ORIGINAL ARTICLE

Comparison of Remineralization Effect of Casein Phosphopeptide Amorphous Calcium Phosphate and Sodium Fluoride on Enamel Surface after Orthodontic Debonding: An In vitro study

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OBJECTIVE: To compare the result of "casein phosphopeptide amorphous calcium phosphate" with "sodium fluoride" (900 ppm) in remineralization of enamel after orthodontic debonding.

METHODOLOY: This study was performed on premolars. Teeth were distributed into 3 groups A, B and C. The baseline microhardness of samples was measured. To create artificial demineralization, all samples were dipped in demineralizing solution. Surface microhardness (SMH) was re-measured for demineralization. Then, CPP-ACP was applied on teeth of gp B and NaF (900 ppm) was applied on teeth of gp C for remineralization. All teeth were cycled between deionized water and surface treatment for 12 weeks. The SMH of gp B and gp C was re-measured for remineralization. Paired t-test and Independent t-test were practiced to match MH before and after demineralization while keeping P value ≤ 0.05. One way ANOVA was practiced to match mean difference in baseline MH and after demineralization.

RESULTS: The baseline SMH of enamel decrease after demineralization. After application of test materials, mean SMH increased by 230.2 VHN and 207.5 VHN respectively. The %age of SMHR reveal the recovery rate of 41.2% in CPP-ACP and 19.1% in NaF group.

CONCLUSION: For treating post orthodontic demineralized lesions, a remineralizing cream having casein phosphopeptide stabilized amorphous calcium phosphate is useful with some mineral and aesthetic improvements.

KEYWORDS: Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP), Sodium Fluoride (NaF), Surface Microhardness (SMH), Vickers Hardness Number (VHN), Rmineralization.

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INTRODUCTION

Fixed appliances are mostly the appliance of choice for orthodontic treatment.[1] The advantages of fixed orthodontic treatment are patient comfort and conservative method of bonding.[2] Disadvantages include surface roughness, scratches, discoloration, demineralization and caries.[3] After fixed orthodontic treatment, the most difficult problem is the control of enamel demineralization.[4] The rough surfaces of bands, wires and brackets confine the cleaning activity of cheeks, tongue and lips.[5] Thus, plaque accumulation and cariogenic micro-organisms increase widely during orthodontic treatment.[6] Clinically visible demineralized lesions arise as quickly as 4 weeks following placement of orthodontic appliances with the incidence of 24.9% to 96%.[7,8]

Demineralization is the removal of enamel's inorganic constituents by acids that are produced by bacteria existing in the plaque.[9] These acids soften calcium phosphate content

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of dentine and enamel, and result in demineralization.[10] Few teeth are extra prone to demineralization such as maxillary lateral incisors, canines, especially in the gingival third and first molars in the middle third.[9,11] Demineralization presents an aesthetic concern because it compromises the aesthetics of smile.[12] Regular remineralization via mineral ions existing in saliva occurs simply in the shallow layers of demineralized lesions.[13]

Though, different managements are mentioned to support remineralization. The scientific base of fluoride use is that fluoride be able to enter into crystal-like arrangement of enamel, reduces its solubility plus increase resistance for acids. Fluoride ions substitute hydroxyl assembly of hydroxyapatite, then form fluorapatite.[13] Thus low dose fluoride application is advised for sub surface remineralization.[11]

A new remineralizing agent CPP-ACP, is a derivative of milk casein, has ability to absorb through enamel surface.[14,15] It preserves supersaturated position of enamel mineral, phosphate and calcium in plaque and encourage remineralization,[16] delays the growth of biofilm plus hinders the adhesion of bacteria to tooth surface.[17,18] It works like buffering agent that stop a fall of pH and regulate acid-base balance.[17] So, CPP-ACP has been included in various manufactured goods for example, chewing gums, mouth rinses, sports drinks, glass ionomer cements, topical creams and water based mousse.[19,5]

Therefore, the rationale of this research was to determine the most effective method for remineralization of demineralized enamel which facilitates the clinicians to overcome the demineralization of enamel after orthodontic debonding.

METHODOLOGY

This experimental study of twelve months was performed in Orthodontics department of de'Montmorency College of Dentistry, Lahore. The sample size was calculated by convenient sampling technique followed by randomization, keeping power of study equal to 90% and level of significance equal to 5% using formula.

$$n_1 = \frac{(Z_{1-\beta} + Z_{1-\alpha/2})^2(\sigma_1 2+\sigma_2 2)}{(\mu 1-\mu 2)^2}$$

Forty five extracted premolars with intact buccal surface (no caries, no cracks, no exposure to chemicals, no filling) are collected from department of Oral Surgery, Punjab Dental Hospital, Lahore and divided into 3 groups A, B and C, each of 15 teeth. The labial surface of teeth was "ground wet" by using 200, 400, plus 600 grit paper of silicon carbide (Automata A, JearWirtz, West Germany) and then buffed by alumina suspension (BUEHLER, IL, USA) to represent smooth enamel surface. Self-cured acrylic resin (Mr.Dental, USA) was poured in Customized molds of plastic. The roots of teeth were detached 2 mm beneath the cemento-enamel joint with a diamond disk (3M UnitekCorp. Monrovia, Calif) and coronal portion were fixed horizontally into self-cured acrylic resin. Then teeth were burnished with non-fluoridated pumice, washed with water and dried with oil free air.

The baseline Microhardness of samples was determined using Microvickers Hardness Tester (Wilson Wolpert Microvickers, 402 MVD, Japan) with a force of 100 grams for 15 seconds. 5 indentations, at a distance of 120 micrometer were made and baseline MH of every sample was determined by averaging the value of all five indentations. The objective of baseline MH determination was to evaluate the alterations that occur following demineralization and remineralization.

Then teeth were etched by 37% phosphoric acid gel "(3M/ESPE, St. Paul, MN, USA)" for 30 sec, showered by water, then dry with air for 10 sec. to obtain frosty white appearance. Light cure primer "(Transbond XT of 3M Unitek Monrovia, CA, USA)" was applied on enamel surface. Then resin composite (Transbond XT of 3M Unitek) was coated on bracket base, the bracket was bonded and resin was light cured using high intensity blue light (intensity 5 W, wavelength 420 nm~480 nm, Woodpecker Medical Instrument) for 20 sec. All samples were saved in deionized water for 1 week. The brackets were deboned with a debonding plier (UnitekCorp. Monrovia, Calif) and remaining composite was removed by Sof Lex aluminum oxide finishing disks (3M UnitekCorp. Monrovia, Calif) in low speed hand piece (NSK, Pana Air, Japan) operated at 10,000 rpm. Then the teeth of all groups were stored in separate beakers containing deionized water. The artificial caries solution/demineralizing solution was prepared with the pH of 4.4 [2.2mM/L CaCl2, 2.2 Mm/L NaH2PO4, 50Mm/L acetic acid, 100 Mm/L NaCl (Merck, USA)][20] . In order to create artificial demineralization, all samples were immersed in 100ml demineralizing solution at room temperature for 1 week. The demineralizing solution was replaced every day to avoid accumulation of substances formed through demineralization. After 1 week, they were kept in separate bottles that contain deionized water. After verification of demineralized lesion by visual inspection, SMH of each sample was re-measured for demineralization as described above. After that, surface treatments were carried out.

"CPP-ACP" was applied on the teeth of gp B and NaF was applied on the teeth of gp C two times daily for 3 minutes for remineralization. All teeth were cycled between deionized water and surface treatment with remineralizing agent for 12 weeks. Group A was considered as control gp for assessment of remineralization. The MH of gp B and gp C samples was re-measured for remineralization. The result

of study shows that here is substantial enhancement in microhardness after using CPP ACP and NaF. But group B shows more significant improvement in microhardness than group C. This shows that CPP-ACP is extra efficient remineralizing agent as compared to NaF.

DATA ANALYSIS

The collected information was assessed by IBM SPSS Statistics 20 (Statistical Package for Social Sciences). Mean ± SD were given for quantitative variable (MH). One way ANOVA was applied to compare the mean differences in MH and following demineralization among the groups. Paired t-test was practiced to match the MH prior to and following demineralization. Independent t-test was applied to compare MH among gp B and gp C. P value of ≤ 0.05 was measured statistically significant.

RESULTS

The MH of every group A (Deionized water), B (CPP ACP) and C (NaF) was measured for demineralization and remineralization. The outcome of CPP-ACP and NaF on the alterations in MH of enamel (%age of microhardness retrieval, %VHNR) was concluded by following formula: % VHNR = (remineralized enamel microhardness - demineralized enamel microhardness) / (baseline enamel microhardness - demineralized enamel microhardness) x 100.

Table 1: Enamel Microhardness at Baseline and afterward demineralization among the groups (One way ANOVA was used to compare mean differences in baseline microhardness and after demineralization).

Enamel Microhardness	Group A (Mean ± SD)	Group B (Mean ± SD)	Group C (Mean ± SD)	p-value
At Baseline	290.0 ± 5.2	290.8 ± 4.2	289.8 ± 2.8	0.776
After demineralization	187.8 ± 3.1	187.9 ± 2.6	188.0 ± 2.1	0.979

Results explained that micohardnes at baseline and after demineralization values were not considerably different among three gps.

Table 2: Enamel Microhardness before and after demineralization among groups (Paired t-test was used to match the microhardness prior to and following demineralization).

	Baseline (Mean ± SD)	After Demineralization (Mean ± SD)	Mean Difference (Mean ± SD)	p-value
Group A	290.0 ± 5.2	187.8 ± 3.1	102.3 ± 6.6	< 0.001
Group B	290.8 ± 4.2	187.9 ± 2.6	102.9 ± 3.5	< 0.001
Group C	289.8 ± 2.8	188.0 ± 2.1	101.8 ± 3.8	< 0.001

Results proved that here was a considerable decrease in enamel micohardness among three groups after demineralization (P < 0.001).

Table 3: Enamel Microhardness after remineralization among group B and C (Independent t-test was applied to match the microhardness among group B and group C after remineralization).

After Remineralization	Group B (Mean ± SD)	Group C (Mean ± SD)	p-value
Enamel Microhardness	230.2 ± 4.1	207.5 ± 4.0	< 0.001
Percentage of Microhardness Recovery	41.2 ± 4.9	19.1 ± 4.6	< 0.001

Results proved that here was a considerable difference in enamel micohardness between both groups after remineralization (P < 0.001). Moreover, %age of microhardness recovery increased by 41.2% in gp B and 19.1% in gp C.

The results of the current study shows that CPP-ACP is statistically significant than NaF in increasing MH of demineralized enamel. Thus CPP-ACP is helpful in our clinical practice if used daily during orthodontic treatment as it increases the rate of remineralization.

DISCUSSION

Demineralization is one of the causes of tooth loss that causes molecular alterations in apatite minerals of tooth, to an obvious demineralized area, association of dentin, and ultimate cavitation.[15] Following the end of fixed orthodontic treatment, the most difficult problem is the control of enamel demineralization. Usually, surface treatment of demineralization was concerned about the mineral loss and mineral gain to the tooth. Thus objective is the facilitation of recovering new minerals.[21] Hence, demineralization persist as primary apprehension for both patients and orthodontists. Since the increased incidence of demineralization in orthodontic patients and the importance of esthetics, occurrence of demineralization must be prohibited. Therefore, we assessed the efficiency of NaF and CPP ACP for inhibition of demineralization to find the best effective agent for this goal.[13]

Techniques for delivering calcium and phosphorous to assist remineralization have been backbone of this kind of study.[21] Fluoride becomes incorporated into the tooth, and replace the hydroxyl group of the hydroxyapatite.[15] By the use of low-concentration of calcium and phosphate solution, no remineralization occur because this is not effective in localizing the ions on surface of tooth in large concentrations.[22] The CPP contains multiple phosphoryl sequences with ability to stabilize calcium phosphate complexes in solutions such as ACP.[15] Previously, the researchers had focused on primary prevention of demineralization, but in current study, we have emphasised on secondary prevention, chiefly, management of persisting demineralized lesions. In previous studies,

numerous techniques have been practiced to evaluate the amount of remineralization, comprising quantitative light-induced fluorescence (QLF), DIAGNOdent, x-ray spectrophotometer, polarized light microscope and micro-computed tomography.[15,23,24] In our study, we have used Vickers indenter for measurement of MH of enamel and baseline SMH value range from 290.0 to 289.8 VHN that was related to preceding study by Lussi et al. in 2000. The sharp decrease in SMH occurred after demineralization. The test materials (NaF and CPP ACP) were applied to enamel for remineralization. After that, mean SMH of samples raised by 230.2 VHN and 207.5 VHN in both groups. The percentage of SMHR show maximum improvement of 41.2% in CPP-ACP and 19.1% in NaF group. Thus, NaF encourage remineralization of enamel, but not as efficiently as CPP-ACP.

This study is in line with Kumar et al. Qiong et al. Guçlu et al. and Lopatiene. Kumar described that CPP-ACP is more efficient as compared to fluoride in decreasing lesion depth. Though fluoride causes an increase in enamel resistance, but resulting remineralization, is a self-restricting phenomenon. This explain the increased efficacy of CPP-ACP in increasing MH than that of fluoride.[25] Qiong et al, conducted an in vitro study to compare CPP-ACP crème with 500 ppm NaF solution for prevention of primary caries in childhood. The result showed that CPP-ACP cream is useful for remineralization of premature enamel lesions of deciduous teeth, extra efficient as compared to 500 ppm NaF.[26] Guçlu et al, told that application of CPP-ACP paste can manage demineralized lesions in permanent teeth, and additional application of 5% NaF varnish had no useful results. They alleged that fluoride causes hyper mineralization of superficial layer, which blocks entry of mineralizing ions into subsurface area.[27] In a systematic analysis, Lopatiene proved that ACP as well as fluoride are equally efficient in treatment of demineralized lesions throughout and afterward fixed orthodontic treatment, but CPP-ACP was extra useful in improving lesions.[28]

Farzanegan et al. compared the efficiency of amorphous calcium phosphate (ACP) and fluoride on MH recovery of damaged enamel. Results showed that MH of samples in the ACP and fluoride groups had considerably enhanced afterward treatment as compared to control gp. Conferring to the above study, both 0.05% ACP as well as 0.05% fluoride solutions improved micro-hardness of enamel in the management of demineralized lesions.[29]

Actually, CPP stop the quick conversion of calcium phosphate phase and thus, ions become stable and retained at tooth surface. So, CPP has ability to transport phosphate, fluoride and calcium ions deeply into the subsurface lesions.[30] Nano-sized crystals were organized on body of lesions after application of CPP-ACP with less inter crystalline gaps.[26] The efficiency of CPP-ACP could be improved in mouth when a biofilm is present, that could attach to CPP and used as storage for phosphate and calcium ions.[25] Thus CPP-ACP may be helpful in our clinical practice if used on regular basis during the period of orthodontic treatment.

CONCLUSION

After this study, it is concluded that, a remineralizing cream having CPP-ACP has promising results in remineralization of demineralized lesions as compared to NaF. CPP-ACP increases MH of enamel more efficiently as compared to NaF with the addition of few minerals and has aesthetic benefits for patients.

CONFLICT OF INTEREST

None declared.

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