Potential use of silver diammine fluoride in detection of carious dentin

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This study aimed to examine whether discoloration of carious dentin after silver-diammine-fluoride (SDF) application might be used as a mean to detect demineralized-dentin. Forty specimens were obtained from 20 human permanent teeth. Teeth were sectioned through the center of carious lesions to create 2-halves in which each half was assigned to a treatment group. Specimens were divided into two groups (n=20) (each half was assigned to one group) according to solution, namely Caries Check (CC), or SDF. SDF group was subdivided into 2 groups: light-cured and 2-day storage groups. The specimens were tested using light-microscope, microhardness test and SEM/EDS analysis. Repeated-measures ANOVA was used for statistical analysis. The light-microscope showed superficial discoloration in the CC-group while SDF (2-day storage) group showed deeper discoloration for the lesion area. SDF showed significant increase in the hardness compared with the CC-group. SDF showed potentiality to be used as an assisting-tool for caries detection.

Keywords: Caries detecting dye, Carious lesion, Silver diammine fluoride, Caries check, Discoloration

INTRODUCTION

Dental caries is a complex bacterial mediated disease that causes acids from the cariogenic bacterial metabolism to diffuse into enamel and dentin dissolving the mineral phase of these tissues. The carious dentinal lesion includes two layers which differ in their chemical and ultrastructure characteristics. The outer layer, which has been referred to as the ‘caries-infected dentin zone’, is heavily contaminated with bacteria and its organic matrix is substantially degraded and incapable of being remineralized. The inner layer represents the ‘caries-affected dentin zone’, which is partially demineralized with limited collagen degradation and can be remineralized.

Recently, the International Caries Consensus Collaboration (ICCC) recommended other terminology for the carious tissue removal based on the level of hardness of remaining dentin (soft, leathery, firm and hard dentin). This degree of hardness can offer some guidance for clinicians where soft dentin is corresponding to caries infected dentin layer and leathery dentin is corresponding to caries affected dentin.

The main treatment objective for carious teeth requiring conservative surgical management is ideally the complete removal of the heavily bacteria contaminated layer that cannot be remineralized while preserving the affected dentin that can be remineralized. This treatment philosophy was introduced by Fusayama et al. to conserve as much of the tooth as possible during caries excavation.

Current methods for clinical assessment of carious dentin involve visual (color) and tactile (hardness) procedures. However, tactile assessment is not a reliable guide for determining caries removal clinically. Discoloration (visual) may be reliable only in slowly progressing caries, where the extent of bacterial invasion is closely related to the discoloration front. However, in cases of rapidly progressing caries, the bacterial invasion is diffuse and precedes the discoloration front.

Caries detecting dyes (CCDs) have been used to facilitate the delineation of ‘caries’ dentin from sound dentin during caries removal clinically. Commonly used dyes usually contain 1% acid red in propylene glycol or recently, polypropylene glycol. However, these dyes do not provide a truly objective method for assessment of caries removal and can lead to excessive removal of dentin or incomplete removal of bacteria, as these dyes appear to stain demineralized collagen matrices instead of bacteria.

Silver diammine fluoride (SDF) is a silver compound that is considered as an efficient, cost-effective and non-invasive solution used on deciduous and permanent carious teeth, proven to have a unique anti-bacterial effect and can inhibit further demineralization and increase the surface microhardness of tooth structures. In addition, SDF application to exposed root surfaces is an effective way of preventing caries initiation and progression. The main drawback of SDF is the formation of dark stains on tooth structures, which has led to its limited clinical use.

However, it has been shown that SDF stains...
demineralized dentin more effectively than sound dentin\textsuperscript{29} which can be used as an advantage. Therefore, the aim of this study was to examine whether discolouration of carious dentin after SDF application might be used as a mean of guidance for caries excavation. The null hypothesis in this study was that SDF discolouration cannot differentiate between the demineralized and sound dentin.

**MATERIALS AND METHODS**

This study protocol was approved by the ethics committee of Tokyo Medical and Dental University under identification code “D2013-022-02 (Institutional Research Board approval number: 725).

A total of 40 specimens were obtained from 20 freshly extracted permanent teeth exhibiting occlusal or smooth surface caries without pulpal involvement. Extracted human teeth were collected with patient consent. Teeth with abnormal discolouration, cracks or pulpal involvement were excluded. Teeth were immediately placed in sterile distilled water at 4°C following extraction. Teeth were sectioned through the center of carious lesions to create 2 halves in which each half was assigned to a treatment group. An additional two cuts were made to create a parallel flat surface, one at the end of each half of the specimen using a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under copious water coolant.

The characteristics of the caries-infected dentin were assessed using two parameters: color and hardness. The superficial carious layer with a heavy natural discolouration was removed from each half using a sterile bur (ISO 001/010) mounted on a low-speed hand piece, exposing the underlying lightly-stained soft infected dentin\textsuperscript{29}. Specimens with lesion depth ranging 1,000–1,200 µm “which was confirmed later with microhardness test” were only included in the study, while teeth with more than 1,200 µm lesion depth were excluded. All caries removal procedures were performed by the same operator. Specimens were divided into two groups (n=20) (each half was assigned to one group) according to solution, namely Caries Check (CC; Caries Check, Nippon Shika Yakuhin, Shimonoseki, Japan) containing 1% acid red in polypropylene glycol, or 38% SDF (Saforide, Bee Brand Medico Dental, Osaka, Japan) containing 44,880 ppm F and 253,870 ppm Ag.

The cross-sectioned surfaces of the specimens were covered with nail varnish for protection against solution contamination. Then, the solution was applied onto the excavated lesion according to manufacturer instructions. CC was applied for 3 s and washed with distilled water for 10 s while SDF was applied for 1 min left for 2 min then washed for 30 s. Half of the SDF samples was stored in artificial saliva (prepared within 24 h before use and consisted of Calcium Chloride Dehydrate 0.7 mmol/L, Magnesium Chloride 0.2 mmol/L, Potassium Dihydrogen Phosphate 4.0 mmol/L, HEPES (Acid buffer) 20.0 mmol/L and Potassium Chloride 30.0 mmol/L and buffered to pH 7) at 37°C for 2 days (n=10) (till most of silver reactions are achieved with development of significant discoloration) for a 2-visit treatment protocol, while the other half was light-cured with halogen light (n=10) (Optilux 501, 600 mW/cm², Demetron, Danbury, CT, USA) for 10 s to obtain immediate color change of dentin (based on the results of our pilot study) for a single-visit treatment protocol.

Then, the cross-sectioned surfaces were polished using 1000–2000-grit SiC papers (Fuji Star, Sanky Rikagaku, Saitama, Japan) and polished with diamond paste down to 0.25 µm under running water and then sonicated in distilled water for 3 min to remove the nail varnish layer and expose the underlying tooth structure.

**Light microscope observation**

All Specimens were observed twice, once before solution application and 2 days after solution application (storage time) using a light microscope (LM, Nikon SMZ1000, Nikon, Tokyo, Japan) under magnification of 4.0× [(5×)×(0.8×)], to determine the depth of discolouration.

**Microhardness measurement**

Microhardness was measured using Vickers hardness number (VHN) immediately after a specimen was removed from artificial saliva. The specimen was mounted on a glass microscope slide with double sided adhesive tape for fixation and measured using microhardness testing machine (MKV-5 hardness tester, Akashi Seisakusho, Kanagawa, Japan). The Vickers diamond indenter was applied to the dentin surface with a load of 50 g for 15 s. Microhardness was determined at 10 successive sites below the surface of the tooth from the center of the carious lesion toward the pulp at intervals of 200 µm. For the sound dentin, indentations started from the dentin-enamel junction (DEJ) towards the pulp chamber within the same specimen. Measurements obtained along 5 lines (3 at the carious side and 2 at the sound side) following parallel tracks approximately 150–200 µm apart. The means of VHN measurements for each specimen at each indentation depth were then analyzed.

**SEM/EDS analysis**

After the microhardness measurement, specimens were platinum/palladium sputter-coated and observed using a field emission scanning electron microscope (SEM) (FE-SEM, S-4500, Hitachi, Tokyo, Japan) with operating conditions of 15 kV. SEM images were taken to correlate structure-hardness relationships. Elemental analysis was performed to identify calcium (Ca), phosphorous (P) and silver (Ag) ions via EDS under SEM (JSM-IT100, SEM, JEOL, Tokyo, Japan) with operating conditions of 15 kV.

**Statistical analysis**

Data are presented as mean and standard deviation (SD). Normal distribution was determined using Kolmogorov-Smirnov and Shapiro-Wilk tests. Microhardness showed normal distribution. Repeated measures ANOVA test
was used to compare different tested depths, followed by pairwise comparison with Bonferroni correction. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with IBM® SPSS® (IBM Released 2015, IBM SPSS Statistics for Windows, Version 23.0, IBM, Armonk, NY, USA).

RESULTS

Optical microscopic observation
Figure 1 illustrates that the red-stained area extended to a depth of $350 \pm 8.5 \, \mu m$ in the CC-treated group and was restricted to the superficial part of the lesion area. The pigmented area was deeply stained at surface (dark red) while the rest of the lesion was lightly stained (pink color).

Figure 2 shows that after 2-days storage in artificial saliva, the specimens showed the black-stained area extended to a depth of $1,150 \pm 6.5 \, \mu m$ from the surface of the lesion in the SDF-treated group, almost staining all the lesion area. SDF stained dentin over 3× deeper than CCD. The pigmented area was deeply stained at surface (black) while the discoloration gradually decreased along the rest of the lesion. However, Fig. 3 demonstrates that light-cured SDF specimens showed deep dark stain that was restricted to the surface of both the sound and the carious dentin lesion without deeper penetration into the lesion.

Microhardness measurement
Table 1 and Fig. 4 demonstrate that the CC application did not modify the microhardness values (VHN) of carious lesion, while SDF application greatly influenced the microhardness values (VHN) of carious lesions. At a depth of 200 $\mu m$ from the surface of the lesion (outer zone of the lesion), the VHN values of the SDF group after 2-days storage (50.7±2) and the light-cured SDF (56.7±0.6) were significantly higher than the CC group (27.87±3.58) at $p \leq 0.001$. The VHN values decreased gradually towards the base of the lesion in the 2-days storage SDF group while in the light-cured SDF group there was a sharp decrease in the VHN values beyond the 200 $\mu m$ depth. The VHN values were significantly higher for SDF group until 600 $\mu m$ compared to the CC group. However, the VHN values of the CC group remained significantly lower than the VHN values of the SDF groups till 600 $\mu m$ depth. Then the VHN values became insignificant between both groups.

Figure 5 illustrates the relation of the microhardness values and the discoloration data of SDF group (2-visit) and CC group.

SEM/EDS analysis
Figure 6 shows the morphological analysis for the cross-sectional surfaces showed only irregular surface with degraded collagen fibers (typical carious lesion image without any additional characteristic features).
Table 1  Mean and SD for microhardness for different tested groups for different dentin depth

|        | Control       | SDF            | SDF light-cured | CC            |
|--------|---------------|----------------|-----------------|---------------|
| 200    | 75.2±1.7aDE   | 50.7±2.6bR     | 56.7±0.6dD      | 25.3±2.8dE    |
| 400    | 76.3±1.4cDE   | 48.9±4.1bB     | 42.5±2.9eE      | 28±4.3cDE     |
| 600    | 78.7±2.1aBCDE | 44.7±5.7bC     | 40.6±2.4cDE     | 32.2±2.5cD    |
| 800    | 80.8±2.2aABC  | 40.9±4.2bCD    | 38.7±3.2cEF     | 35.6±0.5cC    |
| 1000   | 83.3±1.5bA    | 34.3±2.7dD     | 36.1±1.9dF      | 35.9±1.5cC    |
| 1200   | 82.2±2.1aAB   | 72.8±3.9cA     | 71.5±3.3bA      | 72.6±3.9bA    |
| 1400   | 80.1±3aBCD    | 70.7±3bA       | 70.3±1bAB       | 71±3.8bA     |
| 1600   | 77.2±2.8bABCDE| 68.8±2.5bA     | 68.4±1.1bABC    | 68.4±3.7bAB   |
| 1800   | 74.8±3.4aE    | 67.7±3.0bA     | 67.3±0.9bcBC    | 66.2±3.5aAB   |
| 2000   | 73.8±3.4bE    | 65.2±2.8bA     | 65.9±0.9bcC     | 63.6±3.2bB    |

Means with same uppercase letter within each column are not significant at $p>0.05$. Means with same lowercase letter within each row are not significant at $p>0.05$.

Fig. 4  Line chart showing the hardness values at different depth points within the lesion.
The SDF group showed significantly higher VHN values than CC group till 600 µm, while SDF light-cure (SDF/LC) group showed high VHN values superficially then dropped significantly.

Fig. 5  Representative illustration showing relation between hardness values and discoloration area.
regarding the CC group. However, Fig. 7 demonstrates that both SDF groups “light-cured group and after 2-day storage group” showed multiple crystal formations on surface. Crystal formations within the dentinal tubules were mainly found in the 2-days storage SDF group. These crystalline formations recorded high peak of silver using EDS analysis, indicating formation of silver compounds.

Figure 8 shows that all groups showed partially obliterated dentinal tubules at the transparent dentin zone near the end of the lesion and just before the normal dentin area.

DISCUSSION

The present study evaluated the possibility of using SDF material as a mean of guidance for caries excavation. Based on our findings in this study, the null hypothesis was partially rejected as SDF discoloration was able to differentiate between demineralized and sound dentin after storage “2-visit treatment protocol” and not in the light-cured group (1-visit treatment protocol).

CDD contains 1% acid red in propylene glycol with a simple mechanism of action, that the dye solution penetrates into the porous mineral-depleted carious dentin where the acid red stains the organic matrix, which is mainly collagen fibrils, irrespective of whether it has been denatured. However, the disadvantage of CDD is that the lightly stained lesion (pale pink staining of the lesion) should be preserved as it does not contain bacteria. Yet, it is very subjective to judge the degree (intensity) of pale pink staining of the dentin lesion clinically.

The CC contains 1% acid red in polypropylene glycol instead of propylene glycol. The molecular weight (MW) of the polypropylene glycol component employed in CC is 300 g/mol, which is considered very high compared to previously developed CCDs (MW of propylene glycol is 76 g/mol). It has been shown that dyes that were dissolved in higher molecular weight carriers exhibited reduced diffusional properties in porous tissues, which was the reason for the restricted penetration of CC stain into the carious lesion showed under light microscope observation in this study.

SDF is a colorless solution that comprises large amounts of silver and fluoride in addition to ammonia \([\text{Ag} (\text{NH}_3)_2 \text{F}]\). Upon application of SDF, it releases a large quantity of free silver ions (Ag\(^+\)). Silver has a high polarizing power as it has a high ratio of ionic charge in relation to the ion radius which in turn facilitates the formation of strong bonds with nitrogen and sulfur groups present in cysteine and histidine in proteins. The application of SDF on exposed collagen leads to a relatively rapid darkening, suggesting a direct reduction of silver ions into metallic silver.
Demineralized dentinal tissue is a highly porous tissue that leads to more silver uptake while the exposed collagen leads to reduction silver ions, resulting in a darker color change within a short time and color difference between demineralized and sound dentin can be seen clearly. In addition, SDF with lower molecular weight (160.9 g/mol) provides deeper penetration than CC, resulting in deeper staining of the SDF into carious lesion as observed under an optical microscope in this study. Silver particles resulted from SDF application can penetrate demineralized dentin and further penetration into underlying sound dentin. The presence of bacteria within dentinal tubules even after the excavation of demineralized dentin may be reduced as silver is known for its potent anti-bacterial effect. Nevertheless, the light-cured SDF group could not differentiate between the demineralized and sound dentin in terms of color as most of the silver ions present on the surface absorbed the light energy and reduced into metallic silver resulting in rapid darkening of all dentin surface.

In this study, there are two reducing factors affecting silver reduction, one is light and other is protein (exposed collagen). In case of light cure, a massive amount of energy was delivered through light causing rapid reduction of silver ions into metallic silver (rapid discoloration). However, in case of 2-visit SDF group the protein factor (exposed collagen) has the upper hand in reduction of silver ions into metallic silver resulting in better detection of demineralized dentin.

Hardness and texture of dentin serve as an indicator for caries detection clinically and a way to track the changes in mineral content in laboratory-based studies. Hardness testing for human teeth has been determined by a variety of methods including Knoop (KHN) and Vickers (VHN) hardness testing methods. Vickers indenter proved to be better than Knoop indenter in measuring tooth hardness, as Vickers indenter’s errors in measurement can be easily detected and avoided.

In this study, the microhardness of the dentinal carious lesion was determined after the application of CC and SDF, respectively. Although the microhardness testing was performed immediately after the specimens were removed from artificial saliva, some tissue contraction could thus have occurred because dehydration was inescapable during measurement.

The variations in hardness measurement were observed within each group of specimens as it may depend on the rate of caries attack and fluoride content. The hardness data in this study showed superficial improvement in carious dentin lesion after application of SDF.

This hardening effect may be attributed to the reaction of silver in contribution to fluoride-mediated remineralization. Reactions of silver with hydroxyapatite results in the formation of silver phosphate mainly and a portion of metallic silver. In addition to silver reaction with proteins within the lesion leading to the formation of silver-protein complex which protects collagen from further degradation. On the other hand, the reaction of silver with the exposed proteins within the carious lesion or reduction of silver on the dentin surface by light exposure may limit the hardening effect to the superficial layer of the lesion. The previous mentioned silver compounds contribute to the black staining of dentin. Additionally, the high concentration of fluoride (44,880 ppm) can enhance remineralization through the formation of firmly attached fluoride compounds like fluoro-hydroxyapatite, or loosely attached fluoride compounds such as calcium fluoride-like particles. This remineralization effect will even prevent further demineralization of the carious lesion. From the relation between the hardness and discoloration in Fig. 5, it can be assumed that SDF group (2-visit) could discolor most of the lesion area compared with CC group. Since SDF light-cure group caused only superficial discoloration, it was not added to this correlation.

SEM images showed multiple discrete crystal formation only in the SDF groups, which is most probably silver crystals as analyzed by EDS and speculated in previous studies. However, the calcium fluoride particles could not be seen, as these precipitates are easily dissolved and disappear over time.

SEM images showed the partially obliterated dentinal tubules in the transparent dentin area within the lesion which can be correlated to the improvement in hardness in that area.

Removal of the surface layer in SDF group might be done for esthetic reasons. Also, the silver-rich surface layer may hinder the adhesion of restorative material to dentin. One of the limitations in this study was that provisional material was not used during the two-visit protocol. However, this limitation would not affect the outcome of this study. The two-visit protocol may require extra chairside time, but in return SDF offers several advantages including remineralization and antibacterial effects.

Despite the higher clinical efficacy of SDF, the resulting black stain is a concerning side effect of SDF application and affects patients’ and/or parents’ acceptance of this treatment. However, SDF discoloration can be used as an assisting mean of guidance for caries detection.

Further studies will be needed to investigate the anti-bacterial properties of the SDF-treated carious dentin structure after removal of the demineralized discolored dentin area.

CONCLUSION

SDF showed potentiality to be used as an assisting tool for caries detection more in the 2-visit than the single-visit protocol (time-dependent), providing several advantages (arresting the carious lesion chemically and biologically) over a currently used CCD.

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CONFLICT OF INTEREST

All authors declare no conflict of interest in this study.

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