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CHEMICAL AND MORPHOLOGICAL ANALYSIS OF HUMAN DEMINERALIZED ENAMEL SURFACE BY ACID AND LASER ETCHING FOLLOWING REMINERALIZATION BY MI PASTE PLUS

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ABSTRACT

Objectives: The aim of the present study was to evaluate the effects of remineralizing agent (MI Paste Plus) on the surface topography and chemical analysis of sound human enamel following acid-etching and laser etching, using Environmental scanning electron microscopic (ESEM) techniques.

Materials and Methods: Fifty human upper premolars extracted for orthodontic reason were used. The teeth were mounted vertically in a self-cure acrylic cylinder. One clinician prepared all the teeth, in each tooth; a 4x4 mm area was treated in middle third of the buccal surface. The teeth were divided into three groups, Group I : Control group no treatment ; Group II: enamel etched with 37% phosphoric acid; Group III: enamel was irradiated with Er,Cr:YSGG laser. Group II and III were subdivided into subgroup according to remineralizing agent (GC MI Paste Plus), (n=10). Remineralizing paste was used as manufacturer's instructions, a single application of approximately 0.5 mm layer for 3 minutes; twice daily. Synthetic saliva was rinsing between remineralizing paste application, followed by synthetic saliva rinsing for 15 days with fresh replenished on a daily basis. Teeth were examined for morphological and chemical characterization using an Environmental Scanning Electron Microscope. All of the data were analyzed by two-way analysis of variance (ANOVA) and Tukey's post hoc test.

Results: Morphological changes of enamel surfaces of acid-etching group showed type 1 etching pattern and laser etching group showed the normal appearance of the enamel prisms (honeycomb-like structure). Comparison of all groups and control group P & Ca weight%, the greatest mean value was recorded in acid etch group before and after remineralization with GC MI Paste Plus.

Conclusion: Within the limits of this in vitro study, Since Ca and P constitute the major part of tooth structure, while other element represent trace element; results emphasize the effect of GC MI Paste Plus on tooth remineralization. Comparison of all groups and control group, acid etch group before and after remineralization recorded the greatest mean value for P & Ca weight%. Laser etching didn't have a significant effect on tooth composition and mineral content.

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INTRODUCTION

Dental caries is a major public health problem. A goal of modern dentistry is to manage non-cavitated caries lesions non-invasively through remineralization in an attempt to prevent disease progression and improve aesthetics, strength, and function. The enamel surface demineralization resulted in Phosphoric acid; it was at depths ranging from 5 μm to 25 μm [1]. Chemical treatment by acid etching enhances the topography of enamel, changing it from a low-reactive surface to a surface that is more susceptible to adhesion. The demineralization is selective because of the morphological disposition of the prisms. Importantly, the acid-etched surface allows the less mineralized underlying enamel to be exposed to a potentially acidic micro-environment [2 -3]. Lasers, such as Er:YAG laser, have been studied as an alternative method to selectively remove oral mineralized tissues for restorative purposes [4].

Remineralization is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel, to produce net mineral gain[5]. Recently, a range of novel calcium-phosphate-based remineralization delivery systems has been developed for clinical application. Recaldent™, which is a complex of casein phosphopeptides and amorphous calcium phosphate (CPP-ACP), has been proclaimed to prevent and even reverse white spot lesions. Casein phosphopeptides (CPP), which are products of milk protein casein, are thought to have the ability to increase the level of calcium phosphate in dental plaque which would depress the demineralization process and raise the remineralization process [6-7-8]. Recaldent is the active ingredient in MI Paste™ and MI Paste Plus™, which are preventive treatment products marketed by GC America (Alsip, IL, USA) to provide a wide variety of benefits. The products are advertised to help prevent demineralization, and enhance remineralization[9-10].

The purpose of this *in vitro* study was to evaluate the effects of remineralizing agent (MI Paste Plus) on the surface topography and chemical analysis of sound human enamel following acid-etching and laser etching, using Environmental scanning electron microscopic (ESEM) techniques.

MATERIALS AND METHODS

Fifty human upper premolars extracted for orthodontic reason were used. The selected teeth should not be treated with any kind of agent in the past; they should not present restorations, caries or fissures due to the pressure of the pliers during the extraction. Teeth were stored in distilled water at room temperature to prevent dehydration. The labial surface of all teeth were cleaned and polished with fluoride - free paste using a rubber cup mounted on a low-speed contra-angle handpiece for 30 s. The surfaces were rinsed with water and dried with air. The teeth were mounted vertically in a self-cure acrylic (Rapid Repair, Detrey Dentsply Ltd, Surrey, U.K.) cylinder. One clinician prepared all the teeth, in each tooth; a 4x4 mm area was treated in middle third of the buccal surface. The teeth were divided into three groups, Group I : Control group no treatment, These control surfaces were not acid-etched, not exposed to remineralizing agent, and not exposed to synthetic saliva (10 teeth); Group II: enamel etched with 37% phosphoric acid (3M Unitek, Monrovia, USA) for 15s, thoroughly rinsed with distilled water for 60 s and gently air dried. (20 teeth); Group III: enamel was irradiated with Er,Cr:YSGG laser ablated with an energy output of 4.5 watt/30hz (Er,Cr:YSGG laser (2.78 μm wavelength) used for 15 sec at 45degree angulations working distance 5mm on a square size 4x4mm on buccal surface (20 teeth). Group II and III were subdivided into subgroup 10 teeth according to remineralizing agent (GC MI Paste Plus), (n=10). Remineralizing paste was used as manufacturer's instructions: a single application of approximately 0.5 mm layer for 3 minutes[11-12]; twice daily with synthetic saliva [Fusayama

Meyer's artificial saliva, which was composed of NaCl (0.400g/l), KCl (0.400 g/l), $CaCl_2 \cdot H_2O$ (0.906g/l), $NaH_2PO_4 \cdot 2H_2O$ (0.690 g/l), $Na_2S \cdot 9H_2O$ (0.005 g/l) and urea (1 g/l) with a pH of 7.1. It was rinsing between remineralizing paste application, followed by synthetic saliva rinsing for 15 days with fresh synthetic saliva replenished on a daily basis[13]. Teeth were examined for morphological and chemical characterization using an Environmental Scanning Electron Microscope (ESEM) (Quanta 200, FEI Company, Philips Electron Optics, Eindhoven, Netherlands) equipped with Electron Dispersive Analytical X-ray (EDAX).

All of the data were analyzed by two-way analysis of variance (ANOVA) and Tukey's post hoc test.

RESULTS

Morphological changes

Teeth were examined at magnifications of X250 & X1500 with ESEM, which showed on control teeth intact surfaces with typical termination of enamel prisms on the enamel surface with central prism cores appearing as slight depressions and the prism peripheries being slightly elevated. Enamel surfaces of the acid-etching group showed type 1 etching pattern with partial loss of the central prism core and retention of the prism periphery (Fig a). Enamel surfaces of the acid-etching followed by GC MI Paste Plus and synthetic saliva rinsing showed surface coatings with a fine granular texture and no obvious porosities (Fig b). Enamel surfaces of the laser group showed the normal appearance of the enamel prisms was maintained in most areas (honeycomb-like structure). However, confluence of the prismatic and inter-prismatic structure was noted in some areas, giving the enamel an irregular appearnce (Fig c).

Under gone laser etching followed by GC MI Paste Plus and synthetic saliva rinsing showed relatively homogenous dense surface coatings with focal areas that were partially obscured by granular to somewhat globular fine deposits (Fig d).

Chemical analysis

Comparison of all groups and control group P & Ca weight%, the greatest mean value was recorded in acid etch group before remineralization, then laser group before remineralization. After remineralization, higher mean weight percent of P and Ca were recorded in acid etch group. Regarding F weight%, this element was only detected in normal enamel. Regarding O & Mg weight%, the greatest mean value was recorded in normal enamel, followed by laser group before remineralization,

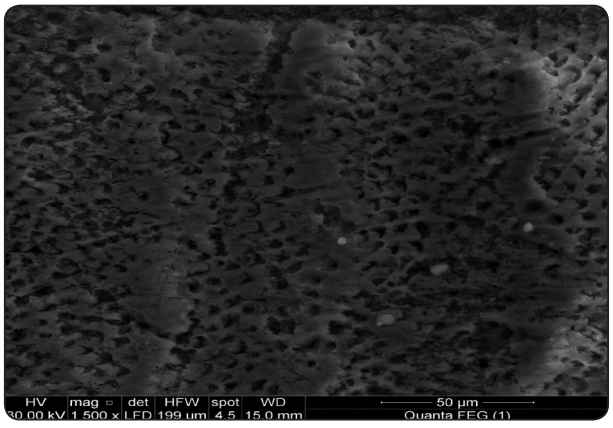

Fig. (a) Acid-etch without treatment

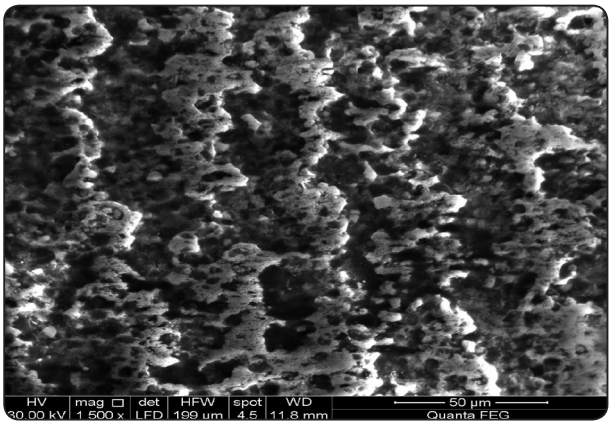

Fig. (b) Acid-etch after treatment

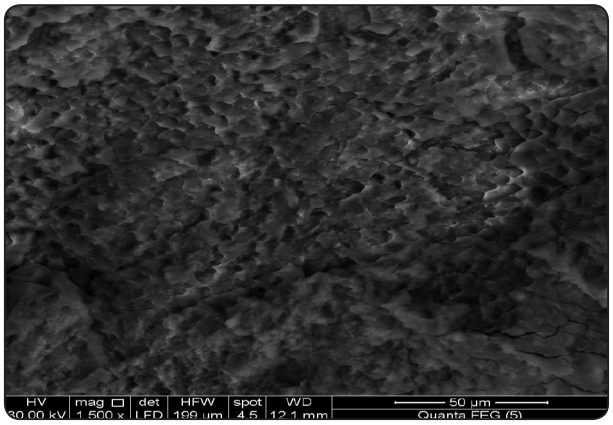

Fig. (c) laser etching without treatment

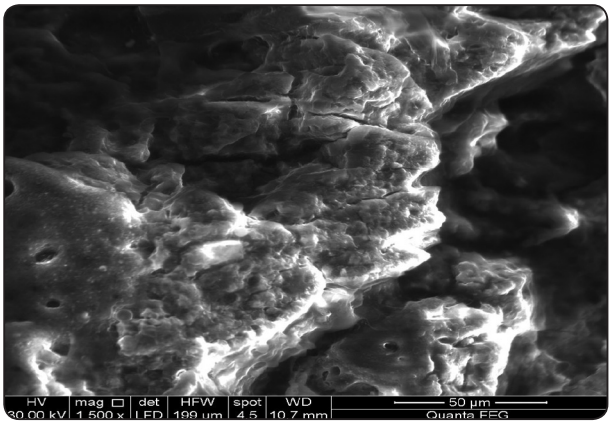

Fig. (d) laser etching after treatment

then acid etch group before remineralization. C weight%, showed the greatest mean value in laser group after remineralization, followed by the acid etch group after remineralization. Regarding Cl weight%, the greatest mean value was recorded in acid etch group after remineralization, then laser group before remineralization. Na weight%, showed the greatest mean value in laser group before remineralization, followed by acid etch group before remineralization, then normal enamel. Regarding Si weight%, this element was only detected in laser group after remineralization. ANOVA test revealed that the difference between all groups was extremely statistically significant (P<0.0001). Tukey's post hoc test revealed no significant difference between both groups after remineralization (Table 1, Fig. e).

TABLE (1) Weight percent of different elements in normal enamel and experimental groups before and after remineralization (ANOVA test)

		Mean	Std. Deviation	Std. Error	Minimum	Maximum	F	Sig.
C	Laser	7.407[b]	.15283	.04833	7.24	7.61		
	Acid etch	8.032[b]	.57548	.18198	7.28	8.68	409.018	<.0001*
	Laser after remineralization	29.173[a]	3.95615	1.25104	23.44	31.63		
	Acid etch after remineraliztion	24.197[a]	1.61432	.51049	22.28	26.23		
	Normal enamel	.0000[c]	.00000	.00000	.00	.00		
O	Laser	28.976[b]	1.43899	.45505	27.89	31.04		
	Acid etch	26.110[c]	.68406	.21632	25.64	27.10	1.609	<.0001*
	Laser after remineralization	21.266[d]	1.74863	.55296	20.18	23.80		
	Acid etch after remineraliztion	21.302[d]	1.05703	.33426	20.55	22.83		
	Normal enamel	56.676[a]	.30104	.09520	56.45	57.11		
Na	Laser	.6570[a]	.09673	.03059	.57	.79		
	Acid etch	.3960[b]	.27330	.08643	.00	.57	38.023	<.0001*
	Laser after remineralization	.0000[c]	.00000	.00000	.00	.00		
	Acid etch after remineraliztion	.0000[c]	.00000	.00000	.00	.00		
	Normal enamel	.3110[b]	.13739	.04345	.22	.51		

		Mean	Std. Deviation	Std. Error	Minimum	Maximum	F	Sig.
Mg	Laser	.2640 [a]	.06851	.02166	.20	.36	23.281	<.0001*
	Acid etch	.1770 [a]	.12284	.03885	.00	.27		
	Laser after remineralization	.0000[b]	.00000	.00000	.00	.00		
	Acid etch after remineraliztion	.0000[b]	.00000	.00000	.00	.00		
	Normal enamel	.2810[a]	.14449	.04569	.18	.49		
Si	Laser	.0000[b]	.00000	.00000	.00	.00	909.893	<.0001*
	Acid etch	.0000[b]	.00000	.00000	.00	.00		
	Laser after remineralization	.5990[a]	.06280	.01986	.56	.69		
	Acid etch after remineraliztion	.0000[b]	.00000	.00000	.00	.00		
	Normal enamel	.0000[b]	.00000	.00000	.00	.00		
P	Laser	18.844[b]	.11325	.03581	18.68	18.92		
	Acid etch	19.780[a]	.08406	.02658	19.69	19.88	396.625	<.0001*
	Laser after remineralization	14.095[d]	.70042	.22149	13.66	15.11		
	Acid etch after remineraliztion	16.930[c]	.59391	.18781	16.32	17.74		
	Normal enamel	13.957[d]	.18227	.05764	13.78	14.21		
Cl	Laser	.5430 [a]	.05794	.01832	.46	.59		
	Acid etch	.4870 [a]	.33787	.10684	.00	.73	21.539	<.0001*
	Laser after remineralization	.4980 [a]	.07729	.02444	.45	.61		
	Acid etch after remineraliztion	.5640[a]	.07633	.02414	.49	.67		
	Normal enamel	.0000[b]	.00000	.00000	.00	.00		
Ca	Laser	43.318[b]	1.55965	.49320	41.07	44.44		
	Acid etch	45.000[a]	.62268	.19691	44.28	45.80	456.216	<.0001*
	Laser after remineralization	34.376[d]	1.36219	.43076	33.53	36.35		
	Acid etch after remineraliztion	37.007[c]	.72610	.22961	36.41	37.85		
	Normal enamel	27.241[e]	.64937	.20535	26.55	27.97		
F	Laser	.0000 [b]	.00000	.00000	.00	.00	6322	<.0001*
	Acid etch	.0000 [b]	.00000	.00000	.00	.00		
	Laser after remineralization	.0000 [b]	.00000	.00000	.00	.00		
	Acid etch after remineraliztion	.0000[b]	.00000	.00000	.00	.00		
	Normal enamel	.0100[a]	.00000	.00000	.01	.01		

**** Significant at P<0.05. Within the same elememt, means sharing the same superscript letter group are not significantly different***

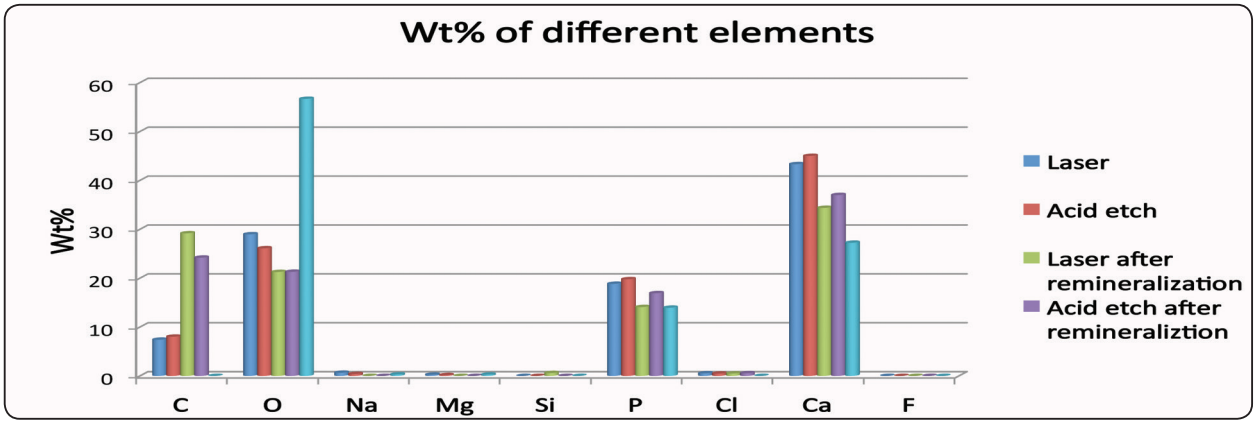

Fig. (e) Column chart showing weight percent of different elements in normal enamel and study groups.

DISCUSSION

Saliva plays a critical role in the prevention and remineralization of enamel and root surface caries. Saliva contains calcium, phosphate, proteins, immunoglobulins, antibacterial substances, and buffers which can neutralize the acids produced by plaque bacteria, raise the pH, reverse the diffusion gradient of calcium and phosphate during demineralization, and enhance remineralization [14]. Hicks demonstrated that synthetic saliva rinsing after acid-etching effectively masked the effects of acid-etching, and may initiate the "remineralizing" process for acid-damaged sound enamel surfaces [13].

The surface topographic changes analyzed by SEM showed that the enamel surface treated by acid etching recorded type 1 etching pattern with partial loss of the central prism core and retention of the prism periphery. Enamel surfaces of the acid-etching followed by GC MI Paste Plus and synthetic saliva rinsing showed surface coatings with a fine granular texture and no obvious porosities. Enamel surfaces of the laser group showed the normal appearance of the enamel prisms was maintained in most areas (honeycomb-like structure). Under gone laser etching followed by GC MI Paste Plus and synthetic saliva rinsing showed relatively homogenous dense surface coatings with focal areas that were partially obscured by granular to somewhat globular fine deposits.

MI Paste Plus™ is a sugar-free, water-based cream paste which was introduced to the American market in April 2007. The active ingredient of MI Paste Plus™ is casein Phosphopeptide-amorphous calcium phosphate with 900ppm Fluoride (CPP-ACPF) [15-16]. In this study, the application of the protective agents enhanced the creation of a superficial mineral layer. As demonstrated by scanning electron microscopy, this protective layer was more homogeneous for the specimens treated with MI Paste Plus after acid etching. POGGIO C. et.al.2015, which tested the efficiency of different remineralizing agents (Remin Pro, Tooth Mousse, MI Paste Plus, and Profluorid Varnish) on bleached enamel surface and they concluded that the use of CPP-ACP (MI Plus Paste) showed complete and homogeneous protective layer which was better in remineralization than the other tested agents [17]. This was in agreement with Jayarajan et al., they concluded that CPPACPF group, enamel rods and prismatic substances are not discernable but areas of calcified deposits are more evident and concentrated along the porous defects. The enamel treated with MI Paste Plus has a homogeneous smooth appearance when compared to MI Paste [18].

Comparison of all groups and control group in this study P& Ca weight%, the greatest mean value was recorded in acid etch group before remineralization, then laser group before remineralization. After remineralization, higher mean weight percent of P and Ca were recorded in acid etch group. The availability of calcium in the oral environment is a key requisite in the remineralization of enamel and dentin. This has led to the development of various calcium based systems that enhance the availability of calcium and phosphate [19-20]. This is in agreement with other studies concluded that remineralization could be estimated in terms of calcium and phosphorous content of enamel also EDAX results indicated that there was a decrease in the wt% of both calcium and phosphorus following the demineralization of samples. Furthermore, after remineralization with MI Paste Plus for15 days [21]. Acid-etching of enamel surfaces created porous enamel surfaces that may provide a more reactive surface for interaction with caries preventive agents [13]. In the current study remineralization after laser etching had less value than remineralization after acid etching this may be due to CPP-ACP molecules need an acidic exposure to get activated and this would separate ACP from the casein. The samples were not acid activated when CPP-ACPF was applied on the tooth surface [22]. Moreover it may be due to a difference in time between the release

of ACP from CPP during the acid challenge and the time required to deposit calcium and phosphate into the lesion during remineralization. Another reason can be due to the short duration of application of the material [23-24].

As regards demineralization process is directly affected by the acidic environment. During demineralization, calcium, phosphate, fluoride, carbonate, sodium, and magnesium ions diffuse out from the enamel surface into the saliva. More the acidic environment more is the outflow of the calcium ion and phosphate ion. Mineral content of surface is higher than the body of the lesion. As calcium and phosphate ions diffuse to the exterior, there is more probability of remineralization at the surface [25-26-27].

In the present study O & Mg weight%, the greatest mean value was recorded in normal enamel, followed by laser group before remineralization, then acid etch group before remineralization. This in agreement with other study that was concluded that the loss of Mg was not statistically significant; however, a relatively large amount of Mg loss may be due to the fact that Mg is among the first elements to be dissolved during the demineralization process. Thus, the loss of Mg could be the first sign of demineralization [28].

In the present study F weight% was detected in normal enamel as a constituent of the tooth structure supplied by tooth pastes, certain food and brevages and topical fluoride application. The absence of this element in acid and laser etch groups denotes that these treatments adversely affect the concentration of this cariostatic element, indicating the need of topical fluoride application after etching to compensate its possible loss and ensure its presence in a beneficial concentration. Fluoride uptake into enamel was influenced by fluoride concentration and pH of topical fluoride solution [29- 30].

Regarding Cl wt%, no significant difference among all the groups was observed as the highest energy density was used for Er:YAG laser irradiation, consistent with the findings of Ca and O, suggesting that this energy density only produces morphological changes on the enamel without structural variations. The differences found between groups could be explained by atomic substitution in apatite, specially chlorapatite (ClAp), as might be expected from the difference in electro-negativities of the elements and the heat generated by laser [31].

The effects of laser and acid etching on the fluorine content of enamel was also studied since fluorine content can be used to verify the remineralization potential. As expected, all treatments significantly decreased the levels of fluorine; however there was no significant difference between the laser and acid etching treated groups [30-32].

Since Ca and P constitute the major part of tooth structure, while other element represent trace element; results emphasize the effect of GC MI Paste Plus on tooth remineralization.

CONCLUSIONS

Under the limitations of this *in vitro* study, the following conclusions can be drawn:

1- Comparison of all groups and control group P & Ca weight%, the greatest mean value was recorded in acid etch group before and after remineralization with GC MI Paste Plus.

2- Laser etching didn't have a significant effect on tooth composition and mineral content.

3- Since Ca and P constitute the major part of tooth structure, while other element represent trace element; results emphasize the effect of GC MI Paste Plus on tooth remineralization.

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