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## IN VITRO PROCENA KAZEIN FOSFOPEPTIDA – AMORFNOG KALCIJUM FOSFATA (CPP-ACP) U PREVENCIJI BELE MRLJE

### IN VITRO ASSESSMENT OF CASEIN PHOSPHOPEPTIDE- AMORPHOUS CALCIUM PHOSPHATE (CPP - ACP) IN PREVENTION OF WHITE SPOT LESIONS

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#### Sažetak

**Uvod:** Ortodonske bravice i drugi ortodontski elementi, otežavaju oralni higijenu olakšavaju akumulaciju dentalnog plaka što povećava rizik od karijesa u toku ortodontskog tretmana.

**Cilj:** ovog istraživanja bio je da se uvrsti koncentracija Ca i Mg u veštačkoj pljuvački posle preventivnog tretmana.

**Metode:** Laboratorijsko ispitivanje (in vitro) obavljeno je na 90 zdravih zuba ekstrahiranih iz ortodontskih razloga. Formirane su tri grupe od po trideset zuba. Zubi iz svake grupe su podeljeni na polovine u buko-lingvalnom pravcu. Na taj način formirani su i ispitivani i kontrolni uzorak, oba od jednog istog zuba. Bravice su zalepljene pomoću GC Fuji OrthoTM LC (GC Amerika Čikago, III), glasjonomer cementom modifikovanim smolom, a zubi su zatim čuvani u veštačkoj pljuvački. Jednom dnevno zubi su premazivani topikalnim gelom - CG Tooth Mousse u trajanju od 5 minuta, a zatim vraćeni u veštačku pljuvačku. Preventivni tretman je sproveden u određenim intervalima od 1, 3 i 6 meseci. Posle svakog perioda ispitivanja, veštačka pljuvačka u kojoj su uzorci čuvani je bila korišćena za određivanje koncentracije Ca i Mg pomoću plamene atomske apsorpcione spektrofotometrije.

**Rezultati:** Rezultati ovog (in vitro) istraživanja pokazali su da su koncentracije Ca i Mg u veštačkoj pljuvački značajno povećane čak i nakon prvog meseca od primene ovog sredstva koje oslobađa Ca i druge minerale (uključujući cement bez fluora koji se koristi za lepljenje bravica) sa maksimalnom vrednošću nakon tri meseca primene. Ove vrednosti su bile značajno niže nakon šest meseci, verovatno kao rezultat njegove apsorpcije u gleđi. Rezultati za Mg su isti kao i za Ca, a vrednost se povećava, kao i njena stabilnost u pljuvački nakon prvog meseca.

**Zaključci:** Rezultati ove in vitro studije jasno ukazuju da preventivni materijali koji se koriste u fiksnom ortodontskom tretmanu inhibiraju demineralizaciju gleđi oko bravica i ortodontskih prstenova. Preporuka za njihovu upotrebu kao dodatnih preventivnih metoda / sredstava je očigledna pored primarne oralne higijene.

**Ključne reči:** zubni karijes, preventivni tretman, koncentracija Ca i Mg

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#### Abstract

**Introduction:** Brackets and various orthodontic elements that are used during the treatment, make the oral hygiene difficult and the accumulation in dental plaque much easier. Increasing the risk of dental caries during orthodontic treatment.

**The aim:** of this study was to determine the concentration of Ca and Mg in artificial saliva after preventive treatment.

**Methods:** The laboratory examination (in vitro) was performed in 90 healthy teeth extracted for orthodontic reasons. Three groups of thirty teeth were formed. The teeth from each group were separated in half in bucco-lingual direction. Thus, the control and test specimens were obtained from the same teeth. The brackets were bonded with GC Fuji OrthoTM LC (GC America Chicago, III), a resin-modified glass ionomer cement and the teeth then were stored in artificial saliva. Once per day, the teeth were coated with topical gel - CG Tooth Mousse in duration of 5 minutes and then returned to artificial saliva. Preventive treatment was at certain intervals of 1, 3 and 6 months. After each study period, the artificial saliva where the samples were stored was used for evaluation of Ca and Mg concentration by flame atomic absorption spectrophotometry.

**Results:** The results of this study (in vitro) showed that the concentrations of Ca and Mg in artificial saliva were significantly increased even after the first month of application of this means which released Ca and other minerals (including fluoride free cement used to bond the brackets) with maximum value after a three-month application. These values were significantly lower after six months, probably as a result of its absorption into the enamel. The results for Mg are the same as for Ca, and the value is increased as well as its stability in saliva after the first month.

**Conclusions:** The results of this in vitro study clearly indicate that preventive materials used in fixed orthodontic treatment inhibit the demineralization of enamel around brackets and orthodontic rings. Evidently, their use as additional preventive methods/tools, besides primary oral hygiene, is recommended.

**Key words:** dental caries, preventive treatment, Ca and Mg concentration

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## Uvod

Veliki broj pacijenata u okviru mlade populacije rešava postojeće funkcionalne i estetske probleme ortodontskim tretmanom. Kontrola demineralizacije u gleđi oko ortodontskih bravica i traka tokom fiksne ortodontske terapije je značajan klinički problem. Bravice i različiti ortodontski elementi (elastični, plastični, naglavci, opruge), koji se koriste tokom tretmana, otežavaju oralnu higijenu i znatno olakšavaju akumulaciju zubnog plaka.

Povećanje broja kariogenih bakterija takođe povećava rizik od karijesa tokom ortodontskog tretmana. Jedno od mogućih rešenja u sprečavanju dekalifikacije gleđi oko bravica tokom fiksno-ortodontskog tretmana je kontinuirana upotreba 10% Kazein fosfopeptid amorfnog kalcijum fosfata - gel za zube GC Tooth Mousse (Dental Cream GC Tooth Mousse). Reynolds je izvestio da je CPP-ACP, koji je dobijen iz mlečnog kazeina, apsorbovan kroz površinu gleđi, uticao na procese demineralizacije-remineralizacije<sup>1</sup>. Nedavno istraživanje pokazalo je da se ovakav efekat delimično može postići proteinom kazeina koji se naziva CPP, koji prenosi kalcijumove i fosfatne jone "zarobljene" u njemu, u obliku APP<sup>2</sup>. Ovaj kompleks CPP-ACP otpušta bioraspoložive jone kalcijuma i fosfata. Postoji mogućnost da antikariogena aktivnost CPP-ACP, ojačana inkorporacijom kompleksa u zubni plak i na površinu zuba, služi kao rezervoar kalcijuma i fosfata. CPP-ACP se vezuje za bakterijski zid i površinu zuba<sup>3</sup>. U slučaju intraoralnog povećanja kiselosti, oslobađaju se kalcijumovi i fosfatni joni, postižući prezasićenost jona u pljuvački i zatim taloženjem kalcijum-fosfatnog jedinjenja na eksponiranoj površini zuba<sup>4</sup>. Pored toga, razgradnja CPP može pomoći da se poveća pH (puffer) proizvodnjom amonijaka; pored toga, može sprečiti adheziju bakterija na površinu zuba i odložiti formiranje biofilma<sup>5</sup>. Ne postoji pregledni rad na Cochran bazi o ulozi CPP-ACP u demineralizaciji i remineralizaciji površine zuba. Ipak, nekoliko *in vitro* studija je pokazalo da proizvodi koji sadrže CPP-ACP smanjuju demineralizaciju i pojačavaju remineralizaciju<sup>6-8</sup>. CPP-ACP se može dodati u žvakaće gume, paste i kreme<sup>9</sup>. U početnoj karijesnoj leziji, sa eliminacijom etiološkog faktora (zubnog plaka), sa jedne strane, i sprovođenjem maksimalnih preventivnih mera sa druge, (oralna higijena i preventivni tretman sa GC

## Background

A large number of patients within the young population solve their existing functional and aesthetic problems with orthodontic treatment. The control of demineralization in enamel around orthodontic brackets and bands during fixed orthodontic treatment is a significant clinical problem. Brackets and various orthodontic elements (elastic, plastic, sleeves, springs) that are used during the treatment, make the oral hygiene difficult and the accumulation in dental plaque much easier.

Increasing the number of cariogenic bacteria also increases the risk of dental caries during orthodontic treatment. One of the possible solutions to prevent the decalcification of enamel around the brackets during the fixed-orthodontic treatment is the continuous use of 10% Casein Phosphopeptide Amorphous Calcium Phosphate (Dental Cream GC Tooth Mousse). Reynolds reported that CPP-ACP, which is derived from milk casein, was absorbed through the enamel surface and affected the demineralization-remineralization processes<sup>1</sup>. Recent research has shown that this is accomplished by a part of the casein protein referred to as CPP, which carries calcium and phosphate ions "stuck" to it, in the form of APP<sup>2</sup>. This complex of CPP-ACP delivers the bioavailable calcium and phosphate ions. It has been suggested that the anticariogenic activity of CPP-ACP relies on the incorporation of nanocomplexes into the dental plaque and on the tooth surface, thereby serving as a calcium and phosphate reservoir. CPP-ACP binds to the bacterial wall and tooth surfaces<sup>3</sup>. In case of an intraoral acid attack, the calcium and phosphate ions are released, reaching a supersaturated state of ions in the saliva and then precipitating a calcium-phosphate compound on the exposed tooth surface<sup>4</sup>. In addition, the breakdown of the CPP can help increase the pH (buffer) by producing ammonia; in addition, it might prevent bacterial adhesion to tooth surfaces and delay formation of biofilms<sup>5</sup>. There is no Cochrane review available on the role of CPP-ACP in demineralization and remineralization. Nonetheless, several *in vitro* studies have shown that CPP-ACP-containing products decrease demineralization and support remineralization<sup>6-8</sup>.

Tooth Mousse), stvaraju se uslovi za dominaciju procesa remineralizacije nad demineralizacijom, čime se zamenjuje deficit minerala<sup>10</sup>. U kiselom okruženju, CPP-ACP jedinjenje oslobađa jone kalcijuma i fosfata, što pomaže supersaturaciji usne duplje. To je potvrđeno rezultatima dobijenim u *in vitro* studiji od strane Zabokova-Bilbilova E.<sup>11</sup>. Naime, dobijene veće vrednosti masenog udela Ca u gleđima, posle jednomesečne primene preventivne dentalne kreme GC Tooth Mousse, potvrdile su ovu tvrdnju. Nakon tri meseca od primene preventivnog stomatološkog gela, gleđ pokazuje značajno veće vrednosti ne samo kalcijuma, već i drugih ispitivanih elemenata (Na, K i Mg).

Cilj ove studije bio je da se nakon preventivnog tretmana sa CCP ACP odredi koncentracija Ca i Mg u veštačkoj pljuvački.

### Metode

Laboratorijsko ispitivanje (*in vitro* uslovima) je sprovedeno na uzorku od 90 zdravih zuba, izvađenih iz ortodontskih razloga. Formirane su tri grupe od po 30 zuba. Zubi iz svake grupe su presečeni na pola u bukolingvalnom pravcu. Prema tome, kontrolni i studijski uzorci su dobijeni od istih zuba. Bravice su za uzorke vezane upotrebom smolom modifikovanog glas jonomer cementa, GC Fuji Ortho<sup>TM</sup> LC (GC America Chicago, III), a uzorci zuba su zatim na sobnoj temperaturi čuvani u veštačkoj pljuvački<sup>12</sup> sastavljenoj od 20 mmol/l NaHCO<sub>3</sub>, 3 mmol/l NaH<sub>2</sub>PO<sub>4</sub>, and 1 mmol/l CaCl<sub>2</sub> neutralne pH vrednosti. Jednom dnevno, zubi su premazivani topikalnim GC Tooth Mousse gelom u trajanju od 5 minuta, a zatim vraćani u veštačku pljuvačku. Preventivni tretman je sproveden u određenim intervalima od 1, 3 i 6 meseci. Nakon svakog perioda ispitivanja, veštačka pljuvačka u kojoj su uzorci čuvani korišćena je za određivanje koncentracije Ca i Mg, primenom plamene atomske apsorpcione spektrofotometrije<sup>13,14</sup>, na sledeći način: kreirali smo kalibracioni diagram, utvrđivali nivo Ca i Mg, izračunali koncentraciju prema formuli.

### Konstrukcija kalibracionog dijagrama

Kalibracioni dijagram je konstruisan korišćenjem metode standardnih rastvora za analizirani element.

CPP-ACP might be incorporated into chewing gums, lozenges, or creams<sup>9</sup>. In the initial carious lesion, with the elimination of the etiological factor (dental plaque), on one hand, and taking of maximum preventive measures, on the other (oral hygiene and preventive treatment with GC Tooth Mousse), conditions are created for the pre-emption of remineralization processes over demineralization, by which the mineral deficit is replaced<sup>10</sup>. In an acidic environment, the CPP-ACP compound releases ions of calcium and phosphate, which helps supersaturation of the oral cavity. This has been confirmed with the results obtained in the *in vitro* study of Zabokova-Bilbilova E.<sup>11</sup>. Namely, the obtained higher values of the mass fraction of Ca in the enamel after one-month application of the preventive dental cream GC Tooth Mousse have confirmed this statement. The enamel after three months of application of the preventive dental cream shows significantly higher values not only of the calcium, but also of the other examined elements (Na, K and Mg).

The objective in this study was to determine the concentration of Ca and Mg in artificial saliva after preventive treatment.

### Methods

The laboratory examination (*in vitro*) was performed in 90 healthy teeth extracted for orthodontic reasons. Three groups of thirty teeth were formed. The teeth from each group were separated in half in buccolingual direction. Thus, the control and test specimens were obtained from the same teeth. The brackets were bonded with GC Fuji Ortho<sup>TM</sup> LC (GC America Chicago, III), a resin-modified glass ionomer cement and the teeth then were stored in artificial saliva<sup>12</sup> consisting of 20 mmol/l NaHCO<sub>3</sub>, 3 mmol/l NaH<sub>2</sub>PO<sub>4</sub>, and 1 mmol/l CaCl<sub>2</sub> at room temperature, neutral pH. Once per day the teeth were coated with topical gel - GC Tooth Mousse in duration of 5 minutes and then returned to artificial saliva. Preventive treatment was at certain intervals of 1, 3 and 6 months. After each study period, the artificial saliva where the samples were stored was used for determination of Ca and Mg concentration by flame atomic absorption spectrophotometry<sup>13,14</sup>, as follows: we created a calibration diagram, determined the level of Ca and Mg and calculated the concentration by the formula.

Regresiona analiza je pokazala funkcionalnu vezu između koncentracije i absorbance Ca i Mg. Za konstrukciju kalibracionog dijagrama korišćen je standardni rastvor Ca i Mg koncentracije 1mg/L. Srednja apsorbansa za svaki standardni rastvor kalcijuma data je u Tabeli 1 i Figuri 1.

Analitička zavisnost od apsorpcije koncentracije Ca data je sledećom jednačinom:  $A = 0,009 \cdot \gamma(\text{Ca}) / \mu\text{g/mL}$   
Koeficijent korelacije je bio: 0,9963.

Analitička zavisnost od apsorpcije koncentracije Mg data je sledećom jednačinom:  $A = 0,1598 \cdot \gamma(\text{Mg}) / \mu\text{g/mL} + 0,0105$

Koeficijent korelacije je bio: 0,994. Kalibracioni dijagram za određivanje koncentracije Mg u pljuvački sa FAAP prikazan je na Figuri 2.

**Tabela 1.** Apsorpcija za odgovarajuće koncentracije Ca u veštačkoj pljuvački

**Table 1.** Absorbance for appropriate concentrations of Ca in artificial saliva

$\gamma(\text{Ca})/\mu\text{g/ml}$	A
0	0
1	0.010
3	0.031
5	0.045
10	0.089

**Tabela 2.** Vrednosti koncentracije Ca u veštačkoj pljuvački ispitivane grupe (mmol/L)

**Table 2.** Values of the concentration of Ca in the artificial saliva in the examined group (mmol/L)

time	$\bar{X}$	SD	N
1 month	3.19	0.86	30
3 months	3.86	1.30	30
6 months	1.89	1.21	30

### Statistička analiza

Za statističku procenu korišćena je analiza varijanse (ANOVA) kako bi se utvrdilo da li postoji statistički značajna razlika između ispitivanih grupa.

### Construction of calibration diagram

Calibration diagram was constructed by using a method of standard solutions for the analyzed element. Regression analysis showed functional relationship between concentration and absorbance of Ca and Mg.

For construction of calibration diagram, standard solutions of Ca and Mg with concentration 1 mg/L were used. Means of absorbance for each calcium standard solution are given in Table 1. and Figure 1.

Analytical dependence on absorbance of Ca concentration was given by the following equation:

$$A = 0.009 \gamma(\text{Ca}) / \mu\text{g/mL}$$

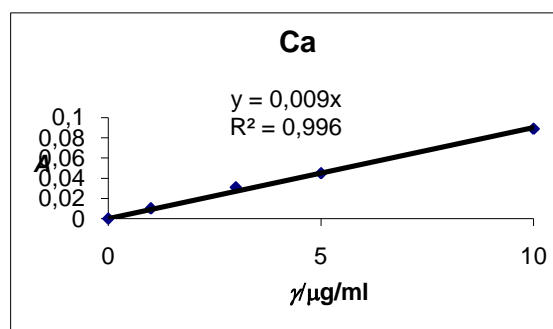
Correlation coefficient was 0.9963.

Analytical dependence on absorbance of Mg concentration was given by the following equation:

$$A = 0.1598 \gamma(\text{Mg}) / \mu\text{g/mL} + 0.0105$$

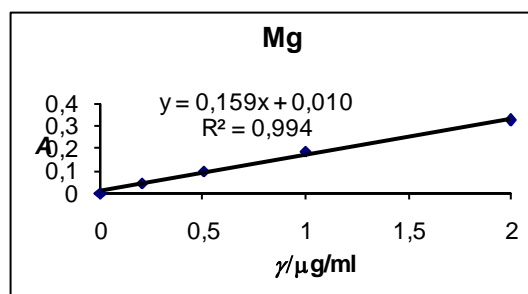
Correlation coefficient was 0.994.

Calibration diagram for determination of Mg in saliva with FAAP is given in Figure 2.



**Figura 1.** Kalibracioni dijagram za određivanje Ca u pljuvački sa FAAP

**Figure 1.** Calibration diagram for determination of Ca in saliva with FAAP



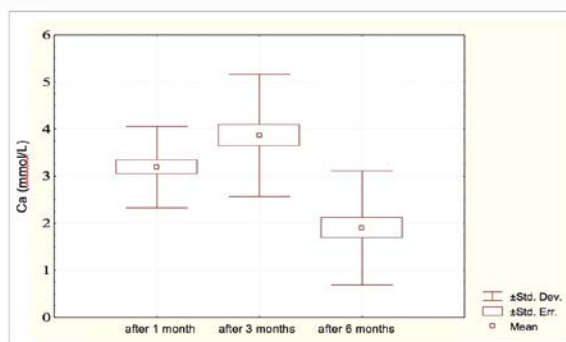
**Figura 2.** Kalibracioni dijagram za određivanje Mg u pljuvački sa FAAP

**Figure 2.** Calibration diagram for determination of Mg in saliva with FAAP

## Rezultati

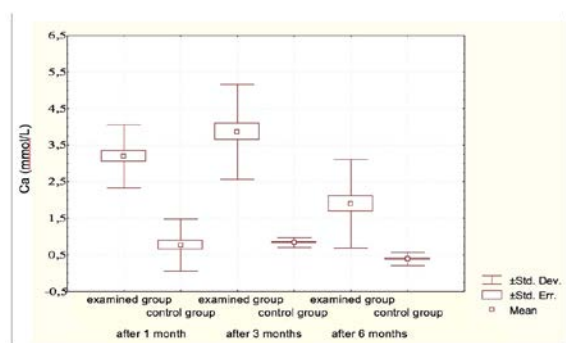
U studijskoj grupi utvrđena je statistički značajna razlika u pogledu srednje vrednosti koncentracije Ca u veštačkoj pljuvački nakon tretmana od 1,3 i 6 meseci (Analiza varijanse:  $F=23,013$ ;  $p=0,00001$ ); (Tabela 2., Figura 3.). Tukey (HSD) test značajnosti je pokazao da nema statistički značajne razlike između tretmana od 1 i 3 meseca, dok je između tretmana od 1 i 6, i tretmana od 3 i 6 meseci, utvrđena statistički značajna razlika (Tabela 3.).

Nakon tretmana od 1,3 i 6 meseci, utvrđena je statistički značajna razlika u pogledu srednje vrednosti koncentracije Ca u veštačkoj pljuvački između ispitivane i studijske grupe (Tabela 4, Figura 4.).



**Figura 3.** Vrednosti koncentracije Ca u veštačkoj pljuvački ispitivane grupe

**Figure 3.** Values of the concentration of Ca in the artificial saliva in the examined group



**Figura 4.** Komparativni prikaz vrednosti koncentracija Ca u veštačkoj pljuvački ispitivane i kontrolne grupe (mmol/L)

**Figure 4.** Comparative overview of the values of the concentration of Ca in the artificial saliva in the examined and control group (mmol/L)

## Statistical analysis

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

## Results

In the study group, there were statistically significant differences in relation to the mean values of Ca concentration in the artificial saliva after treatment of 1, 3 and 6 months (Variance analysis:  $F=23.013$ ;  $p=0.00001$ ) (Table 2., Figure 3.). The Tukey honest significant difference (HSD) test showed that the differences were not significant for treatment of 1 and 3 months, and for treatment of 1 and 6 months, and treatment of 3 and 6 months they were statistically significant (Table 3.).

After treatment of 1, 3 and 6 months, there were statistically significant differences in relation to the mean values of Ca concentration in the artificial saliva between the examined and the control group (Table 4. and Figure 4.).

**Tabela 3.** Razlike između vrednosti koncentracije Ca u veštačkoj pljuvački u ispitivanoj grupi

**Table 3.** Differences between the values of Ca concentration in the artificial saliva in the examined group

time	P
1 - 3 months	0.0634
1 - 6 months	0.00019*
3 - 6 months	0.00010*

\*Tukey (HSD) test

**Tabela 4.** Komparativni prikaz vrednosti koncentracija Ca u veštačkoj pljuvački ispitivane i kontrolne grupe (mmol/L)

**Table 4.** Comparative overview of the values of the concentration of Ca in the artificial saliva in the examined and control group (mmol/L)

time	examined group		control group		t	P
	$\bar{X}$	SD	$\bar{X}$	SD		
1 month	3.19	0.86	0.76	0.71	11.874	0.00001*
3 months	3.86	1.30	0.83	0.13	12.696	0.00001*
6 months	1.89	1.21	0.38	0.17	6.764	0.0001*

\*statistically significant differences

U studijskoj grupi nije utvrđena statistički značajna razlika u pogledu srednje vrednosti koncentracije Mg u veštačkoj pljuvački nakon tretmana od 1,3 i 6 meseci (analiza varijanse:  $F=1,779$ ;  $p=0,1747$ ) (Tabela 5, Figura 5.). Tukey (HSD) test je pokazao razliku između srednje vrednosti koncentracije Mg u veštačkoj pljuvački tokom tretmana za 1,3 i 6 meseci ponaosob (Tabela 6).

Nakon tretmana od 1,3 i 6 meseci, utvrđena je statistički značajna razlika u pogledu srednje vrednosti koncentracije Mg u veštačkoj pljuvački između studijske i kontrolne grupe (Tabela 7. i Figura 6.).

**Tabela 5.** Vrednosti koncentracije Mg u arteficialnoj pljuvački ispitivane grupe

**Table 5.** Values of the concentration of Mg in the artificial saliva in the examined group (mmol/L)

time	$\bar{X}$	SD	N
1 month	0.438	0.164	30
3 months	0.427	0.162	30
6 months	0.368	0.129	30

In the study group, there were no statistically significant differences in relation to the mean values of Mg concentration in the artificial saliva after treatment of 1,3 and 6 months (variance analysis:  $F=1.779$ ;  $p=0.1747$ ) (Table 5., Figure 5.). Tukey honest significant difference (HSD) test showed differences between mean values of Mg concentration in the artificial saliva during treatment for 1, 3 and 6 months alone (Table 6.).

After treatment of 1, 3 and 6 months, there were statistically significant differences in relation to the mean values of Mg concentration in the artificial saliva between the examined and control group (Table 7. and Figure 6.).

**Tabela 6.** Razlike u vrednostima koncentracije Mg u veštačkoj pljuvački u ispitivanoj grupi

**Table 6.** Differences between the values of the concentration of Mg in the artificial saliva in the examined group

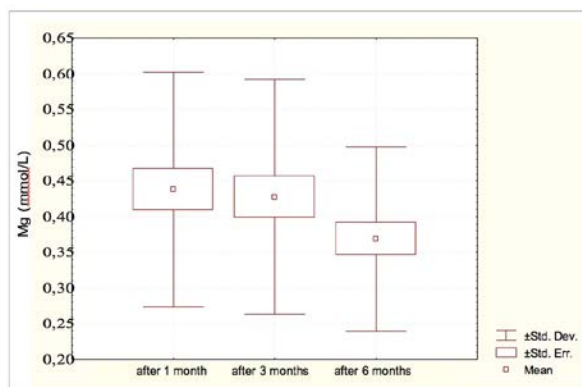
time	p
1 - 3 months	0.9633
1 - 6 months	0.1937
3 - 6 months	0.3018

\*Tukey (HSD) test

**Tabela 7.** Komparativni prikaz vrednosti koncentracija Mg u veštačkoj pljuvački ispitivane i kontrolne grupe (mmol/L)

**Table 7.** Comparative overview of the values of the concentration of Mg in the artificial saliva in the examined and control group (mmol/L)

time	examined group		control group		t	p
	$\bar{X}$	SD	$\bar{X}$	SD		
1 month	0.438	0.164	0.351	0.154	2.112	0.03894*
3 months	0.427	0.162	0.282	0.096	4.174	0.00010*
6 months	0.368	0.129	0.185	0.092	6.316	0.00001*



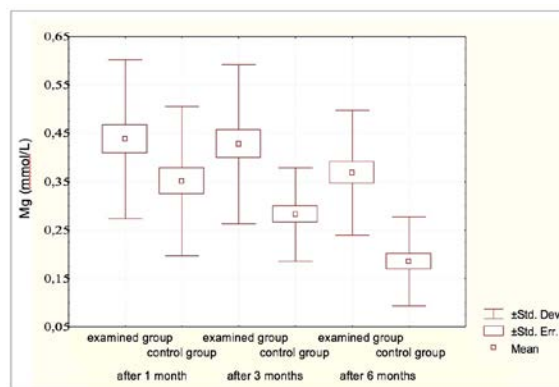
**Figura 5.** Vrednosti koncentracije Mg u arteficialnoj pljuvački ispitivane grupe (mmol/L)

**Figure 5.** Values of the concentration of Mg in the artificial saliva in the examined group (mmol/L)

### Diskusija

Dental krema GC Tooth Mousse ostvaruje svoj antikariogeni efekat uz pomoć CPP-ACP. Mehanizam delovanja CPP-ACP uključuje sjedinjavanje nanokompleksa u zubnom plaku i na površini gingive, delujući kao rezervoar kalcijuma i fosfata. Rejnoldsove studije<sup>1</sup> ukazuju da CPP-ACP inkorporiran u zubni plak može značajno povećati nivo kalcijuma i fosfatnih jona u plaku. Razlog je dejstvo preventivnog preparata GC Tooth Mousse, koji pomaže u sprečavanju demineralizacije. Ivce gleđi remineralizovane zbog izloženosti CPP-ACP otporne su na lateralnu indukciju kiseline u poređenju sa normalnom remineralizovanom gleđi, jer CPP-ACP ima sposobnost da olakša remineralizaciju lezija makule albe na gleđi zbog prisustva hidroksilapatita. Relativno niska nukleotidna regija lezija tretiranih sa CPP-ACP jedinjenjima, takođe može pokazati bolju kristalizaciju i nižu mikrostrukturu od intaktne gleđne mase. To je efekat koji se očekuje u ortodontskom tretmanu fiksnim aparatom koji je potvrđen istraživanjima više autora.

*In vitro* podaci pokazuju da redovna upotreba preventivne stomatološke kreme GC Tooth Mousse smanjuje mogućnost demineralizacije gleđi oko bravica i različitih ortodontskih elemenata koji se koriste tokom ortodontskog tretmana. U isto vreme, ona stimuliše proces remineralizacije, što podrazumeva vraćanje minerala izgubljenih iz hidroksilapatita gleđi.



**Figura 6.** Komparativni prikaz vrednosti koncentracija Mg u veštačkoj pljuvački ispitivane i kontrolne grupe (mmol/L)

**Figure 6.** Comparative overview of the values of the concentration of Mg in the artificial saliva in the examined and control group (mmol/L)

### Discussion

Dental Cream GC Tooth Mousse accomplishes its effect by the use of CPP-ACP. The mechanism of action of CPP-ACP involves the unification of the nanocomplex in the dental plaque and the surface of the gum, acting as a calcium and phosphate reservoir. Studies of Reynolds<sup>1</sup> suggest that CPP-ACP incorporated into the dental plaque can significantly increase the levels of calcium and phosphate ions in the plaque. This is due to the action of preventive dental cream GC Tooth Mousse, which helps in prevention of demineralization of the enamel. The enamel blades remineralized by the surface exposure of CPP-ACP are resistant to lateral acid induction compared to normal remineralized enamel, since CPP-ACP has the ability to help remineralization of white spot lesions on the enamel with hydroxylapatite. The relatively low nucleotide region of the lesions treated with CPP-ACP compounds can also show a better crystallization and lower microstructure than an intact enamel. This is the effect expected in the orthodontic treatment with a fixed apparatus which has been confirmed by the research of several authors.

*In vitro* data show that the regular use of preventive dental cream GC Tooth Mousse reduces the possibility of demineralization of the enamel around the brackets and the various orthodontic elements used during orthodontic treatment. At the same time, it stimulates the process of remineralization, which involves the reverting of minerals lost from the hydroxylapatite of the enamel.

Mnoge laboratorijske studije<sup>15-17</sup>, uključujući i ovu, ukazuju na efekat oslobađanja Ca iz veštačke pljuvačke. Rezultati koncentracije kalcijuma u veštačkoj pljuvački pokazuju više vrednosti posle jednomesečne aplikacije dentalne kreme Tooth mousse GC (3,19 mmol/l) u poređenju sa kontrolnom grupom gde je vrednost bila (0,86 mmol/l). Procena rezultata dobijenih posle tromesečne upotrebe GC Tooth Mousse pokazuje statističku značajnost u poređenju sa kontrolnom grupom, pri čemu su vrednosti Ca u veštačkoj pljuvački bili 3,86 mmol/L, dok je koncentracija Ca u kontrolnoj grupi bila 1,30mmol/L. Posle šestomesečnog perioda vrednost kalcijuma u veštačkoj pljuvački u kojoj su čuvani zubi bila je (1,89 mmol/L). Rezultati izričito ukazuju na smanjenje vrednosti kalcijuma u veštačkoj pljuvački u ovom šestomesečnom periodu, što je rezultat veće gleđne apsorpcije.

Posle preventivne preparacije, sa protokom vremena, uz upotrebu paste GC Tooth Mouss, najveće povećanje je zabelezeno prvom tromesčju, za sve ispitivane mikroelemente (Ca i Mg). Veštačka pljuvačka u maloj meri sadrži Ca i Mg, važne mikroelemente za maturaciju, što je takođe slučaj i u prirodnoj sredini i ima pozitivan efekat na maturaciju gleđi.

Poređenje vrednosti dobijenih za ispitivane elemente (Ca i Mg), u veštačkoj pljuvački u različitim vremenskim intervalima (posle 1,3 i 6 meseci), sa nalazima drugih autora nije urađeno, zbog oskudnosti takvih podataka u raspoloživim naučnim studijama, što govori u prilog originalnim nalazima predstavljenim u ovoj studiji. U fazi inicijalne karijesne lezije sa eliminacijom etiološkog faktora, (zubni plak) na jednoj strain, i uz primenu maksimalnih preventivnih mera (oralna higijena i preventivni tretman sa GC Tooth Mousse) s druge strane, stvaraju se uslovi za remineralizaciju posle procesa demineralizacije, koji kompenzuju deficit minerala i mogu dostići biološku repa raciju<sup>18,19</sup>. U kiseloj sredini, CPP-ACP jedinjenje oslobađa jone kalcijuma i fosfata, koji pomažu supersaturaciji usne duplje.

Many laboratory studies<sup>15-17</sup>, including this study, show the effect of the release of Ca in the artificial saliva. The results of the concentration of Ca in the artificial saliva showed higher Ca values after one-month application of dental cream Tooth Mousse GC (3.19 mmol/L), and they were higher than the concentration in Ca control group (0.86 mmol/L). Evaluation of the data obtained after a three-month application of GC Tooth Mousse showed a statistically significant difference in relation to the control group; the concentration of Ca in the artificial saliva at this time interval was 3.86 mmol/L, compared to the concentration of Ca in the control group (1.30 mmol/L). After a six-month application of the dental cream there was a significantly lower level of calcium in the artificial saliva where the tooth (1.89 mmol/L) was kept. The results pointed out to a significant decrease in Ca in the artificial saliva in this period (six months), as a result of its greater absorption in the enamel.

With the increase in the time interval since the application of the preventative preparation, in this case the dental cream GC Tooth Mousse, the highest increase in three months was observed for all examined microelements (Ca and Mg). The fading of the contents of the artificial saliva with Ca and Mg, important elements for the maturation, suggests that this also occurs in vivo (in the oral environment), with a positive reflection on the maturation of the enamel.

Comparison of the values obtained for the examined elements (Ca and Mg) in the this domain. This, however, speaks in favor of the original findings presented in this study. In the phase of initial carious lesion with the elimination of the etiological factor (dental plaque) on one side, and taking the maximum preventive measures (oral hygiene and preventive treatment with GC Tooth Mousse), on the other hand, conditions are created for the remineralization processes over demineralization, which compensates for the mineral deficit and can reach biological reparations<sup>18,19</sup>. In an acidic environment, the CPP-ACP compound releases ions of calcium and phosphate, which helps supersaturation of the oral emptiness.



## **Zaključak**

Neophodno je očuvati zdravlje zuba i integritet gleđi u periodu fiksne ortodontske terapije primenom odgovarajućih preventivnih mera. Očekivani efekat ovih mera je inhibicija procesa demineralizacije gleđi. Neophodno je da pacijenti obavljaju oralnu higijenu, ali treba naglasiti da je kod ovih pacijenata povremeno potrebno profesionalno uklanjanje plaka.

Rezultati ove studije (*in vitro*) pokazali su da su koncentracije Ca i Mg u veštačkoj pljuvački značajno povećane i nakon prvog meseca primene ovog sredstva, koje oslobađa Ca i druge minerale (uključujući cement bez fluora, koji se koristi za lepljenje bravica), sa maksimalnom vrednošću posle tromesečne aplikacije. Ove vrednosti su bile značajno niže posle šest meseci, verovatno kao rezultat njegove apsorpcije u gleđi. Rezultati za Mg su isti kao i za Ca, pri čemu se vrednost povećava, kao i njena stabilnost u pljuvački nakon prvog meseca upotreba.

Rezultati ove *in vitro* studije jasno pokazuju da preventivni materijali koji se koriste u fiksnom ortodontskom tretmanu inhibiraju demineralizaciju gleđi oko bravica i ortodontskih žica. Pored primarne oralne higijene, preporučuje se njihova upotreba kao dodatne preventivne metode/sredstva.

## **Conclusion**

It is an imperative to preserve the dental health and the integrity of enamel in the period during fixed orthodontic treatment by application of appropriate preventive measures. The expected effect of these measures is the inhibition of the demineralization processes in the enamel. It is essential to practice oral hygiene by the patients, but it has to be emphasized that occasional professional removal of the plaque in these patients is required.

The results of this study (*in vitro*) showed that the concentrations of Ca and Mg in artificial saliva were significantly increased even after the first month of application of this means, which releases Ca and other minerals (including fluoride free cement used to bond the brackets) with maximum value after a three-month application. These values were significantly lower after six months, probably as a result of its absorption into the enamel. The results for Mg are the same as for Ca, and the value is increased as well as its stability in saliva after the first month of use.

The results of this *in vitro* study clearly indicated that preventive materials used in fixed orthodontic treatment inhibited the demineralization of enamel around brackets and orthodontic rings. Evidently, their use as additional preventive methods/tools, besides primary oral hygiene, is recommended.

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