Cumhuriyet Dental Journal: 2020; 23(4)
e-ISSN 2146-2852
Doi:10.7126/cumudj.796823

Original research

REMINERALIZATION OF PRIMARY MOLAR DENTINE WITH SILVER DIAMINE FLUORIDE AND SODIUM FLUORIDE: AN *IN VITRO* STUDY

ABSTRACT

Objectives: Silver diamine fluoride (SDF) offers a minimally invasive and inexpensive alternative to traditional restorative caries treatment. Aim of this study was to evaluate the mineral density value and remineralization effect of 38% SDF solution and 5% sodium fluoride (NaF) varnish on artificial primary tooth dentin caries lesions.

Materials and Methods: 54 extracted sound primary molars were demineralized and allocated into two groups: SDF and NaF groups. After pH-cycling, mineral density values (MDV) were assessed with micro computed tomography (micro-CT) and surface morphology were studied *via* scanning electron microscopy (SEM).

Results: After remineralization agents were apllied and specimens pH-cycled, the MDVs of the Region of Interest (ROI) in lesions increased in both NaF and SDF group. The mean MDV of SDF applied specimens was found to be higher than NaF applied specimens at statistically significant level. In the SEM images, it was observed that the dentinal tubules orifices were occluded almost completely in SDF group while the orifices in NaF group were partially occluded.

Conclusions: The use of 38% SDF demonstrated a great remineralization effect on primary molar dentin than 5% NaF varnish. Further clinical studies are needed to investigate the effect of 38% SDF on primary molars which remains in the mouth longer than incisors and have a great function on chewing.

Key words: Topical fluorides, silver diamine fluoride, microcomputed tomography, tooth remineralization.

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Received : 18.09.2020
Accepted : 22.09.2020

How to Cite: Yilmaz N, Ocak M, Okte Z. Remineralization of Primary Molar Dentine with Silver Diamine Fluoride and Sodium Fluoride: An In Vitro Study. Cumhuriyet Dent J 2020;23:4: 340-347.

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INTRODUCTION

Despite the advance in dental health care in the past few decades, early childhood caries (ECC) is a still widespread condition seen among children throughout the world.[1,2] It is essential to diagnose and treat ECC in early stage, because untreated cavities can lead to severe toothache and development of dental abscesses.[3] Conventional caries treatment involves mechanical removal of the carious lesion by rotary instruments and restoration of the cavity by filling material. However, in most cases it may not be possible to treat ECC with standard methods due to cooperation problems in children and expensive options such as general anesthesia or sedation are needed.[4-6] Therefore alternative treatments for dental caries are neccessary and professionally applied fluoride therapy has been proposed not only to prevent but also to arrest caries.[7] Compared to traditional restorative treatment, arresting caries with fluoride agents is non-invasive, simple to apply and low cost.[8,9]

A number of remineralization agents have been studied in clinical trials or *in vitro* trials to arrest caries lesions.[10] Fluoride varnishes are one of the most widely used topical fluoride treatments. Varnishes are supposed to act as slow-release fluoride reservoirs due to extended contact time between tooth surface and fluoride.[11] One of the most common used varnishes contains 5% sodium fluoride (NaF) and the fluoride concentration is 22.600 parts per million (ppm).[12] However, in recent years the anticaries effects of various formulations have mostly been investigated on initial (enamel) caries but not on dentin.[13]

Silver diamine fluoride (SDF) which has become increasingly popular, is a practical and affordable treatment option for arresting carious lesions.[14] SDF is a translucent solution and the most commonly used SDF concentration is 38% which contains 44.800 ppm fluoride and 255.000 ppm silver ions. Although SDF has been used in some countries in Asia and South America for many years, it has gained considerable interest in the United States after the approval by the Food and Drug Administration (FDA) in 2014.[15,16] However, FDA only approved its use for the management of dental hypersensitivity therefore more evidence is needed for the use of SDF in caries management.

Micro computed tomography (Micro-CT) is a high resolution 3D X-ray imaging tehnique and has been used to determine the mineral density in dental hard tissues, recently. Comparing to transverse microradiography (TMR), Micro-CT is a non-destructive method and does not require complicated and time-consuming specimen preparation procedures.[17,18] In the literature most of the *in vitro* studies evaluating the efficacy of SDF were performed with permanent teeth and in the majority of these studies, mineral density values were not given. Therefore the aim of this study was to evaluate the dentin remineralization of 38% SDF and 5% NaF by comparing mineral density values in primary teeth in *in vitro* conditions. The null hypothesis was that there is no difference between 38% SDF solution and 5% NaF varnish in primary teeth dentin remineralization.

MATERIALS AND METHODS

Preparing specimens with artificial dentine caries

The study protocols were approved by the Ethics Committee of the Faculty of Dentistry, Ankara University (36290600;01). The flow chart in Figure 1 summarises the protocol of this study.

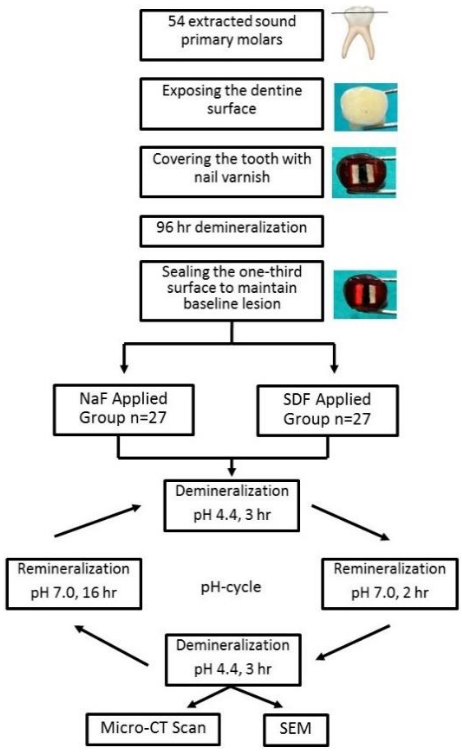

Figure 1. Flowchart of the experiment design.

Extracted sound human primary molars were collected with patient consent. The teeth were thoroughly cleaned and then inspected under a stereomicroscope for visibly observable cracks, hypoplasia and white spot lesions. Gingival tissues at the neck of the tooth were removed manually using hand instruments. Teeth were stored in 0.1% thymol solution at 4 ^{0}C until use and were used within two months of extraction.

Assuming a common standard deviation of 0,1 gHApcm^{-3} and with power at 0.80 and α=0.05, the sample size was at least 27 in each group. 54 extracted sound primary molars were abraded with diamond bur from occlusal surface and dentin was exposed. Then occlusal side was polished by sand papers (600, 800, 1200, 2000 grades). All surfaces except the area of 3x3 mm window on the occlusal surface were sealed with acid resistant nail polish (Revlon Corp., NY, USA). Middle 1x3 mm window of each specimens surface was covered with acid-resistant nail varnish to serve as sound control.

After the varnish had dried completely, each specimen was immersed in 20 mL of demineralizing solution in a small container for 96 hours to produce demineralized lesions approximately 100 microns deep. The solution was changed every 24 hours to ensure the tooth surfaces were in contact with fresh solution. The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M acetic acid and the pH was adjusted to 4.4 with 1 M KOH.[19] Following lesion development, the samples were rinsed thoroughly with deionized water, and the other 1x3 mm window were covered with an acid-resistant nail varnish to maintain the baseline lesion.

Remineralization regimen

The demineralized samples were randomly divided into two groups (n=27) based on treatments: 38% SDF solution (FAgamin, Tedequim S.R.L., Argentina) and 5% NaF varnish (Duraphat; Colgate-Palmolive Co., New York City, NY, USA). A microbrush was used to apply SDF solution and NaF varnish to the uncovered one-third of the dentin surface (demineralized dentin). In the SDF treated group, 2-3 drops of the solution were applied 2-3 minutes to dentin surface according to the manufacturer's instructions. After application, the samples were washed with a flow of deionized water for approximately 30 seconds. In the NaF treated group a thin layer of the varnish was applied using microbrush on demineralized dentin suface and then the samples were immersed in artificial saliva (.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2.2H_2O$, 3 mMNH_4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mMK_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4, and traces of ascorbic acid, pH 6.8) at 25°C for 6 hours.[20] Then varnishes were carefully removed using a surgical blade.

pH-cycling model

To assess the effect of the remineralization agents on dentine demineralization in the treatment groups, the protocol used by Ekambaram *et al.* was employed.[21] The specimens were placed in the pH-cycling system for a period of 7 days. Each cycle involved 3 h of demineralization (2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4 and 0.05 M acetic acid, pH: 4.5) twice daily, with 2 h of remineralization (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4 and 0.15 M KCl, pH: 7) between the periods of demineralization. Samples were then placed in the remineralizing solution over-night (16 hours). All solutions were freshly prepared for each cycle; separate containers were used for each specimen. The pH level of the demineralizing and remineralizing solutions was measured before every cycle.

Microcomputed Tomography (Micro-CT) Measurements

The specimens (n=27 for each group) were scanned by a Skyscan 1172 micro-CT (Bruker, Kontich, Belgium) for mineral density assessment. The X-ray source voltage and current were set at 80 kV and 100 µA, respectively. Specimens were rotated through 360º at 0.6º steps. 0.5 mm aluminium-copper filter used to cut off the softest X-rays. The image pixel size was 13.68 µm. During the scanning, a wet sponge was put into the tube to fix the position of the tooth and to keep the tooth moist.

Two standard mineral cylindrical phantoms (Bruker, Kontich, Belgium) with mineral density values (MDVs) of 0.25 gHApcm^{-3} and 0.75 gHApcm^{-3} were scanned for calibration of the greyscale of the specimens. Phantoms were scanned with the same settings used for the test specimens.

The image analysis software (CTAn, Skyscan NV, Kontich, Belgium) was used to calibrate the greyscale value and calculate it into the mineral density value (MDV, gHApcm−3) using the same software. The MDV of the ROI in each one-third surface of groups were measured. Manually determined ROIs were placed only in the dentin. The ROI was a 1 mm diameter circle in the middle part of the measured surface 50 µm below the exposed tooth surface. Measurements were taken on three locations in each one-third surface and the mean of these measurements were used as the MDV of the related surface.

Surface Morphology

Two specimens in each group were chosen randomly to assess surface morphology. After removing the nail varnish, specimens were dehydrated in a series of ethanol solutions and coated with gold and palladium. The surface morphology of specimens were then observed under scanning electron microscopy (SEM) (FEI Quanta 200, Scanning Electron Microscope, FEI Company, OR, USA).

Data analysis

All of the data were assessed using the 'Kolmogorov-Smirnov (K-S)' test for normality ($p>0.05$). Paired t-test was applied to compare the MDVs of experimental groups. All of the analyses were performed with the software SPSS for Windows version 22.0 (IBM Corporation, Armonk, NewYork, USA). The significance level was set at 5%.

RESULTS

The MDVs of groups are shown in Table 1.

Table 1: MDVs and Standard Deviations of CS, DMS and RMS of Groups

Groups	MDV of CS $g_{HAp}cm^{-3}$	MDV of DMS $g_{HAp}cm^{-3}$	MDV of RMS $g_{HAp}cm^{-3}$
NaF (n=27)	1.361 (0.062) [a1]	0.910 (0.166) [b1]	1.145 (0.120) [c1]
SDF (n=27)	1.376 (0.070) [a1]	0.961 (0.221) [b1]	1.623 (0.171) [c2]

*For each horizontal row: values with identical letters indicate no statistically significance ($p>0.05$)
**For each vertical column: values with identical numbers indicate no statistically significance ($p>0.05$)

In both NaF and SDF groups, the surface covered with nail varnish at the beginning of the experiment was used as a control surface (CS). The surface covered with nail polish after demineralization process was used as demineralization surface (DMS) and the surface that remineralization agents were applied was used as remineralization surface (RMS).

The MDV of CS in NaF and SDF was 1.361 $g_{HAp}cm^{-3}$ and 1.376 $g_{HAp}cm^{-3}$ respectively with no statistically significant difference ($p>0.05$). The MDVs of the DMS were 0.910 $g_{HAp}cm^{-3}$ in NaF group and 0.961 $g_{HAp}cm^{-3}$ in SDF group and there were no statistically significant difference between them ($p>0.05$). After remineralization agents were applied and specimens pH-cycled, the MDVs of the ROI in lesions increased in both NaF and SDF group. The mean MDV of SDF applied specimens were found to be higher than NaF applied specimens at statistically significant level ($p<0.05$).

In NaF group, the MDV of CS was higher than both DMS and RMS at statistically significant level ($p<0.05$). MDV of RMS was also found to be statistically significantly higher than demineralization group ($p<0.05$). Unlike the NaF group, in SDF group the MDV of RMS was found to be statistically significantly higher than the CS, ie the sound dentin surface ($p<0.05$).

SEM images of the groups are displayed in Figure 2a, 2b, 2c, 2d. The CS was covered with dense smear layer with no visible dentinal tubules.

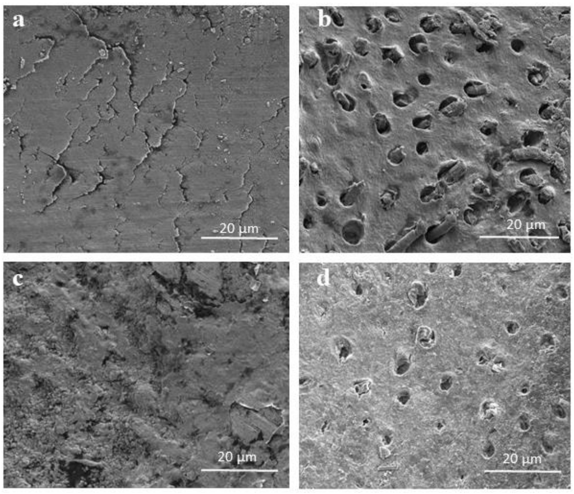

Figure 2. SEM images (x5000 magnification) of surface morphology of CS with smear layer (a), DMS with visible dentinal tubules (b), RMS with SDF (c) and RMS with NaF (d).

In the DMS the dentinal tubules were visibly exposed. The SDF applied surface remained relatively dense and intact compared with NaF applied surface.

DISCUSSION

This study was conducted to compare the effect of SDF and NaF on mineral content of demineralized primary molar dentine. It provides useful information about changes in the minerals of demineralized primary tooth dentine. According to the results of this study, the null hypothesis was rejected. SDF solution showed higher remineralization effect on primary molar dentin than NaF varnish.

In both SDF and NaF groups mean MDV of DMS was found to be lower than CS at statistically significant level which means the demineralization solution was effective to produce artificial dentin caries. However, the MDVs of DMSs in this study were 20–30% higher than natural dentine caries reported in other studies .[18,20] The reason for that may be the MDV of DMS in this study were taken from the superficial layers of the teeth which had a higher mineral density than deeper surfaces of the teeth. It should be noted that natural carious lesions greatly vary in depth depending on the activity of the lesion.[22,23] Additionally, the natural carious lesions occur in a long time period in contrast to artificial caries where the lesion was created in a short time period.

The mean MDV of CS 50 µm below the surface in the varnished area was around 1.36 $g_{HAp}cm^{-3}$ and 1.37 $g_{HAp}cm^{-3}$ in NaF and SDF groups, respectively. These values are similar to other studies with deciduous molars.[18,22,24] The mean MDV of RMSs in both groups were found to be higher than DMS at statistically significant level. So that both SDF solution and NaF varnish showed effective remineralization. Comparing SDF and NaF each other showed that SDF applied RMS presented statistically significantly superior remineralization than NaF group and even CS ie sound dentine. This finding corroborated with Mei *et al.*'s study which reported that density of SDF arrested dentinal lesion was higher than unaffected dentine.[25] It is noteworthy that the fluoride concentrations in 5% NaF and 38% SDF are 22.600 ppm and 44.800 ppm, respectively.[26] The high remineralization effect of SDF on dentine could be explained by its high fluoride concentration and the presence of silver.[27] However, an *in vitro* study reported that the use of 38% SDF demonstared a greater inhibitory effect on dentine demineralization and collagen degradation than 10% NaF and 42% $AgNO_3$ solutions which involves equal amount of fluoride and silver ions, respectively.[10] Similarly, another study compared 38% SDF, 38%SDF + 5% NaF, 5% NaF and water on dentin remineralization and reported that lesion depth in the SDF groups with or without NaF treatment did not show significant differences.[28] SDF and NaF both promote remineralization by formation of insoluble calcium fluoride which dissolves in a salivary environment to realese calcium and fluoride ions. Those released fluoride ions replace with hydroxyl ions of hydroxyapatite to form acid-resistant fluoroapatite. SDF is an alkaline solution and an alkaline environment provides ideal conditions for ion exchange so that formation of fluoroapatite can be promoted.[29,30] Therefore, it should be kept in the mind that the reason for superior remineralization effect of SDF is not only the high fluoride concentration but also the alkalinity of the solution. In this study, greyish black stains were observed on the tooth surfaces treated with SDF even after washing the specimens with deionozied water for 30 seconds according to manifacturer's advisement. In *in vitro* studies, it was determined that silver chloride was formed as a result of chemical reaction between SDF and hydroxyapatite. Also it was concluded that this insoluble silver chloride works as a protective layer on the dentine surface so that the loss of calcium and phosphate from dentine could be decreased.[10,28] In the current study, this silver chloride deposition on the surface was not included in MDV measurements to prevent the high radioopacity effect.

In the SEM images it was observed that the dentin tubules, which were exposed after demineralization, were partially occluded and the diameter of the exposed dentinal tubule orifices were decreased in the NaF treated group. On the other hand, in SDF treated group most dentinal tubule orifices were occluded and dense granular structures were observed. Transmission electron microscopy can be performed to investigate the mineral content of the surfaces.

In the present study, single dentine surface was used for the control group, demineralization group and remineralization group. So that the effect of substrate variability on the hypotheses tested was reduced. Additionally, random allocation of the specimen between the experimental groups further avoided such an effect. To simulate dentine caries, specimens were initially demineralized before SDF and NaF application. After NaF varnish was applied specimens were immersed into artificial saliva for 6 hours. After this period, fluoride varnishes were removed with the help of a surgical blade without touching the dentine surface directly. Thus, this procedure simulated the clinical conditions in which the varnish would probably be removed after some hours via toothbrushing or mastication.[13,20] Since SDF is liquid material and was already washed with a flow of deionized water as recommended by the manufacturer, surgical blade did not used in SDF applied group.

After the demineralization process and application of remineralization agents, there are different studies in which samples are immersed in remineralization solution or subjected to pH-cycle. In daily life, due to the nutrition the pH level never remains constant in oral cavity, therefore it is important to reflect the pH changes to the experimental environment. So that the present study used a pH-cycling model involving combination of demineralization and remineralization on the dentine surface. Although this model is rapid and inexpensive, it was unable to entirely simulate the complex oral conditions such as bacterial biofilm, quality and composition of saliva, oral hygiene practises, eating habits and fluoride uptake.

Many different methods have been used to evaluate the remineralization effect of various agents in enamel and dentin. Chemical analysis, cross-section microhardness testing and transverse microradiography techniques have been widely used. However, these methods are destructive and require complicated and time-consuming specimen preparation procedures.[17,18] Lately, micro-CT has been used to determine the mineral density in dental hard tissue without damaging the tooth specimens. Therefore, to assess the mineral content of primary molar dentin, micro-CT was preferred in the present study.

CONCLUSIONS

Within the limitations of this *in vitro* study, it is concluded that the use 38% SDF demonstrated a great remineralization effect on primary molar dentin than 5% NaF varnish. Further clinical studies are needed to investigate the effect of 38% SDF on primary molars which remains in the mouth longer than incisors and have a great function on chewing.

ACKNOWLEDGEMENTS

This study was supported by Ankara University Scientific Research Projects Funds (No: 16B0234001).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

Gümüş Diamin Florid ve Sodyum Florürün Dentin Remineralizasyonundaki Etkinliğinin In-Vitro Koşullarda Değerlendirilmesi

ÖZ

Amaç: *Bu çalışmada, yapay süt dişi dentin çürük lezyonlarında %38'lik gümüş diamin florid (GDF) çözeltisinin ve %5'lik sodyum florür (NaF) verniğinin remineralize edici etkisi ve mineral yoğunluk değerlerinin karşılaştırılması amaçlanmıştır.* ***Gereç ve Yöntemler:*** *58 adet çekilmiş süt azı dişi okluzal yüzeyden aşındırılarak dentin yüzeyi açığa çıkarılmış ve aside dayanıklı tırnak cilasıyla 1×3 mm'lik 3 adet pencere oluşturulmuştur. Orta üçlüde olan pencere kontrol grubu olarak kullanılmak üzere hiçbir işlem yapılmadan tırnak cilası ile kapatılmış, diğerlerine ise demineralizasyon solüsyonu kullanılarak yapay dentin çürüğü oluşturulmuştur. Daha sonra bir taraftaki pencere, lezyonun ilk halini değerlendirebilmek amacıyla tırnak cilasıyla kapatılmış, diğer taraftaki ise remineralizasyon materyallerine tabi tutulmuştur. Remineralizasyon ajanı olarak sırasıyla; GDF ve NaF uygulanmıştır. Ardından örnekler 7 günlük pH döngüsüne sokulmuştur. Örnek yüzeylerindeki mineral değişimi ölçümleri Mikro-BT ile; yüzey morfolojisindeki değişimler ise SEM ile değerlendirilmiştir.* ***Bulgular:*** *Demineralizasyon* *yüzeyine göre mineral yoğunluk değerleri hem NaF hem de SDF grubunda artmıştır. SDF uygulanan örneklerin ortalama mineral yoğunluk değerleri, istatistiksel olarak anlamlı düzeyde NaF uygulanan örneklerden daha yüksek olduğu bulunmuştur. SEM görüntülerinde, dentin tübüllerinin GDF grubunda neredeyse tamamen tıkalı olduğu, NaF grubunda ise kısmen tıkalı olduğu gözlenmiştir.* ***Sonuçlar:*** *GDF'nin organik içeriği yüksek süt dişi dentininde etkili bir remineralizasyon gösterdiği ve bu nedenle uzun yıllardır altın standart olarak kabul edilen floride alternatif olabileceği düşünülmektedir. Kesici dişlerden daha uzun süre ağızda kalan ve çiğneme üzerinde büyük bir işlevi olan süt azı dişleri üzerinde %38'lik GDF'nin etkisini araştırmak için daha fazla klinik çalışmaya ihtiyaç vardır.* ***Anahtar Kelimeler:*** *Topikal florür, mikrobilgisayarlı tomografi, diş remineralizasyonu.*

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