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COMPARATIVE EVALUATION OF REMINERALIZING EFFECT OF CASEIN PHOSPHOPEPTIDE – AMORPHOUS CALCIUM PHOSPHATE FLUORIDE AND SILVER DIAMINE FLUORIDE ON DEMINERALIZED ENAMEL SURFACES (AN IN VITRO STUDY)

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Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP).
Silver diamine fluoride (SDF).
Energy dispersive x-ray spectroscopy (EDX).

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ABSTRACT

Introduction Caries initiation is associated with demineralization of the tooth enamel surface. Calcium and phosphorous are lost from the subsurface enamel, resulting in the formation of a subsurface lesion. **Aim**: this study aimed to investigate and compare the enamel surface ultramorphology and minerals content by Environmental Scanning Electron Microscope (ESEM) and Energy dispersive x-ray spectroscopy (EDX) and to assess micro-hardness (Vickers test) of demineralized enamel surface treated with CPP-ACP casein Phosphopeptide-Amorphous Calcium Phosphate Flouride(MI Paste Plus) or SDF (FAgamine). **Materials and methods**: This study was conducted on twenty eight human premolars ,were collected from the outpatient clinic of Maxillofacial Department, Faculty of Dentistry, Suez Canal University. The selected premolars distributed into three groups: Group I: consisted of 7 intact sound premolars,(control group) Group II : consisted of 7 demineralized premolars,(demineralized group) while, Group III consisted of 14 premolars (each one divided into 28 halves mesiodistally) then all 28 specimens of group III were demineralized and half of them were treated by MI Paste plus (subgroup IIIa) and other were treated by SDF(subgroup IIIb). All Groups I,II&III were placed in artificial saliva for 7days at 37^{o}c. **Results:** There was a statistically significant difference between calcium and phosphorus values of the three groups; subgroup IIIa showed the highest value followed by subgroup IIIb & group I and finally the least value was related to group II .The highest value of fluoride content related to subgroup IIIb. There was not statistically significant difference between microhardness values of group I and group III while group II revealed the lowest microhardness value. **Conclusion:** Both CPP-ACPF and SDF show areas of mineralized deposits and improvement of enamel ultrastructure. Also both CPP-ACPF and SDF are efficient remineralizing agent, but CPP-ACPF is more efficient as remineralizing agent than SDF. CPP-ACPF shows better microhardness results than SDF.

INTRODUCTION

Caries initiation is associated with demineralization of the subsurface tooth enamel. Calcium and phosphorous are lost from the subsurface enamel, resulting in the formation of a subsurface lesion[(1)]. At this early stage, the caries lesion is reversible via a remineralization process involving the diffusion of calcium and phospharous ions into the subsurface lesion to restore the lost tooth structure [(1)]. Remineralization helps in regaining the lost calcium, phosphate, and fluoride ions of the tooth structure and it is replaced in the form of fluorapatite crystals, which are more resistant to acidic dissolution and substantially larger than the original crystals[2,3]. Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP), (Tooth Mousse, GC India) was introduced as a agent in the year 1998 [3,4].Caseins are a heterogeneous family of proteins predominated as phosphorylated casein-derived peptides produced by digestion of casein[5].

In 2014, Silver Diamine Fluoride (SDF) was cleared for sale in the United States by the Food and Drug Administration as class II medical device[5]. SDF [Ag(NH3)2F] has an anticariogenic effect because it releases fluoride ions rapidly to help remineralization. It is recommended by dentist to prevent the progress of caries mostly in Japan, Australia, and the United States[6].

Among the remineralization techniques available, the relatively products, CPP-ACPF and SDF are commercially available. With the advent of all these systems there is great need to quantitatively evaluate the amount of remineralization and one of the techniques to assess the changes in tooth's mineral content is Scanning Electron Microscope (SEM) equipped with EDAX micro-analyser. This study aimed to investigate and compare the enamel surface ultramorphology and minerals content by Environmental Scanning Electron Microscope (ESEM) and Energy dispersive x-ray spectroscopy (EDX) and to assess micro-hardness (Vickers test) of demineralized enamel surface treated with CPP-ACP (GCMI Paste Plus) or SDF (FAgamine).

MATERIALS AND METHODS

The present study was carried out after waived from the approval of the Research Ethics Committee of Faculty of Dentistry, Suez Canal University (125/2018). Since it was conducted on twenty eight unidentified extracted premolars.28 human permanent premolars were collected from the outpatient clinic of Maxillofacial Department, Faculty of Dentistry, Suez Canal University and the selected premolars were distributed into three groups: Group I: (control group) consisted of 7 intact premolars premolars, Group II: (demineralized group) consisted 7 demineralized premolars, while, Group III consisted of 14 premolars (divided into 28 specimens mesiodistally) then all specimens of group III were demineralized and half of them were treated by MI Paste plus (subgroupIIIa) and other were treated by SDF(subgroupIIIb). All Groups I,II&III were placed in artificial saliva for 7days at 37^{o}c. Teeth were examined using environmental scanning electron microscope (ESEM) and subjected to energy dispersive X-ray spectroscopy (EDX) to perceive the ultramorphology of enamel surface and to measure the values of Ca and PO4 content (weight %), Ca: PO4 ratio, and values of fluoride(F)(weight%) respectively in all teeth, in the three groups. Vickers microhardness test was done to sound enamel, after demineralization, post treatment with GCMI Paste Plus and FAgamine using a digital microhardness tester with a load of 100 g applied on the labial surface of the tooth for 10 seconds. Three indentations were made for each specimen in the occlusal, middle and cervical thirds of the tooth and then were averaged.

To ensure accuracy of the measurements, indentations were done on the flattest points of the enamel surface. This flattest point on the testing surface was determined using the microscope attached along with the microhardness tester at 50x magnification.

After selecting the surface, the indent was made and was again observed under microscope for measuring the diagonals of the indentations under same magnifications. These diagonals were marked with the VHN (Vickers Hardness Number) software, and, hence, the VHN values were determined (The tests were done at National Research Centre, Dokki, Egypt).

RESULTS

The results of the research were divided into:

I. Environmental Scanning Electron Microscope (ESEM) results.

II. Energy dispersive x-ray spectroscopy (EDX) (Minerals content) results.

III. Microhardness results (Vicker test).

ESEM test results: Group I (control): showed typical morphology of the enamel surface layer with honey comb appearance. Smooth and uniform structural shape was shown as a whole with bands of normal enamel rods and interprismatic regions fig(1) .Group II (Demineralized) showed aragged rough profile that showed a destruction of prismatic pattern in all sample. Closer examination with higher magnification of the prismatic pattern of demineralized enamel showed that the integrity of the enamel rods was severely affected with extensive pitting (pores & holes) fig(2). The Scanning Electron micrograph of enamel of subgroup IIIa showed the interprismatic substances with porosities and areas of remineralization also seen fig(3). Higher magnification of the prismatic pattern of the same subgroup displayed thick and more frequent lines of remineralization along the prismatic borders; certain areas of calcifications were evident along the porosities fig(3). TheScanning Electron micrograph of subgroup IIIb after application of SDF showed evenly more distributed opaque colors on the micrographs which indicating an increase in mineral density fig(4). **II) EDX (Minerals content) results: Ca & Po_4 weight ratio**.There was a statistically significant difference between Ca & P weight % values of the three groups at P-value 0.05. Pair-wise comparisons reaveled that subgroup IIIa showed the increase in Ca & P weight % value .There was no statistically significant difference between group I& subgroup IIIb P-value 0.05 While Group II showed the decrese in mean Ca & Po_4 weight %.(fig 5,6). **Ca:Po_4 ratio,** There was a statistically significant difference between Ca:Po_4 ratio in the three groups fig(7). In the three groups, using Pair-wise comparisons revealed that there was no statistically significant difference between group I and subgroup IIIa, subgroup IIIb; all showed the highest mean Ca:Po_4 ratio, While Group II showed the lowest mean Ca: PO_4 ratio. **F weight**, there was a statistically significant difference between F weight % values of the three groups at P-value 0.05< fig(8). Pair-wise comparisons revealed that sub group IIIb showed the highest F weight % value while Group II showed the lowest mean F weight %.

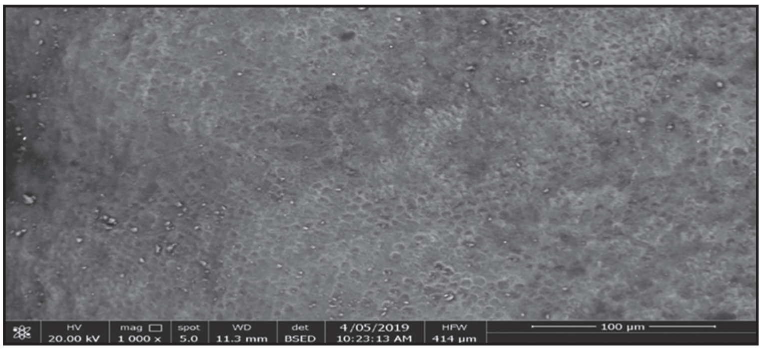

Fig. (1) Scanning Electron micrograph of untreated enamel from GroupI shows smooth surface with enamel rod ends appearing in some areas with honey comb appearance (X1000).

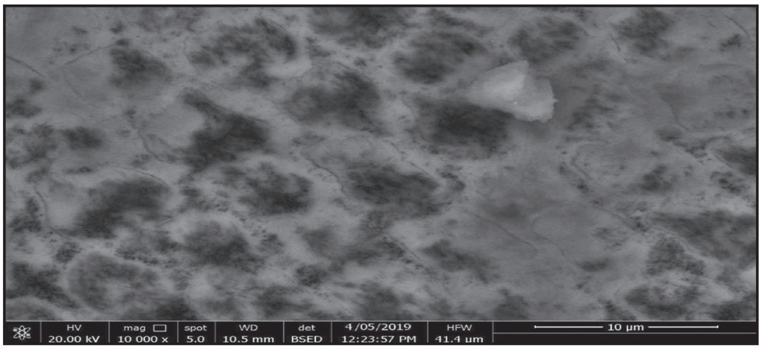

Fig 2: Scanning Electron micrograph of demineralized enamel from Group II shows alterations between rodless enamel and porous areas at higher magnification (X10000).

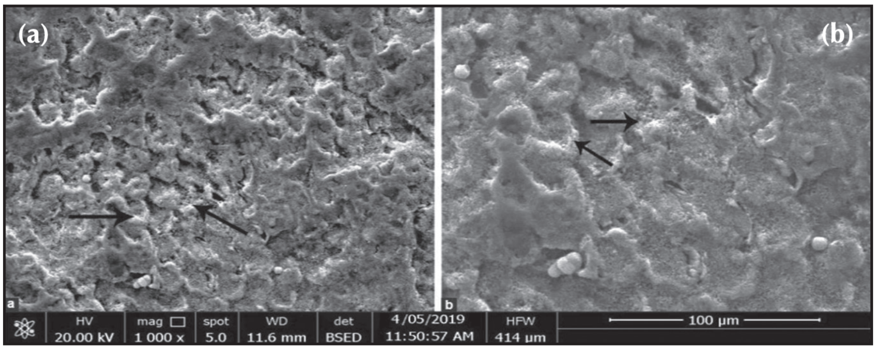

Fig 3 (a&b): Scanning Electron micrograph of enamel from subgroup IIIa shows porosities and areas of remineralization spaces (x1000,2000)

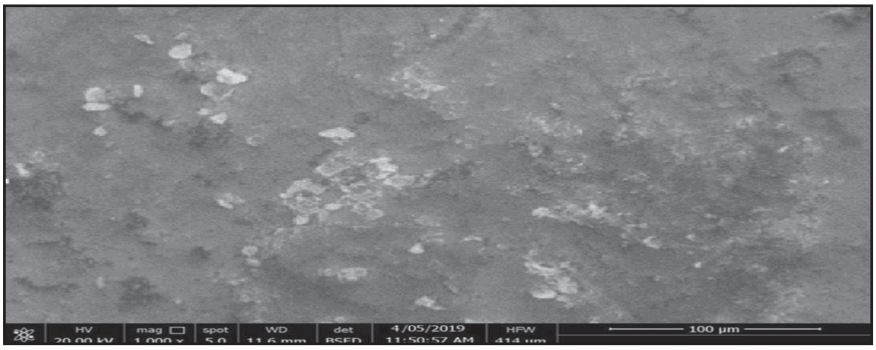

Fig 4: Scanning Electron micrograph of enamel from subgroup IIIb shows scattered mineralized deposits (x1000).

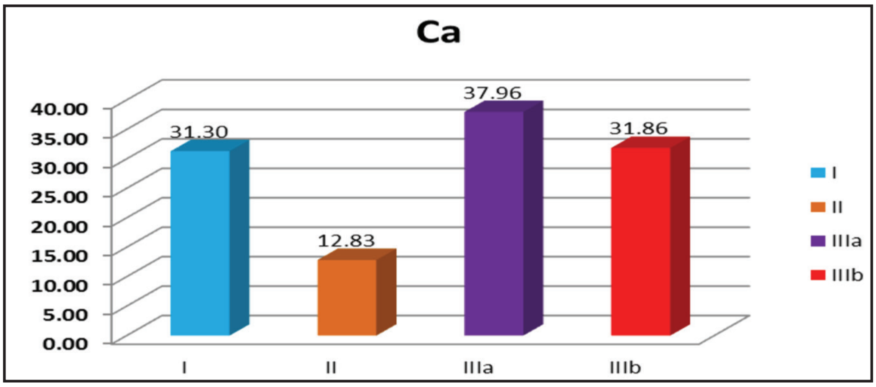

Fig (5): Bar chart representing mean and standard deviation values for Calcium weight % of the three groups.

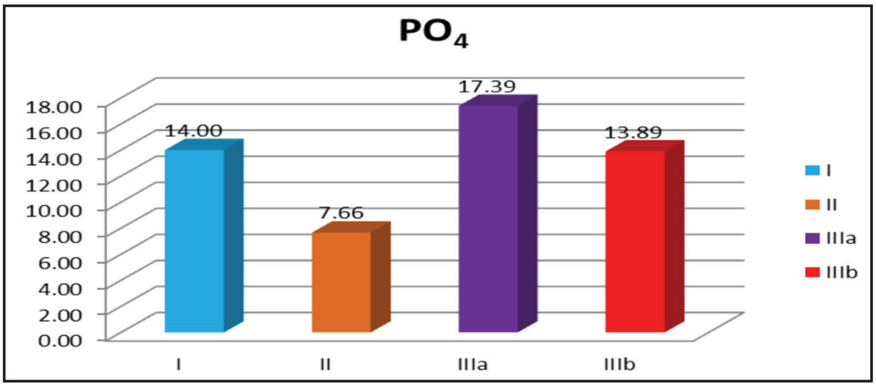

Fig (6): Bar chart representing mean and standard deviation values for Phosphorus weight % of three groups

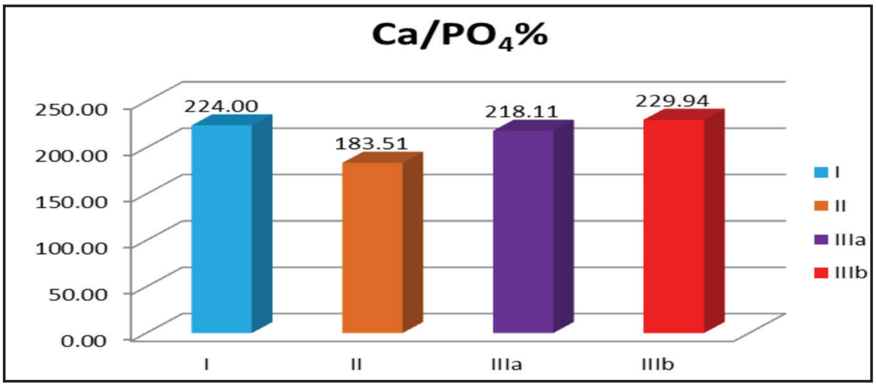

Fig (7): Bar chart representing mean and standard deviation values for Calcium Phosphorus weight ratio% of three groups

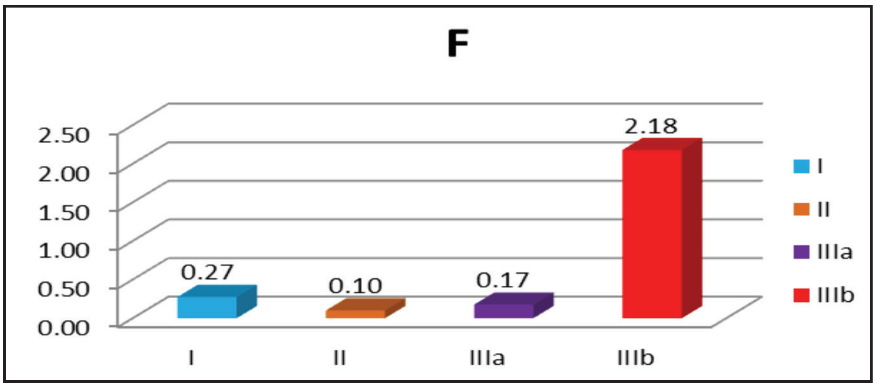

Fig (8): Bar chart representing mean and standard deviation values for Fluoride weight % of three groups.

Microhardness results (vicker test): There was a statistically significant difference between microhardness values of the three groups at P-value 0.05< for load. Pair-wise comparisons revealed that there was no statistically significant difference between group I and subgroup IIIa, subgrupIIIa; showed higher mean values while group II showed lowest mean value microhardness. fig (9)

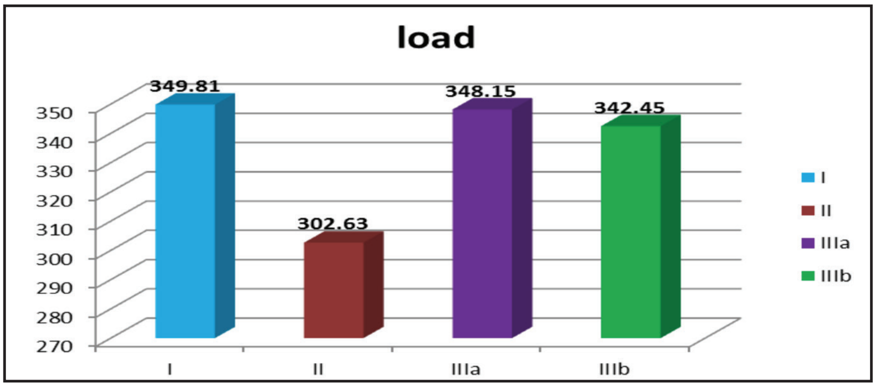

Fig (9): Bar chart representing mean and standard deviation values for microhardness of the three groups.

DISCUSSION

Twenty-eight sound extracted maxillary and mandibular premolars chosen in this study as that. Teeth are most likely to be extracted for orthodontic purpose from patients aged from 11-14 years. They were thoroughly inspected to be free from any caries, restorations, cracks, anatomical or morphological abnormalities therefore no natural alteration influenced the remineralzing action and microhardness [7]. The selected premolars should be freshly extracted and they were stored in normal saline or distilled water at room temperature not more than one month to prevent dehydration. The storage media should be changed weekly to prevent any bacterial growth[8]. Among the methods used to induce caries-like lesions, pH cycles were chosen because it was possible to reproduce the dynamic process of caries lesion development, alternating demineralization and remineralization periods being widely applied to assess dentifrices[9-12]. All samples were placed in artificial saliva after applying the treated materials to simulate the environment of the oral cavity to provide the calcium and phosphorous necessary to promote naturally occurring remineralization[13,14].The environmental scanning electron microscope (SEM) was used to evaluate and record the ultra-structural changes of the enamel surface as it is a non-destructive characterization technique, which requires little or no sample preparation[15] .Regarding to microscopic results of SEM, the samples treated with CPP-ACPF showed that the prismatic pattern displayed thick and more frequent lines of remineralization along the prismatic borders; certain areas of calcifications were evident along the porosities, these results were consistent with the proposed remineralization mechanism of CPP-ACPF and were in accordance with the one obtained by **Reynold *et al*,**[16]. who demonstrated that CPP-stabilized calcium phosphorous solutions remineralized subsurface lesions in human enamel *in vitro*. Samples treated with SDF revealed improvements of the enamel ultrastructure. The specimens manifested a smoother enamel surface with decreased number and size of previously formed pores compared to demineralized enamel indicating an increase in mineral density. Energy dispersive x-ray spectroscopy **(EDX)** was used for assessing Ca, Po_4 and F weight percentage in different groups since the main components of hydroxyapatite are Ca and Po_4 so these elements were main objects of this study, also monitoring Ca/Po_4 ratio changes was done for all samples in the three groups[17.18].Regarding the EDX results, there was an improvement in Ca and PO_4 weight percentage related to subgroups IIa treated with

GCMI Paste plus and FAgamine compared to group II (demineralized group).

Regarding to Ca and Po_4 weight percentage the highest values were recorded in Casein subgroup. The results of this study were consistent with the proposed remineralization mechanism of CPP-ACPF and were in accordance with the results obtained by many previous studies, [19-21] that demonstrated, that, CPP-stabilized calcium phosphate solutions on remineralized subsurface lesions in human enamel *in vitro*.

According **to Yamaguchi *et al*[22] , Oshiro *et al*,[23] and Hegde *et al***,[24] the inorganic components contained in high concentrations in CPP-ACPF acted to enhance remineralization of the demineralized enamel ,these results consistent with the results of the present study. These increase in Ca and Po_4 concentration in subgroup IIIb demonstrated that the concentration of calcium in the remineralization solution (artificial saliva), was found to be reduced as SDF promotes absorption of calcium leading to inhibition of calcium dissolution from enamel. Also these results are in accordance with **Yihong *et al*, ,**[25]who revealed that silver particles could penetrate through the pellicle complex, along with the rod sheaths into the demineralized enamel rods and the dentinal tubules, and form silver-enriched barriers surrounding the carious lesions at depths up to 2,490.2 μm within the dentinal tubules of the carious lesions so it inhibited demineralization and promoted remineralization . The lowest value of F was recorded in group II followed by casein and control groups while the highest value was recorded at SDF subgroup .The results of this study are consistent with the proposed remineralization mechanism of SDF and in accordance with the results obtained by **Mei *et al*,** [26]. One of the objectives of this study was to evaluate the micro-hardness of an enamel treated surface as hardness means the resistance of a material or tooth surface against indentation or penetration, which is considered an important mechanical characteristic of the material or the surface because the resistance to abrasion, friction, erosion, or wear or any other substance, in general, increases with increasing hardness[27]. In the present study Group I (control) showed the highest microhardness value. This was in agreement with many studies done by **Schmitt *et al*,** [28] and **Borges *et al*,**[29].There is a significant decrease in microhardness in group II . This was in agreement with many studies [30-33] as minerals, primarily calcium and phosphate, leak out from the hydroxyapatite crystals during demineralization and in situations where demineralization outpaces remineralization, this leads to the development of subsurface lesions[34].While in subgroup IIIa, in the samples which were treated with CPP-ACPF, there is a marked significant increase in microhardness, this was in agreement with previous studies done by **Agnihotri *et al*,** [35] This could be related to the presence of 'Recaldent' that combines casein phosphopeptide (CPP) from milk with amorphous calcium phosphate (ACP). Moreover, the results showed that the samples treated with SDF had a marked increase in microhardness significant difference from samples treated with CPP-ACPF, this was in agreement with the study done by **Irene *et al*,** [36] **.**This result may be due to formation of hydroxyapatite and fluoroapatite on the exposed organic matrix, along with the presence of silver chloride and metallic silver. The treated lesion increases in mineral density and hardness while the lesion depth decreases**.**

CONCLUSION

Both CPP-ACPF and SDF show areas of mineralized deposits and improvement of enamel ultrastructure. Both are efficient remineralizing agents, but CPP-ACPF is more efficient as remineralizing agent than SDF. CPP-ACPF shows better microhardness results than SDF.

1. The collection of teeth that met the inclusion criteria was a difficult

2. Difficulties in creation of the demineralization window.

3. It is not possible to reconstruct the real oral cavity characteristics to evaluate the actual effects of examined materials.

4. For group III the creation of the“demineralization window” was attempted during the initial pilot study, but it was unsuccessful, because it was difficult to isolate the small area of each tooth.

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