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COMPARATIVE EVALUATION OF DESENSITIZING AND REMINERALIZING EFFECT OF SILVER DIAMINE FLUORIDE AND CPP-ACP ON DEMINERALIZED DENTIN SURFACE: AN IN VITRO STUDY

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ABSTRACT

Background: This study aimed to compare the effect of Silver diamine fluoride (SDF) and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on the desensitization and remineralization of dentin.

Method: Twenty-eight dentin discs were divided into four groups with seven discs each. In group I, discs were immersed in 17% EDTA for 5 minutes only, while group II, III, and IV were immersed in 17% EDTA for 72 hours. Group III was then treated with SDF (Toothmate, Mansoura, Egypt) and group IV was treated with CPP-ACP (MI Paste Plus, GC, Tokyo, Japan) following the manufacturers' instructions. Finally, all groups were immersed in artificial saliva for 72 hours. The discs were collected and examined under a scanning electron microscope (SEM) to analyse the number of opened dentinal tubules and the percentage of calcium level in each group. Collected data were analyzed using One-way ANOVA and Tukey HSD post hoc tests ($p < 0.05$).

Results: Statistically significant differences were found between all four groups in terms of the number of opened dentinal tubules and calcium level ($p<0.05$). Regarding opened dentinal tubules count, the highest mean was found in group II (80.71±9.39), while the lowest mean value was observed in group III (9.14±2.54). For calcium level analysis, the highest mean value was found in group III (35.34±1.37). In contrast, the lowest mean value was observed in group II (14.47±1.26).

Conclusion: SDF had a better effect than CPP-ACP on demineralized dentin surface in terms of desensitization and remineralization.

KEYWORDS: SDF; CPP-ACP; Dentinal tubules; Desensitization; Remineralization.

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INTRODUCTION

Conservative dentistry involves diagnosing, treating, and prognosticating defects in teeth that do not require full-coverage restorations for correction [1]. Teeth are constantly exposed to many deficiencies within life habits, which can lead to caries, hypersensitivity, and demineralization [2]. The hypersensitivity of the dentin is characterized by sharp and short pain from dentinal tubule exposure to different stimuli [3]. However, dentin hypersensitivity is caused by the movement of dentinal fluid inside the dentinal tubules of exposed dentin, according to the hydrodynamic theory [4]. Therefore, a desensitizing agent that blocks neural transmission or occludes the dentinal tubules can be used conservatively to treat hypersensitive dentin [5]. On the other hand, demineralization of both enamel and dentin is initiated mainly by caries; however, calcium and phosphorus are decreased, resulting in subsurface lesion formation [6]. The demineralization process in dentin is more accessible than that in enamel, possibly because dentin crystals increase in carbonate substances compared with those in enamel, and the demineralization process is related to the breakdown of collagen [7]. Therefore, many remineralization materials have been studied for their ability to arrest caries and remineralization-affected dentin [8].

One of these materials is silver diamine fluoride (SDF); however, it is used in desensitization or remineralization. Silver, fluoride, and ammonium ions are combined in a colorless and odorless solution called SDF, which is stabilized by ammonia. The concentration of fluoride ions in SDF (38%) was greater than 44,000 ppm. When placed on carious tooth tissue, a series of chemical reactions occur that promote carious lesion arrest by dentinal tubule blockage, bacterial death, remineralization of the demineralized tooth, inhibition of dentinal collagen degradation, and tooth desensitization by dentinal tubule occlusion [9]. The first country in which SDF was used for carious lesions arrested in primary teeth was Japan in 1969 [10]. Later, in 2014, the Food and Drug Administration (FDA) accepted 38% SDF as a treatment for dentin hypersensitivity; in contrast, it does not result in pulp tissue irritation [9][11]. However, silver is a direct antibacterial agent because it interacts with the thiol groups of amino acids and nucleic acids to disrupt bacteria's metabolic and reproductive pathways that cause cell death. As SDF becomes more well-known as a cariogenic arresting agent, the medical world is becoming increasingly interested in its use [12].

Another material commonly used for desensitization and remineralization of dentin is casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) [13m14]. This material is made from the protein found in milk and contains a phosphoryl sequence, which contributes to the stability of amorphous calcium phosphate [15]. The mechanism of decreasing dentin hypersensitivity might be attributed to the remineralizing effect of CPP-ACP and the participation of hydroxyapatite crystals that occlude dentinal tubule openings[16,17]. Moreover, amorphous calcium phosphate is essential for decreasing dentin sensitivity [18]. The desensitization of dentin is also related to the combination of CPP-ACP, which binds to proteins and continuously deposits calcium and phosphate on exposed dental tubules [19].

Despite the availability of different products used for remineralization and reducing dentin hypersensitivity in the dental market, debate still exists about the superiority of a specific agent in desensitization and remineralization of dentin. Thereover, this in-vitro study investigated and compared the effect of SDF and CPP-ACP on the desensitization and remineralization of demineralized dentin surface. The null hypothesis tested was that there would be no difference in the outcome among the treatment groups.

MATERIALS AND METHODS

Specimens' preparation

Twenty-eight sound-extracted upper permanent premolars were collected from healthy individuals from the Orthodontic clinic, Faculty of Oral and Dental Medicine, Delta University for Science and technology, Gamasa, Egypt. The Faculty of Dentistry Ethics Committee approved this in vitro study under protocol number 0240221002. Tissue remnants were removed using a hand scaler (Zeffiro, Lascod, Florence, Italy), and teeth were then disinfected for 72 hours by storing them in 1% chloramine-T solution [20]. The chosen teeth's crown measurements, obtained via digital calibration, were roughly 9.0 mm to 9.6 mm for bucco-lingual length, 7.0 mm to 7.4 mm for mesio-distal width, and 7.7 mm to 8.8 mm for cervico-occlusal height[21]. The teeth were fixed in acrylic resin blocks and cut horizontally into discs by an automated diamond saw (Isomet 4000, Buehler Ltd., Lake Bluff, IL, USA) under water cooling system. Only one disc was obtained from the middle of the crown of each specimen with a thickness of 2 mm. Then, enamel-covered border of each disc was removed, and dentin surface was polished with microfine 4000-grit abrasive papers.

Study design

The specimens were divided into four groups (n=7). In group I, dentin discs were immersed in 17% EDTA for 5 minutes in order to remove the smear layer and open the dentinal tubules [22]. In group II, III, and IV, dentin discs were immersed in 17% EDTA for 72 hours for demineralization of the dentin [23]. Then, the discs in group III were treated with SDF (Toothmate, Mansoura, Egypt) for 90 seconds, while group IV discs were treated with CPP-ACP (MI Paste Plus, GC, Tokyo, Japan) for 5 minutes following the manufacturers' instructions.[24]. All groups were then washed and stored in artificial saliva for 72 hours [25].

Specimen analysis

The dentin discs were dried, coated with gold, and examined using a scanning electron microscope (JSM-6510LV SEM, JEOL, Tokyo, Japana) at a magnification of 2000×. ImageJ (version 1.51r; NIH, Maryland, USA) software calibrated the SEM images to determine the number of opened dentinal tubules per area [26]. The dentin discs were then investigated using quantitative energy dispersive X-ray analysis (EDX) to assess the calcium levels in the dentin.

Statistical analysis

Data were tabulated and coded using (Microsoft Excel, 2016). The extracted data were analyzed using the Statistical Package for the Social Sciences (IBM-SPSS, version 24, Armonk, NY, USA). The normality of the data was statistically checked by Shapiro–Wilk test. A parametric analysis of variance (One-way ANOVA) followed by Tukey HSD post hoc tests were used for data analysis. The significance level was set at $p \leq 0.05$ within all tests.

RESULTS

Scanning electron microscopy (SEM) analysis

The dentin surface and dentinal tubules of the group I and II showed cleaned cut empty dentinal tubules (arrow head) surrounded by thin bright smooth layer of peritubular dentin and connected by intertubular dentin in a typical manner of normal dentin structure (fig. 1 A&B). In SDF group, nearly complete sealing of the dentin surface and complete dentinal tubule occlusion were observed (fig. 1C). In CPP-ACP group, partial dentinal tubule occlusion was observed (fig. 1D). The highest number of opened dentinal tubules was observed in group II followed by group I and group IV respectively. The SDF group had fewer opened dentinal tubules as presented in table 1. One-way ANOVA test showed that there was statistically significant difference between all groups ($P<0.0001$). The results of Tukey HSD post hoc test indicated a

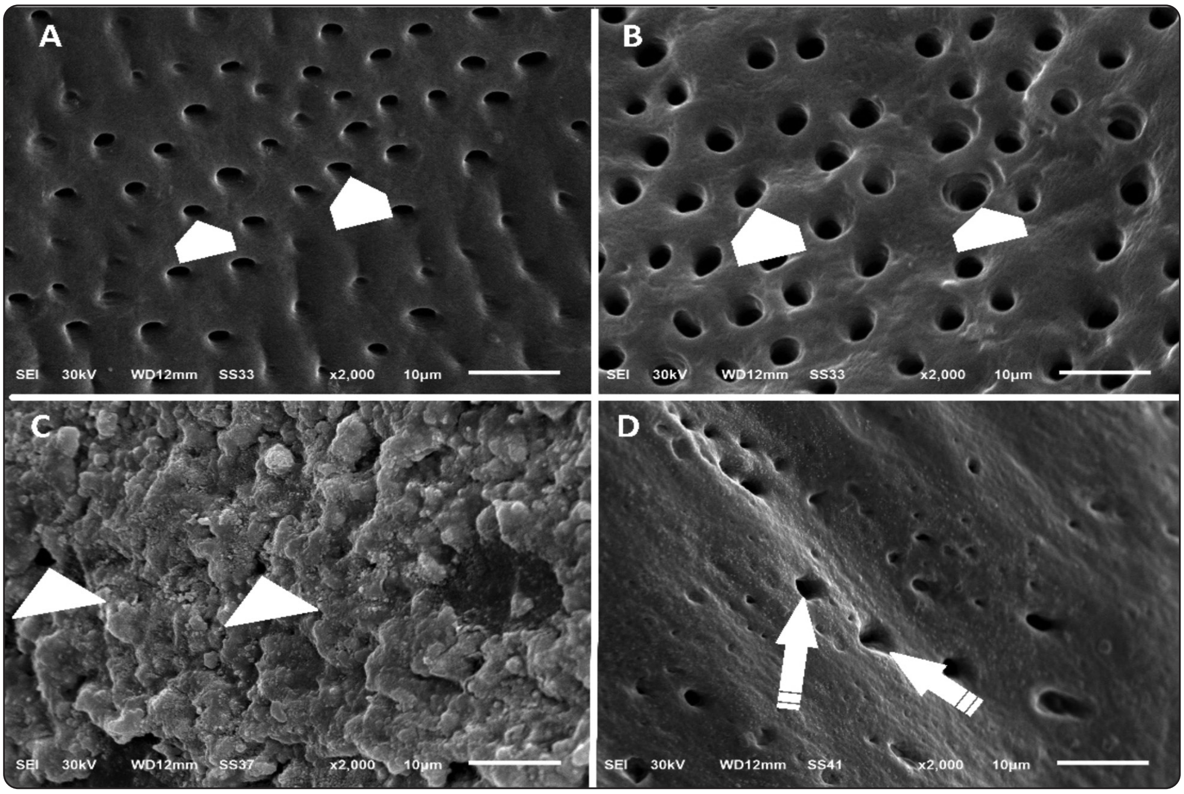

Fig. (1) : SEM photomicrograph of a horizontally cut dentin surface. (A) group I showed opened empty dentinal tubules (arrowhead) and a clean surface without a smear layer. (B) group II showed extremely opened dentinal tubules. (C) group III showed extreme dentinal tubule occlusion by SDF (arrowhead). (D) group IV showed surface coating by CPP–ACP with partial dentinal tubule occlusion (white arrow) (2000X).

statistically significant difference between group II and the remaining groups ($p < 0.05$). Moreover, a statistically significant difference was noted between SDF group and the three other groups ($p < 0.05$). Also, a statistically significant difference was found between the means of SDF group and CPP-ACP group ($p < 0.05$).

Energy dispersive X-ray spectroscopy (EDX) analysis

The mean value and standard deviation of calcium level of each group are presented in Table 1. There was a statistically significant difference among all four groups according to One-way ANOVA ($P < 0.0001$). The highest calcium level was observed in SDF group with a statistically significant difference when compared to the other groups ($p < 0.05$). On the other hand, the lowest calcium level was shown in the group II (14.47±1.26) with a statistically significant difference when compared to the remining three groups ($p < 0.05$). However, there was no statistically significant difference between CPP-ACP group and group I.

TABLE (1) Means and standard deviations (SDS) of opened dentinal tubules and calcium level in dentin for different groups (each, n=7)

Groups	Mean ± SD	
	Opened dentinal tubules	**Ca weight**
Group I	64.86 ±6.15	27.59 ±1.14
Group II	80.71 ±9.39^{a}	14.47 ±1.26^{a}
Group III	9.14 4±2.54^{ab}	35.34 ±1.37^{ab}
Group IV	21.86 ±4.10^{abc}	28.22 ±1.4^{bc}

The values are expressed as the means ± SDs.

The test was one-way ANOVA followed by post hoc Tukey's multiple comparison test.

a: Significance vs. group I, b: Significance vs. Group II, c: Significance vs. SDF at p < 0.05.

DISCUSSION

Remineralization is considered one of the most acceptable methods for the early management of incipient carious lesions following the principles of preventive dentistry [27]. Both of the tested materials in this study had high potential for use as remineralizing agents and for reducing dentin permeability[17,22,25]. However, SDF group showed extreme occlusion of dentinal tubules in addition to the higher calcium level in comparison to the CPP–ACP group. Therefore, the null hypothesis formulated at the beginning of the study was rejected.

Menzel et. al. showed that SDF can occlude the dentinal tubule by participating in silver particles on the dentin surface[28]. Another study showed that the ability of SDF to penetrate the dentinal tubule reaches 500 µm for both types of SDF formulations either solution or gel formulation used in this study[22]. There is scarce of information regarding comparing the effect of CPP-ACP and SDF on dentinal tubule occlusion. Nevertheless, compared with other desensitized materials, such as 8% arginine, which gives a significantly increased dentinal tubule occlusion more than CPP-ACP [24]. Another study by Golriz et al. compared the durability of CPP-ACP to that of sodium fluoride varnish as a desensitizing agent. The study concluded that sodium fluoride had the most rapid effect and was more durable after 30 days than CPP-ACP [29]. The significant difference between the Casein and SDF groups may be due to short-term usage of CPP-ACP; however, it could be more effective for long-term usage [17].

The dentin treated with SDF had a greater remineralization effect on calcium weight than the CPP-ACP-treated dentin. As mentioned, statically significant differences have been observed when SDF group was compared to other tested groups.

It is necessary to know that three conditions are essential for increasing dentin mineralization: primarily, residual crystals from minerals act as growth centers. Second, a mineralization source containing calcium and phosphorus should be found. Finally, the collagen structure must be sturdy to serve as a framework for advancing mineral crystals [8]. This led to the suggestion that fluoride ions added to SDF increase the uptake of calcium found in saliva [30]. Additionally, Mei et al. reported that 38% SDF can remarkably inhibit the degradation of collagen fibers [8]. However, Vinod et al. also agreed with our results, as they compared SDF with CPP-ACP and found that SDF had a greater remineralization effect than CPP-ACP [25]. Another suggestion for increasing calcium levels in the SDF group was the formation of calcium fluoride, which is responsible for caries prevention and carious lesion hardening [31]. This suggestion was further confirmed when Lou et al. mixed hydroxy apatite powder with SDF and obtained calcium fluoride-like material and metallic silver [32]. Also, the calcium level did not increase in the Casein group, unlike in the SDF group, which may be due to casein itself inhibit the mineral deposition on enamel surface [33].

Finally, conservative dentistry uses many materials for desensitization and remineralization. Because of the favourable uses of CPP-ACP on the tooth as the natural effect, it did not affect the dentin surface.

CONCLUSION

It can be concluded that the application of SDF had a positive impact on dentin desensitization by occlusion the opened dentinal tubules higher than CPP-ACP. Moreover, SDF had a greater capacity for dentin remineralization. **Abbreviation**

SDF: silver diamine fluoride; FDA: Food and Drug Administration; CPP-ACP: casein phosphopeptide-amorphous calcium phosphate-containing crème; SEML scanning electron microscope; EDX: energy dispersive X-ray; SD: standard deviation.

Declarations

Ethical approval and consent to participate

Approval was obtained from the Ethical Committee, Faculty of Dentistry, Delta University for Science and Technology (DU:0240221002).

Consent for publication

Not applicable

Availability of data and materials

The data sets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The author declares that they have no competing interests.

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Authors' contributions

MA performed the practical work, wrote, and edited the manuscript, and revised the statistics. AA helped in the practical work and edited and revised the manuscript. AE discussed the practical steps and revised the manuscript. All authors read and approved the final manuscript.

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