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The Repair of Critical–Size Defects with Paste of Tri-Calcium phosphate and Bioglass Containing Magnesium Exposed to Low Level Laser: An Experimental Study in Rabbit Mandible

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ABSTRACT

We would like to sincerely express our gratitude to the Faculty of Veterinary Medicine Research Council of Islamic Azad University, Tabriz branch for approval and financial support for this extensive evaluation of compressive pressure and histomorphologic changes tri-calcium phosphate and bioglass of magnesium paste irradiated with low level laser in repair of experimentally created a critical size defect, middle of bilateral mandibular bones in rabbit. Problems statement: The purpose of this study was to determine the effect of calcium phosphate mixed with bioglass and magnesium and being irradiated with low level laser in rabbit mandibles. Approach: 48 adult New Zealand white rabbits were used. The mandible bone was bilaterally exposed and with help of dental trephine 3 mm in width and 10 mm in depth was made on each side in all rabbits .These rabbits were divided randomly into 4 groups of 12 rabbits in each. In group (I) The defect was left empty as control, group(II) exposed to low level laser irradiation ,whereas In groups (III) the defects were packed with tri-calcium phosphate(40%) and bioglass of magnesium paste (60%), and group IV the defects were filled with same paste and next day was subjected to therapeutic regimen of low level laser with wavelength of 780 nm, with power density of 10 mW intensity and energy density of 6 J/cm^{2,} duration of application 10 minutes for 15 days. The animals were subdivided into two subgroups of 6 rabbits each for 30 and 60 days. At the end of observation period bisected mandibles into two left and right samples from 6 rabbits. A series of samples were used for mechanical properties and other ones used for histomorphological evaluation in all four groups. For mechanical properties using Zwick/Roell MDTL Machine with speed of 0.01 mm/s having Proportional Integral Deferential (PID) controller. In assessing the variation obtained data was analyzed using paired-t-test. Results: Data of the compressive pressure was revealed an average for normal bone 275.0±4 N whereas it was 71.2±12.7N for group I in 1 month duration and that was recorded 108.4±18.2 N for group II and 122.0±10.8 N for group III and 142.0± 18.8 for gro up IV at the end of 1 month. These values were 102.9.±10.2 N for group I and 168.4±6.8 N for group II and 182.6±14.2 N for group III and 220.0 ±8.6 for group IV at the end of two months after implantation. Low level laser therapy beside calcium phosphate paste significantly accelerated healing and increased mechanical properties in mandible defect.

Key Words: Mandibular bone, Low Level Laser, Tri-calcium phosphate, Rabbits

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INTRODUCTION

The materials usually used as bone filler should be easy to use with specific properties that provide structural support in addition to be an osteogenic, biocompatible and even bioresorbable [1,2,3,4]. Low level laser therapy has been investigated and used in clinical practice for approximately 20 years. The initial studies were done in Europe by Mester [2,3] at the beginning of the 1970. There has been growing interest in the effects of laser energy, as shown be the significant quantity of scientific publications with controlled experiments on both animals and humans[5,6,7]. The present study had the objective of analyzing whether there are any differences in final energy transmitted to the tissue having the defect being packed with paste. Bioactive biomaterials such as bioglass[8], and glass ceramic A-W have been known to form a bioactive bone like apatite layer spontaneously on their surfaces and bond to bone through the apatite layer in vivo[9,10]. Therefore, it is generally accepted that an essential prerequisite for an artificial bone biomaterial to directly bond to living bone tissue is the formation of a bone like apatite layer on its surface when implanted in vivo. Laser alone and along with other bone substitutes has been attractive in hard tissue repair because of its good biocompatibility and bioactivity recently; researchers have suggested tri- calcium phosphate in form of pure or combined with other materials as bone filler. Which is suitable for load-bearing orthopedic applications[11,12]. In bone tissue engineering, scaffold serves as the matrices of tissue formation, and play pivotal role in osseointegration and tissue integration, as bone grafting is frequently used to augment bone healing with numerous approaches to reconstructing or replacing skeletal defects. Autologous bone graft remains the most effective grafting materials because it provides the three elements required for bone regeneration; osteoconduction, osteoinduction[13,14]. In this study using tri calcium phosphate (40%) mixed with bioglass and magnesium (60%) was intended to be suitable for defects of mandibular which are constantly under mechanical pressure.

MATERIALS AND METHODS

All animal procedures were carried out according to the guidelines of the Animal Ethics Committee of Islamic Azad University. Forthy-eight New Zealand 40 weeks old and weighing 3.5-4.0 kg male rabbits were used, and divided into four groups (I, II,III,IV) of twelve rabbits each, according to the procedure performed. Animals in each group were subdivided into two subgroups of six rabbits for one month and two months duration. All rabbits were kept in individual cage during the whole experimental period, under strict hygienic conditions and fed with standard ration for rabbits and water ad labium.

Surgical procedure:
Under intramuscular Diazepam (1mg/kg) premedication and intravenous Ketamine hydrochloride (35mg/kg) and Xylazine (5mg/kg) general anesthesia. Bilateral mandibular bone was routinely prepared for surgery. A 4cm longitudinal skin incision was made. The connective tissues and muscles on buccal side was dissected, providing a wide view of mandible bone, a segmental bone defect was created in the middle of the mandibular shaft, 3mm in width and 10 mm in depth , using a delicate orthopedic motor saw . In group I, the defect was left empty, whereas in group II, the defect was subjected to laser irradiation (Mostang 2000:780 nm) 10mW for 10 minutes for 15 days. In group III, the defects were filled up with tricalcium phosphate paste (40%) containing bioglass with magnesium(60%) (Fig. 1) and in group IV the defects were subjected to Laser irradiation and the gap filled with paste of Calcium phosphate (Fig. 2). All rabbits in four groups were divided into two subgroups (one month and two months duration with six rabbits in each one).

Postoperative care:
Antibiotics (penicillin G procaine 40000 IU/kg IM, bid), dexamethasone (0.6 mg/kg, IM), vitamin B.complex (0.2 mg/kg, IM) and analgesic such as Tramadol hydrochloride (5 mg/kg, IM, bid) were administered for 3 post-operative days.

Compressive pressure analysis:
All mechanical testing were performed using a Zwick/ Roell 2005 with a crosshead speed of 0.01 mm/s. A load-distance curve was recorded to obtain the mechanical properties (Figure 3). Load bearing was obtained with maximum load recorded of the linear portion of the load-distance curve. The mechanical properties of the normal radial bone were measured to provide reference values. Six specimens were tested for each condition, and data were represented as mean standard deviations (SD). Statistical analysis was carried out on the load bearing data using one way analysis of variance with the software program SPSS for Windows, version 19 (SPSS Inc.,

Chicago, IL, USA). P<0.05 was considered to be statistically significant. Tukey HSD multiple comparison testing was used to determine the significance of the deviations in the mechanical property of each sample for different times.

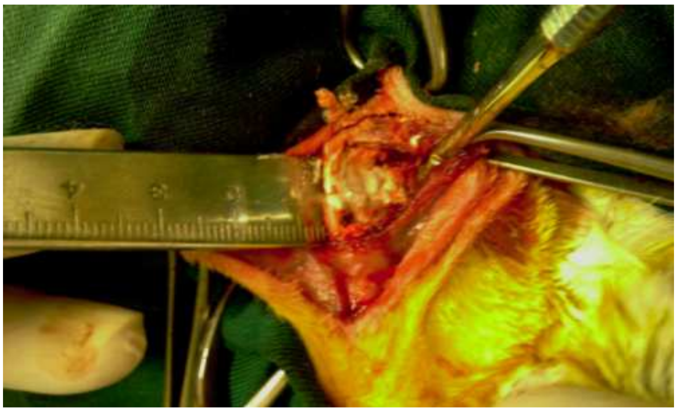

Figure 1. The gap filled with tri-calciumphosphate paste

Figure 2. Rabbits being subjected toLow Level laser Irradiation

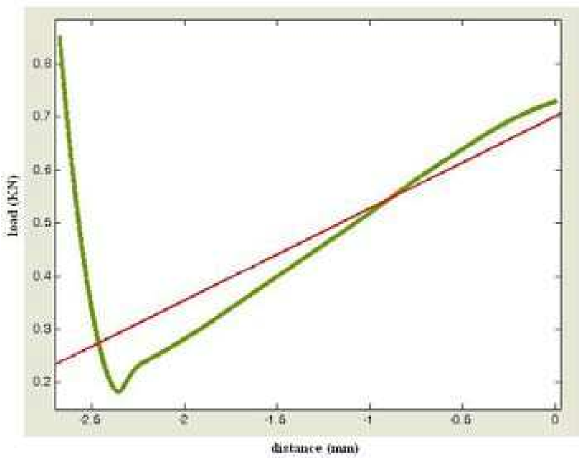

Figure 3. Typical load-distance curve for mechanical properties of specimen. The green line is principle but the mechanical engineer determines & analysis graph with Mat lab software and red line is Customized Functions with State.

RESULTS

No operative or postoperative complications were encountered. All of the rabbits tolerated surgery well and survived until the final experimental time. No wound opening or infections were observed. At sacrifice the macroscopic evaluation revealed maintenance of correct position of the paste in the defect site. There was a significant increase in mechanical properties of mandibular bone in group III and IV in 1st and 2nd months as compared to first two groups (I, II Table 1) . The mechanical property and the mean load for fracturing normal mandibular bone was 275.0 ±4 N whereas it was 71.2±12.7 N for group I which was recorded 108.4±18.2 N for group II and 122.0±10.8 N for group III and 142.0± 18.8 for group IV at the end of 1 month. These values were 102.9.±10.2 N for group I and 168.4±6.8 N for group II and 182.6±14.2N for group III and 220.0 ±8.6 N for group IV at the end of two months after implantation. Newton (N) was recorded values of mechanical pressure respectively. The results of this study show that tri calcium phosphate paste provided irradiated with low level laser is good choice for the healing of segmental mandibular bone defects, as increased and attained almost normal mechanical property or compressive pressure that of normal one (Table 1, Figure 4).

Table 1. Load bearing of normal, groups I, II, III & IV (Mean ± SD).

Time of implantation	Load bearing (N)				
	Normal	Group I	Group II	Group III	Group IV
1st month	275.0±4	71.2±12.0	108.4±18.2	122.0±10.8	142.0±18.8
2nd month	275.0±4	102.9±10.2	168.4±6.8	182.6±14.2	220.0±8.6

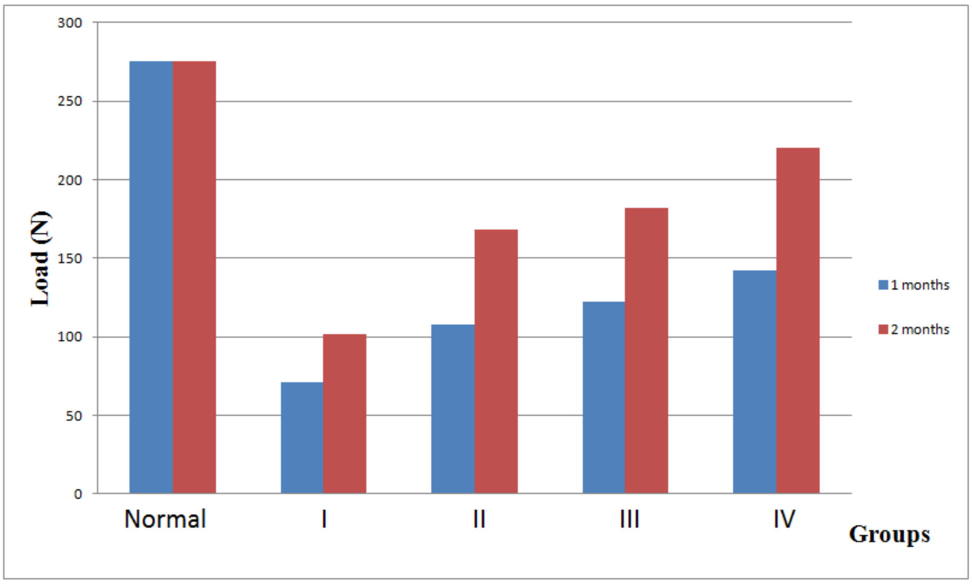

Figure 4. Result of load bearing of group normal, I, II, III and IV at one and two months after implantation.

DISCUSSION

The results of this experiment indicated the laser with and without tri calcium phosphate paste were able to maintain strength and stiffness while improving the incorporation process. This was especially true in the group III in which Tri calcium phosphate (40%) mixed with bioglass containing magnesium (60%) were used to pack the defects. One the most interesting observations from this study was the differences between control and other three groups using laser and bone fillers, but there was no fundamental differences between III and IV implanted groups. There was very good osteoconduction in both the implanted groups and stiffness was proper clinical sign of very rapid resorption and replacement of implant by living bone especially in group III and IV as the significantly increased in mechanical properties in all rabbits in these groups. An ideal bone graft substitute should have osteoconductive, osteoinductive and osteogenic properties. Tri calcium phosphate with bioglass was compatible materials because it provides all of these properties [15,16,17]. Tri calcium phosphate paste has been the implant of choice for most of the orthopedic procedures. However, might have some limitations too, such as donor-site infection, pain, and disease transfer. Because of these limitations, biosynthetic bone graft substitutes are being investigated. As a result, some investigators used mixtures of synthetic scaffolding biomaterials and osteoinductive organic agents to achieve better results and optimum tissue biomaterial contact necessary for stimulating bone in growth [18,19]. Cancellous rib bone was osteoinductive material being used as a bone graft substitute, and combination with tricalcium phosphate alone and provided irradiated with low level laser would probably create a composite with potentiated osteoinductive properties. The results achieved in this investigation indicated that this graft covered with really stimulates a favorable reaction of long bones and the best fracture healing was observed. The osteoconductivity of this graft promotes bone healing and helps in regaining the strength of the defect site faster. The addition of laser creates better conditions for bone formation; the increased osteogenesis may well be attributable to the stimulatory effect of tri calcium phosphate on local cells involved in bone regeneration such as osteoblasts, mesenchymal progenitor cells and endothelial cells [20,21]. As to cover the large gap in the body of long bones the scaffolding materials for bone tissue engineering should be osteoconductive so that osteoprogenitor cells can adhere and migrate on the scaffolds, differentiate and finally form new bone. In this investigation, we consider the tricalcium phosphate with laser can be a good choice for the healing of segmental bone defects, and provides a more rapid regeneration with almost normal mechanical property of bone defects.The results of the present study showed that irradiation with a low-power red laser at the fixed levels affected the cell physiological and molecular properties specially osteoblasts. The frequency of the laser used in irradiation can also affect cell proliferation. In 2001, Coombe et al. [22] used a GaAlAs laser at a wavelength of 830 nm to treat osteosarcoma cells with a single dose or daily irradiation doses of 0.5, 1, 2, or 5 J. They found no significant difference in cell count or MTT activity between the laser-irradiated and control groups over a period of 10 days but in this study results obtained at the end of 1 and 2 months periods. Daily irradiation of low power laser was applied in this study. No apparent cytotoxic effect was observed from the parameters used in this study. Ozawa etal. [23] indicated that significant cell proliferation occurred at the early stages of culture (approximately six days after laser irradiation). After estimating the effect on proliferation at days 1, 3, and 5, they found that LPLI significantly enhanced cell proliferation on day 5. There extensive reports as ALP is a biomarker that is used to evaluate bone metabolism; staining methods are also commonly used to visualize bone nodule formation. Fukuhara et al.[24] observed that irradiation with a GaAlAs laser (905 nm) at an energy density of 3.75 J/cm^2 significantly increased bone nodule area, as measured by von Kossa staining and subsequent determination of the number of ALP-positive colonies of calvarial cells. Khrdra et al. [25] showed that ALP activity increased after exposure to 3 J/cm^2 GaAlAs irradiation (830 nm). Similar results were also observed in our study when we assessed ALP activity and Alizarin Red S staining. A remarkable dose-dependent enhancement of osteogenic induction was observed at both 10 and 14 days. However, based on our quantification of Alizarin Red S staining, the increase at 14 days was not as striking as the induction at 10 days; we attribute this effect to the saturation of osteogenic induction due to the limited cell growth area. But after 1 month there were significant changes in tensile mechanical property III and IV, indicated the positive effect of laser in improved trend of healing. The mechanisms of LPLI promotion of proliferation and differentiation of cells are still not fully understood. Several growth factors, such as PDGFα, TGFβ, IGF1, and BMP2 have been reported to regulate stem cell proliferation and differentiation[26,27,28,29,30]. Our results indicated that LPLI induced higher gene expression of IGF1 than PDGFα and TGFβ. Furthermore, both the anti-IGF1 and anti-BMP2 antibodies were used in our study to verify which growth factor was important in LPLI-induced cell proliferation and differentiation. We found that treatment with these two antibodies reduced osteogenic differentiation, but only IGF1 neutralizing antibodies could reduce LPLI-induced cell proliferation. It is an interesting phenomenon that LPLI can regulate different growth factors under different cellular physiological conditions. After LPLI at 4 J/cm^2, the inhibitory effects of the antibodies on osteogenic differentiation were significantly decreased but were still higher than the inhibitory effects observed in cells to which no antibodies were added. The data indicate that LPLI may regulate proliferation and osteogenic differentiation via IGF1 and BMP2 signaling pathways, but other factors may also be involved. Further studies are necessary to understand the precise signaling pathways involved. Tissue engineering is a very promising approach for the repair of damaged tissues and organs. The ability of stem cells to proliferate and differentiate plays a critical role in their clinical application. LPLI is an economical and non-contact-based method that can be used to manipulate the activity of cells. Recently, several studies examined the use of LPLI in several animal models and found beneficial effects on tissue healing and regeneration[31,32]. Here, our results indicate a potential mechanism underlying the LPLI-mediated effects on stem cells and suggest a clinical application for LPLI in stem cell therapy and bone fracture healing. In conclusion, our results reveal that low power laser irradiation at not only had no cytotoxic effects but also promoted proliferation of host bone cells leading to remarkable changes in mechanical properties[33,34]. Therefore, our results provided a potential cellular mechanism of low level laser beside paste of calcium phosphate with bioglass containing magnesium in early packing up the gap and increasing quality of callus formation as far as mechanical pressure at the site is concerned.

CONCLUSION

The low Level laser with tri Calcium Phosphate is a viable option for obliteration for early stiffness and strength in mandibular defects.

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