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Effect of Laser on the Bone Defects Healing in Rats: An Experimental Study

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SUMMARY

Introduction The stimulating effect of low-power laser on the process of wound healing is characterized by proliferation of fibroblasts, collagen production, and enhanced enzyme activity. The aim of this study was to evaluate the possible enhancing effect of low-power laser on the rate of the healing artificially created osseous defects in rats.
Material and methods Ten albino Wister rats were involved in this experimental study. Round defects (3 mm wide 2 mm deep) were made in each rat on both femurs. The right side was experimental while the left side was control. Osseous defects on the experimental side were daily treated with Galium, Aluminium, Arsenid (GaAlAs) low-power laser (Medicolaser 637, Technoline, Belgrade, Serbia), at the energy output of 4 J/cm² with constant power density of 50 mW, and a wave length 637 nm (visible red light) per defect during seven days. Defects on the control side healed spontaneously. The effects of laser were evaluated two and three weeks postoperatively.
Results Histological analysis showed the powerful osteoblastic activity on the bone defects of the experimental side two weeks after surgery. On the control side, a new bone formation was noticed at the periphery of the bone defects but fibroblastic tissue with no signs of new bone was presented in the central areas. Three weeks after surgery, on the experimental side, bone defects were completely filled with spongious lamellar bone while non-treated bone defects were characterized by mature lamellar bone at the peripheral areas and immature bone at the central areas.
Conclusion The results showed that the use of low-power laser could have a significant influence on the speed of curing bone defects in rats.

Keywords: low-power laser; GaAlAs laser; bone defect; bone healing; osteoblastic activity; experimental study

INTRODUCTION

Bone healing includes the following steps: induction of acute inflammation, regeneration of parenchymal cells, migration and proliferation of parenchymal and connective tissue cells, synthesis of protein and extracellular matrix, remodelling of connective tissue and parenchymal component and collagen forming [1]. Tooth extraction and removal of the chronic periapical lesion can cause the defects in the jaw bone. Healing of the bone socket is similar with bone healing in general and includes the following stages [2]: formation of a blood clot, organisation of the clot, making the epithelial cover of the surface of the wound, formation of the woven bone in the connective tissue filling, and replacement of the woven bone (which is characterized by lacunae not arranged in parallel rows) by the trabecular bone (the mature form of bone where collagen fibers in the bone matrix are organized into successive sheets or lamellae oriented in the same direction) and remodelling the socket [3].

Non-disturbed healing of bone defects and filling the wound with bone tissue is the main prerequisite for normal bone function. A period of approximately six months after creating a bone defect in humans (after tooth extraction or periapical surgery) is necessary for the formation of the lamellar bone and even more is needed following surgery for a defect to completely fill with mature bone tissue.

An ideal technique for wound healing stimulation, inducing more rapid bone regeneration, has not been presented yet. The regenerative procedure has not been accelerated even with new bone substitute materials [4]. Numerous data from literature shows the stimulating effect of soft (low-power) lasers on the process of wound healing [5, 6]. This effect is characterized by proliferation of fibroblasts, faster collagen production and enhanced enzyme activity. However, there are no precise data concerning the influence of low-power lasers on the speed and degree of bone regeneration.

The aim of this study was to evaluate the possible enhancing effect of the GaAlAs low-power laser on the rate of the healing artificially created osseous defects in rats.

MATERIAL AND METHODS

The study was carried out on 10 albino Wister-rats, weighing 440±31 g. Procedures were performed under intraperitoneal anaesthesia with Nembutal (0.1 ml per 100 g of body weight). The skin was shaved under the right (experimental side) and left (control side) femur region. The skin was cut and the femur was exposed by blunt preparation. The periosteum was cut, and round defects (3 mm wide 2 mm deep) were made in the bone at both sides using a round steal bur (Figure 1). Efforts were made not to open the bone marrow space. The periosteum, soft tissue and skin were sutured with catgut (4-0) thereafter.

Immediately after the operation, defects from the experimental (right) side were treated daily with

Galium, Aluminium, Arsenid (GaAlAs) low-power laser (Medicolaser 637, Technoline, Belgrade, Serbia) at the energy output of 4 J/cm^2, with constant power density of 50 mW, and a wave length 637 nm (visible red light) per defect during seven days. The treated area included the defect and the skin around it. Bone defects from the control (left) side were allowed to heal spontaneously.

Half of the experimental animals (5 animals) were sacrificed 2 weeks after surgery and the rest of them (5 animals) after 3 weeks. The femurs were completely extracted and fixed in 10% formaldehyde; following by 20% ethylenediaminetetraacetat (EDTA) for a period of 2 weeks. Decalcified material was embedded in paraffin blocks. The samples were stained with haematoxylin-eosin and analysed under optical microscope. Five sections per each defect were evaluated: one from the central area of the defect, two from the periphery, and two from the areas between them.

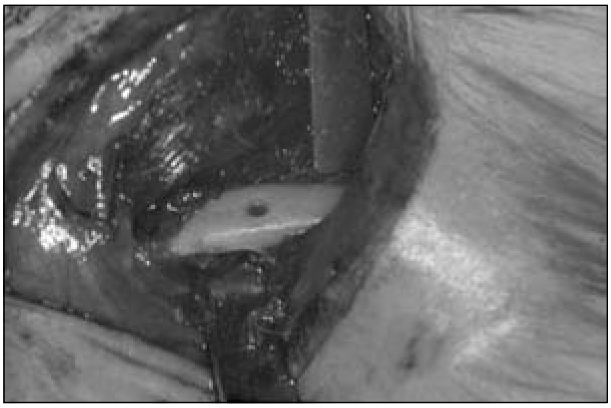

Figure 1. Bone defects of rat's femur (2×3 mm)
Slika 1. Oštećenje butne kosti pacova (2×3 mm)

RESULTS

Microscopic findings of bone defects from all the experimental rats exhibited signs of bone repair and neither inflammatory infiltrates nor fibrous alteration were presented. Histological analysis showed osteoblastic activity two weeks

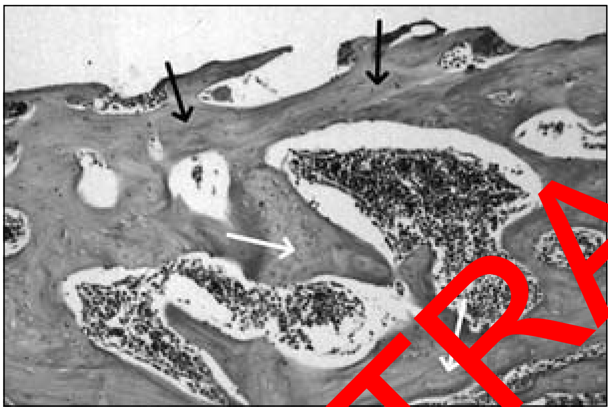

Figure 2. Defects of femur treated with low-power laser showing powerful osteoblastic activity with considerably more mature bone (black arrow) than immature (white arrow) after two weeks (H.E. 33×)
Slika 2. Oštećenja butne kosti tretirana laserom male snage koji pokazuju snažnu osteoblastnu aktivnost sa znatno zrelijom kosti (crna strelica) nego nezrelom (bela strelica) posle dve nedelje (H.E. 33×)

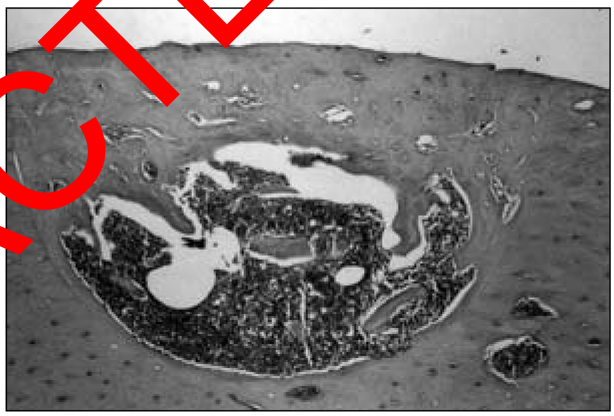

Figure 4. Three weeks after surgery, experimental bone defects completely filled with spongious, lamellar bone with sparse areas of bone marrow (H.E. 20×)
Slika 4. Tri nedelje posle operacije eksperimentalna oštećenja kosti su potpuno ispunjena sunđerastom lamelarnom kosti s retkim oblastima koštane srži (H.E. 20×)

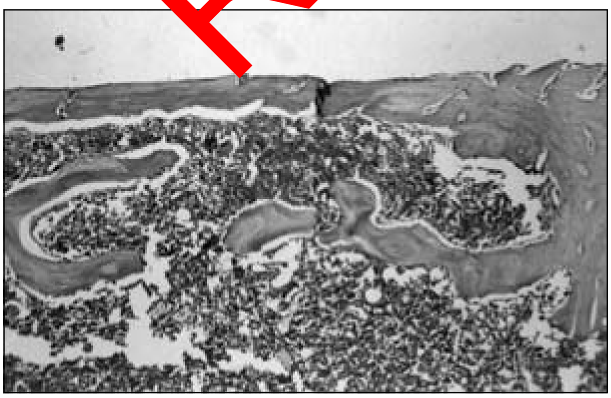

Figure 3. Defects of the control group, two weeks after surgery, osteoblastic activity is noticed. Newly formed bone sprouts are at the periphery of bone defects, and fibroblastic tissue with no signs of new bone formation in the central area of the bone defects (H.E. 33×).
Slika 3. Oštećenja kosti pacova kontrolne grupe dve nedelje nakon operacije sa osteoblastnom aktivnošću. Uočavaju se novoformirani koštani izdanci na periferiji, a u centralnom delu oštećenja je fibroblastno tkivo bez znakova stvaranja nove kosti (H.E. 33×).

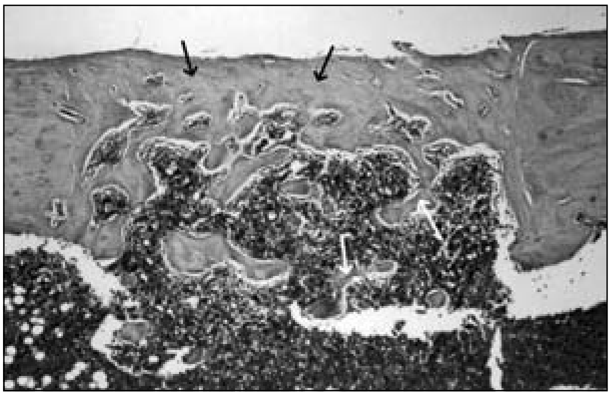

Figure 5. Non-treated bone defects revealed mature lamellar bone at the periphery areas, and immature bone (white arrow) at the central areas after three weeks (H.E. 20×)
Slika 5. Posle tri nedelje nelečena oštećenja kosti sa zrelom lamelarnom kosti u perifernim delovima i nezrelom kosti (bela strelica) u centralnim delovima (H.E. 20×)

after surgery in all the investigated areas of bone defects of the experimental side. Defects of the femur treated with the low-power laser were filled to a great extent by mature lamellar bone of trabecular nature (Figure 2). Two weeks after surgery, in the defects of the control group, a new bone formation activity was noticed. Newly formed bone sprouts were found at the periphery of bone defects while fibroblastic tissue with no signs of new bone formation was noticed in the central area of the bone defects (Figure 3).

Three weeks after surgery, experimental bone defects were completely filled with cancellous, lamellar bone with sparse areas of bone marrow (Figure 4). Non-treated bone defects revealed mature lamellar bone at the periphery areas and still immature bone, characterized by thin bony trabeculae, at the central areas (Figure 5).

DISCUSSION

Healing and regeneration of bone defects is still an important phenomenon, of special interest to surgeons. Bone response to trauma generally follows basic wound healing processes. Injury initiates well-defined cascade of tissue reaction in order to produce a new matrix for repairing bone continuity and architecture. The order of these events is bone-specific and depends on the functional differences between various bones [7].

Experimental studies on the effect of a surgical intervention per se have been studied on different experimental models, mainly on long bones. The general impression is that the diameter of the defect greatly influences the rate, speed, and quality of healing [8, 9]. In animal models, defects greater than 3 mm in diameter tend to heal slower than smaller defects. Having that in mind, the defects created in our study could be considered as being relatively large.

After some surgical procedures which create bone defects in the maxilla or the mandible, the coagulum retraction inside the defect might cause the appearance of “dead spaces”, and proliferation of connective tissue into the defect. This can slow down formation of new bone tissue and cause anatomic deformity and functional disturbance [10].

The stimulating effect of low power laser on connective-tissue and bone regeneration has been established both clinically [5, 6, 11, 12] and experimentally [12, 13, 14]. Nagasawa [12] in his study used the low power laser (GaAlAs) for radiation of bone defects on the rat’s femur and found active formation of cancellous bone with trabeculae. In the group without laser treatment, he found only a few osteoclasts and some cancellous bone and trabeculae. Jovanović et al [14] studying the effect of the same low power laser on wound healing after tooth extraction in dogs, found a favourable effect of laser irradiation, reflected in quicker epithelium regeneration, creation of granular tissue and fibroplasia. However, there is a strong belief that experimental animal studies do not reflect the human situation [15].

The results of this study on rats showed that the use of a 637 nm GaAlAs low-power laser with the power output of 50 mW and 4 J/cm^2 per defect had an obvious influence on the speed of healing of bone defects. The histopathologic investigations carried out in rat femurs pointed out the fact that laser enhances bone regeneration. A positive effect was present especially in early stages of wound healing (2-3 weeks after the creation of bone defects), accelerating the process of bone healing for approximately a week. The strong bio-stimulating effect of low power laser on the process of healing bone defects, noticed earlier has been confirmed also in this experimental model [5]. Additionally, to confirm this laser effect in clinical situations, further investigations are needed.

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RETRACTED

Uticaj lasera male snage na zarastanje oštećenja kosti: eksperimentalno istraživanje

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KRATAK SADRŽAJ

Uvod Laseri male snage utiču na proces zarastanja rana, koji odlikuju proliferacija fibroblasta, brže stvaranje kolagena i pojačana aktivnost enzima. Cilj ovog rada je bio da se proceni efekat lasera male snage na zarastanje veštački izazvanih oštećenja kosti laboratorijskih pacova.

Materijali i metode rada U istraživanju je korišćeno 10 albino vister pacova. Kod svakog od njih su na obe butne kosti načinjena okrugla oštećenja (3 mm široka i 2 mm duboka), s tim da je desna bila eksperimentalna strana, a leva kontrolna. Eksperimentalne strane su svakodnevno tretirane galijum-aluminijum-arsenid (GaAlAs) mekim laserom (Medicolaser 637, Technoline, Belgrade, Serbia) s izlaznom snagom od 50 mW, koristeći 4 J/cm² po oštećenju tokom sedam dana. Kontrolne strane su ostavljene da spontano zarastu. Efekti ozračenosti laserom su procenjivani dve i tri nedelje posle operacije.

Rezultati Dve nedelje nakon operacije histološkom analizom oštećenja kosti eksperimentalne strane uočena je snažna aktivnost osteoblasta. U isto vreme na kontrolnoj strani zapaženi su novoformirani koštani izdanci na periferiji oštećenja kosti, odnosno fibroblastično tkivo bez znakova stvaranja nove kosti u centralnom delu oštećenja. Tri nedelje nakon operacije eksperimentalna oštećenja kosti su potpuno bila ispunjena sunđerastom lamelarnom kosti, dok su netretirana oštećenja kosti odlikovale zrela lamelarna kost na perifernim delovima i nezrela kost u centralnim delovima.

Zaključak Dobijeni nalazi pokazuju da korišćenje lasera male snage može značajno uticati na brzinu zarastanja oštećene kosti kod laboratorijskih životinja.

Ključne reči: laser male snage; GaAlAs-laser; oštećenje kosti; zarastanje kosti; aktivnost osteoblasta

UVOD

Zarastanje kosti je fiziološki proces koji se sastoji od sledećih faza: indukcije akutnog zapaljenja, regeneracije parenhimskih ćelija, migracije i proliferacije parenhimskih i ćelija vezivnog tkiva, sinteze proteinskog i vanćelijskog matriksa, preoblikovanja vezivnog tkiva i parenhimske komponente i stvaranja kolagena [1]. Vađenje zuba i uklanjanje hronične periapikalne lezije ponekad dovodi do oštećenja vilične kosti. Slično opštem obrascu, i zarastanje koštane šupljine nakon vađenja zuba se odvija u fazama [2]. Zarastanje počinje stvaranjem krvnih ugrušaka (koji ispunjavaju šupljinu), organizacijom ugruška, epitelizacijom površine rane, a zatim dolazi do stvaranja tzv. *woven* kosti u vezivnom tkivu, zamene nezrele kosti (koju odlikuju šupljine bez paralelnih redova) trabekularnom kosti (zreli oblik kosti gde su kolagena vlakana u matriksu organizovana u pločice ili lamele usmerene u istom pravcu) i preoblikovanja šupljine [3].

Glavni preduslov za normalno funkcionisanje kosti je neometano zarastanje njenih oštećenja i ispunjavanje rane koštanim tkivom. Nakon stvaranja oštećenja kosti kod ljudi (posle vađenja zuba ili periapikalne hirurške intervencije) potrebno je oko šest meseci za stvaranje lamelarne kosti, a znatno duži vremenski period da se oštećenje potpuno ispuni zrelim koštanim tkivom.

Danas ne postoji idealna tehnika za stimulaciju zarastanja rana i koja podstiče bržu regeneraciju kosti. Regenerativni postupak nije ubrzan čak ni sa novim materijalima za zamenu kosti [4]. Mnogi podaci iz literature ukazuju, međutim, na stimulišuće dejstvo tzv. mekog lasera (slabe snage) na proces zarastanja rana [5, 6]. Njegovo dejstvo odlikuju proliferacija fibroblasta, brže stvaranje kolagena i pojačana aktivnost enzima. Nema, međutim, preciznih podataka o uticaju lasera male snage na brzinu i stepen regeneracije kosti.

Cilj ovog rada je bio da se proceni efekat galijum-aluminijum-arsenid (GaAlAs) lasera male snage na brzinu zarastanja kosti kod veštački stvorenih oštećenja kosti laboratorijskih pacova.

MATERIJAL I METODE RADA

Istraživanje je urađeno na 10 albino pacova soja vistar, prosečne težine od 440±31 gram. Postupak je izveden pod intraperitoneumskom anestezijom s nembutalom u dozi od 0,1 ml na 100 g telesne težine. Ispod desne butne kosti, koja je bila eksperimentalna strana, i leve butne kosti, kontrolne strane, obrijana je koža, potom isečena, a butna kost eksponirana tupim ekarterom. Isečen je periost, a zatim su pomoću čeličnog stvrdla (Slika 1) s obe strane napravljena okrugla oštećenja u kosti (3 mm široka i 2 mm duboka). Pri stvaranju oštećenja vodilo se računa da se ne eksponira prostor koštane srži. Nakon toga su ketgutom (4-0) ušiveni periost, meko tkivo i koža.

Odmah po operaciji oštećenja s eksperimentalne (desne) strane su svakodnevno tretirana mekim 637 nm GaAlAs laserom (Medicolaser 637, Technoline, Belgrade, Serbia), s izlaznom snagom od 50 mW, koristeći 4 J/cm² po oštećenju. Lečenje je trajalo sedam dana, a tretirana oblast je obuhvatala kožu oko oštećenja i samo oštećenje. Oštećenja kosti s kontrolne (leve) strane su ostavljena da spontano zarastu.

Polovina laboratorijskih životinja (pet pacova) je žrtvovana dve nedelje posle operacije, a druga polovina (četiri pacova) posle tri nedelje. Butne kosti su potpuno izvađene i fiksirane u desetoprocentnom formalinu, a zatim ostavljene u dvadesetoprocentnoj etilendiamin-tetraosetnoj kiselini (EDTA) tokom dve nedelje. Dekalcifikovani materijal je ukalupljen u parafinske blokove. Uzorci su bojeni hematoksilinom i eozinom i analizirani na optičkom mikroskopu. Vršena je evaluacija pet preseka po svakom oštećenju: jedan iz centralnog dela oštećenja, dva s periferije, a dva preseka su uzeta između centralnog i perifernih oštećenja.

REZULTATI

Mikroskopski nalazi oštećenja kosti svih laboratorijskih pacova su pokazivali znakove reparacije kosti bez izraženih zapaljenjskih infiltrata i fibroznih promena. Na eksperimentalnoj strani histološka analiza dve nedelje nakon operacije je kod svih životinja otkrila aktivnost osteoblasta u svim ispitivanim oblastima oštećenja kosti. Oštećenja lečena laserom male snage su bila popunjena u velikoj meri zrelom lamelarnom kosti trabekularne prirode (Slika 2). Dve nedelje nakon operacije kod na kontrolnoj strani tela pacova takođe je primećeno stvaranje nove kosti. Zapaženi su novoformirani koštani izdanci na periferiji oštećenja, a u centralnom delu je uočeno fibroblastno tkivo bez znakova stvaranja nove kosti (Slika 3).

Tri nedelje posle operacije oštećenja kosti na eksperimentalnoj strani su bila potpuno ispunjena sunđerastom lamelarnom kosti s retkim oblastima koštane srži (Slika 4). Kod nelečenih oštećenja uočene su zrela lamelarna kost na perifernim delovima i nezrela kost sa tankim koštanim trabekulama u centralnim delovima (Slika 5).

DISKUSIJA

Zarastanje i regeneracija oštećene kosti je i dalje značajan fenomen koji posebno zanima hirurge. Odgovor kosti na traumu je vid procesa obnavljanja tkiva. Kao posledica povrede se javlja dobro definisana kaskada reakcija u tkivu koje stvaraju novi matriks, kako bi vratili kontinuitet i arhitekturu kosti. Tok ovih reakcija je specifičan za svaku kost, a zavisi od funkcionalnih razlika kosti [7].

Eksperimentalna istraživanja uticaja hirurške intervencije i stvaranja oštećenja kosti na spoljnoj koštanoj ploči, kao i praćenje procesa zarastanja, izvođeno je na raznim eksperimentalnim uzorcima, ali se uglavnom izvođenja na dugim kostima. Uočeno je da prečnik oštećenja u velikoj meri utiče na stepen i brzinu zarastanja kosti [8, 9]. Smatra se da na životinjskim uzorcima oštećenja prečnika većeg od 3 mm zarastaju sporije nego manja. Imajući to na umu, oštećenja koja su načinjeni za potrebe ove studije se mogu smatrati relativno velikim.

U kliničkoj praksi oralne hirurgije vlada značajno interesovanje za ubrzanje procesa zarastanja kosti. Posle hirurških zahvata koji dovode do oštećenja koštanog tkiva u maksili i mandibuli, povlačenje ugruška unutar oštećenja može da izazove pojavu tzv. mrtvih prostora i proliferaciju vezivnog tkiva u oštećenju. Ovo može da uspori stvaranje nove kosti i izazove anatomski deformitet i funkcionalni poremećaj [10].

Stimulišuće dejstvo lasera male snage na vezivno tkivo i regeneraciju kosti je potvrđeno i kliničkim [5, 6, 11, 12] i eksperimentalnim istraživanjima [12, 13, 14]. Nagasava (*Nagasawa*) [12] je u svom istraživanju koristio laser male snage (GaAlAs) za zračenje oštećenja na butnoj kosti pacova i zapazio aktivno stvaranje sunđeraste kosti sa koštanim trabekulama. U grupi koja nije bila podvrgnuta laserskom tretmanu primetio je samo nekoliko osteoklasta i malo sunđeraste kosti i trabekule. Jovanović i saradnici [14] su, proučavajući dejstvo istoga lasera male snage na zarastanje rana posle vađenja zuba kod pasa, uočili povoljan efekat laserskog zračenja koji se ogledao u bržoj regeneraciji epitela, stvaranju granularnog tkiva i fibroplaziji. Međutim, osnovni i nemali problem je to što se eksperimentalna istraživanja na životinjama ne mogu jednostavno preneti na ljudsku situaciju [15].

Rezultati našeg istraživanja na pacovima pokazuju da je korišćenje 637 nm GaAlAs lasera male snage, s izlaznom snagom od 50 mW i 4 J/cm² po oštećenju, imalo pozitivan uticaj na brzinu izlečenja oštećene kosti. Histopatološka ispitivanja obavljan na butnim kostima pacova ukazala su i na činjenicu da je regeneracija kosti bila brža pod uticajem lasera. Pozitivan efekat zračenja bio je značajan naročito u ranim fazama zarastanja rane (dve-tri nedelje od stvaranja oštećenja kosti) i značajno je ubrzao proces zarastanja kosti za oko jednu nedelju. Snažni biostumulišući efekat lasera male snage na proces zarastanja kosti koji je ranije primećen potvrđen je i u ovom eksperimentalnom modelu [5]. Ali da bi se ovaj efekat potvrdio i u kliničkim uslovima, neophodna su dodatna klinička istraživanja.