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Remineralizing Effect of Self-Assembling Peptide on Micro-morphological Analysis of Artificial Enamel Carious Lesions: in-vitro study

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ABSTRACT:
Objective: To assess the remineralizing potential of self-assembling peptide (SAP) fluoride plus on the micro-morphological analysis of artificially induced early enamel lesion.**Materials and Methods**: In the current study, a total of 15 sound human molars was selected and prepared by removal of the roots and sectioning the crowns mesiodistally into twohalves resulting in 30 specimens. Demineralization was carried out by immersion of the specimens into demineralizing solution for 72 hours. The specimens were assigned equally into two groups (n=15 for each); Group1: control (no intervention), Group2: SAP fluoride plus. Specimens were stored in artificial saliva for 28 days. Micro-morphological analysis of the specimens was done at baseline, following 72 hours of demineralization, and after 28 days ofstorageusing scanning electron microscope (SEM). **Results**:SEM showed that SAP fluoride plus resulted in better remineralization pattern compared to control group after 28 days of remineralization.**Conclusion:**SAP fluoride plus showed higher remineralizing efficacy than thatof control group.

Keywords: Enamel regeneration, Biomimetic remineralization, Self-assembling peptide, Micro-morphological analysis

I. INTRODUCTION

Dental caries pathophysiology is not simply a continual cumulative loss of tooth minerals, but rather a dynamic process characterized by alternating periods of demineralization and remineralization[1].Demineralization begins at the atomic level on the crystal surface inside the enamel or dentin[2].The earliest phases of demineralization are identified as incipient lesions. The goal of modern dentistry is to manage non-cavitated caries lesions non-invasively through remineralization in an attempt to prevent disease progression and improve esthetics, strength, and function[3].

In the past, fluoride was thought to be the gold standard for halting mineral loss from enamel, However, it was found that fluoride alone couldarrest the early enamel carious lesion instead of reversing it[1].The explanation of this suggestion is that fluoride has the ability to block the superficial porous layer, and this consequently limits further ionic exchange at this layer. This may restrict the remineralization process to the superficial layers making it difficult for full remineralization to the body of the lesionto be achieved[1].

A new approach for reversing and repairing early enamel lesions has been suggested which is called biomimetic remineralization in which enamel is formed as it would, during tooth formation and amelogenesis[4].The application of the self-assembling peptides (SAPs); P11-4, has been advocated for enamel regeneration. Self-assembling peptide is a rationally designed peptide, the monomers of which undergo well characterized self-assembly into a three-dimensional fibrillar scaffold, which mimics the enamel matrix, in response to specific environmental triggers. Enamel crystals are produced around this matrix from the calcium and phosphate of saliva[5].

Recent studies have been conducted to examine SAPs in combination with fluoride varnish resulting in promising results on early enamel lesions remineralization[6, 7]. This was clarified by the synergistic anticariogenic effect of SAPandfluoride varnish[6, 7]. So, SAP fluoride plus, in which SAP and sodium fluoride are incorporated, hasbeen introducedin the market. As

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a result of lack of data examining SAP fluoride plus, so it was found beneficial to evaluate its remineralizing efficacy usingscanning electron microscope. The null hypothesis wasthat there was no significant difference in the micro-morphological analysisof early enamel carious lesions, whether they were treated withSAP fluoride plusor left without intervention.

II. MATERIALS AND METHODS

Material

Table 1. Material used in the study.

Material	**Composition**	**Manufacture**	**Lot number**
Curodont Repair Fluoride Plus	Self-assembling peptide P11−4 and Sodium fluoride 0.05% (0.02% W/V fluoride ion)	Credentis, Windisch, Switzerland	CH220252-882

Methods

Specimen preparation

A total of 15 sound human molars was collected from Oral Surgery clinic at Faculty of Dentistry, Mansoura University. The teeth used for this current study were collected according to the Faculty of Dentistry's institutional ethics committee's regulations. An ultrasonic scaler was used to clean the teeth to eliminate any debris. Teeth disinfection was carried out by immersion of the teeth for 24 hours in 0.5% chloramine-T and then they were immersed in 0.1% thymol solution and refrigerated at 4 C° until use.The roots of the selected teeth were removed using a low-speed diamond saw (Isomet; Buehler, Lake Bluff, IL, USA) under running water and the crowns were cut by alow-speed diamond saw into two halves (buccal and lingual), producing 30 specimens. Each specimen was placed in a block of acrylic resin with the buccal or lingual surfaces facing upward. An adhesive tape (4×4 mm^2 in size) wasplaced in the buccal or lingual surfaces of each specimen followed by application of two layers of acid-resistant nail varnish. Once the nail varnish had dried, the tape was removed exposing 4×4 mm^2 enamel window.

Demineralization

Demineralization was carried out by immersion of the specimens in demineralizing solution for three days at 37 C° in incubator[8].The demineralizing solution was prepared according to the formula of Ten Cate and Duijsters[9] (2.2 mM calcium chloride, 2.2 mM potassium hydrogen phosphate, 0.005 M acetic acid, and 1 M potassium hydroxide) with 4.4 pH. The specimens were then rinsed and kept in deionized water.

Remineralization

Artificial saliva was prepared according to the formula of Ten Cate and Duijsters[9] which contained 1.5 mM calcium chloride, 0.9 mM monosodium phosphate, and 0.15 M potassium chloride at pH 7.0.

The Specimens were randomly allocated intotwo groups (n = 15). Group 1 (control group): no treatment was applied, and the specimens were kept in daily renewed artificial saliva for 28 days at 37 C° in incubator. Group 2 (SAP fluoride plus group): The specimens were dried and etched with 37% phosphoric acid for 20 seconds and then rinsed with deionized water for 20 seconds. The agent was applied for five minutes. It was applied once at the beginning of the study. The specimens were then stored in daily renewed artificial saliva for 28 days at 37 C° inincubator[10, 11].

Scanning electron microscope examination (SEM)

The specimens were left in dry environment, sputter-coated with gold, and mounted on SEM stud. They were examined for micro-morphological characterization using Scanning Electron Microscope (SEM)(SEM; JEOL Ltd., USA) at 2000x magnification. The specimens were examined at baseline, after demineralization, after 28 days of remineralization.

III. RESULTS

SEM Morphological Characters

Representative SEM images revealing morphological features of the enamel surface at baseline, after demineralization, and after remineralization in each group are shown in **Figure 1**(A-D).Baseline enamel showed an intact, homogenous smooth appearance(**Fig.1A**). After demineralization, uneven, rough surface with

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increased porosities was evident (**Fig.1B**).Control group(artificial saliva) showed no morphology alteration or change significant from the demineralized enamel after 28 days of storage (**Fig.1C**). SAP fluoride plus group showed pronounced remineralized patternand the enamel prisms became hidden by mineral deposition (**Fig.1D**).

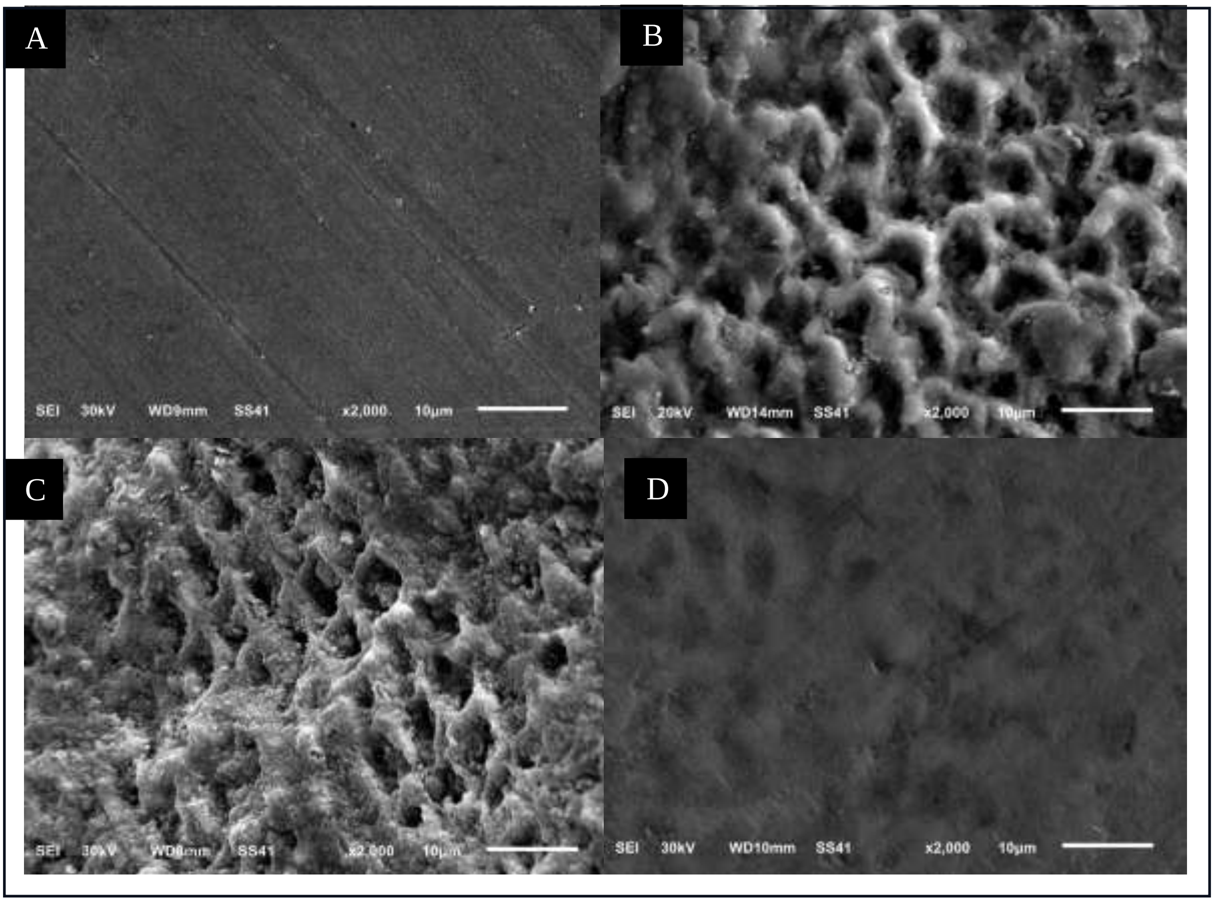

Figure 1(A – D): SEM imageof sound enamel surface at baseline (A), after demineralization (B), after remineralization in control group (C), and after remineralization in SAP fluoride plus group (D).

IV. DISCUSSION

Remineralization is a non-invasive approach to manage non-cavitated carious lesions with the aim of halting the course of the disease and enhancing the strength, appearance, and function of teeth.The requirement for novel and extremely effective technology for enamel remineralization is a crucial component of this treatment philosophy, and it led to the development of several modern remineralizing agents[12]. Biomimetic strategy that simulates the natural process of enamel formation may result in regenerating the damaged enamel surfaces and increasing the tooth longevity[13].Accordingly, this study was conducted to evaluate the remineralizing efficacy of SAP fluoride plus on the micro-morphological analysis of artificially induced carious lesions.

Scanning electron microscope (SEM) is one of the most accurate, time-tested methods to evaluate the demineralization and remineralization of the carious lesions in vitro as demonstrated in earlier studies[14, 15]. SEMaids in illustrating the surface ultra-morphological alterations resulted from application of various remineralizing agents[11].SEM examination is mostly preferred in surface topo-graphic examinations of remineralization investigations because it allows for evaluation without any preparation on enamel surfaces[16].

Based on the findings of the current study, the null hypothesis was rejected. After remineralization, micro-morphological analysis showed that SAP fluoride plus resulted in more pronounced remineralized pattern compared to control group (artificial saliva).Results showed that

SAP fluoride plus group reversed the demineralized enamel surface by occluding defects and regaining the flattened homogenous surface similar to that of the sound baseline enamel. On the other hand, SEM showed that the control group failed to occlude the defects with the demineralized surface pattern remaining even after 28 days.

The better remineralization pattern resulted from SAP fluoride plus could be attributed to that self-assembling peptides promote subsurface remineralization due to its designed application in a monomeric, low viscosity liquid that allows its deep diffusion into the lesion body followed by a rapidly driven 3D gel self-assembly promoting in-depth remineralization[17].Additionally, theeffect of SAP fluoride plusmight be enhanced due to the complementary mechanism of the incorporated fluoride which has the ability to create fluorapatite crystals and also to attract calcium and phosphate ions[4].

The control group failed to occlude the defects of the induced early carious lesion.Although saliva had a role in remineralization[18],it fails to initiate the process of increasing the levels of calcium and phosphate delivery. So, it showed surface only remineralization due to low concentration of calcium and phosphate ions entering the lesion from saliva[18].

One limitation of the current study was the difficulty with simulation of oral conditions and factors that influence early caries lesions in the laboratory setting, because of variations in biofilm and salivary secretion among patients. However, the teeth were maintained in artificial saliva and the material was accurately applied in accordance with the manufacturer's instructions. Other limitations include the lack of studies that have assessed the efficacy of SAP fluoride plus so further in vitro and randomized clinical trials are required.

Through this study, it could be concluded that SAP fluoride plus is more effective in enamel remineralization compared to artificial saliva, providing a novel approach for enamel regeneration.

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