School of Dental Medicine

**Evaluating the Effectiveness of Different Light-Cured SDF Application Techniques in Primary Teeth: An Ex Vivo Study**

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in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Dental Research

by

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# ABSTRACT

## Aim

This ex vivo study evaluated the effect of different light-curing 38% silver diamine fluoride (SDF) application techniques on the hardness of infected dentin, the penetration depth of SDF, and silver ion precipitation at different depths of the dentinal carious lesions.

## Materials and Methods

Eighty extracted carious primary maxillary anterior teeth were randomized into four groups (N = 20). The groups were as follows: (1) “Control” with first and second applications of SDF. (2) “Test 1” with the first application of SDF and the second application of light-cured SDF. (3) “Test 2” with the first application of light-cured SDF and the second application of SDF. (4) “Test 3” with first and second applications of light-cured SDF. Dentin hardness was measured using a Vickers hardness test. Sliced samples’ images were analyzed using OmniMet software to measure the depth of SDF penetration. A scanning electron microscope (SEM) was used to perform elemental analysis using energy dispersive spectroscopy (EDS) at different lesion depths. Statistical analysis was evaluated via Welch’s ANOVA with post-hoc comparisons performed using the Games-Howell test.

## Results

Welch’s ANOVA showed statistically significant differences between all groups’ mean penetration depth and hardness values ($p < .001$).

For all samples, the highest mean penetration depth of SDF (± standard deviations) was recorded for the “control” (225.5 ± 39.6 µm), followed by “test 1” (168.3 ± 27.8 µm), then

“test 2” (96.5 ± 6.6 µm), and lastly by “test 3” (54.0 ± 11.9 µm). SDF penetrated infected, affected, and sound dentin in all samples.

On the contrary, the highest mean of dentin hardness (± standard deviations) was in “test 3” (80.0 ± 8.7 kgf/ $mm^2$), followed by “test 2” (55.4 ± 6.6 kgf/ $mm^2$), then by “test 1” (39.6 ± 5.5 kgf/ mm2), and then by the “control” group (23.7 ± 2.2 kgf/ $mm^2$). In post-hoc comparisons using the Games-Howell test, the four groups had statistically significant differences in mean penetration depth and hardness ($p < .001$).

EDS analysis showed that oxygen, calcium, and phosphorus were the main elements detected in the center of infected, affected, and sound dentin for each specimen in all groups. Silver weight percentage for the center of infected, affected, and sound dentin in all groups ranged between 0.84% - 2%.

**Conclusion**

Light curing SDF after the first and second applications showed a significant increase in the hardness of infected dentin and decreased SDF penetration into sound dentin. These results might be due to the higher silver precipitation on the surface, which could indicate a higher caries arrest effect compared with the other mentioned SDF application techniques.

# DEDICATION

This work is dedicated to my parents, Abdullah and Hind, who have always been my encouragement and strength source. Thank you for your unconditional love and prayers. And for always supporting me no matter what. May God always protect you. I hope always to make you proud.

To my sisters Norah and Ghadah, who have shared this journey with me. Thank you for being there for me whenever I needed you. I feel blessed to have you both as my sisters and best friends.

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# Evaluating the Effectiveness of Different Light-Cured SDF Application Techniques in Primary Teeth: An Ex Vivo Study

# INTRODUCTION

## **Dental Caries in Pediatric Dentistry**:

Dental caries is a multifactorial disease that results in localized dissolution and destruction of the calcified tissue.[1] Without treatment, the carious lesion may advance and result in infection of the dental pulp and surrounding periapical tissues. The main microorganisms involved in the initiation and development of dental caries are *Streptococcus mutans, Lactobacillus acidophilus, and Actinomyces viscosus*.[2]

Dental caries is one of the most prevalent childhood chronic diseases worldwide.[3] [4] In the U.S., more than 40 percent of children have caries by the time they reach kindergarten.[5] Epidemiologic data indicate that early childhood caries (ECC) is highly prevalent in poor and near-poor U.S. preschool children.[6] 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.[7] ECC remains a rising global public health burden despite numerous advancements in dental research and interventional approaches. Oral health-related quality of life, school attendance and performance, speaking, self-esteem, growth, and body weight can be affected if ECC remains untreated.[8] [9] [10] [11] The conventional dental treatment protocol for the management of caries lesions requires surgical intervention removing the infected, demineralized tooth structure followed by placement of a restorative material. Some barriers to children's oral health care are behavioral issues due to age or limited cooperation, high treatment costs, lengthy operating room (OR) waiting lists, and transportation issues.[12] These barriers call

for other alternative caries management modalities to arrest or slow the progression of caries lesion.

Research in cariology has brought about a shift in the understanding of the caries process and its management. Thus, multiple alternative preventive interventions to the traditional methods of restorative care have been suggested by systematic reviews of human randomized controlled trials in arresting or slowing the progression of caries lesions in primary and permanent teeth.[13] [14] [15] One of these interventions is silver diamine fluoride (SDF) that has been used to arrest carious lesions in primary teeth for over 400 years in Japan[16] and many other countries such as Brazil [17], China [18], Australia, and Argentina.[19]

## Silver in medicine and dentistry:

Silver ion (Ag) is antimicrobial at very low concentrations.[20] It has been used for thousands of years. Silver ion is not an essential nutrient and has no physiologic role in human biology. However, it is found at low levels in the body due to ingestion and inhalation of silver from natural sources.[21] Silver is not a known carcinogen or mutagen.[22] Chronic inhalation or ingestion of colloidal silver or other silver preparations can lead to deposition of silver particles in the skin (argyria), eye (argyrosis) and other organs. These are not life-threatening but are considered cosmetically undesirable.[23] Silver is absorbed into the human body and enters the systemic circulation as a protein complex to be eliminated by the liver and kidneys.[23] True allergy to silver is uncommon, and only a few known reports of silver allergy are documented.[24] [25] [26]

Silver nitrate is one of the most common silver salts. It is a colorless and odorless solution. Silver nitrate solution has antibacterial properties, which have been widely used in medicine because of its broad spectrum of antibacterial activity, low toxicity, and lack of bacterial resistance. It has been used as a cauterizing agent in medicine for treating wounds, especially burned wounds. [27]

The use of silver in dentistry dates back as early as 659 A.D in China, not only for its material properties but also for its antimicrobial effects.[27] Dentists used silver nitrate (AgNO3) to arrest carious lesions.[27] Silver nitrate remained a popular caries-arresting medication through the era of G.V. Black. In 1917 and until the 1950s, ammonia was added to the silver nitrate (AgNH3NO3) and marketed as Howe's antimicrobial solution that could penetrate affected dentin to sterilize lesions after cavity preparation and, although not proven clinically, was used as a disinfectant in root canal therapy.[25 28] In 1969, SDF was pioneered by Nishino and Yamaga. They reported a 74% reduction in dental caries in school-aged children with the use of SDF. [25 29]

In a series of clinical trials at the School Dental Service of Western Australia in the 1970s, it was found that silver fluoride is effective in arresting carious lesions on the proximal and occlusal surfaces in primary teeth. Since the 1970s, SDF has been accepted as a therapeutic agent by Japan's Central Pharmaceutical Council of the Ministry of Health and Welfare.[25 29]

## Silver Diamine Fluoride (SDF):

Silver diamine fluoride (SDF) ((5 percent F 44,800 ppm F)) is a safe, noninvasive, inexpensive, effective, and efficient agent that appears to be almost twice as effective as fluoride varnish for caries arrest. [14] It was approved by the United States Food and Drug Administration in 2014 as a dentinal hypersensitivity treatment for adults, and off-label use for caries arrest is permissible and appropriate for patients.[30]

In 2016, Fung et al. conducted a randomized clinical trial that showed SDF to be more effective in arresting dentin carious lesions at 38% than 12% concentration with the biannual application.[31] The 38 percent SDF solution is the only commercial formula available in the United States and approved by the Food and Drug Administration (FDA). The American Academy of Pediatric Dentistry (AAPD) developed guidance and an evidence-based recommendation regarding the application of 38% SDF that supports its use for carious lesion arrest in primary teeth.[32]

Silver diamine fluoride consists of 25% silver, 5% fluoride, and 8% ammonia with a pH of 10.[33] On January 1, 2016, the Code on Dental Procedures and Nomenclature (CDT) Code Maintenance Commission approved the use of code, D1354, for "interim caries arresting medicament application" The code definition is "Conservative treatment of an active, non-symptomatic carious lesion by topical application of caries arresting or inhibiting medicament and without mechanical removal of sound tooth structure." [30]

## Mechanism of action of SDF

The exact mechanisms of action for SDF are not fully understood; however, caries arrest likely results because of its chemical components. SDF associates the remineralization of the dental structures provided by fluoride ions with the antimicrobial effect on the caries microorganisms by the action of silver nitrate. Also, silver salt stimulates sclerosis and calcification of dentin.[34]

Silver and fluoride have a synergic effect rather than a pure addition effect on arresting dentine caries.[35] The combined effect of silver and fluoride in SDF can reduce cariogenic bacteria's growth by reducing the CFU (Colony Forming Units) counts of mono-species strains of S. mutans and Actinomyces naeslundii [36], hamper collagen degradation in dentin through the inhibition of proteolytic peptidases in dentine and saliva, promote tooth desensitization and carious lesion arrest by blocking dentinal tubule, inhibit demineralization and promote remineralization of both enamel and dentin.[37] [38]

SDF enhances the mineral content of dental hard tissues by reacting with hydroxyapatite to form calcium fluoride ($CaF_2$) and silver phosphate to form fluorapatite, which is less soluble than hydroxyapatite in an acidic environment. Thus, carious dental lesions treated with SDF increase in mineral density and have a significantly higher surface microhardness while the lesion depth decreases. [38] [39]

Topical application of SDF in the treatment of sensitive dentin-decayed surfaces results in the development of a squamous layer of the silver-protein conjugate on the exposed dentin, partially plugging the dentinal tubules. This layer will result in increased

resistance to acid dissolution and enzymatic digestion.[30] Silver diamine fluoride is an alkaline solution; SDF's alkaline property contributes to the neutralization of the acidity and therefore inactivates collagen degradation enzymes, such as matrix metalloproteinases (MMPs), cathepsins, and bacterial collagenases.[35] Silver ions act directly against bacteria in lesions by breaking membranes, denaturing proteins, and inhibiting DNA replication.[30]

Dark staining is a common side effect following the application of SDF due to the formation of a silver phosphate layer and a silver sulfide precipitate on carious dentin.[29] Black staining can be reduced by using potassium iodide (KI), which reacts with the remaining free silver ions to form silver iodide, giving a white creamy reaction product.[40] Aside from that, SDF has minimal documented adverse effects limited to rare transient mucosal lesions.[30]

## SDF effectiveness against carious lesions

Silver diamine fluoride (SDF) clinical success is well-documented in multiple clinical studies. A 2017 review concluded that SDF is very effective for preventing and arresting caries in pediatric, adolescent, and elderly populations.[41] Some of the indications for using SDF are for high caries risk patients, difficult-to-treat dental carious lesions, and patients with challenging medical or behavioral complications, patients without access to dental care, or patients who require multiple treatments visits.[38]

It has been shown in an “ex vivo” study by Mei et al. that applying SDF on a carious dental lesion exhibited a remineralization zone rich in calcium and phosphate, similar to arrested carious lesions.[42] Multiple clinical studies have demonstrated the effectiveness of SDF in caries prevention and arrest. Knight et al. compared SDF-treated demineralized dentin to SDF-treated non-demineralized dentin. It was demonstrated that more silver and fluoride are deposited in demineralized dentin and hence more resistant to caries bacteria than treated sound dentin.[43]

SDF reacts with the hydroxyapatite of the carious dentin through the following formula:[44]

$$Ca_{10}(PO_4)_6(OH)_2 + Ag(NH_3)_2F \rightarrow Ag_3PO_4 + CaF_2 + NH_4OH$$

Calcium, phosphate, and fluoride ion concentration after SDF application are essential for the remineralization of the carious lesion.[45] $CaF_2$ act as a fluoride reservoir that releases fluoride when the pH is low and form fluorapatite. $Ag_3PO4$ is another major reaction product that reacts with alkaline chloride and forms AgCl. AgCl has low solubility that helps in the antimicrobial property of SDF. Also, AgCl plays a major role in surface hardening.[46]

Silver chloride is the main precipitant in treated dentin with SDF, as chloride is not a common component of dentin or silver diamine fluoride. It may come from the saliva. Thus, saliva seems to play a crucial role in the SDF caries remineralization progress. When comparing rates of carious lesion arrest in elderly patients with pediatric patients, elderly patients have lower rates of arrest. Elders tend to have less abundant and less functional saliva. Whereas, In the pediatric population, a higher rate of caries arrest was

noted for the buccal and lingual surfaces (surfaces mostly covered by saliva ) and anterior teeth.[30] Saliva has a buffering mechanism as it is saturated with calcium and phosphate ions which are important as they form part of the hydroxyapatite cell. In a study by Mei et al., it was demonstrated that SDF reacts with calcium and phosphate from the saliva to form fluorohydroxyapatite.[47]

A study by Suzuki et al.[48] reported that silver and fluoride ions could penetrate to a depth of approximately 25 microns into the enamel. Chu et al.[49] corroborated the penetration depth of silver ions being 50-200 microns into intact and healthy dentin. In a study by Li et al.[50] on extracted carious primary incisors, the authors reported a penetration depth of $744 \pm 448.9$ µm. This unanticipated 10-fold increase in penetration depth may be because the deep carious lesions reached the pulp, which showed that in the deep cavitated lesions, the silver precipitation could be observed in the pulp chamber, suggesting that the application of a highly concentrated SDF solution on deciduous teeth should be used with caution for various carious lesions.[51]

Effectiveness of a single SDF application ranges from 47% to 90%, depending on the size of the carious lesion and tooth location.[52] [53]Anterior teeth had higher carious lesion arrest rates than posterior teeth, as shown in a randomized clinical trial by Zhi et al.[54] and Fung et al.[38]. Crystal et al.[53] suggested that this may be due to the fact that the anterior teeth surfaces are exposed to natural light, which may lead to more active silver precipitation. This led practitioners to believe that light-curing SDF after its application can provide clinical evidence of carious lesion arrest after SDF application.

Silver ions are sensitive to light. Light curing oxidizes SDF, which precipitates the silver out of the solution. This theoretically results in fewer silver ions available to penetrate through the depth of the lesion. In an ex vivo study by Toopchi et al.[51], it was shown that dental light curing of SDF for 40 seconds after application would decrease the SDF penetration depth in sound dentin and increase hardness in the infected dentin as dental light curing causes increased precipitation of silver ions in the infected dentin layer of the carious lesion, which could improve the antibacterial effect.[55]

There are continuous attempts to achieve better clinical outcomes after SDF application. Specifically, the influence of light-curing on SDF. Based on Toopchi et al.'s [51] observations, this ex-vivo study aimed to compare the hardness of infected dentin, the penetration depth of SDF, and the silver ion precipitation at different depths of dentinal carious lesions of different light-cured SDF application techniques in primary anterior teeth in an environment similar to that of the human mouth.

## Hardness Test

The arrested carious lesion is mostly inactive microbiologically and hypermineralized to approximate sound dentin's hardness.[56] SDF application treatment is considered successful when the carious lesion is hard on probing.[36] Thus, the dentin hardness test can be considered an acceptable method to assess the mineral content of dentin.[57] [58] It is preferred to use micro-indentation methods as there is significant variation in the hardness of enamel and dentin.[59] Multiple studies showed that there is a direct relationship between microhardness of dentin and its mineral content.[60]

## Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) is a microscope that uses a focused beam of electrons instead of light to create high magnification, high-resolution images for analysis.[61] Knoll demonstrated the SEM principle in 1935 and 1939.[62] [63] Then, Von Ardenne developed SEM.[64] Charles Oatley in 1950s and 1960s continued developing the modern commercial SEM.[65]

Images produced by SEM result from interactions of the electron beam with atoms at various depths within the sample. The electron beam has an energy ranging from 0.2 keV to 40 keV that is focused by one or two condenser lenses. These images give information about the topography of the surface and its composition by producing very high-resolution images revealing details less than one nanometer.[61] [66]

## Energy-dispersive X-ray spectroscopy (EDX, EDS, or EDAX)

Energy dispersive X-ray spectroscopy (EDX, EDS, or EDAX) is an analytical technique that gives additional information about the sample during SEM analysis. EDX provides quantitative result by generating X-rays from the sample through electron beams to identify and characterize the elemental composition of a sample as well as their distribution and concentration.[67] If the sample emit low-energy X-rays, it can be increased by using higher beam currents, increasing imaging times, or increasing EDS detector solid angle.[68]

# AIMS/HYPOTHESIS

## Aims

The primary aims of this ex vivo study were to evaluate the effect of light-curing SDF after the first and/or second application in primary teeth on:

1- The **hardness of infected dentin**.
2- The **penetration depth of SDF**.

A secondary aim was to evaluate the **silver ion precipitation at different depths** of dentinal carious lesions.

## Hypothesis

Light curing SDF after the first and/or second application can enhance the chance of arresting dentinal carious lesions by improving dentin hardness, lessening silver ion penetration depth, and increasing silver ion precipitation in the outer layer.

## SIGNIFICANCE OF THE STUDY (LIGHT-CURING SDF)

Silver Diamine Fluoride ($Ag(NH_3)_2F$; SDF) has been used as an alternative treatment for caries prevention and arrest.[69] Several articles have recommended using a 38% concentration for the prevention and arrest of dental caries in children.[55] SDF's ability to arrest carious lesions is theorized to be due to the formation of silver phosphate precipitate to restore the mineral content, thus increasing the microhardness of the carious dentin surfaces. Moreover, the formation of calcium fluoride, from which the fluoride is available for remineralization.[70] [71] In vivo and ex vivo studies have shown the effectiveness of SDF semiannual applications on cavitated extracted teeth in arresting carious lesions.[70]

SDF solution is sensitive to light. Exposing SDF to light causes rapid oxidization of silver ions and precipitates silver out of the solution. Thus, the manufacturers do not recommend light-curing SDF due to the lack of research on its efficacy.[72]

Based on Crystal et al.'s observation, SDF is not recommended on teeth with exposed pulp and needs careful follow-up when applied to deep carious lesions.[32] This indicates the need to limit the penetration of SDF in deep carious lesions approaching the pulp. A study by Toopchi et al.[51] showed that adding an additional step of dental light curing SDF for forty seconds after application will decrease the SDF penetration depth in sound dentin. This could be beneficial in deep dentinal caries approaching the pulp. It was also shown that light-curing SDF increases the hardness of the infected dentin. Increasing dentin hardness indicates remineralization of carious dentinal lesions, caries arrest, and a further reduction in acid attacks and recurrent caries formation.[50]

Moreover, in an in vitro study by Lau et al., it was demonstrated that exposing 38% SDF to light cure did not affect the penetration depth of silver into the non-carious dentin of primary teeth with various surface treatment approaches.[73] In a pilot study by Wilson et al., SDF antimicrobial properties on oral microbes were evaluated with and without light curing and found no significant differences between these treatments.[74]

Based upon the limited evidence regarding light curing SDF, the results of this ex vivo study will aid in evolving SDF application protocols for this increasingly common dental practice to achieve the best clinical outcome for treating carious lesions in primary teeth by evaluating the effectiveness of different light-cured SDF application techniques.

# MATERIALS AND METHODS

## Study Design and Procedures

This ex vivo study was conducted in Gavel Laboratory at Tufts University School of Dental Medicine (TUSDM), Boston, MA, USA. Eighty extracted human primary maxillary anterior teeth were obtained according to human subjects' regulations in the pediatric dentistry department, Tufts University School of Dental Medicine, MA, USA. Teeth were collected in a de-identified manner with no way to link the teeth to the patient they came from. The collected teeth were wiped and cleansed of residual tissues attached to the tooth surface with a 2*2 piece of gauze immediately after extraction and stored in a container of saline solution which was kept in a secure area within the pediatric department for extracted and exfoliated teeth to be collected during the day. An investigator picked them up daily and transported them within 24 hours for storage and the disinfection process at 4° Celsius in the Gavel Lab. All extracted teeth were stored in a 10% aqueous bleach solution for five days, then in an artificial saliva solution (KH2PO4+NaN3+KCL+CaCl2+MgCl2) before the start of the study. A protocol for this study was adopted from that conducted by Toopchi et al.[51], 2021, who evaluated the effect of light-curing on SDF.

## Inclusion criteria

1- Extracted primary maxillary anterior teeth.
2- Extracted due to dental caries into the dentin (cavitated) and diagnosed as code five, according to the ICDAS II (the International Caries Detection and Assessment System) caries diagnosis criteria.[75]

**Exclusion criteria**

1. Teeth with extensive dental caries extending into pulp clinically, corresponding to code six, according to the ICDAS II. [75]
2. Teeth with previous restorations.
3. Teeth with previous SDF applications.
4. Teeth with developmental anomalies or dentinal defects (e.g., amelogenesis/ dentinogenesis imperfacta, dentin hypoplasia, etc.).

**Sample Size Calculation**

A sample size calculation was conducted using the software nQuery Advisor v. 7.0 (Statistical Solutions Ltd., Cork, Ireland). The calculation was performed for the primary outcome of the study, which was Vickers hardness. Based on the results of Toopchi et al.[51] , the mean hardness for Group 1 was assumed to be 558.07 kgf/mm$^2$, and the mean hardness for Group 4 was assumed to be 702.26 kgf/mm$^2$. The mean hardness for Groups 2 and 3 was assumed to be the average of the means for Group 1 and Group 4. Furthermore, the within-group standard deviation was assumed to be 132.46 kgf/mm$^2$ based on the results of Toopchi et al. Under these assumptions, a sample size of n=20 per group was adequate to obtain a power of 80% in conjunction with a Type I error rate of 0.05.

### Randomization

A total of 80 extracted teeth that meet the inclusion criteria were numbered (Figure 1) and then randomly divided into four groups of 20 teeth using the “sample” function of R 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria) (Figure 2). This randomization was done to minimize potential allocation bias and ensure that each sample had an equal chance of being assigned to any group.

### Silver Diamine Fluoride Application

Before 38 % SDF application (38% Ag [NH3]2F; Advantage Arrest ™, Elevate Oral Care, West Palm Beach, FL, USA) (Figure 3), teeth were dried with a gentle flow of compressed air for one minute. 38% SDF was dispensed into a disposable dental plastic dappen dish, followed by one minute of SDF application to the carious lesion using a disposable micro brush applicator (Henry Schein, Inc., Melville, NY, USA) that was saturated with the solution. It was then dried with a gentle flow of compressed air. The excess SDF was removed with a cotton pellet. The SDF was allowed three minutes to dry after being applied without rinsing with water. The second application was made after two weeks for all groups. A guideline workgroup formed by the American Academy of Pediatric Dentistry recommended a follow-up at two to four weeks after the initial treatment to check the arrest of the lesions treated. Reapplication of SDF may be indicated if the treated lesions do not appear arrested.[32]

Each group was further prepared as follows:

Group 1 (Control group):

***1st application:*** 38% SDF application only (Figure 4), and teeth were placed in an artificial saliva solution ($KH_2PO_4+NaN_3+KCL+CaCl_2+MgCl_2$) after 3 minutes of soaking time.

***2nd application (after two weeks):*** A second application of 38% SDF was made, and teeth were placed in an artificial saliva solution after 3 minutes of soaking.

Group 2 (Test Group 1):

***1st application:*** 38 % SDF application only, and teeth were placed in an artificial saliva solution after 3 minutes of soaking.

***2nd application (after two weeks):*** 38% SDF was applied for 1 minute in addition to the use of dental light-curing (Bluephase, Ivoclar Vivadent, N.Y., USA) (Figures 5 and 6) for 40 seconds after 3 minutes of SDF application.

Group 3 (Test Group 2):

***1st application:*** 38% SDF was applied for 1 minute in addition to using dental light-curing for 40 seconds after 3 minutes of SDF application.

***2nd application (after two weeks):*** 38% SDF application only, and teeth were placed in an artificial saliva solution after 3 minutes of soaking.

Group 4 (Test Group 3):

***1st application:*** 38% SDF was applied for 1 minute in addition to using dental light-curing for 40 seconds after 3 minutes of SDF application.

***2nd application (after two weeks):*** 38% SDF was applied for 1 minute in addition to using dental light-curing for 40 seconds after 3 minutes of SDF application.

During the entire study period, the teeth were stored in artificial saliva made in the lab ($KH_2PO_4+NaN_3+ KCL+CaCl_2+MgCl_2$) (Figure 7). They were placed in an incubator (Thermo Fisher Scientific $CO_2$ incubator, Waltham, Mass., USA) at 37˚C to resemble the oral environment (Figure 8). The storage solution was changed weekly to minimize contamination.

**Specimen preparation and manipulation**

Two weeks after the second application[32 51], each tooth was prepared as follows: A large plastic mold was positioned at the top of the table with the cap at the bottom to hold the self-curing acrylic resin material (Ortho-Jet™ for Orthodontic Appliances) (Figure 9), which was poured into the mold until desired coverage was obtained. The tooth was pressed into the resin with the apical surface facing down until it reached the mold's lid, and the mounted samples were removed as soon as the resin had been set (Figures 10,11,12 and 13). Samples were sliced vertically with a thickness of 2 mm mesiodistally (for proximal caries) or bucco-lingually (for facial caries) into two halves, depending on the location of the lesions by slow-speed saw with a water-cooled diamond blade IsoMet 1000 (Buehler, Lake Bluff, IL., USA) (Figure 14 and 15).

**Blinding**

Samples were then re-randomized so that the investigator in charge of recording outcome data of the treatment was blinded to avoid bias, but not initially while preparing the samples (Figures 16, 17, and 18).

**Test for Hardness**

The Vickers hardness test was performed using a microhardness indenter machine (MicroMet 2104, Buehler, Lake Bluff, IL, USA) (Figure 19) under a load of 50 gf for 15 s for each specimen[76] for the infected dentin layer (layer one). Microhardness in Vicker's number (HV) was determined at three successive sites below the tooth's surface from the center of the carious lesion toward the pulp. [49] The measurements were calculated based on the following formula:

$$HV=0.102\ F/S=0.102\ (2F\sin 0\ /2)/d^2=0.1891\ F/d^2$$

Vickers hardness is determined by dividing the force F in Newtons (N) by contact area S ($mm^2$) between the sample and an indenter (a diamond square pyramid), which is calculated by diagonal length d (mm), the mean of two-directional lengths of the indenter, into the specimen by using the same amount of force F in Newtons (N) for all samples. The mean of the three measurements was considered the microhardness of that surface. Indentations were measured using the DiaMet™ hardness testing software to ensure that the indentation on a softer tissue, such as carious dentin, is measured reliably

and accurately. Measurements were taken for all samples under 40× magnification so that the margins of the indent were visible.[77]

### Test for Depth of SDF Penetration

Depth of SDF penetration was measured using an Olympus SZX 16 (Olympus, Tokyo, Japan) stereomicroscope with a digital camera (Ueye 3.90, Obersulm, district of Heilbronn, Germany) at 0.8× magnification. Images were captured and analyzed using OmniMet software (image capture and analysis solution, Buehler) (Figures 20 and 21).

Depth of penetration measurement was taken from the base of the affected dentin (layer two) to the deepest point in sound dentin (layer three). The penetration depth was measured using OmniMet software. Multiple measurements were taken 2 mm apart along the base of the lesion. The three highest measurements were considered the most accurate. The mean of these three measurements was calculated for each sample and compared among the four groups.

**Scanning electron microscope (SEM) and Energy dispersive X-ray spectroscopy (EDX, EDS, or EDAX)**

For qualitative SEM evaluation, a specimen was selected from each group. Before the examination, specimens were completely dried and sputter coated with gold/palladium alloy at 50: 50 ratios to make the samples electronically conductive. A 15 keV operating voltage was selected.

Prepared samples were analyzed by Scanning Electron Microscope (SEM, Amray 3300, North Billerica, Mass.). SEM was recorded by the IXFR system to perform chemical analysis using EDS (Energy Dispersive Spectroscopy) to detect carbon (C), phosphorous (P), calcium (Ca), oxygen (O), silver (Ag), and fluoride ($F^-$) ion levels. The elemental analysis was performed in three areas for each specimen:

- The center of the infected dentin (area one)
- The center of the affected dentin (area two)
- The center of sound dentin (area three).

Readings were expressed as a relative percentage of the weight of the detected element as part of the total weight of the tested area of the sample.

**Statistical Analysis**

Data were analyzed for each group using descriptive statistics (means, medians, standard deviations, interquartile ranges, minima, and maxima). The data's normality and homogeneity of variance were assessed via the Shapiro-Wilk test and Levene's test, respectively.

The Shapiro-Wilk test showed no significant evidence of non-normality ($p > .05$). However, Levene's test showed significant evidence that the assumption of homogeneity of variances was violated ($p < .001$). Since only the assumption of normality was satisfied, statistical significance was evaluated via Welch's ANOVA with post-hoc comparisons performed using the Games-Howell test.

The significance level was set at $\alpha = 0.05$. The statistical software SPSS v. 26 (IBM Corp., Armonk, NY, USA) was used in the analysis.

# RESULTS

## Dentin Hardness

Descriptive statistics for each group (means and standard deviations of Vickers hardness) are presented in Table 1.

Test groups mean dentin hardness were higher than that of the control group. The highest mean of dentin hardness (± standard deviations) was in test group 3 (80.0 ± 8.7 kgf/ $mm^2$ "Kilogram-force per square millimeter "), followed by test group 2 (55.4 ± 6.6 kgf/ $mm^2$), followed by test group 1 (39.6 ±5.5 kgf/ $mm^2$), and then the control group (23.7 ± 2.2 kgf/ $mm^2$).

The comparison between the groups using Welch's ANOVA test determined that the difference in mean value was statistically significant when comparing the readings for all groups ($p < .001$). In post-hoc comparisons via the Games-Howell test, significant differences were found between the control group and test group 1 ($p < .001$), control group and test group 2 ($p < .001$), control group and test group 3 ($p < .001$), test group 1 and test group 2 ($p < .001$), test group 1 and test group 3 ($p < .001$), test group 2 and test group 3 ($p < .001$). Side-by-side boxplots are presented in (Figure 22).

**Depth of SDF Penetration**

On the contrary, for all samples, the highest mean penetration depth of SDF (± standard deviations) was recorded for the control group, followed by test group 1, then test group 2, and then test group 3 (Table 2).

The mean penetration depth of SDF beyond the affected dentin (± standard deviations) for the control group (225.5 ± 39.6 µm) was higher than test group 1 (168.3 ± 27.8 µm), test group 2 (96.5 ± 6.6 µm), and test group 3 (54.0 ± 11.9 µm), respectively. It is important to mention that SDF penetrated all three layers (infected, affected, and sound dentin) in all samples.

The comparison of groups via Welch's ANOVA test showed statistically significant differences ($p < .001$). Further, post-hoc comparisons via the Games-Howell test showed significant differences between the control group and test group 1 ($p < .001$), control group and test group 2 ($p < .001$), control group and test group 3 ($p < .001$), test group 1 and test group 2 ($p < .001$), test group 1 and test group 3 ($p < .001$), test group 2 and test group 3 ($p < .001$). Side-by-side boxplots are presented in (Figure 23).

**Energy dispersive X-ray spectroscopy (EDX, EDS, or EDAX) Elemental Analysis**

SEM images of the samples from the four groups are displayed in (Figures 24 to 46). The mineral content analysis via EDS for all four groups is presented in (Table 3).

EDS analysis was done in three areas for each specimen:

- The center of the infected dentin (area one)
- The center of the affected dentin (area two)
- The center of the sound dentin (area three)

EDS analysis showed that oxygen, calcium, and phosphorus were the main elements detected in all three areas (center of infected, affected, and sound dentin) for each specimen in all groups (Table 3). The minor elements detected in the three areas were sodium, carbon, silver, fluoride, magnesium, aluminum, chlorine, silicon, and nitrogen.

Silver weight percentage for areas 1-3 (center of infected, affected, and sound dentin) in all groups ranged between 0.8 % - 2 %. Further, fluoride ion weight percentage ranged between 0.3 % - 0.9 % for the three areas in all groups.

# DISCUSSION

In this ex vivo study, human primary incisor teeth were used to evaluate the effect of light-curing SDF after first and/or second application on the hardness of infected dentin, the penetration depth of SDF, and silver ion precipitation at different depths of dentinal carious lesions. Based on this study's results, the null hypothesis was rejected since statistically significant differences were observed between different light-curing SDF application techniques, which can enhance the chance of arresting dentinal carious lesions by increasing dentin hardness and decreasing silver ion penetration depth. To the authors' knowledge, this is the first study to assess different light-curing SDF application techniques in primary teeth with a natural carious lesion. This study added value to the limited database on the effects of light-curing SDF.

In clinical settings, to verify if the carious lesion is arrested after SDF application, tactile hardness assessment of the carious lesion is used[77 78 54]. Based on the evidence-based clinical practice guidelines on non-restorative treatments for carious lesions published by the American Dental Association (ADA), the carious lesion is considered arrested when it turns hard to clinical tactile probing. [79] Thus, the microhardness test can be considered an indication of the remineralization and caries arrest effect of different SDF application techniques.

In a clinical trial by Chu et al., it was shown that regular application of 38% SDF for 30 months increased microhardness in the outermost surface of the arrested dentinal carious lesion compared to the active carious lesion.[49] Seto et al. reported that the increased dentin hardness after 38% SDF application is due to silver microwires. [80]

The dentin microhardness of arrested carious lesions has been studied in multiple studies, ranging from 25 to 65 KHN (245 to 638 MPa).[49] However, at a distance of 225 µm or more from the lesion surface, arrested and soft dentinal carious lesions have similar microhardness values.[49] Thus, the microhardness test in this study was performed on the infected dentin.

Since different preparation methods (some studies suggested the use of 100% ethanol to minimize dentin specimens' shrinkage due to drying. Others have suggested that dentin specimens be examined directly without ethanol dehydration[49]) and varied amounts of the load of the microhardness test between the studies (1.5 gf – 500 gf) have been used [81] [82], the results of microhardness from these studies cannot be directly compared. Hardness values of sound dentin ranged from 50 to 70 KNH depending on the location and mineralization of intertubular dentin.[83] In a study by Soekanto et al.[76], the mean Vickers hardness number of sound dentin was 64.8 ± 8.2, and demineralized dentin was 10.3 ± 2.3. Toopchi et al. reported that light curing SDF increased the hardness of infected dentin to 702.3 ± 144.6 kgf/mm$^2$ compared to SDF application only, which was 558.1 ± 119.1 kgf/mm$^2$. Likewise, in this study, the highest mean of dentin hardness was with light-curing SDF after the first and second application (test group 3) (80.0 ± 8.7 kgf/ mm$^2$), followed by light-curing SDF after the first application only (test group 2) (55.4 ± 6.6 kgf/ mm$^2$), then by light-curing SDF after second application only (test group 1) (39.6 ±5.5 kgf/ mm$^2$), and lastly with SDF applications only (control group) (23.7 ± 2.2 kgf/ mm$^2$).

It is important to note that all values of dentin hardness in the present study were higher than that of demineralized dentin reported in the Soekanto et al. study.[76] Also, some Vickers harness numbers ( test groups 2 and 3) reported in this study were in the range of sound dentin.[76 83]

The microhardness results of the present study showed that light-curing SDF increases the microhardness significantly. The possible reason could be that light curing SDF increases silver precipitation in the dentinal carious lesion outermost layer (infected dentin) as it allows a shorter time for the penetration of SDF.[51] This might also explain the increase in microhardness and decreased penetration depth when light-curing SDF is used after the first application only compared to after the second application. The increase in silver precipitation in the carious lesion outermost layer after light curing SDF in the first application prevents further silver penetration in the second application, resulting in more precipitation of the silver ions in the outermost layer.

Light curing SDF allows a rapid reduction process and shorter penetration time for silver ions.[84] Results of the depth of penetration of SDF in the present study were in general agreement with the outcomes of that reported in Toopchi et al.[51] study, which ranged from (80 to 180 µm) for the SDF application only and (50 to 70 µm) for the light cured SDF group.

In this study, the depth of penetration of SDF was the highest for SDF applications only (control group) ($225.5 \pm 39.6$ µm), followed by light-curing SDF after the second application only (test group 1) ($168.3 \pm 27.8$ µm), then light-curing SDF after the first

application only (test group 2) (96.5 ± 6.6 µm), and then light-curing SDF after the first and second application (test group 3) (54.0 ± 11.9 µm).

Results of penetration depth of SDF varied between studies due to the differences in teeth selected, depth of the carious lesion, and specimen preparation.[49] Studies by Yamaga et al. [85]and Willershausen et al. [86] reported a depth of 20–100 µm silver penetration into SDF-treated dentin. Chu and Lo reported an SDF penetration depth of 25–200 µm.[49] Li et al.[50] reported increased silver ion deposit in the innermost demineralized lesion to an average penetration depth of 744 µm and concluded that SDF penetration might be affected by the amount of lesion demineralization.

The authors acknowledge that a limitation of the present ex vivo study is that the lack of movement of the dentinal fluid under pulpal pressure might affect the depth of penetration measurements clinically.

In addition to determining the effect of different light-curing SDF applications technique on the hardness of infected dentin and the penetration depth of SDF. A secondary aim of this study was to measure silver ion precipitation at different depths of dentinal carious lesions. The mineral content analysis via EDS investigated the inorganic composition of SDF-treated dentinal carious lesions. It showed that oxygen, calcium, and phosphorus were the main elements detected in all three areas (center of infected, affected, and sound dentin) for each specimen in all groups. Moreover, the minor elements detected in the three areas were sodium, carbon, silver, fluoride, magnesium, aluminum, chlorine, silicon, and nitrogen.

Yamaga et al. suggested that SDF form silver phosphate, calcium fluoride, and fluorapatite on treated lesions, and silver phosphate causes black staining on treated lesions.[85] Likewise, Mei et al. reported that the reason for dark staining after SDF application is silver compounds.[35] Presence of oxygen, phosphorus, sulfur, and chlorine in SDF-treated carious lesions incorporate silver and form silver phosphate, silver oxide, and silver sulfide that turn silver ions to metallic silver nanoparticles after light exposure contributing to the dark staining after SDF application.[50]

In the present study, the authors detected a low level of silver (≤ 2% ) and fluoride (≤ 0.9%) in the center of infected, affected, and sound dentin layers between study groups. The findings of this study were consistent with UCSF protocol for caries arrest using SDF, which states that silver and fluoride ions may penetrate up to 50–200 microns into dentin.[30] In a study by Willershausen et al., it was reported that silver deposits on the covering dentin layer were (1.7%) and could be found in the dentinal tubules to a depth of 20 μm but diminished after 40μm.[86] This also explains Chu's study, which showed that the fluoride remineralization effect helps to increase dentin hardness in deciduous teeth only to a certain depth, beyond which it is insignificant.[49]

Toopchi et al. reported a higher percentage of silver in the infected dentin layer only (outermost layer), which was (24.6 ±14.7 %) in the light-cured SDF samples compared to (9.3 ± 3.5) in the non-light cured SDF group. However, silver ion precipitation at the affected dentin and sound dentin measured depths did not differ between the two groups and was (≤ 10.3 %) for the affected dentin and (≤ 3.5 %) for sound dentin.[51] The findings of this study and Toopchi et al. study might indicate that light-curing SDF did not have

significant penetration into the deeper dentinal carious layers beyond the surface layer of the infected dentin. This explains McDonald et al. study, which suggested the limited potential of SDF to penetrate deep carious lesions and showed no observable difference in the percentage of silver ions detected between light and non-light-cured samples.[87]

The authors acknowledge that a limitation of the present study is that it did not analyze the mineral content of the specimen's surface, as it was not possible due to the natural carious lesion surface roughness that would affect the accuracy of the results. As for specimens with rough surfaces, the accuracy may be as bad as 50%.[88]

The presence of a low level of silver ion in sound dentin in all groups regardless of SDF application technique and the potential pulpal injury associated with SDF treatment is not fully understood and needs further investigation. Englader et al. showed the presence of silver in the pulp after SDF application and reported an adverse pulpal reaction.[89] However, in a study by Gotjamanos, the presence of silver in the pulp of deciduous teeth treated with SDF did not show any adverse pulpal reaction.[90] Although no severe pulpal reaction after SDF application has been reported, a guideline by the American Academy of Pediatric Dentistry (AAPD) regarding the application of 38% SDF in primary teeth does not recommend SDF application on exposed pulp and advises careful follow-up when SDF is applied to the deep dentinal carious lesion.[32] A systematic review by Zaeneldin et al. concluded that direct SDF application causes pulp necrosis. In contrast, indirect SDF application is generally biocompatible with the dental pulp tissue with a mild inflammatory response.[91]Further long-term studies are needed to investigate

the effect of this low silver level on the pulp of teeth with deep carious lesions and if it could irritate the pulp and cause an adverse pulpal reaction.

The findings of this study provide new evidence for the increasingly common practice of light-curing SDF application. It concluded that light-curing SDF in the first and second applications resulted in the highest dentin hardness, followed by light-curing SDF after the first application only, then by light-curing SDF after the second application only, and lastly, without light-curing SDF. Conversely, the highest penetration depth was without light-curing SDF, followed by light-curing SDF after the second application only, then by light-curing SDF after the first application only, and lastly least depth of penetration was with light-curing SDF after both the first and second applications. It is also important to note that SDF penetrated all three layers (infected, affected, and sound dentin) in all samples. However, a low level of silver percentage was detected beyond the surface in all groups.

Since SDF use in pediatric dentistry is favored for its ease of application, the addition of light curing might increase the chair time for uncooperative patients.[87] Thus, the results of this study suggest that in the case of uncooperative patient light curing SDF after the first or second visit only might be better than SDF application only. However, the outcomes of this study cannot be extrapolated straight to the clinical setting, and caution is recommended when interpreting the results.

Some limitations of this ex vivo study are that clinically the presence of factors such as pulpal response ( intrapulpal pressure and pulpal reparative dentin formation), apposition

of calcified tissue within the dentin, diet, and oral microbiome might cause some differences that could influence the effect of SDF. However, using artificial saliva as a storage medium at 37°C helped simulate the oral environment. Moreover, using 10% bleach as a disinfectant agent for five days could affect the accuracy of the result by influencing the effect of SDF on the carious dentinal lesion.

More studies are needed to analyze the mineral content of the dentinal carious lesion treated with different light-cured SDF. Also, comparisons between different light-curing devices and curing time might affect the effectiveness of light-cured SDF. Moreover, future clinical studies with long-term follow-ups are needed to investigate the clinical relevance of the effect of different light-cured SDF application techniques.

# CONCLUSION

Within the limitations of this ex vivo study, we concluded that:

1- Light curing silver diamine fluoride (SDF) after the first and second applications showed a significant increase in the hardness of infected dentin, which could indicate higher caries arrest effect than the other SDF application techniques mentioned in this study.

2- Conversely, light curing SDF after the first and second applications showed significantly less SDF penetration into sound dentin, which might be due to the higher silver precipitation on the surface.

3- Light curing SDF after the first or second visit showed a significant increase in the hardness of infected dentin and decreased SDF penetration depth compared to SDF application only.

**APPENDIX A**

**Table 1: Vickers Hardness (kgf/$mm^2$) (N=20 per group)**

| Group | Mean | SD* | p** |
|---|---|---|---|
| **Control Group** | 23.7 | 2.2 | <.001 |
| **Test Group 1** | 39.6 | 5.5 | |
| **Test Group 2** | 55.4 | 6.6 | |
| **Test Group 3** | 80.0 | 8.7 | |

* Standard deviation

** Welch's ANOVA. In post-hoc comparisons via the Games-Howell test, significant differences were found between the control group and test group 1 ($p < .001$), control group and test group 2 ($p < .001$), control group and test group 3 ($p < .001$), test group 1 and test group 2 ($p < .001$), test group 1 and test group 3 ($p < .001$), test group 2 and test group 3 ($p < .001$).

**Table 2: Penetration Depth (μm) of Silver Diamine Fluoride (SDF) Into Dentin (N=20 per group)**

| Group | Mean | SD* | p** |
|---|---|---|---|
| **Control Group** | 225.5 | 39.6 | <.001 |
| **Test Group 1** | 168.3 | 27.8 | |
| **Test Group 2** | 96.5 | 6.6 | |
| **Test Group 3** | 54.0 | 11.9 | |

* Standard deviation

** Welch's ANOVA. In post-hoc comparisons via the Games-Howell test, significant differences were found between the control group and test group 1 ($p < .001$), control group and test group 2 ($p < .001$), control group and test group 3 ($p < .001$), test group 1 and test group 2 ($p < .001$), test group 1 and test group 3 ($p < .001$), test group 2 and test group 3 ($p < .001$).

**Table 3: Mineral Content Analysis Via Energy Dispersive X-ray Spectroscopy (EDS) in the Center of Three Different Dentinal Layers for a sample from each Test Group (Wt.%)**

| | Concentration (Wt.%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control group | | | Test Group 1 | | | Test Group 2 | | | Test Group 3 | | |
| **Element** | Infected dentin | Affected dentin | Sound dentin | Infected dentin | Affected dentin | Sound dentin | Infected dentin | Affected dentin | Sound dentin | Infected dentin | Affected dentin | Sound dentin |
| **O** | 37.2 | 42.8 | 41.1 | 41.1 | 41.9 | 41.6 | 39.9 | 40.7 | 41.0 | 40.5 | 40.5 | 40.0 |
| **Ca** | 33.9 | 32.0 | 31.4 | 30.1 | 30.8 | 30.4 | 31.2 | 30.8 | 30 | 29.4 | 29.7 | 29.5 |
| **P** | 18.3 | 15.3 | 17.7 | 16.6 | 16.6 | 16.6 | 17.2 | 16.9 | 16.6 | 16.4 | 16.7 | 16.6 |
| **Na** | 0.5 | 0.4 | 0.4 | 0.5 | 0.4 | 0.3 | 0.5 | 0.4 | 0.3 | 0.4 | 0.4 | 0.4 |
| **C** | 6.2 | 6.0 | 6.3 | 4.0 | 3.3 | 3.9 | 3.2 | 3.5 | 4.0 | 4.8 | 4.4 | 4.8 |
| **Ag** | 2 | 1.6 | 1.2 | 1.0 | 1.0 | 0.8 | 1.6 | 1.3 | 1.2 | 1.1 | 1.3 | 1.4 |
| **$F^-$** | 0.8 | 0.6 | 0.4 | 0.3 | 0.5 | 0.3 | 0.9 | 0.7 | 0.7 | 0.4 | 0.5 | 0.4 |
| **Mg** | 0.6 | 0.9 | 1.1 | 0.7 | 0.7 | 0.9 | 0.5 | 0.7 | 0.7 | 0.6 | 0.7 | 0.7 |
| **Al** | 0.2 | 0.2 | 0.3 | 0.3 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **Cl** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0 |
| **Si** | 0.1 | 0.1 | 0.1 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **N** | 0 | 0 | 0 | 5.2 | 4.4 | 4.8 | 4.6 | 4.7 | 5.3 | 6.1 | 5.7 | 5.9 |

# APPENDIX B

*Figure 1:The collected sample of primary maxillary teeth*

*Figure 2: Sample randomization using the "sample" function of R 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).*

*Figure 3: 38% Silver Diamine Fluoride (Elevate Oral Care, West Palm Beach, FL, USA).*

*Figure 4: Example of the application of 38% SDF only.*

*Figure 5: LED light-cure unit (Bluephase, Ivoclar Vivadent, N.Y., USA).*

*Figure 6: Example of light curing 38% SDF after application.*

*Figure 7: Teeth were stored in artificial saliva made in the lab ($KH_2PO_4$+$NaN_3$+ KCL+$CaCl_2$+$MgCl_2$).*

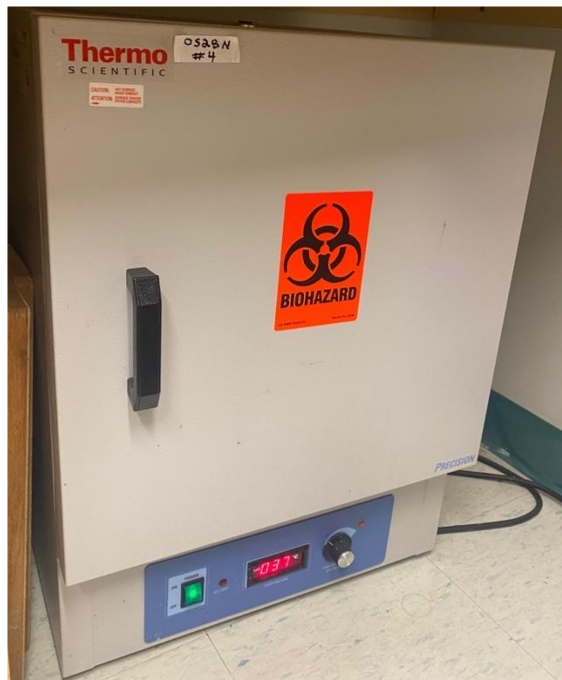



*Figure 8: teeth were placed in an incubator (Thermo Fisher Scientific $CO_2$ incubator, Waltham, Mass., USA) at 37˚C to resemble the oral environment.*

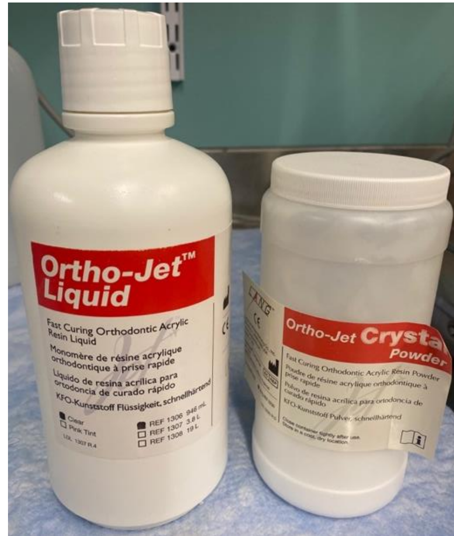
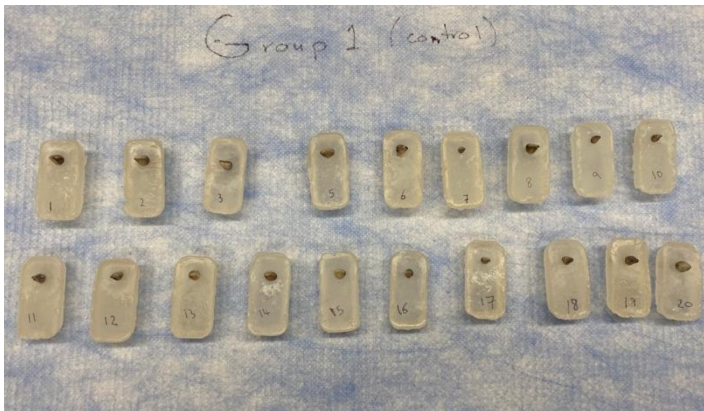
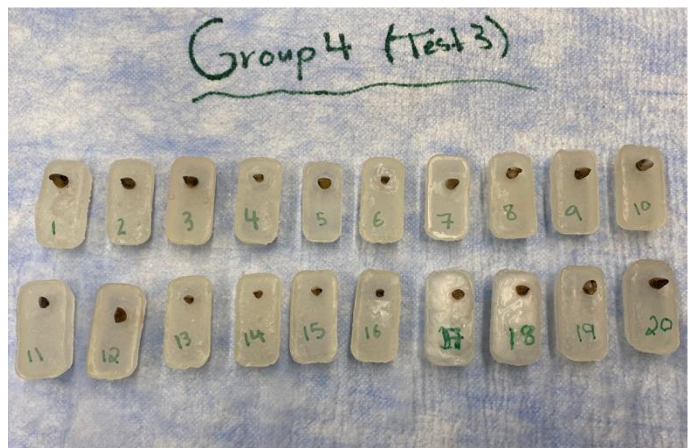
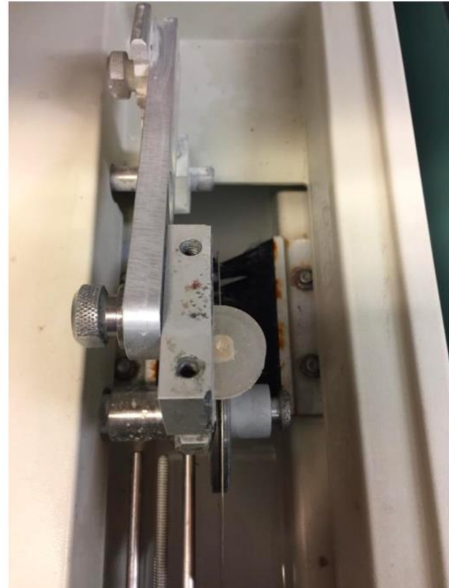



*Figure 9: self-curing acrylic powder/liquid resin material (Ortho-Jet™ for Orthodontic Appliances).*

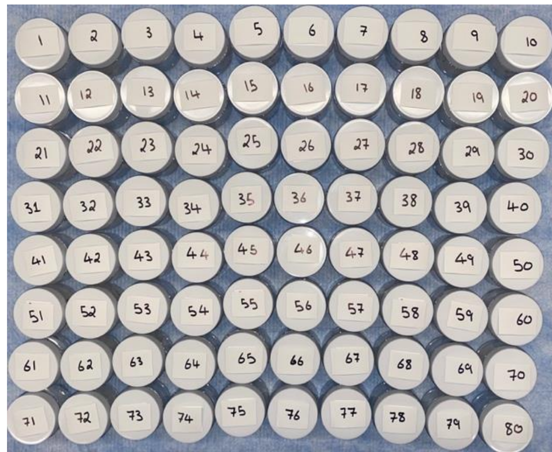


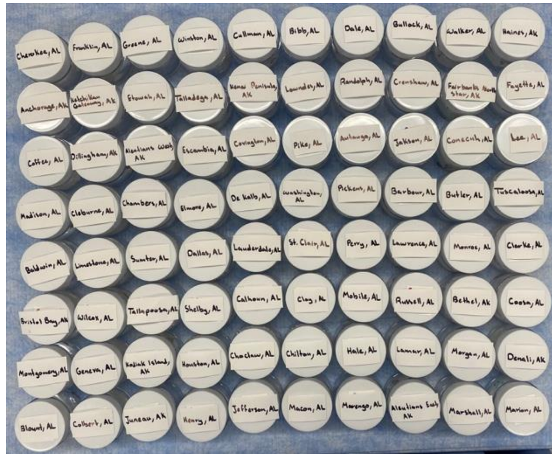



*Figures 10, 11, 12, and 13: Mounted group samples in acrylic.*



*Figures 14, and 15: IsoMet 1000 saw (Buehler, Lake Bluff, IL, USA) used to split samples.*




*Figures 16, 17, and 18: Samples were re-randomized by giving group samples numbers from 1- 80. Then using the "sample" function of R 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria), each number was assigned to a different city name to de-identify sample.*

*Figure 19: A microhardness indenter machine (MicroMet 2104, Buehler, Lake Bluff, IL, USA) used to measure dentin hardness (VHN).*

*Figure 20: Olympus SZX 16 (Olympus, Tokyo, Japan) stereomicroscope with a digital camera (Ueye 3.90, Obersulm, district of Heilbronn, Germany).*

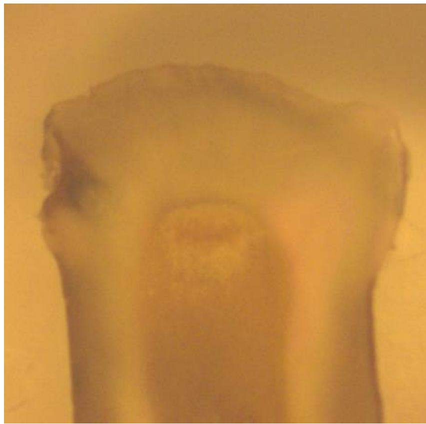


*Figure 21: Each tooth sectioned was inspected under the Stereomicroscope.*

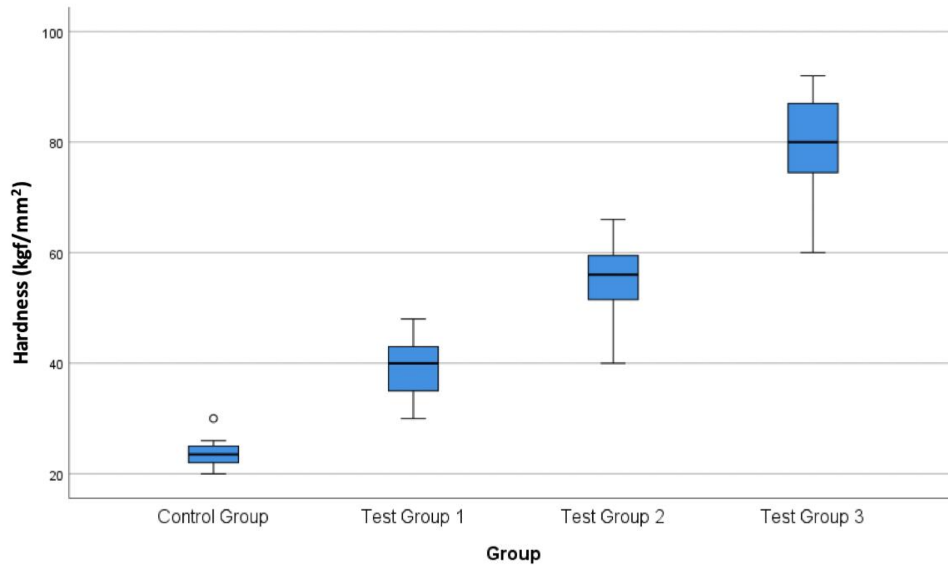



*Figure 22: Side-by-side boxplots comparing test groups' hardness (kgf/mm$^2$)*

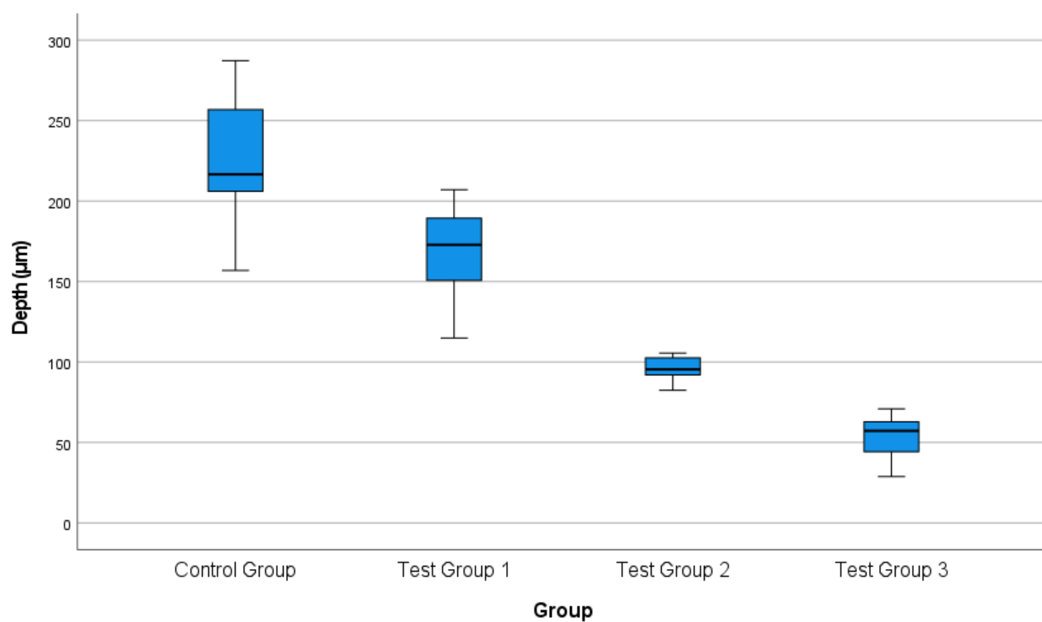
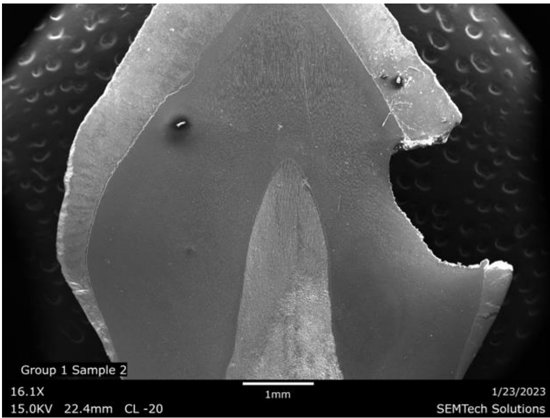
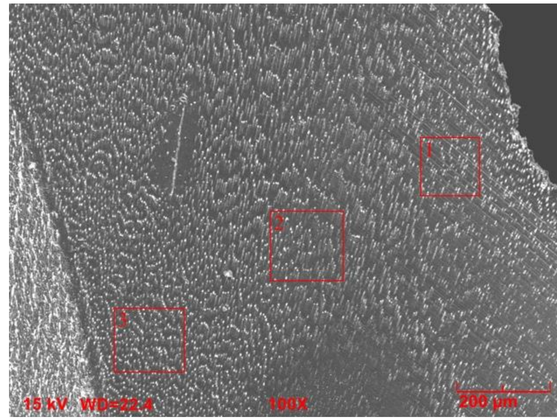
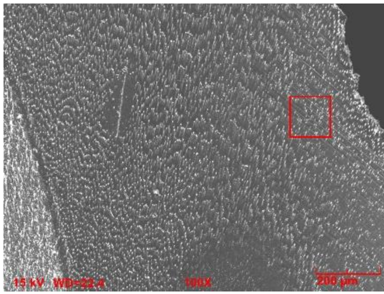
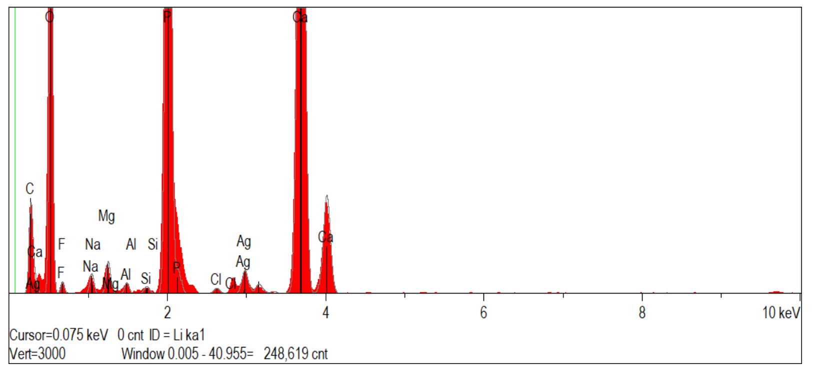


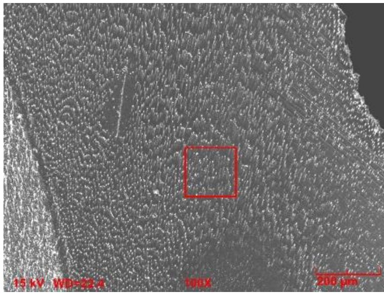

*Figure 23: Side-by-side boxplots comparing the depth of silver diamine fluoride (SDF) penetration (µm)*

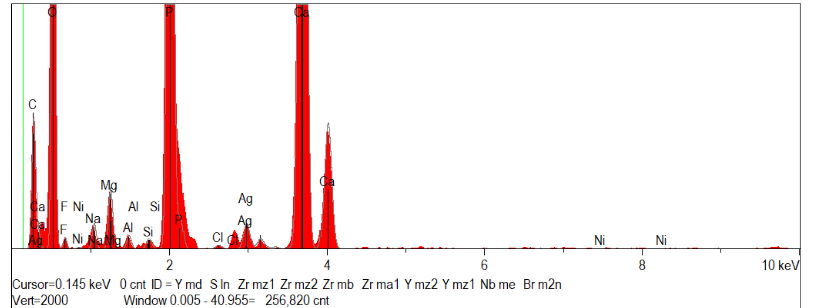


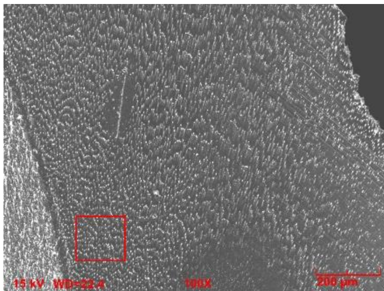



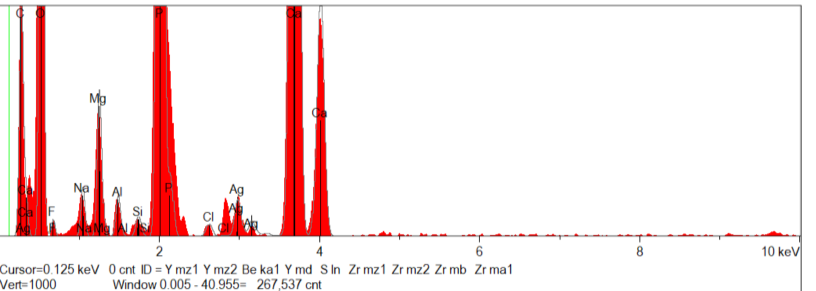





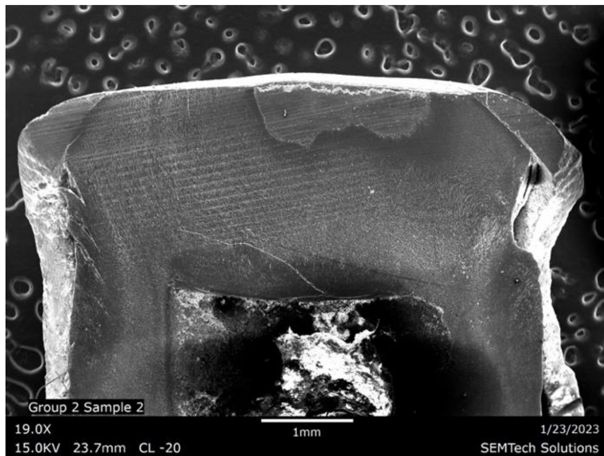



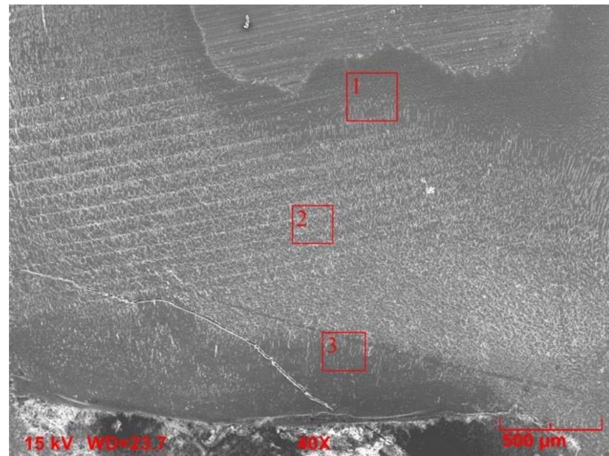


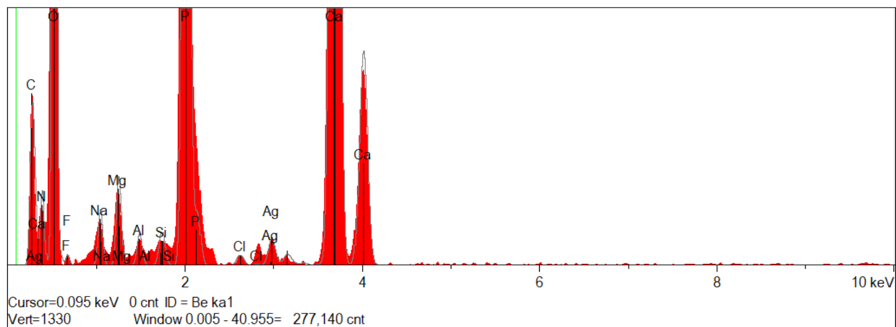



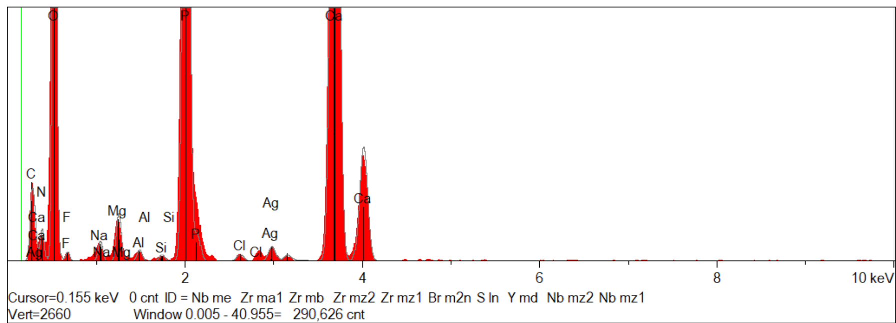


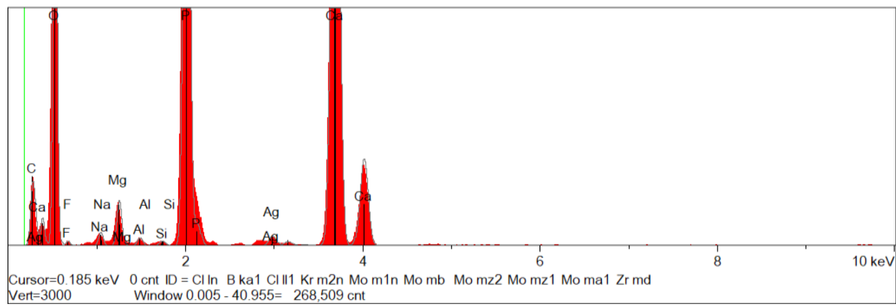

*Figures 24, 25, 26, 27, 28, 29,30 ,and 31 shows SEM images of a sample from the Control (Group 1) and energy dispersive X-ray spectroscopy (EDS) analysis for the center of three layers (infected, affected, and sound) of the treated lesion.*



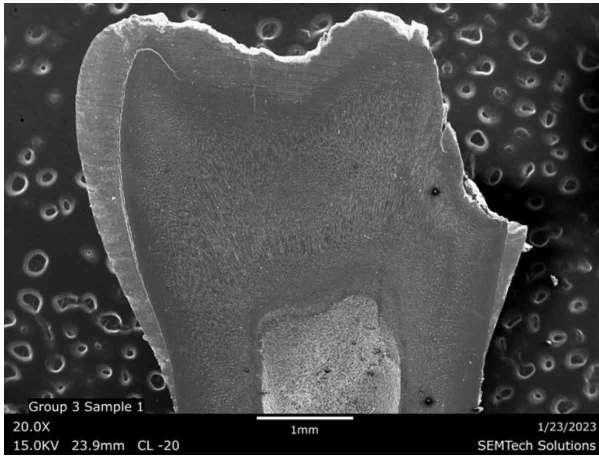



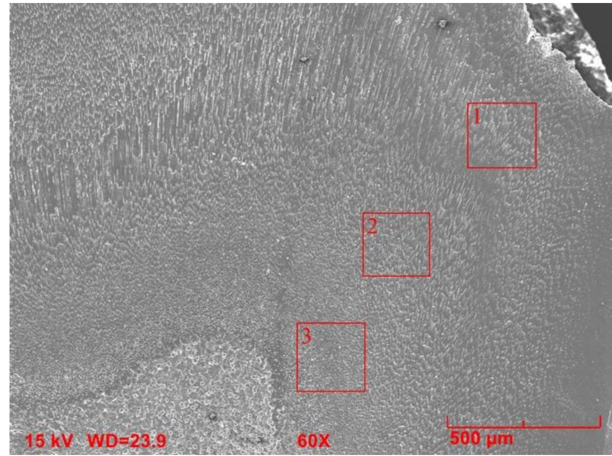



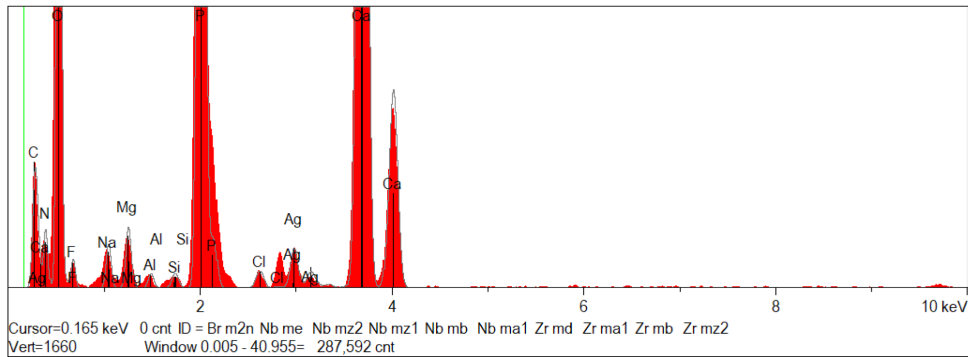



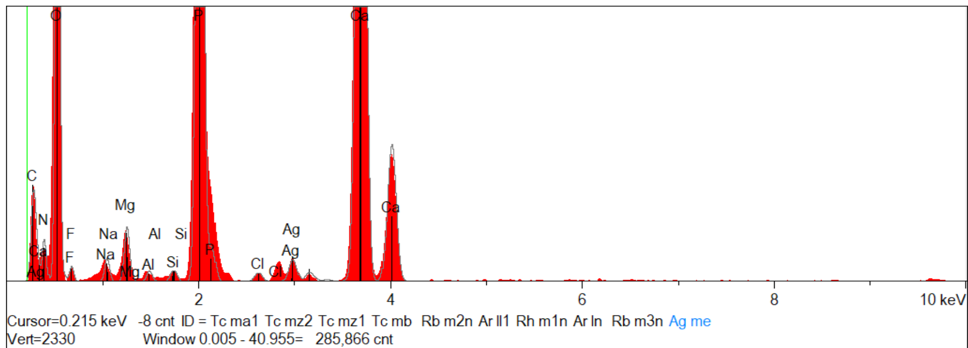



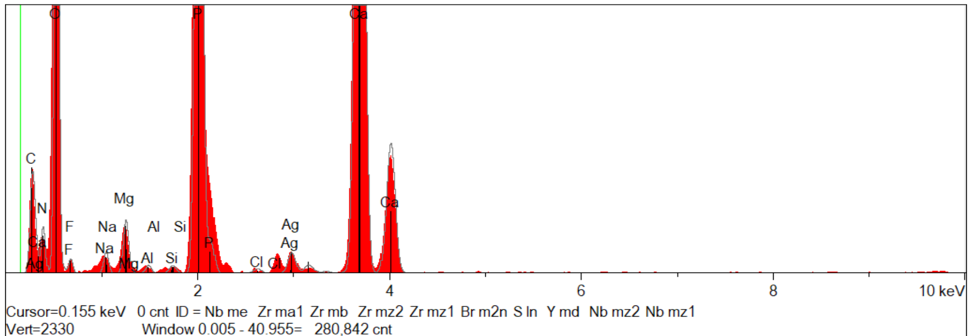

*Figures 32, 33, 34, 35 ,and 36 : shows SEM images of a sample from the test 1 (Group 2) and energy dispersive X-ray spectroscopy (EDS) analysis for the center of three layers (infected, affected, and sound) of the treated lesion.*



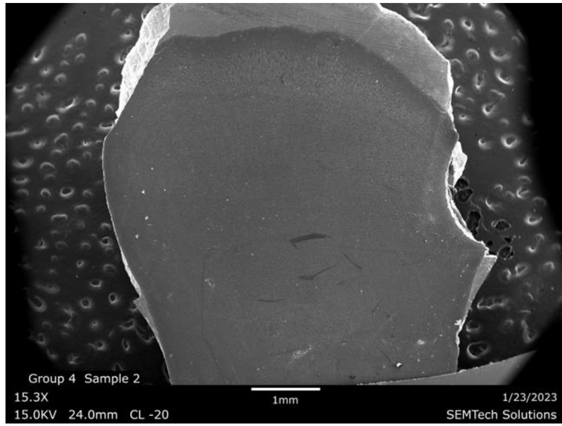



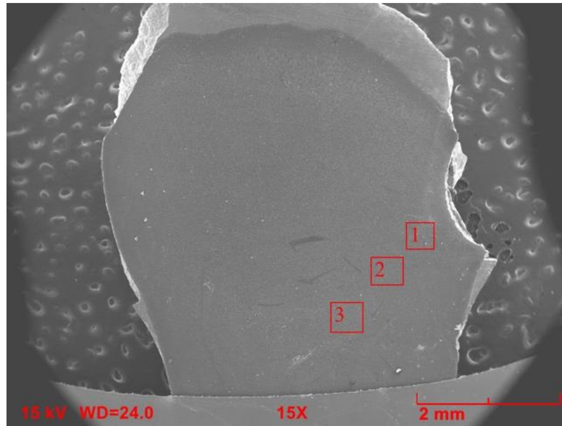



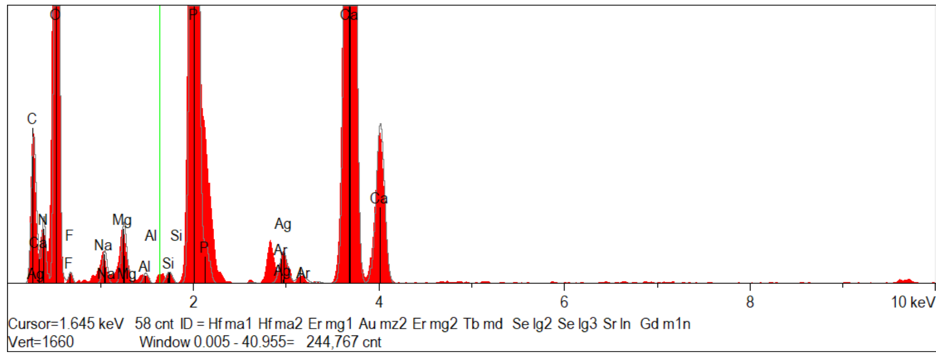



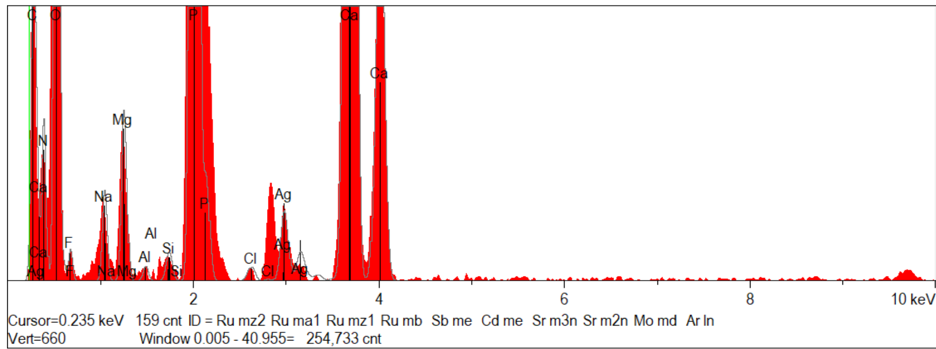



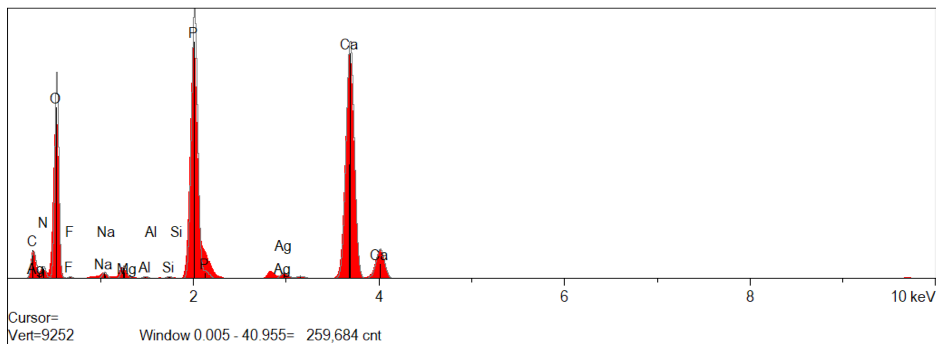

*Figures 37,38 ,39, 40 ,and 41: shows SEM images of a sample from the test 2 (Group 3) and energy dispersive X-ray spectroscopy (EDS) analysis for the center of three layers (infected, affected, and sound) of the treated lesion.*




*Figures 42, 43, 44, 45 ,and 46 shows SEM images of a sample from the test 3 (Group 4) and energy dispersive X-ray spectroscopy (EDS) analysis for the center of three layers (infected, affected, and sound) of the treated lesion.*

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