Utjecaj mliječne kiseline na dentinsko tkivo različite dobi i različitoga lokaliteta

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Sažetak

U ovom je radu ispitivana težina ekstrahiranoga kalcija iz komadića dentina zdravih zubi mladih i starih osoba djelovanjem mliječne kiseline (pH 3,3). Korištena je metoda AAS, a rezultati su izraženi $\mu g/mm^2/h$.

Također je prikazan koeficijent smjera inicijalne brzine otapanja kalcijevih iona mladih i starih uzoraka različitoga lokaliteta.

U obje skupine uzoraka najveća se količina izdvojila u prvome satu. U mladim uzorcima taj se proces odvijao brže, ali je samo u trećem ispitivanju ta razlika bila statistički vjerodostojna ($p<0,05$).

Srednje vrijednosti početnih brzina također pokazuju da je inicijalna brzina otapanja kalcijevih iona veća u mladim nego u starim uzorcima.

Razlike u topljivosti pojedinih uzoraka koje smo dobili u našim rezultatima pokazuju, da svaki uzorak ima svoj dijagram topljivosti u kiseloj sredini, što se povezuje s rasporedom i omjerom kalcijeve soli u uzorcima.

Ključne riječi: *dentin, ekstrakcija kalcijevih iona, inicijalna brzina, mliječna kiselina*

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Uvod

Tijekom rada s pacijentima primjećena je razlika u otpornosti tvrdih zubnih tkiva na demineralizaciju između starih i mladih osoba. Tvrda zubna tkiva starijih osoba pokazuju veću otpornost spram demineralizacijskome učinku mliječne kiseline koja je jedan od glavnih produkata dento-bakterijskoga plaka i zato ima glavnu ulogu u razaranju tvrdih zubnih tkiva. Osim toga na pH vrijednost dento-bakterijskog plaka utječe i kakvoća sline. Zato slina može biti jedan od pokretača demineralizacije tvrdih zubnih tkiva. Istraživanja pokazuju veću incidenciju karijesa u djece i mladih osoba, a to se povezuje s različitim sustavom zubnoga tkiva (1-13).

Potpuno djelovanje kiselina na dentin također je vazano s problematikom adhezivnih tehnika kompozitnih i staklenoionomernih dentalnih restorativnih materijala (14-17).

U ovome radu bila je svrha ispitati težinu ekstrahiranih kalcija iz dentina zdravoga zuba različitih dobnih skupina i različitog lokaliteta, pod djelovanjem mliječne kiseline (pH 3,3). Također smo željeli odrediti inicijalnu brzinu otapanja kalcijevih iona u mladih i starih osoba.

Materijali i metode

U radu smo se koristili kalcijevim laktatom, mliječnom kiselinom, lantanovim sulfatom, standardnim pufer radiometrom pH 4, destiliranom, deioniziranomu ili tetra vodom. Od instrumenata i pribora upotrebljavali smo atomski apsorcijski spektrofotometar (AAS) Jarell ASH 82-500, (18-26) radiometar model PHM 64, Mettlerovu petodecimalnu vagu, mjerni mikroskop Houte-Paris, sušionik, eksikator, vakuum pumpu, kremene kušalice, stomatološke klasične i visokoturažne vrtaljke, dijamantna i ostala brusila, gumice za poliranje, finirere i mikrometarski vijak.

Uzorke dentina uzimali smo od svježe ekstrahiranih, zdravih prednjih zuba osoba od dobi od 8 do 9 i 60 do 80 godina. Zubne smo krune poprečnim rezanjem odvojili od korijenova, a zatim uzdužnim i poprečnim brušenjem dijamantnim brusilicama odstranili caklinu uz neprekidno hlađenje destiliranom vodom.

Transverzalnim (T) longitudinalnim (L) smjerom dobili smo komadiće dentina i razvrstali ih o lokalitetu u cirkumpulpalne (CP) i periferne (P) dijelove. Na taj smo način iz jedne krune zuba dobili 2-5 uzoraka dentina. Sve smo uzorke dentina oprali u destiliranoj vodi i krtako ih osušili u sušioniku pri temperaturi od 40°C. Površinu uzoraka mjerili smo milimetarskim papirom, mikroskopom i mikrometarskim vijkom. Debljinu uzorka odredili smo pomičnom mjerkom. Uzorke smo osušili u vakuumu i izvagali ih na Mettler petodecimalnoj vagi. Težina uzoraka izražena je u mg (Tablice 1 i 2).

U svaku smo kušalicu stavili po jedan uzorak dentina, ulili 9 ml otopine mliječne kiseline pH 3,3, hermetički ih zatvoriti i staviti u ultratermostat. U njemu je bila destilirana voda temperature 37 °C ± 0,05 °C. Sobna temperatura bila je oko 26 °C. Ukupno je bilo uzoraka, 8 starih i 8 mladih, a sedamnaesta kušalica bila je slijepa proba s otopinom mliječne kiseline. Uzorci su bili podvrgnuti demineralizacijom u trajanju od 51 sat.

Raščlambe kalcijevih iona obavili smo na AAS u 4 različita razdoblja, 3, 9, 27 i 51 sat, uzimajući

Tablica 1. *Uzorci staroga dentina*
Table 1. *Old dentin samples*

Uzorak Sample	Zub Tooth	Lokalitet dentina Location of dentin	Težina uzorka/mg Weight of sample/mg	Površina uzorka/mm^2 Surface of sample/mm^2
A-1	13	Periferni dentin, longitudinalni rez, (PF-L).	15,39	34,78
A-2		Cirkumpulpni dentin, transverzalni rez, (CP-T).	15,83	16,78
A-3		Cirkumpulpni dentin, longitudinalni rez, (CP-L).	9,55	32,72
A-4		Cirkumpulpni dentin, komore transverzalni rez, (CP-L).	11,46	24,55
A-5		Periferni dentin, transverzalni rez, (PF-T).	10,68	27,52
B-1	22	Cirkumpulpni dentin, transverzalni rez, (CP-T).	7,3	20,07
B-2		Cirkumpulpni dentin, transverzalni rez, (CP-T).	2,88	11,17
B-3		Periferni dentin, longitudinalni rez, (PF-L).	7,66	19,17

Tablica 2. *Uzorci mladoga dentina*
Table 2. *Young dentin samples*

Uzorak Sample	Zub Tooth	Lokalitetr dentina Location of dentin	Težina uzorka/mg Weight of sample/mg	Površina uzorka/mm² Surface of sample/mm²
I-1	21	Periferni dentin, longitudinalni rez, (PF-L).	4,21	24,07
I-2		Cirkumpulpni dentin, transverzalni rez, (CP-T).	8,51	17,73
I-3		Cirkumpulpni dentin, transverzalni rez, (CP-T).	7,17	20,85
II-1	42	Cirkumpulpni dentin, transverzalni rez, (CP-T).	8,58	12,8
II-2		Cirkumpulpni dentin, transverzalni rez, (CP-T).	5,74	13,9
II-3		Periferni dentin, transverzalni rez, (PF-T).	8,72	18
III-1	43	Cirkumpulpni dentin, longitudinalni rez, (CP-L).	8,39	16,2
III-2		Periferni dentin, transverzalni rez, (PF-T).	10,32	23,1

svaki put kušalice po 1 ml otopine uzorka. Zbog različite koncentracije kalcijevih iona uzorke smo razrijedili 5, 10 i 15 puta 5% otopinom lantana koja na sebe veže fosfor (La-fosfat). Tim se postupkom dobija više vrijednosti kalcija, a izražene su u µg (18).

Nakon obavljenih raščlamba uzorci su oprani u deioniziranoj vodi, sušeni u sušioniku dva sata, a zatim u vakuumu pet sati. Svi su uzorci ponovno izvagani.

Korekcija nejednakih površina uzoraka provedena je tako što je masa otopljenog kalcija u mg podijeljena s površinom uzorka u mm² i tako svedena na relativne vrijednosti. Tim postupkom izjednačeni su uzorci različite površine pa se svi rezultati odnose na otopljeni kalcij s jedinice površine uzorka.

Kao pokazatelj brzine kalcija uveli smo *inicijalnu brzinu*. U kemijskoj kinetici uobičajen je način grafičkog prikazivanja *ovisnosti* promjene koncentracije o *reakcijskom* (mjernom) vremenu.

Na temelju eksperimentalnih podataka konstruiran je dijagram koji pokazuje porast koncentracije kalcija u otopini, a u ovisnosti u reakcijskome vremenu.

Za kvantitativnu usporedbu uzoraka uzeta je kvantitativna obrada, i to samo *početnoga* dijela krivulje. Prema tome postupku pravac je povučen iz početnoga dijela krivulje koji bi u idealnom slučaju trebao biti tangenta na krivulju i predstavljati *početnu brzinu* reakcije. No, pravac nije uvijek tangenta, već sekanta, ali je ipak omogućena kvantitativna usporedba ispitivanih uzoraka. Koeficijent smjera pravca (tangenta) koji je označen s *a* dobra je aproksimacija za razmjernu usporedbu početnih brzina otapanja ispitivanih uzoraka. Iz toga proizlazi: što koeficijent smjera a ima veću vrijednost, to je njegova *početna brzina* veća, a dobije se ako vrijednost s ordinate podijelimo pripadajućom vrijednosti na apscisi, odnosno koncentracije s vremenom.

Budući da smo očekivali mikrogramske vrijednosti kalcija, izrađene su standardne otopine i baždarni pravac. Statističku odradu dobivenih podataka proveli smo ***Student***-testom na IBM računalu s programom ***Statograf***.

Rezultati

Na Tablicama 3 i 4 prikazane su vrijednosti kalcija izdvojenoga iz staroga i mladoga dentinskoga tkiva. Na Tablici 3 prikazali smo težinu kalcija koji se je ekstrahirao po mm² površine uzorka staroga dentina u jednome satu pri temperaturi od 37 °C.

Tablica 3. *Težina otopljena kalcija starog uzorka*
Table 3. *Weight of dissolved calcium of an old dentin sample*

µg $Ca^{2+}/mm^2/h$

Uzorak Sample	A A	Uzorak Sample	B B	Uzorak Sample	C C	Uzorak Sample	D D
B-2	1,61	B-2	0,864	B-2	0,621	A-2	0,37
B-1	1,33	A-2	0,807	B-3	0,545	B-2	0,33
A-2	1,26	B-3	0,762	B-1	0,44	B-3	0,32
A-3	1,12	B-1	0,65	A-4	0,388	B-1	0,31
B-3	1,07	A-1	0,634	A-2	0,387	A-4	0,287
A-1	1,056	A-4	0,62	A-5	0,35	A-3	0,225
A-4	0,898	A-5	0,585	A-1	0,283	A-5	0,22
A-5	0,866	A-3	0,56	A-3	0,254	A-1	0,196

Legenda: *A - Prvo ispitivanje*
B - Drugo ispitivanje
C - Treće ispitivanje
D - Četvrto ispitivanje

Legende: *A - First examination*
B - Second examination
C - Third examination
D - Fourth examination

Tablica 4. *Težina otopljena kalcija mladog uzorka*
Table 4. *Weight og dissolved calcium of an young dentin sample*

µg $Ca^{2+}/mm^2/h$

Uzorak Sample	A-1 A-1	Uzorak Sample	B-1 B-1	Uzorak Sample	C-1 C-1	Uzorak Sample	D-1 D-1
II-1	1,957	II-1	1,336	III-1	1,073	II-1	0,554
III-1	1,546	III-1	1,01	II-1	0,948	III-1	0,53
II-3	1,457	I-2	0,81	I-2	0,66	I-2	0,41
I-2	1,379	II-3	0,743	II-3	0,63	II-2	0,391
I-3	1,0144	III-2	0,683	II-2	0,624	II-3	0,378
II-2	0,993	II-2	0,681	I-3	0,569	III-2	0,295
III-2	0,987	I-3	0,665	III-2	0,502	I-3	0,23
I-1	0,623	I-1	0,526	I-1	0,312	I-1	0,205

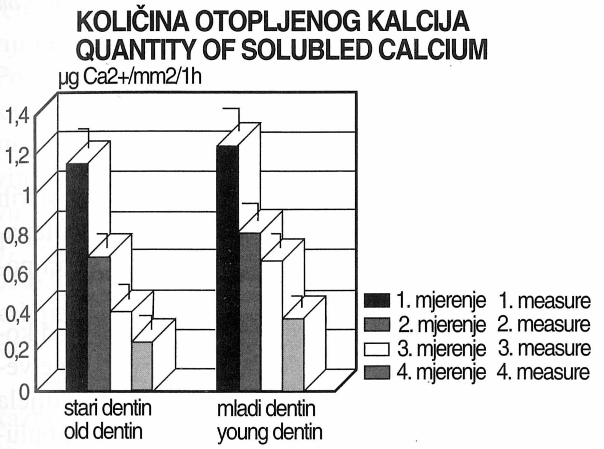
Legenda: *A-1 - Prvo ispitivanje*
B-1 - Drugo ispitivanje
C-1 - Treće ispitivanje
D-1 - Četvrto ispitivanje

Legende: *A-1 - First examination*
B-1 - Second examination
C-1 - Third examination
D-1 - Fourth examination

Vrijednosti kalcija prikazane su u µg. Rezultatae izdvojenoga kalcija iz uzoraka poredali smo po veličini.

U prvom se ispitivanju najviše kalcija izdvojilo iz starih uzoraka B-2, B-1 i A-2. Sva su tri uzorka podrijetlom iz cirkumpulpalnoga dijela dentina. Ovdje je potrebno izdvojiti uzorak B-2, koji je imao najmanju površinu, a po količini izdvojenoga kalcija pokazao je gotovo najmanju otpornost gotovo svim ispitivanjima. Najmanje se kalcija izdvojilo iz uzorka A-5 u prvome ispitivanju. Taj je uzorak imao razmjerno veliku površinu, a po lokalitetu je bio iz perifernoga dijela dentina. U zadnjem ispitivanju najviše se kalcijevih iona izdvojilo iz uzoraka A-2 i B-2, a najmanje od uzoraka A-1 (Tablica 3). Vrijednost otopljenoga kalcija od mladog uzorka prikazuje Tablica 4. Dobivene smo vrijednosti poredali po veličini. Najviše se kalcija izdvojio iz uzoraka II-1 i III-1 u svim ispitivanjuma. Oba su uzorka iz cirkumpulpalnoga dijela dentina s razmjerno malom površinom. Najmanje se kalcija iz izcvojilo iz uzorka I-1 u sva četiri mjerenja. Taj uzorak podrijetlom je iz perifernoga dijela dentina, a ima najveću površinu i najmanju težinu. U svim mladim uzorcima najviše se kalcija izdvojilo u trećem ispitivanju, a nakon toga topljivost znatno opada.

Tijekom ispitivanjn nešto se više kalcija otopilo u mladim uzorcima nego u starijm (p>0,0), ali je samo u trećem ispitivanju ta razlika bila statistički vjerodostojna (p>0,05). Srednje vrijednosti i standardnu devijaciju izdvojenoga kalcija prikazali smo na Slici 1.



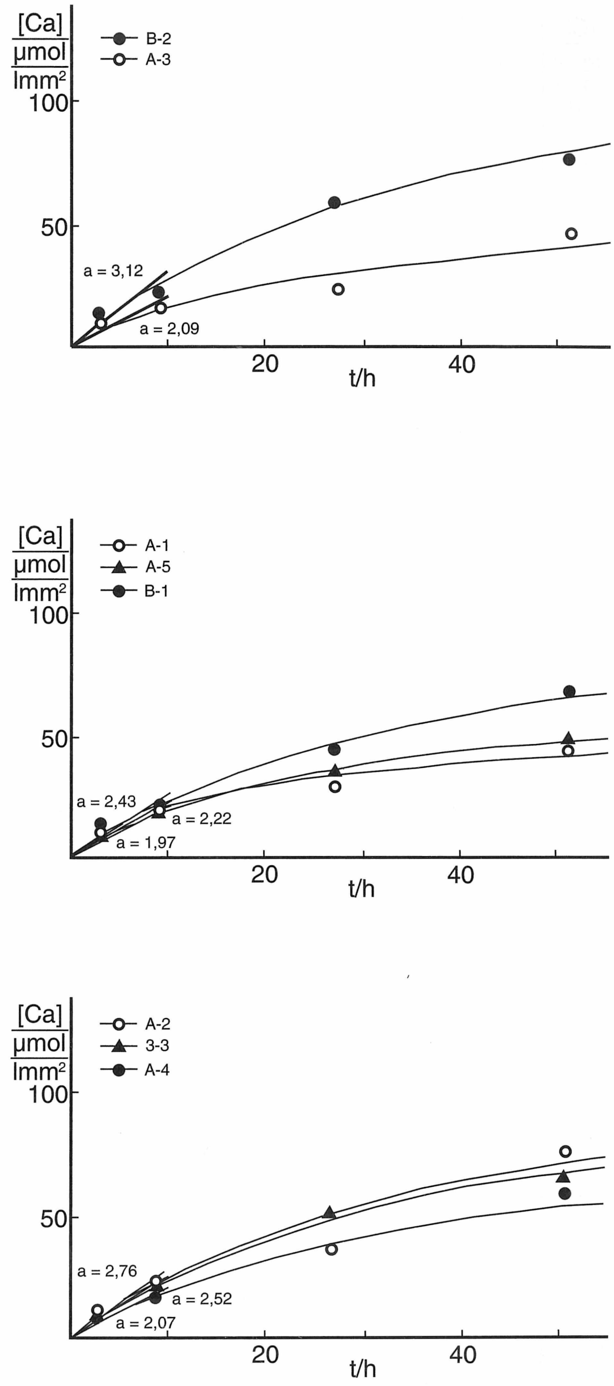
Slika 1. *Srednje vrijednosti otopljena kalcija u jedinici vremena i standardne devijacije.*

Figure 1. *Mean values of dissolved calcium per hour and standard deviation.*

Realniju sliku izdvajanja kalcija iz tih uzoraka pokazuje *inicijalna brzina.* Na dijagramima se mogu pratiti razlike otpornosti pojedinih uzoraka u kiselome mediju, to jest porast koncentracije kalcija u otopini za ekstrakciju.

Najveću *inicijalnu brzinu* pokazao je uzorak B-2 cirkumpulpalnoga dijela staroga dentina. Koeficijent smjera a bio je 3,12 (Slika 2). Taj uzorak ima najmanju površinu i najmanju težinu. Najmanji koeficijent smjera imao je uzorak A-1, *a* 1,97 (Slika 3) s najvećom težinom i površinom. Podrijetlom je bio od perifernoga dijela dentina. Nešto veći koeficijent smjera imali su uzorci A-3 i A-4 s razmjerno velikom površinom (Slike 2 i 4).

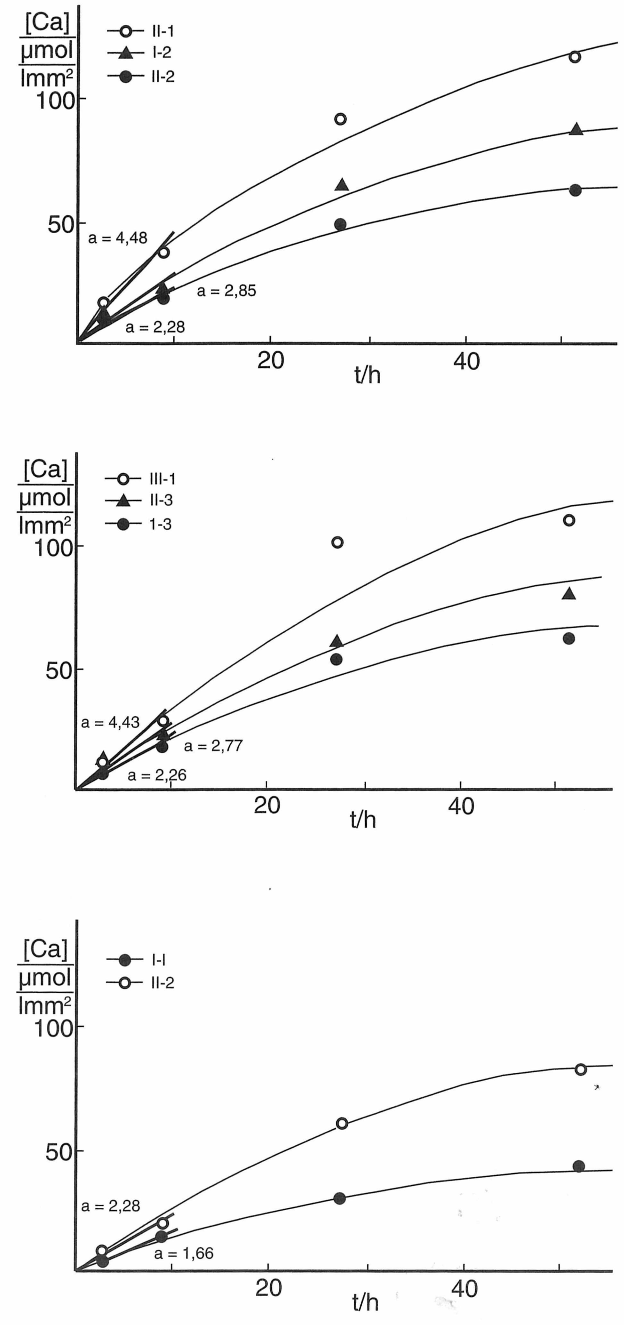
Najveću početnu brzinu od mladih uzoraka ima uzorak II-a s koeficijentom smjera *a* 4,48 (Slika 5).



Slike 2,3,4. *Ovisnost inicijalne brzine ekstrakcije kalcijevih iona o vremenu. Uzorci staroga dentina*

Figure 2,3,4. *Calcium initial velocity dependence on time. Samples taken from old dentin*

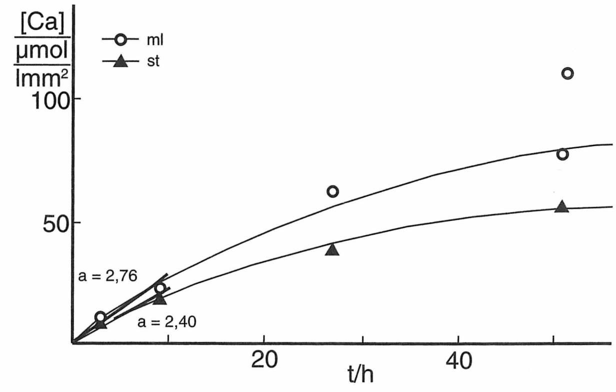
Manji koeficijent smjera imao je uzorak III-1 *a* 2,26 (Slika 6). Oba su ta uzorka od cirkumpulpnoga dijela dentina. Najmanji koeficijent smjera bio je *a*



Slike 5,6,7. Ovisnost inicijalne brzine ekstrakcije kalcijevih iona o vremenu. Uzorci mladoga dentina

Figure 5,6,7. Calcium ions initial velocity dependence on time. Samples taken from young dentin

1,66 od uzorka I-1. Taj je uzorak od perifernoga dijela dentina (Slika 7). Srednja vrijednost svih početnih brzina pokazuje da je *inicijalna brzina* otapanja kalcijevih iona veća u mladim nego u starim uzorcima (Slika 8).



Slika 8. *Ovisnost prosječne inicijalne brzine kalcijevih iona o vremenu u mladih i starih uzoraka*

Figure 8. *Calcium ions averge initial velocity dependence on time in younger and older samples*

Rasprava

Raščlambom koeficijenta smjera mladih i starih uzoraka opažena je najveća *inicijalna brzina* otapanja kalcija u mladim uzorcima, a stari su uzorci pokazali manju brzinu (Slika 8).

Uzorci koji su imali veću početnu brzinu i od kojih se izdvojilo više µg kalcija po mm² površine većim dijelom podrijetlom iz cirkumpulpnoga dijela dentina. Taj dio dentina ima šire dentinske tubuluse i jače mineralizirani peritubularni prostor, a u doticaju s kiselom sredinom prije se otapa.

Marshall (10) nalazi jednaaku vrijednost topljivosti peritubularnoga i intertubularnoga dentina djelovanjem gel mliječne kiseline pH 4. Kinney (27) u svojoj studiji navodi daje početna brzina otapanja peritubularnoga dentina veća od brzine intertubularnoga dentina. Osim toga on pronalazi da je dubina demineraliziranoga sloja ovisna o početnomu dodiru kiseline s površinom dentina. Također je ovisna o koncentraciji otopljenih soli u mediju i nazočnom intertubularnome matriksu (28).

Razlika u početnoj brzini ekstrakciji kalcija između starih i mladih uzoraka u našem ispitivanju tumačimo različitim ***omjerom i rasporedom*** kalcijevih soli dentinu, što ima važne ulogu u cijelome procesu.

Zubno tkivo, odnosno dentin većim je dijelom građen iz hidroksil-apatita (HA), ta je sol najmanje otporna na kiselu sredinu, za razliku od dikalcijevea fosfata (DCP) koji je otporniji u takvoj okolini, što je u skladu s ispitivanjem Ishikawae (29, 30).

Budući da je inicijalna brzina u ladih uzoraka bila veća, držimo da su bili više građeni ih HA, za razliku od starih uzoraka koji manju brzinu otapanja jer su većim dijelom građeni iz DCP (Slika 8). To znači da će brzina otpuštanja kalcijevih iona ovisiti ne samo o *rasporedu i omjeru* kalcijevih soli u dentinu nego i o sposobnosti svakoga pojedinog uzorka da se zaštiti u kiseloj sredini. U našim smo rezultatima primijetili nepravilnost u izdvajanju kalcija iz pojedinih uzoraka, zato pretpostavljamo da svaki pojedini uzorak ima vlastiti dijagram topljivosti u kiselome mediju.

Zanimljivo je spomenuti da se je najviše kalcija izdvojilo iz uzoraka mladoga dentina u prvome mjerenju ($p<0,05$; Slika 1), dok se u jednome dijelu starijih uzoraka to događa tek nakon trećega mjerenja. Poslije prvoga mjerenja, topljivost kalcija iz uzoraka naglo se smanjuje, što bi se moglo povezati s postojanjem interdifuzijske zone koju u svojim radovima navode Eick (14) i Kinney (28). Tu smo pojavu također opazili u našim prijašnjim radovima (3, 4, 24) pa smo u ovome radu raščlambu uzoraka obavljati češće. Na Slici 1. pratimo ukupnu težinu kalcijevih iona izdvojenih iz mladih i starih uzoraka.

Naši neobjavljeni rezultati radova na dentinu pokazali su razmjerno veliku količinu fosfora (čak više od 50%) koji se izdvojio iz mladih uzoraka. Kako je molski odnos kalcija u DP 1,0, a u HA 1,67, to također govori o većem udjelu HA u mladim uzorcima.

Različita topljivost peritbularnoga i intertubularnoga dentina i u kiseloj sredini također govori u prilog našemu razmišljanju.

Zaključak

Ispitivanje težine otopljenoga kalcija iz zdrava dentina mladih i starih uzoraka, te različitoga lokaliteta i dobnih skupina, pokazuje da je količina kalcijevih iona izražena u $\mu g/mm^2/h$ u objema skupinama bila najveća u prvome satu (Tablice 3 i 4).

U mladim uzorcima taj je proces tekao brže, ali je samo u trećem ispitivanju ta razlika bila statistički vjerodostojna ($p<0,05$).

Srednja vrijednost svih početnih brzina pokazuje da je *početna brzina* otapanja kalcijevih iona veća u mladim nego u starim uzorcima. Možemo pretpostaviti da je to posljedica razlike u ***omjeru i rasporedu*** kalcijevih soli u prvoj i drugoj skupini uzoraka.

Količina kalcijevih soli koja se ekstrahira iz uzoraka nije ovisna samo o ukupnoj težini i površini nego i o drugim čimbenicima, kao što su starosna dob, histološka, morfološka i kristalografska struktura dentinskoga tkiva.

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Influence of Acid Lactic on Dentin Tissue of Different Age and Different Locality

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Summary

In this study the amount of extracted calcium from pieces of healthy tooth dentin was escamined taken from young and old persons under lactic acid demineralization challenge (pH 3.3). AAS method was used and results expressed in µg/mm²/h.

Initial speed direction coefficient was also performed, to show calcium ions dissolution of the samples of different age and locality.

The greatest loss of calcium ions was observed in the first hour of measurement in each group sample. The younger dentin samples released calcium faster than the older ones, although this difference was statistically relevant only after the third measurement (p<0.05).

Mean values of initial speed were also higher in younger than in older samples.

The difference in the dissolution ability of some of the samples indicates that each sample has its own dissolution diagram in the our environment. This fact has been connected with the arrangement and ratio of calcium salts in the samples.

Key words: *dentin, extraction of calcium ions, initial speed, lactic acid*

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Introduction

During our practice with patients we have noticed a difference in the resistance of hard dental tissues towards demineralization challenge between younger and older persons. The hard dental tissues of older persons show stronger resistance towards demineralization challenge of lactic acid, which is one of the most important products of dental plaque. Beside this the pH value of dental plaque is influenced by the quality of saliva. Research has show a higher incidence of dental caries in children and adolescents which is connected with the different content of dental tissue (1-13).

The prograding action of acids on dentin is also important because of the adhesion of composite and glass ionometer restoratives (14-17).

The aim of this study was to examine the mass of extracted calcium from the dentin of healthy teeth of different age groups and locality under demineralization challenge of lactic acid (pH 3.3), and to determine the *initial speed* of calcium ions dissolution in younger and older persons.

Materials and Methods

In this study we used calcium lactate, acid lactic, lantan sulfate, standard buffer radiometer pH 4, distilled deionised or tetra water. Instruments and equipment included an atomic absorption spectrophotometer (AAS) Jarell-ASH 82-500, (18-26), radiometer model PHM 64, Mettler five decimal scale, measuring microscope Houte-Paris, dryer, exsicator, vacuum pump, quartz test tubes, dental classical and high RPM drills, diamond and other sanding methods, finishing and polishing gums, micrometer measuring device.

Dentin samples were taken from recently extracted, healthy frontal teeth of patient aged 8-9 and 60--80. We then separated tooth crowns from their roots by transversal cutting. Then we removed the enamel by using turbine drills and constant cooling with distilled water.

Pieces of dentin were obtained by transversal (T) and longitudinal (L) cutting, and classibied by locality as circumpulpal (CP) and peripheral (P) parts. Thus two to five dentin samples from were obtained from a single tooth crown. All the dentin samples were washed in distillate and dried for a short period in the dryer at 40 °C. The surface was measured by using a micrometer device and measuring microscope. The samples were dried in vacuum and their weight measured on a Mettler five decimal scale. The weight of the samples is given in mg (Tables 1 and 2).

In every test tube 9 ml of lactic acid dissolution pH 3.3 was added to a single dentin sample. The test tubes were hermetically sealed and placed in an ultrathermostate at a temperature of 37 °C ± 0.05 °C. Room temperature was 26 °C. A total of 16 samples, 8 "young" and 8 "old". The seventeenth test tube was a blind probe with lactic acid dissolution. Samples were subjected to demineralization for 51 hours.

Calcium ions were analyzed ions at AAS in four different time periods: 3, 9, 27 and 51 hours. According to this time frame 1 ml of dissolution was taken on for occasions from each test tube. Because of different calcium ions concentration, each sample was dissolved 5, 10 and 15 times with 5% lantane dissolution. This process gives higher values of calcium expressed in μg (18).

After analyses samples were washed in deionised water, dried in the dryer for 2 hours and then held in vacuum for 5 hours. The weight of the samples was again measured.

The correction of unequal sample surfaces was made by relativisation of extracted calcium mass with the surface of the sample. This procedure equalized samples with the unequal surface amount, and consequently so all of the results are related to the mass of extracted calcium from the unit of a sample surface.

Initial speed was introduced as a calcium dissolving velocity indicator. Graphic presentation of concentration change *dependence* on the reaction time is usual in chemical kinetics.

On the basis of experimental information a diagram was constructed representing the increase in calcium concentration in dissolution, depending on the time of the reaction.

For quantitative sample comparison we took quantitative processing of the initial part of the curve only was used. According to this procedure the line drawn from the initial part of the curve should be a tangent on the curve and represents the *initial reaction speed.* However, but the line is not always the tangent but also the secant. In spite of this, quantitative comparison of the samples was possible. Direction coefficient (tangent) a presents a adequate approximation for comparison of the analyzed samples of initial velocities.

According to the foregoing in can be concludec that direction coefficient a value is proportionally related to *initial speed* value. This can be estimated if the ordinate value is divided by the aspics value.

Respectively to microgramic expected calcium values we produced standard solutions and adjusting directions were produced. Statistic processing was performed through ***Student*** test on an IBM computer with ***Statograph*** program.

Results

Tables 3 and 4 show the values of extracted calcium from old and young dentin samples. Table 4 shows the weight of calcium extracted from the surface of the old dentin given in mm^2 during one hour at a temperature of 37 °C. Values of calcium are given in μg and the results are clasified according to size.

In the first research, the higher value of the extracted calcium was from the old samples B-2, B-1 and A-2. All three samples were from the circumpulpal part of the dentin. It is necessary to emphasize that sample B-2 had the smallest surface and the least resistance to demineralization challenge in almost all analyses. The most resistant sample was A-5 in the first analysis. This sample had a relatively large surface and by its locality was from the peripheral part of the dentin. The last analysis showed that most of the extracted ions were from samples A-2 and B-2. Sample A-1 gave the smallest amount of ions (Table 3). Table 4 presents the values of dissolved calcium from the young samples. Most of the calcium extracted was in this case was from samples II-1 and III-1 in all analyses. Both these samples were from the circumpulpal part of the dentin with a relatively small surface. In all four measurements the smallest amount of calcium was from sample I-1 with the largest surface and the smallest weight. In all the young samples most of the calcium extracted was in the third measurement after which solubility reduced significantly.

During the study more calcium was dissolved from the young samples than from the old samples ($p>0.05$). The difference was statistically relevant only in the third measurement ($p<0.05$). Mid values and standard deviation of extracted calcium is presented in Figure 1.

Initial speed gives a more realistic picture of calcium extraction from these samples. Differences in resistance towards demineralization of all samples can be followed in the diagrams.

The highest *initial speed* was been determined for sample B-2 from the circumpulpal part of the old dentin. Direction coefficient a̲ was 3.12 (Figure 2). This sample had the smallest surface and smallest weight. Sample A-1 had the smallest coefficient of direction a which was 1.97 (Figure 3). This sample had the most weight and largest surface and originated from the peripheral dentin. Somewhat larger a̲ was shown by A-3 and A-4 samples with relatively large surface (Figures 2 and 4).

The highest initial speed of the young samples was shown by II-1 sample, where a̲ was 4,48 (Figure 5). Sample III-1 performed an *a* 2.26. Both samples were from thecircumpulpal part of the dentin. The smallest *a* was 1.66 by sample I-1. This sample was taken from the peripheral dentin (Figure 7).

Average initial speed shows that the *initial speed* of dissolving ions from dentin was higher greater in younger than in older samples (Figure 8).

Discussion

Direction coefficient analysis of young and old samples showed that calcium dissolving *initial speed* was higher greater in younger than in older samples. (Figure 8).

Samples with higher initial speed are mostly from circumpulpal dentin. They released more calcium ions from the surface unit. Circumpulpal dentin has wider dentine tubules and stronger mineralized peritubular space. Such dentin has less resistance to demineralization challenge.

Marshall (10) established that pH 4 gelled acid lactic equally demineralizes peritubular as well as intertubular dentin. Kinney (27) reports that peritubular dentin has higher dissolving initial speed than intertubular dentine. He also found that the depth of the demineralized dentin depends on initial contact between the dentin surface and acid. It also depends on the amound of dissolved salts concentration in milieu and the present intertubular matrix (28).

The difference in the initial speed of calcium extraction between young and old samples has escplained by the different ***ratio and arrangement*** of calcium salts in dentin, which plays an important role in the whole process.

Dental tissue, including dentin, consists primarily of hydroxy-apatite (HA); a salt which is less resistant to demineralization in comparison with dicalcium phosphate (DCP), which is more resistant to demineralization. This conclusion agrees parallel with the results of Ishikawa (29,30).

According to the higher initial speed in the younger samples the assunption is that they consist of more HA. On the contrary, the older samples consist of more DCP (Figure 8). This means that the velocity of the released the calcium ions will not only depend on calcium salts *ratio and arrangement* but also on the ability of every sample to protect itself from demineralization in the surrounding acid. In this study every sample had its own solubility diagram in the acid milieu. It is interesting to note that most of the calcium has been extracted from the young dentin samples in the first measurement ($p<0.05$; Figure 1), while some of the older samples have that characteristics by the third measurement. After the first measurement, solubility dropped abruptly which might be connected with the interdiffusion zone that Eick (14) and Kinney (28) mentioned in their papers. Exactly the same phenomenon was noticed in our research (3,4,24). According to that sample analysis was carried outs more frequently. Figure 1 presents the total weight of calcium ions extracted from the young and old samples.

The unpublished results of our research on dentin show a relatively high quantity of phosphorus (more than 50%) extracted from young samples. Since the molic ratio of calcium in DCP is 1.0 and in HA 1.67, this also shows that younger dentine consists mainly of HA.

The different solubility of pritubular and intertubular dentin also corroborates this statement.

Conclusion

Analyses of dissolved calcium weight from healthy young and old dentin samples, including the different localities from which the samples had been taken, show that the amount of calcium ions expressed in μg/mm/h was emphasized most in the first hour (Tables 3 and 4).

In the young samples this process was faster, but the difference was statistically relevant the difference was statistically relevant only in the third measurement ($p<0.05$).

The mean value of all initial velocities showed that the *initial speed* of calcium ions dissolving was higher in the younger samples. The assumption is that this is a consequence of the difference in ***ratio and arrangement*** of calcium salts in the first and second group of samples.

The amount of calcium salts extracted from the samples does not only depend on the total weight and surface of the samples but also on other factors like age, histological, morphological and crystallographical structure of the dentin tissue.