BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

Prevention of Enamel Demineralization During Orthodontic Treatment: An *In Vitro* Study Using GC Tooth Mousse

SUMMARY

One of the most difficult problems in orthodontic treatment with fixed appliances is the control of enamel demineralization around the brackets. The bands/brackets and the different orthodontic elements that are used (elastics, plastic, sleeves, springs) make the patient's dental hygiene more difficult and the accumulation of plaque easier. The purpose of this study was to measure the percentage of Ca, Na, K and Mg in the enamel before and after application of the topical gel - GC Tooth Mousse.

In this study, 40 healthy extracted premolars without any clinical sign of decalcification were selected. All teeth were cleaned and cut in half in the bucco-lingual direction with a diamond disc. Thus, the control and test specimens were obtained from the same teeth. Orthodontic brackets were bonded with Fuji Ortho LC. They were divided in 2 groups according to the period of monitoring (14 days and 1 month). Then, they were coated with a topical gel - Tooth Mousse (GC Corp, Japan), for 5 minutes each day, and stored in artificial saliva until analyzing. We have measured the percentage of Ca, Na, K and Mg in the enamel by using the method of flame atomic absorption spectrometry, with a Varian Spectra AA 55 B atomic absorption spectrometer.

The results obtained in this study refer to high percentage of Ca in enamel in the study group for the first examined period. The percentages of Ca in enamel were remarkably higher after 1 month from application of dental mousse. This indicates that, with an in vitro tooth-brackets model, inhibition of creating white spot could be achieved with the use of resin modified glass ionomer cement, supplemented with topical gel exposure. The mineral balance in the oral environment is accomplished by application of the Recaldent™ CPP-ACP in the form of GC Tooth Mousse.

Keywords: Dental Caries; Enamel; Demineralization; Remineralization

E. Zabokova-Bilbilova [1], T. Stafilov [2], A. Sotirovska-Ivkovska [1], F. Sokolovska [2]

St. Cyril and Methodius University
Skopje, FYROM
[1]Department of Pedodontic Dentistry
School of Dentistry
[2]Institute of Chemistry, Faculty of Natural Sciences and Mathematics

ORIGINAL PAPER (OP)
Balk J Stom, 2008; 12:133-137

Introduction

One of the most difficult problems in orthodontic treatment with fixed appliances is the control of enamel demineralization around the brackets. The bands/brackets and the different orthodontic elements that are used (elastics, plastic, sleeves, springs) make the patient's dental hygiene more difficult and the accumulation of plaque easier[2,15]. After the use of fixed appliances, decalcification marks are more pronounced at the gingival part of the teeth, were higher plaque accumulation usually occurs. These decalcification marks are seen as early as 4 weeks after band/bracket placement.

Several studies report different finding concerning possible decalcification of teeth. Mizrahi found that the maxillary incisors and mandibular first molars are most likely to exhibit signs of decalcification[13]. Trimpeneers found the maxillary central incisors to be most susceptible and Gorelick's study reported the maxillary laterals[11,24]. One study measured the frequency of white spot formation on the teeth and ranked them from most common to least common. The ranking is: maxillary lateral, mandibular second premolar, maxillary canine and maxillary first

premolar[26]. The resistance of the mandibular segments has been linked to the buffering capacity of saliva[9].

Much research has been focused on reducing the occurrence of decalcification during orthodontic treatment. Researchers have turned their attention toward appliance design, bonding materials, use of fluorides, sealants and improving oral hygiene[7]. Many products have been developed to prevent demineralization of enamel surface, like casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).

CPP-ACP can be found in multiple products. Recaldent™ is a unique complex containing amorphous calcium phosphate (ACP) and casein phosphopeptide (CPP), obtained from milk casein. The preparation is recommended in need for hard tissue remineralization. The manufacturer compares the material to "liquid enamel". CPP-ACP complex make a strong binding with a bio-film on teeth and form calcium and phosphate reservoir. They are then incorporated into the surface of enamel and dentine[21]. The effect of GC Tooth Mousse, with CPP-ACP complex is part of the new and modern approach to caries prevention. The CPP-ACP complex contained in Recaldent™ is hence an ideal system for transporting free calcium and phosphate ions, and GC Tooth Mousse, containing this novel active ingredient, is the world's first product for professional use in the dental practice[12].

The proposed anticariogenic mechanism of CPP-ACP involves the incorporation of the nanocomolexes into dental plaque and onto the tooth surface, thereby acting as a calcium and phosphate reservoir. Studies have shows that CPP-ACP incorporated into dental plaque can significantly increase the levels of plaque calcium and phosphate ions. This mechanism is ideal for the prevention of enamel demineralization as there appears to be an inverse association between plaque calcium and phosphate levels and measured caries experience[19].

Several *in vitro* and *in vivo* studies have shown that treatment with CPP-ACP corresponds with a reduction in demineralization and increases in remineralization[5,14,22]. A clinical study by Iijima et al[6], who used sugar free chewing gum containing 18.8 mg CPP-ACP, showed that CPP-ACP increased resistance to demineralization, increased remineralization and created remineralized enamel that was more resistant to subsequent demineralization. Cai et al[3] found that the used of sugar-free lozenges containing CPP-ACP significantly increased remineralization of enamel subsurface lesions *in situ*, with 18.8 and 56.4 mg of CPP-ACP increasing remineralization by 78 and 176% respectively. One study found that the treatment with 0.1% CPP-ACP, applied twice daily, resulted in a 14% decrease in smooth surface caries, and with 1.0% CPP-ACP resulted in a 55% decrease[18].

The purpose of this study was to measure the percentage of Ca, Na, K and Mg in the enamel before and after application of the topical gel - GC Tooth Mousse.

Material and Method

In this study, 40 healthy extracted premolars without any clinical sign of decalcification were selected. All teeth were cleaned and cut in half in the bucco-lingual direction with a diamond disc. Thus, the control and test specimens were obtained from the same teeth. Orthodontic brackets were bonded with Fuji Ortho LC (GC America Chicago, III), a resin-modified glass ionomer cement. The teeth were divided in 2 groups according to the period of monitoring (14 days and 1 month). Then, they were coated with a topical gel - GC Tooth Mousse for 5 minutes each day, and stored in artificial saliva until analyzing.

We have measured the percentage of Ca, Na, K and Mg in the enamel by using the method of flame atomic absorption spectrometry (FAAS), with a Varian Spectra AA 55 B atomic absorption spectrometer.

Determination of the Content of Calcium, Magnesium,Potassium and Sodium in Human Tooth Enamel

Determination of Ca, Mg, K and Na by FAAS with Varian Spectra AA 55 B atomic absorption spectrometer is already described[10,25]. Hollow cathode lamps were used as a source of electromagnetic radiation for each element. Lamps are optimized for 15 min before analysis. A mixture of acetylene and air was used for flame. Before the analysis, instrumental parameters for better precision and sensibility on analysis were optimized (Tab. 1).

Table 1. Optimal instrumental parameters for Ca, Na, K and Mg determination by FAAS

Parameters	Ca	Na	K	Mg
Wavelength/nm	422.7	589.0	766.5	285.2
Slit/nm	0,5	0,5	1,0	0,2
Lamp current/mA	10	5	5	4

The content of the investigated elements in the tooth enamel was determined by FAAS after mineralization in a microwave digestion system from Milestone, model Ethos Touch Control. The mineralization program is presented in table 2.

Table 2. Teeth sample mineralization programme

Step	Temperature/°C	Time/min	Power/W	Pressure/bar
1	160	10	300	15
2	210	10	450	15

Preparation of Teeth for Analysis

Teeth samples are stored in artificial saliva, then washed in de-ionized water and dried on room temperature for 2-3 hours. After crushing, powdered tooth (0.1 g) were placed in Teflon vessel and subjected to a wet mineralization in a closed system with 2 ml nitric acid.

Teflon vessels were placed in microwave oven and were mineralized by programme given in table 2 after the second step system ventilations (20 min). The mineralization product was transferred quantitatively into 25 ml and de-ionized water was added. The samples could by subjected to FAAS after this treatment.

Construction of Calibration Dagram

Calibration diagram is constructed by using a method of standard solutions using regression analysis, where functional relationships between mass concentration and absorbance of Ca, Na, K and Mg were obtained. For the construction of calibration diagram standard solutions of Ca, Na, K and Mg, with concentration of 1 mg/L, were used. Means of absorbance for each calcium standard solution are given in table 3.

Table 3. Absorbance for appropriate mass concentrations of calcium in tooth enamel

γ(Ca)/μg/ml	A
0	0
10	0,086
20	0,165
30	0,224

Analytical dependence on absorbance of concentration of Ca is given by equation:
$A = 0{,}0078 \cdot \gamma(Ca)/\mu g/ml$
Correlation coefficient is 0,992.

For statistical evaluation, a one-way analysis of variance (ANOVA) was initially used to see if there was a significant difference between groups.

Results

Table 4 shows the percentage of Ca in enamel in the experimental group of teeth 14 days after application of the topical gel - GC Tooth Mousse. Average value of Ca in the examined group of teeth was 22.38%, and 20.06% in the control group. For this time period, statistically significant difference was found between mass fractions of Ca in the tooth enamel between groups.

Table 4. Values on the mass fraction of Ca in enamel 14 days after application of the topical gel

group	n	$\bar{x}$	SD	t	*p*
test	30	22.38	4.58		
				-2.23	0.033*
control	30	20.06	3.78		

Table 5 shows mass fraction of Na, K and Mg in enamel in the group of examined teeth compared with the control group 14 days after application of the topical gel. For this time period, no statistically significant difference was found between mass fractions of K, Mg in the enamel between both groups. Mass fractions of Na in the enamel displayed increased value, with 0.89% in the examined group and 0.60% in the control group, the difference being statistically significant.

Table 5. Values on the mass fraction of Na, K and Mg in enamel 14 days after application of the topical gel

group	parameters	n	$\bar{x}$	SD	t	*p*
test	Na	30	0.89	0.05		
					2.37	0.02*
control	Na	30	0.60	0.06		
test	K	30	0.042	0.018		
					1.88	0.06
control	K	30	0.04	0.009		
test	Mg	30	0.27	0.08		
					-0.33	0.74
control	Mg	30	0.23	0.07		

Table 6. Values on the mass fraction of Ca in enamel 1 month after application of the topical gel

group	n	$\bar{x}$	SD	t	*p*
test	30	23.04	4.51		
				3.21	0.003**
control	30	21.02	3.84		

Table 6 shows the mass fraction of Ca in enamel in the group of examined teeth compared to the control group of teeth 1 month after application of the topical gel. It shows highly significant statistical difference of the values (23.04% in the examined group, compared to 21.02% in the control group). Values of the mass fraction of Na, K and Mg in enamel of both groups of teeth, 1 month after application of the topical gel, is shown in table 7. These values were higher in the examined group compared to the control group of teeth.

Table 7. Values on the mass fraction of Na, K and Mg in enamel 1 month after application of the topical gel

group	parameters	n	$\bar{x}$	SD	t	*p*
test	Na	30	0.91	0.14		
					-8.96	0.000000**
control	Na	30	0.67	0.05		
test	K	30	0.042	0.017		
					19.09	0.000000**
control	K	30	0.017	0.007		
test	Mg	30	0.29	0.09		
					-2.3	0.02*
control	Mg	30	0.25	0.04		

Discussion

Enamel decalcification around orthodontic bands and brackets has long been a concern. Studies show that orthodontic appliances increase the accumulation and adherence of plaque in mouth. *Streptococcus mutans* and *Lactobacillus* concentrations in the oral cavity increase in conjunction with orthodontic treatment and fixed appliances. These and other bacteria ferment carbohydrates to produce organic acids. These acids can, over time, lead to the dissolution of calcium and phosphate ions from the enamel surfaces. This process of decalcification may lead to white spot lesions and even capitation in as little as 4 weeks[16,20,23].

Clinical experience shows that the use of fixed appliances in orthodontic treatment increases the risk of enamel demineralization, especially in conjunction with compromised oral hygiene. Together with topical gel GC Tooth Mousse applications, the development and use of fluoride-releasing orthodontic materials may reduce the risk of enamel demineralization during orthodontic treatment.

In this *in vitro* study we examined the percentage of calcium, its effect on enamel demineralization, and the alterations that are observed on the enamel surface after the use of fluoride-releasing orthodontic bonding system (Fuji Ortho LC).

Enamel lesion which have been remineralized with topical exposure to CPP-ACP have been shown to be more resistant to subsequent acid challenge, and capable to promote remineralization of enamel subsurface lesions with hydroxyapatite. In addition, the relatively low carbonate environment of the CPP-ACP treated crystalline and lower micro strain than might be found in normal tooth enamel[17].

Enamel demineralization *in vitro* was inhibited to a certain degree in our study. Similar decalcification prevention has been reported by many authors for other fluoride-releasing materials[1,4,8]. However, significant difference in demineralization inhibition was observed between 2 periods of monitoring.

The results obtained in this study refer to high percentage of Ca in enamel in the study group for the first examined periods. The percentages of Ca in enamel were remarkably higher after 1 month from application of dental mousse. The finding from this *in vitro* study indicate that fluoride-releasing adhesives may inhibit enamel decalcification adjacent to orthodontic brackets during the examined period by forming a protective deposit of calcium fluoride-like particles on the enamel surface.

Conclusions

Demineralization during orthodontic treatment is a significant clinical problem.

The results of this study indicate that with an *in vitro* tooth-brackets model, the creation of white spot inhibition could be achieved with the use of resin modified glass ionomer cement, supplemented with topical gel exposure. The effects of CPP-ACP have so far shown promising dose-related increases in enamel remineralization within already demineralized enamel lesion. The mineral balance in the oral environment is accomplished by application of the Recaldent™ CPP-ACP in the form of GC Tooth Mousse.

References

1. *Artun J, Brobakken BO*. Prevalence of caries white spots after orthodontic treatment with multibonded appliances. *Eur J Orthod*, 1986; 8:229-234.
2. *Basdra EK, Huber H, Komposch*. Fluoride released from orthodontic bonding agents alters the enamel surface and inhibits enamel demineralization in vitro. *Am J Orthod Dentofac Orthop*, 1996; 109:466-472.
3. *Cai F, Shen P, Morgan MV, Reynolds EC*. Remineralization of enamel subsurface lesions in situ by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate. *Aus Dent J*, 2003; 48(4):240-243.
4. *Corry A, Millett DT, Creanor SL, Foye RH, Gilmour WH*. Effect of fluoride exposure on cariostatic potential of orthodontic bonding agents: an in vitro evaluation. *J Orthod*, 2003; 30(4):323-329.
5. *Chang HS, Walsh LJ, Freer TJ*. Enamel demineralization during orthodontic treatment. Aetiology and prevention. *Aus Dent J*, 1997; 42(5):322-327.

6. *Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC*. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. *Caries Res*, 2004; 38(6):551-556.
7. *Featherstone JD*. The science and practice of caries prevention. *J Am Dent Assoc*, 2000; 131:887-899.
8. *Featherstone JD, Glena R, Sharaiti M, Shields CP*. Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. *J Dent Res*, 1990; 69:620-625.
9. *Ferguson DB*. Salivary electrolytes. In: Tenovuo J, ed. Human Saliva: clinical chemistry and microbiology. Vol. 1. Boca Raton, FL: CRC Press, 1989; pp 75-99.
10. Flame Atomic Absortion Spectrometry, Analitical Methods, Varian, Australia Pty Ltd, Publication No 85-100009-00 Revised March 1989.
11. *Gorelick L, Geiger AM, Gwinnett AJ*. Incidence of white spot formation after bonding and banding. *Am J Orthod*, 1982; 81(2):93-98.
12. *Manton D, Shen P, Cai F, Cocharne NJ, Reynolds C, Messer LB, Reynolds EC*. Remineralization of White Spot Lesions in situ by Tooth Mousse. Abstract 185-84th General Session of the IADR, 28 June - 1 July, 2006, Brisbane, Australia.
13. *Mizrahi E*. Enamel demineralization following orthodontic treatment. *Am J Ortod*, 1982; 82(1):62-67.
14. *Mellberg JR*. Remineralization A status report. Part III. *Am J Dent*, 1:85-89.
15. *Ögaard B, Rolla G, Arends J, ten Cate JM*. Orthodontic appliances and enamel demineralization. Part 2: prevention and treatment of lesions. *Am J Orthod Dentofac Orthoped*, 1988; 94:123-128.
16. *Reich E, Lussi A, Newbrun E*. Caries-risk assessment. *Int Dent J*, 1999; 49:15-26.
17. *Reynolds EC*. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. *J Dent Res*, 1996; 76(9):1587-1595.
18. *Reynolds EC*. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. *Spec Care Dentist*, 1998; 18(1):8-16.
19. *Reynolds EC, Cai F, Shen P, Walker GD*. Retention in plaque and remineralization of enamel lesion by various forms of calcium in a mouthrinse or sugar-free chewing gum. *J Dent Res*, 2003; 82:206-211.
20. *Robinson C, Shore RC, Brookes SJ, Strafford S, Wood SR, Kirkham J*. The Chemistry of Enamel Caries. *Crit Rev Oral Biol Med*, 2000; 11(4):481-495.
21. *Rose RK*. Effects of an anticariogenic casein phosphatide on calcium diffusion in streptococcal model dental plaques. *Arch Oral Biol*, 2000; 45(7):569-575.
22. *Schupbach P, Neeser JR, Golliard M, Rouvet M, Guggenheim B*. Incorporation of caseinoglycomacropeptide and caseinophosphopeptide into the salivary pellicle inhibits adherence of mutans streptococci. *J Dent Res*, 1996; 75(10):1779-1788.
23. *Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC*. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phospopeptide - amorphous calcium phosphate. *J Dent Res*, 2001; 80(12):2066-2070.
24. *Trimpeneers LM, Dermault LR*. A clinical evaluation of the effectiveness of a fluoride-releasing visible light-activated bonding system to reduce demineralization around orthodontic brackets. *Am J Orthod Dentofacial Orthop*, 1996; 110(2):218-222.
25. *Tsalev DL, Zaprinov ZK*. Atomic Absorption Spectrometry in Occupational and Environmental Health Practice. Volume I. Analytical Aspects and Health Significance,. Boca Raton, Florida: CRC Press, 1983.
26. *Vorhies AB, Donly KJ, Staley RN, Wefel JS*. Enamel demineralization adjacent to orthodontic brackets bonded with hybrid glass ionomer cements: an in vitro study. *Am J Ortod Dentofacial Orthop*, 1998; 114(6):668-674.

Correspondence and requests for offprints to:

Dr. Efka Zabokova-Bilbilova
Department of Pedodontic
School of Dentistry
Vodnjanska 17
1000 Skopje
E-mail: efka_zabokova@hotmail.com

Dr. Trajce Stafilov
Dentistry Institute of Chemistry
Faculty of Natural Sciences and Mathematics
St. Cyril and Methodius University
1000 Skopje
E-mail: trajcest@iunona.pmf.ukim.edu.mk