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EFEKAT KAZEIN FOSFOPEPTID-AMORFNOG KALCIJUM FOSFATA (CPP-ACP) NA DEMINERALIZACIJU GLEĐI OKO ORTODONTSKIH APARATA: IN VITRO STUDIJA

EFFECT OF CASEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM PHOSPHATE (CPP-ACP) TOPICAL AGENTS ON ENAMEL DEMINERALIZATION AROUND ORTHODONTIC BRACES: AN IN VITRO STUDY

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Rezime

Uvod Ortodontski tretman ima brojne prednosti, uključujući i poboljšanje dentalne i facijalne estetike kod pacijenata.

Cilj Cilj rada bio je da se evaluiraju in vitro efekti kazein fosfopeptid-amorfnog kalcijum fosfata (CPP-ACP), sredstva za lokalnu aplikaciju, koje smanjuje demineralizaciju gleđi oko ortodontskih aparata.

Materijal i metode Ispitivanje je obuhvatilo 60 zdravih ekstrahiranih premolara koji nisu imali nikakve kliničke znake demineralizacije. Svi zubi podeljeni su u tri grupe: jedna kontrolna (20 zuba) i dve ispitivane grupe (40 zuba). Zubi (40) su bili podeljeni u 2 grupe prema periodu praćenja – jedna nakon mesec dana, a druga nakon 6 meseci. Zubi su potom premazivani gelom – GC Tooth Mousse u trajanju od 5 minuta tri puta dnevno, a do analiziranja su čuvani u veštačkoj pljuvački. Nakon toga, uzorci su pripremljeni za SEM analizu (JEOL JSM 5300). Spektrometrijom (flame atomic absorption spectrometry – FAAS) je meren procenat Ca i Mg u gleđi. SEM analiza je korišćena za praćenje kiselinom nagrižene površine gleđi tretirane ili netretirane sa CPP-ACP. Dvadeset premolara korišćeno je za praćenje nagriženih površina gleđi pomoću SEM.

Rezultati Prosečne vrednosti sadržaja Ca u gleđi bile su uvećane u ispitivanoj grupi u poređenju sa kontrolnom grupom zuba jedan mesec nakon postavljanja ortodontskog aparata i lokalne primene CPP-ACP. Rezultati su pokazali da SEM analiza detektuje veliki potencijal za remineralizaciju u uzorcima zuba koji su svakodnevno bili izloženi lokalnom dejstvu gela GC Tooth Mousse.

Zaključak: CPP-ACP ima potencijal da remineralizuje dekalcfifikovane delove gleđi kod ortodontskih pacijenata. Primena CPP-ACP može da poboljša mineralizaciju zuba tokom ortodontskog tretmana fiksnim aparatima.

Ključne reči: gleđ, ortodontske bravice, demineralizacija, remineralizacija

Abstract

Introduction Orthodontic treatment has many benefits including the improvement of patient's dental and facial esthetics.

Aim The aim of this study was to evaluate the in vitro effects of a casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) topical agent in reducing enamel demineralization around orthodontic braces.

Material and method This study comprised 60 healthy extracted premolars without any clinical sign of decalcification. The teeth (40) were divided in two groups according to the period of monitoring, after 1 and 6 months. Then they were coated with a topical gel - GC Tooth Mousse for 5 minutes thrice daily, and they have been stored in artificial saliva until analyzing. We measured the percentage of Ca and Mg in the enamel by using the method of flame atomic absorption spectrometry (FAAS). SEM was used to observe the acid-etched enamel surfaces treated with or without CPP-ACP. Twenty premolars were used for ultrastructural examination of the etched enamel surfaces by SEM. The two examined groups were coated with a topical gel - GC Tooth Mousse for 5 minutes thrice daily, and they were stored in artificial saliva for one and six months. After that, the samples were prepared for SEM analysis (JEOL JSM 5300).

Results The mean values of Ca content in enamel were increased in the examined group, compared to the control group of teeth one month after the braces were bonded and topical application with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). The results indicated that SEM analysis detected the highest remineralization potential in the tooth samples exposed daily to topical gel - GC Tooth Mousse.

Conclusions CPP-ACP has the potential to remineralize areas of enamel decalcification in orthodontic patients. The application of CPP-ACP can improve the mineralization of the teeth during orthodontic treatment with fixed appliance.

Key words: enamel, braces, demineralization, remineralization

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Uvod

Ortodontski tretman ima brojne pozitivne efekte, uključujući poboljšanje dentalne i facialne estetike pacijenata. Ipak, idealni estetski ishod može biti kompromitovan razvojem demineralizacije oko aparata tokom ortodontskog tretmana. Demineralizacija gleđi koja prati fiksne ortodontske aparate ispituje se godinama i još uvek je problem, uprkos poboljšanju i materijala i tehnika. Tokom korišćenja fiksnih aparata u ortodontskom tretmanu, formiranje belih mrlja čest je propratni efekat. Ortodontske bravice povećavaju retenciju plaka i ostataka hrane na glatkim površinama zuba, koje bi verovatno imale nisku prevalenciju karijesa¹.

Dostupna literatura ukazuje na znatno variranje prevalencije demineralizacije i razvoja belih mrlja tokom fiksnog ortodontskog tretmana. Gorelick i sar.² su ustanovili da prilikom uklanjanja bravica 49.6% pacijenata ima bele mrlje bar na jednom zubu, dok su Sudjalim i sar.³ dobili širok raspon varijacija u svom istraživanju, te je prevalencija belih mrlja bila u rasponu od 2% do 96%. Bez obzira na prevalenciju, postoji slaganje oko toga da demineralizacija i razvoj belih mrlja predstavljaju problem tokom ortodontskog tretmana. Pored toga, ustanovljeno je da bele mrlje mogu biti prisutne godinama. Danas se zna da CPP-ACP može sprečiti demineralizaciju gleđi i podstići remineralizaciju subpovršinskih lezija gleđi. Kako se pokazalo, kompleks kazein fosfopeptid – amorfni kalcijum (CPP-ACP), derivat mlečnih proteina, ima antikariogeni efekat^{4,5,6}. On sprečava demineralizaciju gleđi i podstiče remineralizaciju i deluje puferski na jone slobodnog kalcijuma i fosfata^{7,8}, inkorporirajući na taj način ACP u plak i na površinu zuba⁹. Remineralizovana gleđ ima povećanu rezistenciju na naknadna dejstva kiseline u poređenju sa netretiranom gleđi. Upotreba CPP-ACP u ortodontskoj kliničkoj praksi predložena je za prevenciju belih mrlja i profilaksu karijesa pre postavljanja ortodontskih aparata¹⁰. Kompleksi CPP-ACP deluju kao rezervoar kalcijuma i fosfata, pomažući da se održi stanje super-zasićenja ovim mineralima, što može da podstakne remineralizaciju gleđi. U ovom momentu, kompleksi CPP-ACP dostupni su u različitim gelovima, kremama i penama, a mogu se inkorporirati i u žvakaće gume¹¹.

Introduction

Orthodontic treatment has many benefits including the improvement of patient's dental and facial esthetics. However, the ideal esthetic outcome can be compromised by development of demineralization around the appliances during orthodontic treatment. Enamel demineralization associated with fixed orthodontic appliances has been observed for years and continues to be a problem in spite of the advances in materials and techniques. While using fixed appliances for orthodontic treatments, the formation of white spot lesions is an unaesthetic and common side effect. Bands and braces increase the retention of plaque and food on smooth tooth surfaces that would otherwise tend to have a low prevalence of caries¹.

Published literature shows wide variation in the prevalence of demineralization and white spot development during fixed orthodontic treatment. Gorelick et al.² observed that, at debonding, 49.6% of patients showed white spot formation on at least one tooth, while Sudjalim et al.³ reported a wide variation within their study, with overall prevalence of white spot lesions ranging between 2% and 96%. Irrespective of prevalence, it is agreed upon that demineralization and development of white spot lesions is a problem during orthodontic treatment. Moreover, it is established that white spot lesions may persist for years. In the light of the present state of knowledge, it has been shown that CPP-ACP can prevent enamel demineralization and promote remineralization of enamel subsurface lesions. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex, a milk protein derivative, has been proven to have anticariogenic effects^{4,5,6}. It prevents enamel demineralization and promotes remineralization through buffering free calcium and phosphate ion activities^{7,8} thereby incorporating ACP into plaque and onto tooth surface and maintaining a state of supersaturation with respect to tooth enamel⁹. Remineralized enamel has an increased resistance to subsequent acid challenge than untreated enamel. The use of CPP-ACP in orthodontic clinical practice has been proposed for white spots prevention and caries prophylaxis before braces bonding procedure¹⁰. The CPP-ACP complexes act as calcium and phosphate reservoir helping to maintain a state of super saturation of these minerals, which can enhance enamel remineralization. Presently, CPP-ACP complexes are available in a variety of gels, creams, and mousses and may also be incorporated into chewing gums¹¹.

Cilj rada bio je da evaluiira in vitro efekat lokalno aplikovanog CPP-ACP u redukciji demineralizacije gleđi oko ortodontskih bravica.

Materijal i metode

Ispitivanjem je obuhvaćeno 60 zdravih, ekstrahiranih premolara, koji nisu imali kliničke znake demineralizacije. Svi zubi podeljeni su u tri grupe: jedna kontrolna (20 zuba) i dve ispitivane grupe (40 zuba). Svi zubi su očišćeni i presečeni na pola u buko-lingvalnoj ravni pomoću dijamantskog diska. Kontrolni i testirani uzorci, prema tome, potekli su od istih zuba. Adheziv koji je korišćen za postavljanje bravica bio je Con Tec LC (Dentaurum, Germany). Zubi (40) su podeljeni u dve grupe prema periodu praćenja, nakon jednog i nakon 6 meseci. Zubi su potom lokalno premazani gelom - GC Tooth Mousse u trajanju od 5 minuta tri puta dnevno, a onda su do analiziranja čuvani u veštačkoj pljuvački (20 mmol/l NaHCO₃, 3 mmol/l NaH₂PO₄ i 1 mmol/l CaCl₂, neutralan pH). Procenat Ca i Mg u gleđi meren je spektrometrijom (flame atomic absorption spectrometry – FAAS) u aparatu Varian Spectra AA 55 B^{12,13}. Pre analize, optimizirani su instrumentalni parametri radi bolje preciznosti i osetljivosti (Tabela 1). Sadržaj istraživanih elemenata u zubnoj gleđi određivan je pomoću FAAS nakon mineralizacije u mikrotalasnom sistemu firme Milestone, model Ethos Touch Control (Tabela 2).

The aim of this study was to evaluate the in vitro effects of a casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) topical agent in reducing enamel demineralization around orthodontic braces.

Material and method

This study comprised 60 healthy extracted premolars without any clinical sign of decalcification. All teeth were cleaned and cut in half buccolingually with a diamond disc. Thus, the control and test specimens were obtained from the same teeth. The adhesive used for bonding braces was Con Tec LC (Dentaurum, Germany). The teeth (40) were divided in two groups according to the period of monitoring, after 1 and 6 months. Then they were coated with a topical gel - GC Tooth Mousse for 5 minutes thrice daily, and they have been stored in artificial saliva (20 mmol/l NaHCO₃, 3 mmol/l NaH₂PO₄ and 1 mmol/l CaCl₂, neutral pH) until analyzing. We measured the percentage of Ca 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^{12,13}. Before the analysis, instrumental parameters for better precision and sensibility were optimized (Table 1). The content of the investigated elements in the teeth enamel was determined by FAAS after mineralization in a microwave digestion system from Milestone, model Ethos Touch Control (Table 2).

Tabela 1. Optimalni instrumentalni parametri za određivanje Ca i Mg pomoću FAAS
Table 1. Optimal instrumental parameters for Ca and Mg determination by FAAS

Parametri/element Parameters/ element	Ca	Mg
Talasna dužina/nm Wavelength/nm	422.7	285.2
Otvor-prorez/nm Slit/nm	0,5	0,2
Struja lampe /mA Lamp current/mA	10	4

Tabela 2. Program mineralizacije uzoraka zuba
Table 2. Tooth sample mineralization program

Faza/korak Step	Temperatura/°C Temperature/°C	Vreme/min Time/min	Snaga/W Power/W	Pritisak/bar Pressure/bar
1	160	10	300	15
2	210	10	450	15

Konstruisanje kalibracionog dijagrama

Kalibracioni dijagram je konstruisan korišćenjem metode standardnih rastvora. Korišćenjem regresionu analizu, dobijeni su funkcionalni odnosi između masene koncentracije i apsorpcije Ca i Mg. Korišćeni su standardni rastvori Ca i Mg sa koncentracijom 1 mg/L. Analitička zavisnost koncentracione apsorpcije od Ca je data jednačinom: $A = 0,0078 \cdot \gamma(\text{Ca})/\mu\text{g/ml}$. Koeficijent korelacije bio je 0,992.

SEM analiza

SEM analiza je korišćena za opserviranje kiselinom nagriženih površina gleđi, tretiranih ili netretiranih sa CPP-ACP. Za ultrastrukturno ispitivanje nagriženih površina gleđi pomoću SEM korišćeno je 20 premolara. Krunice su odsečene od ostatka zuba dijamantskim svrdlom na spoju cementa i gleđi, a svaka krunica presečena je longitudinalno u okluzogingivalnom pravcu da bi se dobile dve bukalne površine gleđi. Svaka od površina dobijenih od istog zuba nasumično je stavljena u jednu od dve eksperimentalne grupe. Zubi u dve ispitivane grupe premazivane su gelom - GC Tooth Mousse tokom 5 minuta tri puta dnevno, a onda čuvani u veštačkoj pljuvački jedan mesec i 6 meseci. Nakon toga, uzorci su pripremljeni za SEM analizu (JEOL JSM 5300) korišćenjem tehnike prskanja (sputter) u vakuumskom evaporatoru. Odgovarajući deo površine gleđi analiziran je da bi se utvrdile mikromorfološke promene u strukturi gleđi na mestu na kojem su bile fiksirane bravice.

U statističkoj analizi, najpre je korišćena jednostrana analiza varijance (ANOVA), a nakon nje i Tukey test, da bi utvrdili da li među grupama postoje značajne razlike.

Rezultati

U ispitivanim grupama zabeležene su statistički značajne razlike u srednjim vrednostima sadržaja Ca u gleđi nakon tretmana posle jednog meseca i posle 6 meseci (analiza varijance $F=3,710$; $p=0,0284$) (Tabela 3).

Construction on calibration diagram

Calibration diagram was constructed by using of the method of standard solutions. Using regression analysis, functional relationships between mass concentration and absorbance of Ca and Mg were obtained. Standard solutions of Ca and Mg with concentration of 1 mg/L were used. Analytical dependence on concentration absorbance on Ca is given by the equation: $A = 0,0078 \cdot \gamma(\text{Ca})/\mu\text{g/ml}$. Correlation coefficient was 0,992.

SEM Observations

SEM was used to observe the acid-etched enamel surfaces treated with or without CPP-ACP. Twenty premolars were used for ultrastructural examination of the etched enamel surfaces by SEM. The crowns were sectioned from the roots with a diamond bur at the buccal cement enamel junction, and each crown was cut longitudinally in an occlusogingival direction to obtain two buccal enamel surfaces. Each surface obtained from the same tooth was randomly allocated to one of two experimental groups. The two examined groups were coated with a topical gel - GC Tooth Mousse for 5 minutes thrice daily, and stored in artificial saliva for one and six months. After that, the samples were prepared for SEM analysis (JEOL JSM 5300), using sputter technique in a vacuum evaporator. The appropriate area of enamel surface was analyzed in order to determine micro morphology changes in the structure of the enamel, on the place of previous braces fixation.

For statistical evaluation, one-way analysis of variance (ANOVA) followed by Tukey's test were initially used to see if there was a significant difference between the groups.

Results

In the examined group of teeth (Table 3) there were statistically significant differences of Ca content mean values in enamel after treatment at one and six months (Analysis of variance $F=3,710$; $p=0,0284$).

Tabela 3. Procenat Ca u gleđi u ispitivanim grupa
Table 3. Percentage of Ca in enamel in the examined group

Vreme time	$\bar{X}$	SD	N
1 mesec 1 month	25,19	2,02	30
6 meseci 6 months	24,57	2,45	30

F=3,710; p=0,0284

Srednje vrednosti sadržaja Ca u gleđi ispitivane i kontrolne grupe zuba jedan mesec nakon postavljanja proteze i lokalne primene CPP-ACP prikazane su u Tabeli 4.

The mean values of Ca content in enamel of the examined and control group of teeth one month after the braces were bonded and topical application with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) are shown in Table 4.

Tabela 4. Poređenje sadržaja Ca u gleđi u ispitivanim i kontrolnoj grupi (%)
Table 4. Comparison of Ca content in enamel between the examined and control group (%)

Vreme time	Ispitivana grupa examined group		Kontrolna grupa control group		t	p
	$\bar{X}$	SD	$\bar{X}$	SD		
1 mesec 1 month	25,19	2,02	23,32	2,34	3,318	0,00156*
6 meseci 6 months	24,57	2,45	23,45	2,41	1,777	0,0807

*statistički značajne razlike / *statistically significant differences

Vrednosti su bile veće u ispitivanim grupama u poređenju sa kontrolnom grupom zuba. Nakon mesec dana, primećena je statistički značajna razlika u vrednostima Ca u gleđi između ispitivanih i kontrolne grupe. Nakon 6 meseci tretmana, beleži se razlika, mada ne statistički značajna, u srednjim vrednostima Ca u gleđi između ispitivanih i kontrolne grupe.

U ispitivanim grupama nije bilo statistički značajnih razlika u srednjim vrednostima sadržaja Mg u gleđi nakon tretmana posle jednog meseca i posle 6 meseci (analiza varijance F=5,031; p=0,0085) (Tabela 5). Srednje vrednosti sadržaja Mg u gleđi ispitivanih grupa i kontrolne grupe zuba jedan mesec nakon postavljanja proteze i lokalne aplikacije CPP-ACP prikazane su u Tabeli 6. Nakon mesec dana, nije bilo statistički značajne razlike u srednjim vrednostima Mg u gleđi u ispitivanoj i kontrolnoj grupi. Nakon 6 meseci tretmana, nije bilo statistički značajnih razlika u srednjim vrednostima Mg u gleđi u ispitivanoj i kontrolnoj grupi.

Reprezentativni SEM prikazi uzoraka nagrižene gleđi prethodno tretiranih sa i bez CPP-ACP predstavljeni su na Figurama 1-3.

These values were increased in the examined group, compared to the control group of teeth. After one month, a statistically significant difference of Ca values in enamel between the examined and control groups was noticed. After six-month treatment were differences, but not statistically significant of the Ca mean values in enamel between the examined and control group.

In the examined group of teeth (Table 5), there were statistically significant differences of Mg content mean values in enamel after treatment at one and six months (Analysis of variance F=5,031; p=0,0085). The mean values of Mg content in enamel of the examined and control group of teeth one month after the braces were bonded and topical application with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) are shown in Table 6. After one month, no statistically significant difference of the Mg mean values in enamel between the examined and control group was found. After six-month treatment, there were statistically significant differences of the Mg mean values in enamel between the examined and control group.

Table 5. Procenat Mg u gleđi u ispitivanim grupama
Table 5. Percentage of Mg in enamel in the examined group

Vreme time	$\bar{X}$	SD	N
1 mesec 1 month	0,323	0,074	30
6 meseci 6 months	0,388	0,078	30

F=5,031; p=0,0085

Table 6. Poređenje sadržaja Mg u gleđi u ispitivanim i kontrolnoj grupi (%)
Table 6. Comparison of Mg content in enamel between the examined and control group (%)

Vreme time	Ispitivana grupa examined group		Kontrolna grupa control group		t	p
	$\bar{X}$	SD	$\bar{X}$	SD		
1 mesec 1 month	0,323	0,074	0,306	0,069	0,914	0,36449
6 meseci 6 months	0,388	0,078	0,357	0,055	2,012	0,04884*

*statistički značajne razlike / *statistically significant differences

U kontrolnoj grupi (veštačka pljuvačka), konfiguracija topografije gleđi očigledno je imala porozne defekte (Slika 1a; uvećanje 1500x). Pri još jačem uvećanju (3500x), poroznost je bila još evidentnija, a oko poroznih polja mogle su se uočiti linije mineralizacije (Slika 1b).

Representative SEM images of the etched enamel specimens pretreated with and without CPP-ACP are shown in Figures 1 - 3.

In the control group (artificial saliva), the configuration of enamel topography was presented apparent with certain porous defects (Figure 1a; 1000× magnification). At a higher

Slika 1a. Porozni defekti gleđi, kontrolna grupa.
SEM (uvećanje x1500)

Figure 1a. Porous defects of enamel, control group.
SEM (magnification x1500)

Slika 1b. Porozni defekti gleđi, kontrolna grupa. SEM
(uvećanje x3500)

Figure 1b. Porous defects of enamel, control group.
SEM (magnification x3500)

U ispitivanoj grupi (nakon jednomesečnog tretmana sa CPP-ACP), evidentna je poroznost interprizmatične supstance, kao i polja remineralizacije (Slika 2a; uvećanje 1500x). Pri jačem uvećanju (3500x), u istoj grupi su se uz poroznost gleđi mogle videti određene oblasti kalcifikacije (Slika 2b).

magnification of 2000×, the porosities were more evident and faint lines of mineralization can be seen in the same group and around the porosities (Figure 1b).

In the examined group (after treatment of one month with CPP-ACP), interprismatic substances with porosities and areas of remineralization were evident (Figure 2a; 1500× magni-

U ispitivanoj grupi (nakon šestomesečnog tretmana sa CPP-ACP), evidentne su oblasti kalcifikovanih depozita uz porozne defekte (Slika 3a; uvećanje 3500x). Pri jačem uvećanju (5000x), u istoj grupi su se uz poroznost mogle primetiti oblasti mineralizovanih depozita i polja kalcifikacije (Slika 3b).

At a higher magnification of 3500 $\times$, certain areas of calcifications along the porosities were seen in the same group (Figure 2b).

In the examined group (after the six-month treatment with CPP-ACP), areas of calcified deposits concentrated along the porous defects were evident (Figure 3a; 3500 $\times$ magnification). At a higher magnification of 5000 $\times$, discernable areas of mineralized deposits and certain areas of calcifications along the porosities were seen in the same group (Figure 3b).

Slika 2a. Polja remineralizacije porozne interprizmatične supstance, ispitivana grupa nakon jednomesečnog tretmana sa CPP-ACP. SEM (uvećanje x1500)

Figure 2a. Interprismatic substances with porosities and areas of remineralization, examined group after treatment of 1 month with CPP-ACP. SEM (magnification x1500)

Slika 2b. Polja remineralizacije porozne interprizmatične supstance, ispitivana grupa nakon jednomesečnog tretmana sa CPP-ACP. SEM (uvećanje x3500)

Figure 2b. Interprismatic substances with porosities and areas of remineralization, examined group after treatment of 1 month with CPP-ACP. SEM (magnification x3500)

Slika 3a. Kalcifikovan depozit uz porozne defekte gleđi, ispitivana grupa nakon šestomesečnog tretmana sa CPP-ACP. SEM (uvećanje x3500)

Figure 3a. Areas of calcified deposits concentrated along the porous defects, examined group after treatment of 6 months with CPP-ACP. SEM (magnification x3500)

Slika 3b. Kalcifikovan depozit uz porozne defekte gleđi, ispitivana grupa nakon šestomesečnog tretmana sa CPP-ACP. SEM (uvećanje x5000)

Figure 3b. Areas of calcified deposits concentrated along the porous defects, examined group after treatment of 6 months with CPP-ACP. SEM (magnification x5000)

Diskusija

Opšte je prihvaćeno da postavljanje fiksnih ortodontskih aparata stvara retenciona mesta za plak i otežava čišćenje zuba. Neravne površine bravica, traka, žica i drugih elemenata, takođe ograničavaju prirodne mehanizme čišćenja kao što su pokretanje oralne muskulature i pljuvačke. U prisustvu ugljenih hidrata u ustima, snižava se pH plaka i ubrzava njegova akumulacija i sazrevanje. Te promene, pogoduju kolonizaciji acidogenih bakterija, kao što su *Streptococcus mutans* i *Lactobacilli*. Međutim, nivo bakterija se značajno smanjuje nakon 6-15 nedelja od uklanjanja fiksnog ortodontskog aparata.

U jednom od prvih kliničkih ispitivanja CPP-ACP kreme, korišćene specijalno za tretman belih mrlja nastalih nakon nošenja fiksnih ortodontskih aparata, Bailey i sar.¹⁴ su zaključili da korišćenje CPP-ACP kreme pospešuje regresiju bele mrlje u poređenju sa placebom. Ipak, u novijim kliničkim istraživanjima došlo se do rezultata koji nisu tako dobri. U svojoj kliničkoj studiji na 54 ispitanika, Beerens i sar.¹⁵ porede CPP-ACP pastu koja sadrži fluorid sa kontrolnom pastom. Nakon tromesečnog perioda tretmana, ne nalaze nikakvu prednost korišćenja fluoridne CPP-ACP paste u odnosu na redovnu oralnu higijenu u smislu regresije bele mrlje, mereno pomoću QLF. CPP-ACP kompleks deluje kao „nosač“ kalcijuma i fosfata, pri čemu ih transportuje do površine zuba i skladišti u plak i pelikulu. Redukcija pH dovodi do oslobađanja jona kalcijuma i fosfata, čime se stimuliše remineralizacija¹⁶.

Kazein fosfopeptidi (CPP) sadrže multifosfoserilne sekvence, koje imaju sposobnost da stabilizuju kalcijum fosfat u nano-komplekse u rastvoru, u obliku amorfnog kalcijum fosfata (ACP). Ovi nano-kompleksi (CPP-ACP) ostaju veoma mali i imaju dve funkcije: mogu se lokalizovati na površini zuba i sprečiti demineralizaciju gleđi, a takođe su dovoljno mali da se probiju duboko u poroznu gleđ i remineralizuju površinu gleđnih kristala. U isto vreme, CPP-ACP stupa u interakciju sa dostupnim jonima fluorida i produkuje amorfnu kalcijum fluorid fosfat, koji se pomoću CPP može taložiti na površini zuba. Rezultat primene CPP-ACP jeste duboka remineralizacija, čime se eliminiše poroznost gleđi. Ova studija je urađena kako bi se utvrdio remineralizacioni potencijal CPP-ACP. Molekuli CPP sadrže klaster fosfoseril ostataka

Discussion

It is generally accepted that the insertion of fixed orthodontic appliances creates stagnation areas for plaque and makes tooth cleaning more difficult. The irregular surfaces of braces, bands, wires, and other attachments also limit naturally occurring self-cleansing mechanisms, such as the movement of the oral musculature and saliva. This in turn encourages a lower plaque pH in the presence of carbohydrates and accelerates the rate of plaque accumulation and plaque maturation. These changes in the local environment appear to favor colonization of aciduric bacteria such as *Streptococcus mutans* and *Lactobacilli*. However, such microbial levels were found to decrease significantly to levels comparable with age matched controls, 6 to 15 weeks into a retention phase, after the removal of fixed appliances.

In one of the first clinical trials of CPP-ACP cream used specifically for treatment of post-orthodontic WSLs, Bailey et al.¹⁴ reported that use of CPP-ACP cream enhanced the regression of WSLs compared with placebo. However, more recent clinical investigations show less promising results. In a clinical study, Beerens et al.¹⁵ compared a fluoride-containing CPP-ACP paste with a control paste in 54 subjects. After a three-month treatment period, they found no advantage for use of the fluoridated CPP-ACP paste over regular oral hygiene in WSLs regression as measured by QLF. The CPP-ACP complex acts as a vehicle for calcium and phosphate, transporting it to the tooth surface and localizing it in plaque and salivary pellicles. A reduction in pH results in a release of calcium and phosphate ions, which act to promote remineralization¹⁶.

Casein phosphopeptides (CPP) contain multiphosphoryl sequences and these have the ability to stabilize calcium phosphate in nano-complexes in solution in the form of amorphous calcium phosphate (ACP). These nano complexes (CPP-ACP) remain very small and they have two effective functions. They can localize at the tooth surface and prevent enamel demineralization and they are also small enough to be able to penetrate deep into the porous enamel and remineralize the surface of the enamel crystals. At the same time, the CPP-ACP will interact with available fluoride ions to produce an amorphous calcium fluoride phosphate and

koji znatno povećavaju očiglednu rastvorljivost kalcijum fosfata stabilizujući amorfnu kalcijum fosfat u neutralnim i alkalnim uslovima. CPP može da stabilizuje preko 100 puta više kalcijum fosfata nego što je normalno moguće u vodenim rastvorima pri neutralnom ili alkalnom pH, pre nego što dođe do spontane precipitacije. Uz to, CPP-ACPF poseduje fluorid. Istraživanja su pokazala da je remineralizacija malih lezija niskim dozama fluorida efikasnija¹⁷.

U ovoj in vitro studiji ispitivan je procenat kalcijuma, njegov efekat na demineralizaciju gleđi i promene koje se opažaju na površini gleđi nakon postavljanja fiksnog ortodontskog aparata (Con Tec LC Dentaurum) i lokalne aplikacije GC Tooth Mousse. Rezultati dobijeni u radu ukazuju na veoma visok procenat Ca u gleđi u ispitivanoj grupi za prvi ispitivani period (mesec dana). SEM prikazi ove tri grupe ukazuju na remineralizaciju sa CPP-ACP, uz najveću količinu mineralnih depozita nakon 6 meseci tretmana sa CPP-ACP.

Zaključak

CPP-ACP ima potencijal da remineralizuje dekalcifikovanu gleđ kod pacijenata sa fiksnim ortodontskim aparatima. Međutim, kako su pacijenti sa demineralizovanom gleđi uglavnom oni sa lošom oralnom higijenom, motivacija takvih osoba za održavanjem pravilne oralne higijene od ključnog je značaja u prevenciji demineralizacije gleđi, te se CPP-ACP može koristiti kao dopunska terapija. Primena CPP-ACP može poboljšati mineralizaciju zuba tokom ortodontskog tretmana fiksnim aparatima.

this can be stabilized by the CPP on the tooth surface. The result from the application of CPP-ACP is remineralization in depth, thus eliminating the porosity in the enamel and restoring the light properties and translucency. This study was undertaken to determine the remineralization potential of CPP-ACP. The CPP molecules contain a cluster of phosphoserine residues that markedly increase the apparent solubility of calcium phosphate by stabilizing amorphous calcium phosphate under neutral and alkaline conditions. CPP can stabilize over 100 times more calcium phosphate than is normally possible in aqueous solution at neutral or alkaline pH before spontaneous precipitation occurs. CPP-ACPF has, in addition, fluoride. Studies have shown that remineralization of small lesions with low-dose fluoride therapy is more efficient¹⁷.

In this in vitro study we examined the percentage of calcium, its effect on enamel demineralization, and the alterations that were observed on the enamel surface after the use of orthodontic bonding system (Con Tec LC Dentaurum) and topical application of dental cream GC Tooth Mousse. The results obtained in this study showed a remarkably high percentage of Ca in enamel in the examined group for the first examined period (at one month). The SEM images of the three groups suggest remineralization with the CPP-ACP showing the greatest amount of mineral deposits, after the six-months treatment with CPP-ACP.

Conclusions

CPP-ACP has the potential to remineralize areas of enamel decalcification in orthodontic patients. However, since patients with enamel decalcification are generally those with poor oral hygiene, good oral hygiene instruction is essential in preventing enamel decalcification in orthodontic patients, with CPP-ACP used as an adjunct therapy.

The application of CPP-ACP can improve the mineralization of the teeth during orthodontic treatment with fixed appliance.

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