Do low-level laser therapy and platelet-rich plasma affect bone calcium content and mechanical properties at tooth extraction sites? An experimental study in the rat

Alireza Sharifi
Shiraz University of Medical Sciences
Tahereh Talaei-Khozani
Shiraz University of Medical Sciences
Nader Tanideh
DVM, MPH, Shiraz University of Medical Science
Hossein Khaje Zadeh
Shiraz University of Medical Science
Meysam Haghighat
Shiraz University of Medical Science
Sheila Shahsavari Pour
Shiraz University of Medical Sciences
Saeid Tavanafar (s.tavanafar@gmail.com)
Birjand University of Medical Sciences

Research Article

Keywords: Low-level laser therapy, Platelet-rich plasma, Bone, Tooth

Posted Date: April 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1463125/v1

Abstract

Background: Both low-level laser therapy (LLLT) and platelet-rich plasma (PRP) have been suggested to improve the repair of mandibular defects. This study investigated the mechanical properties and calcium content at the tooth extraction site in the rat model exposed to LLLT (λ=808 nm) with or without PRP.

Methods: In this experimental rat model, the maxillary left first molar teeth were extracted in twenty male rats. Then, they were divided randomly into four groups. Group one: the extraction sockets were treated by 0.9 W gallium-aluminum-arsenide (GaAlAs) diode laser irradiation for five minutes every 72 hours after extraction for the next 12 days (4 times in overall); group two: PRP was placed in the extraction sockets; group three: a combination of both treatments (LLLT+PRP) was done; group four: untreated extraction socket as the control group. All rats were sacrificed 30 days post-operative. All bone blocks of the extracted socket were prepared for mechanical strength and calcium content analyses.

Results: The compressive strength in the laser group was significantly higher than in both the control and PRP-treated groups (p=0.0001 and 0.00044, respectively). Although a combination of both PRP and LLLT elevated the mechanical strength compared to the control and PRP groups, it was statistically similar to LLLT/PRP group. Calcium content did not change by any of the treatments.

Conclusions: Mechanical and chemical analyses on the bone blocks demonstrated that LLLT improved bone healing; however, PRP alone or combined with LLLT did not show a synergistic impact.

Background

Bone healing is a sophisticated biological process and can be clinically challenging [1–3]. Bone defects in the jaws may result from congenital or developmental malformation, tumor resection, cyst enucleations, trauma, infections, and tooth removal. Tooth extraction is one of the most common procedures performed in the dental office. Healing of such wounds is uneventful in most cases, although some wounds require a longer time to heal and may accompany significant pain or cause bone defects.

Previous studies have demonstrated several methods to accelerate bone repair, including treating the tooth sockets with various bioactive materials [4], using specific growth factors such as bone morphogenetic proteins (BMPs) [5], vitamin D [6], calcium phosphate [7], hormones, plant extracts [8], low-intensity ultrasound mechanical stimulation [9, 10], electromagnetic fields [11] and LLLT [12, 13]. LLLT is also known as "photo-bio-modulation therapy" and has extensive applications in many clinical and experimental studies. Several studies have elucidated that LLLT can be useful for several specific applications in dental practice [14]. LLLT accelerates repair with or without administration of various osteoinductive and osteoconductive biomaterials [15]. Lasers impact numerous phenomena in tissues, such as hemostasis, microbial decontamination, tissue ablation, and vaporization, and influence biological processes, including bio-stimulation (photo-bio-modulation), which promote numerous beneficial therapeutic effects [16]. One of the promising uses of LLLT is the acceleration of bone metabolism. The laser applies a directional non-ionizing electromagnetic radiation which is

monochromatic and coherent [17], and its wound healing stimulation has been used with various power densities. Laser energy could enhance osteoblastic, epithelial cells and fibroblast proliferation, collagen synthesis by fibroblasts, lymphatic system activation, angiogenesis, and bone formation [18]. LLLT has also been shown to boost the osteogenic properties of some biomaterials as well.

Bone healing takes a long time; besides, it is hard to repair spontaneously if the bone defects are more extensive than a critical size. Therefore, researchers also study the osteoinductive and osteoconductive influence of different biological and chemical agents to accelerate this process. One of these biological agents is platelet-rich plasma (PRP) [19–21]. The growing interest in using PRP for bone repair is due to a reduction in the recovery time of injured tissue. Likewise, PRP assists bone healing through the effects of bone-promoting agents, such as specific proteins, cytokines, chemokines, and several growth factors present in the plasma and platelets. Platelets perform various functions such as wound repair, re-epithelialization, and homeostasis. It also releases several growth factors, stimulates angiogenesis, and promotes fibroblast proliferation, increasing collagen synthesis [22].

Platelet-rich Plasma contains fibrin and several growth factors such as vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF- β), epithelial growth factor (EGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) that have beneficial effects on bone growth [23] and can be gelified to form a scaffold and fill the impaired tissues [24]. Further, some growth factors in PRP, such as PDGF, motivate the mesenchymal cells of adjacent tissues to proliferate, migrate and differentiate into osteoblasts. Previous reports show that PRP has improved the migration of osteoblasts and periodontal ligament cells [25]. Besides, the synergistic influence of PRP and some other biomaterials and therapeutic procedures have been studied previously [26, 27].

With regard to the above considerations, the present study aimed to find any synergic impact of biogenic materials such as platelet-rich plasma with LLLT on the healing of maxillary bone defects in the rat model.

Methods

Exclusion criteria

Animals presenting abnormal health conditions were excluded from the experiment. Anesthetic methods and all of the medications were the same for all samples.

Ethical statement

The ethical committee of the Shiraz University of Medical Science approved the study protocol, and all procedures were per ethical principles of animal handling and treatment (IR.SUMS.DENTAL.REC.1400.020). The ARRIVE guidelines were followed in reporting the present study.

Preparation of PRP

Three mL of the rat blood was injected into a tube containing citric acid as an anti-coagulant and centrifuged at 5000 rpm for 15 minutes. A micropipette was used to remove the plasma and buffy coat and placed it into a sterile tube. Plasma was centrifuged for the second time at 2000 rpm for 10 min, and the bottom one/third was considered PRP. In order to gelatinize PRP and activates platelets, it was fortified with 2.5% CaCl2 immediately after transferring into the tooth sockets.

Laser device

The laser device was GaAlAs (OSRAM LD 808, Germany), and its parameters were set as follows: 808 nm wavelength,; 0.9 W output; 1459 J/cm2dose; continuous radiation mode for five minutes.

Study design and surgical procedure

Twenty healthy Sprague–Dawley male rats, approximately 80-90 days old and weighing 180-200 g, were ordered from Animal Laboratory at the Shiraz University of Medical Science. All rats were kept in a large well with adequate ventilation at standard conditions, with food and water freely accessible.

An intramuscular injection of ketamine (Vetased, Romania) and xylazine (Sedaxyl, Belgium) was used to anesthetize rats. After the onset of anesthesia, the maxillary left molar teeth of all animals were extracted. [Fig 1] All animals were divided randomly into one control group (n=5) and three experimental (case) groups: laser- (n=5), PRP- and laser-PRP-treated (n=5) group. In PRP- and laser-PRP-treated groups, approximately 100 µL of PRP (after preparation) was transferred into the socket immediately after extraction, and then, CaCl2 was added to jellified PRP.

The tooth sockets of both the laser group and laser-PRP group were then exposed to gallium aluminum arsenide diode laser every 72 h after extraction for the next 12 days (4 times overall). Rats were labeled and allocated by laboratory personnel; therefore, authors were blind to samples during and at the end of the experiment.

All rats were euthanized by CO2 70% 30 days after the extraction. The maxilla was harvested, and the overlying soft tissue was removed using a scalpel. Tooth sockets with a rim of peripheral bone were separated and fixated in 10% formalin. The length and width of all bone blocks were 4 ± .1mm and 3mm ± .1mm, respectively. The thickness of blocks is variable due to the different amounts of bone formation. All blocks were stored at -20ºC until used.

Mechanical test assessment:

All samples were simulated in terms of mass and volume. A scale with an accuracy of 0.1 mg was used to measure the mass. Archimedes' principle of solids' volume was used to evaluate the samples' volume.

The removed bone samples were fixed longitudinally in place for mechanical testing and exposed to mechanical tests using Zwick/Roell machine (Germany) at a speed of 0.5mm/min.

Calcium content assessment:

After the mechanical test performing, we collected all pieces of bone blocks (due to breaking blocks caused by the mechanical test) and prepared them to measure calcium content. This preparation procedure started by ashing the pieces of bone blocks at 520 ºC for 20 h in the oven (Gallenkamp Muffle Furnace, England). After that, the ashed bone was powdered, and 0.03-gram of bone powder was dissolved in 250 µL HCl and diluted with 31 mL distilled water. The Calcium contents were measured with atomic absorption (Thermo scientific iCE 3500, USA) at a wavelength of 422.7 nm.

Statistical Analysis

Mechanical strength was statistically analyzed by the Mann-Whitney test. Bone Calcium contents were analyzed by a one-way ANOVA test. Intergroup statistical differences were done by LSD as post hoc tests. A P value less than 0.05 was considered significant. All analyses were performed by SPSS 16.0 for Windows, and the graph was depicted by graph pad 5.

Results

All rats tolerated the extraction procedure without apparent inflammation and could eat a regular diet postoperatively.

Mechanical test

After harvesting the maxilla and removing soft tissue, the thickness of bone at the tooth extraction site was measured. The thickness of bone formation in laser and laser/PRP groups (Range=1.2-1.4 mm) was higher than the control group (Range=.7-.8 mm). This indicates that bone formation at the tooth extraction site was better in these groups than in the control group.

As fig 2 indicates, the compressive strength in the laser group was significantly higher than in the control group (P=0.0001). Also, a significant difference was observed between the laser/PRP and control group (P=0.00044). On the other hand, the results showed that the mechanical strength of both PRP-treated and control groups was the same statistically. Therefore, laser and PRP showed no synergistic effects [Fig 2].

Calcium content finding:

Fig 3 displays the amount of calcium content of the reparative bone in tooth sockets of the different groups. The data showed that neither laser nor PRP significantly impacts Ca content. Besides, the combined laser and PRP administration also had no significant influence on Ca content [Fig 3].

Discussion

Postoperatively, bone regeneration is observed progressively filling the defect in the eventuate period. The bone healing process usually requires four to six months, and a longer time is needed for bone remodeling [28].

Recent studies comparing the bone-healing process in rats and humans found numerous similarities, despite this process being faster in rats [29]. This was why rats were preferred as the experimental model for the present study (principles of the 3Rs; Replacement, Reduction, and Refinement) [30]. The previous study indicates differences between human bone and small rodents, which have basic bone structures and lack Haversian canals [31].

Degranulation of the PRP promotes the discharge of several growth factors and substances such as VEGF, TGF beta-1, PDGF, FGF, connective tissue growth factor, transforming growth factors such as insulin or stimulatory (IGF-1), epidermal growth factor, platelet thromboplastin, calcium, serotonin, and fibrinogen hydrolytic enzymes [32, 33]. Previous studies show that PRP could promote bone regeneration by releasing growth factors such as TGF-β1 and PDGF [21] after platelet degranulation [2].

Several limitations are encountered while using LLLT, such as no established optimization and a standard protocol for exposure time, power intensity, and wavelength. Additionally, different studies used different experimental models and a wide range of exposure duration, complicating comparisons between the obtained results. LLLT effect has shown to be dose-dependent, and a single exposure has no significant effect on bone repair [1, 34]. Here, we have applied four consecutive doses of LLLT. Despite using four-dose doses, we also showed that LLLT had no impact on Ca content.

Previous in vivo and in vitro studies showed that LLLT affects osteoblast proliferation and differentiation. Low-level laser-like GaAlAs at830nm wavelength stimulates osteoblastic cell growth, increased alkaline phosphatase (ALP), and osteocalcin in osteogenic cell line culture [35]. Both ALP activity and osteoclastic expression are associated with the mineralization of newly formed bone. The viability of osteocytes around dental implants has been higher after low-level laser therapy [36]. Although literature showed a higher number of osteoblast, higher ALP activity, and osteocalcin expression level, our data revealed that calcium content did not increase by LLLT administration. Therefore, the increase in mechanical strength seems unrelated to calcium content, increased osteoblast mineralization capacity, or ALP activity. A previous study found that LLLT boosts bone healing in irradiated tooth sockets of albino rats [37]. It has also stimulated bone formation in large calvaria defects of ovariectomized rats [3]. In contrast to our data, Nicola et al. found that applying LLLT increases mineral apposition rate and bone volume [38].

The study of photo-bio-modulation therapy should thrive to reach a standardized protocol for a specific wavelength and radiation dose. The wavelength of 808 nm (used in the present study) penetrates the tissue surface (mucosa), reaching the underlying bone (maxilla); thus, it is more suitable for bone-related applications [39]. Earlier studies have shown that several regenerative strategies, biomaterials, and additional therapies, such as LLLT and PRP, accelerate bone repair and growth [1]. Our study also indicates that LLLT administration led to an increase in mechanical strength of repaired bone; however, it had no impact on Ca content.

In the present study, we showed that LLLT enhances bone strength. In addition, no improvement in bone strength was observed in the animals treated with PRP compared to control groups. Reasonably, one would expect that the combination of PRP and LLLT could reinforce bone regeneration and be notably

superior to each of the treatments alone [3, 21]. However, no significant increase in bone strength was detected in our study using a combination of LLLT/PRP treatment. The data showed that PRP combined with LLLT had no significant synergic impact on osteogenesis. In the present study, the rationale behind increased compressive strength of newly formed bone might be related to the local effects of laser stimulating the differentiation of mesenchymal stem cells and the proliferation of fibroblast and osteoblasts [39].

In conclusion, mechanical and chemical analyses of the bone blocks demonstrated that PRP treatment does not improve bone strength. However, LLLT may contribute to the reinforcement of bone strength. Calcium content analysis elucidated that PRP and LLLT, either alone or combined, do not make significant differences.

Limitations

As PRP preparation is vital in its effects on bone repair, one of our study limitations can be using just one PRP preparation method. The other limitation is the evaluation of bone-specific markers such as those involved in mineralization.

Declarations

Ethics approval and consent to participate: The local ethical committee evaluated and approved the study protocol (IR.SUMS.DENTAL.REC.1400.020). All methods were performed in accordance with the ARRIVE guidelines and **Declaration of Helsinki**.

Consent for publication: Not applicable.

Availability of data and materials: The datasets generated during and analyzed during the current study are not publicly available since the manuscript has not been accepted yet, but are available from the corresponding author on reasonable request.

Competing interests: The authors state that they have no financial or non-financial conflicts of interest regarding this research.

Funding: The study was funded by investigators and did not receive external funding.

Authors' contributions: "AS, HKZ, ST Methodology, investigation, writing- original draft preparation, TTK, SSP, MH, and NT resources, writing-review and editing, project administration, funding acquisition, formal analysis. All authors have read and approved the final version of the manuscript."

Acknowledgments: We would like to thank the vice-chancellor of Shiraz University of Medical Sciences for supporting this work financially. The authors wish to thank Dr. Vahedi and the other personnel in the center of comparative and experimental medicine, Shiraz University of Medical Sciences. We appreciate

the collaboration of the personnel in the chemistry department of Shiraz University. This manuscript was extracted from a thesis by AR Sharifi as a part of fulfillment to obtain an MD degree.

References

1. Obradović RR, Kesić LG, Peševska S. Influence of low-level laser therapy on biomaterial osseointegration: A mini-review. Lasers in Medical Science. 2009;24:447–51. doi:10.1007/s10103-008-0573-z.
2. Mariano R, Messora M, de Morais A, Nagata M, Furlaneto F, Avelino C, et al. Bone healing in critical-size defects treated with platelet-rich plasma: a histologic and histometric study in the calvaria of diabetic rat. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2010;109:72–8.
3. Garcia VG, Da Conceição JM, Fernandes LA, De Almeida JM, Nagata MJH, Bosco AF, et al. Effects of LLLT in combination with bisphosphonate on bone healing in critical-size defects: A histological and histometric study in rat calvaria. Lasers Med Sci. 2013;28:407–14. doi:10.1007/s10103-012-1068-5.
4. El-Ghannam A. Bone reconstruction: From bioceramics to tissue engineering. Expert Review of Medical Devices. 2005;2:87–101. doi:10.1586/17434440.2.1.87.
5. McCarthy TL, Ji C, Centrella M. Links among growth factors, hormones and nuclear factors with essential roles in bone formation. Critical Reviews in Oral Biology and Medicine. 2000;11:409–22. doi:10.1177/10454411000110040201.
6. Doetsch AM, Faber J, Lynnerup N, Wätjen I, Bliddal H, Danneskiold-Samsøe B. The effect of calcium and vitamin D3 supplementation on the healing of the proximal humerus fracture: A randomized placebo-controlled study. Calcif Tissue Int. 2004;75:183–8. doi:10.1007/s00223-004-0167-0.
7. Higgins TF, Dodds SD, Wolfe SW. A biomechanical analysis of fixation of intra-articular distal radial fractures with calcium-phosphate bone cement. J Bone Jt Surg - Ser A. 2002;84:1579–86. doi:10.2106/00004623-200209000-00010.
8. Işler SC, Demircan S, Çakarer S, Çebi Z, Keskin C, Soluk M, et al. Effects of folk medicinal plant extract ankaferd blood stopper® on early bone healing. J Appl Oral Sci. 2010;18:409–14. doi:10.1590/S1678-77572010000400015.
9. Hasuike A, Sato S, Udagawa A, Ando K, Arai Y, Ito K. In vivo bone regenerative effect of low-intensity pulsed ultrasound in rat calvarial defects. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2011;111:e12–20. doi:10.1016/j.tripleo.2010.09.061.
10. Findik Y, Baykal T. Effects of low-intensity pulsed ultrasound on autogenous bone graft healing. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;117:e255–60. doi:10.1016/j.oooo.2012.05.023.
11. Kooistra BW, Jain A, Hanson B. Electrical stimulation: Nonunions. Indian J Orthop. 2009;43:149. doi:10.4103/0019-5413.50849.
12. Kazancioglu HO, Ezirganli S, Aydin MS. Effects of laser and ozone therapies on bone healing in the calvarial defects. J Craniofac Surg. 2013;24:2141–6. doi:10.1097/SCS.0b013e3182a244ae.

13. Nagata MJH, Santinoni CS, Pola NM, De Campos N, Messora MR, Bomfim SRM, et al. Bone marrow aspirate combined with low-level laser therapy: A new therapeutic approach to enhance bone healing. J Photochem Photobiol B Biol. 2013;121:6–14.
14. Walsh LJ. The current status of low level laser therapy in dentistry. Part 1. Soft tissue applications. Aust Dent J. 1997;42:247–54. doi:10.1111/j.1834-7819.1997.tb00129.x.
15. Jonasson TH, Zancan R, de Oliveira Azevedo L, Fonseca AC, Silva MC da, Giovanini AF, et al. Effects of low-level laser therapy and platelet concentrate on bone repair: Histological, histomorphometric, immunohistochemical, and radiographic study. J Cranio-Maxillofacial Surg. 2017;45:1846–53. doi:10.1016/j.jcms.2017.08.008.
16. Aoki A, Sasaki KM, Watanabe H, Ishikawa I. Lasers in nonsurgical periodontal therapy. Periodontology 2000. 2004;36:59–97. doi:10.1111/j.1600-0757.2004.03679.x.
17. Schindl A, Schindl M, Pernerstorfer-Schon H, Schindl L. Low-intensity laser therapy: A review. Journal of Investigative Medicine. 2000;48:312–26. https://europepmc.org/article/med/10979236. Accessed 3 Mar 2022.
18. Kawasaki K, Shimizu N. Effects of low-energy laser irradiation on bone remodeling during experimental tooth movement in rats. Lasers Surg Med. 2000;26:282–91. doi:10.1002/(SICI)1096-9101(2000)26:3<282::AID-LSM6>3.0.CO;2-X.
19. Nikolidakis D, Meijer GJ, Oortgiesen DAW, Walboomers XF, Jansen JA. The effect of a low dose of transforming growth factor β1 (TGF-β1) on the early bone-healing around oral implants inserted in trabecular bone. Biomaterials. 2009;30:94–9. doi:10.1016/j.biomaterials.2008.09.022.
20. Wiltfang J, Kloss FR, Kessler P, Nkenke E, Schultze-Mosgau S, Zimmermann R, et al. Effects of platelet-rich plasma on bone healing in combination with autogenous bone and bone substitutes in critical-size defects: An animal experiment. Clin Oral Implants Res. 2004;15:187–93. doi:10.1111/j.1600-0501.2004.00980.x.
21. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85:638–46. doi:10.1016/S1079-2104(98)90029-4.
22. Monto RR. Platelet rich plasma treatment for chronic Achilles tendinosis. Foot Ankle Int. 2012;33:379–85. doi:10.3113/FAI.2012.0379.
23. Oryan A, Alidadi S, Moshiri A. Platelet-rich plasma for bone healing and regeneration. Expert Opin Biol Ther. 2016;16:213–32. doi:10.1517/14712598.2016.1118458.
24. Sadeghi-Ataabadi M, Mostafavi-pour Z, Vojdani Z, Sani M, Latifi M, Talaei-Khozani T. Fabrication and characterization of platelet-rich plasma scaffolds for tissue engineering applications. Mater Sci Eng C. 2017;71:372–80. doi:10.1016/j.msec.2016.10.001.
25. Aoki A, Mizutani K, Schwarz F, Sculean A, Yukna RA, Takasaki AA, et al. Periodontal and peri-implant wound healing following laser therapy. Periodontol 2000. 2015;68:217–69.
26. Shahsavari-Pour S, MSc, Aliabadi E, MSc, Latifi M, PhD, et al. Evaluation of the Possible Synergic Regenerative Effects of Platelet-Rich Plasma and Hydroxyapatite/Zirconia in the Rabbit Mandible

Defect Model. Iran J Med Sci. 2018;43:633. /pmc/articles/PMC6230930/. Accessed 3 Mar 2022.

27. Tschon M, Veronesi F, Contartese D, Sartori M, Martini L, Vincenzi F, et al. Effects of pulsed electromagnetic fields and platelet rich plasma in preventing osteoclastogenesis in an in vitro model of osteolysis. J Cell Physiol. 2018;233:2645–56. doi:10.1002/JCP.26143.
28. Del Fabbro M, Bortolin M, Taschieri S. Is autologous platelet concentrate beneficial for post-extraction socket healing? A systematic review. Int J Oral Maxillofac Surg. 2011;40:891–900. doi:10.1016/J.IJOM.2011.04.009.
29. Elsubeihi ES, Heersche JNM. Quantitative assessment of post-extraction healing and alveolar ridge remodelling of the mandible in female rats. Arch Oral Biol. 2004;49:401–12.
30. Çırak E, Özyurt A, Peker T, Ömeroğlu S, Güngör MN. Comparative evaluation of various low-level laser therapies on bone healing following tooth extraction: An experimental animal study. J Craniomaxillofac Surg. 2018;46:1147–52. doi:10.1016/J.JCMS.2018.05.012.
31. Nunamaker DM. Experimental models of fracture repair. Clin Orthop Relat Res. 1998;355 355 Suppl. doi:10.1097/00003086-199810001-00007.
32. Debus ES, Schmidt K, Ziegler UE, Thiede A. [The role of growth factors in wound healing]. Zentralbl Chir. 2000;125 Suppl 1 SUPPL. 1:49–55. https://europepmc.org/article/med/10929647. Accessed 17 Mar 2022.
33. Shanaman R, Filstein MR, Danesh-Meyer MJ. Localized ridge augmentation using GBR and platelet-rich plasma: case reports. Int J Periodontics Restorative Dent. 2001;21:345–55. http://www.quintpub.com/userhome/prd/prd_21_4_shanaman2.pdf. Accessed 3 Mar 2022.
34. Garcia VG, Sahyon AS, Longo M, Fernandes LA, Gualberto Junior EC, Novaes VCN, et al. Effect of LLLT on autogenous bone grafts in the repair of critical-size defects in the calvaria of immunosuppressed rats. J Craniomaxillofac Surg. 2014;42:1196–202. doi:10.1016/J.JCMS.2014.02.008.
35. Ozawa Y, Shimizu N, Kariya G, Abiko Y. Low-energy laser irradiation stimulates bone nodule formation at early stages of cell culture in rat calvarial cells. Bone. 1998;22:347–54. doi:10.1016/S8756-3282(97)00294-9.
36. Dörtbudak O, Haas R, Mailath-Pokorny G. Effect of low-power laser irradiation on bony implant sites. Clin Oral Implants Res. 2002;13:288–92. doi:10.1034/J.1600-0501.2002.130308.X.
37. Korany NS, Mehanni SS, Hakam HM, El-Maghraby EMF. Evaluation of socket healing in irradiated rats after diode laser exposure (histological and morphometric studies). Arch Oral Biol. 2012;57:884–91. doi:10.1016/J.ARCHORALBIO.2012.01.009.
38. Nicolau RA, Jorgetti V, Rigau J, Pacheco MTT, Dos Reis LM, Zângaro RA. Effect of low-power GaAlAs laser (660 nm) on bone structure and cell activity: an experimental animal study. Lasers Med Sci. 2003;18:89–94. doi:10.1007/S10103-003-0260-Z.
39. Batista JD, Sargenti-Neto S, Dechichi P, Rocha FS, Pagnoncelli RM. Low-level laser therapy on bone repair: is there any effect outside the irradiated field? Lasers Med Sci. 2015;30:1569–74. doi:10.1007/S10103-015-1752-3.

Figures

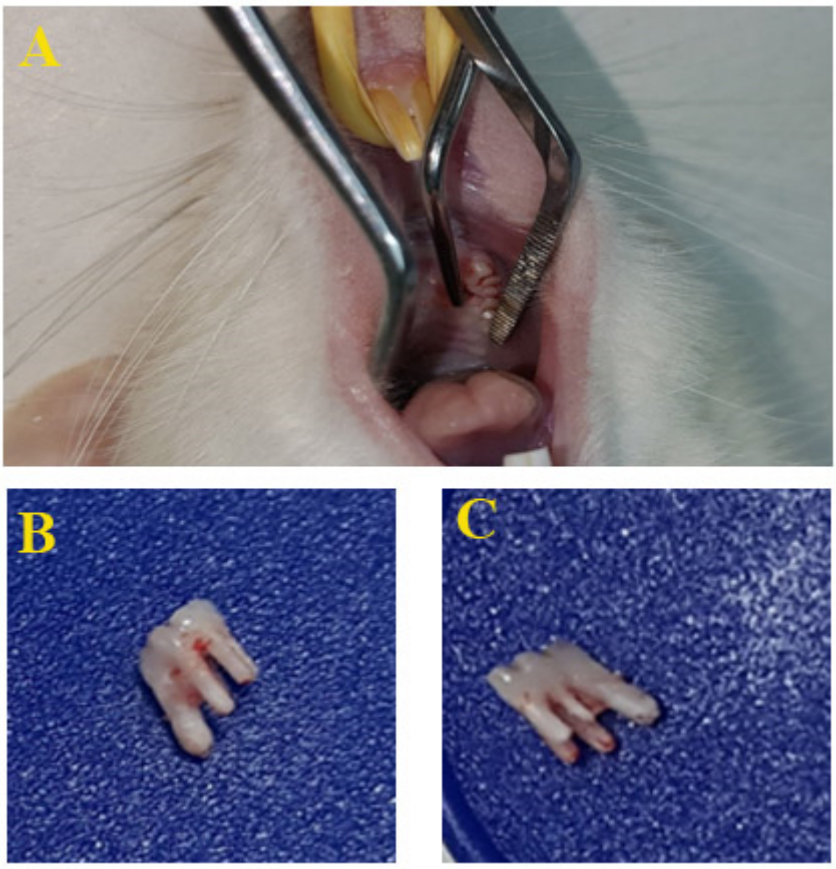

Figure 1

Extraction of maxillary left first molar (A). Extracted tooth from buccal (B) view and palatal view (C).

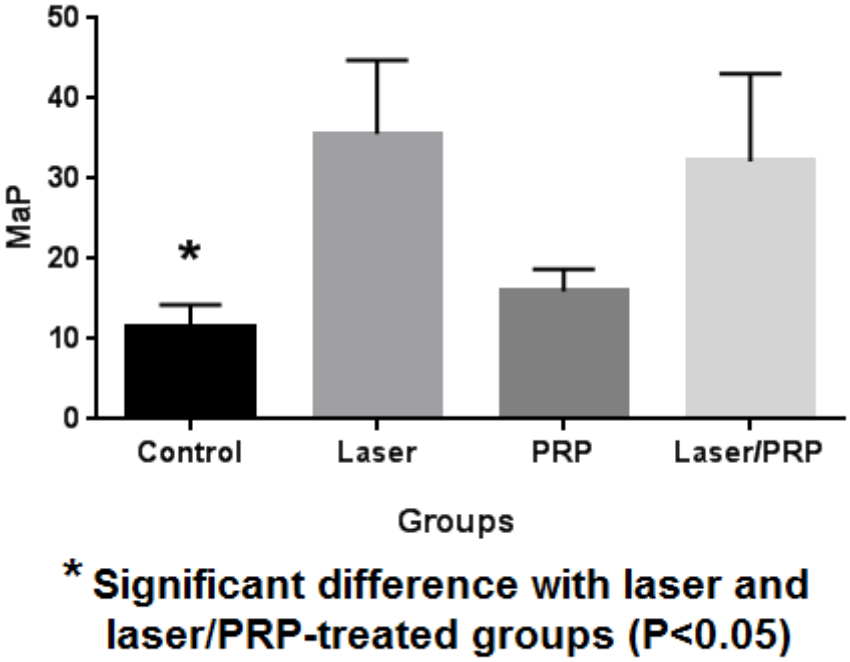

Figure 2

The comparison of the compressive strength of the reparative bone in tooth sockets treated with different methods.

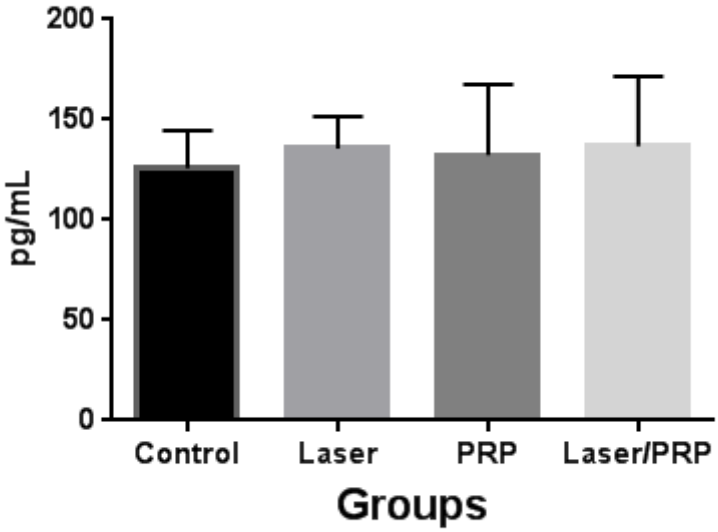

Figure 3

The comparison of the calcium content between the different groups.