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REMINERALIZATION OF PERMANENT MOLAR ENAMEL EARLY CARIOUS LESIONS VIA SILVER DIAMINE FLUORIDE AND SODIUM FLUORIDE VARNISH IN VITRO

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ABSTRACT

Introduction: The choice of enamel early carious lesion best remineralizing agent shows great debate. This study aimed to evaluate the remineralizing effect of silver diamine fluoride (SDF) and sodium fluoride (NaF) varnishes on early carious lesions of permanent molars enamel.

Materials and methods: Twenty non-carious permanent molars were divided into two equal groups 1 and 2. A square shape in the center of the buccal surface of enamel (3x3 mm) subjected to the demineralizing solution (sodium carboxy-methylcellulose gel with lactic acid buffer at PH: 4.8) for 4 weeks creating artificial carious lesion (demineralization cycle). Both SDF and NaF varnishes were applied on carious lesions and preserved in formalin (10%) for 4 weeks (remineralization cycle). The teeth were evaluated after each cycle by scanning electron microscope (SEM) and electro-dispersive spectroscopy (EDS) analysis.

Results: Silver diamine fluoride showed significantly better surface remineralization by SEM images in comparison with NaF varnish. Both materials showed a significant increase in fluoride mineral content on the enamel surface. On the other hand, SDF showed a significant silver increase in comparison with (ca & p) of NaF varnish as a remineralizing agent by EDS analysis.

Conclusion: Silver diamine fluoride and NaF varnishes can remineralize early carious lesions with better enamel remineralization by silver ions of SDF.

KEYWORDS: Silver diamine fluoride, Fluoride varnish, Enamel remineralization.

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INTRODUCTION

Dental care showed a great advancement through the last few decades but, dental caries is still a world health problem affecting children and adults. Almost half of the world's population experience dental carious lesions, making it the most prevalent disease of all health conditions. Everyone is at risk of caries, but children and adolescents have the higher probability. It affects teeth over time by acid by-products which in early stages show no symptoms [1].

Contemporary caries management is focused on creating medical management of disease progression. After the caries process has been halted or arrested, causative factors need to be evaluated and each individual treatment regimen installed to prevent the occurrence of new caries disease. For successful non-invasive management, the lesions have to be detected as early as possible so, they can be handled in a non-operative way [2].

Many clinical trials confirmed the efficacy of fluoride varnishes in the prevention of caries [3]. Fluoride ions promote the remineralization of the tooth surface by inhibiting the action of bacterial enzymes and have the ability to precipitate inside enamel prisms in place of calcium and phosphate, transforming the hydroxyapatite to fluoro-hydroxyapatite, a more resistant phase to acid attack [4]. Sodium fluoride (NaF) is a commonly used fluoride varnish in combination with a calcium phosphate-based delivery system that promotes remineralization and improves fluoride uptake [5].

Silver has been utilized for many years due to its antimicrobial effect and in SDF provided in combination with fluoride. A literature review explains through various studies that SDF can be a medical modality of managing and preventing dental caries [6, 7]. The study aimed to evaluate the remineralizing effect of silver diamine fluoride and sodium fluoride varnishes on the early carious lesion of permanent molar enamel by SEM & EDS.

MATERIALS AND METHODS

The research had been approved by the ethical committee of scientific research of faculty of dentistry (Mansoura University) with the code number: 02051217. Twenty non-carious permanent molars were collected from private dental clinics in Mansoura city (the cause of permanent molars extraction was due to periodontal disease). The sample size of this study was determined according to a previous study Mohd Said et al. [8]

Teeth preparation

The teeth were preserved in formalin (10%) until the samples for the study were collected, then properly cleaned by ultrasonic scaler and examined by the magnifying lens for cracks, decay, or enamel defects. The teeth were divided randomly into two equal groups of 10. Another examiner decides blindly the groups 1 (SDF group) and 2 (NaF varnish group). All teeth were cut mesiodistally, covered with adhesive tapes with dimensions (3×3 mm) were placed on middle third of buccal surface. Two acid resistance nail varnishes of a different color (red and blue) for groups 1 & 2 in the same order. The adhesive tapes are then removed.

Demineralization cycle

Each group was immersed separately in sodium carboxymethylcellulose gel with lactic acid buffer at ph. (4.8) for 4 weeks at room temperature. The buffered gel was changed at two weeks intervals to prevent saturation of the gel. Teeth were removed from the gel and then rinsed with distilled water and finally dried with air. The nail varnish coat was removed using acetone and the prepared teeth. All samples were examined visually and showed white spot lesion formation on center of buccal surface.

Remineralization cycle:

The two varnishes, SDF (Riva Star, SDI, Australia) (group 1) and NaF varnish combined with amorphous calcium phosphate (group 2) (Enamel Pro varnish-Premier Dental-USA) were applied on examined areas of buccal surfaces. Teeth were left without varnishes removal for 6 hours according to manufacturer instruction for (enamel pro) varnish. Silver diamine fluoride group was left for the same amount of time for standardization. Varnishes excess were removed by 5 strokes of toothbrush in cervico-occlussal direction. Teeth were preserved in formalin (10%) for 4 weeks.

Scanning electron microscope & electro-dispersive spectroscopy

All teeth were evaluated by SEM (JEOL-JSM-6510LV) & EDS analysis (Oxford Instruments' x-max 20) (Figure 1 & 2) after each cycle (demineralization and remineralization). To describe the surface characterization of SEM images of enamel, a scale was designed in table (1) as follows:

Statistical analysis and data interpretation

Data were fed to the computer and analyzed using IBM SPSS software package version 22.0. Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) for non-parametric data and mean, standard deviation for parametric data after testing normality using Shapiro–Wilk test. Significance of the obtained results was judged at the (0.05) level.

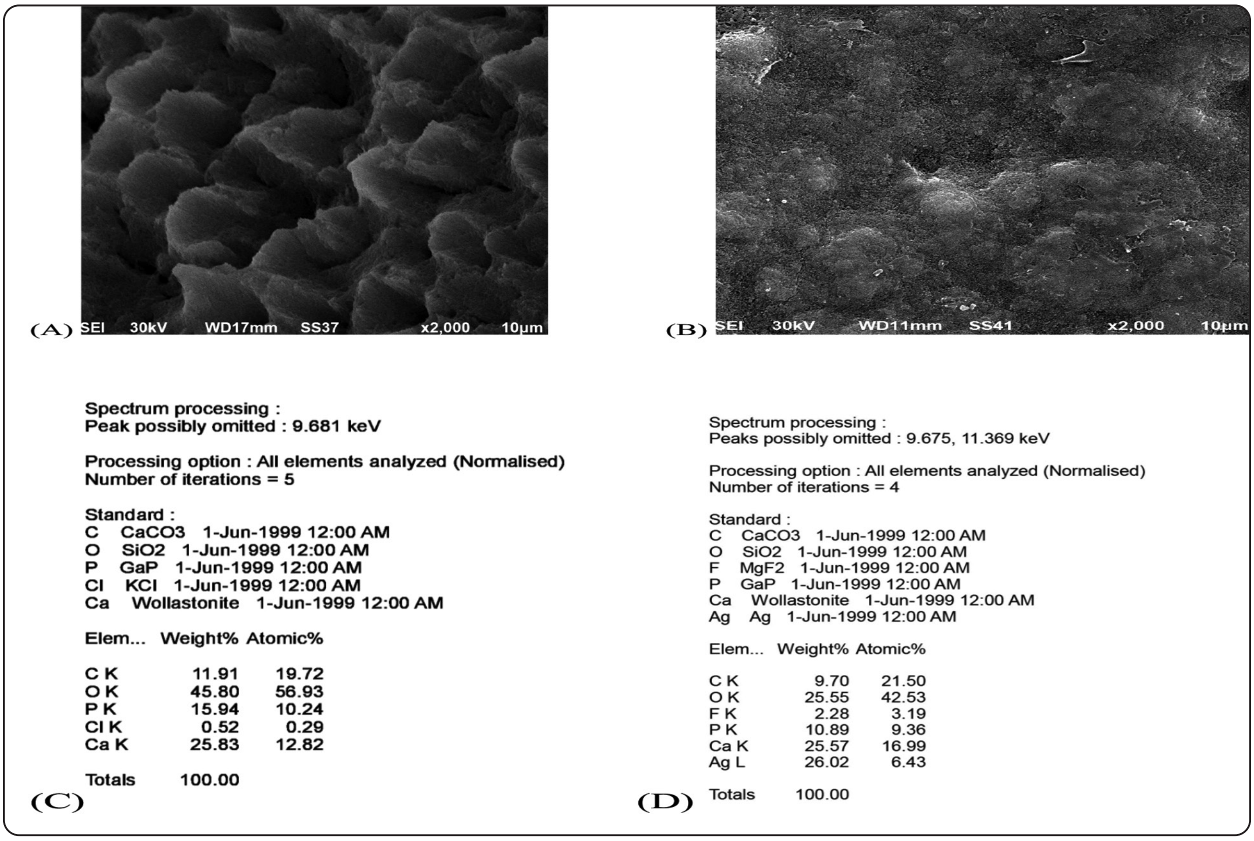

Fig. (1) Group 1 (A) SEM image of demineralized enamel (B) SEM image of remineralized enamel of by SDF (C) EDS of demineralized enamel (D)EDS of remineralized enamel by SDF.

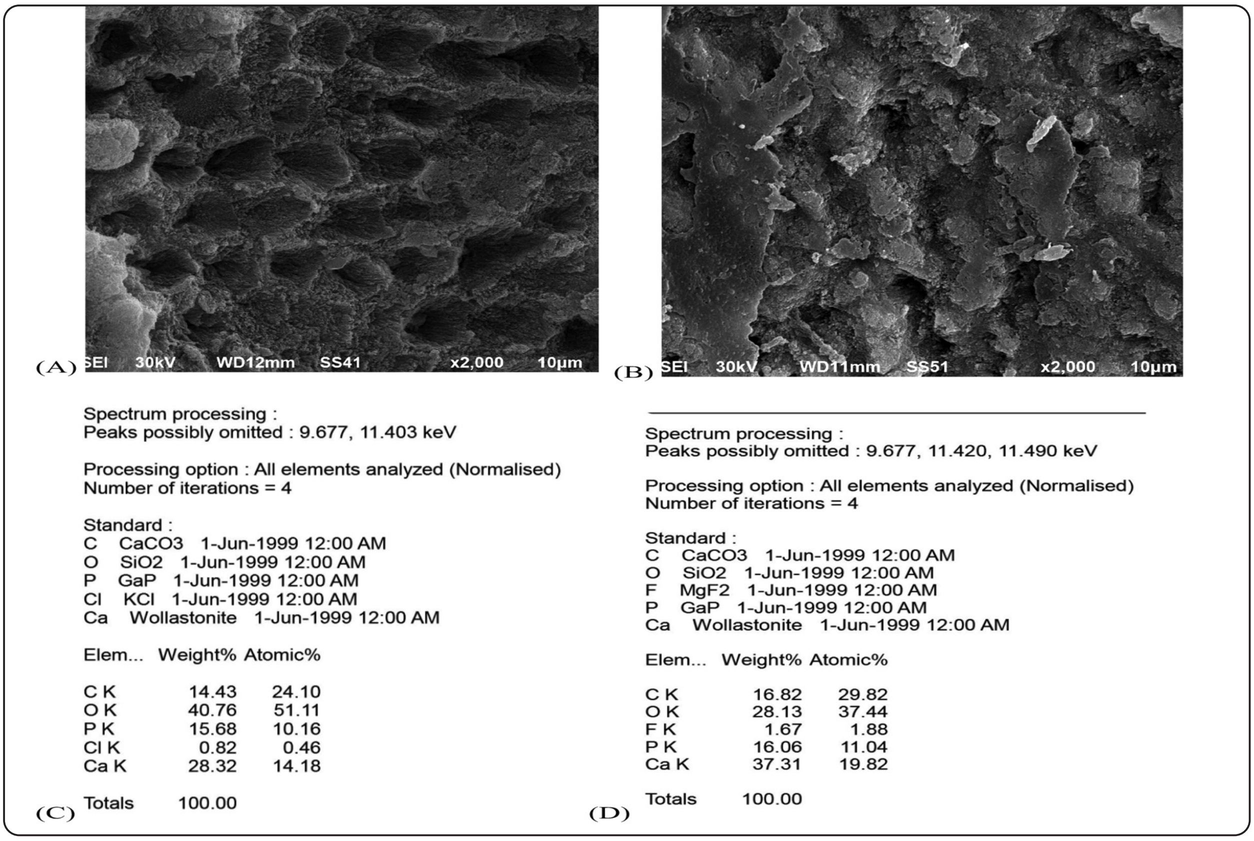

Fig. (2) Group2: (A) SEM image of demineralized enamel (B) SEM images remineralized enamel by NaF varnish (C) EDS of demineralized enamel (D) EDS of remineralized enamel by NaF varnish.

SDF	Silver diamine fluoride
SEM	Scanning electron microscope
Ca	Calcium
NaF	Sodium fluoride
EDS	Electro-dispersive spectroscopy
P	Phosphorus

TABLE (1) SEM images scale for enamel demineralization and remineralization:

0	Normal enamel surface with normal enamel prisms morphology (9).
1	Demineralized enamel surface porosity with open prisms appear in a normal structure (with craters, cracks or not) (9-11).
2	Demineralized enamel surface porosity with deformity in prisms structure (with wide cracks, craters, or not) (9-11).
3	Remineralized enamel surface without prisms closure (presence of mineral particles attached to surface not closing enamel prisms) (12-14).
4	Remineralized enamel surface with partial prisms closure (mineral particles not only attached to surface but also closing some of the prisms) (12-14).
5	Incomplete enamel surface coverage with mineralizing agent (most of the prisms are closed with mineral content, cracks are minimal or absent (12-14).
6	Complete enamel surface remineralization (all prisms are completely remineralized with absence of craters or cracks).

RESULTS

Enamel SEM images of demineralization and remineralization process:

Table 2: showed that no significant difference in surface characterization of demineralized enamel in the two groups. After remineralization, there was a significant improvement in surface characterization in group 1(P=0.004) and group 2 (P=0.002) with better surface remineralization in group 1 with significant difference (p=0.001).

TABLE (2) Enamel surface demineralization and remineralization scale results:

Demineralization and remineralization score	SDF N=10(%)	NAF N=10(%)	Chi-Square test
After demineralization			
1	5(50.0)	7(70.0)	P=0.16
2	5(50.0)	3(30.0)	
Mean±SD	1.5±0.53	1.3±0.48	
After remineralization			
3	0	2(20.0)	p=0.01*
4	0	5(50.0)	
5	10(100.0)	3(30.0)	
Mean±SD	5.0±0.0	4.10±0.74	
Stewart Maxwell test	SM P=0.004*	SM P=0.002*	

Fluoride content

Electro-dispersive spectroscopy analysis of SEM images showed that fluoride content (table 3) was significantly increased in the two groups but no significant difference between the two groups.

Mineralizing elements other than fluoride

In group, 1 silver showed a significant increase with P=0.005 but on the other hand, calcium and phosphorus show no significant increase with a percent of change of 14.5% and 4.6 in the same order. The correlation between these minerals and fluoride in each group showed that increasing Ca &P increase fluoride content in remineralized enamel with P=0.03 but increasing silver doesn't correlate with increasing fluoride content.

TABLE (3) Fluoride content change by SDF and NaF varnishes :

Fluoride content	SDF N=10	NAF N=10	Mann Whitney U test
Before remineralization Median (Min-Max)	0.0(0.0-0.0)	0(0-0)	p=1.0
After remineralization Median (min-max)	2.98 (0.79-10.03)	2.14 (0.16-4.95)	Z=1.36 P=0.17
Wilcoxon signed rank test	z=2.80 p=0.005*	z=2.80 p=0.005*	

DISCUSSION

The biological treatment as a modality of managing early carious lesions has been the treatment of choice for the previous two decades through various materials like NaF varnishes and SDF. Selection of permanent molars because of higher susceptibility to decay at a very young age like MIH especially first permanent molar and absence of hard evidence of caries arrest in permanent teeth [15]. Use of teeth sectioning to allow the flat surface to fit SEM stub and avoid the use of enamel slices to prevent heat effect on enamel surface characterization.

Scanning electron microscope (SEM) and EDS were used to provide three-dimensional images of surface and subsurface layer and evaluate the change of mineral content as a result of both remineralizing agents. Silverstone et al, (1976) divided its demineralized enamel according to topography into five types that resemble only different shapes after demineralization but don't provide severity of destruction to enamel [16]. In this study, we designed a scale to give numerical evaluation describing images according to the degree of defect on enamel and degree of remineralization. Significant surface topographic improvements were provided by SDF & NaF varnishes with a significant upper hand in surface characterization after remineralization delivered by SDF.

Regarding fluoride content, both materials (NaF & SDF) showed a significant rising in fluoride providing an effective remineralization process, and similar findings were provided by Lou et al (2011) and Majithia et al (2016) [17,18]. Lou et al, found that adding SDF to hydroxyapatite provides mineralization with fluoride forming CaF2 but it washes out soon. In our study, EDS illustrated that fluoride remains in the enamel surface in the form of MgF2 [17].

Regarding other mineralizing elements, silver (group 1) manifested a significant remineralizing effect which coincide with the results of savas et al (2016) [19]. On the other hand, Ca & P (group 2) illustrated no significant rise in mineral content but showed a significant correlation with increasing fluoride uptake which supports considering Ca & P as a vehicle [5]. The limitation of this study show through not resemble the dynamic process in the oral cavity.

CONCLUSION

Silver diamine fluoride and sodium fluoride varnish can be considered a treatment modality of early carious lesion of permanent teeth with higher remineralization and better improvement of surface topography by SDF due to higher remineralization effect by silver ions.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

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