Brief Retrospect on the Use of Photobiomodulation (PBM) Therapy for Augmented Bone Regeneration (ABR)

As technology advances at a rapid rate, innovations in regenerative medicine will eventually include the use of energy-based therapeutics, such as low intensity-pulsed ultrasound stimulation (LIPUs), pulsed electromagnetic field stimulation (PMFs), and low-level laser/light therapy (LLLt) or photobiomodulation therapy (PBMt). Among these treatments, LLLt/PBMt attracted significant attention by the turn of the century, as evidenced by the numerous publications compared to LIPUs and PMFs, particularly for augmented bone regeneration (ABR). This is a testament of how the maturation of technology and scientific knowledge leads to latent compounded applications, even when the value of a technique is reliant on empirical data. This article reviews some of the notable investigations using LLLt/PBMt for bone regeneration published in the past decade, focusing on how this type of therapy has been utilized together with the existing regenerative medicine landscape.

Key words
Low-level laser/light therapy; Photobiomodulation therapy; Laser therapy; Augmented bone regeneration
INTRODUCTION

Bone defects, either fractures or lack thereof, are among the most common disorders encountered in the medical field. This condition can be brought about by numerous conditions such as injuries, cancer (and related treatments), surgery, infectious and autoimmune diseases, and even genetics. Variations with regards to cause of different bone defects also equates disparities between appropriate treatments. Decades of research have already contributed to the development of ever more advancing therapeutics seeking one vital purpose—guided bone regeneration (GBR). Although it is well known that bone can regenerate like any other tissue in the human body, bone requires a particular set of conditions to proceed. It follows an extended scheme of regeneration that involves several overlapping processes. Since bone tissue is the main support structure that influences stability of almost all surrounding soft tissue, it’s crucial that bone heals according to its form and associated function.

Stabilization of bone fractures is one of the most important considerations when addressing bone defects. Callus formation in broken bones progress slowly and only occurs within a very short range of distance. Minimizing the distance and mobility of the fractured bone tissue limits the occurrence of non-union. This has led medical and clinical scientists to develop different devices and techniques for fracture immobilization with the aim of GBR.

Aside from immobilization, another aspect to be considered is proper ossification. Bone is a hard tissue which contains high amounts of minerals compared to other soft tissues. This necessitates the presence of a mineral source to support calcification of the newly formed dense tissue. Hence, this led to medical and material scientists to examine biomaterials not only as immediate mineral source for tissue calcification but as proper tissue scaffolds mimicking the stability and structural form of bone tissue. Copious implantable materials for GBR based on natural sources (demineralized/acellular bone), synthetic materials (polymers/mineral-based/metals), and related composites have already been successfully utilized as viable medical products.

Biological mechanisms have also been elucidated with the aim of using cells and exploiting biomolecular pathways to improve previously developed techniques for GBR. The introduction of cell, protein, and genetic-based biomolecular analyses was accompanied by enormous interest in using biological molecules to promote augmented bone regeneration (ABR). Notwithstanding the advancements in GBR generated from recent investigations, the use of energy-based medical therapeutics such as pulsed electromagnetic field stimulation, ultrasound stimulation, and laser application for ABR has garnered traction lately.

Initial studies mainly utilized these aforementioned energy-based technologies for the purpose of observation and visualization of organs and tissues. Early investigations regarding the use of energy for tissue regeneration have been scarce and far in between. This could possibly be due to the persistent changes in understanding the fundamental nature of the different forms of energy. Unlike biomaterials and biologics, energy not only exists in different forms but also in a broad spectrum and is highly transferrable across various states of matter. This complicates not only the direct application of energy for therapeutic purposes but also imposes the promotion of proper safety precautions for both the practitioner and patient alike. In addition to this, technological advancements in the form of hardware capable of measuring, generating, and harnessing the potential of different forms of energy became a limiting factor in pursuing energy-based therapeutics early on. Nevertheless, the knowledge, policies, and machinery/devices have already reached the level of maturity enough to merit investigations several energy-based technologies for regenerative applications.

As previously mentioned, pulsed magnetic field stimulation (PMFs) is one of the energy-based therapy that is currently being investigated for ABR. Initial studies attributed the efficacy of PMFs to the two factors; the fast-tracked synthesis of bone matrix thru the induced weak electric currents, and the subsequent bone loss matrix downregulation. Later studies have revamped this theory by suggesting that PMFs boosts cell proliferation and step-up the osteogenic process. This led to the approval of PMFs for supplementing post-osteotomy recovery and as treatment for both osteoarthritis and pseudoarthrosis.

Low intensity pulsed ultrasound stimulation (LIPUs) similarly went through the same inquiries concerning its potential for ABR using animal models and through clinical studies alike. A number of studies delving into the mechanism by which LIPUs leads to ABR had reported that ultrasound induction influences the c-fos, cyclooxygenase-2, prostaglandin-2, nitric oxide (NO), osteocalcin, and osteopontin levels in osteoblasts as a result of amplified calcium influx into the osteoblasts. This occurs through signal transduction across the cytoskeleton proteins, actin filaments, and extracellular matrix connected via adhesive contacts during LIPUs. Ultrasound stimuli travels between adherent cells setting off stretch-
activated calcium channels; this in turn increases cell surface expression of integrins leading to formation of stress fibers via reorganization of actin cytoskeleton.15-17

Likewise, Low-level Laser therapy (LLLT); the use of non-ionizing light energy for regenerative medicine, has also been a prominent topic for ABR as of late. Compared to PMFs and LIPUs, application of LLLT for bone regeneration is a more recent and pervasive topic that picked up greater traction by the end of the 20th century. Based on current publication search, information regarding the use of PMFs and LIPUs dates back as far as mid 1970s and has relatively fewer number of publications compared to LLLT, this indicative of the major interest and relative greater potential of using lasers for bone regeneration therapy. Thus, this article will be a retrospect on select publications with notable developments concerning the use of LLLT for bone regeneration in the past decade.

PHOTOBIOIMODULATION THERAPY (PBMT) FOR BONE REGENERATION

Low-level laser/light therapy entails exposure of cells and tissues to light energy ranging within the red and near-infrared (600-1070 nm) wavelength of the electromagnetic spectrum.18 The presence of chromophores like hemoglobin and melanin, which have effective absorption below 600 nm, can be efficiently utilized to treat superficial tissues while longer wavelength ranging from 780-950 nm are used for addressing deeper tissues like bone.18 This energy-based therapy is delivered in lower power density need to generate heat thus the term “cold laser” became associated with LLLT.19

In fact, a recent publication concerning naming scheme for the said procedure have led to the promotion of “Photobiomodulation therapy” (PBMT) as an alternative term for LLLT.20 This was to improve consolidation of published articles making use of low-level/low-power laser/light treatments that do not induce ionizing or thermal effects on cells and tissues nonetheless stimulate certain biological response. The term “photobiostimulation” was derived from the photochemical effect resulting from the absorption of light energy promoting certain chemical changes that affect biological processes in living organisms. Thus, for the remainder of this article, LLLT will be referred to as PBMT.

Although abundant studies have been conducted regarding the use of PBMT for regenerative purposes, the mechanism by which it enhances the recovery of damaged tissue is yet to be fully elucidated. Based on existing literature, improvement on bone regeneration through PBMT is attributed to at least two modes of action. The first mechanism is ascribed to the absorption of the light energy by the respiratory chain terminal enzyme-cytochrome C oxidase leading to the increase of mitochondrial membrane potential which consequently elevates adenosine triphosphate, cyclic adenosine monophosphate, NO, and reactive oxygen species levels.21 This increased mitochondrial activity causes interactions with the cell nucleus that translating to modifications in gene expression related to dense tissue formation and ossification.22-24 Through this effect, PBMT has been known to influence not only cell viability but also the expression of collagen 1, osteocalcin, bone morphogenetic protein-2, alkaline phosphatase, bone sialoprotein in osteoblasts.25-27 The second mechanism relates to the effect of PBMT on macrophage polarization where in the same alteration in mitochondrial activity affects the proinflammatory and anti-inflammatory protein expressions in macrophage cells. Studies have shown that PBMT is capable favoring the transformation of macrophages into M2 phenotype which alleviate inflammation and promotes tissue repair and remodeling.1,26

Current literature regarding the use of PBMT for bone regeneration is mainly sourced from orthodontic applications reporting the suitability of the procedure for reducing pain and inflammation,26-27 periodontitis managemen,26,28 and bone remodeling during tooth movement.29,30 However, independent studies are still being conducted regarding the use of PBMT for regenerating critical sized bone defects in animal models,31-33 enhancing results of distraction osteogenesis,34,35 regenerating irradiated bone,36 repair bone lesions,37 manage osteoporosis,38,39 and resolve osteonecrosis.40,41

PBMT COMBINED WITH OTHER ENERGY BASED THERAPEUTICS

Studies regarding the use of PBMT in conjunction with other energy-based therapy have also been conducted for ABR to a limited extent. The concept of combined energy-based therapeutics mimics the trend in biomaterial development in which combinations thereof or composites are tested to determine possible advantages within the same context. For the most part, PBMT is paired with LIPUs and more commonly compared with PMFs. This is probably due to the fact that PBMT and PMFs are both electromagnetic radiations that both exists in a spectrum although at drastically different wavelengths. On the other hand, LIPUs is high frequency vibrations that travel across tissues and does not carry photon
energy, making it a suitable complementary physical stimulation for tissues and cells.

The combination of LiPUs and PBMt have been studied as cumulative alternating treatment for improving bone consolidation during distraction osteogenesis, remodeling bone during tooth movement and post-ostectomy tibial bone regeneration. These studies primarily concentrate on the synergistic effect of both treatments with LiPUs improving cell infiltration and viability; and PBMt promoting osteogenesis and reducing inflammation.

**PBMT COMBINED WITH IMPLANTABLE MATERIALS**

Aside from the combined application of PBMt with either LiPUs or PMFs, it has also been tested out together with synthetic and biologically derived implantable materials for bone regeneration. This was brought about by the initial success from the use of PBMt for treating disease and wound in different tissue types. Since the start of the 21st century, PBMt has been applied as a supplementary procedure in some studies that makes use of biomaterials, drugs, grafts, and cell seeded tissue scaffolds for bone regeneration.

Relatively recent publications applying PBMt on bone implants focuses on both the stimulatory effect on the bone defect and the photothermal effect on the implant material. Case in point, the use of magnetic particles [strontium hexaferrite] and hydrogenated black titanium oxide coating both improve bone regeneration and simultaneously ablate cancer cells through hyperthermia upon exposure to near-infrared laser. Under this context the researchers makes use of a non-ionizing light energy to promote bone tissue repair whilst killing tumor cells with heated implanted biomaterial. The use of black phosphorous together with PBMt as bone implants has also been investigated. Subsequent publications have reported the usability of near-infrared light not only as stimulation for tissue repair but also as a photothermal release mechanism for strontium chloride and microsphere drug carriers for bone regeneration.

Osteoporosis treatment through use of simvastatin and alendronate have also been tested together with PBMt where in reports indicated enhanced cell viability and bone formation. Similarly, studies about post-surgery PBMt of bone defects implanted with autogenous bone grafts, non-autogenous bone implants, and fibrin sealants confirm the benefits of the said treatment in terms of bone regeneration and implant integration.

The use of biologics such as stem cells and platelet concentrate alongside PBMt were also explored for ABR in independent studies. The observed increased bone regeneration benefited from PBMt enhancing stem cell viability whilst improving anti-inflammatory response in animals treated with platelet concentrate respectively.

**FUTURE OF LLLT/PBMT FOR AUGMENTED BONE REGENERATION**

The considerable number of studies conducted using PBMt has expanded the coverage of this energy-based therapeutic to include bone tissue. Fig. 1 summarizes the notable treatments combined with PBMt for augmented bone regeneration. However, the abundant investigations appear to symptomatic of several issues that still needs to be addressed within the experimental framework of using...
PBMt for regenerative medicine. This becomes particularly noticeable with regards to examining factors such as light/laser wavelength, dosimetry, and hardware used for PBMt. Possessing a wide-range tunable laser would be ideal for verifying the effects of the different laser/light wavelengths at specific doses but then again this would probably significantly raise the cost of conducting such experiments. This is probably why this type of definitive investigation is yet to be conducted. As a result, the parameters for assessing the PBMt is yet to be established and until then investigators would be limited to relying on existing literature to pick out experimental settings. In addition, corroborating the results of independent studies is also rather complicated since the efficacy of PBMt is chiefly reinforced by empirical/observational data. Granting that probable mechanisms have been proposed as to how PBMt enhances tissue regeneration, these investigations will remain handicapped until a more conclusive intracellular process can be directly linked PBMt. Even so, the extensive queries regarding the added value of using red to infrared spectrum of photonic energy for regenerative purposes will continue to grow with biomaterials, pharmaceuticals, and treatment technique further develop. As technology mature and hardware for energy-based therapeutics become more available, widespread adaptation of these types of treatments for regeneration is inevitable even for deep seated hard tissue like bone.

REFERENCES

1. Schlundt C, El Khassawna T, Serra A, Dienelt A, Wendler S, Schell H, et al. Macrophages in bone fracture healing: their essential role in endochondral ossification. Bone 2018;106:78-89.
2. Friedenberg ZB, Bright CT. Bioelectric potentials in bone. J Bone Joint Surg Am 1966;48:915-23.
3. Grace KL, Revell WJ, Brookes M. The effects of pulsed electromagnetism on fresh fracture healing: osteochondral repair in the rat femoral groove. Orthopedics 1998;21:297-302.
4. de Haas WG, Watson J, Morrison DM. Non-invasive treatment of ununited fractures of the tibia using electrical stimulation. J Bone Joint Surg Br 1980;62-B:465-70.
5. Tsai MT, Chang WH, Chang K, Hou RJ, Wu TW. Pulsed electromagnetic fields affect osteoblast proliferation and differentiation in bone tissue engineering. Bioelectromagnetics 2007;28:519-28.
6. Sun LY, Hsieh DK, Lin PC, Chiu HT, Chiou TW. Pulsed electromagnetic fields accelerate proliferation and osteogenic gene expression in human bone marrow mesenchymal stem cells during osteogenic differentiation. Bioelectromagnetics 2010;31:209-19.
7. Midura RJ, Ibiwoye MO, Powell KA, Sakai Y, Doehring T, Grabiner MD, et al. Pulsed electromagnetic field treatments enhance the healing of fibular osteotomies. J Orthop Res 2005;23:1035-46.
8. Otter MW, McLeod KJ, Rubin CT. Effects of electromagnetic fields in experimental fracture repair. Clin Orthop Relat Res 1998;355 Suppl:S90-104.
9. Ciombor DM, Aaron RK. Influence of electromagnetic fields on endochondral bone formation. J Cell Biochem 1993;52:37-41.
10. Wang SJ, Lewallen DG, Bolander ME, Chao EY, Ilstrup DM, Greenleaf JF. Low intensity ultrasound treatment increases strength in a rat femoral fracture model. J Orthop Res 1994;12:40-7.
11. Cook SD, Ryaby JP, McCabe J, Frey JJ, Heckman JD, Kristiansen TK. Acceleration of tibia and distal radius fracture healing in patients who smoke. Clin Orthop Relat Res 1997;337:198-207.
12. Heckman JD, Ryaby JP, McCabe J, Frey JJ, Kilcoyne RF. Acceleration of tibial fracture-healing by non-invasive, low-intensity pulsed ultrasound. J Bone Joint Surg Am 1994;76:26-34.
13. Hadjiargyrou M, Lombardo F, Zhao S, Ahrens W, Joo J, Ahn H, et al. Transcriptional profiling of bone regeneration. Insight into the molecular complexity of wound repair. J Biol Chem 2002;277:30177-82.
14. Reher P, Harris M, Whiteman M, Hai HK, Meghji S. Ultrasound stimulates nitric oxide and prostaglandin E2 production by human osteoblasts. Bone 2002;31:236-41.
15. Pavalko FM, Norvell SM, Burr DB, Turner CH, Duncan RL, Bidwell JP. A model for mechanotransduction in bone cells: the load-bearing mechanosomes. J Cell Biochem 2003;88:104-12.
16. Yang RS, Lin WL, Chen YZ, Tang CH, Huang TH, Lu BY, et al. Regulation by ultrasound treatment on the integrin expression and differentiation of osteoblasts. Bone 2005;36:276-83.
17. Lee HS, Millward-Sadler SJ, Wright MO, Nuki G, Salter DM. Integrin and mechanosensitive ion channel-dependent tyrosine phosphorylation of focal adhesion proteins and beta-catenin in human articular chondrocytes after mechanical stimulation. J Bone Miner Res 2000;15:1501-9.
18. Chung H, Dai T, Sharma SK, Huang YY, Carroll JD, Hamblin MR. The nuts and bolts of low-level laser (light) therapy. Ann Biomed Eng 2012;40:516-33.
19. Escudero JSB, Perez MGB, de Oliveira Rosso MP, Buchaim DV, Pomini KT, Campos LMG, et al. Photobiomodulation therapy (PBMT) in bone repair: a systematic review. Injury 2019;50:1853-67.
20. Anders JJ, Lanzafame RJ, Arany PR. Low-level light/laser therapy versus photobiomodulation therapy. Photomed Laser Surg 2015;33:183-4.
21. de Freitas LF, Hamblin MR. Proposed mechanisms of photo-
20. Karu TI. Mitochondrial signaling in mammalian cells activated by red and near-IR radiation. Photochem Photobiol 2008;84:1091-9.

21. Pyo SJ, Song WW, Kim IR, Park BS, Kim CH, Shin SH, et al. Low-level laser therapy induces the expression of BMP-2, osteocalcin, and TGF-β1 in hypoxic-cultured human osteoblasts. Lasers Med Sci 2013;28:543-50.

22. Frozanfar A, Ramezani M, Rahpeyma A, Khajehahmadi S, Arbab HR. The effects of low level laser therapy on the expression of collagen type I gene and proliferation of human gingival fibroblasts (Hfg3-Pi 53): in vitro study. Iran J Basic Med Sci 2013;16:1071-4.

23. Manzano-Moreno FJ, Medina-Huertas R, Ramos-Torrecillas J, García-Martínez O, Ruiz C. The effect of low-level diode laser therapy on early differentiation of osteoblast via BMP-2/TGF-J, García-Martínez O, Ruiz C. The effect of low-level diode laser therapy on early differentiation of osteoblast via BMP-2/TGF-β1. J Stomatol Oral Maxillofac Surg 2018;119:699-76.

24. El-Maghraby EM, El-Rouby DH, Saafan AM. Assessment of the effect of low-energy diode laser irradiation on gamma irradiated rats’ mandibles. Arch Oral Biol 2013;58:796-805.

25. Pejicic A, Mirkovic D. Anti-inflammatory effect of low level laser treatment on chronic periodontitis. Med Laser Appl 2011;26:27-34.

26. Celebi F, Turk T, Bicakci AA. Effects of low-level laser therapy and mechanical vibration on orthodontic pain caused by initial archwire. Am J Orthod Dentofacial Orthop 2015;148:608-17.

27. Dalvi SA, Hanna R, Gattani DR. Utilisation of antimicrobial photodynamic therapy as an adjunctive tool for open flap debridement in the management of chronic periodontitis: a randomised controlled clinical trial. Photodiagnostics and Photodyn Ther 2019;25:440-7.

28. Kim KA, Choi EK, Ohe JY, Ahn HW, Kim SJ. Effect of low-level laser therapy on orthodontic tooth movement into bone-grafted alveolar defects. Am J Orthod Dentofacial Orthop 2015;148:608-17.

29. Milligan M, Arrudchelvan Y, Gung SG. Effects of two wattages of low-level laser therapy on orthodontic tooth movement. Arch Oral Biol 2017;80:62-8.

30. de Almeida AL, Medeiros IL, Cunha MJ, Sbrana MC, de Oliveira PG, Esper LA. The effect of low-level laser on bone healing in critical size defects treated with or without autogenous bone graft: an experimental study in rat calvaria. Clin Oral Implants Res 2014;25:1131-6.

31. Garcia VG, Sahyon AS, Longo M, Fernandes LA, Gualberto Junior EC, Novaes VC, 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.

32. Bosco AF, Faleiros PL, Carmona LR, Garcia VG, Theodoro LH, de Araujo NJ, et al. Effects of low-level laser therapy on bone healing of critical-size defects treated with bovine bone graft. J Photochem Photobiol B 2016;163:303-10.
thermal therapy against tumors. Nanomedicine 2018;14:811-22.
47. Zhang W, Gu J, Li K, Zhao J, Ma H, Wu C, et al. A hydrogenated black TiO$_2$ coating with excellent effects for photothermal therapy of bone tumor and bone regeneration. Mater Eng C Mater Biol Appl 2019;102:458-70.
48. Wang X, Shao J, Abd El Raouf M, Xie H, Huang H, Wang H, et al. Near-infrared light-triggered drug delivery system based on black phosphorus for in vivo bone regeneration. Biomaterials 2018;179:164-74.
49. Tong L, Liao Q, Zhao Y, Huang H, Gao A, Zhang W, et al. Near-infrared light control of bone regeneration with biodegradable photothermal osteoimplant. Biomaterials 2019;193:1-11.
50. Fallahnezhad S, Amini A, Hajihossainlou B, Chien S, Dadras S, Rezaei F, et al. Combined effects of photobiomodulation and alendronate on viability of osteoporotic bone marrow-derived mesenchymal stem cells. J Photochem Photobiol B 2018;182:77-84.
51. de Miranda JR, Choi IGG, Moreira MS, Martins MD, Cortes ARG, Yoshimoto M. Histologic evaluation of early bone regeneration treated with simvastatin associated with low-level laser therapy. Int J Oral Maxillofac Implants 2019;34:658-64.
52. de Almeida JM, de Moraes RO, Gusman DJ, Faleiros PL, Nagata MJ, Garcia VG, et al. Influence of low-level laser therapy on the healing process of autogenous bone block grafts in the jaws of systemically nicotine-modified rats: a histomorphometric study. Arch Oral Biol 2017;75:21-30.
53. Torquato LC, Suárez EAC, Bernardo DV, Pinto ILR, Mantovani LO, Silva TIL, et al. Bone repair assessment of critical size defects in rats treated with mineralized bovine bone [Bio-Oss®] and photobiomodulation therapy: a histomorphometric and immunohistochemical study. Lasers Med Sci. In press 2021.
54. Magri AMP, Fernandes KR, Kido HW, Fernandes GS, Fermino SS, Gabbi-ARMelin PR, et al. Bioglass/PLGA associated to photobiomodulation: effects on the healing process in an experimental model of calvarial bone defect. J Mater Sci Mater Med 2019;30:105.
55. de Oliveira Gonçalves JB, Buchaim DV, de Souza Bueno CR, Pomini KT, Barraviera B, Júnior RSF, et al. Effects of low-level laser therapy on autogenous bone graft stabilized with a new heterologous fibrin sealant. J Photochem Photobiol B 2016;162:663-8.
56. Pomini KT, Buchaim DV, Andreo JC, Rosso MPO, Della Coletta BB, German IJS, et al. Fibrin sealant derived from human plasma as a scaffold for bone grafts associated with photobiomodulation therapy. Int J Mol Sci 2019;20:1761.
57. Kim H, Choi K, Kweon OK, Kim WH. Enhanced wound healing effect of canine adipose-derived mesenchymal stem cells with low-level laser therapy in athymic mice. J Dermatol Sci 2012;68:149-56.

How to cite this article: Padalhin AR. Brief retrospect on the use of photobiomodulation (PBM) therapy for augmented bone regeneration (ABR). Med Laser 2021;10:15-21. https://doi.org/10.25289/ML.2021.10.1.15