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Assessing the Impact of Treatment Duration and Demineralization on Enamel Fluoride Uptake

Procjena o utjecaju trajanja tretmana i demineralizacije na unos fluorida u caklinu

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Abstract

Objectives: This study aimed to determine the effect of time span between exposing the enamel to calcium lactate and sodium fluoride and of a demineralized surface on the uptake of alkali-soluble fluorides. **Materials and Methods:** A total of 20 non-carious human wisdom teeth, were used. First 10 teeth, were cut into four slabs each and allocated to one of four groups: two treatment groups A) Calcium lactate followed by sodium fluoride with minimal delay (just drying between the two treatments); B) Calcium lactate followed by sodium fluoride with a delay of one hour; one of two control groups C) Sodium fluoride, as the positive control; D) Negative control group without treatment. The other 10 teeth were used for the second part of the research, each tooth was cut into 4 slabs and divided into one of two groups - the first group underwent two weeks of demineralization liquid treatment, the second group, as a control, underwent two weeks in deionized water. After two weeks, all slabs were treated with calcium lactate and followed with sodium fluoride 226ppm F-.Enamel fluoride uptake was determined by the fluoride extraction method by Caslavska and analyzed using a fluoride ion-specific electrode. **Results:** Enamel fluoride uptake showed no statistical difference regarding various durations of treatment between exposing enamel slabs to calcium lactate and sodium fluoride, as well as demineralization of the surface of the enamel. **Conclusions:** Time span between exposing enamel to calcium lactate and sodium fluoride and the demineralization process did not significantly affect the enamel uptake of alkali-soluble fluorides.

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Introduction

Dental caries, a complex disease caused by the interaction of dietary sugars, oral bacteria, and the host's oral environment, continues to pose a major public health challenge worldwide. Despite progress in dental care, a significant proportion of the population still suffers from caries, resulting in discomfort, tooth loss, and a lower quality of life. Implementing effective preventive strategies is crucial to reduce the incidence of this condition and improve overall oral health (1-3).

Preventive strategies are essential to protect against dental caries and maintain oral health. The use of fluoride is a widely recognized approach to strengthen enamel, making it more resistant to demineralization while also aiding in the remineralization process (4-8). Fluorides help prevent dental caries by suppressing the activity of bacterial enzymes that

Uvod

Zubni karijes, složena bolest prouzročena međudjelovanjem šećera iz hrane, oralnih bakterija i oralnoga okoliša domaćina, i dalje je velik izazov za javno zdravstvo diljem svijeta. Unatoč napretku u stomatološkoj skrbi još znatan dio stanovništva pati od karijesa, što rezultira nelagodom, gubitkom zuba i lošijom kvalitetom života. Provedba učinkovitih preventivnih strategija ključna je za smanjenje učestalosti toga stanja i poboljšanje cjelokupnoga oralnog zdravlja (1 – 3). Preventivne strategije ključne su za zaštitu od zubnog karijesa i održavanje oralnog zdravlja. Upotreba fluorida široko je priznat pristup u jačanju cakline zato što je čini otpornijom na demineralizaciju, a istodobno pomaže u procesu remineralizacije (4 – 8). Fluoridi pomažu u prevenciji zubnog karijesa tako što suzbijaju aktivnosti bakterijskih enzima koji proizvode kiseline i potiču pojavu kristala fluorapatita ko-

produce acids and encouraging the development of fluorapatite crystals, which are more resistant to acid dissolution than hydroxyapatite. Recent research has examined the potential for enhancing fluoride's cariostatic effects by combining it with other agents. (9).

One such agent is calcium, which has been shown to work synergistically with fluoride to enhance enamel remineralization as well, thereby offering better protection against caries (10, 11). Researchers have explored how calcium application prior to fluoride exposure can enhance fluoride uptake by dental enamel, thereby increasing the overall efficacy of fluoride treatments in preventing dental caries. This approach leverages calcium's chemical affinity for fluoride, potentially leading to better protection against tooth decay (12). However, the optimal time span between applying calcium and fluoride treatments to maximize fluoride uptake remains unclear.

Studies have shown that using a calcium pre-rinse before applying a low-concentration fluoride rinse (0.05% NaF, 226 ppm F) can significantly increase fluoride levels in saliva and dental biofilm, reduce fluoride toxicity, minimize mineral loss, and promote the formation of calcium fluoride-like deposits in dental plaque (13).

This study aimed to investigate the effect of demineralized surface and the time span between exposing enamel slabs to calcium lactate and sodium fluoride on the uptake of alkali-soluble fluorides.

Null Hypothesis (H0): The time span between calcium and fluoride exposure and the demineralization process does not significantly alter the enamel's ability to uptake fluoride when compared to the control.

Materials and methods

All experimental procedures in this blind and randomized, *in vitro* study were carried out in accordance with the Declaration of Helsinki, which outlines ethical principles for medical research involving human subjects. The study received approval from the Ethics Committee of the School of Dental Medicine, University of Zagreb, under protocol number (05-PA-30-17-4/2023).

The study included 20 human non-carious wisdom teeth extracted for orthodontic reasons, from Department of Oral Surgery of the School of Dental Medicine, from patients aged 21 to 30. All patients signed written informed consent of donation of biological materials to scientific purposes, with prior approval of the Ethics Committee. After the extraction, teeth were mechanically cleaned from the debris, disinfected with 5% NaOCl, and stored in 0,25 % chloramine solution, in sealed cup, refrigerated on 4 – 7 °C until the experimental procedures. Each tooth was brushed with manual toothbrush and universal polishing fluoride-free paste, and thoroughly washed with tap water and deionized water afterwards. Each tooth crown was inspected for sound and smooth enamel surface and then cut and divided into four slabs marked with a number and letter (a, b, c, d) and randomly allocated into one of the treatment groups. Each slab was covered in laboratory dental wax (Iskra ltd chemi-

ji su otporniji na otapanje kiseline od hidroksiapatita. Autori nedavnih istraživanja ispitivali su kako bi se mogli poboljšati kariostatički učinci fluorida kombiniranjem s drugim tvarima (9). Jedna takva tvar jest kalcij za koji se pokazalo da sinergijski s fluoridom djeluje na poboljšanje remineralizacije cakline, čime se omogućuje bolja zaštita od karijesa (10, 11). Također je istaknuto da primjena kalcija prije izlaganja fluoridu može poboljšati unos fluorida u zubnu caklinu, čime se povećava ukupna učinkovitost tretmana fluoridom u prevenciji zubnog karijesa. Tim se pristupom iskorištava kemijski afinitet kalcija prema fluoru, što potencijalno poboljšava zaštitu od karijesa (12). No ostaje nejasan optimalni vremenski raspon između primjene tretmana kalcijem i fluoridom kako bi se maksimizirao unos fluorida. U studijama je istaknuto da uporaba sredstva za prethodno ispiranje s kalcijem, prije primjene sredstva za ispiranje s niskom koncentracijom fluorida (0,05 % NaF, 226 ppm F), može znatno povećati razinu fluorida u slini i zubnom biofilmu, smanjiti toksičnost fluorida, minimizirati gubitak minerala i pospješiti stvaranje naslaga sličnih kalcijevu fluoridu u zubnom plaku (13). Tom je istraživanju svrha bila utvrditi učinak demineralizacije površine i vremenskog raspona između izlaganja pločica cakline kalcijevu laktatu i natrijevu fluoridu na unos fluorida topljivih u alkalijama.

Nulta hipoteza (H0): Vremenski raspon između izlaganja kalciju i fluoridu te proces demineralizacije ne mijenjaju značajno svojstvo cakline da unese fluorid u usporedbi s kontrolom.

Materijali i metode

Svi eksperimentalni postupci u ovoj slijepoj i randomiziranoj studiji *in vitro* provedeni su u skladu s Helsinškom deklaracijom u kojoj su navedena etička načela za medicinska istraživanja u koja su uključeni ljudi. Studija je dobila suglasnost Etičkog povjerenstva Stomatološkog fakulteta Sveučilišta u Zagrebu pod brojem protokola (05-PA-30-17-4/2023). Istraživanjem je obuhvaćeno 20 nekarijesnih umnjaka pacijenata u dobi od 21 do 30 godina izvađenih iz ortodontskih razloga, a dobiveni su od Zavoda za oralnu kirurgiju Stomatološkog fakulteta. Svi pacijenti potpisali su informirani pristanak za donaciju biološkog materijala u znanstvene svrhe, uz prethodno odobrenje Etičkog povjerenstva. Poslije ekstrakcije zubi su mehanički očišćeni, dezinficirani 5-postotnim NaOCl-om i pohranjeni do pokusa u 0,25-postotnoj otopini kloramina u zatvorenoj posudi, u hladnjaku na temperaturi od 4 do 7 °C. Svaki zub opran je ručnom četkicom za zube i univerzalnom pastom za poliranje bez fluorida, a poslije toga temeljito je ispran vodom iz slavine i deioniziranom vodom. Svaka zubna kruna pregledana je da se ustanovi ima li zdravu i glatku površinu cakline, a zatim je izrezana i podijeljena na četiri pločice označene brojem i slovom (a, b, c, d) te nasumično raspoređena u jednu od skupina za tretiranje. Svaka pločica prekrivena je laboratorijskim stomatološkim voskom (Iskra d.d. kemijska industrija, Sv. Ivan Zelina,

cal industry, Sv. Ivan Zelina, Croatia), leaving only the upper enamel surface open. Orthodontic wire was placed at the back of each slab for easier handling and covered with transparent neutral varnish to prevent any interaction with experimental solutions. Experimental solutions used in this study included calcium lactate 150mM (Farmalabor, GLJZ - Croatia), sodium fluoride 226 ppm F (Farmalabor, GLJZ - Croatia), and deionized water (E&H Renfert, Singen, Germany). The solution used for demineralization purposes was as described by Buskes et al. 1985)

Enamel fluoride uptake subject to the duration of the treatment

The first treatment group "a" was pretreated with calcium lactate 150mM followed by sodium fluoride 226 ppm F (pH – 7.6) with minimal delay (just a short drying between the two treatments). The second treatment group "b" was pretreated with calcium lactate 150mM followed by sodium fluoride 226 ppm F with a delay of one hour (leaving time for calcium to eventually adhere or penetrate the enamel) between those two treatments. The third treatment group "c" was treated with sodium fluoride only, as the positive control. The last group "d" was the negative control group – the group without treatment. Treatments with calcium lactate and/or sodium fluoride were done by slow shaking in 5 mL of selected solution and lasted for 5 minutes each.

After the treatments, the slabs were placed in polystyrene plastic cups with lids and alkali-soluble fluoride extraction was conducted by immersing the enamel slabs in a 1 M KOH solution (0.25 ml per sample) for 24h under agitation (160 rpm) at room temperature, using the method outlined by Caslavska (14). After the 24h period, all extracts were buffered with 0.5 ml of TISAB III solution containing 1.0 M HCl, and the samples were carefully removed from the tubes

Fluoride uptake in the solutions were measured using a fluoride ion-selective electrode (ISE) (Thermo Scientific™ Orion™), coupled with an ion analyzer (Orion Dual Star; Thermo Scientific) that had been previously calibrated using fluoride standards prepared to match the samples. Fluoride uptake was expressed in micrograms of fluoride per square centimeter (μg F/cm²) of enamel surface. The surface area of each slab was measured prior to treatment. The study design of the research is schematically presented in Table 1.

Enamel fluoride uptake subject to demineralization of the surface

The second part of the study was conducted using another 40 prepared enamel slabs divided into two groups - the first group of 20 slabs underwent the demineralization process for two weeks in demineralization liquid (procedure described by Buskes et al.) (15). The second group of 20 slabs were left in deionized water for two weeks and served as a control. After two weeks, all slabs were treated with calcium lactate and followed with sodium fluoride 226ppm F-. After the treatments, alkali-soluble fluoride extraction was conducted in the same way as described in the first part of this study.

Hrvatska), a ostavljena je otvorena samo gornja površina cakline. Ortodontska žica postavljena je sa stražnje strane svake pločice radi lakšeg rukovanja i prekrivena prozirnim neutralnim lakom da bi se spriječila bilo kakva interakcija s eksperimentalnim otopinama. Eksperimentalne otopine korištene u ovom istraživanju bile su kalcijev laktat 150 mM (Farmalabor, GLJZ – Hrvatska), natrijev fluorid 226 ppm F (Farmalabor, GLJZ – Hrvatska) i deionizirana voda (E&H Renfert, Singen, Njemačka). Otopina korištena za potrebe demineralizacije bila je kao što su opisali Buskes i suradnici. (1985.) (15).

Unos fluorida u caklinu ovisno o trajanju tretmana

Prva skupina „A" najprije je tretirana kalcijevim laktatom od 150 mM, a zatim natrijevim fluoridom od 226 ppm F (pH – 7,6) uz minimalnu odgodu (samo kratko sušenje između dvaju tretmana). Druga skupina „B" najprije je tretirana kalcijevim laktatom od 150 mM poslije čega je slijedio natrijev fluorid od 226 ppm F s odgodom od jednog sata između tih dvaju tretmana (ostavljeno je vrijeme da se kalcij eventualno prilijepi ili prodre u caklinu). Treća skupina „C" tretirana je samo natrijevim fluoridom kao pozitivnom kontrolom. Posljednja skupina „D" bila je negativna kontrolna skupina – skupina bez tretmana. Tretmani s kalcijevim laktatom i/ili natrijevim fluoridom rađeni su polaganim miješanjem u 5 mL odabrane otopine i trajali su po 5 minuta. Poslije tretmana pločice su stavljene u polistirenske plastične čaše s poklopcima i provedena je ekstrakcija fluorida topljivog u alkalijama uranjanjem caklinskih pločica u 1 M otopinu KOH-a (0,25 mL po uzorku) tijekom 24 sata uz miješanje (160 okretaja u minuti) na sobnoj temperaturi, korištenjem metode koju je opisala Caslavska (14). Poslije 24 sata svi su ekstrakti puferirani s 0,5 mL otopine TISAB III koja je sadržavala 1,0 M HCl-a, a uzorci su pažljivo uklonjeni iz čašica. Otpuštanje fluorida u otopine mjereno je fluoridnom ionselektivnom elektrodom (ISE) (Thermo Scientific™ Orion™) spojenom s ionskim analizatorom (Orion Dual Star; Thermo Scientific) koji je prije toga kalibriran korištenjem fluoridnih standarda pripremljenih da odgovaraju uzorcima. Unos fluorida izražen je u mikrogramima fluorida po četvornom centimetru (μg F/cm²) površine cakline. Površina svake pločice izmjerena je prije obrade. Protokol istraživanja shematski je prikazan u tablici 1.

Unos fluorida u caklini u odnosu prema demineralizaciji površine

Drugi dio studije proveden je korištenjem još 40 pripremljenih pločica cakline podijeljenih u dvije skupine – prva skupina od 20 pločica podvrgnuta je procesu demineralizacije dva tjedna u tekućini za demineralizaciju (postupak opisali Buskes i sur.) (15), a druga skupina, također od 20 pločica, bila je dva tjedna u deioniziranoj vodi i služila je kao kontrola. Poslije dva tjedna sve pločice su tretirane kalcijevim laktatom i zatim natrijevim fluoridom 226 ppm F-. Poslije tretmana provedena je ekstrakcija fluorida topljivog u alkalijama na jednaki način kao što je to opisano u prvom dijelu ove studije. Svi prikupljeni podatci uneseni su i složeni u Microsoftovu datoteku Office Excel. Provjerena je normal-

Table 1. Schematic representation of the study design of exploring the comparative efficacy of calcium pre-treatment in modulating enamel reactivity with the time span from exposure to calcium lactate to fluoride.
Tablica 1. Shematski prikaz dizajna istraživanja usporedne učinkovitosti predtretmana kalcijem u modulaciji reaktivnosti cakline s vremenskim rasponom od izlaganja kalcijevu laktatu do fluorida

Group a (n = 10)	Group b (n = 10)	Group c (n = 10)	Group d (n = 10)
Pretreated with calcium lactate followed by sodium fluoride with minimal delay (just drying between the two treatments) • Prethodno tretiran kalcijevim laktatom nakon čega slijedi natrijev fluorid s minimalnom odgodom (samo sušenje između dvaju tretmana)	Pretreated with calcium lactate followed by sodium fluoride with a delay of one hour (leaving time for calcium to eventually adhere or penetrate the enamel) • Prethodno tretiran kalcijevim laktatom, a zatim natrijevim fluoridom s odgodom od jednog sata (ostavljeno je vrijeme da se kalcij eventualno zalijepi ili prodre u caklinu)	Treated with sodium fluoride only, as the positive control • Tretirano samo natrijevim fluoridom kao pozitivnom kontrolom	Negative control group – the group without treatment. • Negativna kontrolna skupina – skupina bez tretmana
Fluoride extraction was conducted by immersing the enamel slabs in a 1 M KOH solution for 24h under agitation at room temperature. After the 24h period, all extracts were buffered with TISAB III solution containing 1.0 M HCl. • Ekstrakcija fluora provedena je uranjanjem caklinskih pločica u 1 M otopinu KOH-a tijekom 24 sata, uz miješanje na sobnoj temperaturi. Nakon 24 sata svi su ekstrakti puferirani otopinom TISAB III koja je sadržavala 1,0 M HCl-a The extracts were analyzed using fluoride ion-specific electrode • Ekstrakti su analizirani elektrodom specifičnom za fluoridne ione			

Table 2. Schematic representation of the study design of exploring the comparative efficacy of calcium pre-treatment in modulating enamel reactivity during the enamel demineralization process
Tablica 2. Shematski prikaz dizajna istraživanja komparativne učinkovitosti predtretmana kalcijem u modulaciji reaktivnosti cakline tijekom procesa demineralizacije cakline

Group I (n=20)	Group II (n=20)
Treated for two weeks in demineralization liquid. After two weeks, all slabs were treated with calcium lactate and followed with sodium fluoride 226ppm F^- • Tretirana dva tjedna u tekućini za demineralizaciju. Nakon dva tjedna sve su pločice tretirane kalcijevim laktatom i zatim natrijevim fluoridom 226 ppm F-	The second group was two weeks in deionized water, as a control. After two weeks, all slabs were treated with calcium lactate and followed with sodium fluoride 226ppm F^- • Druga skupina bila je kao kontrola dva tjedna u deioniziranoj vodi . Nakon dva tjedna sve pločice su tretirane kalcijevim laktatom i zatim natrijevim fluoridom 226 ppm F-
Fluoride extraction was conducted by immersing the enamel slabs in a 1 M KOH solution for 24h under agitation at room temperature. After the 24h period, all extracts were buffered with TISAB III solution containing 1.0 M HCl • Ekstrakcija fluora provedena je uranjanjem caklinskih pločica u 1 M otopinu KOH-a tijekom 24 sata, uz miješanje na sobnoj temperaturi. Nakon 24 sata svi su ekstrakti puferirani otopinom TISAB III koja je sadržavala 1,0 M HCl-a The extracts were analyzed using fluoride ion-specific electrode • Ekstrakti su analizirani elektrodom specifičnom za fluoridne ione	

All collected data were entered, imputed and arranged in a Microsoft Office Excel file. The normality of data distribution was checked, and alkali-soluble fluoride data was transformed using $\log_{10}$ Wilcoxon Matched Pairs Test and Friedman ANOVA was employed to analyze the impact of both the substrate and treatments. The study design of this part of the research is schematically presented in Table 2.

Results

Treatment duration

In all comparisons of observed enamel slab groups (0.635±0.443vs0.661±0.838vs 0.382±0.18vs 0.07052±0.0241 µg F/cm2, for groups Ca lactate with minimum delay - NaF, Ca lactate with delayed NaF, NaF only, no treatment) there were no statistically significant differences observed between any pair of treatment groups as indicated by the p-values being greater than 0.05. The only statistically significant difference was observed between enamel slabs treated with sodium fluoride only(group c) and those in the negative control group(group d), with a p-value of 0.028, which was expected effect of sodium fluoride.

The mean value and standard deviation (SD) of alkali-soluble fluoride after evaluating the impact of the time span

nost distribucije podataka, a podatci o fluoridu topljivom u alkalijama transformirani su korištenjem log10 Wilcoxonova testa parova i Friedmanova testa ANOVA za analizu utjecaja i supstrata te tretmana. Studijski dizajn toga dijela istraživanja shematski je prikazan u tablici 2.

Rezultati

Trajanje tretmana

U svim usporedbama promatranih skupina caklinskih pločica (0,635 ± 0,443 vs. 0,661 ± 0,838 vs. 0,382 ± 0,18 vs. 0,07052 ± 0,0241 µg F/cm^2, za skupine Ca laktat s minimalnom odgodom – NaF, Ca laktat s odgođenim NaF-om, samo NaF bez tretmana) nije bilo statistički značajne razlike uočene između bilo kojeg para tretiranih skupina, kao što je naznačeno p-vrijednostima koje su veće od 0,05. Jedina statistički značajna razlika zabilježena je između caklinskih pločica tretiranih samo natrijevim fluoridom (skupina C) i onih u negativnoj kontrolnoj skupini (skupina D), s p -vrijednošću od 0,028, što je očekivani učinak natrijeva fluorida. Srednja vrijednost i standardna devijacija (SD) fluorida topljivog u alkalijama poslije procjene utjecaja vremenskog raspona izme-

between exposing enamel slabs to calcium lactate and sodium fluoride are presented in **Figure 1**.

Demineralization process

The comparison between the control group and demineralized samples showed that the demineralization process did not significantly alter the enamel's ability to uptake fluoride when compared to the control (15.1696±4.1059 vs 12.9853 ± 6.0061µg F/cm2) since there was no significant difference found. The mean value and standard deviation (SD) of alkali-soluble fluoride after investigating the comparative efficacy of calcium pretreatment, followed by fluoride, all following the demineralization process of enamel are presented in **Figure 2**.

đu izlaganja pločica cakline kalcijevu laktatu i natrijevu fluoridu prikazani su na slici 1.

Proces demineralizacije

Usporedba između kontrolne skupine i demineraliziranih uzoraka pokazala je da proces demineralizacije nije značajno promijenio svojstvo cakline da apsorbira fluorid u usporedbi s kontrolom (15,1696 ± 4,1059 prema 12,9853 ± 6,0061µg F/cm^2) zato što nije pronađena značajna razlika. Srednja vrijednost i standardna devijacija (SD) fluorida topljivog u alkalijama poslije ispitivanja komparativne učinkovitosti predtretmana kalcijem praćenoga fluoridom, a sve nakon procesa demineralizacije cakline, prikazani su na slici 2.

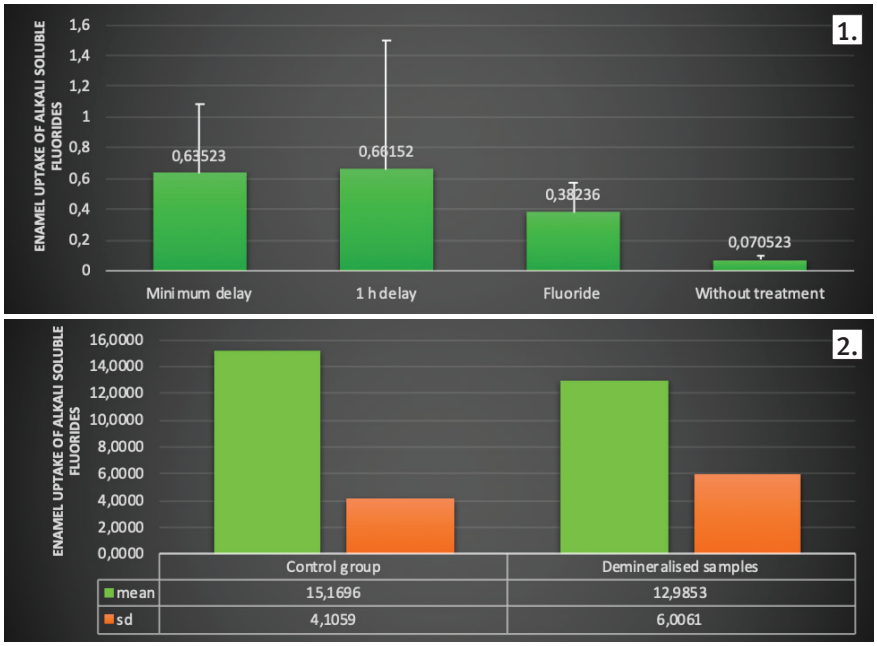

Figure 1. The effect of treatment duration between exposing enamel slabs to calcium lactate and to sodium fluoride, on enamel uptake of alkali soluble fluorides.
Slika 1. Učinak trajanja tretmana između izlaganja pločica cakline kalcijevu laktatu i natrijevu fluoridu na unos alkalno topljivih fluorida
Figure 2. Impact of demineralization process on enamel fluoride uptake.
Slika 2. Utjecaj procesa demineralizacije na unos fluorida u caklinu

Discussion

Fluoride plays a vital role in preventing dental caries, which remains the most prevalent preventable chronic disease globally. Its key function lies in halting the process of demineralization and enhancing remineralization of tooth enamel. Recognizing the growing burden of dental caries, the World Health Organization (WHO) promotes fluoride use as a cornerstone of its Global Oral Health Strategy for the 21st century. This approach includes population-wide fluoridation measures, such as water fluoridation, which WHO endorses as one of the most cost-effective methods to reduce tooth decay. Additionally, the regular use of fluoride-containing toothpastes complements these efforts, particularly in areas with limited access to dental care (16, 17).

The present study investigated the efficacy of calcium pretreatment followed by fluoride application on enamel fluoride uptake, focusing on the impact of the demineralization process of the surface and the treatment period.

The results showed in all comparisons of observed enamel slab groups (0.635±0.443vs0.661±0.838vs 0.382±0.18vs 0.07052±0.0241µg F/cm2, for groups Ca lactate with min-

Rasprava

Fluorid je vitalno važan u prevenciji zubnog karijesa koji diljem svijeta ostaje najčešća kronična bolest, a može se spriječiti. Njegova ključna funkcija jest zaustavljanje procesa demineralizacije i pospješivanje remineralizacije zubne cakline. Prepoznajući sve veći teret zubnog karijesa, Svjetska zdravstvena organizacija (WHO) promiče upotrebu fluorida kao temeljni kamen svoje Globalne strategije oralnoga zdravlja za 21. stoljeće. Taj pristup obuhvaća mjere fluoridacije za cijelu populaciju, kao što je fluoridacija vode koju WHO podupire kao jednu od najisplativijih metoda za smanjenje karijesa. Uz to, redovita uporaba pasta za zube koje sadržavaju fluorid nadopunjuje te napore, osobito u područjima s ograničenim pristupom stomatološkoj skrbi (16, 17).

Usredotočujući se na utjecaj procesa demineralizacije površine i razdoblja tretmana, autori ove studije istraživali su učinkovitost predtretmana kalcijem praćenoga primjenom fluorida na unos fluorida u caklinu. Rezultati su pokazali da u svim usporedbama promatranih skupina pločica cakline (0,635 ± 0,443 vs. 0,661 ± 0,838 vs. 0,382 ± 0,18 vs. 0,07052 ± 0,0241µg F/cm^2, za skupine Ca laktat s minimalnom od-

imum delay - NaF, Ca lactate with delayed NaF, NaF only, no treatment) there were no statistically significant differences observed between any pair of treatment groups. This is consistent with previous research (18), which also reported no significant differences in fluoride uptake with varying time intervals between calcium and fluoride treatments. Both studies suggest that the timing of calcium application relative to fluoride does not critically influence fluoride uptake. These findings offer valuable insights for clinicians in preventive dentistry, thus emphasizing that the timing of fluoride application is less important than the use of calcium pretreatment and the correct application sequence. Such confirmed results are particularly beneficial for patients at higher risk of caries and non-cooperative patients who cannot apply fluoride over a specific period, providing a more adaptable approach to caries prevention.

However, our study found a statistically significant difference between enamel slabs treated with sodium fluoride only (group C) and those in the negative control group (group D), with a p-value of 0.028. This expected result aligns with the study by Mellberg and Chomicki (19), which demonstrated a significant increase in fluoride uptake in enamel treated with sodium fluoride compared to untreated controls. The effectiveness of sodium fluoride in promoting enamel remineralization is well-documented also in the literature (4), (20).

On the demineralization part of the study, findings showed no significant difference in fluoride uptake between the control group and the demineralized samples. This suggests that the demineralization process did not significantly alter the enamel's ability to incorporate fluoride. This result is consistent with the study by González-Cabezas et al. (2012) (21), which also found that demineralized enamel can effectively uptake fluoride, supporting the potential of fluoride treatments in re-mineralizing early carious lesions. We expected an increased uptake due to a significant lack of minerals in demineralized enamel, but this was not the case since the uptake is regulated by the presence of calcium ions, the integrity of the enamel surface, and the concentration of fluoride in the surrounding environment. Calcium ions are essential for forming stable calcium-fluoride complexes on the enamel surface (22).

These findings hold important clinical relevance. They indicate that sodium fluoride on its own is effective at increasing fluoride uptake in enamel, and the timing of calcium lactate application does not play a major role in this process. This insight can be used to refine fluoride treatment protocols in dental practice, maximizing remineralization outcomes for patients, which is key for long-term prevention and sustained oral health. Modern fluoride protocols in dentistry aim to prevent dental caries by using various ion-releasing dental materials and fluoride products that cater to different patient needs (23, 24). Common fluoride protocols include fluoride toothpastes, fluoride mouth rinses, fluoride gels and varnishes, and fluoridated water (25, 26). Silver diamine fluoride (SDF) is a new protocol, which is used to arrest active dental caries. It is commonly used in patients who cannot undergo traditional restorative procedures, especially in children and elderly populations (27).

godom – NaF, Ca laktat s odgođenim NaF-om, samo NaF bez tretmana) nije bilo statistički značajnih razlika između bilo kojeg para tretiranih skupina. To je u skladu s dosadašnjim istraživanjem (18) čiji su autori također istaknuli da nema znatnih razlika u unosu fluorida s različitim vremenskim intervalima između tretmana kalcijem i fluoridom. U obje studije pokazano je da vrijeme primjene kalcija u odnosu na fluorid ne utječe kritično na unos fluorida. Ta otkrića omogućuju kliničarima dragocjene uvide u preventivnoj stomatologiji, te se ističe da je vrijeme primjene fluorida manje važno od upotrebe predtretmana kalcijem i pravilnoga slijeda primjene. Takvi potvrđeni rezultati posebno su korisni za pacijente s većim rizikom od karijesa i one nesuradljive koji ne mogu primijeniti fluorid tijekom određenog razdoblja zato što omogućuju prilagodljiviji pristup prevenciji karijesa.

No mi smo u našoj studiji otkrili statistički značajnu razliku između pločica cakline tretiranih samo natrijevim fluoridom (skupina C) i onih u skupini negativne kontrole (skupina D) s p-vrijednošću od 0,028. Taj očekivani rezultat u skladu je sa studijom koju su proveli Mellberg i Chomicki [19] i u kojoj su zabilježili značajno povećanje unosa fluorida u caklini tretiranoj natrijevim fluoridom u usporedbi s netretiranom kontrolom. Učinkovitost natrijeva fluorida u poticanju remineralizacije cakline dobro je dokumentirana i u drugoj literaturi (4), (20).

Kad je riječ o dijelu studije o demineralizaciji, nalazi nisu pokazali značajnu razliku u unosu fluorida između kontrolne skupine i demineraliziranih uzoraka. To sugerira da proces demineralizacije nije značajno promijenio svojstvo cakline da unese fluorid. Taj je rezultat u skladu sa studijom González-Cabezasa i suradnika (2012.) (21) koji su također otkrili da demineralizirana caklina može učinkovito apsorbirati fluorid i tako poduprijeti potencijal tretmana fluoridom u remineralizaciji ranih karijesnih lezija. Očekivali smo povećani unos zbog značajnog nedostatka minerala u demineraliziranoj caklini, ali to nije bio slučaj jer je unos reguliran prisutnošću kalcijevih iona, cjelovitošću površine cakline i koncentracijom fluorida u okolnom okolišu. Ioni kalcija prijeko su potrebni za stvaranje stabilnih kompleksa kalcija i fluorida na površini cakline (22).

Ti su nalazi klinički veoma važni. Naime, oni pokazuju da je natrijev fluorid sam po sebi učinkovit u povećanju unosa fluorida u caklinu, a vrijeme primjene kalcijeva laktata nema važnu ulogu u tom procesu. Ovaj se uvid može koristiti za usavršavanje protokola liječenja fluorom u stomatološkoj ordinaciji zato što maksimizira kod pacijenata rezultate remineralizacije, što je ključno za dugoročnu prevenciju i održivo oralno zdravlje. Suvremenim protokolima s fluorom u stomatologiji se želi spriječiti zubni karijes korištenjem različitih dentalnih materijala koji otpuštaju ione i proizvoda s fluorom koji zadovoljavaju različite potrebe pacijenata (23, 24). Uobičajeni protokoli s fluorom uključuju paste za zube s fluorom, sredstva za ispiranje usta s fluorom, fluoridne gelove i lakove te fluoridiranu vodu (25, 26). Srebrov diamin-fluorid (SDF) noviji je protokol koji se primjenjuje za zaustavljanje aktivnoga zubnog karijesa. Obično se koristi kod pacijenata koji se ne mogu podvrgnuti tradicionalnim restauracijskim postupcima, osobito kad je riječ o djeci i starijoj populaciji (27).

This study has several limitations. Since it was conducted *in vitro*, the results may not entirely reflect the conditions of the oral cavity. Factors such as saliva flow, oral microbiota, and dietary habits can affect fluoride absorption and enamel remineralization in vivo (28). Additionally, the study assessed the treatments over a short duration, and further long-term research is needed to evaluate the sustainability and enduring effects of these treatments on enamel remineralization.

Conclusions

The time span between exposing enamel slabs to calcium lactate and sodium fluoride did not significantly affect enamel uptake of alkali-soluble fluorides. Based on these findings, it is suggested that the sodium fluoride products may be applied immediately after calcium lactate. Findings from demineralization processes indicate that the demineralization process did not significantly alter the enamel's ability to uptake fluoride when compared to the control.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: All experimental procedures in this blind and randomized, *in vitro* study were carried out in accordance with the Declaration of Helsinki, which outlines ethical principles for medical research involving human subjects. The study received approval from the Ethics Committee of the School of Dental Medicine, University of Zagreb, under protocol number (05-PA-30-17-4/2023).

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Ova studija ima nekoliko ograničenja. Budući da je provedena *in vitro*, rezultati možda neće potpuno odražavati stanje usne šupljine. Čimbenici kao što su protok sline, oralna mikrobiota i prehrambene navike mogu utjecati na apsorpciju fluorida i remineralizaciju cakline *in vivo* (28). Uz to, u studiji su procijenjeni tretmani u kratkom trajanju, a daljnja dugotrajna istraživanja nužna su za procjenu održivosti i trajnih učinaka tih tretmana na remineralizaciju cakline.

Zaključak

Vremenski raspon između izlaganja pločica cakline kalcijevu laktatu i natrijevu fluoridu nije značajno utjecao na unos alkalno topljivih fluorida. Na temelju tih nalaza predlaže se da se proizvodi s natrijevim fluoridom mogu primijeniti odmah nakon kalcijeva laktata. Nalazi poslije procesa demineralizacije pokazuju da sam proces demineralizacije nije značajno promijenio svojstvo cakline da apsorbira fluorid u usporedbi s kontrolom.

Sukob interesa: Autori nisu bili u sukobu interesa.

Etičko odobrenje: Svi eksperimentalni postupci u ovoj slijepoj i randomiziranoj studiji *in vitro* provedeni su u skladu s Helsinškom deklaracijom u kojoj su navedena etička načela za medicinska istraživanja u koja su uključeni ljudi. Studija je dobila suglasnost Etičkog povjerenstva Stomatološkog fakulteta Sveučilišta u Zagrebu pod brojem protokola 05-PA-30-17-4/2023.

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Doprinos autora: **F. K. S.** – prikupljanje podataka, formalna analiza i interpretacija, odgovornost za vizualizaciju i pisanje izvornog nacrta, odobrio konačnu verziju teksta; **K. S.** – koncept i dizajn, formalna i statistička analiza, interpretacija podataka; **K. P.** – koncept i dizajn, nadzor, interpretacija podataka, kritički osvrt i redaktura, odobrio konačnu verziju teksta; **I. Š.** – sudjelovao u ideji i dizajnu, vodio studiju, kritički osvrt i uređivanje, odobrio konačnu verziju teksta; **K. B.** – pridonio provođenju istraživanja, kritički osvrt i redakcija, odobrio konačnu verziju teksta

Sažetak

Cilj rada: Ovim se istraživanjem želio utvrditi učinak vremenskog raspona između izlaganja cakline kalcijevu laktatu i natrijevu fluoridu te demineralizacije površine nakon ugradnje alkalno topljivih fluorida. **Materijali i metode:** Korišteno je ukupno 20 nekarijesnih ljudskih umnjaka. Svaki od prvih 10 zuba izrezan je u četiri pločice i raspoređen u jednu od četiriju skupina – u dvije tretirane skupine: A) kalcijev laktat nakon čega slijedi natrijev fluorid s minimalnim odgodom (samo sušenje između dvaju tretmana) i B) kalcijev laktat nakon čega slijedi natrijev fluorid s odgodom od jednog sata, te u dvije kontrolne skupine: C) natrijev fluorid kao pozitivna kontrola i D) negativna kontrolna skupina bez tretmana. Ostalih 10 zuba korišteno je za drugi dio istraživanja – svaki zub izrezan na 4 pločice dodijeljen je u jednu od dviju skupina. U prvoj su pločice zuba bile dva tjedna podvrgnute tretmanu tekućinom za demineralizaciju, a u drugoj, kao kontroli, dva su tjedna bile u deioniziranoj vodi. Nakon dva tjedna sve su pločice tretirane kalcijevim laktatom i zatim natrijevim fluoridom od 226 ppm F-. Unos fluorida u caklinu određen je metodom ekstrakcije fluorida prema Caslavskoj i analiziran s pomoću elektrode specifične za fluoridne ione. **Rezultati:** Apsorpcija fluorida u caklini nije pokazala statističku razliku s obzirom na različita trajanja tretmana između izlaganja pločica cakline kalcijevu laktatu i natrijevu fluoridu, kao ni demineralizacija površine cakline. **Zaključak:**Vremenski raspon između izlaganja cakline kalcijevu laktatu i natrijevu fluoridu te proces demineralizacije nisu značajno utjecali na ugradnju alkalno topljivih fluorida u caklinu.

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