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INVESTIGATION OF HeNe LASER THERAPY INFLUENCE ON BCP/PLGA OSSEOINTEGRATION – EXPERIMENTAL STUDY

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Summary. *The efficiency of HeNe laser application in treatment of artificially caused bone defects in mandible of rats filled with biocomposite BCP/PLGA (bicalcium phosphate/polylactide–co-glycolide) was analyzed. Animals were divided in two separate groups. The first group (A) was the control one. In the second group (B) the implantation site was submitted to HeNe laser irradiation. Animals were sacrificed two, six, eight and twelve weeks after laser therapy. Histological samples were decalcified and submerged in paraffin blocks. Samples were colored with HE. Morphometrical analysis consisted of measuring area fraction, area, integral optical density, mean density and density variation. Results showed beneficial effect of laser treatment in reparation of alveolar bone after two and six weeks. After eight and twelve weeks no beneficial laser effect on biocomposite osseointegration could be noticed.*

Key words: *Bicalcium phosphate/polylactide-co-glycolide, HeNe laser, osseointegration*

Introduction

The development of low power lasers is an important step in current medicine. Low power lasers have powerful biostimulative effect, which can be seen as enlargement of cell metabolism and microcirculation that induces larger number of epithelial, connective and osseous cells mitosis (1,2). Laser irradiation induces proliferation of fibroblasts and production of collagen, increases enzyme activity and vascularisation of treated area. They stimulate spongy bone metabolism and through activation of osteoblast activity, induce faster formation and maturation of young bone, i.e. formation of callus (3).

Advanced destruction of alveolar bone is an important problem in dental therapy. In these cases, reparative potentials of bone are distinguished and reparation of bone cannot be easily achieved by means of conservative therapeutic methods. Regenerative procedures occur after application of bone implants (4). Their role is to opturate bone defect mechanically and to induce osteogenesis at the same time (5,6). Today, there is a large number of biomaterials which are used as bone substitutes. Bioresorbable composite implant BCP/PLGA (bicalcium phosphate/polylactide–co-glycolide) has achieved a great success in modern bone defects therapy (7,8).

According to the studies reported up to date which have showed efficiency of BCP/PLGA (7,8), and low power lasers (9-17), a stimulative influence of low power laser therapy on biomaterial BCP/PLGA (bicalcium phosphate/polylactide–co-glycolide) integration in surrounding bone tissue was investigated in this study.

The Aim of the Investigation

The aim of investigation was:

- To investigate the influence of HeNe low power laser on bone reparation after BCP/PLGA implantation.
- To morphometrically and patohistologically evaluate the efficiency of BCP/PLGA and HeNe low power laser in stimulation of regenerative bone mechanisms.

Materials and Methods

The research was carried out on 80 white female rats of Wistar syngenic type, age 10 weeks. Animals were prepared for intervention applying Diazepam (Bensedin, ICN Galenika, Serbia and Montenegro) and anesthetized with Ketamin hydrochloride USP (Ketalar, Rotexmadica Gmbh, Trittau, Germany). Defects 1,4 mm in diameter and 1,6 mm deep were made in animals in the region between medial line and foramen mental on the left side of the mandible and in thus made defect BCP/PLGA was implanted.

Animals were divided in two equal groups: control group A (40 animals) and experimental group B (40 animals):

- Control group (A): BCP/PLGA was implanted in artificially made defect of mandible;
- Experimental group (B): after implantation of BCP/PLGA in artificially made defect of mandible, the implantation site was submitted to HeNe (Melles Griot 05-LLR-831) laser irradiation (5 days, 5 minutes per day).

Postoperative period was with no adverse effects.

Bone histomorphometrical investigation

Animals were sacrificed two, six, eight and twelve weeks after laser therapy had been finished. Bone samples of mandible, from the medial line to the foramen mental (region where artificial defect was made and implantation executed) were taken. They were cut in the vestibule-oral direction. Samples were washed in physiological solution, fixed in 10% formaldehyde and then decalcified chemically and by electrolysis. Chemical decalcification was done in 15% solution of nitric acid. After decalcification, bone tissue was dehydrated in alcohol and then moulded into paraplast, dried and dyed. Thus obtained histological sections, 2-4 μm thick, were dyed routinely by hematoxylineosin (HE) and PAS methods, and patohistologically analyzed. Obtained histological preparations were histomorphologically analyzed by a Lucia 3.2 G system for image analysis (Laboratory Imaging, Prague, Czech Republic), on a NU-2 microscope (Carl Zeiss, Jena, Germany).

After the test area was defined, following values were measured:

- area fraction (the percent of bone tissue at each visual area; expressed in %);
- area (bone area which can be seen at each visual area; expressed in μm^2);
- integral density (integrally collected optical density of investigated bone part; expressed in arbitral units of measurement);
- mean density (mean value of optical density; expressed in g/cm^2);
- density variation (density variation of bone tissue, newly formed bone).

T-test was used for analysis of obtained results. Values were shown as a mean value and standard deviation.

Results

- *Results after two weeks*

Histological analysis of cortical alveolar bone of B experimental group where BCP/PLGA after implantation was submitted to HeNe laser irradiation, revealed intensive enlargement of bone tissue with numerous cement lines and decrease of Hawersian canals compared to the control group (Fig. 1, 2). Similar results were noticeable on histological preparations of spongy alveolar bone of B experimental group compared to the control group (Fig. 3, 4).

Fig. 1. Two weeks after finished HeNe laser therapy intensive osteogenesis of compact bone after application of BCP/PLGA(HE, x25)

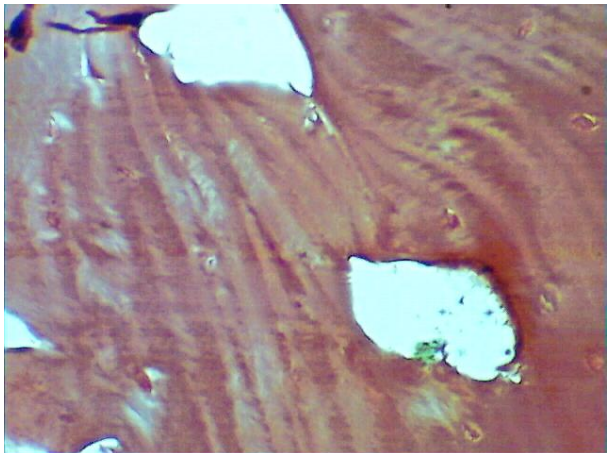


Fig. 2. At the same time osteogenesis of compact bone in control group (HE, x25)

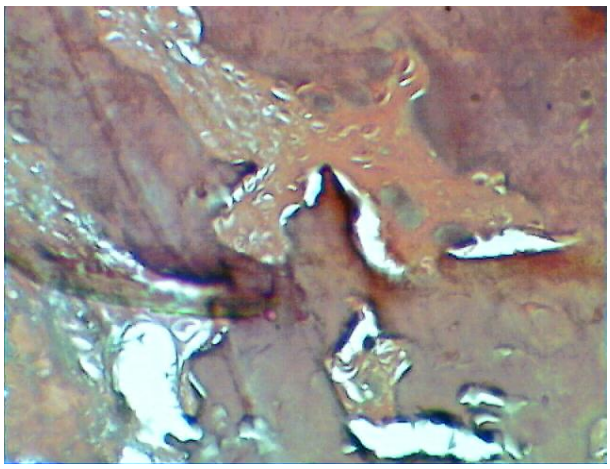


Fig. 3. Two weeks after finished HeNe laser therapy intensive osteogenesis of spongy bone after application of BCP/PLGA (HE, x25)

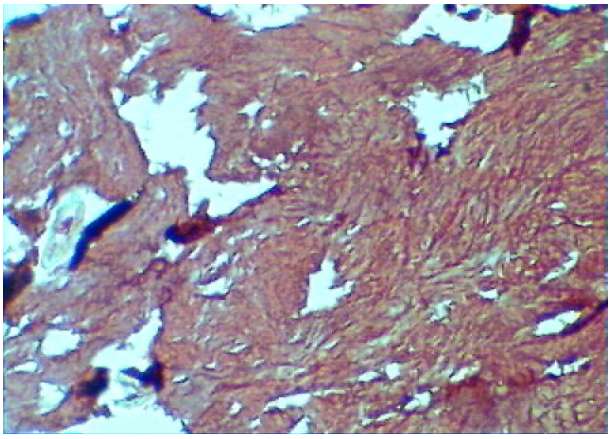


Fig. 4. At the same time osteogenesis of spongy bone in control group. (HE,x25)

Morphometrical analysis of bone showed statistically higher values of area fraction, area and integral density in experimental B group ($p < 0.05$). Density variation was higher in control group ($p < 0.05$) (Graph 1 and 2, table 1).

• *Results after six weeks*

Histological analysis of cortical bone revealed no difference between two groups. *Histological analysis* of spongy alveolar bone of B experimental group revealed intensive enlargement of bone tissue with numerous cement lines and decrease of Hawersian canals compared to the control group.

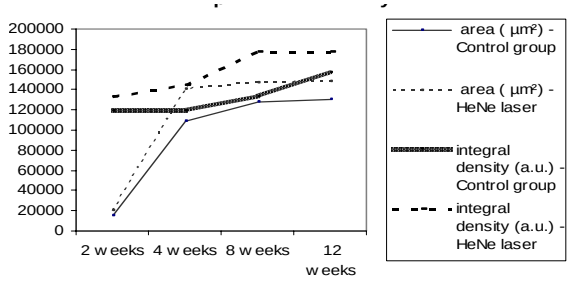
Morphometrical analysis of bone showed statistically higher values of area fraction, area and integral density in experimental B group ($p < 0.05$) (Graph 1 and 2, table 1).

• *Results after eight weeks*

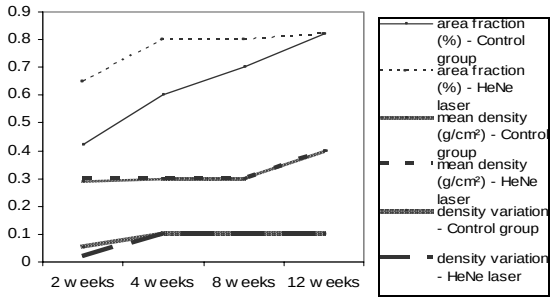
Histological analysis of cortical and spongy alveolar bone revealed no difference between two groups. *Morphometrical analysis of bone* revealed no statistically important difference between groups after eight weeks (Graph 1, 2).

• *Results after twelve weeks*

Histological analysis of cortical and spongy alveolar bone revealed no difference between two groups. *Morphometrical analysis of bone* revealed no statistically important difference between groups after twelve weeks (Graph 1, 2).



Graph 1.Morphometrical values (area and integral density)



Graph 2. Morphometrical values (area fraction, mean density, density variation)

Discussion

Advanced bone loss is very difficult problem in dental therapy. In such cases bone reparation mechanisms could be stimulated by the use of bone substitutes (5). Today, there is a large number of different biomaterials which are used as bone fillers. Biphasic calcium phosphate/poly-DL-lactide-co-glycolide (BCP/PLGA) achieved a significant success in modern stomatological therapy. In current literature advanced properties of BCP/PLGA are proposed: biocompatibility, high reparation at the site of implantation and successful integration in surrounding bone tissue (7,8). In this manner, reparative mechanisms are developed and results of therapy can be significantly improved. These advanced properties of BCP/PLGA are the main reason for its usage in the present study. In the current investigation the possibility of biomaterial osseointegration improvement was investigated.

Lomnitski and Babiashevic (3) investigated the influence of HeNe laser on healing of artificially caused bone defects in rabbit mandible. They concluded that laser light induced significant reparatory effects. HeNe laser therapy of artificially caused rat bone fractures

Table 1. Mean morphometrical values of experimental bone after two and six weeks.

Variables	2 weeks		6 weeks		p
	Control group	HeNe laser	Control group	HeNe laser	
Area fraction (%)	0.42 ± 0.17	0.65 ± 0.13	0.6 ± 0.15	0.8 ± 0.11	<0.05
Area (μm^2)	11094.31 ± 3071.48	16687.58 ± 3138.15	108511.9 ±26455.49	140285.8 ±18322.13	<0.05
Integral optical density (a.u.)	82746.4 ±10222.94	109219.26 ±15485.95	1100490 ±28079.01	143909.7 ±26741.40	<0.05
Mean density (g/cm^2)	0.29 ± 0.03	0.33 ± 0.04	0.3 ± 0.02	0.3 ± 0.03	n.s.
Density variation	0.05 ± 0.02*	0.02 ± 0.01*	0.1 ± 0.02	0.1 ± 0.02	<0.05*

induced faster healing of treated areas; group submitted to laser irradiation showed better vascularisation and faster bone formation (2). These histological results are similar to findings from this study which emphasize biostimulative effect of low power laser irradiation on bone reparation, compared to reparatory mechanisms which occurred after biomaterial implantation without beneficial laser's effects.

Satiah and al (18) used HeNe laser and analyzed bone. They discovered that the concentration of calcium-hydroxyapatite was enlarged 15 days after operation. Authors thought that this positive observation was the result of higher osteoblast activity, and in this way was the sign of larger formation of organic matrix and higher mineralization. In this investigation similar results were noticed. In experimental group treated with HeNe laser there was enlargement of new formed bone at the site of BCP/PLGA implantation.

In the present study bone morphometrical analysis (of newly formed tissue at preparation site and surrounding bone tissue) was done. The morphometrical analysis revealed statistically higher values of area fraction, area and integral density in experimental group submitted to HeNe laser irradiation after two and six weeks ($p<0.05$).

Based on obtained results, it was noticed that low power lasers had beneficial effects at both early time intervals (two and six weeks). Area, area fraction, and integral optical density, had higher values in the group submitted to laser irradiation ($p<0.05$).

After eight and twelve weeks, the difference in obtained values between experimental and control group could not be noticed. Based on these results, it could be concluded that low power lasers have biostimulative effect at shorter time intervals. It has to be noticed that beneficial effect was more intense at shorter time interval (two weeks). These findings are similar to literature data which emphasize that low power laser therapy, if applied at inflammatory time period of bone reparation, enlarges normal cell activity (resorption and formation of bone) (1,2).

Conclusion

The findings from this study demonstrate that low power lasers have biostimulative effect on early reparatory mechanisms in bone defects, and that they help integration of implanted biomaterial.

Morphometrical analysis revealed significantly higher values of area fraction, area and integral density in group submitted to HeNe laser irradiation at early reparatory time.

Based on earlier described results, it can be concluded that HeNe low power laser is efficient in early bone defect therapy and can be useful as a stimulus of early stage of biomaterial osseointegration.

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ISPITIVANJE UTICAJA HeNe LASEROTERAPIJE NA BCP/PLGA OSEOINTEGRACIJU – EKSPERIMENTALNI RAD

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Kratak sadržaj: *Ispitan je uticaj terapije HeNe laserom na arteficijalno izazvane defekte mandibule pacova koji su ispunjeni sa biokompozitnim materijalom BCP/PLGA (bifazni kalcijumfosfat/polilaktidkoglikolid). Životinje su podeljene u dve grupe. Prva grupa (A) je bila kontrolna grupa. U drugoj grupi (B), mesto implantacije biomaterijala je podvrgnuto dejstvu HeNe lasera. Životinje su žrtvovane nakon dve, šest, osam i dvanaest nedelja nakon završene laseroterapije. Histološki preparati su dekalcifikovani i ukalupljeni u parafinske blokove. Preparati su bojeni HE. Morfometrijska analiza se satojala iz merenja arealne frakcije, areje, integrisane optičke gustine, srednje gustine i varijacije gustine koštanog tkiva. Rezultati su pokazali blagotvoran uticaj laseroterapije u reparaciji koštanog tkiva nakon dve i šest nedelja. Nakon osam i dvanaest nedelja nije uočen blagotvorni uticaj lasera na oseointegraciju biokompozita.*

Ključne reči: *Bifazni kalcijumfosfat/polilaktidkoglikolid, HeNe laser, oseointegracija*