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Biomimetic Enamel Remineralization: A Comparative in vitro Study of Self-Assembling Peptide, Fluoride Varnish, and their Combination at Different Times by Surface Roughness Test.

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Abstract

Aims: This study aimed to evaluate the efficacy of self-assembling peptide, fluoride varnish, and their combination to remineralize the artificial carious lesion in vitro. **Materials and methods:** Sixty extracted sound premolars were randomly divided into four groups. The artificial carious lesion was created by immersion the specimens in the demineralizing solution. The enamel surface was treated with: Group1: (n=15) artificial saliva alone, group2: (n=15) fluoride varnish, group3: (n=15) self-assembling peptide, group4: (n=15) self-assembling peptide + fluoride varnish. The enamel surface was assessed by a profilometer at the baseline, after demineralization, 1-week, and 4-weeks after remineralization. **Results:** all four groups exhibited high statistically significant differences between the different times of test periods, In 1 and 4 weeks after remineralization, the least surface roughness mean value was seen in the self-assembling peptide + fluoride varnish group followed by self-assembling peptide group then fluoride varnish group while the artificial saliva showed the greatest surface roughness mean value with a highly statistically significant difference between groups. **Conclusions:** self-assembling peptide superior to fluoride varnish in remineralization ability and this potential is boosted when they combined together.

الخلاصة

الأهداف: هدفت هذه الدراســـة الى تقييم فعالية البيتيد الذاتي التجميع، ورنيش الفلورايد، ومزيجهم لإعادة تمعدن الآفة الصناعية في المختبر. **المواد وطرائق العمل:** تم تقسيم ستين ضاحكًا سليمًا بشكل عشوائي إلى أربع مجموعات. تم إنشاء الآفة النخرية الاصطناعية عن طريق غمر العينات في محلول إزالة المعادن. تمت معالجة سطح المينا المعالج بـ: المجموعة ١: (١٥ عينة) اللعاب الاصـــطناعي وحده ، المجموعة ٢: (١٥ عينة) ورنيش الفلورايد ، المجموعة ٣: (١٥ عينة) البيتيد ذاتي التجميع ، المجموعة ٤: (١٥ عينة) البيتيد ذاتي التجميع + ورنيش الفلورايد. تم تقييم سطح المينا بواسطة جهاز اختبار الخشـــونة عند خط الأســـاس ، بعد ازالة المعادن ، لمدة أســـبوع ، و ٤ أســـابيع بعد إعادة التمعدن. **النتائج:** أظهرت جميع المجموعات الأربع فروق ذات دلالة إحصـــائية عالية بين الأوقات المختلفة لفترات الاختبار ، في ١ و ٤ أســـابيع بعد إعادة التمعدن ، شوهدت اقل قيمة لمتوسط خشونة السطح في مجموعة البيتيد ذاتي التجميع + ورنيش الفلورايد متبوعة بمجموعة البيتيد ذاتي التجميع ثم مجموعة ورنيش الفلورايد بينما أظهر اللعاب الصناعي أكبر قيمة متوسط لخشونة السطح مع وجود فروق ذات دلالة إحصـائية عالية بين المجموعات. **الاستنتاجات:** ببتيد ذاتي التجميع متفوق على ورنيش الفلورايد في القدرة على إعادة التمعدن وهذه الإمكانية تتعزز عندما تتحد معًا.

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INTRODUCTION

Dental Caries is initiated by a combination of multifactor. The main etiological factors are the host (teeth and saliva); the microorganism (bacteria and plaque); the substrate (dietary sugar); in addition to the time [1]. The disease resulting from an ecologic convey within the dental biofilm, from a well-balanced microbiological population to a cariogenic, and acidogenic population of micro-organisms initiated and continued by frequent consumption of fermentable dietary carbohydrates. The resulting activity of this shifting in the biofilm is related to an imbalance between demineralization and remineralization, resulting in a net loss of minerals within dental hard tissues, and causing the sign and symptom of a carious lesion [2 & 3]. The early sign of dental caries is a white lesions which develop as a result of plaque accumulation on the affected surface, commonly due to inadequate oral hygiene[4 & 5]

Roughness is a fundamental and important property of tooth surfaces which is related to the attachment of foreign materials, stains and, a plaque to their surfaces. Surface roughness influences the quality, color, biofilm colonization and, performance of surfaces in the oral cavity [6]. The most commonly reported surface roughness limit for adherence of dental biofilm is 0.2 μm so, increase of roughness above this value enhance the colonization and retaining of bacteria which lead to initiate dental caries [6 & 7], so the controlling of enamel surface roughness plays an essential role in dental caries prevention or enhance enamel remineralization by inhibiting the bacterial adhesion on the enamel surface [8]. The main purpose of this study is to evaluate the efficacy of self-assembling peptide (SAP_{11-4}), fluoride varnish (FV) and their combination (SAP_{11-4}+FV) on the enamel roughness of permenant premolars after their application on artificially induced carious lesions.

MATERIALS AND METHODS

This study was conducted in the University of Mosul / College of Dentistry / Department of Paedodontics, Orthodontics, and Preventive Dentistry after getting approval from the research ethics committee. REC reference no. UoM.Dent/H.L.16/21.

Materials.

1. CURODONT Repair™ from Credentis AG, Switzerland.
2. Enamelast, fluoride varnish from Ultradent, USA.
3. Demineralizing solution consists of $CaCl_2$ (2.2 mM), NaH_2PO_4 (2.2mM), and acetic acid (0.05 M), pH (4.4) were adjusted with (1M) KOH, 20 ml/tooth [9].
4. Artificial saliva contains NaCl (0.40g), KCl (0.40g), $CaCL_2.2H_2O$ (0.79g), $NaH_2PO_4.2H_2O$ (0.78g), $NaS_9.H_2O$ (0.005g), $CO(NH2)_2$ Urea

1g, in 1000 ml of Distilled water, at pH (7) [10].

Methods.

Sample Collection.

Sixty freshly sounds human permanent premolars extracted for orthodontic treatment were collected from the private clinics in Mosul City. The teeth were cleaned with tap water and stored in 0.1% thymol solution in a closed container at room temperature until their use. The specific inclusion criteria of selecting the teeth was sound teeth, each tooth that has an enamel defect (hypoplastic lesion), stain (extrinsic or intrinsic), cracks, white spot lesion, fluorosis, and restorations was excluded.

Sample Preparation.

Attached soft tissue and calculus were removed from the tooth by a surgical blade No.15, and the enamel surfaces were polished using a rubber cup and non-fluoridated pumice (PD, Germany) with a contra-angled low-speed hand-piece, then the roots were cut from 2mm below the cemento-enamel junction with abundant water irrigation using a diamond disc (Nti, Germany) in low speed handpiece after that, each tooth crown was poured in a cylindrical plastic model (20mm diameter, 10mm depth) by auto polymerized acrylic resin (Shanghai New Century Dental Materials, China) in a way that the buccal surface appeared upwards parallel to the floor of the ring. The buccal surfaces were polished using finishing and polishing disks (Top BM, Russia) in coarse, medium, fine and superfine respectively 10 second for each grade by a low-speed contra-angle hand-piece, a circular 6*6 mm of adhesive tape was placed on the middle of the buccal surface in each sample, the remaining surface was painted with acid-resistant nail varnish, the tape was removed after drying the varnish leaving a window of exposed enamel [9 & 11].

Initial Carious Lesion Formation.

The total samples in the main study were immersed in 20 ml of the demineralizing solution separately in a single plastic volumetric container for 60 consecutive hours until a white spot lesion created as a modification to the method described by Kamal *et al*. [9] some of the studies and researchers used the same demineralization procedure for 2 days [12 & 13].

Grouping and Surface Treatment.

The total number of the samples in the main study were (60) teeth which were randomly divided into (4) groups, (n=15) samples in each group.

Group 1 (Control group): After the demineralization, there was no treatment carried out, the samples were stored in daily renewed artificial saliva only for 4 weeks.

Group 2 (FV group): After the demineralization, the samples in this group were dried and a thin, layer of the fluoride varnish which contains sodium fluoride 5% was painted on the enamel window in a

single painting motion as in figure (1, A) and were allowed to be dried. The samples were stored in daily renewed artificial saliva for 4 weeks [9 & 14].

Group 3 (SAP_{11-4} group): After the demineralization, curodont repair was applied on the samples according to the manufacturer's instructions, each sample was moistened with 20% sodium hypochlorite for 20 sec. then etched for 20 sec. with phosphoric acid 37%, and rinsed with water thoroughly, the surfaces were air-dried; later (SAP_{11-4}) was applied on the exposed enamel window as in figure (1, B) and allowed to absorb for 5 min. The samples were stored in daily renewed artificial saliva for 4 weeks [9, 14].

Group 4 (SAP_{11-4} + FV group): After the demineralization, (SAP_{11-4}) was applied firstly as in group 3 after that, fluoride varnish was painted as in group 2. The samples were stored in daily renewed artificial saliva for 4 weeks [9]

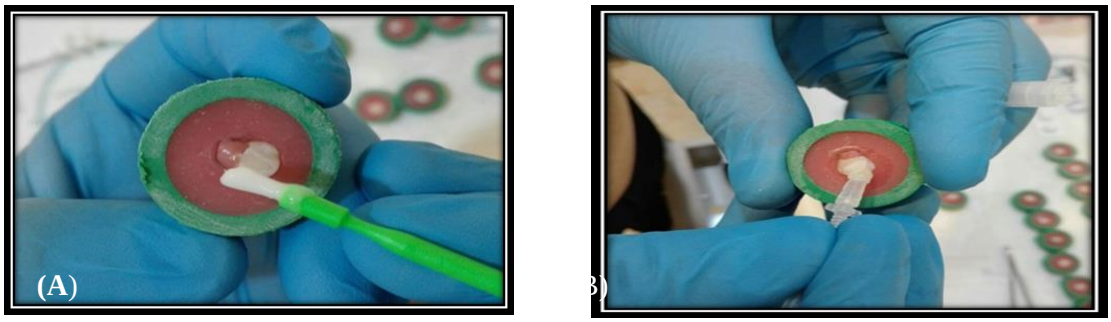

Figure (1) the Surface Treatment (A): Varnish Application. (B): Curodont Repair Application.

Surface Roughness.

Surface roughness (SR) can be characterized by the aberration in the direction of the normal wave of the actual surface from its optimal shape [15]. To evaluate enamel surface texture, surface roughness test was carried out for each sample in the experimental group and were measured at baseline, after demineralization, one and four weeks after remineralization respectively using a profilometer with magnification 50X (MITUTOYO, Tokyo/Japan). The surface roughness of the specimens was measured based on the parameter of maximum roughness height by measuring the distance between the highest peak and lowest valley in y-direction along the central line of the area and the resulted value was expressed in μm [16]. Three values of surface roughness for each specimen were taken and the average of these values was considered.

RESULTS

The results of this study in **table (1)** represents one-way analysis of variance (ANOVA) in the comparison of mean SR values between the four different groups control, FV, SAP_{11-4} and, SAP_{11-4}+FV in each time of test period baseline, after demineralization, 1-week and 4-weeks after the remineralization. In the baseline the results showed that there was no statistically significant difference between

groups in SR values of the enamel, while there was a statistically significant difference between groups in SR values after the demineralization and, there was a highly statistically significant difference in 1-week and 4-weeks after remineralization between the four group types $p \leq 0.01$. **Table (2)** represents the comparison of mean SR values of teeth enamel between the different times of test period for each group type by one-way analysis of variance (ANOVA) test, all four groups showed a highly statistically significant difference between the different times of test period ($p \leq 0.01$). **Table (3)** explains means, standard deviation, minimum, maximum values and Duncan's multiple range test of mean SR values of the enamel between and within the groups. In the baseline, the results of measurement showed that the mean SR values were from (0.30) to (0.46) for the different groups. After demineralization all four groups showed significant elevation in mean SR values that were from (0.57) to (0.90) for the different groups. After 1-week of remineralization, all groups showed a significant reduction in mean SR values except the control group that exhibited the highest SR mean value (0.67) followed by FV group (0.54), then SAP_{11-4} group (0.42) with no significant difference with baseline, while the least SR mean value was found in SAP_{11-4}+FV group (0.38) with no significant difference with baseline. After 4-week of remineralization, all the groups showed a reduction in the mean SR values but not significantly compared with the first week after remineralization except the SAP_{11-4}+FV group which had the least SR mean (0.34) followed by SAP_{11-4} group (0.40), then FV group (0.51), the control group was also showed a significant reduction (0.63) which did not showed a significant reduction at the first week of remineralization.

Table (1) ANOVA Test of Mean SR Values between the Different Groups.

		Sum of Squares	df	Mean Square	F	Sig.
Baseline	Between Groups	.005	3	.001806	1.535560	.215
	Within Groups	.066	56	.001176		
	Total	.071	59			
After Demineralization	Between Groups	.075	3	.025091	4.741841	.005
	Within Groups	.296	56	.005291		
	Total	.372	59			
1-week After Remineralization	Between Groups	.745	3	.248233	256.193062	.000*
	Within Groups	.054	56	.000969		
	Total	.799	59			
4-weeks After Remineralization	Between Groups	.721	3	.240484	398.395326	.000*
	Within Groups	.034	56	.000604		
	Total	.755	59			

df: degree of freedom. *Highly statistically significant difference at $p \leq 0.01$.

Table: (2) ANOVA Test for Mean SR Value within Each Group.

		Sum of Squares	Df	Mean Square	F	Sig.
Control	Between Groups	.911	3	.303829	146.188720	.000*
	Within Groups	.116	56	.002078		
	Total	1.028	59			
FV	Between Groups	.829	3	.276251	143.099696	.000*
	Within Groups	.108	56	.001930		
	Total	.937	59			
SAP_{11-4}	Between Groups	1.660	3	.553322	253.914340	.000*
	Within Groups	.122	56	.002179		
	Total	1.782	59			
SAP_{11-4}+FV	Between Groups	1.623	3	.541139	292.159923	.000*
	Within Groups	.104	56	.001852		
	Total	1.727	59			

df: degree of freedom. *Highly statistically significant difference at $p \leq 0.01$.

Table (3): Means, Standard deviation, Minimum, Maximum Values and Duncan's Multiple Range test of Mean SR Values between and within the Groups.

Groups		Baseline	Demineralization	1 week	4 week
Control	Mean	.3840 a, C	.6907 c, A	.6707 a, A	.6320 a, B
	N	15	15	15	15
	Std. Deviation	.03869	.05738	.05675	.01740
	Minimum	.31	.60	.57	.61
	Maximum	.44	.78	.75	.67
FV	Mean	.4053a, C	.7320 b c, A	.5433 b, B	.5160 b, B
	N	15	15	15	15
	Std. Deviation	.01959	.07580	.01988	.03460
	Minimum	.37	.59	.51	.45
	Maximum	.44	.85	.59	.56
SAP_{11-4}	Mean	.3920a, B	.7893 a, A	.4220 c, B	.4040 c, B
	N	15	15	15	15
	Std. Deviation	.04246	.08004	.00902	.02063
	Minimum	.30	.57	.40	.36
	Maximum	.46	.90	.44	.44
SAP_{11-4}+FV	Mean	.3807a, B	.7493 a b, A	.3873 d, B	.3457 d, C
	N	15	15	15	15
	Std. Deviation	.03195	.07564	.01335	.02211
	Minimum	.30	.61	.37	.30
	Maximum	.42	.84	.41	.38

N: Number of the specimens, Std. Deviation: Standard Deviation, Different Small letters indicate statistical significant difference within the same column (vertically). Different capital letters indicate statistically significant difference within the same row (horizontally) $p \leq 0.05$.

DISCUSSION

Measuring enamel surface roughness is a practical and effective method for assessing the efficiency of the preventive agents and stage of development of the carious lesion, [8] because the changes in enamel-surface roughness enhances the bacterial colonization and retention as well as the accumulation of the stains [6]. Self-Assembling Peptide is a monomeric solution in contrast to fluoride, it can diffuse deeply and undergo subsurface remineralization of the primary lesions [17]. SAP_{11-4} is an intelligently designed peptide that able to undergo spontaneous hierarchical self-assembly triggered with specific environmental factors shaping de Novo hydroxyapatite crystals and catching calcium and phosphate ions from saliva forming a 3D matrix to replace the degraded enamel matrix with the biomimetic matrix [9, 18 & 19]. The results of this study showed an excellent roughness reduction potential of self-assembling peptide alone and also in combination with the fluoride varnish compared to fluoride varnish alone after application on artificially induced enamel lesion, in all four groups the enamel surface roughness was significantly increased after demineralization compared to the baseline due to the same protocol was used for demineralization, a statistically significant difference between the different groups after the demineralization might be due to the different source of teeth collection and from different ages explaining the different ability of teeth to resist acid attacks. The results of the current study showed that FV, SAP_{11-4}, and SAP_{11-4}+FV groups have the ability to reduce the surface roughness after 1-week of remineralization except control group but, the highly statistically significant difference between them indicates the different ability of each treatment option in decreasing the surface roughness which in turn inhibit the progression and promote remineralization of a carious lesion by decrease bacterial adhesion. The least mean value of surface roughness was recorded in the combined SAP_{11-4}+FV group refer to the best ability to reduce the surface roughness followed by self-assembling peptide alone then by fluoride varnish alone. The effect of self-assembling peptide together with fluoride varnish and calcium phosphate from saliva may provide a continuous minerals supply to the tooth which deeply filled the micro porosities that formed after demineralization may explain the best results of the SAP_{11-4}+FV group however, there were no studies to compare our best results of combining two remineralizing agents on reducing the surface roughness of enamel. The superiority of self-assembling peptide over fluoride varnish in the ability to reduce the roughness of enamel surface might be due to the low viscosity that enabling it to deeply penetrate the micropores of the enamel surface triggering the self-assembly process and forming a scaffold that influences the minerals deposition, our result was agreed with Metwally *et al.* [20] who compared the ability to reduce the enamel surface roughness between self-assembling peptide and fluoride varnish after single and double application, they concluded the same results of superiority as in our study with no differences in single and double application,

this results were also agreed with Ceci *et al.* [21] who used curodont protect and they concluded that the use of self-assembling peptide had a protective effect on the demineralization of enamel. The result of fluoride varnish may due to acting as a reservoir of fluoride ion supplying the tooth slowly with minerals from artificial saliva forming Calcium Fluoride (CaF2) which filled in porosities that formed after demineralization of enamel surface and enhancing remineralization [22], however it did not reach the baseline value of surface roughness even after 4-weeks. In the fourth week of remineralization, only the combined SAP_{11-4}+FV group was able to induce an additional significant reduction in surface roughness, however no studies were explaining the effect of time on surface roughness to compare our results. The artificial saliva in the control group did not induce a significant roughness reduction after 1-week of remineralization and was able to significantly reduce the surface roughness after 4-weeks of remineralization, this result refers to the limited ability of artificial saliva to reduce the surface roughness of enamel which was in agreement with many studies that used artificial saliva as a control group such Mohammed, *et al.* [23], according to their results, the artificial saliva had the highest surface roughness after 2 and 4 weeks remineralization of the previously bleached enamel surface.

CONCLUSIONS

Within the limitations of the study, the remineralizing effect of self-assembling peptide combined with fluoride varnish is excellent in reducing the surface roughness of artificially induced enamel lesion which in turn is superior to the remineralizing effect of fluoride varnish alone.

Conflicts of interest:

Authors declare that they have no conflict of interest real or perceived, financial or non-financial in this article.

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