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Učinak fluoridnih gelova i lakova na demineralizaciju/ remineralizaciju cakline u usporedbi s kompleksom CPP-ACP

Effect of the Fluoride Gels and Varnishes Comparing to CPP-ACP Complex on Human Enamel Demineralization/ Remineralization

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Sažetak

Svrha: Cilj ovog in vitro istraživanja bio je utvrditi učinak fluoridnih gelova i lakova u odnosu na CPP-ACP kompleks na sprječavanje demineralizacije cakline. **Materijali i Metode**: Caklinski blokovi su ispolirani, podijeljeni u osam grupa i izloženi dnevnom cikličkom režimu. Tri skupine su tretirane 10 minuta s fluoridnim gelovima: Fluorogal, Fluor Protector Gel and Cervitec Gel, jedna je tretirana samo s GC Tooth Mousse i jedna je tretirana s GC Tooth Mousse (Recaldent CPP-ACP 10.0%).. Preostale tri grupe su tretirane fluoridnim lakovima: Fluoridin Gel N5, Bifluorid 12 i Fluor Protector. Oni su premazivani jedanput na tjedan prije demineralizacijskog razdoblja. Svi uzorci su čuvani u umjetnoj slini između i nakon ciklusa. Površinska mikrotvrdoća (SMH) uzoraka je mjerena na početku i nakon 12 dana koristeći HMV-2000 (Shimadzu, Japan). Postotak promjene SMH (% SMH) je izračunat nakon cikličkog režima. Podaci su analizirani t-testom za individualne usporedbe ($p<0,05$). **Rezultati:** Statistička analiza t-testom pokazala je značajnu razliku između SMH prije i nakon tretmana fluoridima u svim skupinama. Sve skupine tretirane fluoridnim gelovima, lakovima i GC Tooth Mousseom pokazale su povećanje SMH. Nije bilo statistički značajne razlike između postotaka promjene SMH cakline između skupina. Nije bilo statistički značajne razlike između fluoridnih gelova, lakova i GC Tooth Moussea. **Zaključak:** Rezultati dobiveni u ovom istraživanju pokazali su da fluoridni lakovi, gelovi i Tooth Mousse učinkovito sprječavaju demineralizaciju u eksperimentalnim uvjetima.

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Ključne riječi
fluoridacija, površinska; zubna caklina; tvrdoća; zub, demineralizacija

Uvod

Terapija fluorom dulje je od pet desetljeća okosnica preventivnih strategija protiv karijesa, zapravo otkako se počelo s fluoridacijom vode (1). Zaštitni čimbenici u usnoj šupljini, a i dodatno uzimanje sredstava kao što su fluoridi te bioraspoloživi kalcij i fosfati, potiču remineralizaciju cakline (2, 3).

Stomatolozi se u protukarijesnoj preventivi često lokalno koriste fluoridima. Njihov najvažniji učinak jest djelovanje na dodirnu površinu zuba/plaka, poticanje remineralizacije rane karijesne lezije i smanjivanje topljivosti cakline (4, 5).

Lakovi su razvijeni s namjerom da se poboljša djelovanje između fluorida i zubne cakline jer u tankom sloju dulje ostaju na površini zuba (12 ili više sati) i sprječavaju trenutačni gubitak fluorida nakon aplikacije. Nakon nanošenja lak djeluje kao da je spremnik iz kojega se sporo otpuštaju fluoridi (6-9). Fluoridni ion može se ugraditi u hidroksilapatitnu strukturu zubne cakline zamjenom hidroksilne sku-

Introduction

Fluoride therapy has been the centerpiece of caries-preventive strategies since the introduction of water fluoridation schemes over five decades ago (1). Protective factors are present in the oral environment, an extrinsic supply of these factors such as fluoride and bioavailable calcium and phosphate will favor an equilibrium shift towards enamel remineralization (2, 3).

Topically applied fluoride products are used extensively as an operator-applied caries-preventive intervention. The most important anti-caries effect of fluoride is considered to result from its action on the tooth/plaque interface, by promotion of remineralization of early caries lesions and reducing tooth enamel solubility (4,5).

Varnishes were originally developed to prolong the contact time between fluoride and dental enamel, as they adhere to the tooth surface for longer periods (12 hours or more) in a thin layer, and prevent the immediate loss of fluoride after pine ili otopljenog hidroksilapatita manje topljivim oblikom fluorida, primjerice, fluorapatitom ili fluorhidroksilnim apatitom. I u kliničkim istraživanjima dokazano je da topikalna fluoridna sredstva smanjuju demineralizaciju cakline *in vitro* (10).

Kalcij i fosfatna tehnologija ugrađena u stomatološke proizvode uključuju amorfni kalcijev fosfat (ACP), kazein-fosfopeptid i amorfni kalcijev fosfat (CPP-ACP), kalcijev natrijev fosfosilikat (CSPS) i tri-kalcijev fosfat (TCP). Svim tim preparatima nastoji se povećati količina dostupnog kalcija i fosfata, obično zajedno s fluoridom. U profesionalne proizvode, kao što su ACP, CSPS i TCP, tehnološki su različito ugrađeni i trenutačno se nalaze u restorativnim materijalima, pečatnim smolama, ortodontskim cementima, profilaktičkim pastama, fluoridnim lakovima i gelovima za profesionalnu primjenu (11, 12).

Osim lakova i kompleks CPP-ACP-a u obliku paste ima snažan antikariogeni učinak zahvaljujući fosfoproteinu kazeinu i kalcijevu fosfatu (13). Kazein-fosfopeptid (CPP) sadržava sekvenciju Ser(P)-Ser(P)-Ser(P)-Glu-Glu koja stabilizira kalcijev fosfat u otopini vežući amorfni kalcijev fosfat (ACP) na svoj višestruki ostatak fosfoserina (14). Antikariogenost nanokompleksa CPP-ACP-a dokazana je na životinjama i kod karijesnog modela *in vitro* (15-17). Daljnja istraživanja provedena na humanim karijesnim modelima *in situ* pokazala su da CPP-ACP može spriječiti demineralizaciju cakline i potaknuti remineralizaciju (14, 18, 19, 20).

Korištenje CPP-ACP-a s fluorom i njegov učinak na remineralizaciju cakline pripisuje se stvaranju CPP-stabiliziranog amorfnog kalcijeva fluoridnog fosfata (21), što je rezultiralo povećanim brojem fluoridnih iona u plaku te većom koncentracijom biodostupnih iona kalcija i fosfata. Ovaj sinergijski učinak CPP-ACP-a i fluorida na smanjenje karijesa istraživan je na životinjama te u pokusima *in vitro* i *in situ* (16, 21, 22)

Svrha ovog istraživanja bila je ustanoviti učinak fluoridnih gelova i lakova na površinsku mikrotvrdoću rane caklinske lezije u pH-cikličkom modelu cakline (demineralizacija/remineralizacija), u odnosu na kompleks CPP-ACP-a.

Materijali i metode

Opis istraživanja

Izvađeni zdravi zubi čuvali su se u 2-postotnoj otopini formaldehida (pH 7,0) na sobnoj temperaturi. Nakon što su im uklonjeni korijeni i pulpe, izbrusci zdrave cakline izrezani su dijamantnim diskom s obrazne i jezične strane zuba. Nakon toga položeni su u akrilnu masu za ulaganje (Acryl Fix Kit – Struers) kako bi se stvrdnjavali tijekom noći. Površina caklinskih blokova, hlađena vodom, postupno je polirana karborundnim diskovima (320, 600 i 1200 grit s Al_2O_3 papi-

application. After application the varnish acts as a slow-releasing reservoir of fluoride (6-9). Fluoride ion can be incorporated into the hydroxylapatite structure of tooth enamel by the replacement of hydroxyl groups or by redeposition of dissolved hydroxyl-apatite as less soluble fluoridated forms, such as fluorapatite or fluorhydroxyl-apatite. Topical fluoride agents have been shown to decrease enamel demineralization in vitro and in clinical studies (10).

Calcium and phosphate technologies currently incorporated into dental products include amorphous calcium phosphate (ACP), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), calcium sodium phosphosilicate (CSPS) and tricalcium phosphate (TCP). The overall intent of these technologies is to increase the amount of available calcium and phosphate, typically together with fluoride. In the case of professional products, ACP, CSPS and TCP technologies have variously been incorporated and are currently available in restorative materials, sealants, orthodontic cement, prophylaxis pastes, in-office fluoride varnishes and in-office fluoride gels (11, 12).

Apart from the varnishes, the CPP-ACP complex in the form of paste has a strong anticariogenic effect which was attributed to the phosphoprotein casein and calcium phosphate (13). Casein phosphopeptide (CPP) containing the sequence Ser(P)-Ser(P)-Ser(P)-Glu-Glu has the ability to stabilize calcium phosphate in solution by binding amorphous calcium phosphate (ACP) with their multiple phosphoserine residues (14). The anticariogenicity of the CPP-ACP nanocomplexes has been demonstrated in animal and *in vitro* caries models (15-17). Further studies using human *in situ* caries models have shown that the CPP-ACP could prevent enamel demineralization and promote remineralization (14, 18, 19, 20).

The use of CPP-ACP with fluoride and its synergistic effect on enamel remineralization have been attributed to the formation of CPP-stabilized amorphous calcium fluoride phosphate (21), resulting in the increased incorporation of fluoride ions into plaque, together with increased concentrations of bioavailable calcium and phosphate ions. This synergistic effect of CPP-ACP and fluoride in reducing caries was investigated in animals, in *in-vitro* and *in situ* studies (16, 21, 22).

The aim of this study was to determine the effect of the fluoride gels and varnishes comparing to CPP-ACP complex on surface microhardness of early enamel lesions in pH-cycling model enamel (demineralization/remineralization).

Materials and Methods

Study design

Extracted sound human teeth were stored in 2% formaldehyde solution (pH 7.0) at room temperature. 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 surface of the blocks is ground flat with water-cooled carborundum discs (320, 600 and 1200 grit of Al_2O_3 papers; Buehler, Lake Bluff, IL, rom; Buehler, Lake Bluff, IL, SAD). Ti su postupci obavljeni kako bi se postigla paralelna ravna površina za Vickersov test mikrotvrdoće. Uzorci su rehidrirani u deioniziranoj vodi najmanje 60 minuta prije korištenja. Za standardizaciju blokova najprije su odabrani uzorci za početnu mikrotvrdoću (pet mjerenja u različitim područjima blokova, 50g – 490,3 mN ili 0,05 Hv), 10s, HMV–2000; Shimadzu Corporation, Tokio, Japan). Caklinski blokovi s prosječnom mikrotvrdoćom površine između 142 i 462 VHN-a nasumce su razvrstani u osam skupina.

Ukupno 34 zdrave caklinske pločice podijeljene su u eksperimentalnu i kontrolnu skupinu. Od osam skupina, šest je imalo po četiri pločice, a dvije po tri. Kontrolna skupina sadržavala je četiri pločice i nisu imale nikakav tretman u istraživanju. Korišteni su sljedeći fluoridni gelovi i lakovi: Fluoridin Gel N5 (Fluoride gel; Voco-GmbH, Cuxhaven, Njemačka), Fluorogal forte (NaF 8400 ppm, FH_2 2600 ppm, Galenika, Beograd, Srbija), Fluor Protector Gel (1450 ppm, Ivoclar Vivadent), Bifluorid 12 (NaF 27100 ppm, CaF 29200), Fluoride gel (Voco-GmbH, Cuxhaven, Njemačka), Fluor Protector varnish (fluorsilane, NaF 22600 ppm, Ivoclar Vivadent AG FL-9494 Schaan), Cervitec Gel (900 ppm F, Ivoclar Vi-

USA). These procedures are conducted to form parallel planar surfaces for the Vickers microhardness tests. Specimens were re-hydrated in deionised water for at least 60 minutes prior to use. 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 142 and 462 VHN were randomly divided into eight experimental groups.

A total of 34 sound enamel slabs were divided into experimental and control groups. Within eight experimental groups, six contained four slabs each, and two of them contained three slabs. The control group contained four slabs and did not receive any treatment during the experiment. The following fluoride gels and varnishes were applied: Fluoridin Gel N5 (Fluoride gel; Voco-GmbH, Cuxhaven, Germany), Fluorogal forte (NaF 8 400 ppm, FH_2 2600 ppm, Galenika, Belgrade, Serbia), Fluor Protector Gel (1450 ppmF, Ivoclar Vivadent), Bifluorid 12 (NaF 27 100 ppm, CaF 29 200) (Fluoride gel;Voco-GmbH, Cuxhaven, Germany), Fluor Protector varnish (fluorsilane, NaF 22.600 ppm, Ivo-

Tablica 1. Sastav fluoridnih gelova, lakova i GC Tooth Moussa testiranih u istraživanju mikrotvrdoće
Table 1 Formulation details for fluoride gels, varnishes and GC Tooth Mousse tested in microhardness study

Naziv preparata • Test Product	Broj proizvoda • Batch Numbers	Fluorid, koncentracija (ppm) • Fluoride source Concentration (ppm)	Sastav • Ingredients
Fluoridin Gel N5 (Voco)	LOT N 0813150	NaF 2.26 %	Hidratizirane smole, etanol, natrij ciklamata, okus maline • Hydrated colophony, Ethanol, Sodium cyclamate, Raspberry flavor
Bifluorid 12 (Voco, GmbH, Cuxhaven, Germany)	LOT 0912036	NaF+CaF(27.100+29.200)	Etil acetat, celuloza nitrat s alkoholom, izopentil propionat, natrij fluorid • Ethyl acetate, Cellulosenitrate with alcohol, isopentyl propionate,sodium fluoride
Fluor Protector Gel (Ivoclar, Vivadent)	LOT N 27055	1450ppm F	Voda, ksilitol, hidroksietilceluloza, alkohol, kalijev fluorid, kalcij glicerofosfat, lauret-23, pantenol, metilparaben, aroma, natrij saharin • Aqua, Xylitol, Hydroxyethylcellulose, Alcohol, Potassium fluoride, Calcium glycerophosphate, Laureth-23, Panthenol, Methylparaben, Aroma, Sodium sacharin
Cervitec Gel	LOT N 01189	900 ppmF	Voda, hidroksietilceluloza, lauret-23, klorheksidin diglukonat, natrijev fluorid, aroma, natrij saharin • Aqua, Hydroxyethylcellulose, Laureth-23, Chlorhexidine digluconate,Sodium fluoride, Aroma, Sodium sacharin
Fluorogal forte (Galenika, Beograd, Srbija)	Ser.no. 1150	NaF 8400, HF 2600	Natrijev fluorid 18.50 mg(8400 ppmF), hidrofluorna kiselina (FH) 6.82 mg (2600 ppmF) • Sodium fluoride 18.50 mg(8400 ppmF), fluorhydric acid (FH) 6.82 mg (2600 ppmF)
GC Tooth Mousse	LOT 090619V		Glicerol, propilen glikol, Recaldent CPP-ACP (kazein fosfopeptid-amorfni kalcijev fosfat), D-glucitol, koloidni silicij, natrij karboksil metil celuloza (CMC-Na), titan dioksid ksilitol, guar guma, fosforne kiseline, natrijev saharin, cinkov oksid, magnezijev oksid, etil 4-hidroksibenzoat, propil 4-hidroksibenzoat • Glycerol, Propylene glycol, Recaldent CPP-ACP(casein phosphopeptide-amorphous calcium phosphate), D-glucitol, Colloidal Silica, Sodium carboxyl methyl cellulose (CMC-Na), titanium dioxide xylitol, Guar Gum, Phosphoric acid, Sodium saccharin, zinc oxide, magnesium oxide, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate
Fluor Protector varnish (Vivadent)	LOT F29756	Fluorsilane, NaF, 22 600	1g Fluor Protector (=0,92 ml) sadrži: Bis (4-[2-(difluorhidroksilil etil]-2-metoksicikloheksil)[N,N-(trimetihekseksan-1,6-diil) dikarbamat](9 mg)(fluorsilan) • 1g Fluor Protector (=0,92 ml) contains: Bis (4-[2-(difluorhydroxysilyl ethyl]-2-methoxycyclohexyl)[N,N-(trimethyhexexane-1,6-diyl) dicarbamate](9 mg)(fluorsilane)

vadent) i Tooth Mousse (GC Corporation, Itabashi-Ku, Tokio, Japan.) (tablica 1). Tri skupine tretirale su se fluoridnim lakovima (Fluoridin Gel N5, Bifluorid 12 i Fluor Protector). Caklinske pločice premazivale su se jedanput na tjedan prije demineralizacije. Preparati su ostavljeni netaknuti pet minuta na površini zuba. Nakon 12 dana pH cikličkog režima, fluoridni sloj pažljivo je skinut skalpelom. Zubi su destiliranom vodom očišćeni od svih zaostalih naslaga.

Od ostalih pet ispitivanih skupina tri su tretirane fluoridnim gelom (Fluorogal, Fluor Protector Gel i Cervitec Gel), jedna samo GC Tooth Mousseom, a kod posljednje su upotrebljeni GC Tooth Mousse i Cervitec Gel. Svih pet eksperimentalnih skupina tretirano je deset minuta, osim one s GC Tooth Mousseom i Cervitec Gelom. Taj je tretman trajao dulje, što znači deset minuta GC Tooth Mousseom i dvije minute Cervitec Gelom. Za proučavanje ciklusa demineralizacije i remineralizacije cakline koji nastaje ispod zubnog plaka, prije toga je razvijen laboratorijski ciklički pH-model. Na taj se način pokušalo oponašati proces djelovanja kiseline (demineralizacije) i remineralizacije slinom u ustima (23). Humani caklinski uzorci bili su podvrgnuti svakodnevnom cikličkom režimu koji se sastojao od dva desetominutna tretmana fluoridnim gelom – jedan prije i jedan poslije demineralizacijskog razdoblja od šest sati koristeći se demineralizacijskom otopinom i 18 sati remineralizacije u umjetnoj slini. Dnevni ciklički režim pH ponavljao se 12 dana. Plan pokusa bio je postavljen tako da oponaša dnevnu izmjenu od šest sati demineralizacije i 18 sati remineralizacijskog oporavka u slini. Tri eksperimentalne skupine jedanput na tjedan tretirane su fluoridnim lakom. Nakon pH-cikličkog režima fluoridni sloj je pažljivo uklonjen skalpelom. Sve pločice isprane su destiliranom vodom 15 sekundi prije i poslije promjene DM/RM-otopine ili tretmana te osušene mekim upijajućim papirom. Zatim su svi uzorci 18 sati bili uronjeni u remineralizacijsku otopinu s pH 8,0 na temperaturi od 37°C (Cultura Vivacare Diagnostic Line-Vivadent).

SMH je određen mjerenjem duljine udubine (μm) koristeći se sustavom za analizu slike. Uzorci su ispitani mikroskopom Olympus BH-2. Slika je prenesena videokamerom na monitor spojen na računalo IBM-XT (Olympus CUE-2 software). SMH svakog bloka cakline određen je na početku i ponovno nakon 12 dana pH cikličkog režima.

De/remineralizacijska otopina

Demineralizacijska otopina sastoji se od natrijeva acetata (0,1 mM CH_3COONa), kalijeva klorida (150 mM KCl_2), kalcijeva klorida (1,5 mM $CaCl_2$) i kalijeva dihidro-

clar Vivadent AG FL-9494 Schaan, Cervitec Gel (900 ppm F, Ivoclar Vivadent) and Tooth Mousse (GC Corporation, Itabashi-Ku, Tokyo, Japan) (Table 1). Three groups were treated with fluoride varnishes (Fluoridin Gel N5, Bifluorid 12 and Fluor Protector).They were coated once a week before the demineralization period. Agents were left undisturbed for five minutes on the tooth surfaces. After 12 days, pH cycling regime the fluoride film was carefully removed with a scalpel. Also, the teeth were cleaned of any remaining resinous sediments with distilled water.

From the remaining five experimental groups, three of them were treated with fluoride gels (Fluorogal, Fluor Protector Gel and Cervitec Gel), one was treated only with GC Tooth Mousse and the last group was treated with GC Tooth Mousse and with Cervitec Gel. All the five experimental groups were treated within the period of 10 minutes, with the exception of the group which was treated with GC Tooth Mousse and Cervitec Gel. This treatment lasted longer, which means 10 minutes of treatment with GC Tooth Mousse and 2 minutes with Cervitec Gel. To study the cycles of demineralization and remineralization of enamel that occur 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 remineralization by saliva in the mouth (23). Human enamel specimens were subjected to a daily cycling regime comprising: two ten minutes treatments with fluoride gels, one before and one after the demineralization period of 6 hours using demineralization solution and 18 hours remineralization in artificial saliva. Daily pH cycling regime was repeated during 12 days. The test scheme was designed to model a daily challenge of a 6-hour demineralization and an 18-hour remineralisation repair by artificial saliva. Three experimental groups received one fluoride varnish treatment per week. Following the pH-cycling regime, the fluoride film was carefully removed with a scalpel. All slabs were rinsed with distilled water for 15s before and after any DM/RM solution change or treatment 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).

SMN was determined by measuring the lengths of the indentations (μm) using the following image analysis system. Samples were examined with reflected light using an Olympus BH-2 microscope. The image was transmitted via a video camera to a monitor connected to an IBM-XT computer (Olympus CUE-2 software). The SMH of each enamel block was determined at baseline and again after the 12 days pH cycling regime.

The final SMH minus the baseline SMH gives the change in hardness (Δ SMH) as change in indentation length (μm). The indentation length was used as a direct measure of change rather than the VHN which is derived from, and inversely proportional to, the square of the indentation length.

De/remineralising solutions

The composition of demineralizing solution was: sodium acetate (0.1 mM CH_3COONa), potassium chloride (150 mM KCl_2), calcium chloride (1.5 mM $CaCl_2$) and potassigenfosfata (0,9 mM KH2PO4). Vrijednost pH ugođena je na 4,5 s pomoću hidrokloridne kiseline (0,1 mol/l). Blaga odstupanja ispravljena su hidrokloridnom kiselinom 0,1 mol/l kako bi se u demineralizacijskom razdoblju zadržala konstantna pH-vrijednost između 4,35 i 4,65. Umjetna slina (BSI – 24) sadržava natrijev klorid (0,50 g/l), natrijev bikarbonat (4,2 g/l), natrijev nitrat (0,03 g/l) i kalijev klorid (0.20 g/l), a prikazana je u tablici 2. Umjetna slina imala je pH 8,0. U demineralizacijskoj i remineralizacijskoj otopini vrijednosti pH mjerene su svaki dan pH-metrom (HI 8014, HANNA instruments, Bioblock Scientific, Illkirch, Francuska) (24).

um dihydrogen phosphate (0.9 mM KH2PO4). The pH was adjusted to 4.5 using hydrochloric acid (0.1 mol/l). Slight elevations were corrected with hydrochloric acid 0.1 mol/l to maintain a constant pH value between 4.35 and 4.65 during the demineralization period. The artificial saliva (BSI-24) contained: sodium chloride (0.50 gr/l), sodium bicarbonate (4.2 g/l), sodium nitrate (0.03 g/l) and potassium chloride (0.20 g/l), shown in Table 2. The pH of artificial saliva was 8.0.The pH values of demineralization and remineralization solutions were measured every day using pH meter (HI 8014, HANNA instruments, Bioblock Scientific, Illkirch, France)(24).

Tablica 2. Sastav umjetne sline
Table 2 Composition of artificial saliva

Sastojci • Component	Koncentracija • Concentration (gl^{-1})
NaCl	0.50
$NaHCO_3$	4.20
$NaNO_2$	0.03
KCl	0.20

Postupak testiranja

Tvrdoća u vickersima (VHN) određena je na temelju srednjih vrijednosti dobivenih tijekom šest mjerenja na površini svakog uzorka. Mikrotvrdoća površine cakline izmjerena je prije pH-cikličkog režima i nakon njega u svakoj testiranoj skupini. Dobiveni rezultati obrađeni su komercijalno dostupnim softverom (Sigma Stats, SPSS) koristeći se Studentovim t-testom za zavisne uzorke sa stupnjem pouzdanosti od 95 posto. Razlike između različitih materijala testirane su jednosmjernim testovima ANOVA i Tukeyevim HSD-om. Za analizu je korišten komercijalno dostupni softver (Sigma Statistika, SPSS).

$$VHN = F^{x}\ 1{,}85/d^2 \qquad d = \text{mean diameters}$$

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 on SMH change before/after treatment in each group were analyzed using Student t-test for dependent samples at 95% level of confidence. Differences between the different groups of materials were tested with one-way ANOVA and Tukey HSD test. Commercially available software (Sigma Stats, SPSS) was used for the analysis.

$$VHN = F^{x}\ 1.85/d^2 \qquad d = \text{mean diameters}$$

Rezultati

Srednja vrijednost i standardna devijacija SMH-a nalaze se tablici 3. Nakon dvanaestodnevnog ciklusa uzorci tretirani fluoridnim gelom, preparatom Tooth Moussea i kombinacijom Tooth Moussea i Cervitec Gela pokazali su statistički veći SMH nego kontrolna skupina ($p<0{,}05$). Analiza SMH-a pokazala je da je bila veća mikrotvrdoća cakline nakon tretmana s kombinacijom Tooth Moussaa i Cervitec Gela u usporedbi s mikrotvrdoćom površine caklinskih blokova tretiranih samo Tooth Mousseom, ali ne i statistički značajna ($p>0{,}05$). ANOVA nije pokazala statistički značajne razlike u utjecaju na remineralizaciju između fluoridnih gelova i lakova te kompleksa CPP-ACP-a ($p> 0{,}05$).

Results

The means and standard deviations for the SMH are presented in Table 3. After 12 days cycling, specimens treated with fluoride gels, dental crème Tooth Mousse and combination of Tooth Mousse and Cervitec Gel exhibited statistically higher SMH than the control group ($p<0.05$). SMH analysis showed that enamel microhardness after treatment with combination of Tooth Mousse and Cervitec Gel in comparison with the surface microhardness of the enamel blocks treated only with Tooth Mousse was higher but it was not statistically significant ($p>0.05$). ANOVA did not show statistically significant differences in the effect on remineralization between fluoride containing gels and varnishes and CPP-ACP complex ($p>0.05$).

Tablica 3. Statistička analiza promjene mikrotvrdoće površine cakline tijekom 12 dana pH-ciklusa (p-vrijednost, Student t-test)
Table 3 Statistical analysis of rates of enamel surface microhardness change during 12 days pH cycling (p-value, Student t-test)

Fluoride gels, fluoride varnishes and GC Tooth Mousse	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 (p<0.05)
		Skupina 1 Group 1	Skupina 2 Group 2	Skupina 1 • Group 1	Skupina 2 • Group 2			
Control	30	294.04	303.33	10.74	12.47	-2.03	24	0.053
GC Tooth Mousse	30	231.3	281.5	46.6	47.7	-2.91	28	0.000008
Fluoridin N5	40	173.5	243.7	29.9	27.8	-7.69	38	0.000001
Bifluorid 12	40	161.1	195.7	20.7	23.3	-4.95	38	0.000025
Fluor Protector	40	158.6	213.3	17.7	31.4	-6.77	38	0.000000
GC Tooth Mouse + Cervitec Gel	40	237.4	301.2	70.8	98.0	-2.35	38	0.000002
Fluorogal-Galenika	40	172.9	219.8	30.7	46.1	-3.78	38	0.000014
Fluor Protector Gel	30	167.7	199.4	22.0	18.4	-4.28	28	0.000016
Cervitec gel	40	210.7	257.1	62.6	66.5	-2.27101	38	0.000000

Najveći postotak SMH-a ustanovljen je za Fluoridin N5, a najmanji za Fluor Protector Gel. • The highest values of percentage SMHR were observed for the Fluoridin N5 and the lowest with Fluor Protector Gel.

Tablica 4. Postotak promjene mikrotvrdoće kao razlika između mjerenja SMH-a prije pH-cikličkog režima i 12 dana poslije
Table 4 The percentage of microhardness changes as a difference between SMH measurements before and after 12 days pH cycling regime

Testirani proizvod • Test product	SMH (početna) • (Initial)	SMH_1 (nakon tretmana) • (after treatment)	% SMH
Control	294.04	303.33	3.16%
GC Tooth Mousse	231.3	281.5	21.7%
Fluoridin N5	173.5	243.7	40.49%
Bifluorid 12	161.1	195.7	21.48%
Fluor Protector	158.6	213.3	34.45%
GC Tooth Mousse + Cervitec gel	237.4	301.2	26.88%
Fluorogal	172.9	219.8	27.16%
Fluor Protector Gel	167.7	199.4	18.92%
Cervitec gel	210.7	257.1	22.02%

$\% SMH = \{[SMH_1 - SMH]/SMH\} \times 100$

Rasprava

Minerali, u prvom redu kalcij i fosfati, otpuštaju se tijekom demineralizacije iz hidroksiapatitnih kristala i u slučaju da demineralizacija potisne remineralizaciju to rezultira razvojem potpovršinske lezije. To inicijalno zahvaća samo caklinu i često rezultira pojavljivanjem bijelih točaka jer je određena količina potpovršinskih minerala izgubljena, pa se mijenja izgled tvrdoga zubnog tkiva.

Slina je prezasićena kalcijem i fosfatom i to pomaže u sprječavanju demineralizacije kada se postigne kritični pH tijekom djelovanja kiseline. Više od desetljeća pojedini istraživači i stručnjaci preporučuju profesionalnu primjenu topikalnih fluorida za osobe s umjerenim do visokim rizikom od karijesa (8, 11, 25)

Utjecaj topikalnih fluorida dobro je poznat u prevenciji karijesa i jačanju otpornosti minerala zuba, kad je riječ o kariogenim promjenama. Mehanizam za smanjenje demineralizacije i za lakšu remineralizaciju uključuje promjenu mineralne strukture cakline stvaranjem kalcijeva fluorida i fosfatnih minerala te povećanjem fluorida na površini cakline.

Discussion

Minerals, primarily calcium and phosphate, leak out from the hydroxyapatite crystals during demineralization and in situations where demineralization outpaces remineralization, this leads to the development of subsurface lesions. These initially involve only the enamel and often result in the appearance of white spots where sufficient subsurface mineral content has been lost to alter the optical properties of the dental hard tissues.

Saliva is oversaturated with calcium and phosphate that helps to prevent demineralization until the critical pH is reached during an acid attack.

For more than a decade, individual investigators and expert panels have recommended that professional topical fluoride use be limited to those individuals with moderate-to-high caries risk (8, 11, 25).

The role of topical fluoride in caries prevention and enhancing tooth mineral resistance to a cariogenic challenge is well known. The mechanism for reducing demineralization and facilitating remineralization involves modification of the

Manja količina fluorida djeluje na transformaciju manje stabilne, više topljive mineralne faze (di-kalcijev fosfat dihidrat [DCPD], okta-kalcijev fosfat [OCP], tri-kalcijev fosfat [TCP]) u stabilniju, manje topljivu mineralnu fazu hidroksiapatita [HAP], fluorhidroksiapatita [FHAP] i fluorapatita [FAP] .

Izloženost razmjerno visokom sadržaju fluorida, najčešće na površini cakline, potiče stvaranje kalcijeva fuorida. Tretmanom caklinskih pločica zaštitnim fluoridnim gelom, fluoridi te kalcijevi i fosfatni ioni postaju dostupni, što može biti važno za transformaciju površinskog kalcijeva fluorida u FHAP-u.

Postupno otpuštajući preparati, kao što su lakovi, mogu dugo profilaktički djelovati. Učinkovitost ovisi o stupnju i količini otpuštanja iz osnovnog materijala. Fluoridni lakovi vrlo su dobri (8, 21). Postupno otpuštajući sustavi, uključujući i lakove, općenito su inicijalno aktivni – brzo otpuštaju aktivne tvari, a zatim slijedi spora faza otpuštanja (26).

Jedna studija *in vitro* s dva 5-postotna natrij-fluoridna laka provedena je 2001. i autori ističu da lakom prekrivena caklina uronjena u pufersku otopinu kalcijeva fosfata (kako bi se oponašao intraoralni okoliš), nastavlja otpuštati fluoride još pet do šest mjeseci. (27).

U ovom istraživanju korišten je klorheksidin gel sam (Cervitec gel) ili se kombinirao sa zubnom pastom Tooth Mousse.

U istraživanju A. P. Erdema vrjednovan je i uspoređivan učinak *in vitro* dvaju fluoridnih lakova i jednog laka s fluorid klorheksidinon na *Streptococcus mutans* i *Streptococcus sobrinus* i na formiranje biofilma (28). U tom je istraživanju Bifluorid 12 (6 % naF i 6 % Ca F_2) imao najmanji inhibitorni učinak u eksperimentalnom razdoblju, iako ima najveću koncentraciju fluorida u usporedbi s lakom Fluor Protectorom (1% difluorsilana). Te razlike između fluoridnih lakova mogu se objasniti svojstvima različitih lakova i načinima djelovanja. Bifluorid 12 ima veću viskoznost od drugih ispitivanih lakova, pa se može zadržati u debljem sloju na caklinskim izbruscima. Fluor Protector sadržava poliuretanski sastojak difluorosilan, ima niski pH i na površini cakline stvara tanki prozirni film. Ima i manju koncentraciju fluorida negoli Bifluorid 12, no remineralizacijski učinak je bio bolji, što se može objasniti dodanim silanom.

Cervitec gel korišten u ovom istraživanju sadržava klorheksidin koji je prema kemijskom sastavu bis-biguanid s antibakterijskim, antikariogenim i remineralizacijskim djelovanjem i manjim toksičnim djelovanjem. Ako se Cervitec gel doda nakon tretmana caklinskih pločica zubnom pastom Tooth Mousse povećava se učinak remineralizacijskog efekta Tooth Mousseom nakon demineralizacije. I pH vrijednosti testiranih lakova i gelova parametri su koje treba uzeti u obzir.

Inhibicija demineralizacije i promocija remineralizacije zahtijevaju dovoljne količine kalcija, fosfata i fluorida. Ako se na površini zuba može održavati viša razina tih minerala prije djelovanja kiseline i tijekom toga procesa, njihova povećana koncentracija sprječava migraciju kalcija i fosfata iz zuba. Poznato je da prezasićenost kalcijem i fosfatom u ustima rezultira povećanom otpornošću na demineralizaciju i da je tada slina prezasićena tim ionima. Tijekom remineralizacije

mineral structure of enamel with creation of fluoridated calcium and phosphate mineral phases, and increasing the surface enamel fluoride content. The presence of low-level fluoride influences the transformation of less stable, more soluble mineral phases (dicalcium phosphate dehydrate [DCPD], octacalcium phosphate [OCP], tricalcium phosphate [TCP] to more stable, less soluble mineral phases hydroxyapatite [HAP], fluorhydroxyapatite [FHAP], fluorapatite [FAP].

The exposure to a relatively high fluoride content most likely led to calcium fluoride formation initially on the enamel surface. With the subsequent treatment of the enamel slabs with fluoride protective gel, fluoride, calcium and phosphate ions became available, which may have allowed for transformation of the surface calcium fluoride to FHAP.

Sustained-release vehicles such as varnishes may exert a long-term prophylactic effect. The agent's efficacy depends on its degree and rate of release from the carrying material. Fluoride varnishes have been found to be effective (8, 21). Sustained-release systems, including varnishes, generally show an initial burst, with rapid release of the active agent, followed by a slower phase osf release (26).

One *in vitro* study of two 5% sodium fluoride varnishes conducted in 2001 found that varnish coated on enamel slabs was then immersed in buffered calcium phosphate solution (to mimic the intraoral environment) and continued to release fluoride for five to six months (27).

In this study, chlorhexidine gel was used (Cervitec gel) alone or in combination with dental crème Tooth Mousse.

In the study of Erdem AP, the effect of two fluoride varnishes and one fluoride/chlorhexidine varnish on Streptococcus mutans and Streptococcus sobrinus on biofilm formation, was evaluated and compared *in vitro* (28). In that study, bifluoride 12 (6% naF and 6% Ca F_2) had the lowest inhibitory effect during the experimental period, although it had the highest fluoride concentration compared to Fluor Protector varnish (1% difluorsilan). These differences between the fluoride varnishes may be explained by the characteristics of the different varnishes and mechanisms of action. Bifluoride 12 has a higher viscosity than the other tested varnishes, which may have resulted in a thicker layer on the enamel slabs. Fluor Protector contains the polyurethane-based compound difluorosilane, has a low pH and forms a thin transparent film on the enamel surface. Fluor Protector contained a lower fluoride concentration than Bifluoride 12, its remineralization effect was better, and this may be explained by the silane content.

Cervitec gel used in our study contains chlorhexidine, which is chemically bis-biguanide with antibacterial, anticariogenic and remineralizing actions and few toxic effects. The addition of Cervitec gel after treatment of enamel slabs with dental crème Tooth Mousse increased the remineralisation effect of Tooth Mousse against demineralization. Also, pH values of the test varnishes and gels are parameters that should be considered.

The inhibition of demineralization and the promotion of remineralization both require the presence of sufficient quantities of calcium, phosphate and fluoride. If a higher level of these minerals can be maintained at the tooth surface prior to and during an acid attack, their increased concentration helps prevent migration of calcium and phosphate from fluorid je na površini demineralizirane cakline, na površini demineraliziranih caklinskih kristala i privlači kalcij i fosfate te se na taj način remineraliziraju kristali.

Nakon primjene topikalnih fluorida, kalcijevi se fluoridi oblikuju poput kuglica na površini zuba. Osim toga formira se i površinski pokrov fosfata kako bi smanjio topljivost kalcijevih fluoridnih depozita u slini. Kalcijevi fluoridi, poput kuglastih depozita, vjerojatno stvaraju spremnik fluorida s naknadnim oslobađanjem kalcija, fosfata i fluorida.

Viša koncentracija topikalnih fluorida i produžena primjena povećavaju količinu fluorida koja se otpušta te onu koja se odlaže, ali i njihovu dostupnost (29, 30).

Također je ustanovljeno da je količina depozita kalcijeva fluorida povezana s dostupnošću kalcija i fluoridnih iona na površini zuba.

Djelomice vezani fluorid poznat je i kao KOH-topljivi ili alkalno topljivi fluorid i inhibira demineralizaciju caklinskih kristala. Kalcijeve fluoridne kuglice i ionski fluoridi dostupni intraoralno, slabo su vezani fluoridi. Druga kategorija je čvrsto vezani fluor koji je također poznat kao alkalno netopljivi fluorid, KOH-netopljivi fluorid ili apatitno vezani fluorid koji je ugrađen u apatitne kristale. Cruz i njegovi kolege pronašli su da je to minimalno u zdravoj caklini nakon kratke izloženosti topikalnim fluoridima tijekom ispitivanja *in vitro* (31).

Attin i suradnici ističu da su u jednoj studiji demineralizirani uzorci tretirani 5-postotnim natrij-fluoridnim lakom postigli na fluoridiranim mjestima i topljivi i netopljivi KOH-fluorid (32). Čvrsto vezani fluorid nastaje nakon nekog vremena, a pritom se najprije pojavljuje difuzija dostupnih fluorida u caklinu.

U ovom istraživanju bilo je potrebno poliranje caklinskih blokova zbog relevantnosti testiranja SMH-a. Na taj test ne utječe pretjerano inherentnost poroznosti caklinskih blokova i nije toliko osjetljiv na tehniku operatera. SMH-test dovoljno je osjetljiv da se njime može otkriti rana demineralizacija cakline. Dakle, taj test, kako se čini, manje je osjetljiv na operaterove pogreške i ne utječe pretjerano na osnovnu poroznost caklinskih blokova, pa nije potrebno pregledati mnoštvo uzoraka kako bi se dobilo nekoliko prihvatljivih. (33).

Opterećenje od 50 grama izabrano je zato što se udubljenje nakon takvog opterećenja pokazalo dovoljno osjetljivim za mjerenje promjene minerala kod testiranja mikrotvrdoće isječaka (34). U dosadašnjim studijama u kojima se rabio SMH-test autori su se uglavnom koristili većim opterećenjima, primjerice od 500 g za Vikersov test tvrdoće za ocjenu promjene mineralnog sadržaja. Budući da je fokus našeg istraživanja bio na početnoj leziji, odabrali smo 50-gramsko opterećenje za procjenu SMH na temelju iskustva stečenog u našoj ranijoj studiji (35). Opterećenje od 50 g pokazalo se adekvatno kod praćenja gubitka minerala. Smatramo da je korištenje većeg opterećenja, kao što je 500 g kojim se koriste Koulourides i suradnici, previsoko za točno razlikovanje u promjeni tvrdoće koja nastaje tijekom 12 dana pH-cikličkog režima s dnevnim 6-satnim djelovanjem kiseline (36).

SMH-test ne može se rabiti za točne podatke o promjeni tvrdoće koja se događa ispod površine cakline (34). Tim se testom može izmjeriti promjena tvrdoće na različitim mjestima i na više malih površina cakline. Učinkovitost topikalne

the tooth. It is known that supersaturation with calcium and phosphate ions intraorally results in increased resistance to demineralization and that saliva is supersaturated with these ions. During remineralization, fluoride is present on the surface of the demineralized enamel on the surface of the demineralized enamel crystals and attracts calcium and phosphate ions, thereby aiding remineralization of the crystals.

Following the application of topical fluorides, calcium fluoride-like globules are formed on the tooth surface. In addition, the surface coatings of phosphates on these calcium fluoride-like deposits have been found to reduce their solubility in saliva. The calcium fluoride-like globular deposit is believed to create a fluoride reservoir, with subsequent release of calcium, phosphate and fluoride.

A higher concentration of topical fluoride and a more prolonged application increase the amount of fluoride released as well as the deposition and availability of these globules (29, 30).

The amount of calcium fluoride-like deposit has also been found to be related to the availability of calcium and fluoride ions on the tooth surface.

Partially bound fluoride is also known as KOH-soluble or alkali-soluble fluoride, and inhibits demineralization of the enamel crystals. The calcium fluoride-like globules and ionic fluoride available intraorally are loosely bound fluoride. The other category is firmly bound fluoride, which is also known as alkali-insoluble fluoride, KOH insoluble fluoride or apatitically bound fluoride, which is the fluoride, incorporated into the apatite crystals. Cruz et al. found this to be minimal in sound enamel during *in vitro* testing following brief exposures with topical fluorides (31).

Attin et al. found that demineralized samples treated with 5% sodium fluoride varnish in one study acquired both KOH-soluble and KOH-insoluble fluoride subsequent to fluoride application, at the fluoridated sites (32). Firmly bound fluoride also requires time for its acquisition, which first involves diffusion of available fluoride into the enamel.

In this study, it was necessary to polish the enamel blocks flat for reliable SMH testing. The SMH test does not appear to be overly influenced by the inherent porosity of the enamel blocks and is not as sensitive to operator technique. The SMH test has sufficient sensitivity to detect the early stages of enamel demineralization. Overall, the SMH test appears to be less susceptible to operator-related error, and is not overly influenced by the baseline porosity of the enamel blocks, thus eliminating the necessity for screening large numbers of enamel blocks to obtain a few acceptable blocks (33).

A 50-gram load was chosen because indentations made with this load have previously been shown to be a sensitive measure of mineral change in cross-sectional microhardness testing (34). Previous studies using the SMH test have generally used higher loads, such as 500 g, applied to the Vickers diamond to evaluate changes in mineral content. Since the focus of our study was on the incipient lesion, we chose a 50-gram load to evaluate SMH based on experience with 50-gram load in our previous study (35). The 50-gram load proved to be an acceptable means of following mineral loss. We consider the use of a higher load, such as the 500-gram load used by Koulourides et al. to be too high to accurately differentiate changaplikacije koncentriranog fluorida ovisi o procesu difuzije i količini ugrađenoj u caklinu tijekom određenog vremena.

Fluoridni gelovi korišteni u ovom istraživanju su: Fluorogal, Fluor Protector gel i Cervitec gel te fluoridni lakovi: Fluoridin gel N5, Bifluorid 12 i Fluor Protector. Svi su komercijalno dostupni i koriste se u mnogim zemljama. Fluoridni lakovi topikalno su se primjenjivali jedanput na tjedan kako bi se oponašala profesionalna primjena u kliničkoj praksi. Porast mikrotvrdoće dobiven u skupinama tretiranima fluoridnim gelovima ili fluoridnim lakovima upućuje na to da su i gelovi i lakovi u velikoj mjeri spriječili demineralizaciju cakline *in vitro*. Ovi rezultati u skladu su s onima iz ranijih studija (37, 38, 39) u kojima se ispitivao učinak fluoridnih gelova i lakova fluora na demineralizaciju cakline.

Budući da je u fluoridnim lakovima veća koncentracija fluorida i dulje ostaju u doticaju sa zubima, bolje su ih štitili u slučaju demineralizacije.

Fluoridin N5 bio je najučinkovitiji – oporavio je 40,49 % caklinske tvrdoće (SMH %). Caklina tretirana jedanput na tjedan Bifluoridom 12 imala je tijekom pH-ciklusa niži postotak promjene SMH-a od ostalih skupina.

Tooth Mousse se ispitivao u mnogim studijama. Kazeinfosfopeptid i amorfni kalcijev fosfat (CPP-ACP) uključuju se u nanokomplekse u zubnom plaku i na površini zuba te tako djeluju kao spremnik kalcija i fosfata (37). Istraživanja su pokazala da ugradnja CPP-ACP-a u zubni plak može znatno povećati razinu kalcija i fosfata (40, 41). Terapija pastom Tooth Mousse usmjerena na ispravljanje poremećene demineralizacijsko-remineralizacijske ravnoteže i temelji se na koncentraciji kalcijeva fosfata u plaku, tekućini plaka i slini koja je važna u prevenciji karijesa. Mineralni ioni izgubljeni zbog demineralizacije moraju se zamijeniti ionima istog oblika, veličine i električnog naboja. Otkako je za CPP-CP otkriveno da na karijesnom modelu *in situ* kod čovjeka povećava razinu kalcija i fosfata u plaku do pet puta (17, 41), predloženo je da se iskoristi kao kalcij-fosfatni spremnik jer utječe na djelovanje slobodnih kalcijevih i fosfatnih iona u tekućini plaka pomažući održavati prezasićenost s obzirom na caklinske minerale, čime smanjuje demineralizaciju cakline i potiče njezinu remineralizaciju (17).

Uysala i suradnici u svojoj su studiji *in vitro* procijenili gubitak minerala na poprečnom presjeku (37). U tim ispitivanjima *in vivo* i *in vitro* ispitanici su nasumce bili podijeljeni u dvije skupine, a svaki od njih dobio je samo jedan ispitivani materijal (Aegis Ortho ®ACP-ortodontski kompozit ili Concise® smolom modificirani kompozit) jer su osnovni klinički i radiološki pregledi, pregledi sline i laserska fluorescencija pokazali da su svi pacijenti bili u istoj opasnosti od karijesa i demineralizacijske aktivnosti. U spomenutoj studiji rezultati mikrotvrdoće pokazuju da zubi tretirani ACP-ortodontskim kompozitom znatno manje gube minerale iz cakline u usporedbi sa zubima spojenima konvencionalnim kompozitnim smolama u skupini adolescenata, ortodontskih pacijenata.

Rezultati ovog istraživanja pokazuju da je zubna pasta Tooth Mousse (10 % CPP-ACP) uspjela povećati površinsku tvrdoću cakline i remineralizirati površinsku leziju u caklini *in vitro.* Korištenje Cervitec gela u kombinaciji s Tooth Mousseom u ovom je istraživanju pokazalo povećanje SMH-a od 26,88 % u usporedbi s korištenjem samo Tooth Mou-

es in hardness occurring during 12 pH cycling regime with daily 6-hour acid challenge in our experiment (36).

The SMH test cannot be used to give accurate details of the hardness changes occurring below the enamel surface (34). SMH test gives a measure of the change in hardness occurring at multiple minisites at different locations on the enamel surface. The effectiveness of the topical applications of concentrated fluorides is dependent on the diffusion process and how much of the agent is loaded into the enamel during a given amount of time.

The fluoridated products assessed in the present study are the following, fluoride gels: Fluorogal, Fluor Protector Gel and Cervitec Gel and fluoride varnishes: Fluoridin Gel N5, Bifluorid 12 and Fluor Protector which are commercially available and commonly used in many countries. Topical application of the fluoride varnishes was done once a week to simulate professional application by a dentist. The increase of microhardness observed in the groups who received fluoride gels or fluoride varnishes indicates that both fluoride gels and varnishes were able to significantly inhibit the demineralization of enamel *in vitro*. These results are in accordance with those of previous studies (37, 38, 39) that investigated the effect of fluoride gels and fluoride varnishes on enamel demineralization.

Because fluoride varnishes had a greater concentration of fluoride and was left in contact with the teeth for a longer period of time, they had a better protective effect against demineralization, which is not statistically significant.

Fluoridin N5 was the most efficient recovering 40.49% of the enamel hardness (% SMH).The enamel pretreated with Bifluorid 12 every week during the pH cycling had a lower percentage SMH than the other groups.

Tooth Mousse has been investigated in many studies. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) involves the incorporation of the nano-complexes into dental plaque and onto the tooth surface, thereby acting as a calcium and phosphate reservoir. (37). Studies have shown that CPP-ACP incorporated into dental plaque can significantly increase the levels of plaque calcium and phosphate ions (40, 41). The therapy with dental crème Tooth Mousse directed at correcting demineralization-remineralization imbalances is based on the promise that calcium phosphate concentrations in plaque, plaque fluid and saliva play an important role in caries prevention. The lost mineral ions due to demineralization must be replaced with ions of the same shape, size and electrical charge. Since CPP-CP have been found to increase the levels of calcium and phosphate in plaque up to fivefold in human *in situ* caries models and short-term mouthwash studies (17, 41), the proposed mechanism of their anticariogenicity is that they act as a calcium-phosphate reservoir, buffering the activities of free calcium and phosphate ions in the plaque fluid helping to maintain a state of supersaturation with respect to enamel minerals, thereby depressing enamel demineralization and enhancing remineralisation (17).

Many authors have found that CPP-ACP can reduce the size of demineralized areas and promote the remineralization of enamel, but a combined application with fluoride strengthens the effect.

In the study of Uysal et al., (37) mineral loss was assessed *in vitro* by cross-sectional microhardness, a recognized anassea koji je povećao SMH-a za 21,7 %. Dobiveni rezultati u skladu su s nalazima Sudjalima i suradnika te Kargula i njegovih kolega (42, 43).

Prema Sudjalimu i suradnicima, četverosatna primjena površinskog preparata odgovara otprilike aplikaciji dva puta na tjedan (42). Caklinski izbrusci iz kontrolne skupine u našem su istraživanju uronjeni u demineralizacijsku/remineralizacijsku otopinu i nisu bili tretirani Tooth Mousseom ili fluoridnim preparatima.

Vrlo je vjerojatno da i fluoridni gelovi i fluoridni lakovi koji se rabe u studijama *in vitro* mogu znatno i dugotrajno povećati razinu fluorida u slini i zubnom plaku. Fluoridni lakovi korišteni u ovom istraživanju mogu se samo povremeno profesionalno aplicirati u ordinaciji dentalne medicine.

Trenutačno postoje tri načina remineralizacije na bazi kalcijeva fosfata koji potiču remineralizaciju: CPP-ACP, amorfni kalcijev fosfat (ACP) i sintetički minerali – sastoje se od kalcija, natrija, fosfora i silicija koji otpušta kristalni hidroksilni karbonat-apatitni (HCA), sloj koji je kemijski i strukturno isti kao mineral zuba.

Zaključak

Rezultati ovog istraživanja, dobiveni u eksperimentalnim uvjetima, pokazuju da gelovi i lakovi s velikom količinom fluorida te Tooth Mousse sam ili u kombinaciji s Cervitec gelom učinkovito inhibiraju demineralizaciju cakline. Potrebne su kliničke studije kako bismo provjerili jesu li mogući slični rezultati u složenijem okružju usne šupljine. Daljnja istraživanja trebala bi biti provedena i zbog razvoja strategije za korištenje takvih proizvoda kako bi se spriječio zubni karijes.

Zahvale

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Izjava

Autori ističu da nisu bili ni u kakvom sukobu interesa.

lytical method. In his *in vivo* and *in vitro* study the subjects were randomly divided into two equal groups and each subject received only one tested material (Aegis Ortho ®ACP-containing orthodontic composite or Concise ® resin-based composite) because the baseline clinical, radiological, salivary and laser fluorescence examinations showed that the patients were equal in regards to caries risk or demineralization activity. In this study, microhardness results show that teeth bonded with ACP-containing orthodontic composite have significantly less enamel mineral loss when compared with teeth bonded with conventional composite resin in a group of orthodontic adolescent patients.

The results of this study show that dental crème Tooth Mousse (10% CPP-ACP) was able to increase the surface hardness of enamel and remineralize subsurface lesions in human enamel *in vitro*. The use of Cervitec gel combined with Tooth Mousse in our study demonstrated 26.88% increase of SMH compared with the used of Tooth Mousse alone demonstrating 21.7% increase of SMH. Our findings are in agreement with the findings of Sudjalim et al. and Kargul et al. (42, 43).

According to Sudjalim et al., four-hourly application of topical medicaments corresponds to approximately twice-weekly applications. Enamel slabs from the control group in our study were immersed in demineralization/remineralization solution without exposure to any treatments with Tooth Mousse or fluoride products (42).

It is likely that both fluoride gels and fluoride varnishes used within the current *in vitro* study could provide a substantial and prolonged increase in fluoride levels within saliva and dental plaque in the oral environment. With the fluoride varnishes used in this study, only periodic professional application at the dental office would be possible.

There are currently three calcium phosphate-based remineralization technologies that claim to promote remineralization: CPP-ACP; amorphous calcium phosphate (ACP); and synthetic minerals composed of calcium, sodium, phosphorus and silica that release a crystalline hydroxyl-carbonate apatite (HCA) layer that is chemically and structurally the same as tooth mineral.

Conclusion

The results of the present study obtained under experimental conditions show that high fluoride gels, high fluoride varnishes, and Tooth Mousse alone and in combination with Cervitec gel effectively inhibit enamel demineralization. Clinical studies are required to check whether similar results can be obtained in the more complex oral environment. Further investigation should be carried out to develop strategies for using such products to prevent dental caries.

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Declaration

The authors deny any conflict of interesr.

Abstract

Aim: This *in vitro* study was conducted to investigate the effect of fluoride gels and varnishes comparing to CPP-ACP complex on the inhibition of enamel demineralization. **Material and Methods:** Enamel blocks were ground flat, allocated into eight groups and subjected to a daily cycling regime. Three groups were treated within the period of 10 minutes with fluoride gels: Fluorogal, Fluor Protector Gel and Cervitec Gel, one was treated only with GC Tooth Mousse and one was treated with GC Tooth Mousse (Recaldent CPP-ACP 10.0%) The remaining three groups were treated with fluoride varnishes: Fluoridin Gel N5, Bifluorid 12 and Fluor Protector. They were coated once a week before the demineralization period. All specimens were stored in artificial saliva between and after cycles. The surface microhardness (SMH) of the specimens was determined at baseline and after 12 days using HMV-2000 (Shimadzu, Japan).The percentage of SMH change (% SMC) was calculated before and after cycling regime. Data were analyzed by t-test for individual comparisons ($p<0.05$). **Results:** Statistical analysis by t-test showed significant difference between SMH before and after fluoride treatment in all groups. All the groups treated with fluoride gels, varnishes and GC Tooth Mousse showed increase in SMH. There was no significant statistical difference between the percentages of SMH of the enamel between groups. There was no statistically significant difference between the fluoride gels, varnishes and GC Tooth Mousse. **Conclusion:** The results obtained in the present study showed that high fluoride varnishes, gels and Tooth Mousse effectively inhibit demineralization under experimental conditions.

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Fluorides, Topical; Dental Enamel; Hardness ; Tooth Demineralization

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