

# Uticaj sredstava za irigaciju na odnos kalcijuma i fosfora u dentinu korena zuba

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## The effect of irrigating solutions on the Ca/P ration in root dentine

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### KRATAK SADRŽAJ

**Cilj** ovog istraživanja je bio da se ispita uticaj Na-EDTA i NaOCl na mineralni sastav dentina korena zuba (Ca/P) primenom SEM i EDS mikroanalize. **Materijal i metod:** Korišćeno je 22 uzorka dentina srednje trećine korena prvih premolara koji su ekstrahovani iz ortodontskih razloga. Uzorci su polirani i podeljeni u četiri eksperimentalne grupe. Prva grupa uzoraka je ispirana fiziološkim rastvorom i služila je kao kontrola. Druga grupa je tretirana pomoću 15% Na-EDTA u trajanju od jednog minuta sa naknadnom irigacijom pomoću fiziološkog rastvora. Treća grupa uzoraka je podvrgnuta irigaciji sa 5,25% NaOCl bez ikakvog pretrretmana, i poslednja, kombinovanom ispiranjem i Na-EDTA i NaOCl. **Rezultati** su pokazali da primena Na-EDTA u kombinaciji sa NaOCl kao završnim ispiranjem i NaOCl posebno, značajno menjaju odnos Ca/P u dentinu korena zuba. Završna irigacija sa NaOCl povećava efektivnost helatnih sredstava.

**Ključne reči:** dentin korena zuba, mineralni sastav, odnos Ca/P, irigacija Na-EDTA, NaOCl, SEM, EDS.

### SUMMARY

**The aim** of this study was to evaluate the effect of Na-EDTA and NaOCl in mineral contents in root dentine using SEM and EDS. **Material and method:** Twenty-two specimens of the middle radicular third obtained from human first molars, extracted for orthodontic reasons, were examined. Specimens were polished and divided into four groups. The first group was treated with saline and used as control. The second group was treated with Na-EDTA for one minute, followed by saline irrigation. The third group was treated with combination of Na-EDTA and NaOCl and the last, fourth group was treated with NaOCl only. **Results** have shown that Na-EDTA combined with NaOCl as the final flush and NaOCl alone, have significantly influenced changes in the Ca/P ration of the root dentine. The use of NaOCl as the final flush altered the effectiveness of chelating agents in root dentine.

**Key words:** root dentine, mineral contents, Ca/P ratio, irrigation Na-EDTA, NaOCl, SEM, EDS.

Dentin je mineralizovano tkivo endodoncijuma koje čini najveći deo zubne mase. Sastoji se iz organske i neorganske komponente. Glavne neorganske komponente tvrdog zubnog tkiva su Ca i P koji su raspoređeni u vidu kristala hidroksiapatita. Odnos Ca/P u hidroksiapatitu dentina iznosi oko 1,67 i zavisi od mnogobrojnih faktora: stepena mineralizacije, tipa kristala, starosti tkiva, anatomske lokacije, kao i tehnika koje služe za određivanje njihovog

Dentine is mineralised tissue of the endodontium and presents the largest part of the dental mass. It is composed of organic and inorganic elements. The main inorganic components of the hard dental tissue are Ca and P distributed in the form of hydroxiapatite crystals. The Ca/P ratio in hydroxiapatite of dentine is approximately 1.67 and is dependent on many factors: mineralisation level, type of the crystals, age of the tissue, anatomical site tested, and

odnosa<sup>1</sup>. Utvrđeno je takođe da neki hemijski agensi, koji se koriste za uklanjanje razmaznog sloja u kanalu korena zuba, mogu da uzrokuju promenu u hemijskoj strukturi humanog dentina i menjaju odnos Ca/P na dentinskoj površini. Promene u relaciji Ca/P mogu da utiču na normalan odnos između organskih i neorganskih komponenti, i na permeabilnost i rastvorljivost dentina, a što se može odraziti i na adheziju materijala za (definitivnu) opturaciju kanala korena<sup>2,3</sup>.

Helatni rastvori se u endodontskoj terapiji, koriste jer olakšavaju biomehaničku preparaciju kanala korena zuba i uklanjaju razmazni sloj. Razmazni sloj (*Smear layer*) nastaje kao posledica instrumentacije zidova kanala korena i sadrži neorganske i organske supstance koje uključuju fragmente odontoblastnih nastavaka, mikroorganizme i nekrotični materijal<sup>4</sup>.

Najčešće korišćena helatna sredstva za irigaciju kanala korena sadrže EDTA<sup>5</sup>. Efikasnost ovih sredstava zavisi od njihove koncentracije, pH vrednosti i vremena aplikacije. Dubina penetracije helatora takođe je determinisana starošću i tvrdoćom dentina, dužinom kanala korena i drugim individualnim morfološkim karakteristikama ovog mineralizovanog tkiva<sup>6</sup>.

Da bi se dobio maksimalni efekat čistih zidova u toku i posle instrumentacije u kanalu korena, neophodno je nakon aplikacije helatnih sredstava primeniti i rastvore za organski deo dentina. Najefikasniji način uklanjanja organskih i neorganskih ostataka razmaznog sloja u kanalu korena je irigacija pomoću EDTA sa naknadnim ispiranjem pomoću NaOCl<sup>6</sup>.

Za kvantitativnu analizu mineralnog sastava dentina koriste se brojne metode

Hemijske metode služe za identifikaciju malih količina konstitucionih minerala kao i za kvantitativnu analizu njihovog odnosa, ali nisu pogodne za praćenje odnosa Ca/P u dentinu korena zuba *in situ*, jer većina dovodi do značajne destrukcije dentinskih konstituenata. Za identifikaciju i semi-kvantitativnu karakterizaciju hemijskih elemenata (kao i odnosa Ca/P) prisutnih u dentinu korena zuba, najčešće se koriste x-zračna energetska-disperzivna spektroskopija (EDS) ili atomska disperzivna spektrometrija (ADS).

**Cilj** ovog rada je bio da se ispita uticaj sredstava za irigaciju kanala korena Na-EDTA i NaOCl na mineralni sastav površine dentina korena zuba (Ca/P odnos), korišćenjem SEM i EDS mikroanalize.

## Materijal i metode

U istraživanjima je korišćeno 11 prvih premolara ekstrahovanih iz ortodontskih razloga. Kod svih zuba odstranjena je krunica i apikalna trećina korena (4 mm od vrha korena), a preostali deo korena je uzdužno presecan na dva jednaka segmenta. Unutrašnje površine 22 uzorka su potom polirane abrazivnim diskovima (od najgrubljih

on techniques implemented for the assessment of their relation. It has been, also, confirmed that some chemical agents used for removal of the smear layer are capable of influencing changes in chemical structure of human dentine and further alterations in the Ca/P ratio on dental surface. Changes in the Ca/P ratio can affect normal relation of organic and inorganic components as well as permeability and solubility of dentine, which is reflected on adhesion of the root canal obturative material.

Chelating solutions are often used in endodontic treatment because they make easier to complete biomechanical procedure of the root canal and the removal of the smear layer. Smear layer is the consequence of instrumentation of the root canal walls and it contains inorganic and organic substances, including odontoblasts fragments, microorganisms and necrotic particles.

The most common chelating agents, used for irrigation of the root canal, are containing EDTA. Efficacy of these agents depends on their concentration, pH values and duration of application. Dentine age and hardness, root canal length and other individual morphological characteristics are determining the penetration depth of chelators.

In order to obtain the highest level of clean walls during and after the root canal instrumentation, it is necessary to apply solutions for the organic part of dentine following chelating agents. The most efficient way of removing organic and inorganic particles of the smear layer in the root canal is irrigation with EDTA and subsequent use of NaOCl.

There are numerous methods for quantitative analysis of mineral contents in dentine.

Small amounts of constitutional minerals can be traced chemically and then quantitatively analysed but the Ca/P ratio in root dentine *in situ* cannot be followed up because dentine components are usually destroyed by chemical impact. For identification and semiquantitative measurements of chemical elements (and of the Ca/P ratio) that are present in root dentine, x-ray-energetic-dispersion spectroscopy (EDS) or atomic dispersion spectrometry (ADS) are used most commonly.

**The aim** of this paper was to evaluate the effect of root canal irrigating agents NaOCl and EDTA on mineral contents in dentine (Ca/P ratio) by use of SEM and EDS microanalysis.

## Material and methods

Total of eleven first premolars, extracted for orthodontic reasons, were used in the study. All teeth had cut crown and apical third of the root (4mm from apex), and remaining part of the root was longitudinally dissected in two equal segments. Inner surface of 22 specimens were then polished with abrasive discs (rough to finest). Dur-

do najfinijih). U toku i posle poliranja, uzorci su ispirani destilovanom vodom, a zatim, sušeni u termostatu na 37°C u trajanju od 30 minuta<sup>7</sup>. Uzorci su potokm podeljeni u četiri grupe, i svaka polovina zuba je tretirana različitim rastvorima uz pomoć šprica.

- I grupa: kontrolni uzorci; 10 ml fiziološki rastvor (4 uzorka),
- II grupa: 15 % Na-EDTA (Complexon III-”Merck”, pH=7,2) 1 minut + 10 ml fiziološki rastvor (6 uzoraka),
- III grupa: 10 ml 5,25% NaOCl (6 uzoraka),
- IV grupa: 15% NaEDTA 1 minut + 10 ml 5,25% NaOCl (6 uzoraka).

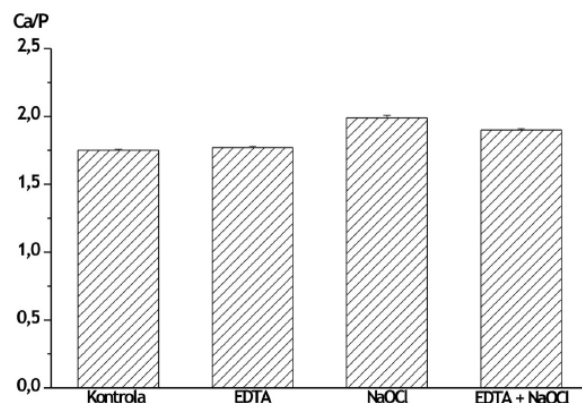
Posle tretmana Na-EDTA i NaOCl, uzorci su sušeni na sobnoj temperaturi i pripremani konvencionalnom metodom za elektronsku mikroskopiju. Nivoi Ca i P u dentinu korena zuba mereni su pomoću SEM i EDS mikroanalize na JEOL-JSM-5300. Zbog nemogućnosti da se upadni elektronski snop usmeri uvek na isto mesto, merenja su urađena na površini dentina na najmanje tri bliske tačke, u cilju određivanja prosečnog sadržaja Ca i P u regijama svakog uzorka. Zabeležene su promene u nivou minerala i razlike između grupa su statistički analizirane.

Statistička analiza podataka obuhvatila je testiranja značajnosti promena srednje vrednosti Ca/P indeksa kod tretiranih grupa u odnosu na kontrolnu grupu (t-testom), kao i između tretiranih grupa analizom varijanse (sa post-hoc analizom).

Skening-mikrografije tretiranih uzoraka urađene su na uvećanju od 1500x.

## Rezultati

Dobijeni rezultati prikazani su na grafikonima 1-3 i mikrofotografijama 1-3. Utvrđeno je da sve tri vrste tretmana dovode do promene Ca/P indeksa u odnosu na kontrolnu grupu. Razlika je bila statistički značajna kod uzoraka II grupe (tretman sa Na-EDTA)  $t=2,530$  ( $p<0,05$ ), a visoko statistički značajna kod uzoraka III grupe (tretman NaOCl)  $t=21,466$  ( $p<0,0001$ ) i uzoraka IV grupe (tretman Na-EDTA+NaOCl)  $t=30,000$  ( $p<0,001$ ).



ing and after the polishing, specimens were irrigated with distilled water and then dried on 37° C during 30 minutes. Specimens were divided in four groups and each half of the tooth was treated with different solution through syringes.

- I group (control): 10ml saline (4 specimens)
- II group: 15% Na-EDTA (Complexon III-”Merck”, pH=7.2) for 1 min + 10ml saline (6 specimens)
- III group: 10ml 5.25% NaOCl (6 specimens)
- IV group: 15% Na-EDTA for 1 min + 10ml 5.25% NaOCl (6 specimens).

After treatment with Na-EDTA and NaOCl, specimens were dried at room temperature and prepared by conventional method for electronic microscopy. Calcium and phosphorus levels in dentine of the root were evaluated by SEM and EDS microanalysis on JEOL-JSM-5300 device.

Due to difficulties in directing the electron beam in the same spot, measurements were attempted in at least three spots close to one another. Subsequently, average values of Ca and P contents were obtained for each specimen.

Statistical analysis consisted of testing the significance of varying mean values of the Ca/P index in all examined groups comparing to control (t-test) and among experimental groups by variance analysis (post-hoc analysis).

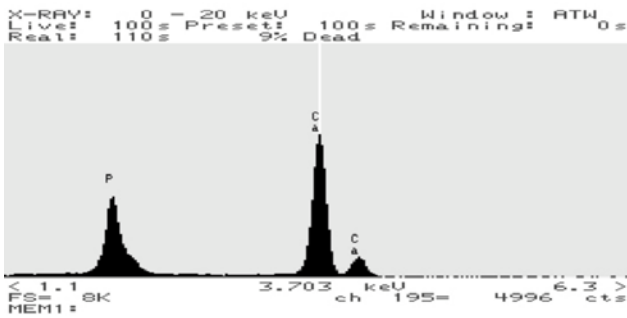
Scanning micrographs of treated specimens were magnified 1500X.

## Results

Obtained results are presented in graphs 1-3 and micro photos 1-3. It was estimated that all three treatment types are influencing changes in the Ca/P index comparing to the control. Discrepancies were statistically significant in specimens of the II group (Na-EDTA),  $t=2.530$  ( $p<0.05$ ) and highly significant in the III (NaOCl),  $t=21.466$  ( $p<0.0001$ ) and the IV group (Na-EDTA + NaOCl),  $t=30.000$  ( $p<0.001$ ).

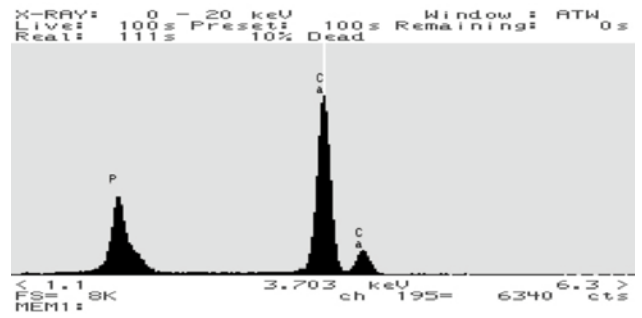
Grafikon 1. Srednje vrednosti Ca/P indeksa u kanalu korena posle tretmana različitim sredstvima ( $\pm$  SD).

Graph 1. Mean values of Ca/P index in root canal after the treatment with different agents.



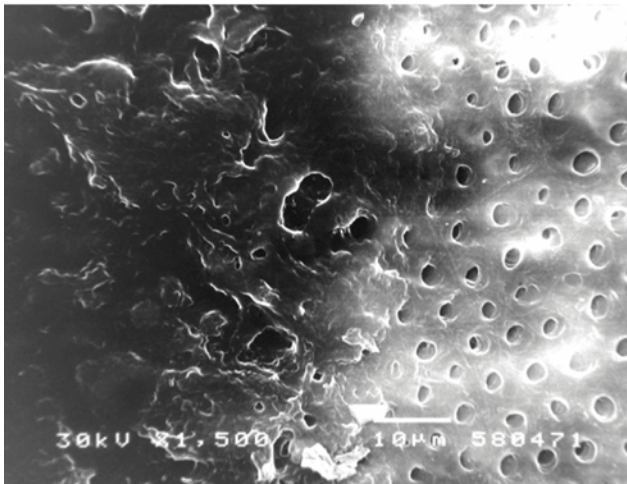
Grafikon 2. Odnos Ca/P jednog merenja posle tretmana kanala korena sa 15% Na-EDTA (1 minut). Vrednost Ca iznosi 4996.

Graph 2. Ca/P ratio after root canal treatment with 15 % Na-EDTA (1 minute). The value of Ca is 4996.



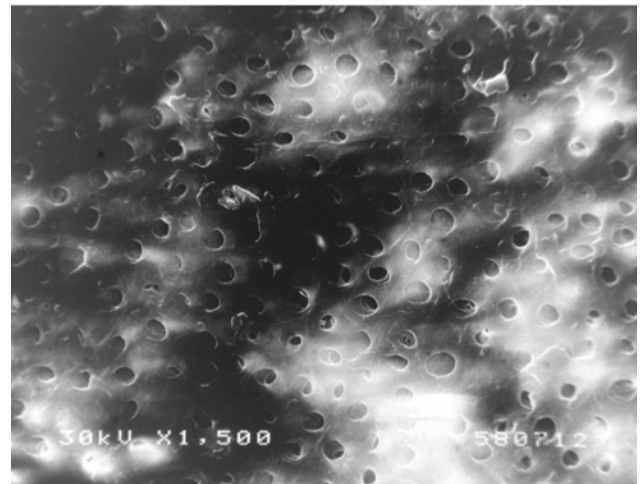
Grafikon 3. Izuzetno visok nivo Ca posle jednog EDS merenja u kanalu korena tretiranog sa 5,25% NaOCl. Vrednost Ca iznosi 6340.

Graph 3. Extremely high level of Ca after one measurement in root canal treated with 5,25% NaOCl. The value of Ca is 6340.



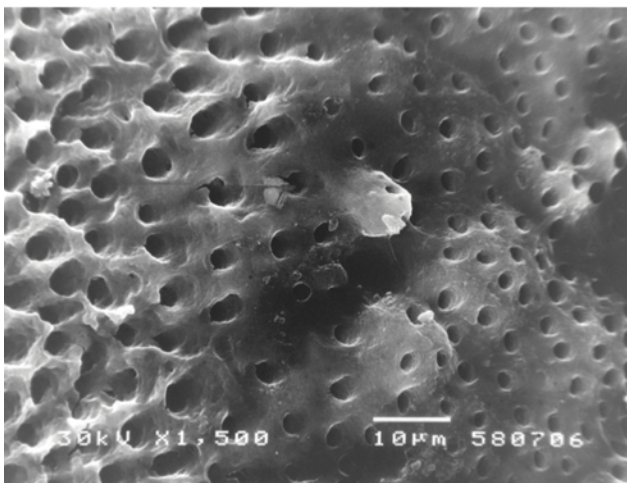
Slika 1. SEM površine dentina tretirane sa 15% Na-EDTA (1 minut): Granična površina između dentinskih tubula kanala i dentinske mase korena

Figure 1. SEM of dentin surface treated with 15% Na-EDTA (1 minute): Interface of dentin tubules and root dentin mass.



Slika 2. SEM površine dentina tretirane sa 5,25% NaOCl. Uočavaju se plitki otvori dentinskih kanalića.

Figure 2. SEM of dentin surface treated with 5,25% NaOCl. Shallow orifices of dentin tubules are observed.



Slika 3. SEM površine dentina tretirane kombinacijom 15% NaEDTA i 5,25% NaOCl: Čista površina dentina unutar kanala korena; razmazni sloj je uklonjen, a neravna dentinska površina pokazuje nemogućnost idealnog poliranja uzorka.

Figure 3. SEM of dentin surface treated with combination of 15% Na-EDTA and 5,25% NaOCl: Clean dentin surface inside root canal; smear layer was removed, a rough dentin surface indicates the impossibility of ideal sample polishing.

Analiza varijanse za tri vrste tretmana je, takođe, ukazala da je razlika bila visoko statistički značajna vrednost  $F=272,255$  ( $p<0,001$ ). Statistički značajne razlike uočene su i između svih tretmana međusobno, a najveće razlike su registrovane između tretmana rastvorom Na-EDTA (II grupa) i tretmana rastvorom NaOCl (III grupa).

Srednje vrednosti Ca/P indeksa u kanalu korena posle tretmana različitim sredstvima ( $\pm$ SD) prikazane su na grafikonu 1.

Korišćenje Na-EDTA u kombinaciji sa NaOCl i primena NaOCl posebno, dovodi do značajne promene odnosa Ca/P u dentinu korena zuba u poređenju sa kontrolnom grupom ( $p<0,001$ ). Međutim, primena samo Na-EDTA, dovodi do promena u Ca/P korenskog dentina sa manjim nivoom značajnosti u odnosu na kontrolnu grupu ( $p<0,05$ ).

Na skening-mikrografiji uzoraka tretiranih Na-EDTA (II grupa), zapažaju se otvori plitkih dentinskih kanalića, na graničnoj površini između obrađenog i neobrađenog dela dentina korena (Slika 1.). Merenja nivoa minerala u ovoj grupi uzoraka bila su najuniformnija, tj. nisu pokazivala nagle skokove ni u količini Ca, ni u količini P. Grafikon 2 prikazuje EDS merenje količine Ca i P posle tretmana sa 15% Na-EDTA.

Tretman uzoraka 5,25% NaOCl (III grupa), pokazala je značajno povećanje količine Ca, koje je zapaženo u svim EDS analizama. Međutim, u nekoliko merenja, nivoi fosfora su imali znatno niže vrednosti, tako da je prosečan odnos Ca/P iznosio 1,99. Na grafikonu 3. prikazane su vrednosti EDS merenja koje verifikuje izuzetno visok nivo Ca i neznatno povećanje fosfora.

Skening mikrografija ovih uzoraka pokazala je nejasno otvorene dentinske kanaliće (Slika 2).

Četvrta grupa uzoraka tretirana je kombinacijom 15% NaEDTA i 5,25% NaOCl. U većini merenja zapažena je povećana količina Ca dok su nivoi fosfora bili slični kao u prethodnim grupama, ili su pokazivali neznatno povećanje. Ukupan Ca/P odnos bio je izmenjen i prosečno je iznosio 1,9.

Skening mikrografija uzoraka tretiranih kombinacijom Na-EDTA i NaOCl pokazuje otvorene dentinske kanaliće i čistu površinu dentina (Slika 3).

## Diskusija

Ispitivanje materijala pomoću EDS metode zasniva se na bombardovanju uzorka upadnim elektronskim snopom i emisiji x-zračenja iz uzorka. Emitovano x-zračenje ima karakterističnu energiju za različite hemijske elemente, što omogućava analizu hemijskog sastava površine ispitivanog uzorka<sup>8</sup>. EDS mikroanaliza zahteva idealno polirane površine, jer poroznost tkiva može izazvati sekundarnu difrakciju. Poliranjem se, međutim, formira razmazni sloj, koji, s druge strane, može biti odgovoran za detekciju različitih elemenata na površini tkiva. Kada se ispituje sastav mineralizovanog (i poroznog) tkiva kao što je dentin, rezultati EDS metoda zavise i od varijabilnosti rezulta-

Variance analysis of the treatments effects has shown that the difference was highly statistically significant  $F=272,255$  ( $p<0,001$ ). Further significant differences were observed among each type of treatment, and the highest values were recorded between the II (Na-EDTA) and the III (NaOCl) group.

Mean values of the Ca/P index in the root canal following treatments with different agents (SD) are presented on graph 1.

On scanning micrograph of the specimens treated with Na-EDTA (II group), shallow openings of dentine tubules can be observed, on the interface of instrumentated and non-instrumentated part (fig.1). Measurements of minerals levels in this group of specimens were normalized, which means that no picks in Ca content, nor in P content have appeared. The measurement of Ca and P contents following treatment with 15% Na-EDTA, is presented on graph 2.

After treating specimens with 5.25% NaOCl (III group), significant increase in Ca amount was observed in all EDS test. However, several measurements have exposed significantly lower phosphorus levels with the consequent mean Ca/P ratio of 1.99. Graph 3 is presenting EDS measurements values by which an extremely high Ca level and slightly increased phosphorus were verified. On the scanning micrograph of these specimens unclearly opened dentine tubules can be seen (fig.2).

The IV group of specimens was supplied with combination of 15% Na-EDTA and 5.25% NaOCl. In most of the measurements, increased Ca amount was noticed and phosphorus levels have coincided with the previous measurements in other groups or were slightly increased. Total Ca/P ratio was altered on average – 1.9. On the scanning micrograph of the specimens treated with combined Na-EDTA and NaOCl, opened dentine tubules and clean surface of dentine can be seen.

## Discussion

Analysis of materials using EDS (Energy Dispersive Spectroscopy) is achieved by bombarding the sample with electrons and detecting emitted X-ray radiation. Emission of x-rays is carrying energy, which is typical, for different chemical elements and that is enabling analysis of chemical contents at the surface of a specimen. For EDS microanalysis it is necessary that all tested surfaces be perfectly polished because tissue porosity can lead to the secondary diffraction. However, on a polished surface a smear layer is formed which can influence detection of various elements on the tissue surface. The results of EDS methods depend on variability of data related to mineralisation

ta koji zavise od stepena mineralizacije odnosno anatomске lokacije u uzorku<sup>9</sup>. Naravno, rezultati zavise i od same metodologije, to jest pripreme uzoraka za istraživanja. Priprema materijala i obrada uzoraka u ovom istraživanju urađena je prema metodologiji koju su predložili Dogan i sar. (2001)<sup>7</sup>. Ovi istraživači, međutim, nisu prikazali tačnu starost uzoraka. I pored poliranja, SEM mikrografije u ovom istraživanju pokazale su otvore plitkih dentinskih kanalića, što ukazuje na nemogućnost dobijanja idealno ravnih uzoraka. Da bi procena mikroanalize bila što realnija, u ovom radu korišćeni su zubi približno iste starosti, a vrednosti očitavane samo u srednjoj trećini korena i uvek od strane samo jednog istraživača.

Dobijeni rezultati pokazuju da primena EDTA u kombinaciji sa NaOCl može promeniti mineralni sastav dentina korena, a korišćenjem EDTA bez NaOCl dolazi do minimalnih promena u sastavu dentina korena zuba. Razlog relativnog "očuvanja" dentina pri upotrebi EDTA može biti posledica ograničenih mogućnosti EDS mikroanalize koja ne može da otkrije promene na površini dentina zbog prisustva fibrozne komponente razmaznog sloja. Smatra se da se uticaj EDTA zasniva na uklanjanju neorganskog dela razmaznog sloja, pri čemu se na zidu kanala korena zadržava organska fibrozna komponenta<sup>5</sup>. Drugo objašnjenje moglo bi se potražiti u realno nepromenjenom sastavu zbog prisustva organskog matriksa razmaznog sloja koji predstavlja ograničavajući faktor za rastvaranje dentina, što ima za posledicu nepromenjen sastav minerala<sup>7</sup>. Jedan od razloga mogao bi biti i kratkotrajno delovanje EDTA na dentin. Primenjen je 15% Na-EDTA u trajanju od samo jednog minuta. Ovaj kratkotrajni efekat se ne može porediti sa rezultatima Dogana i sar. koji su tretirali uzorke 17% EDTA u trajanju od 15 minuta. Osim toga, velika molekulska masa EDTA i specifična struktura njegovog molekula najverovatnije utiču na sporiji prodor supstance kroz tkivo. Tako, u ovom slučaju izostaje efekat koji kompleksirajući agens izaziva pri kraćem eksponiranju dentina. Neke studije ukazuju da već petominutna aplikacija EDTA može izazivati izraženu dekalifikaciju peritubularnog dentina<sup>10</sup>.

U ovom istraživanju, ustanovljeno je i da pojedinačna primena NaOCl dovodi do značajne promene mineralnog sastava dentina kanala korena. Podaci iz literature ukazuju da tretman NaOCl-om uzrokuje akumulaciju minerala u dentinu korena zuba povećavajući količinu karbonata i smanjujući nivo fosfata<sup>7</sup>. Ova studija je pokazala da je kalcijum uglavnom, bio znatno povećan u svim merenjima, dok je nivo fosfora bio različit i varirao je od znatnog smanjenja do znatnog povećanja. Ukupan odnos Ca/P je ipak bio viši nego kod ostalih grupa (zbog porasta Ca), pri čemu je teško objasniti zašto su nivoi fosfora u nekim regijama dentina rasli. Činjenica je takođe da upotreba NaOCl kao irigansa, dovodi do rastvaranja organske komponente *razmaznog sloja* i ostavlja samo mineralizovani deo ovog sloja. Postojanje mineralizovanog sloja na površini dentina posle irigacije NaOCl-om može biti razlog za povećani nivo kalcijuma u dentinu kanala korena.

level, or to anatomical site in specimen when mineral containing (and porous) tissue like dentine is examined. Results, of course, are dependent on the research methodology, particularly of procedures in specimen preparation. Material was prepared and the specimens in this investigation processed according to the methodology suggested by Dogan et al. (2001). Nevertheless, they have not presented exact age of the tissue of specimens tested. In order to develop a reliable microanalysis, teeth of approximately the same age were used and date was recorded in the middle radicular third by the same examiner. On SEM micrographs shallow orificies of dentinal tubules were observed in this study despite thorough polishing procedure. Obtained results have shown that application of EDTA in combination with NaOCl is altering mineral contents of the root dentine, and the use of EDTA without NaOCl is influencing only minor changes in dentine components of the root canal. Possible reasons for a relatively "preserved" dentine following use of EDTA are thought to be for EDS microanalysis limited properties in detecting alterations on dentinal surface in presence of the smear layer's fibrous component. It is considered that EDTA removes inorganic part of the smear layer while its organic, fibrous component remains on the root canal walls. Other explanation might be that organic matrix of the smear layer presents limiting factor for dentine solubility, thus making possible mineral content to stay unaffected. Also, EDTA is used and acts on dentine during a relatively short time. Here, 15% Na-EDTA was applied for one minute. Short-term effects cannot compare to the results of Dogan et al. who treated specimens with 17% EDTA for over 15 minutes. Moreover, big molecular mass of EDTA and the specific structure of the molecule are more likely to influence its penetration through the tissue. That is why the effect of a complexing agent evoking in dentine in this short-term exposure is absent. Some studies have shown that five-minutes-application of EDTA can already provoke significant decalcification of peritubular dentine.

In this research, it was assessed that single use of NaOCl significantly alters mineral contents of the root canal dentine. Reported data confirm that application of NaOCl causes accumulation of minerals in radicular dentine by increasing carbonates and decreasing phosphates. In our study, results have shown that calcium levels were significantly higher in all measurements and phosphorus levels have varied from a significant decrease to the significant increase. Total Ca/P ratio was higher than in other groups (because of the Ca increase) but it remains difficult to explain why phosphorus levels were rising in some parts of dentine. It is, also, known that use of NaOCl as the irrigating agent is leading to solubility of the smear layer's organic component and leaving only mineralised part. Existence of the mineralised layer on the dentine surface after irrigation with NaOCl might be the reason for increased Ca levels in dentine of the root canal.

## Zaključak

Na osnovu dobijenih rezultata, može se zaključiti da tretman površine dentina 15% rastvorom Na-EDTA, 5.25% NaOCl i kombinacijom ova dva rastvora značajno menja odnos Ca/P u dentinu korena. Kratkotrajni tretman kanala korena rastvorom Na-EDTA u trajanju od 1 minuta statistički značajno menja Ca/P odnos dentina korena zuba ( $p < 0,05$ ). Tretman kanala korena NaEDTA i NaOCl statistički značajno povećava Ca/P odnos dentina korena zuba ( $p < 0,001$ ). Završna irigacija NaOCl pojačava efektivnost helatnih sredstava. Tretman kanala korena NaOCl-om takođe, statistički značajno menja mineralni sastav dentina korena zuba i povećava odnos Ca/P u dentinu korena zuba.

## Conclusion

Based on the obtained results, it can be concluded that treating dentine surface with 15% Na-EDTA, 5.25% NaOCl and combination of the two agents, significant alterations in the Ca/P ratio occur. Short-term application of Na-EDTA over one minute influences statistically significant changes in the Ca/P ratio ( $p < 0.05$ ). Application of Na-EDTA and NaOCl leads to the significant increase of the Ca/P ratio ( $p < 0.001$ ). Final flush with NaOCl enhances efficacy of chelating agents. Application of NaOCl in a statistically significant way causes changes in mineral contents of the radicular dentine and increases the Ca/P ratio in root canal dentine.

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