EFFECT OF AMORPHOUS CALCIUM PHOSPHATE CASEIN PHOSPHOPEPTIDE REMINERALISING AGENT ON ENAMEL DEMINERALIZATION ADJACENT TO ORTHODONTIC BRACKETS – AN IN VITRO STUDY

Rajat Gandhi,[1] Sudheer Kumar, [2] Mukesh Kumar, [3] Abhay Kumar Jain,[4] Akram Ansari,[5] Parag Bohra[6]

Post Graduate Student, [1] Professor & Head,, [2] Professor, [3] Reader, [4] Reader, [5] Post Graduate Student [6]

1-6 Department of Orthodontics and Dentofacial Orthopedics, Teerthanker Mahaveer Dental College & Research Centre, Moradabad.

Abstract

Objective: This extensive study was conducted to examine the effects of Amorphous Calcium Phosphate-Casein Phosphopeptide (ACP-CPPF) with Sodium Fluoride (GC Tooth Mousse plus) (TM plus) on the demineralization of enamel adjacent to the brackets used in the orthodontic treatment.

Materials and method: 30 non carious, human maxillary premolars with no visible enamel defects were collected. For all thirty samples, pre adjusted edge wise stainless steel premolar brackets were bonded on the teeth with light cure composite resin. The teeth were randomly assigned into two groups of 15 teeth each and coded with unique colored nail varnish leaving a rectangular window extending occlusally. The teeth in each group were immersed separately in an artificial saliva solution for 11 hours and an acid solution for 1 hr maintained at room temperature. After each acid challenge the surface layers in the exposed enamel windows in both groups were removed by brushing with a soft tooth brush. Group 1, control group didn't receive any application and group 2 received GC Tooth mousse plus respectively. The teeth were immersed alternately in the saliva and acid solution for 31 days. After 31 days the brackets were removed and the teeth mounted in polyester resin. The teeth were sectioned longitudinally through the buccal window. Two sections, each approximately 0.5 mm thick were obtained from each specimen. The sections were mounted in water and photographed with a polarised light microscope at 10x magnifications.

Results: The results of this study showed that amorphous calcium-phosphate casein phosphopeptide fluoride (ACP-CPPF) (GC Tooth mousse plus) on daily application will provide maximum protection against the enamel demineralization in orthodontic patients, by reducing the lesion formation and simultaneously remineralizing the demineralized area by providing calcium phosphate ions and fluoride ions constantly.

Conclusion: Amorphous calcium-phosphate casein phosphopeptide fluoride (GC Tooth mousse plus) when applied has a definite benefit in preventing the enamel demineralization adjacent to orthodontic brackets over the controls. Amorphous calcium-phosphate casein phosphopeptide fluoride (GC Tooth mousse plus) showed better efficiency in reducing the demineralization and enhancing the remineralization around the orthodontic brackets with good patient compliance.

Keywords: Tooth Mousse plus, Remineralization, Demineralization

Introduction

It has been observed lately that there has been an ever increasing demand for orthodontic treatment from both adolescents and adults. The expectations from such treatment have also increased over the time with respect to the result oriented treatment outcomes, specifically towards aesthetics, and functional stability. The most common and unaesthetic side effect pertaining to the usage of fixed appliances for orthodontic management includes development of white spot lesions.[1] These lesions are referred to as "subsurface enamel porosity from carious demineralization" and they are observed as a milky white opacity when present on smooth surfaces".[2]

The demineralization of enamel adjoining brackets used during orthodontic therapy is a considerable clinical problem, and this has been reported to have significantly increased the severity and prevalence of demineralization of enamel following orthodontic management as compared to the subjects who were untreated. In the orthodontic patients, the prevalence of white spot lesions has been accounted in the range of 2% and 96%.[3]

Following factors contribute to the increase in prevalence of these lesions in patients with orthodontic treatment.

- Fixed orthodontic appliances used during the process of treatment form a niche that supports
- inhabitation for plaque, by evading normal oral hygiene practices.
- Orthodontic brackets surrounded with residual adhesives provides roughened surface that allows bacteria to adhere the brackets.[4]
- In the typical orthodontic patients, the newly erupted teeth is possibly more liable to acid attack.[5]

Demineralised surface enamel is considered to be the precursor / early lesion of enamel caries and is primarily caused due to the action of acids. During the orthodontic treatment, these acids may come mainly from two sources, namely usage of cement for retaining of the orthodontic bands and due to the breakdown of the food debris products.[6]

In the recent studies, a product derived from milk casein which is referred as amorphous calcium phosphate-casein

phosphopeptide (ACP-CPP), has been described to possess topical anticariogenic property.[7] It is observed that incorporation of fluoride within the ACP-CPP complex (ACP-CPPF) promotes remineralization; the mineral which is deposited is constant with fluorapatite.[8]

The aim of this extensive study was to examine the effects of Amorphous Calcium Phosphate-Casein Phosphopeptide Fluoride (GC Tooth Mousse plus) (TM plus) on the demineralization of enamel adjacent to the brackets used in the orthodontic treatment.

Materials and Method

Lesion Preparation

Thirty (30) non-carious human premolars with no visible enamel defects extracted for orthodontic treatment were collected. The premolars were enrolled according to the following criteria: 1) Premolars extracted after taking the consent from the patients. 2) Malformed, decayed, restored and attrited teeth were excluded. Any soft tissue, calculus and/or bone remaining on the teeth following extraction were removed with a dental scalar and the teeth stored in deionized water until required.

For all thirty teeth, pre-adjusted edgewise MBT stainless steel premolar (3M Unitek) brackets of 0.022" slot were bonded on the teeth with light cure composite resin, Transbond XT (3M Unitek). The enamel was conditioned for 30 seconds with etchant containing 37% phosphoric acid. The teeth were then washed and dried for 15 seconds with the help of moisture and oil free air spray. The enamel surface exhibited a dull frosty white appearance indicating a successful etch. A thin layer of Transbond XT light cure adhesive primer was painted over the etched enamel surface with a brush tip and was light cured for 20 seconds with a light curing unit. The Brackets were placed immediately after all the etched surfaces were coated with a thin layer of light cure composite resin, Transbond XT (3M Unitek). The brackets were positioned on the buccal surface at the height of contour mesiodistally and in the middle 1/3 occlusogingivally parallel to the long axis of the tooth. Excess bonding agent was removed with an explorer tip and the brackets were light cured for 40-60 seconds.

Masking tape was used to cover the occlusal 1/3 rd on buccal surface area (4x1mm) adjacent to brackets and acid resistant varnish (nail varnish) was used to paint the rest of the tooth surfaces. The teeth were randomly assigned into two groups of 15 teeth each. Each group coded with unique coloured nail varnish.

Masking tape was removed from the occlusal 1/3 rd on buccal surface area adjacent to brackets, and the exposed enamel of each tooth was treated with application of respective materials.

Group 1: Control group did not receive any application color coding red.

Group 2: Tooth Mousse Plus was applied to the exposed enamel windows, using applicator brush, and was allowed to dry for 3 minutes before it was immersed in saliva solution color coding pink. (Figure 1)

The teeth in each group were immersed separately in an artificial saliva solution (neutral pH and containing 20mmols/L Potassium carbonate, 3mmol/L potassium dihydrogen phosphate and 1mmol/L Calcium Chloride) for 11 hours and in acid solution (50 mmol/L acetic acid at 4.4 pH) for 1 hour for 31 days.

Figure 1 Control Group color coding Red & Tooth mousse plus color coding pink.

Both solutions were agitated constantly and maintained at room temperature. The pH cycle was performed by immersing the teeth in saliva for 11 hours and after 11 hours the teeth were removed from the artificial saliva and immersed in the acid solution for 1 hour. After each acid challenge, the surface layers in exposed enamel windows in all the four groups were removed by brushing the teeth with assigned materials for 5 seconds with a soft tooth brush. The solutions were changed twice a week and pH of each solution was monitored.

Sectioning and Microradiography

After a period of 31 days, the brackets were removed and the teeth were mounted in acrylic (metha-acrylate resin) cylindrical blocks of 2.5 cm diameter and 2 cm height. The teeth were sectioned longitudinally through the buccal windows with a hard tissue microtome. A specimen of 0.5 mm thickness was obtained by sectioning through the middle of the teeth. The acrylic surrounding the thin specimens was removed and mounted on glass slide using water. The sections were evaluated with polarized light microscopy. The depth of demineralised lesions was measured using ProGres C3 2.5 image analysis software. The depths of the demineralised enamel in each section were measured at three sites. The first site was near the Occlusal third, the second site was Middle third and the third site was near the Gingival third close to the bracket. Microphotographs of the occlusal half of the buccal surface were taken with fixed magnification of 10 times.

Data Collection

The data collected (depth of demineralized lesion) from all the groups and sites were first entered to Microsoft Excel (Microsoft, Redmond, Wash). All data were visually screened for any missing data or outliners and for validity of distribution assumptions. The data obtained were subjected to following statistical analysis.

Statistical Analysis

Descriptive statistical analysis includes mean, standard deviation, minimum and maximum values were calculated for each test group with software SPSS (statistical package for social sciences, version 21.0). Results on continuous measurements were presented on Mean ± SD and results on categorical measurements are presented in Number (%). Significance for all statistical tests were performed (P<0.05). Analysis of variance (ANOVA) was done to find out if there is any statistical significance difference between the four groups. Pair wise comparison (Post-hoc Tukey test) was done using Bonferroni method to find out if there is any statistical significance difference between the Groups and the Sites.

Results

Comparison of depth (in micro meters):

In this experiment we have two factors influencing depth i.e. Group and Site. Group are of two types – Control and GC Tooth Mousse plus and Site is of three types – Occlusal third, Middle third and Gingival third.
The mean depth recorded among different groups and their sites, and the mean depth recorded at different sites of each group is tabulated (Table.1). The ANOVA test (Table.1, Graph.1) has shown that the Group is a significant factor in influencing the depth of demineralisation and the difference in mean depth recorded between the groups are found to be statistically significant (P<0.001). It was observed, that Control group recorded higher mean depth of enamel loss of 42.49 ± 2.88 (Figure.2). The lowest mean depth was recorded in TM+ group 23.10 ± 1.34 (Figure.3).

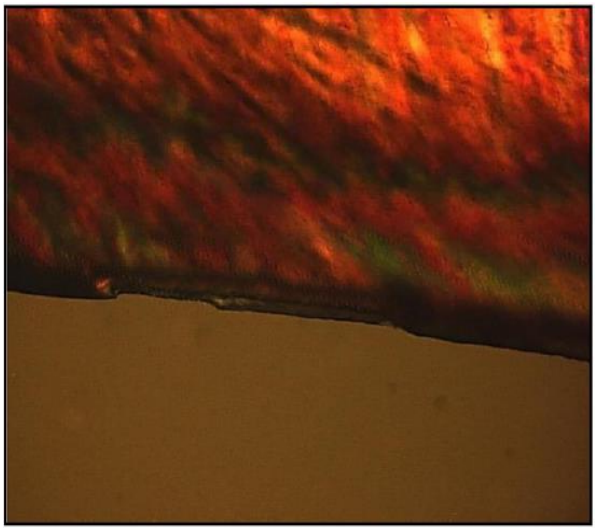


Figure.2. Demineralized area - control group

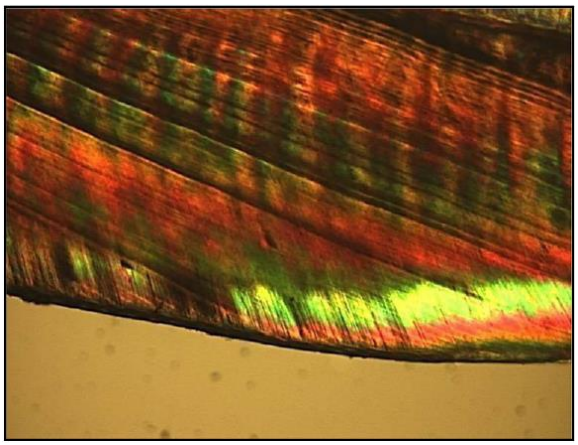


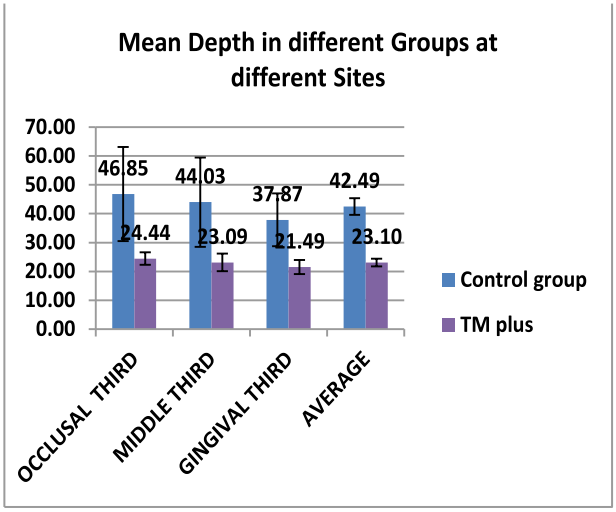
Figure.3. Demineralized area – tooth mousse plus

Among the different sites examined, Occlusal third recorded higher mean depth 34.75 ± 12.52 µm followed by Middle third 33.11 ± 11.81µm. Lowest mean depth 29.21 ± 8.06 µm was recorded in gingival third which was found to be statistically significant (P<0.001). Post-hoc test was carried out to find the pairwise significance and the results are given.
There was difference in mean depth between the Control and GC Tooth Mousse plus Group which was statistically significant (P<0.001). The difference in mean depth recorded at gingival third and occlusal third was statistically significant (P<0.001). The difference in mean depth recorded at gingival third and middle third and the difference in mean depth recorded at Middle third and occlusal third was also statistically significant (P<001). GC Tooth Mousse plus Group recorded the lowest mean depth 23.10 ± 1.34 to all the other groups at all the sites.
When pair wise comparison was done between control and GC Tooth Mousse plus groups for depth of demineralization, it was found that mean depth of demineralization is statistically significant in control group. However, within GC Tooth Mousse plus Group, the mean depth of demineralization is not statistically significant

Table 1: Descriptive statistics for mean depth recorded at different Site of each Group

Group	Site	Mean	Std. dev.	Median	Min	Max
1.CONTROL	Occlusal Third	46.85	16.34	44.56	23.79	75.90
	Middle Third	44.03	15.49	40.99	23.46	69.67

	Gingival Third	37.87	9.15	40.76	23.46	46.79
2. TM +	Occlusal Third	24.44	2.15	24.46	20.96	29.46
	Middle Third	23.09	3.03	22.87	18.89	28.46
	Gingival Third	21.49	2.41	20.79	18.98	28.98



Graph 1: Comparison of mean depth of demineralization recorded in different Groups at different Sites of the teeth studies

Discussion

Fixed appliances are an inseparable part of contemporary orthodontic treatment but a major disadvantage of fixed mechanotherapy is measurable and significant amount of enamel demineralization that might occur adjacent to orthodontic bracket even within one month of bonding. Decalcification of the surface of enamel near fixed orthodontic appliance is a vital and widespread iatrogenic effect of orthodontic treatment. The banding and bonding of the orthodontic appliances to teeth raise the amount of plaque retention sites, consequently making oral hygiene difficult. As degree of mineralization is directly associated to the enamel translucency, initial demineralization of enamel is generally seen as a white spot lesion clinically. Lesions like this are clinically induced within 4 weeks time, which is typically the time span between one orthodontic appointment and the next.[9]

In highly cariogenic environment these lesions can advance rapidly. They may produce carious cavitations that will require suitable restoration, if left untreated. Therefore, the prevention, diagnosis, and treatment of WSL are critical to reduce tooth discoloration which compromises the esthetics of the patient and to prevent decay as well.[2]

Reynolds EC[7] introduced amorphous calcium phosphate-casein phosphopeptide (ACPCPP), a product which is a derivative of milk casein, has been reported to have topical anti-cariogenic effects by preventing the precipitation of calcium phosphate. The mechanism of action which is proposed for ACP-CPP is associated to its localization at the surface of tooth, where the buffering of free calcium and phosphate ion activities takes place. It prevents demineralization by maintaining a condition of super saturation with respect to enamel, thereby facilitating remineralization of enamel. It acts as Calcium & Phosphate ion reservoir which increases the level of plaque calcium and phosphate ions, thereby limiting enamel demineralization and enhancing re-mineralization. It was found that the remineralized enamel was more resistant to decalcification when compared with untreated enamel. Effect of enamel remineralization was not restricted to chewing gums as a vehicle.[10, 11, 12, 13 and 14]

ACP-CPP reduces depth of the lesion irrespective of its use as a topical coating or toothpaste.[15, 16] Studies on immunolocalization confirmed that ACP-CPP causes the formation of a less-cariogenic plaque. If ACP-CPP is incorporated into the pellicle in exchange for albumin, it is shown to inhibit the adherence of Streptococcus Mutans and Streptococcus Sobrinus.[17]

ACP-CPP (Tooth Mousse) has found to have superior capacity for neutralization of acids than toothpaste containing fluoride. With the addition of fluoride, enamel which is exposed to ACP-CPP shows increased acid resistance.[16, 18, 19 and 20] That is perhaps because of the capability of ACP-CPP to interact with fluoride ions to generate an additional anticariogenic effect by the formation of a stabilized amorphous calcium fluoride phosphate phase.[9] It also demonstrated superior remineralizing potential when it was used in combination of fluoridated toothpaste than when used alone. The combined advantage of ACP-CPP and fluoride in reducing the demineralization and enhancing remineralization was proved by many studies.[15, 16]

To get full benefits clinically, patient compliance is major concern, that to be when patient has to use both. Considering the knowledge of both fluoride and ACP-CPP, a new product has been developed where fluoride 900 ppm is combined with ACP-CPP (Tooth Mousse plus). This in vitro study aims at evaluating the effect of Tooth Mousse plus in reducing the demineralization adjacent to orthodontic brackets (ACP-CPP 900ppm Fluoride 0. 2%w/w).In this study non fluoridated composite resin was used as an adhesive to eliminate the influence of fluoride on results providing fluoride protection of enamel despite patient noncompliance and delivering the fluoride in a

sustained manner over a longer period of time. GC Tooth Mousse Plus (TM+) is water based creams containing 1% Casein Phosphopeptide-Amorphous Calcium Phosphate. In ACP-CPPF the level of fluoride is 0.2% w/w (900ppm), which approximates that of adult strength toothpastes.

The pH cycling system[16] has been illustrated to work effectively in assessment of progression of lesions and mineral alteration in enamel lesions. Artificial early caries like lesions of enamel have been effectively used to study the demineralization of enamel in vitro, as they demonstrate all of the major histological features of natural caries. The buffered remineralizing and the demineralizing solutions were prepared by combination of analytical-grade chemicals and deionized water. The demineralizing solution, consisted of 2.2 mM CaCl2, 2.2 mM KH2PO4, and 0.05M acetic acid, the solution had the pH adjusted to 4.4 with 1 M KOH. The remineralizing solution, consisted of 1.5mM CaCl2, 0.9mM NaH2Po4, and 0.15 M KCL had the pH of 7.0. This solution was in accordance with the super saturation of apatite minerals found in saliva.

The samples were divided into two Groups– Control and GC Tooth Mousse plus Group. Each specimen was studied for the extent of demineralisation at three Sites - occlusal third, middle third and gingival third. From the statistical analysis it was observed that Group and Sites were significant factors which influences depth of demineralisation (P<0.001).

It was observed, that control group recorded higher mean depth of enamel loss. This demineralisation was a result of plaque activity which will alter the microbial environment so that proliferation of the facultative bacterial population is increased. This increases the chances of decalcification. The lowest mean depth was recorded in GC Tooth Mousse plus Group. The difference in mean depth between the different groups was found to be statistically significant (P<0.001) The mean lesion depths (occlusal, middle and gingival combined) showed a statistically significant difference between the two groups (p< 0.0001).There is a statistically significant difference in the mean depth recorded between control and GC Tooth Mousse plus Group (TM+) (P<0.001). The results of TM+ showed statistically significant difference which indicates the added advantage of fluoride with ACP-CPP on preventing demineralization than when used alone. These results are in accordance with the study conducted by Theresia Rini Sudjalim et al. They found that the application of TM, NaF, or TM/NaF can significantly reduce the demineralization, but better results were seen with combined application of TM and NaF[15]. Navid Karimi Nasab et al have shown that there was a 50 % reduction in the enamel demineralization when ACPCPP preparation was used alone.[21] The results of present study showed that TM+ Group (23.10 ± 1.34 µm mean depth) had the maximum effect on inhibition of demineralization of enamel adjacent to orthodontic brackets. They used topical coating of CPP-ACP after the use of a fluoridated tooth paste. The results were better when TM was used than NaF used alone. The results were further improved when TM/NaF was used and similar results were found with TM+ group. This suggests the synergetic action of fluoride with ACP-CPP.[15, 16, 21]

Among the different sites, occlusal third recorded higher mean depth (34.75 µm) followed by Middle third (33.11µm). Lowest mean depth (29.21 µm) was recorded in gingival third which is in contrast with the study by Bishara SE et al[2] and Soumya KM et al.[22] The difference in mean depth recorded at the three different sites was found to be statistically significant (P<0.001).

TM+ group recorded the lowest mean depth compared to all the other groups at all the sites. This result supports the ability of ACP-CPP to interact with fluoride ions to produce an additive anticariogenic effect through the formation of a stabilized amorphous calcium fluoride phosphate phase and also the synergistic effect as reported by studies.[18, 20, 21, 23]

The results of this study showed that ACP-CPPF on daily application will provide maximum protection against the enamel demineralization in orthodontic patients, by reducing the lesion formation and simultaneously remineralizing the demineralized area through providing calcium phosphate ions and fluoride ions constantly.[24,25] For high caries risk and poor oral hygiene patients ACP-CPPF have maximum benefit compare to ACPCPP with good compliance. However, since TM and TM+ contain casein, it is contraindicated in patients allergic to milk and milk products.

Conclusion

1. ACP-CPP and sodium fluoride when applied alone has a definite benefit in preventing the enamel demineralization adjacent to orthodontic brackets over the controls. But not many differences were found between ACP-CPP and sodium fluoride.
2. Addition of fluoride to ACP-CPP will enhance the prevention of demineralization by two times.
3. ACP-CPPF showed better reducing efficiency compared to sodium fluoride and ACPCPP, suggesting the probable synergistic action of ACP-CPP with fluoride in reducing the demineralization and enhancing the remineralization around the rthodontic bracket with good patient compliance.
4. In a non compliant patient application of sodium fluoride varnish will help in prevention of WSLs.

References

1. Chapman JA, Roberts EW, Eckret GJ, Kula KS, Gonzalez-Cabezas C. “Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances”. Am J Orthod Dentofacial Orthop 2010; 138: 188- 194.
2. Bishara SE, Ostby AW. White Spot Lesions: Formation, Prevention and Treatment. Semin Orthod 2008; 14: 174-182.

3. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. Am J Orthod Dentofacial Orthop 1982; 81: 93-98.
4. Gwinnett AJ, Ceen RF. Plaque distribution on bonded brackets: a scanning microscope study. Am J Orthod Dentofacial Orthop 1979; 75: 667-677.
5. Garcıa-Godoy F, Hicks MJ. Maintaining the integrity of the enamel surface: the role of dental biofilm, saliva and preventive agents in enamel demineralization and remineralization. J Am Dent Assoc 2008; 139: 25S-34S.
6. Mizrahi E. Enamel demineralization following orthodontic treatment. Am J Orthod Dentofacial Orthop 1982; 82: 62-7.
7. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res 1997; 76(9): 1587-1595.
8. Cochrane NJ, Saranathan S, Cai F, Cross KJ, Reynolds EC. Enamel subsurface lesion remineralization with casein phosphopeptide stabilized solutions of calcium, phosphate and fluoride. Caries Res 2008; 42: 88-97.
9. O'Reilly MM, Featherstone JD. Demineralization and remineralization around orthodontic appliances; An in vivo study. Am J Orthod Dentofacial Orthop 1987; 92(1): 33-40.
10. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J Dent Res 2003; 82: 206-211.
11. Reynolds EC. The prevention of sub surface demineralization of bovine enamel and change in plaque composition by Casein in an Intra-oral Model. J Dent Res 1987; 66(6): 1120-1127
12. Shen P, Cai F, Nowickil A, Vincent J, Reynolds EC. Remineralization of Enamel Subsurface Lesions by Sugar-free Chewing Gum Containing Casein Phosphopeptide-Amorphous Calcium Phosphate. J Dent Res 2001; 80(12): 2066-2070.
13. Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Remineralization of enamel subsurface lesions in situ using sugar free lozenges containing casein phosphopeptide amorphous calcium phosphate. Aust Dent J 2003; 48: 240–243.
14. Lijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of Enamel Subsurface Lesions by a Sugar –Free Chewing Gum containing casein phosphopeptide Amorphous Calcium Phosphate. Caries Res 2004; 38: 551-556.
15. Sudjalim TR, Woods MG, Manton DJ, Reynolds EC. Prevention of demineralization around orthodontic brackets in vitro. Am J Orthod Dentofacial Orthop. 2007; 131: 705.e1-705.e9.
16. Kumar VLN, Itthagarun A, King NM. The effect of casein phosphopeptide amorphous calcium phosphate on remineralization of artificial caries-like lesions. An in vitro study. Aust Dent J 2008; 53: 34–40.
17. Schupbach P, Neeser JR, Golliard M, Rouvet M, Guggenheiml B. Incorporation of Caseinoglycomacropeptide and Caseinophosphpeptide into the Salivary Pellicle Inhibits Adherence of Mutans Streptococci. J Dent Res 1996; 75(10): 1779-1788.
18. Reynolds EC, Cain CJ, Webber FL, Black CL, Riley PE, Johnson IH, Perich JW. Anticariogenicity of Calcium Phosphate Complexes of Tryptic Casein Phosphopeptides in the Rat. J Dent Res 1995; 74(6): 1272-1279.
19. Kariya S, Sato T, Sakaguchi Y, Yoshii E. Fluoride effect on acid resistance capacity of CPP-ACP containing material. 82nd General Session of the IADR 2004, Honolulu, Hawai.
20. Lennon AM, Pfeffer M, Buchalla W, Becker K, Lennon S, Attin T. Effect of a Casein/Calcium Phosphate-Containing Tooth Cream and Fluoride on Enamel Erosion in vitro. Caries Res 2006; 40: 154–157.
21. Nasab NK, Kajan ZD, Balalaie A. Effect of Topical C5 on enamel adjacent to orthodontic brackets. An in-vitro study. Aust Orthod J 2007; 23: 46-49.
22. Soumya KM, Hegde N, Reddy VP, Chandrashekar BS, Koushik SRH, Raju AS. Evaluation of remineralising agent on enamel around orthodontic brackets – an in-vitro study. J Dent Oral Bio. 2011; 2(2): 1-6.
23. Mazzaoui SA, Burrow MF, Tyas MJ, Dashper SG, Eakins D, Reynolds EC. Incorporation of phosphopeptied-Amorphous Calcium phosphate into a Glassinomer cement. J Dent Res 2003; 82(11): 914-918.
24. Roslyn J,Mayne Nathan J,Cochrane, Eric C. Reynolds .Invitro study of the effect of casein phosphopeptide amorphous calcium fluoride phosphate on iatrogenic damage to enamel during orthodontic adhesive removal Am J Orthod Dentofacial Orthop:2011;139(6):e543-e51.
25. Guotao Wu, Xinqiang Liu, and Yongfu Hou. Analysis of the effect of CPP-ACP tooth mousse on enamel remineralization by circularly polarized images. Angle Orthod: 2010; 80(5): 267-72.

Corresponding Author
Dr.Rajat Gandhi
Post Graduate Student
Department of Orthodontics and Dentofacial Orthopedics,
TMDCRC, Moradabad