Chapter

Alternative Strategies for Stem Cell Osteogenic Differentiation

Carla Cristina Gomes Pinheiro and Daniela Franco Bueno

Abstract

Discovering strategies that increase the osteogenic differentiation potential of mesenchymal stem cells (MSCs) can lead to new perspectives for bone disease treatments. The possibility to associate the mesenchymal stem cells with scaffolds and to use them in bone regeneration as well as the number of studies to understand the signaling pathway of osteogenesis are increasing. Identifying osteogenic induction factors is extremely important and crucial for the success of bone regeneration. Studies have shown that proteins, such as bone morphogenetic proteins (BMPs), trichostatin A and IGF-1, can be efficiently used for osteogenic regeneration. However, the use of these proteins increases the treatment cost. Fortunately, low-level laser therapy (LLLT) may be a new alternative for adjuvant therapy to treat bone regeneration because it has biostimulatory effects on the conversion of mesenchymal stem cells into osteoblasts and on the induction of ex vivo ossification. The principle of tissue photobiomodulation with LLLT was first described in dermatology for healing wounds; however, other applications have been described, with anti-inflammatory and anti-edema effects and cellular proliferation and differentiation. Following this way, we will discuss some alternative strategies for osteogenic differentiation and suggest that the low-power lasers can be an innovative instrument for cell differentiation.

Keywords: osteogenesis, mesenchymal stem cells, low-level laser therapy, low-power laser, osteogenic differentiation

1. Introduction

Bone transplantation is one of the most common tissue transplants in the world, second only to blood transplant. There are approximately 15 million bone fractures per year worldwide and about 10% of those will experience no tissue regeneration, potentially leading to complications such as infections and pain [1]. Technological advances and increase in life expectancy of the global population have sparked interest in and use of alternative strategies in regenerative medicine.

Tissue bioengineering is an interdisciplinary field where engineering and biological science strategies are applied jointly in order to develop biological substitutes to restore, maintain, and/or increase the function of damaged tissues [2, 3].

In concern to bone tissue engineering different medical areas as well as dentistry areas have developed bone tissue engineering strategies (stem cells (SCs), biomaterials, and growth factors) to rehabilitate congenital malformations and craniofacial syndromes associated with bioengineering [3, 4]. Therefore, the main
goal of bioengineering is to overcome limitations imposed by current conventional treatments, which are based on reconstructive surgery or organ transplant. Above all, it aims at being able to produce substitutes for organs and tissues with immune tolerance, so that transplantation can be achieved without the risk of rejection by the organism [5].

Three elements are necessary for bone tissue bioengineering: osteoconduction, osteoinduction, and osteogenesis; together, these three elements form the basis for obtaining a new, functional bone tissue [6, 7]. Given the increase in regenerative medicine studies and the need to find a biological source to promote tissue formation, that is, osteogenesis, stem cells appear to be a potentially unlimited biological source [8].

Stem cells (SCs) can be defined as cells that are capable of: (1) proliferation and self-renewal and (2) answering to external stimuli and giving rise to different specialized cell lines. Consequently, they are considered important for regenerative medicine [8]. Stem cells are classified based on their source and plasticity; hence, they can be divided into three different groups: embryonic stem cells, induced pluripotent stem cells (iPSCs), and adult stem cells.

Embryonic stem cells are those derived from the inner mass of a blastocyst (4 or 5 days after the egg has been fertilized), that are capable of differentiating in the three germ layers (endoderm, ectoderm, and mesoderm). They are known as being pluripotent. However, the therapeutic use of these cells has been questioned by several studies due to teratoma formation after transplantation in animals, potential immune rejection by the host, and strong association with ethical issues [9].

An increasing number of studies have been published about induced pluripotent stem cells (iPSCs). iPSCs are somatic cells—able to differentiate into the same cell type—but genetically altered, with four genes being inserted into their genome: OCT-4, SOX2, c-Myc, and KLF4. This increases their ability to differentiate and decreases their plasticity, changing them from somatic to pluripotent cells [9].

Another type of stem cell is the multipotent stem cell, which includes adult stem cells. They have lower plasticity than pluripotent cells and, although they can differentiate into some types of cells of adult tissues, they are unable to differentiate into germ layers. Adult stem cells are found in the body and are responsible for tissue maintenance and repair [5].

The first adult SCs described in the literature were those found in bone marrow, which have been used in the treatment of several diseases affecting the hematopoietic SCs since the 1950s. Hematopoietic SCs found in bone marrow can give rise to all types of blood cells (lymphocytes, red blood cells, platelets, etc.). In addition, studies about bone marrow transplant have led to the discovery of another important cell type—larger and adherent—that support regeneration of other tissues: the mesenchymal stem cells. Since then, several studies have begun using particularly these stem cells [10, 11].

2. Mesenchymal stem cells (MSCs)

Even after birth and growth, we can still find stem cell niches in different tissues—bone marrow, adipose tissue, skeletal muscle, dental pulp, placenta and umbilical cord, and fallopian tube—usually involved in tissue maintenance and repair [12–17].

Those are known as adult mesenchymal stem cells (MSCs). Their own characteristics are preserved, that is, they remain multipotent and undifferentiated, capable of self-renewal and differentiation into multiple cell lines—under specific in vitro conditions—including osteogenic, chondrogenic, adipogenic, and myogenic lineages [18].
The first three sources are considered key differentiation lineages in determining MSCs’ multipotentiality [19]. In 1976, Friedenstein et al. isolated cells with morphological features that were described as colony-forming unit-fibroblasts (CFU-Fs). Bone marrow stromal cells were first described as bone progenitor cells present in its stromal fraction [12]. In 1991, Caplan named those stromal cells as mesenchymal stem cells with potential for cell expansion while remaining undifferentiated, the cells being a great option in cell therapy for tissue regeneration [11]. Subsequent studies have shown that these cells are able to remain undifferentiated when cultured for prolonged periods of time. Moreover, they have the ability to differentiate into mesodermal cell lineages, including chondrocytes, osteoblasts, adipocytes, and myoblasts [5].

Currently, the definition of MSCs includes several morphological and immunophenotypic factors as well as functional features. According to the International Society for Cellular Therapy (ISCT), MSCs: (i) are plastic-adherent when maintained in in vitro conditions; (ii) show positive expression of the CD13, CD29, CD44, CD54, CD73, CD90, CD105, CD166, and Stro-1 cell surface markers and negative expression of the CD14, CD19, CD34, CD45, and HLA-DR markers; and (iii) are a group of clonogenic cells, capable of differentiating into several mesodermal cell lineages [19].

A range of studies have shown that multipotent MSCs can also differentiate into unrelated germline cells in a process known as transdifferentiation. Thus, in addition to differentiating into mesodermal cells—such as bone, fat, and cartilage—MSCs also have the potential for endodermal and neuroectodermal differentiation [20]. Even though adult MSCs are generally considered to originate from mesoderm, some authors describe embryonic MSCs derived from neuroepithelium and the neural crest, such as MSCs from deciduous dental pulp [20, 21].

Adult MSCs can be isolated from several tissues, with similar membrane receptor functions and expressions. However, none of those membrane receptors is considered a MSC-specific cell surface marker; rather, MSCs show a profile of cell surface markers, with positive and negative expression, varying according to source and cell heterogeneity [22, 23].

Furthermore, important features of MSCs for clinical use are their non-immunogenicity, as described in the literature, and immunomodulatory properties, which can be observed from two different perspectives, namely: (i) immunosuppressive effects of allogeneic MSCs, inducing immune tolerance; and (ii) effect of inflammatory cytokines in MSCs’ activity and differentiation, in cell-to-cell interactions [8, 24–27].

Bone marrow is considered one of the main sources of MSCs, both in experimental studies and clinical use [26]. Yet, bone marrow MSCs are obtained through a painful surgical incision that produces a low number of harvested cells [28], with only about 0.001–0.01% of the total population of nucleated cells being identified as MSCs [5, 29].

Therefore, due to the aforementioned difficulties, alternative sources of MSCs—such as lipospritated adipose tissue, dental pulp, umbilical cord tissue, and skeletal muscle among others—have been studied, as they are often discarded and can be easily procured and manipulated in order to obtain MSCs [16, 22, 30, 31]. Cells obtained from sources other than the bone marrow contribute greatly to the development of cell therapies and consequently to the choice of the best cellular source for clinical uses and better response to target tissue regeneration [6, 16, 17, 25].

The possibility to use a non-invasive source of MSCs in bone tissue engineering has been increased by researches, because of the ease of obtaining the tissue, since they are discarded and do not involve ethical controversy. Since the year 2000, described by Gronthos, mesenchymal stem cells derived from dental pulp (DPSCs) have been studied by other researchers, and the use of DPSCs in vitro and in vivo
has generated a great expectation for the translational use in tissue bioengineering, especially for bone neoformation [8, 30–32]. The profile of DPSCs when compared to stem cells derived from human adipose tissue (hASCs), the DPSCs present an increase in the extracellular matrix formation capacity and presented expression profile for osteogenic genes (RUNX2, BGLAP and ALP) [33]. These comparative results between alternative sources for translational use may help us find the best source of stem cells for each type of tissue to being repaired.

Recently re-emerged as an attractive source of osteogenic progenitor cells (OCPs), the periosteum can be isolated from several locations in the body, such as the anterior tibia, and the spinous process [34]. Periosteal OCPs were involved in bone repair and may also differentiate in response to paracrine signals from mechanically stimulated osteocytes. However, the interconnection of load stimulation with the molecular mechanisms is still unclear. On the other hand, another group of researchers recently described the presence of an immature cell with clonal multipotency and self-renewal characteristics in the long bones and calvarium of mice denominated with periosteal stem cells (PSCs) that are also involved in the support of the bone tissue repair [35]. With the advancement of technology, a new cellular and molecular markers can be innovative therapeutic target to open the best possibilities for promising therapies.

3. Strategies for osteogenic differentiation

A basic premise for a cell to be characterized as MSC is its ability to differentiate into a range of mesenchymal tissues—as mentioned above. Thus, stimulus for osteogenic differentiation must be efficient, resulting in viable and functional cells that produce bone extracellular matrix. This functionality is highly important for cellular characterization and applications in regenerative medicine [36].

In accordance with the basic requirements for carrying out tissue bioengineering, selection and strategy of signs of differentiation (osteoinduction) are other key aspects that should be explored. These are external inducers that promote cell proliferation and differentiation to regenerate the new tissue [36–38].

The biomaterial is not only involved as a structural support but can also be used as an inducer of osteogenic factors depending on its composition. The biomaterial classes most cited in the literature are the active ceramics, biodegradable polymers, and biodegradable metals. The mechanisms of the interaction between the cell and the biomaterial as well as of the osteogenic stimuli have not been clarified yet [39].

Another growing trend in bioengineering is the use of three-dimensional (3D) culture system, this possibility of cell culture is innovative and being explored by researchers, one of the factors that draws attention to this technique is the release of bioincomparable or non-absorbable compounds and the possible customization of the area to be regenerated [40].

Osteogenic induction and differentiation are often achieved via growth factors, which—through molecular mechanisms involving pathways, such as Wnt, BMP, FGF, and PTH, and genes that are essential for osteogenesis [41], such as RUNX 2, COL, ALP, OCN, OP, BGLAP, and SSP1—play a key role in osteogenesis and osteogenic differentiation, as shown in Figure 1 [42–44]. In this context, identifying those factors is crucial for successful tissue regeneration.

3.1 Bone morphogenetic protein (BMP)

Bone morphogenetic proteins (BMPs) are cytokines from the beta family and are used in clinical applications to stimulate bone regeneration [45]. These proteins are involved in the development of the embryo and in skeletal formation.
Manochantr et al. showed that after in vitro stimulation of bone marrow-derived MSCs with 100 ng/ml BMP-2, there was upregulation of the level of expression of genes associated with osteogenic differentiation (RUNX2 and OCN) and increase in alkaline phosphatase (ALP) production [46].

During a regular bone remodeling process, typical of an organism maintaining physiological stability, both BMPs and their antagonists are needed since BMPs induce osteo-precursor cells to proliferate and differentiate, thereby leading to formation of bone tissue. Members of the BMP family have different functions and are primarily related to the formation of bone and cartilage [47].

Upon BMP-receptor activation, receptor-regulated SMADs (R-SMADs) are translocated to the nucleus, where they regulate gene transcription by binding to DNA and interacting with DNA-binding proteins. Additionally, SMADs interact with transcription factors, transcriptional coactivators, and corepressors. The transcription factor associated with Run-Runx is one of the most studied transcription factors for BMP signaling, responsible for regulating processes such as bone formation and hematopoiesis [46, 47].

Runx2 transcription factors cooperatively regulate gene transcription that lead to differentiation of mesenchymal progenitor cells into osteoblasts [48]. Hence, it is widely regarded as a marker for cells committed to the osteochondral lineage and osteoblast differentiation. Runx2 expression is low in mesenchymal cells and is induced by BMP signaling [49].
Osteogenesis and Bone Regeneration

Osterix (OSX) is another example of a transcription factor mediated by BMP/SMAD signaling and likely by MAPK signaling and other pathways [50]. Taken together, Runx2 and Osterix are the most studied transcription factors for BMP signaling involved in the differentiation of MSCs into osteoblasts. Moreover, recombinant BMP-2 (rhBMP-2) has been used for bone induction in humans being treated for long bone fractures and spinal arthrodesis [45]. A clinical study showed improved bone density and quantity formed when compared to the gold standard surgery (anterior iliac crest bone graft), used in maxilla reconstruction in cleft lip and palate patients.

3.2 Insulin-like growth factor type I

Insulin-like growth factor type I (IGF-1) is yet another factor currently being studied as an osteoinducer. IGF-1 is the most abundant growth factor found in the bone matrix and it plays an important role in development and maintenance of skeletal tissue [51]. It has been shown, under in vitro conditions, that IGF-1 is a stimulant for osteogenic differentiation through the increase in expression of ALP, Runx2, and OCN genes in MSCs from molar dental pulp [51].

Previous studies have demonstrated that the stimulant effect of IGF-1 on bone matrix synthesis in cell cultures derived from rat calvaria is a result of at least two distinct regulatory signals: first, the effect on cellular differentiation—osteoprogenitor cells and pre-osteoblasts—in osteoblasts (increased production of bone collagen); and second, the stimulation of osteoprogenitor cells’ proliferation, thereby resulting in an increase in the number of functional osteoblasts. Despite working together to increase production of extracellular matrix, those signals differ in origin and can act synergistically with other factors, such as, for example, BMP-9 [37] and OSX, to promote osteogenic differentiation [50].

Insulin-like growth factors are known for mediating skeletal growth and bone formation [37, 52, 53]. Different studies have shown that IGF-1, in particular, promotes differentiation of bone cells in autocrine and paracrine pathways [52, 53]. Previous in vitro and in vivo studies have used IGF-1 to promote osteogenesis while treating dental pulp-derived osteoblastic cells [53, 54] and in an aged rat model, respectively. On the other hand, studies using rat fracture models show that the use of IGF-1 or PDGF alone does not stimulate OCN expression [55]. Nevertheless, using IGF-1 along with MSCs can cause expression of both factors to increase, as well as a significant upregulation of OCN by ODM in comparison to ODM alone.

The use of those factors for cell proliferation and differentiation is still being tested and is correlated with high treatment costs. On the other hand, low-level laser therapy (LLLT) could be a new alternative adjunct therapy for bone regeneration.

3.3 Low-level laser therapy

In the last 30 years, low-level laser therapy (LLLT) has been used mainly for the treatment of wounds; however, its applicability in pathological conditions such as tissue regeneration, pain relief, and inflammation has increased in different branches of regenerative medicine and dentistry [56, 57].

LLLT consists of exposing cells or tissues to low-level red and infrared lasers at wavelengths of 600–1100 nm and energy output of 1–500 mW and is called “low-level” due to its use of low-density light when compared to other forms of laser therapy. This type of irradiation may be a continuous or pulsed wave comprised of a constant, low-density energy beam (0.04–50 J/cm²). The laser is directed at the target tissue or a monolayer of cells, with power in milliwatts (mW) [36, 58].
LLLT transmits energy at low levels; hence, there is no heat or sound emission nor vibrations. There are no thermal reactions because there is no immediate increase in temperature in the tissue being irradiated by laser. Experiments after low-level laser have shown negligible, immediate heat increase in tissue (±1°C) [36, 59].

Studies with LLLT have proven effective in biostimulation, increasing the rate of cell proliferation, migration, and adhesion. Several different lasers with varying sources of light—including helium-neon (HeNe), ruby, and gallium-aluminum-arsenide (GaAlAs)—have been used in a range of LLLT treatments and protocols [36, 60–63].

As mentioned above, LLLT can promote a range of biological processes, including cell proliferation [59, 64, 65] and differentiation [36, 66]. The effects of LLLT on cell proliferation have been studied in vitro in several types of cells, namely: fibroblasts, endothelium, keratinocytes, myoblasts, and mesenchymal stem cells, among others [36, 66–71]. Nevertheless, the molecular mechanism associated with the stimulatory effects remains unclear.

One possible theory is the ability of LLLT to influence photoreceptors in cells. This mechanism is called photobiology or biostimulation. It has been stated that biostimulation occurs through the electron transport chain in mitochondrial enzymes, inducing high levels of cell respiration by endogenous porphyrin or cytochrome c during tissue stress (lesioned) [62], which increases cell metabolism and function [66]. The response to LLLT’s biostimulation effects is an increase in microcirculation, leading to higher ATP production and subsequent increase in DNA and RNA synthesis, thereby improving cellular oxygenation, nutrition, and regeneration [59, 65].

Similar to any drug treatment, LLLT has its own “active ingredient,” that is, its irradiation parameters, such as wavelength, power, power density, and energy density. Regarding interaction of the laser with matter, the effects of LLLT have been described by Karu [72] as: primary, acting as modulators of cell function, and secondary, relieving pain or inducing healing. Indeed, those effects depend on appropriate irradiation parameters [72].

Several mechanisms that aim at explaining the mitogenic effects of low-level laser therapy have been proposed, including: light absorption by mitochondrial enzymes; photon absorption by flavins and cytochromes in the mitochondrial respiratory chain, affecting electron transfer; singlet oxygen production through photoexcitation of endogenous porphyrins; and photoactivation of calcium channels, resulting in higher intracellular calcium concentrations and cell proliferation [73, 74].

Furthermore, laser therapy alters cell membrane permeability, causing subsequent physiological changes in the target cells. The magnitude of the biostimulation effect will depend on the wavelength used as well as the physiology of cell at the time [69].

It has been suggested that porphyrins and cytochromes, which are part of the mitochondrial respiratory chain, are the first photoreceptors in the visible wavelength range. When energy (photons) is absorbed by the photoreceptors’ cell membrane, a cascade of cellular response occurs, provoking production of reactive oxygen species (ROS), ATP synthesis, changes in cell membrane permeability, and release of nitric oxide. Those effects in turn lead to an increase in cell proliferation; changes in extracellular matrix synthesis; and local effects in components of the immune, vascular, and nervous system. Besides, intracellular pH levels are altered—a change associated with activation of ATPase. Changes in oxidation-reduction status cause higher levels of intracellular Ca2 and stimulate cell metabolism. High levels of intracellular Ca2 promote several biological processes, such as RNA and DNA synthesis, cell mitosis, and secretion of proteins. It
has been observed that Ca uptake by mammal cells can be induced by monochromatic red light (laser), depending on the dosage applied. Most cellular responses to LLLT derive from changes in mitochondrial and membrane activity, including mitochondrial membrane potential, as shown in Figure 2. Despite the positive results that argue for this type of treatment, the underlying action mechanism is yet to be understood [75].

In addition, studies show that ATP can activate P13K signaling pathway (phosphoinositide 3-kinase) through the ERK1/ERK2 genes, a pathway that regulates proliferation of certain types of cells [76]. Studies have also shown that LLLT promotes wound healing, collagen synthesis, nerve regeneration, bone remodeling and repair, and pain relief [57, 59, 77–80].

There are several studies in the literature that state the relationship between osteogenic differentiation, mesenchymal stem cells and LLLT, showing stimulation of matrix production, DNA synthesis, and formation of bone nodules in cultures of osteoblast-lineage cells after LLLT [36, 81, 82]. In 2005, Abramovitch-Gottlieb and colleagues used bone marrow MSCs cultured in 3D coralline (Porites lutea) biomaterial and He-Ne red laser irradiation (wavelength of 632.8 nm) to promote osteogenic differentiation [66]. Samples of biomaterial containing irradiated bone marrow MSCs showed an increase in neoformed bone tissue when compared to

---

**Figure 2.**
The cellular effect of low-level laser therapy (LLLT) on cellular metabolism. LLLT is proposed to act via mitochondria (cytochrome c oxidase) displacing nitric oxide (NO) from the respiratory chain and increasing levels of adenosine triphosphate (ATP) and reactive oxygen species (ROS). These changes act via intermediaries cyclic adenosine monophosphate (cAMP)-activated transcription factors AP-1. The interaction of the ROS and IκB further transcription factor NF-κB. The LLLT can be photoactive of calcium channels, resulting in higher intracellular calcium concentrations. All stimuli resulting in changes in gene expression and subsequent downstream production of chemical messengers implicated in the cellular changes increase cell proliferation, cell differentiation, cell motility, and growth factors production.
non-irradiated samples. This suggests that tissue bioengineering (biomaterial containing mesenchymal stem cell) together with LLLT have biostimulation effects on osteogenic induction.

Osteogenic differentiation in MSCs has also been reinforced by another study using red laser at 647 nm. MSCs were irradiated with LLLT at differing periods of time and energy levels. Non-irradiated cells (control) were kept under the same conditions as irradiated cells. Samples of cells receiving LLLT showed a significant increase in production of extracellular matrix after 4–5 days compared to non-irradiated cells, indicating that red laser promotes osteoblast differentiation. This increase in extracellular production was maintained with daily irradiation (5, 10, and 20 J) for 21 days, which corresponds to the period of differentiation and maturation of MSCs in osteoblasts [36].

Moreover, in a study using a blue laser, MSCs were irradiated (wavelength of 405 nm) for 180 s through a fiber connected to the bottom of the culture plate. The results showed that irradiation with blue laser can promote extracellular calcification produced by MSCs differentiated into osteoblasts, in addition to inducing translocation of CRY1 protein (cryptochrome 1) from the cytoplasm to the nucleus. CRY1 is a regulator for circadian rhythm and extracellular calcification in MSCs [70]. Based on hypotheses described in previous studies, LLLT can act as adjunct treatment in tissue bioengineering, representing a new strategy in bone rehabilitation.

4. Final considerations

The creation of biobanks of mesenchymal stem cells due to the possibility of isolating and manipulating MSCs from a range of tissues as well as storing them in ultralow temperatures for future use as a bioengineering strategy for bone or other tissues’ rehabilitation is of great economic and scientific interest. Yet, strategies and quality management of these biocomponents must still be developed. The ability of MSCs for osteogenic differentiation has been well established in the literature; however, the analysis of the potential for differentiation between in vitro and in vivo sources of MSCs may direct their use in future therapies.

Author details

Carla Cristina Gomes Pinheiro and Daniela Franco Bueno*  
Instituto de Ensino e Pesquisa do Hospital Sírio-Libanês, São Paulo, Brazil

*Address all correspondence to: dbuenousp@gmail.com
References

[1] Salmasi S, Nayyer L, Seifalian AM, Blunn GW. Nanohydroxyapatite effect on the degradation, osteoconduction and mechanical properties of polymeric bone tissue engineered scaffolds. The Open Orthopaedics Journal [Internet]. 2016;10:900-919. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28217213 [Cited: September 24, 2018]

[2] Langer R, Vacanti J. Tissue engineering. Science. 1993 May 14;260(5110):920-926. Review. PubMed PMID: 8493529

[3] Gimbel M, Ashley RK, Sisodia M, Gabbay JS, Wasson KL, Heller J, et al. Repair of alveolar cleft defects: Reduced morbidity with bone marrow stem cells in a resorbable matrix. Journal of Craniofacial Surgery [Internet]. 2007;18(4):895-901. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17667684 [Cited: November 6, 2016]

[4] Yousefi AM, James PF, Akbarzadeh R, Subramanian A, Flavin C, Oudadesse H. Prospect of stem cells in bone tissue engineering: A review. Stem Cells International [Internet]. 2016;2016:6180487. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26880976 [Cited: November 6, 2016]

[5] Shanti RM, Li WJ, Nesti LJ, Wang X, Tuan RS. Adult mesenchymal stem cells: Biological properties, characteristics, and applications in maxillofacial surgery. Journal of Oral and Maxillofacial Surgery [Internet]. 2007;65(8):1640-1647. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0278239107004715

[6] Raposo-Amaral CE, Bueno DF, Almeida AB, Jorgetti V, Costa CC, Gouveia CH, et al. Is bone transplantation the gold standard for repair of alveolar bone defects? Journal of Tissue Engineering [Internet]. 2014;5:2041731413519352. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24551445%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3924878

[7] Dimitriou R, Jones E, McGonagle D, Giannoudis PV. Bone regeneration: Current concepts and future directions. BMC Medicine [Internet]. 2011;9:66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21627784 [Cited: November 6, 2016]

[8] de Mendonca CA, Bueno DF, Martins MT, Kerkis I, Kerkis A, Fanganiello RD, et al. Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. The Journal of Craniofacial Surgery. 2008;19(1):204-210

[9] Collas P, Cibelli JB, Stice SL, Golueke PJ, Munsie MJ, et al. Dedifferentiation of cells: New approaches. Cytotherapy. 1998;9(3):236-244

[10] Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luriá EA, et al. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. Experimental Hematology [Internet]. 1974;2(2):83-92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4455512 [Cited: November 6, 2016]

[11] Caplan AI. Mesenchymal stem cells. Journal of Orthopaedic Research [Internet]. 1991;9(5):641-650. Available from: http://doi.wiley.com/10.1002/jor.1100090504 [Cited: November 6, 2016]

[12] Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors
in normal and irradiated mouse hematopoietic organs. Experimental Hematology [Internet]. 1976;4(5):267-274. Available from: http://www.ncbi.nlm.nih.gov/pubmed/976387 [Cited: November 6, 2016] [13] Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Molecular Biology of the Cell. 2002;13:4279-4295 [14] da Silva Meirelles L, Chagastelles PC. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. Journal of Cell Science. 2006 Jun 1;119(Pt 11):2204-2213. [Epub 2006 May 9]. PubMed PMID: 16684817 [15] Jazedje T, Perin PM, Czeresnia CE, Maluf M, Halpern S, Secco M, et al. Human fallopian tube: A new source of multipotent adult mesenchymal stem cells discarded in surgical procedures. Journal of Translational Medicine [Internet]. 2009;7:46. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2714040&tool=pmcentrez&rendertype=abstract [16] Bueno DF, Kerkis I, Costa AM, Martins MT, Kobayashi GS, Zucconi E, et al. New source of muscle-derived stem cells with potential for alveolar bone reconstruction in cleft lip and/or palate patients. Tissue Engineering Part A [Internet]. 2009;15(2):427-435. Available from: http://www.liebertonline.com/doi/abs/10.1089/ten.tea.2007.0417 [Cited: November 9, 2016] [17] Zucconi E, Vieira NM, Bueno CR Jr, Secco M, Jazedje T, Costa Valadares M, et al. Preclinical studies with umbilical cord mesenchymal stromal cells in different animal models for muscular dystrophy. Journal of Biomedicine and Biotechnology [Internet]. 2011;2011:715251. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=21785565 [18] Shi S, Bartold P, Miura M, Seo B, Robey P, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthodontics & Craniofacial Research [Internet]. 2005;8(3):191-199. Available from: http://doi.wiley.com/10.1111/j.1601-6343.2005.00331.x [Cited: November 6, 2016] [19] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy Position Statement. Cytotherapy [Internet]. 2006;8(4):315-317. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16923606 [20] Kolf CM, Cho E, Tuan RS. Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: Regulation of niche, self-renewal and differentiation. Arthritis Research & Therapy. 2007;9(1):204 [21] Kerkis I, Caplan AI. Stem cells in dental pulp of deciduous teeth. Tissue Engineering, Part B. Reviews [Internet]. 2012;18(2):129-138. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22032258 [Cited: November 6, 2016] [22] Secco M, Zucconi E, Vieira NM, Fogaça LLQ, Cerqueira A, Carvalho MDF, et al. Mesenchymal stem cells from umbilical cord: Do not discard the cord! Neuromuscular Disorders. 2008;18(1):17-18 [23] Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV, et al. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs [Internet]. 2006;184(3-4):105-116. Available from: http://www.karger.com/doi/10.1159/000099617 [Cited: November 6, 2016]
[24] Collart-Dutilleul PY, Chaubron F, De Vos J, Cuisinier FJ. Allogenic banking of dental pulp stem cells for innovative therapeutics. World Journal of Stem Cells [Internet]. 2015;7(7):1010-1021. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26328017 [Cited: November 6, 2016]

[25] Fernandes TL, Shimomura K, Asperti A, Pinheiro CCG, Caetano HVA, Oliveira CRGCM, et al. Development of a novel large animal model to evaluate human dental pulp stem cells for articular cartilage treatment. Stem Cell Reviews 2018 Oct;14(5):734-743. DOI: 10.1007/s12015-018-9820-2. PubMed PMID: 29728886; PubMed Central PMCID: PMC6132738

[26] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science [Internet]. 1999;284(5411):143-147. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10102814 [Cited: November 6, 2016]

[27] Leyendecker A, CCG P, Amano MT, Bueno DF. The use of human mesenchymal stem cells as therapeutic agents for the in vivo treatment of immune-related diseases: A systematic review. Frontiers in Immunology. 2018;9(SEP):1-50

[28] Hibi H, Yamada Y, Ueda M, Endo Y. Alveolar cleft osteoplasty using tissue-engineered osteogenic material. International Journal of Oral and Maxillofacial Surgery. 2006;35(6):551-555

[29] Fanganiello RD, FAA I, Kobayashi GS, Alvizzi L, Sunaga DY, Passos-Bueno MR. Increased in vitro osteopotential in SHED associated with higher IGF2 expression when compared with hASCs. Stem Cell Reviews and Reports [Internet]. 2015;11(4):635-644. Available from: http://link.springer.com/10.1007/s12015-015-9592-x [Cited: July 5, 2018]

[30] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2000;97(25):13625-13630. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.240309797 [Cited: August 30, 2018]

[31] Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: Stem cells from human exfoliated deciduous teeth. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2003;100(10):5807-5812. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=156282&tool=pmcentrez&rendertype=abstract

[32] Leyendecker A Junior, Gomes Pinheiro CC, Lazzaretti Fernandes T, Franco Bueno D. The use of human dental pulp stem cells for in vivo bone tissue engineering: A systematic review. Journal of Tissue Engineering [Internet]. 2018;9:2041731417752766. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29375756 [Cited: September 24, 2018]

[33] Fanganiello RD, Ishiy FAA, Kobayashi GS, Alvizzi L, Sunaga DY, Passos-Bueno MR. Increased in vitro osteopotential in SHED associated with higher IGF2 expression when compared with hASCs. Stem Cell Reviews and Reports. 2015;11(4):635-644

[34] Moore ER, Zhu YX, Ryu HS, Jacobs CR. Periosteal progenitors contribute to load-induced bone formation in adult mice and require primary cilia to sense mechanical stimulation. Stem Cell Research & Therapy. 2018 Jul 11;9(1):190. DOI: 10.1186/s13287-018-0930-1. Erratum in: Stem Cell Res Ther. 2018 Aug 28;9(1):229. PubMed PMID: 29996901; PubMed Central PMCID: PMC6042447
[35] Debnath S, Yallowitz AR, McCormick J, Lalani S, Zhang T, Xu R, et al. Discovery of a periosteal stem cell mediating intramembranous bone formation. Nature [Internet]. 2018;562(7725):133-139. Available from: http://www.nature.com/articles/s41586-018-0554-8

[36] Pinheiro CCG, de Pinho MC, Aranha AC, Fregnani E, Bueno DF. Low power laser therapy: A strategy to promote the osteogenic differentiation of deciduous dental pulp stem cells from cleft lip and palate patients. Tissue Engineering Part A [Internet]. 2018;24(7-8):569-575. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28699387 [Cited: August 13, 2018]

[37] Chen L, Zou X, Zhang RX, Pi CJ, Wu N, Yin LJ, et al. IGF1 potentiates BMP9-induced osteogenic differentiation in mesenchymal stem cells through the enhancement of BMP/Smad signaling. BMB Reports. 2016;49(2):122-127

[38] Alonso N, Risso GH, Denadai R, Raposo-Amaral CE. Effect of maxillary alveolar reconstruction on nasal symmetry of cleft lip and palate patients: A study comparing iliac crest bone graft and recombinant human bone morphogenetic protein-2. Journal of Plastic, Reconstructive & Aesthetic Surgery. 2014 Sep;67(9):1201-1208. DOI: 10.1016/j.bjps.2014.05.014. Epub 2014 May 20. PubMed PMID: 24909628

[39] Gao C, Peng S, Feng P, Shuai C. Bone biomaterials and interactions with stem cells. Bone research. 2017 Dec 21;5:17059. DOI: 10.1038/ boneres.2017.59. eCollection 2017. Review. PubMed PMID: 29285402; PubMed Central PMCID: PMCPMC5738879. Available from: www.nature.com/boneres [Cited: October 29, 2018]

[40] Paino F, La Noce M, Giuliani A, De Rosa A, Mazzoni S, Laino L, et al. Human DPSCs fabricate vascularized woven bone tissue: A new tool in bone tissue engineering. Clinical Science [Internet]. 2017;131:699-713. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5383003/pdf/cs1310699.pdf [Cited: October 19, 2018]

[41] Bessa PC, Casal M, Reis RL. Bone morphogenetic proteins in tissue engineering: the road from the laboratory to the clinic, part I (basic concepts). Journal of Tissue Engineering and Regenerative Medicine [Internet]. 2008;2(1):1-13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18293427 [Cited: August 30, 2018]

[42] Viti F, Landini M, Mezzelani A, Petecchia L, Milanesi L, Scaglione S. Osteogenic differentiation of MSC through calcium signaling activation: Transcriptomics and functional analysis. PLoS One. 2016;11(2):1-21

[43] Cho YD, Yoon WJ, Kim WJ, Woo KM, Baek JH, Lee G, et al. Epigenetic modifications and canonical wingless/int-1 class (WNT) signaling enable trans-differentiation of nonosteogenic cells into osteoblasts. The Journal of Biological Chemistry. 2014;289(29):20120-20128

[44] Chen Q, Liu W, Sinha KM, Yasuda H, de Crombrugghe B. Identification and characterization of microRNAs controlled by the osteoblast-specific transcription factor osterix. PLoS One. 2013;8(3):e58104. DOI: 10.1371/journal.pone.0058104. [Epub 2013 Mar 5]. PubMed PMID: 23472141; PubMed Central PMCID: PMC3589352

[45] Canan LW, da Silva FR, Alonso N, Tanikawa DYS, Rocha DL, Coelho JCU. Human bone morphogenetic protein-2 use for maxillary reconstruction in cleft lip and palate patients. Journal of Craniofacial Surgery [Internet]. 2012;23(6):1627-1633. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23147291
[46] Manochantr S, Marupanthorn K, Tantrawatpan C, Kheolamai P, Tantikanlayaporn D, Sanguanjit P. The effects of BMP-2, miR-31, miR-106a, and miR-148a on osteogenic differentiation of MSCs derived from amnion in comparison with MSCs derived from the bone marrow. Stem Cells International. 2017;2017

[47] James AW. Review of signaling pathways governing MSC osteogenic and adipogenic differentiation. Scientifica (Cairo) [Internet]. 2013;2013:684736. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3874981&tool=pmcentrez&rendertype=abstract

[48] Zhang Y-W, Yasui N, Ito K, Huang G, Fujii M, Hanai J-I, et al. A RUNX2/PEBP2αA/CBFA1 mutation displaying impaired transactivation and Smad interaction in cleidocranial dysplasia. Proceedings of the National Academy of Sciences of the USA. 2000 Sep 12;97(19):10549-10554. Published online: 2000 Aug 29. DOI: 10.1073/pnas.180309597. PMCID: PMC27062

[49] Maeda S, Hayashi M, Komiya S, Imamura T, Miyazono K. Endogenous TGF-b signaling suppresses maturation of osteoblastic mesenchymal cells. The EMBO Journal [Internet]. 2004;23:552-563. Available from: www.embojournal.org [Cited: August 13, 2018]

[50] Celil AB, Hollinger JO, Campbell PG. Osx transcriptional regulation is mediated by additional pathways to BMP2/Smad signaling. Journal of Cellular Biochemistry [Internet]. 2005;95(3):518-528. Available from: http://doi.wiley.com/10.1002/jcb.20429 [Cited: August 13, 2018]

[51] Alkharobi H, Al hodhodi A, Hawsawi Y, Alka faji H, Devine D, El-Gendy R, et al. IGFBP-2 and -3 co-ordinately regulate IGF1 induced matrix mineralisation of differentiating human dental pulp cells. Stem Cell Research. Nov 2016;17(3):517-522. DOI: 10.1016/j.scr.2016.09.026

[52] Wang S, Mu J, Fan Z, Yu Y, Yan M, Lei G, et al. Insulin-like growth factor 1 can promote the osteogenic differentiation and osteogenesis of stem cells from apical papilla. Stem Cell Research [Internet]. 2012;8(3):346-356. Available from: http://dx.doi.org/10.1016/j.scr.2011.12.005

[53] Feng X, Huang D, Lu X, Feng G, Xing J, Lu J, et al. Insulin-like growth factor 1 can promote proliferation and osteogenic differentiation of human dental pulp stem cells via mTOR pathway. Development, Growth & Differentiation [Internet]. 2014;56(9):615-624. Available from: http://doi.wiley.com/10.1111/dgd.12179

[54] Siddals KW, Allen J, Sinha S, Canfield AE, Kalra PA, Martin Gibson J. Apposite insulin-like growth factor (IGF) receptor glycosylation is critical to the maintenance of vascular smooth muscle phenotype in the presence of factors promoting osteogenic differentiation and mineralization. The Journal of Biological Chemistry. 2011;286(19):16623-16630

[55] Commons GW, Longaker MT. Regulation of human adipose-derived stromal cell osteogenic differentiation by insulin-like growth factor-1 and platelet-derived growth factor-alpha. 2011;126(1):41-52

[56] Conlan MJ, Rapley JW, Cobb CW. Biostimulation of wound healing by low-energy laser irradiation. Journal of Clinical Periodontology [Internet]. 1996;23:492-196. ISSN: 0303-6979. Available from: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1600-051X.1996.tb00580.x [Cited: July 23, 2018]

[57] Nanami T, Shiba H, Ikeuchi S, Nagai T, Asanami S, Shibata T. Clinical applications and basic studies of laser in dentistry and
oral surgery. The Keio Journal of Medicine [Internet]. 1993;42(4):199-201. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8126980 [Cited: November 6, 2016]

[58] Stein A, Benayahu D, Maltz L, Oron U. Low-level laser irradiation promotes proliferation and differentiation of human osteoblasts in vitro. Photomedicine and Laser Surgery [Internet]. 2005;23(2):161-166. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15910179 [Cited: November 6, 2016]

[59] Conlan MJ, Rapley JW, Cobb CM. Biostimulation of wound healing by low-energy laser irradiation. A review. Journal of Clinical Periodontology [Internet]. 1996;23(5):492-496. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8783057 [Cited: November 6, 2016]

[60] Tajali SB, Macdermid JC, Houghton P, Grewal R. Effects of low power laser irradiation on bone healing in animals: A meta-analysis. Journal of Orthopaedic Surgery and Research. 4 Jan 2010;5:1. DOI: 10.1186/1749-799X-5-1. PubMed PMID: 20047683; PubMed Central PMCID: PMC2829511

[61] Fekrazad R, Sadeghi Ghuchani M, Eslaminejad M, Taghiyar L, Kalhori KA, Pedram M, et al. The effects of combined low level laser therapy and mesenchymal stem cells on bone regeneration in rabbit calvarial defects. Journal of Photochemistry and Photobiology B: Biology. 2015 Oct;151:180-185. DOI: 10.1016/j.jphotobiol.2015.08.002. [Epub 2015 Aug 11]. PubMed PMID: 26298068

[62] Karu T, Pyatibrat L, Kalendo G. Irradiation with He Ne laser increases ATP level in cells cultivated in vitro. Journal of Photochemistry and Photobiology B: Biology. 1995;27(3):219-223

[63] Yu H-S, Chang K-L, Yu C-L, Chen J-W, Chen G-S. Low-energy helium-neon laser irradiation stimulates interleukin-la and interleukin-8 release from cultured human keratinocytes. Journal of Investigative Dermatology. 1996 Oct;107(4):593-596. PubMed PMID: 8823366

[64] Stein E, Koehn J, Sutter W, Wendtlandt G, Wanschitz F, Thurnher D, et al. Initial effects of low-level laser therapy on growth and differentiation of human osteoblast-like cells. Wiener Klinische Wochenschrift. 2008;120(3-4):112-117

[65] AlGhamdi KM, Kumar A, Moussa NA. Low-level laser therapy: A useful technique for enhancing the proliferation of various cultured cells. Lasers in Medical Science [Internet]. 2012;27(1):237-249. Available from: http://link.springer.com/10.1007/s10103-011-0885-2 [Cited: November 6, 2016]

[66] Abramovitch-Gottlib L, Gross T, Naveh D, Geresh S, Rosenwaks S, Bar I, et al. Low level laser irradiation stimulates osteogenic phenotype of mesenchymal stem cells seeded on a three-dimensional biomatrix. Lasers in Medical Science [Internet]. 2005;20(3-4):138-146. Available from: http://link.springer.com/10.1007/s10103-005-0355-9 [Cited: November 9, 2016]

[67] Hrnjak M, Kuljić-Kapulica N, Budisin A, Giser A. Stimulatory effect of low-power density He-Ne laser radiation on human fibroblasts in vitro. Vojnosanitetski Pregled [Internet];52(6):539-546. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8644477 [Cited: November 6, 2016]

[68] Góralczyk K, Szymańska J, Łukowicz M, Drela E, Kotzbach R, Dubiel M, et al. Effect of LLLT on endothelial cells culture. Lasers in Medical Science. 2014;30(1):273-278
[69] Yu HS, Chang KL, Yu CL, Chen JW, Chen GS. Low-energy helium-neon laser irradiation stimulates interleukin-1 alpha and interleukin-8 release from cultured human keratinocytes. Journal of Investigative Dermatology [Internet]. 1996;107(4):593-596. PubMed PMID: 8823366. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8823366 [Cited: November 6, 2016]

[70] Kushibiki T, Awazu K. Blue laser irradiation enhances extracellular calcification of primary mesenchymal stem cells. Photomedicine and Laser Surgery [Internet]. 2009;27(3):493-498. Available from: http://www.liebertonline.com/doi/abs/10.1089/pho.2008.2343 [Cited: November 6, 2016]

[71] Kushibiki T, Hirasawa T, Okawa S, Ishihara M. Low reactive level laser therapy for mesenchymal stromal cells therapies. Stem Cells International. 2015;2015

[72] Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. Journal of Photochemistry and Photobiology B: Biology [Internet]. 1999;49(1):1-17. Available from: https://www.sciencedirect.com/science/article/pii/S101113449800219X?via%3Dihub [Cited: September 24, 2018]

[73] 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. Journal of Photochemistry and Photobiology B: Biology. 2013;121:6-14

[74] Marques MM, Diniz IMA, de Cara SPHM, Pedroni ACF, Abe GL, D’Almeida-Couto RS, et al. Photobiomodulation of dental derived mesenchymal stem cells: A systematic review. Photomedicine and Laser Surgery [Internet]. 2016;34(11):500-508. Available from: http://online.liebertpub.com doi/10.1089/pho.2015.4038

[75] Zungu IL, Hawkins Evans D, Abrahams H. Mitochondrial responses of normal and injured human skin fibroblasts following low level laser irradiation—An in vitro study. Photochemistry and Photobiology [Internet]. 2009;85(4):987-996. Available from: http://doi.wiley.com/10.1111/j.1751-1097.2008.00523.x [Cited: September 24, 2018]

[76] Li C-S, Zheng Z, Su XX, Wang F, Ling M, Zou M. Activation of the extracellular signal-regulated kinase signaling is critical for human umbilical cord mesenchymal stem cell osteogenic differentiation. BioMed Research International. 2016;2016:3764372. DOI: 10.1155/2016/3764372. [Epub 2016 Feb 16]. PubMed PMID: 26989682; PubMed Central PMCID: PMC4771893

[77] Tim CR, Bossini PS, Kido HW, Malavazi I, von Zeska Kress MR, Carazzolle MF, et al. Effects of low-level laser therapy on the expression of osteogenic genes during the initial stages of bone healing in rats: A microarray analysis. Lasers in Medical Science. 2015;30(9):2325-2333

[78] Silva GBL, Saccono NT, Othon-Leite AF, Mendonça EF, Arantes AM, Bariani C, et al. Effect of low-level laser therapy on inflammatory mediator release during chemotherapy-induced oral mucositis: A randomized preliminary study. Lasers in Medical Science. 2014;30(1):117-126

[79] Anders JJ, Borke RC, Woolery SK, Van de Merwe WP. Low power laser irradiation alters the rate of regeneration of the rat facial nerve. Lasers in Surgery and Medicine [Internet]. 1993;13(1):72-82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8426530 [Cited: November 6, 2016]
[80] Huang YY, Chen AC, Carroll JD, Hamblin MR. Biphasic dose response in low level light therapy. Dose Response. 1 Sep 2009;7(4):358-383. DOI: 10.2203/dose-response.09-027. Hamblin. PubMed PMID: 20011653; PubMed Central. PMCID: PMC2790317

[81] Tim CR, KNZ P, BRO R, Fernandes K, Matsumoto MA, Parizotto NA, et al. Low-level laser therapy enhances the expression of osteogenic factors during bone repair in rats. Lasers in Medical Science [Internet]. 2014;29(1):147-156. Available from: http://link.springer.com/10.1007/s10103-013-1302-9 [Cited: July 23, 2018]

[82] Pereira LO, JPF L, Azevedo RB, Gronthos S, Mankani M, Brahim J, et al. Laser irradiation did not increase the proliferation or the differentiation of stem cells from normal and inflamed dental pulp. Archives of Oral Biology [Internet]. 2012;57(8):1079-1085. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22469390 [Cited: November 6, 2016]