



## Remineralizing Efficacy of Biomimetic Self-Assembling Peptide on Artificially Induced Enamel Lesions (*In vitro* Study)

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### ABSTRACT

**Background:** The technology of self-assembling peptides have been progressed as an alternative remineralizing agent to fluoride, that assemble into a fibrillar three-dimensional scaffold.

**Aim:** To investigate the effectiveness of self-assembling peptide (P11-4) on enamel remineralization both alone and in combination with fluoride compared to fluoride-based delivery systems and casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF).

**Materials and Methods:** Enamel caries-like lesions were created artificially on the buccal surfaces of 25 extracted human maxillary first premolars of permanent teeth. Specimens were randomly arranged into five groups ( $n = 5$ ) based on the remineralizing agent used: Group 1-control: Artificial saliva, Group 2 - fluoride varnish, Group 3 – CPP-ACPF varnish, Group 4-self-assembling peptide agent, Group 5 - double application group (self-assembling peptide and fluoride). The application of all the materials was according to their producer's instructions and the storage of the specimens were in artificial saliva that renewed daily. Assessment of surface microhardness (SMH) was done at baseline, after demineralization and after 2 weeks of remineralizing agents' application, then the data were analyzed using ANOVA and paired t-test.

**Results:** Although, no significant difference in decrement in enamel microhardness between fluoride, self-assembling peptide and the double application group, while it was significantly higher in these groups when compared to CPP-ACPF. In addition, the control group revealed, no statistically significant difference in mean of enamel microhardness after remineralization compared to that after demineralization.

**Conclusions:** Self-assembling peptide alone and its combination with fluoride varnish, both have the same remineralizing efficacy, showing an encouraging, noninvasive regeneration potential as an alternative remineralizing agent to fluoride.

**Keywords:** Self-assembling peptide, Guided enamel regeneration, Surface microhardness

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### INTRODUCTION

Dental caries is a multifactorial disease, depending on the balance occurring between both protective and pathological factors. The understanding of the need to initiate a healthy balance in the oral environment between these factors gradually shift the treatment principles for dental caries management toward the prevention of disease progression and

enhancement of Remineralization process [1].

The Remineralization process of dental tissues is defined as deposition of calcium and phosphate ions that are delivered from an external source into crystal voids in demineralized enamel that result in a net mineral gain. The cornerstone of the Remineralization process is the fluoride, while its ability in promoting net Remineralization is limited to the availability of both phosphate and calcium ions [2].

There are many materials that are available nowadays for dental Remineralization. Although the fluoride remains the utmost well-known and the most widely used one, many limitations of its

usage have been developed. One of these is that the fluoride (specifically in high concentrations) shows a dominated surface Remineralization when compared to the body of the carious lesion, causing difficulty to achieve full body Remineralization [3].

The efficacy of topical fluoride applications, which act as a cariostatic agents, have been confirmed, and fluoride is considered the most effective method for arresting and preventing the formation of dental caries. Despite of many professional topical fluoride applications have long been used; the most effective kinds of fluoride (F) that inducing remineralization of initial carious lesions still unclear [4].

The CPP-ACP binds easily both to the tooth surface and the bacteria in dental plaque. The major function of CPP-ACPF (CPP-ACP fluoride) is to stabilize a high concentration of calcium and phosphate ions at tooth surface by binding at pellicle and plaque. It come up with a very effective mode that elevating the amount of calcium present in the fluid of dental plaque, forming a desirable condition for increasing the remineralization [5]. The CPP-ACPF and due to the added fluoride content has shown an improvement in the ability to remineralize initial carious lesions [6].

Recently, self-assembling peptides (P11-4; CurodontRepair, Credentis AG, Switzerland) have been evolved to be alternative remineralizing agent to Fluoride [7]. Self-assembling peptides come together into a fibrillary three-dimensional scaffold in the lesion according to the environmental pH and salts concentrations. So that this scaffold can then act as a nucleator for hydroxyapatite, encouraging tissue regeneration from within as shown in Figure 1. Some Studies demonstrated the helpfulness of self-assembling peptides in the natural repair process of enamel tissue, which significantly increase the mineral gain and inhibit the loss of these minerals [8]. Based on these findings, self-assembling peptides may be helpful in mineral behavior modulation during the process of dental tissue engineering.

As an effort to illuminate the potential of remineralization for the biomimetic self-assembling peptide technology, this study was done to evaluate the efficacy of remineralization for self-assembling peptide alone and in

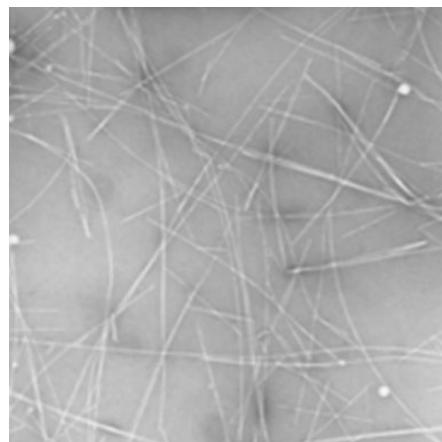


Figure 1: P11-4 peptide matrix.

combination with fluoride compared to fluoride alone and CPP-ACP fluoride (CPP-ACPF), assessed by surface microhardness (SMH) measurements. The null hypotheses suggested that there is no difference in the potential of remineralizing efficacy between self-assembling peptide, self-assembling peptides in combination with fluoride varnish, CPP-ACPF, fluoride alone and artificial saliva using microhardness testing.

## MATERIALS AND METHODS

### Remineralizing agents

The materials that have been tested for the remineralization in this study were as following: (1) ALPHA PRO®(Dental Technologies, INC , USA) as fluoride varnish with the fluoride concentration of 22,600 ppm. (2) MI varnish (GC company, Japan) as CPP-ACPF varnish which consisted of 5% CPP-ACP and 22,600 ppm fluoride. (3) CURODONT Repair (Credentis, Switzerland), that designed Curolox® technology which is based on the self-assembling peptide (P11-4).

### Tooth surface preparation

A total of 25 sound extracted maxillary permanent first premolars teeth were used. All Teeth have been cleaned by ultrasonic scaler, prophylaxis done with rubber cup and pumice then stored for 2 weeks in a thymol solution [9]. Teeth inspection was done to exclude any enamel defects, stains, caries or cracks. Then a mold of self-cured acrylic resin was used for each tooth by embedding the tooth in the mold with its buccal surface directed upward. Then buccal surfaces were polished by Sof-Lex Disks (3M ESPE, USA) in a progressive manner (beginning with the coarse, then medium and fine, ending

with the superfine) using contra-angle slow-speed hand-piece [10]. A window of exposed enamel 2 mm × 2 (in dimension) was created by coating the remaining buccal surface of each tooth with anail varnish (Pank, paris) which is an acid-resistant one.

#### Demineralization

The demineralizing solution was prepared by using the following concentrations: 2.2 mM sodium dihydrogen orthophosphate dehydrate ( $\text{NaH}_2\text{PO}_4$ ), 2.2 mM calcium chloride ( $\text{CaCl}_2$ ) and acetic acid with the concentration of 0.05 M; then adjusting the pH were done to 4.4 by adding 1M potassium hydroxide. Followed by immersion of Each specimen individually in the demineralizing solution for 4 successive days (96 h) to form artificial caries-like lesions on enamel surface [11]. Then washed carefully and kept in storage with deionized water.

#### Sample grouping and Remineralization process

The preparation of artificial saliva was done according to ten Cate and Duijsters' formulation [12]. And consisted of 0.9 mM  $\text{NaH}_2\text{PO}_4$ , 1.5 mM  $\text{CaCl}_2$  and 0.15 M potassium chloride, the pH was adjusted to 7.0. According to the treatments applied, all the Specimens were haphazardly divided into five groups (n=5):

**Group1** (the control group): The specimens were stored in daily renewed artificial saliva without any treatment.

**Group2** (the fluoride group): Each specimen in this group was dried and coated with a uniform, thin layer of the fluoride varnish, then left it for 20 s to allow the varnish to be absorbed, followed by air-dryness.

**Group3** (the CPP-ACPF group): Each specimen in this group was dried and coated with a uniform, thin layer of the MI varnish. Then the varnish was left without any interruption for 20 s.

**Group4** (self-assembling peptide group): The agent was supplied by the manufacure in plastic containers with their own brushes , the application of the material was done on the tooth surface and left intact for 5 min (until all the material disappear), so allowing it to be diffused and self-assembly.

**Group 5 double application group (self-assembling peptide and fluoride):** application of self-assembling peptide and left for 5 min,

allowing it to diffuse (according to manufacture instructions), and then application of fluoride varnish was done, then left it for 20 s to allow the varnish to be absorbed.

After the first 6 h of storage in artificial saliva for specimens in (group 2, 3 and 5), the varnish was removed with cotton and distilled water.

The storage of all Specimens was done by putting each group separately in artificial saliva that renewed daily until SMH test. [9]

#### Assessment of surface microhardness

Twenty-five teeth were used to measure SMH at baseline (sound enamel surface), after demineralization process and then after 2weeks of remineralization. The measurements were accomplished with digital Vickers microhardness tester with a diamond indenter, in the laboratory of metal testing, Department of Metallurgy and Production Engineering, University of Technology. Collecting the measurements was carried out through the application of 500 g load for 30 s directed vertically to the enamel surface. All the measurements were completed by using the same calibrated machine and same examiner. The average of three indentations in each reading was taken, and that represent the hardness value for each specimen.

#### Statistical analysis

Data analysis was done by using the 25th version of Statistical Package for Social Sciences (SPSS). The data presented as mean, standard deviation and ranges. While Categorical data presented by percentages and frequencies. Analysis of Variance (ANOVA) (two tailed) was used to compare the mean of enamel microhardness between groups. Paired t-test was used to compare the mean of enamel microhardness at baseline level, after the demineralization process, and after remineralization. A level of P-value less than 0.05 was considered significant.

## RESULTS

Table 1 shows the comparison between study groups by baseline enamel microhardness of teeth. There was no statistically significant difference ( $P= 0.317$ ) in baseline enamel microhardness of teeth between study groups.

The comparison between enamel microhardness at baseline and after demineralization showed

that means of enamel microhardness at baseline was significantly higher than that after demineralization (364.62 and 218.76, P= 0.001) as presented in table 2.

**Table 1:** Comparison between study groups by baseline enamel microhardness of teeth.

Study Group	Baseline enamel microhardness	F value	P - Value
	Mean ± SD	Range	
Group 1	340.42 ± 26.05	314.2 – 369.05	1.264 0.317
Group 2	385.45 ± 81.95	272.7 – 473.5	
Group 3	384.14 ± 23.29	355.95 – 420.5	
Group 4	338.47 ± 35.44	287.0 – 379.89	
Group 5	374.66 ± 40.17	312.7 – 412.15	

**Table 2:** Comparison between enamel microhardness at baseline and after demineralization.

Enamel microhardness	Baseline	After demineralization	t-test	P - Value
	Mean ± SD	Mean ± SD		
	364.62 ± 47.5	218.76 ± 21.77		

**Table 3:** Comparison in enamel microhardness after remineralization with after demineralization in each study group.

Study group	Enamel microhardness		Paired t-test	P - Value
	After demineralization	After remineralization		
	Mean ± SD	Mean ± SD		
Group 1	212.35 ± 26.58	231.8 ± 15.03	1.406	0.232
Group 2	214.85 ± 16.16	374.2 ± 28.6	8.853	0.001
Group 3	242.81 ± 26.98	339.1 ± 14.02	16.106	0.001
Group 4	208.47 ± 8.59	354.8 ± 30.71	8.547	0.001
Group 5	215.36 ± 11.57	372.3 ± 23.18	20.276	0.001

**Table 4:** Comparison between study groups in percentage of change in enamel microhardness after remineralization compared to demineralization.

Study Group	Percentage of change in enamel microhardness after remineralization compared to demineralization (%)	F value	P - Value	Study groups	
				Group 1	Group 2
				Mean ± SD	Range
Group 1	10.64 ± 16.53	-7.37–31.26			
Group 2	75.38 ± 22.85	42.59–101.91	13.903	0.001	
Group 3	40.49 ± 9.47	26.24–51.1			
Group 4	70.86 ± 21.83	50.81–106.19			
Group 5	72.95 ± 7.71	64.5–83.69			

**Table 5:** Post hoc tests (LSD) to confirm the differences in percentage of change in enamel microhardness occurred between groups after remineralization compared to demineralization.

Percentage of change in enamel microhardness (%)	Study groups					P-Value
	Group 1	Group 2	Group 3	Group 4	Group 5	
Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
10.64 ± 16.53	75.38 ± 22.85	-	-	-	-	0.001
10.64 ± 16.53	-	40.49 ± 9.47	-	-	-	0.011
10.64 ± 16.53	-	-	70.86 ± 21.83	-	-	0.001
10.64 ± 16.53	-	-	-	-	72.95 ± 7.71	0.001
-	75.38 ± 22.85	40.49 ± 9.47	-	-	-	0.004
-	75.38 ± 22.85	-	70.86 ± 21.83	-	-	0.677
-	75.38 ± 22.85	-	-	-	72.95 ± 7.71	0.822
-	-	40.49 ± 9.47	70.86 ± 21.83	-	-	0.01
-	-	40.49 ± 9.47	-	-	72.95 ± 7.71	0.006
-	-	-	-	70.86 ± 21.83	72.95 ± 7.71	0.847

with GER is based on forming a 3D scaffold through the assembling of short hydrophilic peptide into fibers [16].

Different approaches are used to provide proof for mineral gain or loss. The most widely used one is SMH analyses, which assess the demineralization and remineralization changes that have been occurred in enamel. The evaluations of SMH are fast, simple, and easy to measure in a nondestructive pattern. The SMH mechanism of action is depending on quantifying the resistance of the materials' surfaces contrary to plastic deformation from a standard source, so the measurements can be taken from the same specimen repeatedly over a period of time, decreasing the experimental disparity, all these make the evaluation of SMH is a practical choice for mineral changes' estimations [17].

The results revealed that means of enamel microhardness at baseline was significantly higher than that after demineralization.

Data analysis showed that all four treatment procedures significantly enhanced the remineralization of enamel lesions and a significant increase of enamel microhardness have been gained when compared to artificial saliva.

group treated with fluoride showed highest enamel microhardness than other groups followed by the double application group then self-assembling peptide and CPP-ACPF, however the lowermost mean of SMH has been found in artificial saliva.

The comparison between study groups in percentage of change in enamel microhardness after remineralization compared to demineralization. Although It was obvious that this percentage of change in enamel microhardness was higher in fluoride group than that in other groups. But there was no significant difference in decrement in enamel microhardness between groups of fluoride, self-assembling peptide and double application group, that disagree with Kamal D. et al. who found that Self-assembling peptides showed the uppermost remineralizing efficacy when compared to fluoride [18]. These findings could be attributed to fluoride's capability in forming fluorapatite crystals by attracting phosphate and calcium ions that will lead to increase in mineral

content [19].

The findings of the group treated with CPP-ACPF, which is a calcium phosphate-based agent exhibited a significant increase in the mean of SMH in comparison to group of artificial saliva. this may be explained by the formulation of CPP-ACP agent which consist of two parts: CPP and ACP. CPP is a phosphoprotein that is milk-derived and has a notable ability in the stabilization of high ACP concentrations which is asoluble compound, and will prevent them from growing to the serious size that are required for their nucleation and precipitation. CPP-ACP forming nanocomplexes that bind onto dental plaque and enamel surfaces, acting as a reservoir for high level of phosphate and calcium ions that present in a nonstructurally bound patterns favoring the remineralization to be achieved [20].

A statistically significant difference in the mean of SMH have been found between fluoride and CPP-ACPF. These results disagree with studies done by Shetty et al., and Mohd Said et al. [21,22]. And agree with Lata et al., [23] who stated that CPP-ACPF is not as efficient as fluoride in the remineralization of enamel carious lesions. These results may be related to fluoride that shows higher remineralizing potential than CPP-ACPF.

Values of remineralization for the control group revealed the least measurements, and this is coordinate with studies done by Somani et al. [24]. Who found that the control group disclosed the least remineralization values? This could be due to the formulation of the artificial saliva that have been used in this study, which did not have any fluoride ions [25].

## CONCLUSION

Self-assembling peptide and the combination of it with fluoride varnish both have the same remineralizing efficacy. Showing an encouraging and noninvasive regeneration potential to be an alternative remineralizing agent to fluoride.

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