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# ZARASTANJE EKSTRAKCIONE RANE NA EKSPERIMENTALNOM MODELU PACOVA

# HEALING OF EXTRACTION WOUND IN AN EXPERIMENTAL MODEL OF RAT

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***ABSTRACT***

***Uvod****: Proces zarastanja rane nakon ekstrakcije zuba je sa velikom pažnjom istraživan kod nekoliko životinjskih vrsta pod normalnim i eksperimentalnim uslovima koji bi mogli ometati zarastanje. Hronologija zarastanja rane nakon ekstrakcije zuba – molara i inciziva se kod pacova odvija u nekoliko faza i ispitivana je histološkim, radiografskim, dezintrometrijskim, imunohistohemijskim i stereološkim(tačkasta volumetrija) metodama, kao i upotrebom mini kamere za određivanje volumenske frakcije histoloških komponenti*

***Cilj rada****: Na osnovu literature dostupne u elektronskim bazama podataka analizirati histolo-ške i radioloske rezultate o toku zarastanja normalne ekstrakcione rane pacova i vremenskog perioda koji je poterban da rana nakon eksrakcije u potpunosti zaraste.*

***Materijal i metod****: Istraživanjem smo dobili 22 rada koji prate normalan tok zarastanja od kojih je 7 zadovoljilo kriterijume pogledu kompletnog opisa događaja u alveoli nakon ekstrakcije zuba kod pacova.*

***Rezultati****: Drugog dana nakon ekstrakcije rana pocinje da epitelizira. Prvi znaci stvaranja koštanog tkiva javljaju se četvrtog dana posle ekstrakcije kao subperiostalna osteogeneza. Četvrtog i petog dana, kod nekih fibroblasta počinje diferencijacija u osteoblaste. Petog dana vide se delikatne trabekule mlade kosti i rtg prisutna jaća senka u cetralnom delu apikalne trećine alveole. Zavrsetak epitelizacije je osmog dana posle ekstrakcije kada je lamina dura malo manje izražena, dok donji delovi alveole imaju izraženiju rtg senku. Četrnaestog dana u najvećem broju primeraka, alveola je potpuno popunjena retikularnom kosti cija je rtg senka približna okolnoj kosti. Dvadesetog dana mlada kost ispunjava alveolu. Šezdesetog postoperativnog dana alveolu ispunjava lamelarna kost, dok se remodelacija koštanih rubova nastavlja do 112. postoperativnog dana.*

***Zaključak****: Zarastanje ekstrakcione rane kod eksperimentalnih pacova počinje sa pojavom epitela drugog dana posle ekstrakcije. Koštano tkivo počinje da se stvara četvrtog dana posle ektrakcije. Završetak epitelizacije je 8. dana, dok mlada kost ispunjava alveolu 20. postoperativnog dana. Potpuno koštano zarastanje dešava se 60. dana posle ekstrakcije. Metode od kojih je očekivano da ubrzaju zarastanje ekstrakcione rane kao što je primena lasera male snage, koštanog morfogenetskog proteina i polarizovane svetlosti na modelu pacova nisu dale očekivane rezultate.*

***Ključne reči****: ekstrakciona rana, zarastanje, eksperimentalne životinje*

***ABSTRACT***

***Introduction****:The process of wound healing after tooth extraction has been studied in several animal species under normal and experimental conditions that might interfere with the healing. Chronology of wound healing after tooth extraction (molars and incisors), which consists in several phases, has been examined by histological, radiological, immunohistochemistry, stereology (bone volumetry), and densitometry methods and by using of micro-camera to determine the volume fraction of histological components.*

***The aim*** *of this study is to analyse the process of healing of normal extraction wound and to determine the period of time it takes for the postextraction wound to completely heal.*

***Matherial and method****: The research is based on the literature available in electronic databases Pubmed/Medline, Kobson, Googlescholar. The total of 22 papers which follow the normal course of healing were found; 7 papers met the criteria regarding the complete description of the healig process in the socket after tooth extraction in rats.*

***Results****: On the second day after the extraction wound starts with forming epithelium. The first sign of bone tissue has appeared four days after the extraction as subperiostal osteogenesis. On fourth and fifth day, in some fibroblasts differentiation in osteoblasts has already begun.. On the fifih day the delicate trabecules of young bone attached to the walls of the socket in its basal part can be seen, and the signs of ossification are clearly indicated by a darker shadow in the central part of the apical third of the socket present in the x ray. The end of the epitelization is on the eighth day, when the lamina dura was slightly less pronounced, while the lower parts of the socket have a pronounced X-ray shadow. In most of the specimens, the socket has been completely filled with reticular bone on day 14. The socket has been filled with young bone after 20 days.Presense of lamelar bone can be seen 60$^{th}$ day after exraction. Remodelation of aveolar ridges lasts until 112$^{th}$ day.*

***Conclusion****: The healing of normal extraction wounds in rats begins with the appearance of the epithelium of the second postoperative day, and the end of the epitelization is on the eighth day . Formation of bone begins on the fourth day after the exodontia. The intense bone formation lasts until the twentieth postoperative day when the young r bone fills the socket. Lamellar bone is present on 60$^{th}$ days after extraction. Applicaton of bone morfogenetic protein, low power laser and polarized light had no expected effect on the the healing of the extraction wound in experimental rats.*

***Key words:*** *extraction wound, healing, experimental animal*

## *Uvod*

Proces zarastanja rane nakon ekstrakcije zuba je sa velikom pažnjom istraživan kod nekoliko životinjskih vrsta pod normalnim i eksperimentalnim uslovima koji bi mogli ometati zarastanje.

Pacovi imaju ukupno šesnaest zuba, osam u gornjoj i osam u donjoj vilici[1]. Od toga četiri duga, ostra inciziva, po dva u svakoj vilici. Incizivi su specijaliovani za glodanje i mogu se obnavljati tj. ponovo izrastati tokom zivota. Imaju jedan, dug koren sa širokim apikalnim otvorom. Kada bi izrastali bez trošenja-abrazije, incizivi bi bili lučno savijeni pod uglom od 86 stepeni. Stopa erupcije je kod odraslih pacova 2,2 mm nedeljno (0,31-0,32 mm dnevno), donji incizivi u proseku rastu 2,8 mm nedeljno (0,4 mm dnevno). Na taj način stari zub se zameni novim za nekih 40-50 dana, odnosno pacov nema inciziva starijih od 50 dana[1].

Molari kod pacova sluze za mlevenje hrane. Postoji ukupno dvanaest molara, po šest u svakoj vilici, sa svake strane po tri. Molari se ne obnavljaju, tj. kod pacova postoji samo jedan set molara u toku života, zbog toga se nazivaju monofiodonti. Molari imaju dva malo kraća korena, koji ne rastu[1].

Pacovi nemaju ni očnjake ni premolare. Na mestima gde bi trebalo da budu lateralni sekutići, očnjaci i premolari postoje prostrane dijasteme.

Broj i vrsta zuba kod različitih vrsta izražavaju se kao dentalna formula. I n/n C n/n P n/n M n/n , gde je I, C, P, M oznaka za incizive, kaninuse, premolare i molare, dok je n/n- odnos gornji i donjih zuba sa svake stane u vilici. Tako dolazimo do dentalne formule kod pacova koja glasi : I: 1-1, C: 0-0, P: 0-0, M: 3-3 [2].

Hronologija zarastanja rane nakon ekstrakcije zuba – molara i inciziva koja se kod pacova

*Slika 1. Vilice pacova[1]*
*Figure1. A jaws in rats[1]*

## *Introduction*

The process of wound healing after tooth extraction has been studied with great care in several animal species under normal and experimental conditions that might interfere with the healing.

Rats have a sixteen teeth, eight in the upper eight in the lower jaw[1]. There are four long, sharp incisors, two in each jaw. Incisors are specialized for gnowing and can be renewed, i.e, they continiue growing throughout life. Incisors have a long root with a wide apical opening. If grown without abrasion, incisors arch would form an angle of 86 degrees. The rate of eruption in adult rat is 2.2 mm per week for upper incisors (0.31 to 0.32 mm per day), with average 2.8 mm per week for lower incisors (0.4 mm per day). Rats nave no incisor older than 50 days, cause the old tooth is replaced by a new in approximately 40-50 days[1].

Rat molars are used for grinding. There are a total of twelve molars, six in each jaw three on each side. Molars don't get renewed, that is in rats there is only one set of molars in a lifetime, which is why they are colled monofiodonts. Molars have two roots that are a little shorter and they do not grow[1].

Rats have no canines or premolars. There is large diastema in places where there should be lateral incisors, canines and premolars. In different species the number and type of teeth are expressed as a dental formula. I n / n C n / n P n / n M n / n, where I, C, P, M are signify incisors, caninuses, premolars and molars, while n/n- is ratio between the upper and lower teeth on each side in the jaw. The dental formula in rat is: I: 1-1, C: 0-0, P: 0-0, M: 3-3[2].

Wound healing chronology after tooth extraction (molars and incisors), which consists in several phases, has been examined by histological, radiological, immunohistochemistry[5,6] stereology (bone volumetry), and densitometry [3,4], methods and by using of micro-camera to determine the volume fraction of histological components [7].

**The aim** of this study is to analyse the process of healing of normal extraction wound and to determine the period of time it takes for the postextraction wound to completely heal[3–7]. The research is based on the literature available in electronic databases Pubmed/Medline[8], Kobson[9], Googlescholar[10]. The studies which

odvija u nekoliko faza ispitivana je histološkim, radiografskim, dezintrometrijskim[3,4], imunohistohemijskim[5,6] i stereološkim (tačkasta volumetrija) metodama, kao i upotrebom mini kamere za određivanje volumenske frakcije histoloških komponenti[7].

**Cilj rada** je da na osnovu literature dostupne u elektronskim bazama podataka Pubmed/ Medline[8], Kobson[9], Googlescholar[10] analiziramo rezultate o toku zarastanja normalne ekstrakcione rane i vremenskog perioda koji je poterban da rana nakon eksrakcije u potpunosti zaraste[3–7]. Nisu razmatrani radovi u kojima su obrađivani eksperimentalni modeli kod kojih je aplikovan analgetik, antibiotik, hormonska supstanca, i drugi medikamenti, kao i oni kod kojih su odstranjene polne žlezde, pankreas, druga tkiva ili organi, kod kojih je namerno pravljen defekt u kosti vilica i nakon toga prćen tok zarastanja rane.

## *Rezultati*

Istraživanjem smo dobili 22 rada koji prate normalan tok zarastanja od kojih je 7 zadovoljilo kriterijume u pogledu kompletnog opisa događaja u alveoli nakon ekstrakcije zuba kod pacova.

## *Histološka analiza ekstarkcione rane[4,6,11]*

U studijama su uglavnom žrtvovani Wistar pacovi koji su čuvani pod istim uslovima i žrtvovani na isti način. Intraperitonealnom injekcijom pacovi su uvedeni u opštu anesteziju, da bi im zatim bili pažljivo ekstrahovani zubi. Pacovi su žrtvovoani dekapitacijom, vilice su potapane u 10 % rastvor formalina na 48 sati. Posle fiksacije vilice su tangencijalno presecane i potapane u parafin. Longitudinalni preseci debljine po 6nm bojeni su hematoksilinom i eozinom.

1. dan: Alveolu ispunjava koagulum koji se sastoji od mešavine fibrina i ćelija krvi. Ćelije periodontalnog ligamenta su dominantne ćelije u alveoli. Proliferacija ćelija je niska (indeks prebojenosti L = oko 2%) do 16$^{h}$ nakon ekstrakcije zuba, ali dramatično raste na maksimalni nivo 1 dan posle ekstrakcije (L = 23%)[4]. Između 1 i 2 dana, brojni fibroblasti koji potiču iz ostatka periodontalnog ligamenta aktivno naseljavaju koagulum i nastavjaju da se da se

used experimental models with the application of analgetics, antibiotics, hormonal substances and other drugs, as well as those in which genital glands, pancreatic, and other tissues or organs were removed, or in which the defect was deliberately caused into the jaw bone in order for the healing process to be observed, have not been considered in this study.

## *Results*

The total of 22 papers which follow the normal course of healing were found; 7 papers met the criteria regarding the complete description of the healig process in the socket after tooth extraction in rats.

## *Histological analysis of exraction wound [4,6,11]*

Wistar rats used in this studies were kept under the same conditions and sacrificed in the same way. General anesthesia was administered to rats by inraperitoneal injection, and then the teeth were carefully extracted. The rats were sacrefited by decapitation, the jaws were submerged into 10% formalin for 48 hours. After the fixation, jaws were tangentially sectioned and submerged in paraffin. Longitudinal sections 6 nm thick were stained with hematoxylin and eosin.

Day 1. The socket is filled with mash of clot consisting of a mixture of fibrin and blood cells. Cells of periodontal ligament are the dominant cells in the socket. Up until 16 $^{h}$ after tooth extraction cell proliferation was low (index of coloration (L) = 2%), but increases dramatically to the maximum level one day after extraction (L = 23%)[4]. Between 1$^{st}$ and 2$^{nd}$ days, numerous fibroblasts derived from the rest of periodontal ligament actively populate the clot and multiply. Fibroblasts possess a high proliferative activity, but a small number of cellular organelles responsible for synthesis of procollagen, which proves that trey are mostly immature cells[6].

Day 2. Blood clot surface is covered by the necrotic content consisting of degenerated blood cells, fibrin and food debris, and there is epithelial migration whithin which there are glycogen granules made visible by PAS staining[4]. The rest of the periodontal ligament was evident 2 days after extraction and rich in type III collagen fibers, which can form a template

umnožavaju. Fibroblasti poseduju visoku proliferativnu aktivnost, ali imaju mali broj mobilnih organela odgovornih za sintezu prokolagena, tako da su to uglavnom nezrele ćelije[6].

2. dan: Površina koaguluma pokrivena je nekrotičnim sadržajem koji se sastoji od degenerisanih krvnih ćelija, fibrina i debrisa od hrane, kao i prisustvo epitelne migracije unutar koje postoje granule glikogena uočljive PAS bojenjem[4]. Ostatak periodontalnog ligamenta je evidentan 2 dana nakon ekstrakcije i bogat kolagenim vlaknima tipa III koja mogu formirati šablon za stvaranje buduće trabekularne kosti[11]. U ostatku alveole postoji generalizovano bojenje fibronektina što ukazuje na njegovu važnu ulogu u formiranju granulacionog tkiva, tako što stvara matricu za migraciju fibroblasta. U apikalnom regionu alveole mezenhimalne ćelije prožimaju koagulum, dok se na zidovima alveole uočava nekoliko multinukleusnih gigantskih ćelija.

3. dan: Fibroblasti sadrže više mobilnih organela i deponuju više kolagenih vlakana koja zamenjuju koagulum gustim vezivnom tkivom. Razvili su se i fibroblasti iz endosealnog i perivaskularnog tkiva, ali na nižem nivou i u kasnijim vremenskom periodu od fibroblasta iz periodontalnog ligamenta[6]. Enosealni i perivaskularni fibroblasti su samo u malom broju poslužili za dalji proces zarastanja rane.

4. dan: Prvi znaci stvaranja koštanog tkiva počinju kao subperiostalna osteogeneza, sa pojavom osteoida na eksternoj površini bukalne i lingvalne lamele (ekstraalveolarna apozicija kosti), kao i osteoklastna aktivnost koja je dovela do resorpcije bukalnog i lingvalnog alveolarnog ruba. Koagulum je potpuno zamenjen gustim vezivnim tkivom, a zidovi alveole pokriveni su gigantskim ćelijama. Epitelizacija gingive je progresivna, ali nije prekrila površinu alveole. Četvrtog i petog dana, kod nekih fibroblasta počinje diferencijacija u osteoblaste, a proliferacija fibroblasta je drastično smanjena na bazalne vrednosti[6].

5. dan: Alveola je većim delom prekrivena gingivalnim epitelom. Vide se delikatne trabekule mlade kosti prikačene za zidove alveole u njenom bazalnom delu. Ostaci periodontalne membrane naziru se u tragovima, vlakna počinju da gube svoju normalnu orijentaciju. Aktivnost osteoklasta (početak remodelacije) je izražena na rubovima alveole[4,6].

8. dan: U ovom periodu gingivalni epitel u potpunosti pokrio površinu alveole a apikalni deo ispunjen je mladom kosti i osteoidom. Periodontalni ligament nije više prisutan. Nastav-

for creating future trabecular bone[11]. In the rest of the socket there is a generalized staining of fibronectin suggesting its important role in the formation of granulation tissue, by creating a matrix for migration of fibroblasts. In the apical region of the socket the mesenchymal cells penetrate the socket clot, while several multinucleus giant cells can be observed on the walls of socket.

Day 3. Fibroblasts contain several mobile organelles and deposit more collagen fibers that replace the blood clot with dense connective tissue. Fibroblasts from endoseal and perivasculavar tissue have also developed, but at a lower level and later than fibroblasts from periodontal ligament[6]. Endoseal and perivascular fibroblasts have played only a miner role to the further process of wound healing.

Day 4. The first sign of bone formation is subperiostal osteogenesis, with the appearance of osteoid on the external surface of the buccal and lingual lamel (extraalveolar apposition of bone) and osteoclast activity leading to resorption of the buccal and lingual alveolar edge. The clot has been totally replaced by dense connective tissue, and alveolar walls are covered with giant cells. Epitelization of the gums is a progressive, but hasnt still covered the whole surface of the socket. During the fourth and the fifth day, in some fibroblasts differentiation in osteoblasts has already begun, and fibroblast proliferation was drastically reduced to basal values[6].

Day 5. The socket is largely covered by gingival epithelium. The delicate trabecules of young bone attached to the walls of the socket in its basal part can be seen. Remnants of periodontal ligament can be observed in the trace, the fibers begin to lose their normal orientation. Osteoclasts activity (the beginning of remodeling) is emphasised at the edges of the socket[4,6].

Day 8. At this point gingival epithelium has completely covered the surface of the socket while the apical part is filled with young bone and osteoid. Periodontal ligament is no longer present. Deposition of bone continues on the buccal and lingual surfaces so does resorption and remodeling of bone edges.

Day 10. In most of the specimens the socket has been half filled with young bone. The alveolar edges are rounded, partially due to resorption of the lingual and buccal sides, partially because subperiostal apposition.

Day 13. Thin trabecules of young bone occupied most of the socket, while the alveolar

lja se depozicija kosti na bukalnoj i lingvalnoj površini kao i remodelovanje rubova resorpcijom.

10. dan: U najvećem broju primeraka alveola je do polovine popunjena mladom kosti. Rubovi alveole su zaobljeni, delimično usled resorpcije sa lingvalne i bukalne strane, delimično zbog subperiostalne apozicije.

13. dan: tanke, trabekule mlade kosti zauzele su veći deo alveole, dok se remodelovanje alveolarnog grebena i dalje nastavlja. Dobro keratinizirani epitel pokriva površinu alveole u celosti. Kolagena vlakna su paralelena sa epitelnom bazalnom membranom[4].

14. dan: U najvećem broju primeraka, alveola je potpuno popunjena retikularnom kosti. Trabekularna površina pokazuje intenzivnu koštanu aktivnost. Rana je u potpunosti epitelizirala[3].

20. dan: Mlada kost je ispunila alveolu. Osteoblasti prekrivaju površinske trabekule, ali nema korteksa na gornjoj površini kosti. Periost je u potpunosti regenerisan[4]. Tridesetog dana od ekstrakcije aktivni osteoblasti vide se uglavnom na alveolarnom rubu.

60. dan: Alveolu ispunjava lamelarna kost. Kost pokazuje skoro homogeni kontinuitet i gotovo se ne razlikuje od susedne kosti[4].

Nakon ekstrakcije **inciziva** proces zarastanja se odvija se na sličan nači kao kod molara. U prvoj nedelji delikatne koštane trabekule prekrivene osteoblastima su vidljive uglavnom u apikalnom i srednjem regionu, na unutrašnjoj površini bukalnog zida. Postoji obilje vezivnog tkiva i novoformiranih kapilara. Ostaci koaguluma vide se u centralnim regoinima. Do kraja druge nedelje došlo je do progresivnog koštanog zarastanja, pri čemu je alveola jednako popunjena zrelim vezivnim tkivom i koštanim trabekulama. U trećoj nedelji unutrašnjost alveole je popunjena trabekularnom kosti[4].

## *Radiološka analiza ekstrakcione rane[3,4]*

1.-4. dan: na rtg snimku vidi se rasvetljenje na mestu nekadašnjih korenova, lamina dura se vidi kao jasno definisana neprozirna linija.

5. dan: jasno se vide prvi znaci početka osifikacije - rentgenološki prisutna jača senka u cetralnom delu apikalne trećine alveole. Ovaj nalaz je u saglasnosti sa histološkim nalazom iz tog perioida, odnosno sa pojavom koštanih trabekula uz zidove alveole.

ridge remodeling continues. Well-ceratinised epithelium completely covers the alveolar surface. Collagen fibers are parallel with the epithelial basal membrana[4].

Day 14. In most of the specimens, the socket has been completely filled with reticular bone. Trabecular bone surface shows an intensive bone activity. The wound has been completely covered with epithelium[3].

Day 20. The socket has been filled with young bone. Osteoblasts cover the surface of trabeculae, but no cortex on the upper surface of the bone exists. Periosteum is completely regenerated[4]. 30$^{th}$ day after the extraction the active osteoblasts are mainly seen on the alveolar edge.

Day 60. The socket consists of lamellar bone. The bone shows a nearly homogeneous continuity and is almost indistinguishable from the sorounding bone[4].

After the extraction of incisors the healing process goes on in a similar way as with molars. In the first week delicate trabeculas of the bone covered in osteoblasts are visible mainly in the apical and middle region, on the inner surface of the buccal wall. There is an abundance of connective tissue and newly formed capillaries. The remains of blood clot can be seen in the central regions. By the end of the second week the bone has heal progressively, and the socket is equally filled with mature connective tissue and bone trabeculas. In the third week the inside of the socket has been filled with trabecular bone[4].

## *Radiographic analysis of the extraction wound[3.4]*

Day 1-4: X-rays shows the illumination where once there were roots, lamina dura is seen as a clearly defined opaque line.

Day 5.: The first signs of the beginning of ossification are clearly indicated by a darker shadow in the central part of the apical third of the socket present in the x ray. This finding correlates with the histological findings from the same period, i.e. with the appearance of bone trabecules along the alveolar walls.

Day 7. The cortex is barely distinguished, while the fundus of the socket is seen as a much clearer X-ray shadow[3].

Day 8. Lamina dura is slightly less pronounced, while the lower parts of the socket show a more pronounced X-ray shadow, which

7. dan: Korteks se slabo razlikuje, dok se fundus alveole vidi kao jasnija radiološka senka[3].

8. dan: Lamina dura je malo manje izražena, dok donji delovi alveole imaju izraženiju radiološka senku, što se podudara sa histološkim nalazom na kome se vidi da je apikalni deo alveole ispunjen mladom kosti i osteoidom.

10. dan: Lamina dura i dalje je vidljiva ali skoro izjednačena sa okolnom kosti, dok se radiološka senka ne pojačava u ostalim delovima rane[4]. U ovoj fazi histološki nalaz pokazuje da je alveola do polovine popunjena mladom košću.

14. dan: Alveola je popunjena tkivom koje daje gustu i jednaku radiološka senku[3] Alveola je ispunjena sadržajem čija je radiološka senka približna okolnoj kosti, dok se lamina dura jedva nazire. Na histološkom nalazu vidi se jasno solidno formirana retikularna kost koja ispunjava alveolu.

20. dan: Prvobitno radiološka rasvetljenje skoro je u potpunosti zamenjeno senkom koja je po intenzitetu slična okolnoj kosti. Lamina dura se više ne razaznaje . I u ovom periodu radiološki nalaz se poklapa sa histiloškim nalazom- nema korteksa na površini kosti, mlada kost potpuno ispunjava alveolu.

30. dan: Bezuba mesta sasvim su zamenjena tkivom čija je radiološka senka jednaka sa okolinom, tako da skoro nije moguće odrediti gde su zidovi nekadašnje alveole[3]

60. dan: Prazna alveola ima iste radiološke karakteristike kao okolna intaktna kost[3]. Histološki, još u ranijem periodu, 50. dana od ekstrakcije alveolu ispunjava zrela lamelarna kost.

Procenom mineralne gustine kosti i totalnog koštanog volumena nakon ekstrakcije zuba utvrđeno je da je maksimalni porast minerlane gustine od 1-14. dana, pri čemu se nastavlja do 56. dana, dok koštani volumen raste do 56. dana a zatim se smanjuje do 112 dana posle ekstrakcije[12] .

## *Ostale studije od uticaja na praćenje procesa zarastanja ekstrakcione rane na eksperimentalnom modelu pacova*

### *Koštani morfogenetski protein*

Koštani matrks je bogat faktorima rasta među kojima je i koštani morfogenetski protein

is in agreement with the histological findings, which shows that the apical part of the socket filled with young bone and osteoid.

Day 10. Lamina dura is still visible but almost indistinguishable from the surrounding bone, while the X-ray shadow is not amplified in the other parts of the wound[4]. At this stage, histologic findings shows that the socket is half filled with young bone.

Day 14. The socket is filled with tissue that provides a dense and even x ray shadow[3], while the lamina dura is barely visible. A histological findings clearly shows solid formed reticular bone filling the socket.

Day 20. The previous X-ray-illumination has almost complitely been replaced by a shadow similar in intensity to the one of the sorrounding bone. Lamina dura is no longer discernible. In this period, radiographic findings agrees with the histological findings in that there is no cortex on the surface of the bone and that the young bone has complitely filled the socket.

Day 30. Edentulous spots have been completely replaced by tissue with X-ray shadow even to the local area, so that it is almost possible to distinguish the walls of the former alveola[3].

Day 60. At this point the empty socket has the same radiological characteristics as the surrounding intact bone[3]. Histologicaly the socket has been filled with mature lamellar bone at an earlyer period on the 50$^{th}$ day after the extraction.

The assessment of mineral density of the bone and total bone volume after tooth extraction, confirmes that the increase in the mineral density is maximal in the period between the 1$^{th}$ and the 14$^{th}$ day, and that it continues to rise until 56$^{th}$ day, as well as the bone volume which afterwards decreases until the 112$^{th}$ day after the extraction[12].

## *Other studies relevant to the wound healing process in an experimental model of rat*

### *Bone morphogenetic protein*

Bone matrix is rich in growth factors, including the bone morphogenetic protein (BMP), which is synthesized and secreted from the osteoblasts and incorporated into the matrix during bone formation. BMP, which is released during bone resorption can induce differentiation of mesenchymal cells into osteoblasts (osteoin-

(BMP) koji se sintetiše i sekretuje iz osteoblasta i inkorporira u matriks u toku formiranja kosti. BMP koji se oslobađa u toku resorpcije kosti može indukovati diferencijaciju mezenhimalnih ćelija u osteoblaste (osteoindukcija) stimulišući regeneraciju i reparaciju kosti. Eksperimentalne studije ukazale su da je BMP efikasan u lečenju koštanih defekata u kostima različitih prirode [13,14]. Posebno, važni su BMP 7 i BMP 2 (rhBMP-2 i rhBMP-7) koji su korišćeni sa očiglednim uspehom za lečenje koštanih lezija u ortopedskoj i maksilofacijalnoj hirurgiji [15].

BMP je rastvorljiv i difunduje brzo u biološkim medijumima. Da bi se postigla optimalna efikasnost, BMP bi trebalo da ima sa nosač-materijal koji produžava vreme boravka na mestu aplikacije, a koji mora biti biokompatibilan. U te svrhe koriste se hidroksiapatit, mineralizovani i demineralizovani koštani mtriks, kompoziti i biostaklo.

Koštani morfogenetski protein u kombinaciji sa hidroksiapatitom aplikovan posle eksrakcije zuba u koštanu alveolu pacova stimuliše osteogenezu, ali ne ubrzava proces osteointegracije[16]. U drugoj studiji gde je praćeno dejstvo samog hidroksiapatita i u kombinaciji sa koštanim morfogenetskim proteinom, rana je kod pacova koji su tretirani samo hidroksiapatitom brže zarastala do 21. postoperativnog dana, dok je na istom stadijumu zarastanja bila 42. postoperativnog dana kod obe ispitivane grupe[17].

## *Zračenje*

Studija koja ispituje uticaj lokalnog zračenja i zračenja celog tela na pojavu osteoblasta u alveolarnoj kosti pacova nakon ekstrakcije maksilarnog molara pokazala je malo razlike između jedinki koje su bile lokalno zračene dozom zračenja od 6 Gy i ne-zračenih jedinki. Nasuprot tome, u grupi kod koje je zračeno celo telo došlo je do odloženog zarastanja rane. Ultrastrukturna studija je pokazala da je osteoblasta nakon zračenja celog tela bilo manje kao i da su imali slabije razvijene organele[18].

## *Laser male snage*

Laser male snage ima biostimulativni efekat, ali efekat na zarastanje ekstrakcione rane kod

duction) by stimulating bone regeneration and reparation. Experimental studies have shown that BMP is effective in treating bone defects in different types of bones[13,14]. In particular, BMP 7 and BMP 2 (rhBMP-2 and rhBMP-7) used with significant succes in treatment of bone lesions in orthopedic and maxillofacial surgery are important [15].

BMP is soluble and diffuses rapidly in biological media. In order to achieve optimal efficiency, BMP should have the carrier-material which increase the stay at the application spot, and which must be biocompatible. For this purposes hydroxy-apatite, mineralized and demineralized bone matrix, composites and bioglass is used. BMP combined with hydroxyapatite applied after the tooth extraction in the bone socket of the rat stimulates osteogenesis but does not accelerate the process of osteointegration[16]. In another study focusing on the effect of the hydroxy-apatite alone and where combined with BMP shows that the wound in rats treated only with hydroxyapatite heales faster until the 21st postoperative day, while on the 42nd postoperative day the healing was at the same stage with both of the examined group[17].

## *X-ray radiation*

A study on the influence of local irradiation and whole body radiation on the appearance of osteoblasts in rat alveolar bone after maxillary molar extraction showed there was little difference between individuals that were locally irradiated radiation dose of 6 Gy and non-radiated animals. On the other hand, the group exposed to the whole body radiotherapy displayed delayed healing of the wound. Ultrastructural study showed that there were less osteoblasts after whole body radiation and that they had less developed organelles[18].

## *Low power laser (LPL)*

LPL has a stimulative effect, but the effect on healing of extraction wounds in experimental rats is questionable. Some authors state that LPL increases the activity of bone cells (resorption and formation) around the bone defect without changing the structure of the bone[19,20] whereas in another study low power laser does

eksperimentalnih pacova je diskutabilan. Neki autori navode da laser male snage povećava aktivnost koštanih ćelija (resorpciju i formaciju) oko koštanog defekta bez promene strukture kosti[19,20] dok u drugoj studiji laser male snage nije ubrzao epitelizaciju i osteogenezu nakon ekstrakcije zuba kod pacova[21]. U radu autora iz naše institucije[22], laser male snage pokazuje evidentan biostimulativni potencijal na ekstrakcionim ranama na drugom eksperimentalnom modelu. Analizom ekstrakcione rane pasa koja je zračena 7 dana posle ekstrakcije, uočena je značajna razlika u izgledu ekstrakcione rane 7, 14, 21, 28 i 35 dana posle ekstrakcije.

U prvoj nedelji kod ne tretitanih rana sluzokoža je prekrivena hiperplastičnim epitelom, prisutan je snažan subepitelni zapaljenski infiltrat, malo granulacija, intraosealna inflamacija i odsustvo fibroplazije. Odosteoblastična aktivnost izostaje. Kod rana tretiranih laserom male snage sluzokoža pokazuje veći stepen hiperplazije, intraosealni deo rane pokazuje izrazite granulacije i fibroplazije kao i novostvorene koštane gredice. Periferni deo rane pokazuje snažnu osteoblastičnu aktivnost.

U drugoj nedelji, kod rana ne tretiranuh laserom sluzokoža pokazuje blagu subepitelnu inflamaciju, postoji izražena fibroplazija i granulacije. Periferni deo rane pokazuje novostvorene koštane gredice, dok ih u centralnom delu rane nema. U drugoj grupi sluzokoža je u drugoj nedelji normalnog izgleda. Postoji osteoblastična aktivnost u svim segmentima.

Treće nedelje posle ekstrakcije sluzokoža je normalnog izgleda kod nezračenih slučajeva. Periferni delovi rane sadrže zrelu lamelarnu kost, a centralni nezrelu, vlaknastu kost. Posle tretmana laserom male snage u trećoj nedelji sluzokoža je takođe normalne građe, osteoblastična aktivnost je vrlo izražena, tako da ima više zrele nego nezrele kosti.

Posle četiri nedelje intraosealni delovi rane su izgrađeni od gracilnih koštanih gredica. Kod tretiranih rana postoje izraženije koštane gredice.

Posle pet nedelja kod ne tretiranih rana postoje zrele koštane gredice i koštana srž. U centralnim delovima rane postoji fokus ožiljnog vezivnog tkiva. Kod rana tretiranih laserom postoje zrele koštane gredice i koštana srž, bez fokusa ožiljačnog vezivnog tkiva (Tabela 1).

not accelerate epithelialization and osteogenesis after tooth extraction in rats[21]. A study done at our institution[22] documents evident biostimulativing potencial of low power laser on extraction wound of the second experimental model. The analysis of extraction wounds of dogs, which is radiated 7 days after extraction, shows a significant difference in the appearance of extraction wounds 7, 14, 21, 28 and 35 days after extraction.

Mucosa of the untreted wounds in the first week is covered with hyperplastic epithelium, there is a strong subepithelial inflammatory infiltrate, a bit of granulationes, intraosseous inflammation and the absence of fibroplasion. Osteoblastic activity is missing. In wounds treated with low-power laser the mucoses shows a higher degree of hyperplasia, intraosal part of the wound shows pronounced granulationes and fibroplasia as well as newly developed bone trabeculas. Peripheral part of the wound shows strong osteoblastic activity. In the second week, the mucosa of the untreated wound shows mild subepithelial inflamation and there is also a pronounced, fibroplasia and granulation. Peripheral part of the wound shows newly formed bone trabeculas, while there is none in the central part of the wound. In the second group the mucosa looks normal in the second week. There is osteoblastic activity in all segments.

In the third week after the extraction the mucosa appears to be normal in the group hasn't been exposed to radiation. Peripheral parts of the wound contain mature lamellar bone, and central immature, fibrous bone. The mucosa of the wound treated with LPL also appears to be normal in the third week after the treatment, osteoblastic activity is highly pronounced, so there is more of the mature than the immature bone.

After four weeks in untreated gruop intraosal parts of the wound are constructed from graceful bone trabeculas. The treated wound displaced bone trabecula that are more pronounced.

After five weeks there are mature bone trabecula and bone marrow in the untreated wounds.There is also a focus of connective scar tissue In the central parts of the wounds. In the wounds that treated with LPL there are mature bone trabeculas and bone marrow withouth the connective scar tissue focus (Table 1).

*Tabela 1. Biologija zarastanja ekstrakcione rane kod eksperimentalnih životinja zračenih laserom male snage*

| | **Ekstrakcione rane ne tretirane laserom male snage** | **Ekstrakcione rane tretirane laserom male snage** |
|---|---|---|
| **1. nedelja** | Sluzokoža- hiperplastični epitel, subepitelni zapaljenski infiltrat<br>Kost- inflamacija, odsustvo fibroplazije | Sluzokoža- veći stepen hiperplazije,<br>Kost-granulacije, fibroplazija, koštane gredice, prisutna osteoblastična aktivnost |
| **5. nedelja** | Kost- zrele koštane gredice i kostna srž, u centralnim delovima rane fokus ožiljnog vezivnog tkiva | Kost- zrele koštane gredice, kostna srž, nema fokusa ožiljnog tkiva |

*Table 1. Biological events of extraction wounds in experimentl animal treated with LPL*

| | Extraction wounds not treated with LPL | Exraction wounds treated with LPL |
|---|---|---|
| 1. week | Mucosa-hyperplastic epithelium, a strong subepithelial inflammatory infiltrate.<br>Bone-inflamation, osteoblastic activity is missing | Mucosa- higher degree of hyperplasia,<br>Bone- pronounced granulationes and fibroplasia, newly developed bone trabeculas. osteoblastic activity |
| **5. week** | Bone-mature bone trabecula and bone marrow<br>A focus of connective scar tissue | Bone-mature bone trabeculas and bone marrow withouth the connective scar tissue focus |

## *Bioptron lampa*

Ekstrakciona rana koja prirodno zarasta, kod eksperimentalnih pacova počinje da epitelizira drugog dana posle ekstrakcije, a završetak epitelizacije je osmog dana[4]. Pod dejstvom polarizovane svetlosti ekstrakcina rana epitelizira devetog dana. Mikromorfološki nalaz devetog dana posle ekstrakcije pokazuje da je epitel bez epitela regenerisan sa bazalno-ćelijskom hiperplazijom i sa povećenim mitotskim indeksom i hiperhromatizacijom jedara u njegovoj donjoj trećini[22].

## *Zaključak*

Zarastanje normalne ekstarkcione rane kod pacova počinje sa pojavom gingivalnog epitela drugog postoperativnog dana, a završetak epitelizacje je osmog dana. Kost počinje progresivno da se stvara četvrtog postoperativnog dana, a petog postoji vidljiva radiološka senka. Intenzivno stvaranje kosti traje do dvadesetog postoperativnog dana sa jasnom radiološkom senkom u tom periodu. Lamelarna kost prisutna je šezdeset dana posle ekstrakcije, kada se na snimku prazna alveola ne razlikuje od okolne kosti. Faza remodelovanja alveolarnih rubova se nastavlja do 112. dana posle ekstrakcije. Na brže zarastanje ekstrakcione rane kod eksperimentalnih pacova nisu uticali koštani morfogenetski protein (BMP), laser male snage i polarizovana svetlost.

## *Polarized light (Bioptron lamp)*

Naturally healing wound in experimental rats begins epitelization on the second day after the extraction and completes the epitelization at the eight day[4]. Under the influence of the polarized light the extraction wound finish with epitelization on the ninth day. Micromorphological record on the ninth day after extraction shows that the epithelium without epithelium with basal-cell hyperplasia was regenerated and with the increased mitotic index and hiperhromatization of the nucleuses in its lower third[22].

## *Conclusion*

The healing of normal extraction wounds in rats begins with the appearance of the gums epithelium of the second postoperative day, and the end of the epitelization is on the eighth day . The progresive formation of bone begins on the fourth day after the exodontia, and on the fifth there is a visible x-ray shadow. The intense bone formation lasts until the twentieth postoperative day by a clear X-ray shadow whithin that period. Lamellar bone is present is 60$^{th}$ days after extraction, when the empty socket can not be distinguished from the surrounding bone on the X-ray. The phase of the allveolar ridge remodeling continues until 112$^{th}$ days after the tooth extraction. BMP, LPL, and polarized light had no effect on the the healing of the extraction wound in experimental rats.

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