ACTA
STOMATOLOGICA
CROATICA
www.ascro.hr

Acta Stomatol Croat. 2011;45(3):159-165.

IZVORNI ZNANSTVENI RAD
ORIGINAL SCIENTIFIC PAPER

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Učinak fluoridirane paste za zube na remineralizaciju cakline i mikrotvrdoću nakon demineralizacije in vitro

The Effect of Fluoridated Dentifrice Formulations on Enamel Remineralisation and Microhardness after in Vitro Demineralization

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Sažetak

Svrha: Svrha rada bila je procijeniti utjecaj komercijalnih pasta za zube s različitom formulom fluorida na remineralizaciju površine cakline. **Materijali i metode:** Pripremljeni su caklinski blokovi te, uz hlađenje vodom, polirani karborundnim diskovima različite finoće (320, 600 i 1200 grita). Nakon toga su nasumce podijeljeni u sedam skupina. Uzorci su bili izvrgnuti svakodnevnom režimu koji se sastojao od dvaju jednominutnih četkanja – jednim nakon šestosatne demineralizacije i drugim nakon remineralizacije u umjetnoj slini u trajanju od 18 sati. Tijekom izmjene pH-ciklusa svaka od sedam skupina tretirana je različitim pastama za zube: fluoridnim pastama Sensodyne Rapid (1040 ppm NaF), Colgate Total (1100 ppm NaF), Parodontax fluoride (1400 ppm NaF), Sensodyne fluoride (1400 ppm NaF), Pronamel Sensodyne (1450 ppm NaF), Elmex-GABA International (1250 ppm F Amine fluoride) i pastom bez fluorida Detartrine (Septodont). Mikrotvrdoća površine (SMH) uzoraka izmjerena je Shimadzovim mikrodurimetrom HMV–2000 (50 g, 490,3 mN. 10 s) na početku istraživanja i nakon dvanaestodnevne primjene pH-režima. **Rezultati:** U svim skupinama uzoraka tretiranima fluoridnim pastama ustanovljen je porast SMH u odnosu prema kontrolnoj skupini. Pronamel, Sensodyne F, Sensodyne Rapid i Colgate Total statistički su nakon dvanaestodnevnog pH-režima bili superiorniji od ostalih fluoridnih pasta i onih bez fluorida kojima su se koristili sudionici u kontrolnoj skupini. Vrijednosti mikrotvrdoće cakline nakon 12 dana tretmana Parodontaxom u odnosu na početak pokazale su povećanje SMH, ali nije bilo statističke značajnosti (p>0,05). **Zaključak:** Dobiveni rezultati pokazuju da paste s visokim sadržajem fluorida (1450 ppm) u eksperimentalnim uvjetima učinkovito sprječavaju demineralizaciju.

Zaprimljen: 2. svibnja 2011.
Prihvaćen: 7. srpnja 2011.

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Ključne riječi
zubna caklina; fluoridacija; pasta za zube; zub, demineralizacija; testovi tvrdoće

Uvod

Kad je riječ o karijesu, preventivni učinci fluorida poznati su već godinama. U prvom se redu odnose na smanjenu topljivost cakline, što je povezano s ugrađivanjem fluorida u apatitnu rešetku (1, 2). Zubni karijes je određen dinamičkim procesom na temelju demineralizacije i remineralizacije. Bakterije iz plaka proizvode velike količine kiseline, kao što je mliječna iz fermentabilnih ugljikohidrata. Kiseline su zarobljene između površine zuba i biofilma plaka, pa tada pH cakline lako može pasti ispod 5,6 te iz nje počinju nestajati minerali. Suprotno tome, fluoridi iz različitih dodataka mogu stvoriti povoljan okoliš za zaštitu zubnog tkiva od kiselina (3).

U mnogobrojnim istraživanjima istaknuto je da dentobakterijski plak i slina sadržavaju fluorid u rasponu od 0,02 do 0,04 ppm (4–6). Poznato je da imaju važnu zadaću u pre-

Introduction

The caries-preventive effects of fluoride have been well known for years. They are primarily attributed to the reduction of enamel solubility which is due to fluoride incorporation into the apatite lattice (1, 2). Dental caries is determined by the dynamic process based on the demineralization and remineralisation. Plaque bacteria produce a large quantity of acids such as lactic acid from fermentable carbohydrates. Acids are entrapped between the tooth surface and plaque biofilm and then the pH of the enamel easily falls below 5.6, and loss of mineral (decalcification) from enamel is induced. Contrary to these effects, fluoride from different supplements can create favourable environment to protect tooth substances from acidogenic attacks (3).

Numerous studies have shown that dentobacterial plaque and saliva usually contain fluoride in the range 0.02-0.04

venciji demineralizacije tijekom ortodontskog tretmana (7). No, četkanje fluoridnim pastama za zube nekoliko sati znatno povećava količinu fluorida u slini. Tako njegova razina odmah nakon četkanja može dosegnuti 5 ppm (8, 9). Fluoridi iz zubnih pasta mogu pružiti aktivnu zaštitu ako se otpuštaju u topljivom bioraspoloživom obliku. Topikalno primijenjeni fluoridni ioni mogu prodrijeti u pore cakline i ući u demineralizirana područja zuba (10–12).

Paste za zube na tržištu sadržavaju nekoliko fluoridnih spojeva, uključujući natrijev fluorid, natrijev monofluorofosfat (MFP), kositarov fluorid (SnF_2) i organske fluoride poput aminfluorida. Njihovo relativno protukarijesno djelovanje i dalje se smatra vrlo sličnim. U literaturi postoje mnoge proturječnosti u vezi s učinkovitošću različitih fluoridnih formula. Unatoč tomu neki autori ističu da su paste s natrijevim fluoridom djelotvornije od onih s MFP-om (13). To se uglavnom pripisuje činjenici da je ione monofluorfosfata potrebno hidrolizirati u ustima kako bi otpustili slobodne fluoridne ione (1, 12, 14).

Kritični pH za početnu demineralizaciju trebao bi iznositi oko 5,5. U mnogobrojnim studijama in vitro dokazano je svojstvo fluorida u zaštiti cakline i dentina od napada kiseline (10, 15). Očekuje se da ju paste s fluorom mogu aktivno zaštititi i od karijesa i od kiselina (10, 16).

Svrha ovog rada bila je procijeniti učinak in vitro komercijalno dostupnih pasta za zube s različitom koncentracijom fluorida na površinsku mikrotvrdoću ranih caklinskih lezija u modelu pH-ciklusa.

ppm (4-6). It is well known that fluorides play an important role in the prevention of demineralization during orthodontic treatment (7). However, brushing with a fluoride toothpaste results in considerably increased fluoride levels in saliva during several hours. Fluoride levels immediately after brushing can reach 5 ppm (8, 9). Delivered from a dentifrice, fluoride can provide active protection if delivered in a soluble bioavailable form. Topically applied fluoride ions can penetrate the pores of enamel and enter demineralised areas of the tooth (10-12).

Several fluoride compounds are currently available in marketed dentifrices, including sodium fluoride, sodium monofluorophosphate (MFP), stannous fluoride (SnF_2), and organic fluorides, such as amine fluoride. Their relative anti-caries activity is still considered to be very similar. In the literature, there are many controversies regarding the efficacy of different fluoride formulas. In spite of that, some studies suggest that sodium fluoride dentifrices are slightly more effective than those employing MFP (13). This is mainly attributed to the fact that monofluorophosphate ions need to hydrolyze in the mouth to release free-fluoride ions for efficacy (1, 12, 14).

Critical pH for the initial demineralisation should be approximately 5.5. There are numerous in vitro studies demonstrating the ability of fluoride to protect enamel and dentine against acid attacks (10, 15). It is expected that dentifrices with fluoride can offer superior anti-caries and acid protection (10, 16).

The aim of this study is to assess the *in vitro* effect of commercially available toothpastes containing different concentration of fluoride on surface microhardness of early enamel lesions in a pH-cycling model.

Materijali i metode

Opis istraživanja

Izvađeni zdravi humani zubi bili su uronjeni u 2-postotnu otopinu formaldehida (pH 7,0) na sobnoj temperaturi. Ukupno je upotrijebljeno 28 caklinskih pločica. Primijenjene su sljedeće zubne paste: placebo pasta (Detartrine Paste–Septodont) i šest fluoridnih pasta za zube – Sensodyne Rapid (1040 ppm NaF), Colgate Total (1100 ppm NaF), Parodontax fluoride (1400 ppm NaF), Sensodyne fluoride (1400 ppm NaF), Pronamel Sensodyne (1450 ppm NaF) i Elmex-GABA International (1250 ppm Amine fluoride) (tablica 1). Njima su se tretirali zdravi izbrusci humane cakline koji su bili izloženi dvanaestodnevnim ciklusima demineralizacije i remineralizacije.

De/remineralizacijske otopine

Sastav korištene demineralizacijske otopine bio je: natrijev acetat (0,1mM CH_3COONa), kalijev klorid (150 mM KCl_2) i kalijev dihidrogenfosfat (0,9 mM KH_2PO_4). Uz pomoć solne kiseline pH je prilagođen na 4,5 (0,1mol/L). Kiselost demineralizacijske otopine kontrolirala se svaki dan, a blagi otkloni tijekom demineralizacijskog razdoblja korigirani su kloridnom kiselinom 0,1mol/L kako bi pH-vrijednost stalno bila između 4,35 i 4,65.

Materials and Methods

Study design

Extracted sound human teeth were stored in 2% formaldehyde solution (pH 7.0) at room temperature. A total of 28 enamel slabs were used. The following toothpaste were applied: Placebo paste (Detartrine Paste-Septodont) and six fluoride tooth pastes - Sensodyne Rapid (1040 ppm NaF), Colgate Total (1100 ppm NaF), Parodontax fluoride (1400 ppm NaF), Sensodyne fluoride (1400 ppm NaF), Pronamel Sensodyne (1450 ppm NaF) and Elmex-GABA International (1250 ppm Amine fluoride) (Table 1). The toothpastes were applied on sound human enamel slabs which were exposed to twelve daily demineralization/remineralisation cycles.

De/remineralising solutions

The composition of demineralising solution was sodium acetate (0.1 mM CH_3COONa), potassium chloride (150 mM KCl_2), calcium chloride (1.5 mM $CaCl_2$) and potassium dihydrogen phosphate (0.9 mM KH_2PO_4). The pH was adjusted to 4.5 using hydrochloric acid (0.1mol/l). Slight elevations were corrected with hydrochloric acid 0.1mol/l to maintain a constant pH value between 4.35 and 4.65 during the demineralization period.

Tablica 1. Detaljni sastav zubnih pasta testiranih u studiji mikrotvrdoće
Table 1 Formulation details for toothpastes tested in microhardness study

Testirani proizvod • Test Product	Broj proizvoda • Batch Numbers	Fluorid, koncentracija (ppm) • Fluoride Source, Concentration (ppm)	Abrazivni sustav • Abrasive System	Surfaktant • Surfactant	Izmjereni pH zubnih pasta • Formulation pH by our Measurements
Sensodyne Rapid	180B61	NaF, 1040	Silica	Sodium methyl cocoyltaurate	6.68
Colgate Total	(L)9240PL112E	NaF, 1100		Sodium lauryl sulphate	7.33
Elmex	054002	AmF, 1250	Silica	None	4.84
Sensodyne Fluoride	BN 049AL2	NaF, 1400	Silica	Cocamidopropyl Betaine	5.36
Parodontax Fluoride	BN339062	NaF, 1400		Cocamidopropyl Betaine	7.88
Pronamel Sensodyne	0042TKWA	NaF, 1450	Silica	Cocamidopropyl Betaine	7.14
Detartrine (control)	40855	________	Silica	NA	8.61

NA - Nije dostupan podatak • Not available

Remineralizacijska otopina (RM) (umjetna slina) sadržava: natrijev klorid (0,50g/L), natrijev bikarbonat (4,2g/L), natrijev nitrat (0,03g/L) i kalijev klorid (0,20g/L). Njezin pH bio je 8,0. Svaki dan su pH-metrom bile izmjerene pH-vrijednosti demineralizacijske i remineralizacijske otopine (HI 8014, HANNA instruments, Bioblock Scientific, Illkirch, Francuska).

Priprema caklinskih pločica

Nakon što su uklonjeni korijen i pulpa, zdrava caklina izrezana je dijamantnom pločom s obrazne i jezične strane zuba. Isječci su uloženi u akrilnu masu za ulaganje (Akril Fix Kit, Struers) i stvrdnjavali su se tijekom noći. Površina caklinskih blokova postupno je polirana uz vodeno hlađenje karborundnim diskovima (320, 600, 1200 grit s Al_2O_3 papirom; Buehler, Lake Bluff, IL, SAD). Ti se postupci obavljaju kako bi se postigla paralelna ravna površina za Vickersov test mikrotvrdoće.

Eksperimentalni postupak

Za standardizaciju blokova najprije su odabrani uzorci za početnu mikrotvrdoću (pet mjerenja u različitim područjima blokova, 50 g (490,3 mN ili 0,05 HV), 10 s, HMV–2000; Shimadzu Corporation, Tokijo, Japan). Caklinski blokovi s prosječnom mikrotvrdoćom površine između 213 i 368 VHN-a nasumce su uvršteni u sedam skupina (šest eksperimentalnih i jednu kontrolnu). Ukupno dvadeset i osam zdravih caklinskih pločica podijeljeno je u sedam skupina od po četiri pločice u svakoj. Za proučavanje ciklusa demineralizacije i remineralizacije cakline koja nastaje ispod zubnog plaka u ustima, bio je prije toga razvijen laboratorijski ciklički pH-model. Na taj se način pokušavalo oponašati proces napada kiseline (demineralizacije) i remineralizacije slinom u ustima (7). Humani caklinski uzorci bili su podvrgnuti svakodnevnom cikličkom režimu koji se sastojao od dvaju jednominutnih četkanja zubnom pastom – jedan prije i drugi nakon šestosatne demineralizacije u demineralizacijskoj otopini te 18 sati remineralizacije u umjetnoj slini. Dnevni ciklički režim pH ponavljao se 12 dana i u tom razdoblju svaka od sedam

The remineralizing (RM) solution (artificial saliva) contained: sodium chloride (0.50gr/l), sodium bicarbonate (4.2 g/l), sodium nitrate (0.03 g/l) and potassium chloride (0.20 g/l). The pH of artificial saliva was 8.0. The pH values of demineralization and remineralisation solutions were measured every day using pH meter (HI 8014, HANNA instruments, Bioblock Scientific, Illkirch, France).

Preparation of enamel slabs

After removal of the roots and pulp, sound enamel sections were cut from the buccal and lingual sides of the teeth using a diamond saw. The sections were mounted in acrylic resin (Acryl Fix Kit, Struers) and cured overnight. Enamel surfaces of the blocks were progressively polished with water-cooled carborundum discs (320, 600 and 1200 grit of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA). These procedures were conducted to form parallel planar surfaces for the Vickers microhardness tests.

Experimental procedure

For standardization of the blocks, a previous selection of specimens for the initial microhardness was made (five indentations in different regions of the blocks, 50 g (490.3 mN or 0.05 Hv), 10 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). Enamel blocks with a mean surface microhardness between 213 and 368 VHN were randomly divided into seven groups (six experimental and one control).

A total of twenty eight sound enamel slabs were divided into seven groups of four slabs each. To study the cycles of demineralization and remineralisation of enamel that occurs under dental plaque in the mouth, a laboratory pH cycling model was previously developed. This model tries to mimic the process of acid attack (demineralization) and remineralisation by saliva in the mouth (7). Human enamel specimens were subjected to a daily cycling regime comprising: two one-minute brushing with toothpaste; one before and one after the demineralization period of 6 hours using demineralization solution and 18 hours remineralisation in artificial saliva. Daily pH cycling regime was repeated during skupina ručno je četkana pastom za zube. Standardna četkica za zube (Oral-B Medium) upotrijebljena je za ručno četkanje. Shema pokusa bila je postavljena tako da oponaša dnevnu izmjenu od 6 sati demineralizacije i 18 sati remineralizacijskog oporavka u slini. Dva tretmana fluoridom na dan (jedan prije demineralizacije i jedan nakon nje) primjenjivala su se u svim eksperimentalnim skupinama kako bi se procijenio kariostatski učinak zubnih pasta. Sve pločice su se ispirale destiliranom vodom 15 sekundi prije i poslije promjene DM/RM-otopine ili četkanja zubnom pastom. Nakon toga su bile osušene mekim upijajućim papirom. Zatim su svi uzorci na 18 sati uronjeni u remineralizacijsku otopinu s pH 8,0 i na temperaturi od 37°C (Cultura Vivacare Diagnostic Line-Vivadent).

Mjerenje pH-vrijednosti pasta za zube

Deset mililitara deionizirane vode dodano je u pet mililitara svake paste za zube, zatim je sve izmiješano i izmjeren je pH. Među uzorcima pasta u ovom istraživanju šest je komercijalno dostupnih. Detartrine (Septodont) bez fluorida bio je kontrola. Standardna puferska otopina (Solutions tampons techniques 2 X pH= 4,00; 2 X pH= 7,00; 2 X pH= 10,00 (25°C), 10 X 6 ampoules: L 4998; Cat. No. 93150, Biobloch Scientific) s nominalnim pH vrijednostima od 4,0 do 7,0 upotrijebljena je za kalibriranje pH-metra s točnošću od 0,01 jedinica.

Postupak testiranja

Vrijednost tvrdoće prema Vickersu (VHN) određena je iz srednje vrijednosti dobivene tijekom šest mjerenja na površini svakog uzorka. Mikrotvrdoća površine cakline izmjerena je prije cikličkog pH-režima u svakoj testiranoj skupini i nakon njega. Dobiveni podaci analizirani su komercijalno dostupnim softverom (Sigma statistika, SPSS) koristeći se Student t-testom za zavisne uzorke na 95 posto pouzdanosti.

Rezultati

Srednje vrijednosti i standardne devijacije za SMH nalaze se u tablici 2. Nakon 12 dana cikličkog režima, uzorci tretirani s fluoridnim zubnim pastama pokazali su statistič-

12 days. During the pH cycling regime each of those seven groups were manually brushed with toothpaste. Standard toothbrush (Oral-B Medium) was used for manual brushing. The test scheme was designed to model a daily challenge of a 6-hour demineralization and an 18-hour remineralisation repair by saliva. Seven experimental groups received two fluoride treatments per day (one before and one after demineralization) to evaluate the cariostatic effect of the toothpastes. All slabs were rinsed with distilled water for 15 s before and after any DM/RM solution change or toothpaste brushing period and were wiped dry with soft tissue paper. All samples were then immersed in remineralizing solution at pH 8.0 for 18 hours at 37˚C (Cultura Vivacare Diagnostic Line-Vivadent).

Measurements of dentifrices' pH values

Ten mililiters of deionized water were added to 5 ml of each dentifrice, mixed and pH was measured. The sample of dentifrices used in the current study included six commercially available toothpastes. Fluoride free Detartrine paste (Septodont) was used as a control. Standard buffer solutions (Solutions tampons techniques 2 X pH= 4.00; 2 X pH= 7.00; 2 X pH= 10.00 (25°C), 10 X 6 ampoules: L 4998; Cat. No. 93150, Biobloch Scientific) with nominal pH values of 4.0 and 7.0 were used to calibrate the pH meter with an accuracy of 0.01 units.

Testing procedure

The Vickers hardness number (VHN) was determined from the mean values obtained from six indentations on the surface of each specimen. Microhardness of enamel surface was measured before and after pH-cycling regime in each tested group. The obtained data were analyzed using commercially available software (Sigma Stats, SPSS) using Student t-test for dependent samples at 95% level of confidence.

Results

The means and standard deviations for the SMH are presented in Table 2. After 12 days cycling, specimens treated with dentifrices containing fluorides exhibited statistically

Tablica 2. Statistička analiza vrijednosti promjene mikrotvrdoće površine cakline tijekom dvanaestodnevnog pH-ciklusa (p-vrijednost, Student t-test)
Table 2 Statistical analysis of rates of enamel surface microhardness change during 12 days pH cycling (p-value, Student t-test).

Zubna pasta • Toothpaste	N	N	Vickersov test SMH na početku • Mean Vickers SMH baseline	Vickersov test SMH nakon 12 dana • Mean Vickers SMH 12 days	SD	SD	t-vrijednost • t-value	df	p
	Skupina 1 • Group 1	Skupina 2 • Group 2	Skupina 1 • Group 1	Skupina 2 • Group 2	Skupina 1 • Group 1	Skupina 2 • Group 2			
Sensodyne F	24	24	287,1	353,9	39,4	36,3	-6,1094	46	0,000000
Sensodyne Rapid	24	24	288,9	353,0	38,1	38,2	-5,8141	46	0,000001
Colgate Total	24	24	318,4	368,0	51,4	52,3	-3,3148	46	0,001794
Elmex	24	24	280,7	299,9	25,1	25,8	-2,6149	46	0,012026
Parodontax F	24	24	299,6	306,4	46,1	36,3	-0,5705	46	0,571065
Pronamel	24	24	255,4	329,9	22,4	45,4	-7,2217	46	0,000000
Detartrine	24	24	288,9	306,1	37,8	42,1	-1,4835	46	0,144744

$p<0.05$

ki znatno viši SMH od onih tretiranih pastom bez fluorida ($p<0,05$) (Tablica 2). Analiza SMH pokazala je da je mikrotvrdoća cakline nakon tretmana s pastama Pronamel, Sensodyne F i Sensodyne rapid bila znatno veća nego nakon tretmana s drugim fluoridnim pastama ili pastom bez fluora nakon dvanaestodnevnog pH-cikličkog režima. No, vrijednosti mikrotvrdoće cakline na početku i nakon dvanaestodnevnog tretmana Parodontaxom postigle su povećanje SMH, ali to nije bilo statistički značajno ($p>0,05$). Te vrijednosti ne razlikuju se od negativne kontrole ($p>0,05$). Remineralizacijski potencijal u skupini tretiranoj pastom s aminfluoridom pokazao je statistički veliko povećanje mikrotvrdoće nakon 12 dana pH-ciklusa.

Rasprava

Svrha ove demineralizacijsko/remineralizacijske cikličke studije in vitro bila je procijeniti koliko su učinkovite fluoridne paste u zaštiti omekšale caklinske površine u slučaju daljnje demineralizacije. Ispitivanje mikrotvrdoće smatra se relativno jednostavnim i pouzdanim načinom za dobivanje neizravnih informacija o promjeni sadržaja minerala u caklini (17). Mineralni višak ili manjak u caklini zbog demineralizacijskog i remineralizacijskog procesa može se mjeriti kao promjena tvrdoće.

Početne vrijednosti mikrotvrdoće u ovom su istraživanju bile od 255,4 do 318,4 VHN-a. Osnovne vrijednosti mikrotvrdoće cakline dobivene su prije početka procesa demineralizacije.

Cuy i suradnici (18) istaknuli su da tvrdoća cakline varira ovisno o stupnju mineralizacije, lokalnim varijacijama u caklinskim prizmama i čvorovima te povećanoj poroznosti u blizini dentocaklinskog spojišta. Meredith i njegovi kolege (19) dokazali su da se mikrotvrdoća smanjuje od vanjske površine cakline prema dentocaklinskom spojištu. Mjerenje mikrotvrdoće može neizravno dokazati mineralni višak ili manjak/gubitke ili dobitke (20, 21). Nedostatak te tehnike (SMH) jest da se ne može kvantificirati količina viška ili manjka/gubitka ili dobitka minerala. No, to može dati dokaze o kvalitativnim promjenama minerala unutar caklinske površine. Rezultati ovog istraživanja pokazali su da korištenje pasta s većim udjelom fluorida može učinkovito povećati SMH cakline nakon pH-ciklusa. U ovoj studiji mikrotvrdoća cakline izmjerena je na poliranoj caklini.

Dinamički proces obavljen je u mnogobrojnim laboratorijima na različitim pH-cikličkim modelima (15, 22). U ovoj su se studiji ispitivali pH-ciklusi (izmjenična demineralizacija/ remineralizacija) u razdoblju od dva tjedna i to šest sati na dan demineralizacije pri pH 4,5. Demineralizacijska otopina djelomice je bila zasićena kalcijem i fosfatom. Nakon demineralizacije proces remineralizacije trajao je 18 sati svaka 24 sata, u skladu s već opisanim metodama (15).

Analiza površinske mikrotvrdoće (SMH) pokazala je da su Pronamel (1450 ppm F), Sensodyne F (1400 ppm F), Sensodyne rapid (1040 ppm F) i Colgate Total postigli veću vrijednost mikrotvrdoće cakline od ostalih pasta s fluoridom te placebom bez fluorida nakon dvanaestodnevnog pH-cikličkog režima. Moglo bi se zaključiti da je učinkovi-

higher SMH than those treated with the fluoride-free paste ($p<0.05$) (Table 2). SMH analysis showed that the enamel microhardness after treatment with Pronamel, Sensodyne F and Sensodyne rapid was considerably higher than after treatment with other fluoride dentifrices or a fluoride-free control paste after 12 days pH-cycling regime. However, rates of enamel microhardness at baseline and after 12 days cycling treated with Parodontax obtained increase in SMH, but it was not statistically significant ($p>0.05$). These SMH values did not differ from the negative control ($p>0.05$). Remineralisation potential in group treated with toothpaste containing aminfluoride showed statistically significant result after 12 days pH cycling in microhardness increase.

Discussion

The purpose of this in vitro demineralization/remineralisation cycling study was to evaluate the ability of fluoride dentifrices to protect softened enamel surface against further demineralization challenges. Microhardness testing is considered to be a relatively simple and reliable method for the provision of indirect information on mineral content changes in enamel (17). The gain or loss of minerals in enamel as a result of demineralization and remineralisation process can be measured as hardness change.

Initial microhardness values in our study ranged from 255.4 to 318.4 VHN. These baseline values of enamel microhardness were obtained before the process of demineralization was initiated.

Cuy and colleagues (18) found that enamel hardness varies depending on the degree of mineralization of the enamel, local variations in enamel rods and tufts, and increased porosity near the dentoenamel junction. Meredith and colleagues (19) showed that microhardness decreases from the outer enamel surface toward the dentoenamel junction. Microhardness indentation measurements can provide indirect evidence of mineral loss or gain (20, 21). The drawback of this technique (SMH) used in the current study is that it cannot quantify the amount of mineral loss or gain. However, it can provide qualitative evidence on mineral changes within the enamel surface. The findings from this study have shown that the use of dentifrices with higher amount of fluoride can be effective in increasing enamel SMH after pH cycling. In this study enamel microhardness was measured on polished enamel.

The dynamic nature of the process has been modelled in numerous laboratories by various pH cycling models (15, 22). This study examined pH cycling (alternating demineralization/remineralisation) over a period of 2 weeks with 6 hours of demineralization daily in a pH 4.5. Demineralising solution was partially saturated with calcium and phosphate. After demineralization a process of remineralisation was started for 18 hours during every 24 hours, according to methods reported previously (15).

The analysis of surface microhardness (SMH) showed that Pronamel (1450 ppm F), Sensodyne F (1400 ppm F) Sensodyne rapid (1040 ppm F) and Colgate Total have obtained higher value of enamel microhardness than other flutija dostupnost fluorida iz tih triju pasta rezultirala povećanom otpornošću na demineralizaciju i učinkovitijom remineralizacijom. Korištenje procjene površinske mikrotvrdoće za praćenje remineralizacijskog procesa dobro je poznata metoda. U nekoliko istraživanja in vitro opisana je remineralizacija umjetno demineralizirane caklinske lezije (23, 24). U uvjetima stvorenima u ovom istraživanju, neke paste mogu znatno poboljšati proces remineralizacije cakline. Sensodyne rapid, Sensodyne F i Pronamel pokazali su velik antikarijesni potencijal u odnosu prema ostalim pastama korištenima u ovom modelu, što ističe važnost sastava fluorida i formule pomoćnih tvari u postizanju antikarijesnog potencijala u uvjetima in vitro (25). Reintsema i Arends (26) u svojoj studiji sa šest fluoridnih pasta nisu mogli potvrditi znatnu povezanost između razine fluorida i antikarijesnog učinka na površini cakline. Pet od šest ispitanih pasta imalo je 1100 ppm fluorida ili manje. Budući da je istraživanje obavljeno 1988., može se pretpostaviti da povećana koncentracija fluorida stvara povoljno okružje za ugradnju fluora u caklinu. Kemijska formula suvremene paste može snažno utjecati na njihov učinak. Jedan od glavnih uzroka demineralizacije cakline jest pad pH ispod kritične točke za otapanje hidroksilapatita (27, 28). Ravnoteža između njezine demineralizacije i remineralizacije održava caklinsku površinu netaknutom. Rezultati Arnolda i suradnika pokazali su povećanu remineralizaciju na razini pH između 4,5 i 5,1 pod utjecajem aminofluorida. To je dokazalo da fluoridi povećavaju unos minerala tijekom remineralizacije cakline i sprječavaju gubitak minerala tijekom demineralizacije. Stvaranje kalcijeva fluorida ovisi o pH i manje je topljiv ako su pH-vrijednosti niske (27, 28, 29).

Zaključak

Rezultati ovog istraživanja dobiveni u eksperimentalnim uvjetima pokazuju da zubne paste s visokom koncentracijom fluorida (1450 ppm) djelotvorno sprječavaju demineralizaciju cakline. Kliničke studije potrebne su kako bi se provjerilo mogu li se slični rezultati dobiti u mnogo složenijem oralnom okolišu.

Zahvale

Rezultati istraživanja predstavljeni su kao oralna prezentacija na 23. svjetskom kongresu Međunarodne udruge za dječju i preventivnu stomatologiju (IAPD), od 15. do 18. lipnja 2011. u Ateni.

Ovo istraživanje pomogao je Erasmus Mundus External Cooperation Window Project Basileus – Balkans Academic Scheme za internalizaciju učenja u suradnji sa sveučilištima u Europskoj uniji.

oride dentifrices and fluoride-free placebo after 12 days pH-cycling regime. It could be concluded that more effective delivery of fluoride from those three dentifrices resulted in increased resistance to the demineralization and more effective remineralisation. The use of assessment of surface microhardness to monitor the remineralisation process is well established. Several in vitro studies have shown remineralisation of artificially demineralised enamel lesions (23, 24).

Under conditions created in the present study, some dentifrices can significantly enhance the process of enamel remineralisation. The dentifrices Sensodyne rapid, Sensodyne F and Pronamel showed superior anticaries potential compared to other commercial dentifrices in this model, which demonstrates the importance of fluoride compound and formulation excipients on driving anti-caries potential *in vitro* (25). Reintsema & Arends (26) in their study on six fluoride-containing dentifrices could not establish any significant relation between level of fluoride and the anti-caries effect on enamel surface. Five of six tested dentifrices had 1100 or less ppm of fluoride incorporated. Since the study was performed in 1988, it could be assumed that increased concentration of fluoride creates a favourable environment for incorporation in enamel. Chemical formulation of the contemporary dentifrices can strongly influence their effect. One of the main causes of enamel demineralization is the drop of pH below the critical point for hydroxyapatite dissolution (27, 28). The equilibrium between enamel demineralization and remineralisation maintains an intact enamel surface. The results of Arnold et al. showed an increased remineralisation at pH levels between 4.5 and 5.1 under influence of amine fluoride. It has been shown that fluorides enhance mineral uptake during enamel remineralisation and inhibit mineral loss during demineralization. Calcium fluoride formation depends on pH and is less soluble at low pH values. (27, 28, 29)

Conclusion

The results of the present study obtained under experimental conditions show that high fluoride toothpastes (1450 ppm) effectively inhibit enamel demineralization. Clinical studies are required to check whether similar results can be obtained in the more complex oral environment.

Acknowledgements

The results of the present study were presented as an oral presentation during the 23th World Congress of International Association of Dentistry for Children (IAPD); 15–18 June 2011, Athens, Greece.

This research was supported by Erasmus Mundus External Cooperation Window Project Basileus - Balkans Academic Scheme for the Internalization of Learning in cooperation with EU universities.

Abstract
Purpose: The aim of this study is to assess the effect of commercially available dentifrices with different fluoride formula on remineralisation of enamel surface. **Material and Methods**: Enamel blocks were ground flat with water-cooled carborundum discs (320, 600 and 1200 grit) and randomly divided into seven groups. The specimens were subjected to a daily cycling regime comprising two one-minute brushing treatments; one before and one after the demineralization period of 6 hours and 18 hours, and remineralisation in artificial saliva. During the pH cycling regimen each of those seven groups were treated with different dentifrices: fluoride dentifrice Sensodyne Rapid (1040 ppm NaF), Colgate Total (1100 ppm NaF), Parodontax fluoride (1400 ppm NaF), Sensodyne fluoride (1400 ppm NaF), Pronamel Sensodyne (1450 ppm NaF), Elmex-GABA International (1250 ppm F Amine fluoride), and fluoride free Detartrine Paste (Septodont). The surface microhardness (SMH) of those specimens was determined at baseline and after 12 days using HMV-2000 (50 g, 490.3 mN, 10 s) (Shimadzu, Japan). **Results**: All specimen groups treated with fluorides showed increase in SMH compared to control group. Pronamel, Sensodyne F, Sensodyne Rapid and Colgate Total were statistically superior to other fluoride dentifrices and a fluoride-free control after 12 days pH-cycling regime. Rates of enamel microhardness at baseline and after 12 days cycling treated with Parodontax obtained increase in SMH, but it was not statistically significant ($p>0.05$). **Conclusion**: The results obtained in the present study show that high fluoride toothpastes (1450 ppm) effectively inhibit demineralization under experimental conditions.

Received: May 2, 2011
Accepted: July 7, 2011

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Key words
Dental Enamel; Fluoridation; Toothpastes; Tooth Demineralization; Hardness Test

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