Promene u mandibuli usled osteoporoze

Changes in mandible due to osteoporosis

DOI:10.2298/SGS0701016P

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ORIGINALNI RAD (OR)
ORIGINAL ARTICLE

KRATAK SADRŽAJ

Uvod. Osteoporoza može biti jedna od promena koje se javljaju u koštanom tkivu usled starenja.

Cilj. Cilj ovog istraživanja je bio da se ispitaju promene u mandibuli nastale usled osteoporoze.

Metod rada. Kao materijal korišćeni su uzorci kosti izdvojeni iz mandibula kadavera. Bezuba mandibula je bila eksperimentalna kost u istraživanju. U studiji je primenjeno 5 različitih metoda analizovanja strukture kosti iz uzoraka. Izmerene su apsolutne gustine uzoraka. SEM analiza i difrakcija X zracima su primenjene u cilju ispitivanja sastava slojeva kosti mandibula.

Rezultati. Dobijeni rezultati su pokazali da je najmanja gustina od 1.9658 g/cm^3 ustanovljena pri merenjima uzorka bezubog grebena eksperimentalne mandibule. Na osnovu SEM mikrografija utvrđene su redukcije trabekula i smanjenja širina lamela. Statistički značajne razlike (p<0.01) su zabeležene upoređivanjem numeričkih vrednosti širina mikroprostora u spongioznim površinama eksperimentalnih uzoraka. Progresivno povećanje poroznosti je utvrđeno u uzorku bezubog grebena kod 6,35% , i u uzorku bazalnog sloja kod 14,06% eksperimentalne mandibule. Upoređivanjem površina kosti sa različitim oblicima apatita usled izmena oblika kristala utvrđena je značajna povezanost prisustva kiseonika (P=0.046) na erodovanim i oštećenim površinama kosti (p<0.001). Hidroksiapatit, kalcijum-fosfat, tetrakalcijum-pirofosfat i amorfni kalcijum fosfat su sačinjavali neorganske faze u značajno izmenjenim mikrokristalima uzorka eksperimentnog bezubog grebena.

Zaključak. Na osnovu rezultata istraživanja utvrđena je različita degradacija kortikalnog matriksa i spongioznih slojeva bezube mandibule nastalih usled osteoporoze.

Ključne reči: osteoporoza, mandibula, bezubost.

SUMMARY

Introduction: Osteoporosis may occur in bone tissue as a result of ageing.

Aim: The aim of this study was to investigate remodelling of old human edentulous mandibular bone due to osteoporosis.

Mateirals and Methods: Osseous surfaces of one edentulous and one dentate mandible were extracted from a cadaver. This study included 5 methods of analysis of bone structures in experimental and control bony samples of human mandibular bones. Absolute densities of samples were determined. SEM analysis and X-ray diffraction analysis of spectra were used to assess bony layers.

Results: Minimum density 1.9658 g/cm^3 of edentulous ridge site was detected. SEM micrographs indicated reductions of trabecular and lamellar width. Significant differences (p<0.01) in cancellous site width were recorded in experimental basal specimens. Noticeable increase of porosity was observed in 6.35% of edentulous ridge site and 14.06% of experimental basal site. There was a significant correlation between oxygen presence (p=0.046) eroded and defect bone surfaces (p<0.001). Complexes of hydroxyapatite, calcium-phosphate, tetracalcium-pyrophosphate and amorphous calcium-phosphate were inorganic constituents considerable mineral modifications of edentulous ridge site.

Conclusion. Based on the results of the present study, various levels of degradation of cortical matrix and cancellous layers of edentulous mandible may be observed as a result of osteoporosis.

Key words: osteoporosis, mandible, edentulism.

Na osnovu istraživanja o metaboličkim poremećajima, lokalnoj atrofiji i procesima starenja kosti, utvrđeno je da osteoporoza može značajno da ošteti humani skelet [1-3]. Ovakve promene mogu biti uzrok poremećaja i u kostima donje i kostima gornje vilice [4-7].

Various studies have shown metabolic disturbances, local atrophy and aging disorders due to regional or systemic osteoporosis [1-3]. These changes may also affect upper and lower jaw bone tissue [4-7].

U jednom broju studija ispitivana je osteoporoza i razlike u strukturi alveolarnih i bazalnih slojeva mandibule[5,8], što je ukazivalo na izvesnu mogućnost da smanjenje gustine alveolarne kosti usled osteoporoze može biti značajno intenzivnije od promena u bazalnim slojevima tela mandibule. Međutim, za razliku od većine kostiju u ljudskom organizmu, gustina kosti u mandibuli može biti čak i povećana za vreme starenja[6].
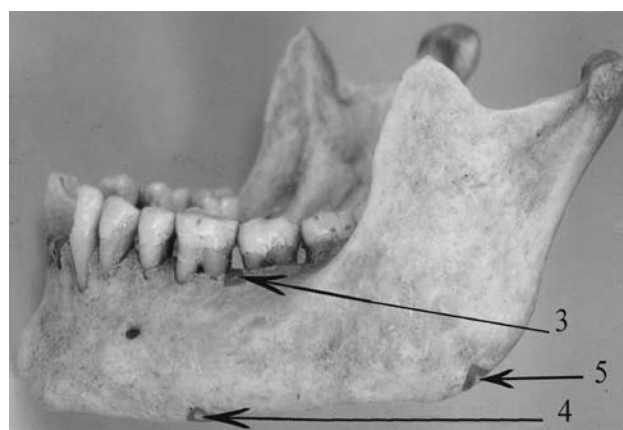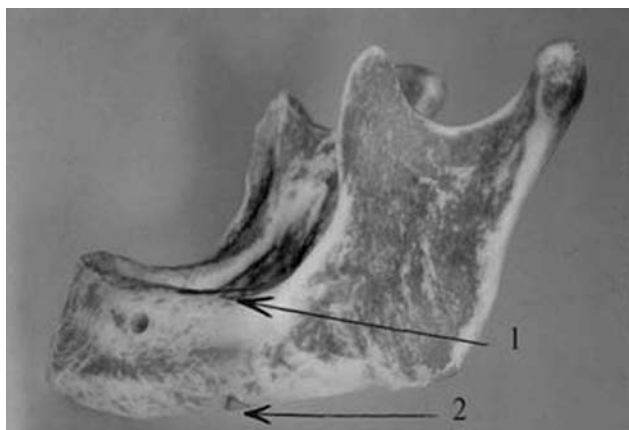
Makroskopski, osteoporoza i degradacija mandibule mogu nalikovati atrofiji koja različito oštećuje površine bezube mandibule[4]. Isto tako, negativna remodelovanja mandibule usled osteoporoze mogu biti uočljiva na mikroskopskom nivou u korteksnim i u spongioznim slojevima[7, 8]. Međutim, bez obzira na očigledne strukturne promene, mineralni sastav osteoporozne madibule do sada nije ispitivan.

Stoga je cilj ovih istraživanja bio da se ispitaju promene u uzorcima kosti iz mandibule kadavera, nastale usled osteoporoze.

Osteoporosis and structural difference in alveolar and basal layers of human mandibles were reported[5, 8], suggesting that decrease of alveolar bone density could be significant when compared with mandibular basal surfaces. Moreover, in contrast to many other bones in human body, density might increase in old human mandibles[6].

Macroscopically, osteoporosis and degradation of mandible could appear as atrophy which affects surfaces of edentulous mandible to different levels[4]. Moreover, negative remodelling of mandible due to osteoporosis seems to be predetermined in microscopic levels of cortical and cancellous parts[7, 8].

However, despite of structural alterations, changes of mineral-crystal forms of osteoporotic mandible have not been analyzed.

The aim of this study was to investigate changes in edentulous mandible due to osteoporosis.

Materijal i metod

Bezuba mandibula kadavera bila je eksperimentalni uzorak. Kontrolna kost je bila neresorbovana mandibula kadavera gde su očuvani zubi.

Na bezuboj mandibuli su označene eksperimentalne površine kosti bezubog grebena i bazalne kosti, a na kontrolnoj mandibuli su analizirane 3 površine ─ površina po alveolarnom grebenu, bazalna kost i površina po angulusu mandibule (Slika 1).

Materials and methods

As an experimental specimen, an edentulous mandible of cadaver was used. The dentate mandible was used as control.

Experimental edentulous ridge and basal bone site were marked on the surface of edentulous mandible. Alveolar ridge site, basal bone site and gonion site were marked on the surface of the control dentated mandible. (Figure 1)

Slika 1. Eksperimentalna bezuba mandibula kadavera i kontrolna mandibula. a) 1-eksperimentalna površina kosti bezubog grebena; 2-eksperimentalna površina bazalne kosti mandibule; b) kontrolna mandibula. 3-površina po alveolarnom grebenu; 4-bazalna kost. 5-površina po angulusu mandibule;
Figure 1. Experimental edentulous cadaveric mandible and control mandible. a) 1-experimental edentulous ridge site; 2- experimental basal bone site; b) 3-alveolar ridge site; 4-basal bone site. 5-gonion site

Radiografisanje je primenjeno na početku istraživanja. Filmovi (Kodak T-MAT G, USA), iz iste serije su korišćeni u radiografisanju (ORTHOPANTOMOGRAPH Siemens 10, serijski broj 01492, Siemens, Germany) pri

X-ray was applied in the beginning of this study. Panoramic dental films (KODAK T-MAT G, USA) of the same batch were taken (ORTHOPANTOMOGRAPH Siemens 10, serial no.01492, Siemens, Germany) at 58 kVp

kondicijama 58 kV i 5 mA; ekspoziciji 20sec. (Slika 2). Metod svetlosne denzitometrije (Transmission Densitometer DTII05; England R.Y. Parry LTD, Newbury Berkshire, The England, UK) korišćen je u cilju ispitivanja optičke gustine površina na ortopantomogramima.

and 5 mA; exposure time 20sec. (Figure 2) Light-beam densitometry (Transmission Densitometer DTII05; England R.Y. Parry LTD, Newbury Berkshire, The England, UK) was used to assess optical densities of samples in panoramic X-rays.
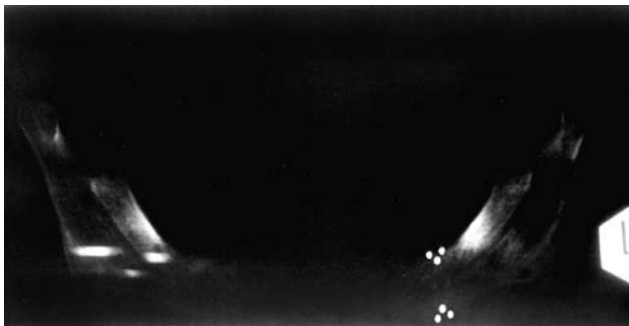
Slika 2. Ortopantomogram a) eksperimentalna bezuba mandibula i metalnim kuglicama ograničene površine od značaja u istraživanju; b) kontrolna mandibula i metalnim kuglicama ograničene tri kontrolne površine kosti.
*Figure 2. Panoramic radiograph; a) Experimental mandible - metal ball-markers indicated borders of areas of interest; b) control mandible and metal ball-markers which indicated borders of 3 areas of interest.*

Posle radiografisanja mandibule, dijamantnim diskom (Diaflex-transvident Horico disc for handpiece, Horico HOPF, Ringleb & Co. GmBH&CIE, Berlin, Germany) usečeni su urezi u kosti. Dijamantno konično svrdlo (B Diasint diamond bur, Dentaurum Pforzheim, Germany) korišćeno je pri odsecanju uzoraka sličnih dimenzija iz eksperimentalne i iz kontrolne mandibule.

U cilju ispitivanja gustine izdvojenih uzoraka kosti korišćen je piknometar (9-11). Tečnost u piknometru je bio benzol pri temperaturi 20º C. Na osnovu formule $\Delta\rho=2\Delta m$ izračunata je greška merenja. Apsolutna gustina svakog uzorka izračunata je na osnovu formule $\Delta\rho=\Delta m(masa)$-greška merenja/$\Delta v(zapremina)$.

After taking X-rays of the mandible, grooves were cut in the bone using diamond discs (Diaflex-transvident Horico disc for handpiece, Horico HOPF, Ringleb & Co. GmBH&CIE, Berlin, Germany). Diamond inverted cone bur (B Diasint diamond bur for straight hand piece, Dentaurum Pforzheim, Germany) was used to cut off samples of similar dimensions from experimental and control mandible.

A pycnometer was used to measure density of obtained samples (9-11). The pycnometer liquid was benzol at 20°C. Standard error was calculated using the following formula: $\Delta\rho=2\Delta m$. Absolute density of each bone sample was calculated using the following formula: $\Delta\rho$ (absolute density) = Δmass – standard error/ Δvolume.
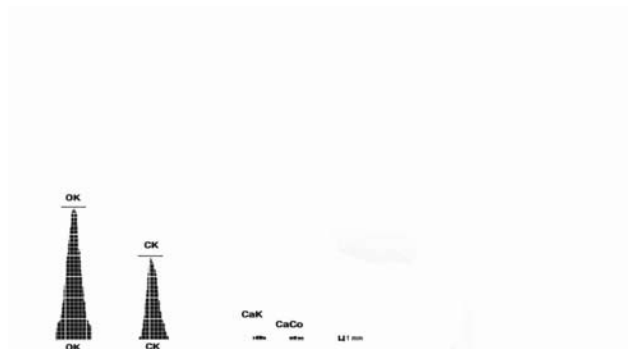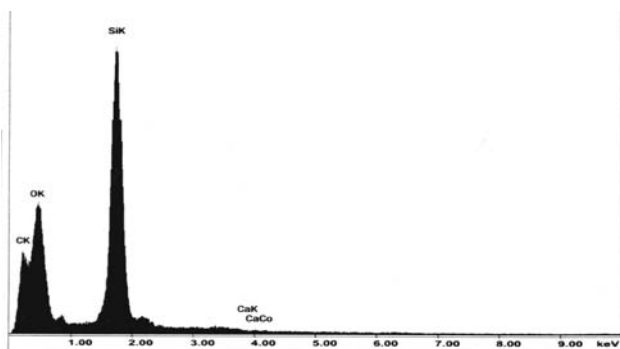
Slika 3. EDAX analiza a) Mikroelementi u spektru uzorka 1; b) Analiza kvantitativne zastupljenosti mikrolemenata u uzorku 1.
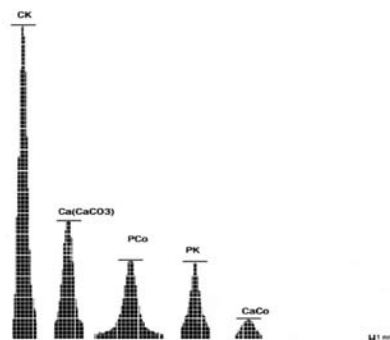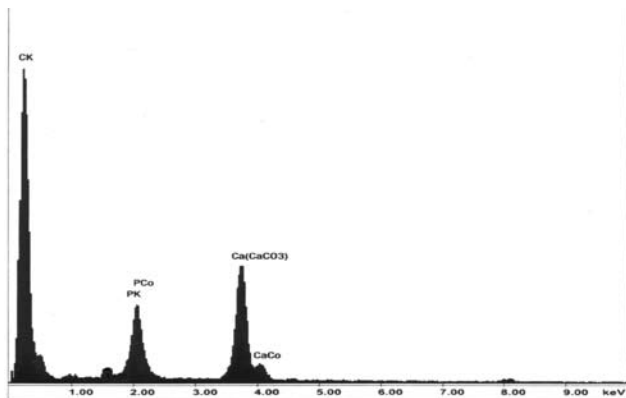*Figure 3. EDAX-RF microanalysis. a) Microelements in sample 1 spectrum; b) Quantitative analysis of microelement presence in sample 1.*

Posle dehidracije uzorci su čuvani u vakumu i pripremljeni za mikroanalizu. Sadržaji mikroelemenata u uzorcima kosti su utvrđeni kvalitativnom disperzivnom mikroanalizom (Philips EDAX energy dispersive X-ray spectrometer 9100, Phillips, The Nederland) (Slike 3 — 4). Zatim je primenjena skening-elektronska mikroskopi-

Dehydrations of bone samples were conducted in ethanol and kept in vacuum prior to microanalysis. Qualitative energy-dispersive microanalysis was used to detect microelements (Philips EDAX energy dispersive X-ray spectrometer 9100, Phillips, The Nederland). (Figures 3—4). Scanning electron microscopy method was applied (Field-emission

ja (Field-emission Scanning Electron Microscope, Philips XL30; Philips Eindhoven, The Nederland) nesprašenih eksperimentalnih i kontrolnih uzoraka kosti pri različitim uvećanjima (Slike 5 ‒ 8). Uzorci su pripremani za analizu u etanolu. U cilju konačnog upoređivanja uzoraka odabrano je uvećanje od 623 puta za sve uzorke (Slika 5b, Slika 6b, Slika 7b, Slika 8b, Slika 9).

Scanning Electron Microscope, Philips XL30; Philips Eindhoven, The Nederland) to examine sections of samples. Bone samples were optimally dehydrated. Samples were irradiated in standardized way. Different magnifications were used for SEM observations of sections of experimental and control sites (Figures 5 ‒ 8). Finally, x623 magnification was used in SEM analysis of each experimental (Figure 5b, Figure 6b) and control specimen (Figure 7b, Figure 8b, Figure 9).


Slika 4. EDAX analiza a) Mikroelementi u spektru uzorka 2; b) Analiza kvantitativne zastupljenosti mikrolemenata u uzorku 2.
Figure 4. EDAX analysis a) Microelements in sample 2 spectrum; b) Quantitative analysis of microelement presence in sample 2
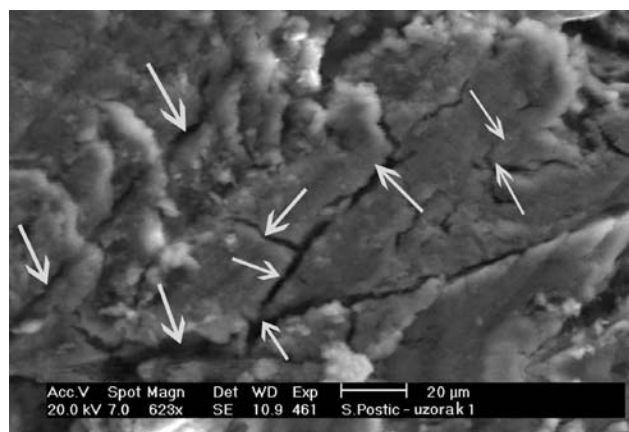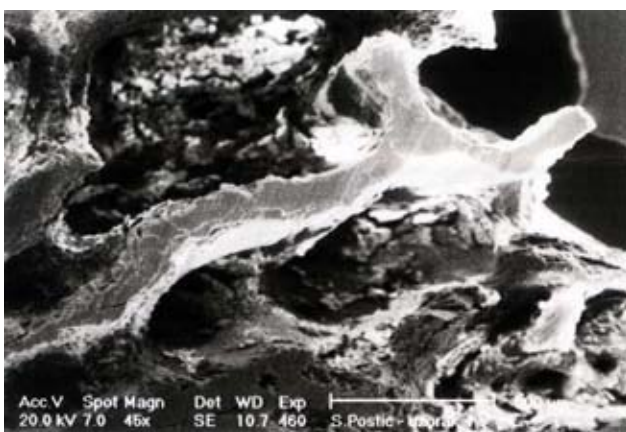
Slika 5. SEM prikaz koštane površine uzorka 1: a) uvećanje 45 puta.; b) uvećanje 623 puta, strelicama označene brojne mikropukotine u osteoporoznoj kosti bezubog grebena.
Figure 5. SEM of sample 1 surface: a) x45 magnification ; b) x 623 magnification, arrows indicate numerous microporosities in osteoporotic bone of the edentulous ridge;
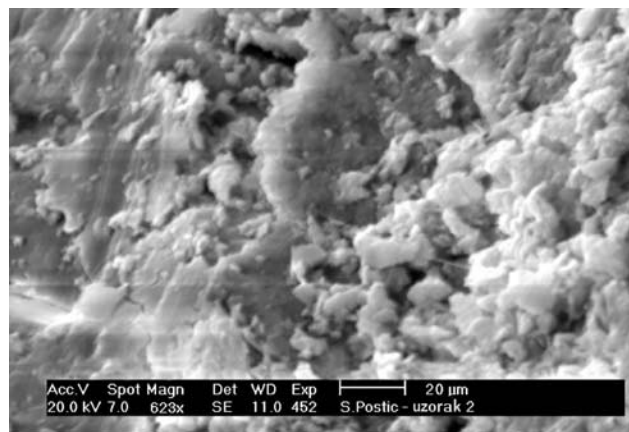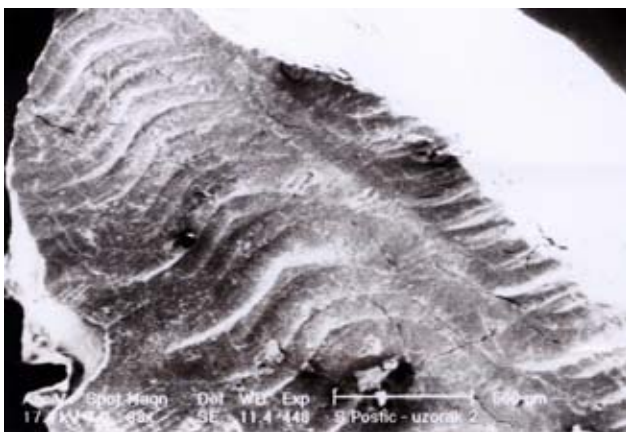
Slika 6. SEM prikaz koštane površine uzorka 2 a) uvećanje 68 puta; b) uvećanje 623 puta.
Figure 6. SEM of sample 2 surface: a) x68 magnification; b) x623 magnification;
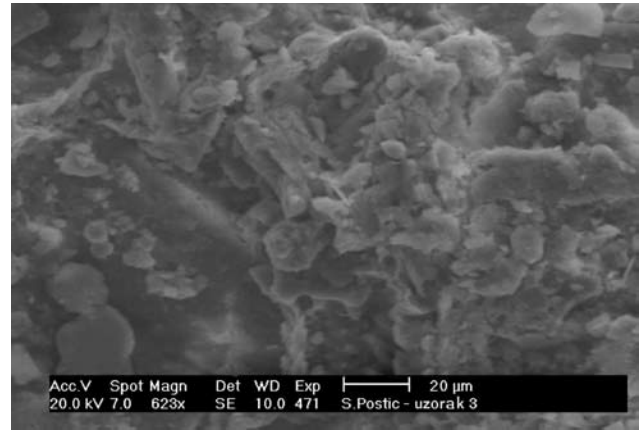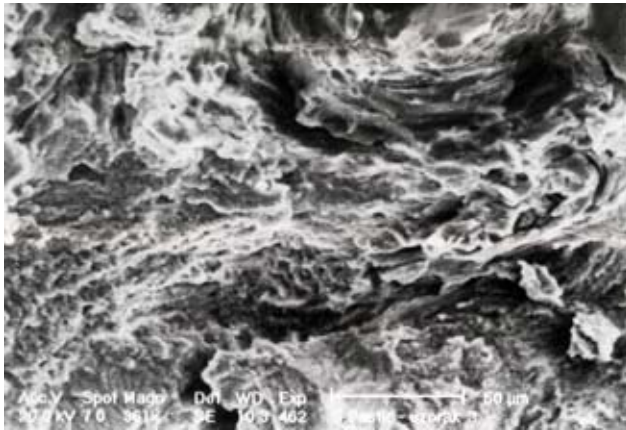
Slika 7. SEM prikaz koštane površine uzorka 3 a) uvećanje 361 puta: b) uvećanje 623 puta.
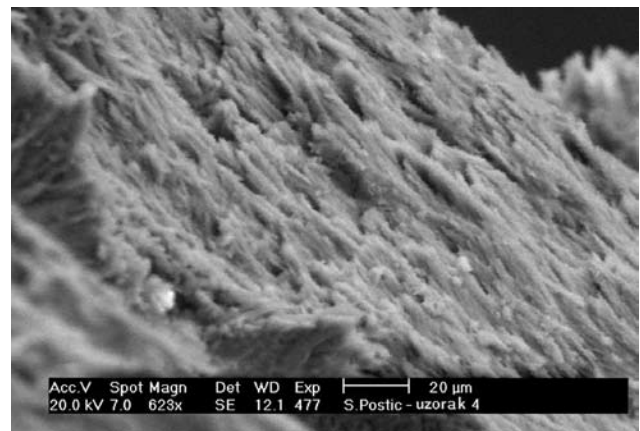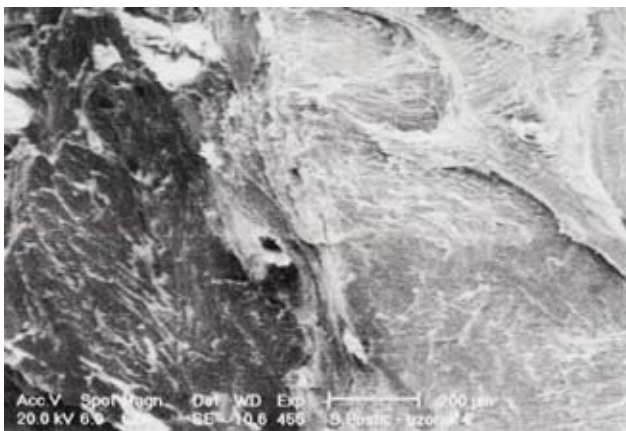*Figure 7. SEM of sample 3 surface: a) x361 magnification ; b) x623 magnification;*

Slika 8. SEM prikaz koštane površine uzorka 4 a) uvećanje 62 puta; b) uvećanje 623 puta.
Figure 8. SEM of sample 3 surface: a) x62 magnification; b) x623 magnification.
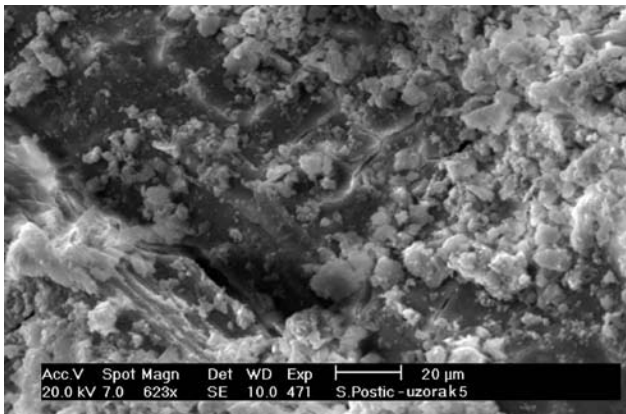
Slika 9. SEM prikaz površine angulusa kontrolne mandibule - uzorak 5 , uvećanje 623 puta.
Figure 9. SEM photomicrograph of control mandible angulus - sample 5, x623 magnification;
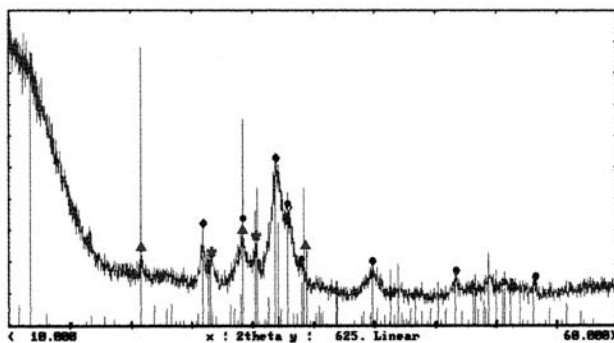
*Slika 10. Neorganski konstituenti mikrokristala u sprašenom uzorku 1; horizontalna referentna linija 2θ(Cu)-maksimalna vrednost 60; vertikalna linija (cps)-najveći intenzitet 1000; • =Ca5(PO4)3(OH);   * =CaHPO4 ; ▲ =CaHPO4x2H2O; ◊= Ca4P2O9;*
*Figure 10. Inorganic constituents of microcrystals in dusty sample 1: horizontal reference line: 2θ(Cu)-maximum 60; vertical reference line: intensity(cps)- maximum 1000. •=Ca5(PO4)3(OH)   *=CaHPO4 ▲ =CaHPO4x2H2O; ◊= Ca4P2O9;*

Slika 11. Neorganski konstituenti mikrokristala u sprašenom uzorku 2; horizontalna referentna linija 2θ(Cu)-maksimalna vrednost 60; vertikalna linija (cps)-najveći intenzitet 1000; • =Ca5(PO4)3(OH); Figure 11. Inorganic constituents of microcrystals in dusty sample 2: horizontal reference line: 2θ(Cu)-maximum60; vertical reference line: intensity (cps)-maximum 1000. .=Ca5(PO4)3(OH);
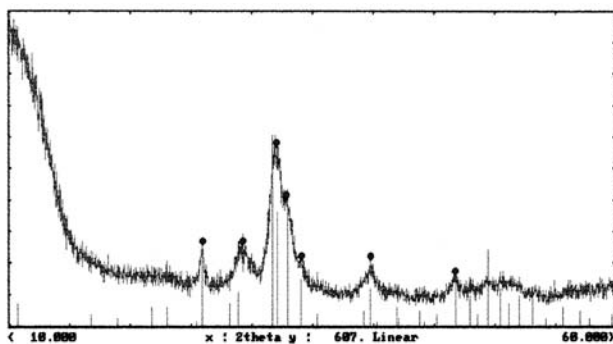
Difrakcija X zracima je primenjena u cilju analizovanja mineralnih sastava sprašenih uzoraka (Crystalloflex difractometer D-500; Siemens, Germany), jačina struje 35KV pri 20mA (Slika 10, Slika 11).

X-ray diffraction method was used for analysis of mineral-phased compositions of samples (Crystalloflex difractometer D-500; Siemens, Germany), at 35KV and 20mA (Figures 10 — 11).

Rezultati

Results

Vrednosti gustine eksperimentalnih uzoraka od 1,9658 g/cm^3 do 2,0748 g/cm^3 izmerene su u piknometru (Tabela 1).Greška merenja je iznosila ± 0.0005 grama pri masi od 0.0531 grama.

Density of 1.9658 g/cm^3 - 2.0748 g/cm^3 of basal site-″S2″ was measured in the pycnometer (Table 1). Standard error was ± 0.0005g per 0.0531g.

Tabela 1. Gustine uzoraka
Table 1. Sample densities.

Uzorci u kostima prikazani na osnovu ortopantomograma Panoramic radiographs of bone samples	Optička-relativna gustina Optic-relative density (U/mm^2)	Izdvojen uzorak kosti Sample	Apsolutna gustina Density (g/cm^3)
Osteoporozni bezubi greben Osteoporotic edentulous ridge site	-2.38	1	1.9658
Osteoporozni bazalni sloj Osteoporotic basal bone site	-2.51	2	2.0748
Alveolarni greben Alveolar ridge site	-0.90	3	2.1296
Bazalna kost Basal bone site	-0.62	4	2.1357
Angulus mandibule Gonion site	-2.52	5	2.1810

EDAX analizom ustanovljena je različita zastupljenost mikrolemenata u eksperimentalnim uzorcima 1 i 2. (Slike 3 — 4). U uzorku 1 mikroelementi su bili zastupljeni redosledom: kalijum, ugljenik, kalcijum (Slika 3b). Najviše je bio zastupljen kalijum.

U uzorku 2 mikroelementi su bili zastupljeni redosledom: kalijum ugljenik, fosfor, kalcijum–karbonat, kalcijum (Slika 4b). Kvantitativno najviše su bili prisutni kalijum i kalcijum karbonat.

U kontrolnim uzorcima utvrđeno je postojanje kalijuma, kalcijum-karbonata, ugljenika, fosfora i natrijuma. U kontrolnim uzorcima je bilo značajno više kalcijuma, fosfora i natrijuma.

EDXRF microanalysis revealed different microelements in experimental samples S1 and S2 (Figures.3 — 4). As for S1, microelements were as follows: potassium, carbon, calcium and cobalt (Figure 3b). The majority of sample substance contained potassium.

In the sample S2, the following microelements were detected: potassium, carbon, phosphorus, calcium-carbonate, cobalt and calcium (Figure 4b). Potassium and carbonates were major constituents of bony substance of S2.

Qualitative energy-dispersive microanalyses of control samples showed the presence of potassium, calcium-carbonate, carbon, phosphorus and sodium microelements. Potassium and carbonates were major constituents of control bone.

Upoređujući prisustvo kiseonika (kiseonik u jonima i kiseonik u kompleksnim jedinjenjima na površinama kosti) i prisustvo kalcijuma u ekperimentalnim uzorcima, izračunata je značajna vrednost koeficijenta korelacije (r=0.626). Kada su upoređivani prisustvo fosfora i kalcijuma (kalcijum-kobalt u paru) u eksperimentalnim uzorcima utvrđena je statistički značajna vrednost koeficijenta korelacije (r=0.547). Vrednost koeficijenta korelacije nije bila statistički značajna (r=0.223) kada su upoređivani fosfor i kalcijum-karbonat u eksperimentalnim uzorcima.

Analizom svih eksperimentalnih uzoraka utvrđeno je značajno prisustvo kiseonika.

Rezultati analize SEM mikrografija su pokazali modifikacije trabekula, razgradnju koštanih slojeva i poroznost eksperimentalnih uzoraka 1 i 2 (Slika 5, Slika 6). Negativno remodelovanje lamela je utvrđeno u uzorku 2. Brojne mikro-pukotine su uočene u uzorku 1. (Slika 5b).

Najveći porast poroznosti – 14,6 % utvrđen je u uzorku 2 (Tabela 3).

Significant coefficient was calculated (r=0.626) correlating oxygen and calcium levels in experimental samples. When phosphorus and calcium (calcium-cobalt pair) in experimental samples were compared, a significant value was calculated (r=0.547). Correlation coefficient was not significant (r=0.223) when phosphorus and calcium carbonate in experimental samples were compared.

EDXRF analyses of experimental samples revealed significant oxygen presence.

SEM micrograph revealed trabecular modifications, porosity and degradation of osseous layers in experimental samples S1 and S2 (Figure 5, Figure 6). Negative remodelling of lamellar parts was seen in S2. Microcracks were shown in experimental sample S1 (Figure 5b).

The greatest increase in porosity -14.6% was recorded in sample S2 (Table 3).

Tabela 2. Prosečne vrednosti debljina lamela
Table 2. Mean values of lamelar thickness

Uzorak Sample	Debljina Thickness(µm)
1	17,9 ± 7,51
2	23.03 ± 8.91
3	9.31 ± 3.89
4	4.06 ± 1.02
5	26.15 ± 7.83

Tabela 3. Procenat poroznosti u eksperimentalnim uzorcima
Table 3. Percentage of sample porosity.

	Uzorak 1 Sample1	Uzorak 2 Sample2	Uzorak 5 Sample 5
Ukupan broj šupljina u spongioznoj kosti / Totals of cancellous sites			17
Broj poroznih mikrošupljina / Totals of porosities	108	29	
Procentualna zastupljenost poroznosti u eksperimentalnim uzorcima Percentage of porosities in samples	183.05%	405.08%	
Procenat povećanja poroznosti Percentage of porosity increase in comparison to cancellous sites in referent sample	6.35%	14.06%	Referentan uzorak referent sample

Utvrđene su statistički značajne razlike (P<0.01; t=2,947) u broju poroznih mikrostruktura uzorka 1 i uzorka 2. Numeričke vrednosti rastojanja između susednih lamela su bile značajno različite (Tabela 4).

Dvosmerna analiza varijansi je primenjena da bi se utvrdilo da li su sve površine uzoraka pogodne za nakupljanje jona kiseonika, i da li su različite razgrađene površine eksperimentalnih uzoraka kosti sa različitim oblicima apatita usled izmena oblika kristala više prijemčive za

There was statistically significant difference ((p<0.01; T test value=2.947) in the number of porous microstructures in S1 and S2. Numerical values for interlamellar distances were significantly different (Table 4).

Two-way ANOVA was applied to assess whether all sample surfaces were suitable oxygen ion adherence, as well as to distinguish oxygen affinity of experimental samples with different types of apatite. Two way ANOVA revealed significant presence of oxygen in

kiseonik. Analiza varijansi je pokazala da postoji značajna povezanost prisustva kiseonika (P=0.046) na erodiranim i razgrađenim površinama kosti (P<0.001).

Difrakcijom X-zracima utvrđeno je da su nestabilna faza hidroksi-apatita, kalcijum fosfata i amorfni kalcijum-fosfat (CaHPO$_4$X2H$_2$O) sačinjavali kristale u sprašenom uzorku 1 (Slika. 10 i Tabela 5).

Nedovoljno čvrsta faza hidroksilapatita je bila većinski sadržaj u sprašenom uzorku 2 (Slika 11) (Tabela 5).

eroded (p<0.001) and decomposed osseous surfaces (p=0.046).

X-ray diffraction analysis showed that the unstable phase of hydroxyapatite, calcium phosphate and amorphous calcium phosphate formed crystals of dust in S1 (Figure 10) (Table 5).

Insufficiently hard phase of hydroxyapatite was predominant in dusted S2 (Figure 11) (Table 5).

Tabela 4. Udaljenja graničnih - terminalnih linija i površina
Table 4. Distances of adjacent terminal lines.

Rastojanja između graničnih linija susednih lamela Distances between terminal lines of adjacent lamellae	
SEM uzorak SEM specimen	Srednja vrednost ± SD Mean ± SD (μm)
osteoporozna bazalna kost osteoporotic basal bone site	7.9 ± 4.06
angulus kontrolne mandibule gonion site	15.25 ± 4.02
kontrolna bazalna kost basal bone site	0.49 ± 0.38
Rastojanja između graničnih linija susednih trabekula Distances between terminal lines of adjacent trabecular surfaces	
SEM uzorak SEM specimen	Srednja vrednost ± SD Mean ± SD (μm)
osteoporozni bezubi greben osteoporotic edentulous-ridge site	9.97 ± 5.39
alveolarni greben alveolar ridge site	3 ± 2.16

Tabela 5. Sadržaji kristala u uzorcima
Table 5. Crystals and mineral phases.

Uzorak Sample	Kristali Crystals	Veličina kristala Sizes of crystals in samples(μm)	Prosečna vrednost±SD kristala Means±SD of bony surfaces with distorted crystals(μm^2)
		kristali slični po veličini uzornim kristalima sizes of crystals similar to referent crystal	
S1	Ca5(PO4)3(OH) CaHPO4 CaHPO4•nH2O	37,0	27.2±13.11
S2	Ca5(PO4)3(OH)	27,2	4.81±2.38
		uzorni-referentni kristali size of referent crystal	
S3	Ca5(PO4)3(OH) Ca4P2O9	22,5	9.27±8.19
S4	Ca5(PO4)3(OH)	24,5	1.35±0.18
S5	Ca5(PO4)3(OH)	22,8	2.68±0.87

Diskusija

Male vrednosti gustina eksperimentalnih uzoraka kosti izmerene su, skoro sasvim sigurno usled osteoporoze. Obzirom na značajnu masu kompaktnih slojeva, bazalna površina mandibule ne bi trebalo da bude predisponirana za oštećenja usled gubitka koštanog tkiva u osteoporozi. Bazalni sloj kosti kontrolne mandibule mogao bi se smatrati referentnom površinom pri izračunavanju stepena poroznosti u eksperimentalnim uzorcima [5, 7, 12-25, 27].

U cilju utvrđivanja posebnih promena u ovom eksperimentu, potrebno bi bilo diskutovati da li su prekidi kontinuiteta trabekula, u literaturi ranije ustanovljen poremećaj prouzrokovan osteoporozom [2,3], bili lokalizovani samo između preostalih umreženih pomoćnih-"sekundarnih" trabekula u uzorku 1 neposredno uz, i prema površini tela bezube mandibule, ili su bili prisutni i u nosećim trabekulama? Analizom SEM mikrografija su utvrđeni ne samo brojni prekidi struktura umreženih sekundarnih trabekula, već i prekidi u nosećim trabekulama. U tom smislu negativno remodelovanje bezubog grebena humane osteoporozne mandibule moglo bi se, sasvim sigurno smatrati osobenim i jedinstvenim poremećajem u humanom skeletu.

Na osnovu rezultata analize SEM mikrografija moglo bi se pretpostaviti da bi prekomerna odstupanja od dimenzija mikroprostora između površina susednih trabekula ukazivala zapravo na sekundarnu hipertrofiju trabekula koja ne bi bila posledica negativnog remodelovanja bezubog grebena usled osteoporoze. Međutim, na osnovu dokumentovanih vrednosti standardnih devijacija, a takođe i prosečnih vrednosti udaljenja graničnih linija po površinama susednih trabekula, sekundarna hipertrofija trabekula je utvrđena u uzorku alveolarnog grebena kontrolne mandibule. Sekundarno hipertrofisanje trabekula u alveolarnom grebenu dokumentovali su i rezultati upoređivanja prosečnih vrednosti i standardnih devijacija mikrokristala. Pored toga, utvrđene prosečne vrednosti i standardne devijacije udaljenja susednih trabekula mogle bi, vrlo verovatno ukazati na varijacije u širini, odnosno debljini trabekula u bezubom grebenu eksperimentalne osteoporozne mandibule. U tom smislu, varijacije u debljini trabekula mogu, skoro bezuslovno govoriti u prilog sporadične hipertrofije trabekula i u bezubom grebenu osteoporozne mandibule.

Uzorak bezubog grebena eksperimentalne osteoporozne mandibule i uzorak alveolarnog grebena između zuba kontrolne mandibule nalazili su se u tkzv. "žvaćnom centru" [26]. Samim tim, treba pretpostaviti da je remodelovanje bilo najintenzivnije upravo u tim uzorcima. Stoga je, u ovom istraživanju bilo posebno interesantno dijagnostikovati da li su sekundarno podebljane trabekule postojale i pre negativnog remodelovanja koštanih slojeva u eksperimentalnom uzorku bezubog grebena. Brojne hipertrofične lamele u alveolarnoj kosti najverovatnije su nastale usled prethodnih opterećenja na greben za vreme mastikacije. Na taj način, sekundarna hipertrofija trabekula ne bi se mogla dovesti u vezu sa osteoporozom.

Na osnovu analize SEM mikrografija utvrđeno je smanjenje broja spongioznih šupljina u trabekularnoj kosti eksperimentalnih uzoraka i postojanje brojnih poro-

Discussion

Low density of edentulous ridge site and detected decrease in density of experimental samples were, most probably, the result of osteoporosis.

Basal bone, resembling compact surface should be less susceptible to destructions. Basal bone in the control sample should be accepted as the reference for calculation of levels of porosity in experimental samples [5,7,12-25,27].

In order to detect specific changes in this experiment, it should be discussed whether trabecullar disruptions, which were summarized as osteoporotic trabecular disorders [2, 3], were located between cross-linking trabecular rods of experimental edentulous ridge site, or were present in stress-bearing trabeculae as well. SEM micrographs documented disruptions in cross-linking trabecular rods as well as in stress-bearing trabeculae. In this respect, negative remodelling of edentulous ridge and edentulous mandible should be considered as unique disorder in human skeleton.

SEM micrograph of overextension of trabecullar adjacent portions could suggest that secondary trabecullar hypertrophy not associated with negative remodeling of edentulous ridge. However, in respect of recorded standard deviations and mean distances of terminal lines of adjacent trabecullae, secondary trabecullar hypertrophy was detected in alveolar ridge site. Secondary hypertrophy of alveolar ridge trabecullae was also documented by comparison of mean and SD values in microcrystals.

Additionally, mean and SD values of distances of adjacent trabecullar portions could suggest diversity and variations of trabecular thickness within edentulous ridge site. Consequently, variations in trabecular thickness could strongly account for sporadic secondary hypertrophy of trabeculae in edentulous ridge site.

Experimental edentulous ridge and control alveolar ridge sites were detected within the so-called "chewing centers" [26]. Therefore, remodeling should have been the most intensive on these sites. In this study, it was particularly interesting to detect whether secondary hypertrophic trabecullae had already existed, prior to negative remodeling of edentulous ridge site.

Numerous hypertrophic trabecullae could have appeared in response to previous masticatory loading. Thus, secondary hypertrophy of trabecullae could not be related to osteoporosis.

SEM micrographs suggested decreased number of cancellous regions in trabecullar bone of experimental specimens ($p < 0, 01$) and numerous porosity sites with the tendency of increasing and negative bone remodelling.

znih šupljina (p<0,01) sa tendencijom povećanja poroznosti i negativnog remodelovanja slojeva kosti. Stoga je vrlo verovatno da je ubrzana resorpcija koštanog matriksa uzrokovana osteoklastičnom aktivnošću dovela do intenzivne poroznosti.

U ovoj studiji utvrđena je povezanost nakupljanja kiseonika na razgrađenim površinama kosti eksperimentalnih uzoraka, koje su oštećene usled osteoporoze, i broja razgrađenih površina. Čini se u potpunosti opravdanom pretpostavka da su porozni prostori bili pogodne površine za nakupljanje jona kiseonika. Joni kiseonika su "označili" ne samo prekinute površine trabekula, već i osteoporozom oštećene lamele.

Uprkos činjenici da je na osnovu SEM mikrografija utvrđeno postojanje lamela u svim uzorcima moglo bi se smatrati da je eksperimentalni uzorak broj 2 u osnovi bio sačinjen od kortikalnih slojeva. Međutim ekperimentalni uzorak bezubog grebena u osnovi nije bio sačinjen od kortikalne kosti. Na osnovu činjenice da su kortikalne lamele kontrolnog uzorka broj 5 iz angulusa mandibule, iz kontrolnog uzorka broj 4 iz baze kontrolne mandibule, kao i lamele iz eksperimentalnog uzorka broj 2 baze bezube mandibule bile pre svega u primarnoj kompresivnoj grupi, a ponegde i u sekundarnoj kompresivnoj grupi lamela, moglo bi se, na prvi pogled pretpostaviti da nije bilo redukcija lamela usled osteoporoze. Pored toga moglo bi se smatrati da, sasvim očigledno nije bilo ni stanjivanja lamela. Takvo zapažanje bi bilo u skladu sa nalazima Boyde i Kingsmill-a [6]. Međutim, SEM mikrografije bazalnih slojeva ekperimentalne mandibule pokazale su modifikacije oblika lamela i redukcije broja lamela što se, sasvim sigurno može pripisati osteoporozi i starenju kosti [8].

Izmenjeni oblici lamela mogli bi se smatrati posledicom nakupljanja kalcijum-karbonata.

Sporadično uvećane lamele iz uzorka 2 bazalne kosti eksperimentalne mandibule mogle bi govoriti u prilog sasvim posebnog procesa remodelovanja [1, 6]. Međutim, značajno različiti oblici lamela bazalnog sloja eksperimentalne mandibule mogli bi ukazivati i na remodelovanje sekundarnim zadebljavanjem korteksa osteoporozne mandibule procesom endokortikalne apozicije, ili udruženim procesima periostalne i endokortikalne apozicije [2].

Na osnovu strukture koštanih slojeva prikazanih SEM mikrografijama, neuobičajnog uvećanja lamela bazalnog sloja eksperimentalne mandibule i haotično diseminovane površine korteksa u uzorku bezubog grebena eksperimentalne mandibule koje ukazuju na neregularnosti u rasporedu koštanih mikropovršina u eksperimentalnoj mandibuli. Zaključuje se da su opisane promene posledica osteoporoze. Pored toga, smanjena debljina koštanih jedinica i redukcija volumena eksperimentalne mandibule svakako bi se mogli smatrati posledicom disbalansa po onim površinama kosti gde su postojali mogući, odnosno uslovno aktivni potencijali za remodelovanje [1-3].

Joni kiseonika bili su najbrojniji na ne-simetričnim površinama koštanih slojeva bezubog grebena (odnosno, mikroskopski na nesimetričnim kristalima) implicitno ukazujući na demineralizaciju, nedostatak dobro mineralizovanog — kristalnog apatita i poroznost u koštanim slojevima.

The resorption of bone matrix could be induced by osteoclastic activity.

In this study, there was a positive correlation between oxygen presence in degraded bone surfaces and the extent of surface degradation. It could be assumed that porous cavities were suitable for positioning oxygen ions. Oxygen ions indicated not only interrupted trabecullar surfaces but also osteoporotic lamellae.

Despite the fact that SEM micrographs showed lamellar forms in all samples, it should be assumed that experimental edentulous basal site essentially had cortical structure. However, experimental edentulous ridge site was not originally only cortical. Based on the finding that cortical lamellae of control gonium, basal bone sites in control and experimental samples were arranged in principally compressive groups and partly within secondary compressive groups of osseous layers, it could be assumed that there was not lamellar reduction due to osteoporosis. Moreover, it could be assumed that there was no thickness reduction of lamellae. Similar findings were discussed in the study of Boyde and Kingsmill [6]. However, SEM micrographs of experimental basal bone layers showed lamellar modification and reduction which indicated osteoporotic and age related changes of mandibular bone [8].

Modified lamellar forms could be the consequence of calcium-phosphate apposition.

Enlarged lamellae of experimental basal bone could verify particular remodelling [1,6]. However, significantly different lamellar forms of experimental basal sites could indicate remodelling by secondary thickening of osteoporotic cortex by endocortical apposition, or by joint periosteal and endocortical apposition [2].

SEM micrograph observations, unusual increases in lamellar forms of experimental basal site and chaotically distanced cortical parts of edentulous ridge site could indicate irregular pattern in osteoporosis. Besides, decreased thickness of bone units, reduction in experimental bone volume and changes of trabecular and cortical experimental portions should be identified as sequellae of osteoporosis and imbalance at remodelling sites [1-3].

Oxygen ions were predominant on asymmetric surfaces of osseous layers of edentulous ridge implied demineralization, the lack of mineralized crystal apatite and porosities in osseous layers.

It should be noted that carbonate presence in complex compounds implied low levels of calcium in hydroxyapatite in experimental osteoporotic bone samples. Unstable "weak" mineral phase of apatite and transformed crystallites were in edentulous ridge site most probably because

Čini se da bi bilo važno napomenuti da je prisustvo karbonata u kompleksnim jedinjenjima zapravo ukazalo na prethodno postojanje kalcijumom deficitarnog hidroksi-apatita u eksperimentalnim — osteoporoznim uzorcima kosti. Nestabilna faza hidroksi-apatita i transformisani kristali najverovatnije su bili u bezubom grebenu usled disbalansa u remodelovanju. Oslabljena kristalna faza hidroksi-apatita, kalcijum karbonat i amorfni kalcijum-fosfat (CaHPO$_4$X2H$_2$O) sigurno su odgovorni za asimetrije mikrokristala kosti u ekspeimentalnom uzorku bezubog grebena [28].

Veoma interesantan nalaz—jedinjenje tetrakalcijum-pirofosfat-Ca4P2O9 i hidroksil-apatit u uzorku bezubog grebena eksperimentalne mandibule trebalo bi najverovatnije posmatrati kao značajno prisustvo vezanih jona kiseonika sa tendencijom kasnijeg otpuštanja. U tom smislu kalcijum, kao posebna frakcija mogao bi biti prenet na oslabljeni hidroksiapatit u ranoj fazi negativnog remodelovanja kosti. Slobodna frakcija kalcijuma i kalcijum fosfat najverovatnije bi trebalo da ukažu na sekundarno mineralizovanje kosti. Stoga bi se moglo, skoro sasvim sigurno tvrditi da bi alveolarni greben mandibule mogao biti prekursor, nagoveštavajući incidence negativnih remodelovanja kostiju [29] i osteoporozu [30].

Hidroksi-apatit i kalcijum sulfat u kompleksnom jedinjenju treba posmatrati kao mineralni kompleks koji ukazuje na dehidrolizu i dominaciju nestabilnih faza apatita u osteoporoznom bezubom grebenu.

Zaključak

Rezultati istraživanja pokazuju sliku različite degradacije kortikalnog matriksa i spongioznih slojeva bezube mandibule uzrokovane osteoporozom.

of remodelling imbalance. Weakened crystal phase of hydroxyapatite, calcium carbonate and amorphous calcium phosphate in edentulous ridge might be responsible for severe distortions of crystal symmetry [28].

An interesting finding – tetracalcium-phyrophosphat and hydroxyapatite in edentulous ridge of experimental bone should be seen as rich oxygen pool with releasing potential. In this respect, calcium fraction could be transferred onto weakened phase of apatite in the early phase of negative remodelling. Calcium fraction and Calcium phosphate could most probably indicate secondary bone mineralization. Thus, alveolar ridge *in vivo* could be the predictor of negative bone remodelling [29] and osteoporosis [30].

Hydroxyapatite and calcium phosphate in a complex compound could indicate dehydrolysis and dominance of unstable apatite phases in experimental dusted edentulous ridge site.

Conclusion

This study has shown different osteoporotic degradation levels of cortical matrix and cancellous parts of edentulous mandible.

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