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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 Microhardness Test.

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Abstract

Aims: This study was 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 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 Vickers surface microhardness 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 greatest surface microhardness 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 group showed the least surface microhardness mean value with a highly statistically significant difference between them. **Conclusions:** self-assembling peptide superior to fluoride varnish in remineralization ability and this potential is boosted when they combined together. Relating to surface microhardness, remineralization is a dynamic process that increases with time.

الخلاصة

الأهداف: الهدف من هذه الدراسة هو تقييم فعالية الببتيد 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 الاصطناعي وحده ، المجموعة ٢: (١٥ عينة) ورنيش الفلورايد ، المجموعة ٣: (١٥ عينة) الببتيد ذاتي التجميع ، المجموعة ٤: (١٥ عينة) الببتيد ذاتي التجميع + ورنيش الفلورايد. تم تقييم سطح المينا بواسطة صلادة سطح فيكرز الدقيقة عند خط الأساس ، بعد ازالة المعادن ، لمدة أسبوع ، و ٤ أسابيع بعد إعادة التمعدن. **النتائج:** أظهرت جميع المجموعات الأربع فروق ذات دلالة إحصائية عالية بين الأوقات المختلفة لفترات الاختبار ، في ١ و ٤ أسابيع بعد إعادة التمعدن ، شوهدت أكبر قيمة لمتوسط الصلادة السطحية في مجموعة الببتيد ذاتي التجميع + ورنيش الفلورايد متبوعة بمجموعة الببتيد ذاتي التجميع ثم مجموعة ورنيش الفلورايد بينما أظهر اللعاب الصناعي أقل قيمة متوسط للصلادة السطحية مع وجود فروق ذات دلالة إحصائية عالية بينهما. **الاستنتاجات:** ببتيد ذاتي التجميع متفوق على ورنيش الفلورايد في القدرة على إعادة التمعدن وهذه الإمكانية تتعزز عندما يتحدان معًا فيما يتعلق بصلابة السطح الدقيقة ، فإن إعادة التمعدن هي عملية ديناميكية تزداد بمرور الوقت.

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INTRUDUCTION

Dental caries is an infectious, multifactorial disease characterized by loss of dental hard tissue elements over time leading to the destruction of the tooth, dental caries occurs due to changes in a balance between re and demineralization, the demineralization process is described as a loss of minerals from tooth structure while the remineralization process is described as re-precipitation of minerals in tooth structure [1, 2]. The initial carious lesions are seen as white spot lesions and are known as subsurface demineralization [3]. The growing goal of modern dentistry is to treat early lesions through non-invasive remineralization with the ultimate goal of preventing lesion progression and promoting strength, beauty and, dental function [4, 5]. A variety of remineralizing agents obtainable for non-invasive management of incipient caries like fluorides but, these agents fail to attain matrix-mediated mineralization similar to the physiologic structure of enamel [6] and do not promote deep remineralization or improve the appearance of these lesions [7, 8]. To overcome this limitation, there is an endeavor to shifting from non-invasive to regenerative therapies, as an alternative approach in the management of non cavitated lesion in which bio-mineralization the affected dental tissues occurs [9]. As a result of these attempts scientists from the University of Leeds evolved the Curolox technology which is a patented technology for guided enamel regeneration [10]. Self-assembling peptide (SAP_{11-4}) is an alternative approach that recently introduced for management of early carious lesions that regenerating demineralized dental tissue by forming de Novo hydroxyapatite crystal in 3D-matrix through demineralized lesions, promoting the bio-mineralization of the enamel lesions [11, 12, 13]. SAP_{11-4} is designed to facilitate in-depth biomimetic remineralization of dental tissue [14, 15] and improve the visual appearance of early carious lesion [16, 17]. So, the purpose of conducting the current study is to evaluate the remineralization potential of a self-assembling peptide (SAP_{11-4}) compared to fluoride varnish (FV) application after an acidic challenge to permanent teeth.

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 [18].

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) [19].

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 (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 [10, 18].

Initial Carious Lesion Formation.

The total samples in the 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 was created as a modification to the method described by Kamal *et al*. (2020) [18].

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. [18, 20].

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 [18, 20].

Group 4 (SAP_{11-4} + FV group): After the demineralization, (SAP_{11-4}) were 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 [18]

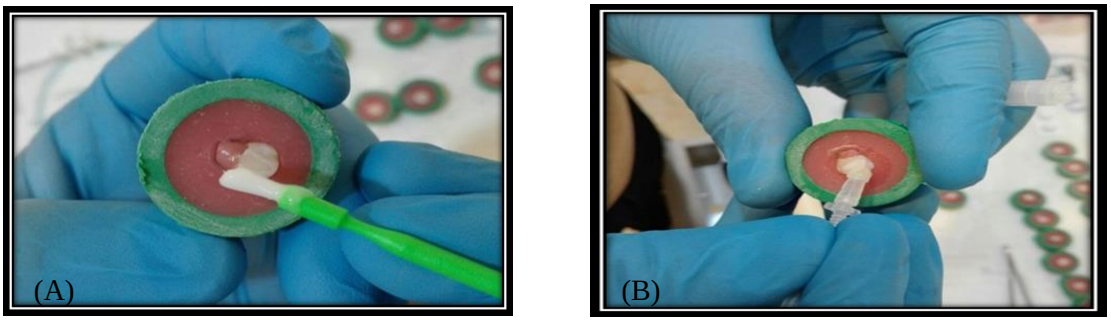

Figure (1) the Surface Treatment (A): Varnish Application. (B): Curodont Repair Application.

Surface Microhardness Test (SMH).

The enamel microhardness of the specimens was measured at baseline, after demineralization, one week and four weeks after remineralization respectively by Vicker's Microhardness Tester (Wolpert, Germany) using 500 gm load for 15 seconds on the exposed enamel window of the specimens that were constant at the whole study for all the specimens [21]. Three indentations were measured and the average value of them was calculated for each specimen to decrease any variation in the indentation values of the different parts of the exposed enamel window of the buccal surface of specimens. The Vickers hardness number (VHN) was obtained by using the following equation: $HV = 1.854 (F/d^2)$ where HV is a Vickers hardness in Kgf/mm^2 (Mpa) F: the indentation load in (Kgf), d: the average of two diagonal lengths of the indentation in (mm) as $d=d1+d2/2$ [22].

RESULTS

Table (1) represents one-way analysis of variance (ANOVA) in the comparison of mean values of SMH between the four different groups in each time of test period. There was no statistically significant difference between the mean SMH values

of different groups in the baseline, while there was a statistically significant difference between groups after 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 SMH values of teeth enamel between the different time 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)** demonstrates means, standard deviation, minimum, maximum values and Duncan's multiple range test of mean SMH of the teeth enamel between and within the groups. The results of the baseline measurement showed that the SMH values were range from (336.82 to 351.51) for the different groups. After demineralization all four groups showed a reduction in the values of SMH and were range from (230.56 to 252.12) for the different groups. After 1-week of remineralization, the four groups showed an increase in SMH values the highest SMH value was found in SAP_{11-4}+FV group followed by SAP_{11-4} group, then FV group, while the lowest SMH mean value was found in the control group that was treated with artificial saliva alone. After 4-week of remineralization, the four groups showed a significant increase in SMH values, SAP_{11-4}+FV group was also represented the highest SMH value followed by SAP_{11-4} group, then FV group, while the lowest SMH mean value was found in the control group.

Table (1): ANOVA Test for Mean SMH Values between the Groups.

		Sum of Squares	df	Mean Square	F	Sig.
Baseline	Between Groups	50.724	3	16.908	1.188	.323
	Within Groups	796.879	56	14.230		
	Total	847.603	59			
After Demineralization	Between Groups	797.704	3	265.901	14.004	.000*
	Within Groups	1063.304	56	18.988		
	Total	1861.008	59			
1-week after Remineralization	Between Groups	43648.554	3	14549.518	761.935	.000*
	Within Groups	1069.348	56	19.095		
	Total	44717.901	59			
4-week after Remineralization	Between Groups	54061.407	3	18020.469	844.053	.000*
	Within Groups	1195.595	56	21.350		
	Total	55257.002	59			

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

Table (2): ANOVA Test for SMH Mean Values within Each Group.

Groups		Sum of Squares	df	Mean Square	F	Sig.
Control	Between Groups	94427.162	3	31475.721	1.988E3	.000*
	Within Groups	886.667	56	15.833		
	Total	95313.829	59			
FV	Between Groups	75015.465	3	25005.155	1.158E3	.000*
	Within Groups	1209.149	56	21.592		
	Total	76224.613	59			
SAP_{11-4}	Between Groups	87246.467	3	29082.156	1.585E3	.000*
	Within Groups	1027.542	56	18.349		
	Total	88274.009	59			
SAP_{11-4}+FV	Between Groups	132167.625	3	44055.875	2.463E3	.000*
	Within Groups	1001.769	56	17.889		
	Total	133169.394	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 SMH between and within the Groups.

Groups		Baseline	Demineralization	1-week	4-week
Control	Mean	344.6040 a, A	237.6080 b, D	263.3667 d, C	273.0587 d, B
	N	15	15	15	15
	Std. Deviation	3.71565	4.16070	4.33568	3.66303
	Minimum	339.82	231.56	256.50	265.87
	Maximum	350.51	243.15	269.30	278.56
FV	Mean	344.1580 a, A	245.4007 a, D	285.6767 c, C	301.3567 c, B
	N	15	15	15	15
	Std. Deviation	3.75514	4.45507	4.70694	5.50124
	Minimum	336.82	239.15	278.37	293.59
	Maximum	351.51	252.12	296.39	312.44
SAP_{11-4}	Mean	345.9420 a, A	244.8693 a, D	305.1480 b, C	327.9860 b, B
	N	15	15	15	15
	Std. Deviation	3.82744	4.45363	3.51207	5.15531
	Minimum	339.82	238.15	300.90	320.87
	Maximum	351.51	251.12	312.09	337.32
SAP_{11-4}+FV	Mean	346.3880 a, B	238.1140 b, D	336.8293 a, C	353.6540 a, A
	N	15	15	15	15
	Std. Deviation	3.78993	4.35389	4.80562	3.89115
	Minimum	340.82	230.56	330.01	347.51
	Maximum	351.51	244.15	342.82	360.60

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

DISCUSSION

Self-Assembling Peptide is an intelligently designed peptide that able to undergo spontaneous hierarchical self-assembly triggered with specific environmental factors shaping three-dimensional fibrillar scaffolds [15, 18]. SAP_{11-4} type that used in this study is a monomeric solution unlike fluoride, it can diffuse deeply and undergo subsurface remineralization of the initial lesions, where it forms a three-dimensional matrix replacing the degraded enamel matrix with a biomimetic matrix [13]. The numerical hardness values refer to the number of mineral contents in the tooth structure therefore, SMH has been utilized to detect the mineral loss and gain in dental tissues [23] reinforcing the fact that SMH evaluations are a feasible and reliable choice to assess the mineral changes of demineralization and remineralization cycles that occur in the enamel. Depending on the results of the current study the null hypothesis was rejected. The results of this study showed promising remineralizing potential in terms of enamel bio-mineralization of self-assembling peptide, and also in combination with the fluoride varnish after application on artificially induced enamel lesion. According to the results of the current study the statistically significant difference between the different groups after the demineralization may be due to the different sources of teeth collection from the different geographic area in Mosul city and from patients with no age limit explaining the variation of resistances between different teeth to acid attacks. In this study, the highest remineralization potential after 1 and 4-weeks of remineralization seen in the combined SAP_{11-4}+FV group followed by the SAP_{11-4} group then FV group, the greatest remineralization ability of SAP_{11-4}+FV group may be due to the presence of high fluoride ions concentration that enhancing the regenerative potential of self-assembling peptide and explaining the additive synergistic advantage of combining two remineralizing agents that boost the remineralization potential, the superiority of SAP_{11-4} group over fluoride varnish in this study was agreed with Üstün and Aktören, (2019); Kamal *et al.*, (2020); and Sezici *et al.*, (2021) [24, 18, 20]. This will reinforce the fact that the self-assembling peptide can produce a hierarchical 3D scaffold within the demineralized lesion and to form de novo hydroxyapatite crystals by the aids of calcium and phosphate ions from saliva. The control group (artificial saliva) has the least remineralization ability even after 4-weeks of remineralization, all groups showed a significant increase in mean SMH values after 1-week and an additional significant increase after 4-weeks of remineralization in comparison with demineralization mean values, these results explains the positive effect of increased storage time on increasing the SMH of enamel indicating the increased remineralization ability of these agents with time, These results were in agreement with Alsamolly (2021), Sindhura *et al.* (2018), Kirkham *et al.* (2007), Kamal *et al.* (2020), Üstün and Aktören (2019), Takahashi *et al.* (2016), kind *et al.*(2017), and Silvertown *et al.*(2017) [5, 6, 15, 18, 24, 25, 26, 27,] those evaluated the time-dependent changes related to the remineralization efficacy following a single application of different remineralizing agents,

the result showed that application of SAP_{11-4} led to the gradual increase in the mineralization. The result of this study was in agreement with Kamal *et al.,* (2020) who concluded that the highest remineralizing potential achieved by combined group self-assembling peptide with fluoride varnish after 4-week of remineralization and even after 1 week followed by self-assembling peptide after 4 and 1 weeks of remineralization which was better than fluoride varnish even after 4-weeks of remineralization which in turn was better than artificial saliva at 1-week and 4-weeks after remineralization.

CONCLUSIONS

Within the limitation of this study, self-assembling peptide superior to fluoride varnish in remineralization ability and this potential is enhanced when they are combined. Relating to surface microhardness, remineralization is a dynamic process that increases with time.

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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