cure glass ionomer cement to dentine. Australian dental journal, 51(1), 42-45.

41) Hamama, H. H., Yiu, C. K., & Burrow, M. F. (2015). Effect of silver diamine fluoride and potassium iodide on residual bacteria In dentinal tubules. Australian dental journal, 60 (1), 80-87.

42) Zhao, I. S., Mei, M. L., Burrow, M. F., Lo, E. C. M., & Chu, C. H. (2017). Effect of Silver Diamine Fluoride and Potassium Iodide Treatment on Secondary Caries Prevention and Tooth Discolouration in Cervical Glass Ionomer Cement Restoration. International journal of molecular sciences, 18 (2), 340.

43) Li, R., Lo, E.C.M., Liu, B.Y., Wong, M.C.M. and Chu, C.H., 2016. Randomized clinical trial on arresting dental root caries through silver diammine fluoride applications in community-dwelling elders. Journal of dentistry, 51, pp.15-20.

44) Craig, G. G., Knight, G. M., & McIntyre, J. M. (2012). Clinical evaluation of diamine silver fluoride/potassium iodide as a dentine desensitizing agent. A pilot study. Australian dental journal, 57(3), 308-311.

45) Featherstone JD, Cutress TW, Rodgers BE, Dennison PJ. Remineralization of artificial caries-like lesions in vivo by a self-administered mouthrinse or paste.

Caries Res 1982;16(3):235-42.

46) Featherstone JD, Duncan JF, Cutress TW. A mechanism for dental caries based on chemical processes and diffusion phenomena during in-vitro caries simulation on human tooth enamel. Arch Oral Biol 1979;24(2):101-12.

47) Featherstone JD, Rodgers BE. Effect of acetic, lactic and other organic acids on the formation of artificial carious lesions. Caries Res 1981;15(5):377-85.

48) ten Cate JM, Duijsters PP. Alternating demineralization and remineralization of artificial enamel lesions. Caries Res 1982;16(3):201-10.

49) McIntyre JM, Featherstone JD, Fu J. Studies of dental root surface caries. 1: Comparison of natural and artificial root caries lesions. Aust Dent J 2000;45(1):24-30.

50) Craig, R.G. and Peyton, F.A., 1958. The microhardness of enamel and dentin. Journal of Dental Research, 37(4), pp.661-668.

51) Atkinson, H. F., and Saunsbury, P.: An Investigation Into the Hardness of Human Enamel, Brit. D. J. 94: 249, 1953.

52) Angker, L., Nockolds, C., Swain, M. V., & Kilpatrick, N. (2004). Correlating the mechanical properties to the mineral content of carious dentine—a comparative study using an ultra-micro indentation system (UMIS) and SEM-BSE signals. Archives of Oral Biology, 49(5), 369-378.

53) Hosoya, Y., Marshall, S. J., Watanabe, L. G., & Marshall, G. W. (2000). Microhardness of carious deciduous dentin. Operative Dentistry, 25(2), 81-89.

54) Pereira, P. N. R., Inokoshi, S., Yamada, T., & Tagami, J. (1998). Microhardness of in vitro caries inhibition zone adjacent to conventional and resin-modified glass ionomer cements. Dental Materials, 14(3), 179-185.

55) Marshall Jr, G.W., Marshall, S.J., Kinney, J.H. and Balooch, M., 1997. The dentin substrate: structure and properties related to bonding. Journal of dentistry, 25(6), pp.441-458.

56) Marshall, G.W., Habelitz, S., Gallagher, R., Balooch, M., Balooch, G. and Marshall, S.J., 2001. Nanomechanical properties of hydrated carious human dentin. Journal of Dental Research, 80(8), pp.1768-1771.

57) Mäkinen, K.K., Chiego Jr, D.J., Allen, P., Bennett, C., Isotupa, K.P., Tiekso, J.

and Mäkinen, P.L., 1998. Physical, chemical, and histologic changes in dentin caries lesions of primary teeth induced by regular use of polyol chewing gums. Acta Odontologica Scandinavica, 56(3), pp.148-156.

58) Llodra, J.C., Rodriguez, A., Ferrer, B., Menardia, V., Ramos, T. and Morato, M., 2005. Efficacy of silver diamine fluoride for caries reduction in primary teeth and first permanent molars of schoolchildren: 36-month clinical trial. Journal of dental research, 84(8), pp.721-724.

59) Chu, C.H., Lo, E.C.M. and Lin, H.C., 2002. Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese pre-school children. Journal of dental research, 81(11), pp.767-770.

60) Lo, E.C.M., Schwarz, E. and Wong, M.C.M., 1998. Arresting dentine caries in Chinese preschool children. International Journal of Paediatric Dentistry, 8(4), pp.253-260.

61) Chu, C.H. and Lo, E.C., 2008. Microhardness of dentine in primary teeth after topical fluoride applications. journal of dentistry, 36(6), pp.387-391.

62) Queiroz CS, Hara AT, Paes Leme AF, Cury JA. pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- and remineralization. Braz Dent J 2008;19(1):21-7.

63) Soekanto, S. (2017). The potential of several fluoride-based varnishes as remineralization agents: Morphological studies, dentin surface hardness, and crystallinity tests. International Journal of Applied Pharmaceutics, 9(Special Issue 2), 60-66.

64) Duangthip D, Chu CH, Lo EC. A randomized clinical trial on arresting dentine caries in preschool children by topical fluorides--18 month results. J Dent 2016;44:57-63.

65) Besinis, A., De Peralta, T. and Handy, R.D., 2014. Inhibition of biofilm formation and antibacterial properties of a silver nano-coating on human dentine. Nanotoxicology, 8(7), pp.745-754.

66) Willershausen I, Schulte D, Azaripour A, et al. Penetration Potential of a Silver Diamine Fluoride Solution on Dentin Surfaces. An Ex Vivo Study. Clin Lab 2015;61(11):1695-701.

67) Pereira, P. N. R., Inokoshi, S., Yamada, T., & Tagami, J. (1998). Microhardness of in vitro caries inhibition zone adjacent to conventional and resin-modified glass

ionomer cements. Dental Materials, 14(3), 179-185.

68) Banerjee, A., Sherriff, M., Kidd, E.A.M. and Watson, T.F., 1999. Cariology: A confocal microscopic study relating the autofluorescence of carious dentine to its microhardnes. British Dental Journal, 187(4), p.206.

69) Seaman, F. and Shannon, I.L., 1979. Fluoride treatment and microhardness of dentin. The Journal of prosthetic dentistry, 41(5), pp.528-530.