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وحماية المستهلك

IMPACT OF AMMONIUM HEXAFLUOROSILICATE ON REMINERALIZATION OF ROOT CARIES

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ABSTRACT

Ammonium hexafluorosilicate solution has gained attention as a fluoride-based compound potentially preventing dental caries and treating dentin hypersensitivity without discoloration. This study aimed to evaluate ammonium hexafluorosilicate's efficacy in remineralizing demineralized human dentin compared to sodium fluoride and deionized water. Fifty-seven sound, permanent human maxillary first premolars collected from Iraqi patients, two teeth for Scanning Electron Microscopy and the remaining divided randomly into 5 groups: control (-ve) represented by deionized water, control (+ve) represented by 2% sodium fluoride, and three study groups with different concentrations 10000, 15000, 20000 ppm of ammonium hexafluorosilicate respectively, one tooth from each group selected for Scanning Electron Microscopy and the other for Energy Dispersive Spectroscopy. The teeth were tested for baseline reading, after demineralization and after treatment with selected concentrations of ammonium hexafluorosilicate, sodium fluoride, and deionized water according to their group's design by using Energy dispersive Spectroscopy in al-Khora company in Baghdad city. Data were analyzed by using Shapiro Wilk, Repeated Measure Analysis of Variance (ANOVA) with Tukey's HSD. There was a significant increase in the weight percentage of (calcium, phosphorous, and fluoride) in the treatment stage as compared with the demineralized dentin in all the study groups. the 20000-ppm group, exhibited the highest increase in these key remineralization elements, confirming previous Scanning Electron Microscopy findings. Compared to the demineralized state, Energy Dispersive Spectroscopy analysis showed dentin treated with ammonium hexafluorosilicate exhibited a significant increase in the weight percentage of key remineralization elements: calcium, phosphorus, and fluoride. The highest positive change was observed in the group treated with 20,000 ppm ammonium hexafluorosilicate; these are supported by Scanning Electron Microscopy findings.

Keywords: Ammonium hexafluorosilicate, Energy Dispersive Spectroscopy, human demineralized dentin, root caries.

تأثير فلوروسيليكات الامونيوم على إعادة التمعدن في تسوس جذور الاسنان

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الخلاصة

لقد جذبت مادة فلوروسيليكات الامونيوم الانتباه كمادة قائمة على الفلورايد والتي يحتمل أن تمنع تسوس الأسنان وتعالج فرط حساسية العاج دون إحداث تلون الأسنان .هدفت هذه الدراسة إلى تقييم فعالية فلوروسيليكات الامونيوم في تعويض المعادن في العاج البشري منزوع المعادن مقارنة بفلوريد الصوديوم والمياه منزوعة الأيونات. تم استخدام 57 سن من الضواحك الأولى العلويه تم جمعها من مرضى عراقيين. تم تخصيص سنين لفحص المجهر الإلكتروني الماسح بينما تم تقسيم الباقي بشكل عشوائي إلى 5 مجموعات:مجموعة ضابطة سالبه, مياه منزوعة الأيونات, مجموعة ضابطة موجبة فلوريد الصوديوم بتركيز2٪,ثلاث مجموعات دراسة بتراكيز مختلفة 10000 و15000 و20000 جزء بالمليون من مادة فلوروسيليكات الامونيوم على التوالي. تم اختيار سن واحد من كل مجموعة لفحص المجهر الإلكتروني الماسح والآخر لفحص طيفية الأشعة السينية المبعثرة. تم أخذ قراءات الأساس قبل نزع المعادن وبعد المعالجة بالمحاليل المخصصة وفقًا لتصميم المجموعة باستخدام طيفية الأشعة السينية المبعثرة في شركة الخوره في مدينة بغداد. تم تحليل البيانات باستخدام اختبار Shapiro Wilk وتحليل التباين ذو المقاييس المتكررة (ANOVA) مع اختبار Tukey's HSD. أظهرت جميع المجموعات المدروسة زيادة ملحوظة في النسبة المئوية لوزن (الكالسيوم والفوسفور والفلور) في مرحلة العلاج مقارنة بالعاج منزوع المعادن في جميع المجموعات الدراسية. أظهرت مجموعات فلوروسيليكات الامونيوم، وخاصة مجموعة 20000 جزء بالمليون، أعلى زيادة في هذه العناصر الأساسية لتعويض المعادن وهذا اكد النتائج السابقة لفحص المجهر الإلكتروني الماسح. تشير هذه الدراسة إلى أن فلوروسيليكات الامونيوم قد يعزز تعويض المعادن في العاج البشري منزوع المعادن بشكل أكثر فعالية من صوديوم الفلورايد والمياه منزوعة الأيونات، مع ملاحظة أفضل النتائج عند تركيز 20000 جزء بالمليون .وقد دعمت نتائج المجهر الالكتروني الماسح هذه النتيجه.

الكلمات المفتاحية: فلوروسيليكات الامونيوم ,طيفية الأشعة السينية المبعثرة ,عاج بشري منزوع المعادن, تسوس جذر السن.

INTRODUCTION

Dental caries, commonly known as cavities, are a widespread problem affecting millions worldwide **(Alwaheb &Alhuwaizi, 2018; Muslim, 2018)**. It is defined as "progressive, irreversible microbial disease of multifactorial nature affecting the calcified tissue of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic portion of the tooth" **(Marya, 2011;Mutlak &Yas, 2017;Al-Ward &Radhi, 2023).**

As populations live longer and retain their teeth more with the possibility of increasing periodontal diseases, they are more likely to develop root caries, this type of caries develops at the exposed root site, most root caries begin in the cervical portion of the root or at the exposed cementum-enamel junction **(Al-Mahmood *et al.*, 2020; Fathallh &Mahmood, 2021)**. Similar to coronal caries root caries results of demineralization-remineralization processes, the root surface exchange minerals with oral fluids, resulting in a higher mineral content in the surface layer **(Takahashi &Nyvad, 2016)**. This is one of the strategies that can be utilized to increase tooth hardness and resist dental caries. One of these minerals is fluoride **(DS, 2011)**, which forms a fluoride-rich surface layer that is acid-resistant and induces hardening of the tooth

surface **(Epple *et al.*, 2022)**. Ammonium hexafluorosilicate solution (AHF) emerges as a promising candidate in the fight against caries, attracting the attention of researchers in Japan and the USA **(Suge &Matsuo, 2013)**. Its effectiveness stems from its dual action: providing fluoride for remineralization and forming a silica-calcium phosphate precipitate that occludes exposed dentin tubules, potentially reducing dentin hypersensitivity **(Hosoya *et al.*, 2013)**.

The goal of the present study was to examine changes in the surface minerals of artificially demineralized root dentin of sound permanent teeth after AHF treatment.

MATERIALS AND METHODS

The Sample

Fifty-seven sound, permanent human maxillary first premolars collected from Iraqi patients, between the ages of 12 and 18 years, which had been extracted for orthodontic purposes. by using a cumine scaler, any soft tissue remnants on the root surface were removed until became free from organic material, and polished by using a handpiece and rubber cup with a non-fluoridated pumice. Then, placed in a plastic container with screw cup with de-ionized water, to which 0.1% crystals of thymol were added, to avoid bacterial growth. Teeth samples were reserved in the fridge at 4˚C till use to avoid dehydration of teeth **(Parry *et al.*, 2001).**

The experimental design:

Teeth were divided as follows: two teeth were tested using Scanning Electron Microscope (SEM) for examination of sound and demineralized dentin (after pH cycling), while the other fifty-five teeth were randomly divided into five groups as follows:

1. The (-ve) control group received none of the study agents (deionized water).
2. The (+ve) control group have received 2% sodium fluoride (NaF).
3. The group of 10000 ppm of AHF.
4. The group of 15000 ppm of AHF.
5. The group of 20000 ppm of AHF.

Every group consist (n=11) teeth, one for Scanning Electron Microscope examination (SEM) while the remaining (10) for Energy Dispersive Spectroscopy (EDS).

Dentin surface preparation:

The buccal surface of each tooth was polished by using 600, grit silicon carbide sandpaper adjusted on grinding machine at a low speed or 15 seconds funder flooding water **(Soares *et al.*, 2017)**. This technique was used to produce a flat tooth surface **(de Oliveira *et al.*, 2007)**. To define the experimental area a nail varnish used to paint the surfaces of the tooth except for a window sized 3×4 mm on the buccal surface of the root at the imaginary line that separate between cervical and middle third, on the buccal surface of the root which represent the experimental area **(Parry *et al.*, 2001)**.

Preparation of Ammonium hexafluorosilicate solution:

Three concentrations of the solution were made by dissolving the ammonium hexafluorosilicate powder in distilled water as follow:

- 10000 ppm ⟶ 2 g (AHF) in 200 ml deionized water.

- 15000 ppm ⟶ 3 g (AHF) in 200 ml deionized water.
- 20000 ppm ⟶ 4 g (AHF) in 200 ml deionized water.

Caries like lesion induction in dentin specimens:

To induce caries lesion on dentin surface, the pH cycling procedure was followed **(Featherstone *et al.*, 1983; Featherstone, 2004; Hasan *et al.*, 2023)**. A demineralizing and remineralizing solutions were prepared as follow and then pH was adjusted.

A- Demineralizing solution comprised:
1) acetic acid (0.075 Mol/L)
2) calcium chloride (1.0 mMol/L)
3) potassium phosphate (2.0 mMol/L)
The pH was adjusted to 4.3 by pH meter at 37˚C.
B- Remineralizing solution comprised:
1) 150 mMol/L potassium chloride
2) 1.5 mMol/L calcium nitrate
3) 0.9 mMol/L potassium phosphate
The pH was adjusted to 7, at 37˚C.

The pH cycling of each tooth was done by dipping separately in 20 ml of demineralizing solution and placed for 6 hrs. at 37˚C in the incubator. Then, each tooth was withdrawn and washed for two minutes with running de-ionized water. After that, the teeth samples were dipped separately in 20 ml of remineralizing solution for 17 hours at 37˚C in the incubator and each tooth sample was withdrawn and washed for two minutes with running de-ionized water before repeating the cycle another time.

The forementioned four steps were repeated for ten days (once a day) and specimens were washed with de-ionized water, then examined using light microscope to detect if there were any microscopical changes related to caries development.

Study steps:

1. Ten teeth from each group were tested for (EDS) analysis (this represent the baseline).
2. All the teeth were undergoing PH cycling, at the eleventh day, teeth were tested for (EDS) analysis (except those of microscopical examination) this represents the reading after caries like lesion induction. SEM of sound dentin and after PH cycling were done.
3. All teeth of the study and control groups were treated according to their group's design; treated with selected concentration of AHF, NaF and deionized water for three minutes by submerged each tooth sample separately in 20 ml of the selected agent solution. Teeth were re-stored in de-ionized water for the following day at temperature of 37˚C in the incubator (this procedure was repeated every day for one week).
4. Finally, at the eighth day, samples were examined again for EDS, this represents the reading after treatment and SEM test was done for teeth selected for microscopical examination.

Energy Dispersive Spectroscopy (EDS)

The weight percentages (wt%) of Calcium (Ca), Phosphorus (P), Oxygen (O) and fluoride (F) were measured using EDS as it's an elemental analysis examination or chemical characterization of a sample. (Ju *et al.*, 2010). Both tests SEM and EDS were done in Al-khora company.

Scanning Electron Microscope (SEM)

SEM was used to examine the morphological changes occur in dentin surface with a focused beam of electrons to scan the surface **(Klein *et al.*, 2012;Shubbar *et al.*, 2023)**. The teeth were coated with gold by a coating machine (NGSTROM ADVANCED, ion sputter) to improve the imaging of samples **(Pretorius, 2011;Hamdia *et al.*, 2023).**

STATISTICAL ANALYSIS

Data description, analysis and presentation were performed using Statistical Package for social Science (SPSS version -22, Chicago, Illionis, USA), minimum, maximum, mean and standard deviation, Shapiro Wilk test, Repeated Measure One Way ANOVA with Tukey HSD.

RESULT AND DISCUSSION

Energy dispersive spectroscopy (EDS) analysis:

Repeated measure ANOVA test was used to compare between the weight percentage of the minerals at the three stages and there were statistically significant differences in weight percentage of calcium and phosphorous, oxygen and fluoride among the three stages in each group (table 1), while, no statistically significant differences were found among groups at either the baseline or after demineralization. But groups' differences in the treatment stage were statistically significant.

Table (1) Descriptive and statistical test of EDX among groups and stages.

Variables.		10000		15000		20000		NaF		D.W		F	P value
		Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD		
Calcium	Baseline	33.840	0.980	34.002	0.912	34.290	1.708	35.320	1.527	33.820	1.069	2.383	0.065
	Demin.	27.190	1.850	28.155	0.862	26.680	1.404	27.510	1.350	26.900	1.147	1.793	0.147
	Treat.	30.310	1.219	31.810	2.033	33.865	1.674	29.600	1.102	24.300	2.394	41.461	**0.000**
F		83.850		66.264		131.398		119.421		145.174			
P value		**0.000**		**0.000**		**0.000**		**0.000**		**0.000**			
Effect size		0.844		0.857		0.868		0.792		0.751			
Phosphorous	Baseline	14.810	0.526	15.310	0.384	15.240	0.405	15.240	0.395	14.860	0.860	1.891	0.128
	Demin.	10.420	2.051	11.310	1.338	12.540	1.911	11.220	1.129	12.410	0.451	1.523	0.057
	Treat.	12.970	2.446	13.845	0.920	15.170	0.799	13.310	1.073	10.350	0.910	16.489	**0.000**
F		49.515		39.223		14.383		43.948		60.698			
P value		**0.000**		**0.000**		**0.000**		**0.000**		**0.000**			
Effect size		0.666		0.692		0.734		0.641		0.395			
Oxygen	Baseline	50.300	1.130	50.480	1.060	49.560	1.852	48.700	1.650	49.820	1.247	2.444	0.060
	Demin.	59.130	2.268	57.815	1.026	60.280	3.614	58.720	0.899	60.090	1.102	2.415	0.063
	Treat.	53.350	.344	51.255	1.511	49.465	2.193	52.330	2.121	63.960	2.561	89.282	**0.000**
F		93.022		61.851		134.914		120.574		299.445			
P value		**0.000**		**0.000**		**0.000**		**0.000**		**0.000**			
Effect size		0.738		0.809		0.932		0.846		0.738			
Fluoride	Baseline	0.550	0.303	0.618	0.204	0.600	0.149	0.790	0.296	0.780	0.230	2.048	0.104
	Demin.	1.100	0.327	1.140	0.560	1.300	0.205	1.400	0.163	1.490	0.269	2.465	0.058
	Treat.	1.690	0.285	1.990	0.357	2.700	0.170	2.100	0.323	1.222	0.268	35.747	**0.000**
F		72.050		102.317		238.989		94.716		26.167			
P value		**0.000**		**0.000**		**0.000**		**0.000**		**0.000**			
Effect size		0.766		0.823		0.916		0.812		0.543			

Tukey HSD test was used for multiple pairwise comparison between groups and stages of each mineral separately; in table (2), multiple comparison between calcium in the treatment stages, and there is a significant difference when comparing concentrations of AHF with D.W at p level 0.05, and between: (10000, 20000) and (20000, NaF).

Table (2) Multiple pairwise comparison of **Calcium** weight percentage in the treatment stage using Tukey HSD.

Groups		Mean Difference	P value
10000	15000	-1.500	0.325
	20000	-3.555	**0.000**
	NaF	0.710	0.893
	D.W	6.010	**0.000**
15000	20000	-2.055	0.083
	NAF	2.210	0.053
	D.W	7.510	**0.000**
20000	NaF	4.265	**0.000**
	D.W	9.565	**0.000**
NaF	D.W	5.300	**0.000**

In table (3) the mean difference of weight percentage of calcium among stages by groups was presented. A significant difference is seen in the mean differences between all the stages at p value < 0.05, except between the baseline and treatment stage of 20000 ppm of AHF, non-significant difference was found.

Table (3): Multiple pairwise comparison of **calcium** among stages using Tukey HSD.

Groups	Stages		Mean Difference	p value
10000	Baseline	Demin.	6.650	**0.000**
		Treat.	3.530	**0.000**
	Demin.	Treat.	-3.120	**0.000**
15000	Baseline	Demin.	5.847	**0.000**
		Treat.	2.192	**0.003**
	Demin.	Treat.	-3.655	**0.000**
20000	Baseline	Demin.	7.610	**0.000**
		Treat.	0.425	0.999
	Demin.	Treat.	-7.185	**0.000**
NaF	Baseline	Demin.	7.810	**0.000**
		Treat.	5.720	**0.000**
	Demin.	Treat.	-2.090	**0.003**
D.W	Baseline	Demin.	6.920	**0.000**
		Treat.	9.520	**0.000**
	Demin.	Treat.	2.600	**0.000**

For the weight percentage of oxygen (O), significant differences were found in the treatment stage among all groups, except between (10000, 15000), (10000, NaF), (15000, 20000), (15000, NaF) non-significant differences were seen (table 4).

Table (4): Multiple pairwise comparison of **Oxygen** in the treatment stage using Tukey HSD.

Groups		Mean Difference	P value
10000	15000	2.095	0.121
	20000	3.885	**0.000**
	NaF	1.020	0.755
	D.W	-10.610	**0.000**
15000	20000	1.790	0.241
	NaF	-1.075	0.718
	D.W	-12.705	**0.000**
20000	NaF	-2.865	**0.013**
	D.W	-14.495	**0.000**
NaF	D.W	-11.630	**0.000**

Table (5) presents the mean difference of weight percentage of oxygen (O) among stages by groups. A significant difference is seen in the mean differences between all the stages at p value < 0.05, except between the baseline and treatment stage of (15000, 20000) ppm of AHF there were non-significant differences.

Table (5): Multiple pairwise comparison of **oxygen** among stages using Tukey HSD.

Groups	Time		Mean Difference (I-J)	p value
10000	Baseline	Demin.	-8.830	**0.000**
		Treat.	-3.050	**0.000**
	Demin.	Treat.	5.780	**0.000**
15000	Baseline	Demin.	-7.335	**0.000**
		Treat.	-0.775	0.759
	Demin.	Treat.	6.560	**0.000**
20000	Baseline	Demin.	-10.720	**0.000**
		Treat.	0.095	0.999
	Demin.	Treat.	10.815	**0.000**
NaF	Baseline	Demin.	-10.020	**0.000**
		Treat.	-3.630	**0.000**
	Demin.	Treat.	6.390	**0.000**
D.W	Baseline	Demin.	-10.270	**0.000**
		Treat.	-14.140	**0.000**
	Demin.	Treat.	-3.870	**0.000**

For the weight percentage of Phosphorous (P) in the treatment stage, table (6) show that there is a significant mean difference when comparing between the groups except between: 10000 with 15000 and NaF, also, between 15000 with 20000 and NaF there was a statically no significant differences The highest mean difference between the study groups was shown significantly between (10000, 20000).

Table (6): Multiple pairwise comparison of **Phosphorous** in the treatment stage using Tukey HSD.

Groups		Mean Difference	P value
10000	15000	-0.875	0.616
	20000	-2.200	**0.007**
	NaF	-0.340	0.981
	D.W	2.620	**0.001**
15000	20000	-1.325	0.215
	NAF	0.535	0.906
	D.W	3.495	**0.000**
20000	NaF	1.860	**0.032**
	D.W	4.820	**0.000**
NaF	D.W	2.960	**0.000**

Table (7) presents the mean difference of weight percentage of Phosphorous (P) among stages by groups. A significant difference is seen in the mean differences between all the stages at p value < 0.05, except between the baseline and treatment stage of 20000 ppm AHF there were non-significant differences.

Table (7): Multiple pairwise comparison of **Phosphorous** among stages using Tukey HSD.

Groups	Time		Mean Difference (I-J)	p value
10000	Baseline	Demin.	4.390	**0.000**
		Treat.	1.840	**0.001**
	Demin.	Treat.	-2.550	**0.004**
15000	Baseline	Demin.	4.000	**0.000**
		Treat.	1.465	**0.013**
	Demin.	Treat.	-2.535	**0.005**
20000	Baseline	Demin.	2.700	**0.000**
		Treat.	0.070	0.999
	Demin.	Treat.	-2.630	**0.003**
NaF	Baseline	Demin.	4.020	**0.000**
		Treat.	1.930	**0.001**
	Demin.	Treat.	-2.090	**0.024**
D.W	Baseline	Demin.	2.450	**0.000**
		Treat.	4.510	**0.000**
	Demin.	Treat.	2.060	**0.027**

Significant differences were found in the weight percentage of Fluoride (F) in the treatment stage among all groups, except between 15000 ppm AHF with each of (10000 ppm AHF and NaF) as non-significant differences were seen (Table 8).

Table (8): Multiple pairwise comparison of **Fluoride** in the treatment stage using Tukey HSD.

Groups		Mean Difference	P value
10000	15000	-0.300	0.154
	20000	-1.010	**0.000**
	NaF	-0.410	**0.021**
	D.W	0.468	**0.006**
15000	20000	-0.710	**0.000**
	NaF	-0.110	0.912
	D.W	0.768	**0.000**
20000	NaF	0.600	**0.000**
	D.W	1.478	**0.000**
NaF	D.W	0.878	**0.000**

Table (9) presents the mean difference of weight percentage of Fluoride (F) among stages by groups. A significant difference is seen in the mean differences between all the stages at p value < 0.05, except between the between demineralization and treatment stage of D.W there were non-significant differences.

Table (9): Multiple pairwise comparison of **Fluoride** among phases using Tukey HSD.

Groups	stage		Mean Difference	p value
10000	Baseline	Demin.	-0.550	**0.000**
		Treat.	-1.140	**0.000**
	Demin.	Treat.	-0.590	**0.000**
15000	Baseline	Demin.	-0.522	**0.000**
		Treat.	-1.372	**0.000**
	Demin.	Treat.	-0.850	**0.000**
20000	Baseline	Demin.	-0.700	**0.000**
		Treat.	-2.100	**0.000**
	Demin.	Treat.	-1.400	**0.000**
NAF	Baseline	Demin.	-0.610	**0.000**
		Treat.	-1.310	**0.000**
	Demin.	Treat.	-0.700	**0.000**
D.W	Baseline	Demin.	-0.710	**0.000**
		Treat.	-0.442	**0.000**
	Demin.	Treat.	0.268	0.090

Microscopic features of the outer enamel surface using SEM:

The goal of the SEM investigation was to assess the surfaces' topographic alterations of one representative sample from each group in addition to one sound tooth and one tooth after demineralization.

In the pattern of the sound dentin surface, SEM was showed that the dentin surface was covered by a uniform smear layer, with evidence of dentinal tubules opening; after demineralization, dentin surface appeared without smear layer, with the dentinal tubules

completely opened. Treating with 10000 ppm of AHF made the dentin surface covered with a uniform smear layer with some evidence of smear layer in the dentinal tubules entrance and some dentinal tubules were occluded with a thin layer of the precipitate, and some scattered precipitations on the surface as a white precipitate, this precipitation and occluding of dentinal tubules increased as the AHF concentration increase. For NaF, SEM showed irregular smear layer of precipitation with some dentinal tubules occluded completely with the precipitate on the surface. while for surfaces treated with D.W, the dentinal tubules still opened and there were no precipitations on the surface as shown in figure (1).

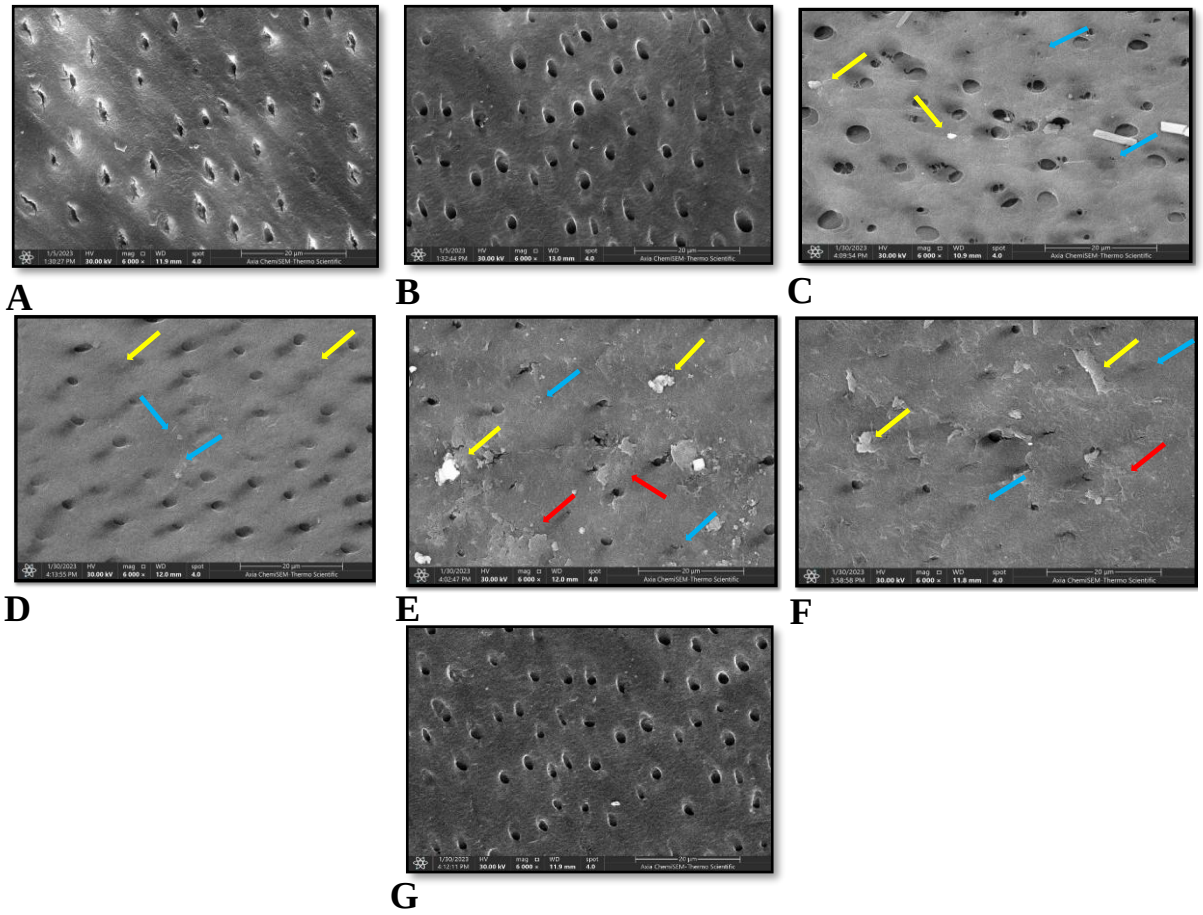

Figure (1): SEM image of dentin after treatment with selected agent comparing with sound and demineralized dentin, the blue arrows show the occluded dentinal tubule, while the yellow ones show the precipitation and the reds show the irregularities of the precipitate on the surface: (A) sound dentin; (B) dentin after demineralization; (C) 10000 ppm AHF; (D) 15000 ppm AHF; (E) 20000 ppm AHF; (F) 2% NaF; (G) deionized water.

Discussion for these findings:

A statistically non-significant differences in minerals' weight percentage for sound teeth and after artificial lesion formation were found among different groups, as there was standardization in the study (the same type of teeth used and the same tooth surface examined) and the same pH cycling method was used.

EDS analysis showed reduction in the weight percentage of calcium and phosphorous after demineralization when compared to the control, and was confirmed by SEM for demineralized dentin surface. But, EDS analysis for the fluoride demonstrated that the weight percentages increased after demineralization this may be due to the bonding of fluoride to fluorapatite crystals is firmer than that of calcium and phosphorous in hydroxyapatite crystals, therefore not easily wearing by pH cycling.

The reduction in minerals' weight percentage is an indication of artificial lesion initiation, since any drop in the environment's pH below a crucial pH (6–6.9) is indicative of the onset of a lesion **(Sung *et al.*, 2016;Neel *et al.*;Delgado *et al.*, 2016)** because of the acidic medium that causes outward movement of minerals of tooth mainly calcium and phosphorous leaving behind micropores **(Li et al., 2014).**

EDS, the finding of which showed that, in all the groups of AHF the weight percentage of calcium, phosphorous and fluoride were increased in the treatment stages as compared to the demineralization stage in addition to that, 20000 ppm of AHF showed the highest mean difference of weight percentage of these minerals between demineralization and treatment stage followed by 15000 and 10000 ppm AHF whereas NaF showed the lowest mean difference and this is supported by SEM findings

The explanation is that fluoride ions from AHF react with the calcium ions liberated after demineralization of apatite crystals in dentin surfaces to form calcium fluoride (CaF_2) or fluorapatite that will precipitate in the porous demineralized surfaces of caries like lesion in dentin surfaces **(Dorozhkin &Epple, 2002)**. In addition to that, AHF is an acidic solution with a pH below 3.4, therefore it demineralized the tooth surface preceding re-precipitating calcium and phosphate as a silica-calcium phosphate precipitate. Silica compounds also cause apatite from artificial saliva and simulated bodily fluids **(Suge *et al.*, 2010;Gelmboldt *et al.*, 2018)**. AHF solution seems to selectively react with peritubular dentin, so, if the silica content of dentinal tubular precipitates is increased, further mineralization of the dentin surface may be expected **(Takagi *et al.*, 1992).**

The EDS analysis of NaF revealed that there was an increase in the mean weight percentage of calcium, phosphorous and fluoride as compared to demineralization stage. The dentin surface treated with NaF was showed irregular smear layer of precipitation with some dentinal tubules occluded completely with the precipitate on the surface as demonstrated by the SEM this attributed to the reprecipitation of minerals to the surface.

Regarding control group (DW), EDS finding showed that weight percentage of calcium, phosphate and fluoride decrease in the treatment stage as compared to demineralization and all these showed a statistically significant difference except the fluoride which showed non-significant difference, this may explained by that when the teeth after pH cycling were stored in DW, they continue in losing of minerals because DW contain no

minerals to enhance remineralization of calcium and phosphate, while fluoride is not easily dissociated.

EDS analysis for the oxygen, demonstrated that the weight percentages increased and decreased in adverse direction to that of calcium, phosphorous and fluoride, which is difficult to be explained because the exact chemical composition of dentin crystals that would be formed after application of different mineralizing agents is not exactly known. However, since the EDS analysis was used to express the elements in dentin surface in weight percentage, therefore the increase in percentage of one element would affect the percentages of other elements in the chemical composition of dentin in order to achieve balanced composition and the total percentage that does not exceed 100%.

CONCLUSION

EDS analysis revealed an increase in the weight percentage was noticed for calcium, phosphorous and fluoride of dentin after treatment with AHF as compared to demineralization stage and the maximum value was recorded with 20000 ppm of AHF group; SEM of dentin surface revealed that ultrastructural changes occurred after treating with AHF showed that the surface was covered with smear layer and dentinal tubules occluded.

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