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Research article

Effects of the association between hydroxyapatite and photobiomodulation on bone regeneration

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Abstract: *Background:* Hydroxyapatite (HA)-based ceramics are widely used as artificial bone substitutes due to their advantageous biological properties, which include biocompatibility, biological affinity, bioactivity, ability to drive bone formation, integration into bone tissue and induction of bone regeneration (in certain conditions). Phototherapy in bone regeneration is a therapeutic approach that involves the use of light to stimulate and accelerate the process of repair and regeneration of bone tissue. There are two common forms of phototherapy used for this purpose: Low-Level Laser Therapy (LLLT) and LED (Light Emitting Diode) Therapy. Understanding the mechanisms of laser therapy and its effects combined with hydroxyapatite has gaps. Therefore, this review was designed based on the PICO strategy (P: problem; I: intervention; C: control; O: result) to analyze the relationship between PBM therapy and hydroxyapatite. *Methods:* The bibliographic search, with the descriptors "hydroxyapatite AND low-level laser therapy" and "hydroxyapatite AND photobiomodulation" resulted in 43 articles in the PubMed/MEDLINE database, of which 1 was excluded for being a duplicate and another 33 due to inclusion/exclusion criteria, totaling 9 articles for qualitative analysis. In the Web of Science database, we obtained 40 articles, of which 7 were excluded for being duplicates, 1 for not having the full text available and another 17 due to inclusion/exclusion criteria, totaling 15 articles for qualitative analysis. *Results:* The most used biomaterial was composed of hydroxyapatite and β-tricalcium phosphate in a proportion of 70%–30%. In photobiomodulation, the gallium-aluminum-arsenide (GaAlAs) laser prevailed, with a wavelength of 780 nm, followed by 808 nm. *Conclusions:* The results indicated that the use of laser phototherapy improved the repair of bone defects grafted with the biomaterial, increasing the deposition of HA phosphate as indicated by biochemical estimators, spectroscopy and histological analyses.

Keywords: low-level laser therapy; hydroxyapatite; bone regeneration; photobiomodulation; bone repair

1. Introduction

Bone defects can be the result of pathological processes (e.g., cancer), post-trauma, post-surgery or even be of congenital origin [1]. Bone regeneration, in most cases, occurs naturally since bone is a dynamic and highly vascularized tissue [2].

However, in some cases, this regeneration does not occur, whether due to poor blood supply in the region, systemic or local pathologies, the presence of infections or also in the case of critical bone defects. In these cases, there is a need for procedures that assist in bone repair, with bone grafting being the most performed procedure, whether in Human Medicine, Veterinary Medicine or Dentistry [3].

Approximately two million procedures involving bone grafts are performed each year around the world [4]. In the United States, bone grafting is second only to blood transfusion when it comes to tissue transplantation, with around 500,000 bone grafts being performed per year [5]. The materials used in bone grafting can be classified according to their properties or according to their characteristics.

According to the properties of the materials used in bone grafting, they can be: Osteoconduction, the so-called osteoconductive materials provide maintenance of the physical framework of the particles that facilitate angiogenesis and cell penetration (interconnectivity); osteoinduction, where osteoinductive materials promote the differentiation of undifferentiated mesenchymal cells in the region into osteoblasts, in the presence of bone morphogenetic proteins (BMPs); osteogenesis, where osteogenic materials have osteogenic cells incorporated into the material (e.g., mesenchymal stem cells, osteoblasts or osteocytes); osteostimulation, where osteostimulating materials upregulate the expression of osteogenic genes or proteins by mesenchymal stem cells; and bioactivity, where bioactive materials form a bone-like mineral layer on their surface, which is intended to assist the osseointegration process [6–8].

However, according to the characteristics of the materials used in bone grafting, they can be: Origin, they are autograft (from the patient), allograft (human donor), xenograft (from a non-human donor) and synthetic (manufactured); immunogenicity refers to how the body reacts to the material, and this includes the risk of disease transmission, inflammatory responses or immunomodulation in the osseointegration process; porosity understands the size and shape of the material's pores; physical characteristics, where the grafts are formulated in liquid form, masses, granules of different sizes and in finishing materials (e.g. sponges); resorption rate, defined by the speed with which a bone graft is reabsorbed by the human body; incorporation, some grafts can be incorporated with bone marrow, blood or platelet-rich plasma; composition, they may contain cells, silicate, bioglass, proteins among others [7].

Hydroxyapatite (HA) is a calcium phosphate compound $Ca_{10}(PO_4)_6(OH)_2$, which is the main mineral component of bone tissue [9]. It is a first-generation xenograft, having been used since the 1950s. It can be obtained in two ways: The first naturally from marine coral (calcium carbonate) or bovine bone, or it can be made synthetically. Synthetic HA was first produced in the 1970s [10]. Its properties include osteoconduction and osteoinduction [11].

HA has several benefits, such as low cost, variety of formulations (from nanoparticles, granules and blocks) and good porosity. This material has high chemical stability and a slow resorption rate, which can impair bone healing and make it difficult to assess the material's osseointegration in radiological examinations [10].

With the aim of accelerating the bone regeneration process for optimized morphophysiological recovery, complementary therapies can be associated, such as low-intensity laser (LLLT). This type of laser therapy can use red or infrared light to stimulate tissues, modulating the repair process, increasing tissue vascularization, reducing pain, increasing the production of mitochondrial ATP among other biostimulatory effects [11,12]. Its non-invasive approach and the ability to accelerate bone recovery make low-level laser therapy a promising option of growing interest in regenerative medicine.

The understanding of the mechanisms of laser therapy and its effects combined with hydroxyapatite has gaps. Therefore, this review was designed based on the PICO strategy (P: problem; I: intervention; C: control; O: result) [13,14] to analyze the relationship between PBM therapy and hydroxyapatite.

2. Materials and methods

This review began by searching the PubMed/MEDLINE and Web of Science databases using the keywords: "hydroxyapatite AND low-level laser therapy" and "hydroxyapatite AND photobiomodulation".

After crossing the keywords, the titles and summaries of all results were read. From there, the manuscripts were separated into included and excluded according to the eligibility criteria. The authors carried out this process impartially and independently.

The inclusion criteria were:

• Therapeutic use of HA and LLLT as complementary therapy;
• Studies on humans;
• Animal studies;
• In vivo studies;
• Case reports;
• Publications only in English and that allowed full access to the text;
• Each article included should present data on the LLLT protocol.

- The exclusion criteria were:

• Duplicate articles;
• When the title/summary was unrelated to the objective;
• Did not use HA;
• Did not use LLLT;
• High power laser used;
• Other languages (except English);
• When access to the full text was not obtained;

• Incomplete data on the type of HA used.
• Letters to the editor;
• Review articles;
• Comments;
• Unpublished abstracts;
• Dissertations or theses from repositories.

The selected articles were read in full and with caution. To minimize study bias, two independent researchers participated in the article selection phase, ensuring that the selection and exclusion criteria were strictly followed.

The data was collected, organized into tables by the reviewers and compared afterwards. The discrepancies were resolved after a new analysis of the study in question. The selection outline, according to the PRISMA flowchart, is shown in Figure 1.

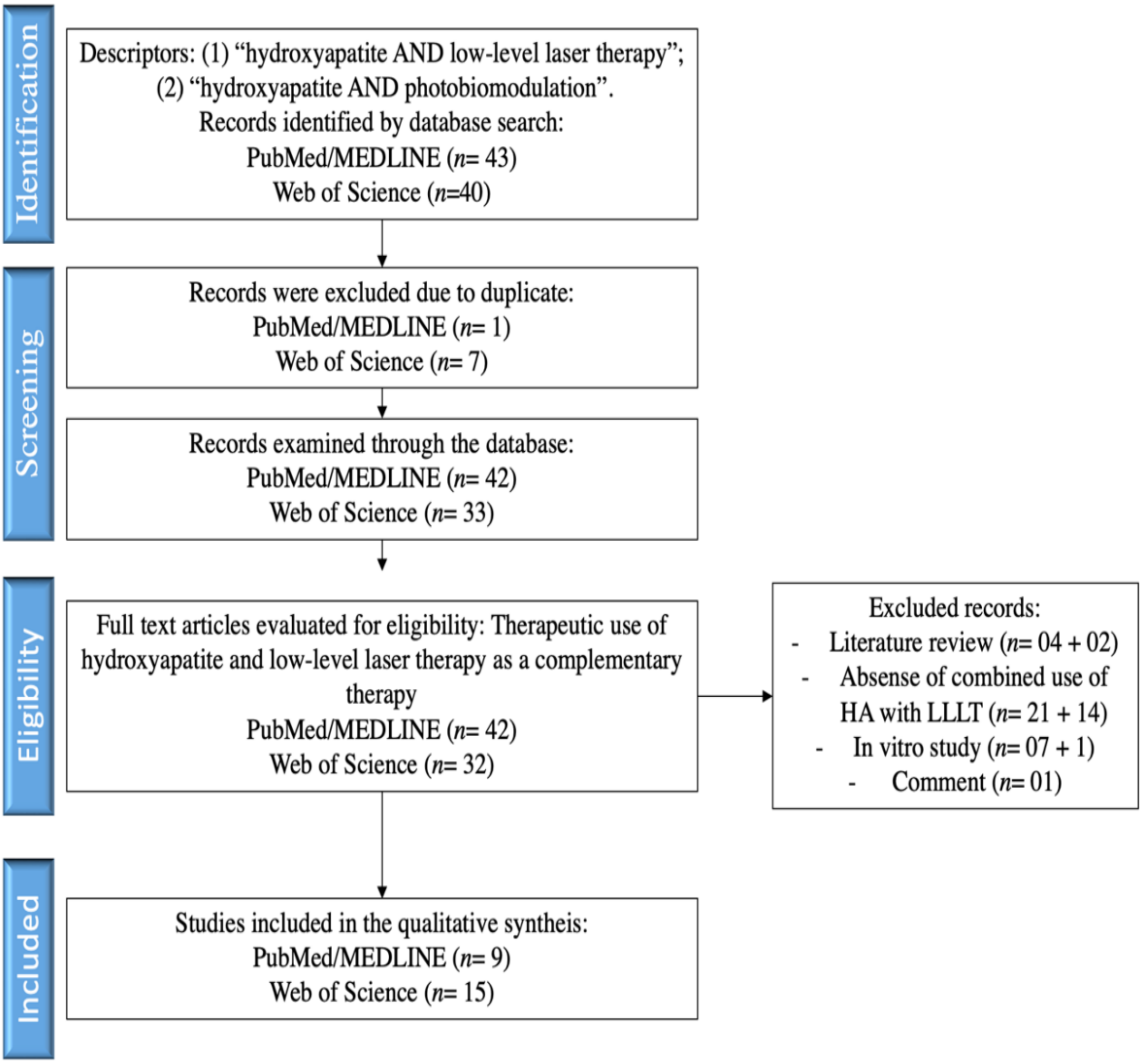

Figure 1. Flow diagram showing study selection [15].

3. Results

The bibliographic search resulted in 43 articles in the PubMed/MEDLINE database, of which 01 was excluded due to being duplicated and another 33 due to inclusion/exclusion criteria, totaling 9 articles for qualitative analysis. In the Web of Science database, we obtained 40 articles, of which 07 were excluded for being duplicates, 01 for not having the full text available and another 17 due to inclusion/exclusion criteria, totaling 15 articles for qualitative analysis. The selection of studies and the details of inclusion and exclusion of manuscripts are described in Figure 1 (flow diagram).

The analysis of the selected studies allows us to observe that, due to its physicochemical properties, hydroxyapatite is widely used in several areas, focusing mainly on regenerative medicine and dentistry. Of the 24 articles that were described in detail in table 1, the most used material was Baumer's GenPhos® HATCP, being present in 17 works. 3 studies used Bone Ceramic®, 1 Cerabone®, 1 HA SIN®, 1 Bego oss® and 1 QualyBone® (Figure 2).

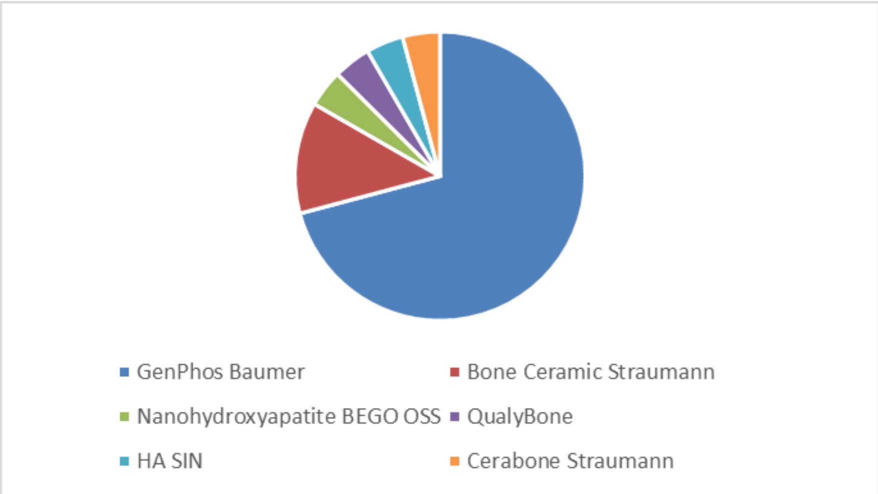

Figure 2. Graphic with the biomaterials used in the studies.

Regarding the laser, the wavelengths of the devices used varied between 780 nm and 850 nm. Three studies compared two types of laser (FisioLed® 850 nm and TwinFlex® Evolution 780nm) and concluded that both improved the repair of bone defects with no statistical difference between them. One study used the Laserpulse® equipment, 4 Thera Lase®, 1 BioWave®, 1 Therapy XT®, 1 CHEESE®, 1 LED® device, 1 Bioset®, 9 TwinFlex® and 2 FisioLed® (Figure 3).

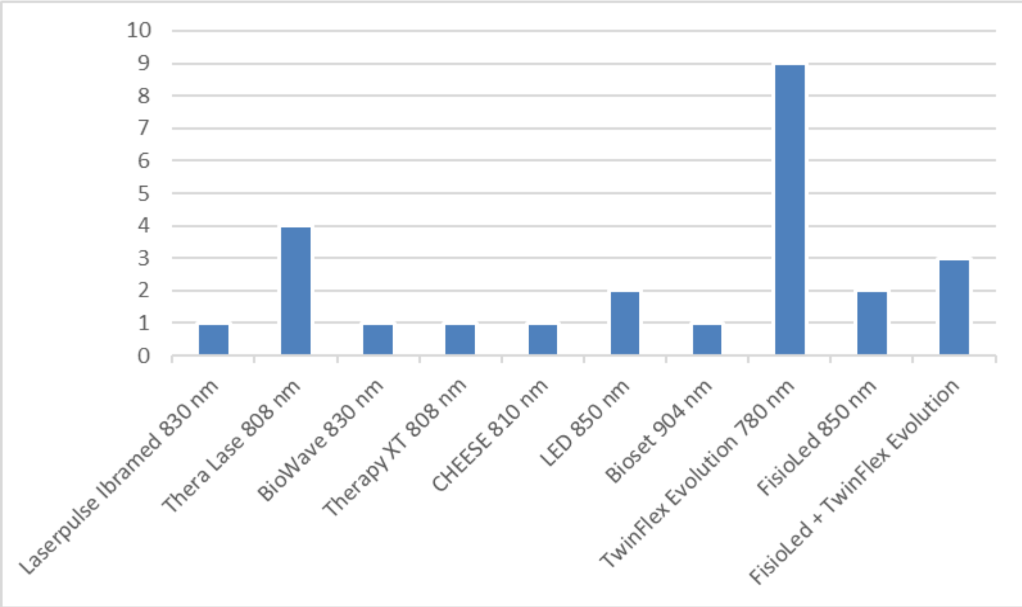

Figure 3. Chart with the laser devices used and their respective wavelengths.

Of the 24 articles examined, 18 used rats, 5 used rabbits and only 1 used human. The articles selected for this review are in Table 1.

Table 1. Studies selected according to eligibility criteria.

Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
De Carvalho et al. 2011 [16]	To evaluate, through Raman spectroscopy, the repair of bone defects or treated not with infrared laser light associated or not with the use of HATCP graft and guided bone regeneration (GBR)	TwinFlex®, MMOptics, São Carlos, São Paulo – Brazil; λ780 nm, output 50 mW, spot size 0.4 cm^2, 16 J/cm^2	Output Power: 50 mW Power Density: - Energy Density: 16 J/cm^2	Irradiated every other day for two weeks	15 rabbits (5 groups, n = 3) Euthanasia: 30 days post-surgery.	Biphasic ceramic bone (Baumer, GenPhos HATCP®) and bovine bone membrane (Baumer, GenDerm®)	It was concluded that Infrared (IR) laser light was able to accelerate fracture consolidation and the association with HATCP and GBR resulted in increased deposition of calcium hydroxyapatite.
Dos Santos Aciole et al. 2011 [17]	Evaluate histomorphometric laser PBM in bone repair of surgical fractures fixed with wire osteosynthesis (WO), whether or not treated with Biphasic Ceramic Bone Graft	TwinFlex®, MMOptics, São Carlos, São Paulo – Brazil; λ780 nm, output 50 mW, spot size 0.4 cm^2, 16 J/cm^2	Output Power: 50 mW Power Density: - Energy Density: 16 J/cm^2	Irradiated every other day for two weeks	15 rabbits (5 groups, n = 3) Euthanasia: 30 days post-surgery.	Biphasic ceramic bone (Baumer, GenPhos HATCP®) and bovine bone membrane (Baumer, GenDerm®)	It was concluded that IR laser light was able to accelerate fracture consolidation and the association with HATCP and GBR resulted in increased HA deposition

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
Soares et al. 2014 [18]	To evaluate, by optical microscopy, the repair of bone defects grafted or not with biphasic synthetic microgranular Calcium Hydroxyapatite (HA) + Beta-TCP associated or not with Laser phototherapy – LPT	TwinFlex Evolution®, MMOptics, São Carlos, São Paulo – Brazil; λ780 nm, output 70 mW, spot size 0.4 cm^2, 20 J/cm^2	Output Power: 70 mW Power Density: - Energy Density: 20 J/cm^2 session 140 J/cm^2 treatment	Irradiated every 48 hours for 2 weeks.	40 rats (4 groups, with 2 subgroups, n = 5) Euthanasia: 15 and 30 days.	Biphasic synthetic micro-granular HA + Beta-TCP (70%/30% respectively; Baumer®, São Paulo, SP, Brazil)	LPT associated with the HA + Beta TCP graft resulted in a more advanced stage of bone repair at the end of the experiment.
Soares et al. 2014 [19]	To evaluate, through optical microscopy, the qualitative description of the repair of bone defects grafted or not with biphasic synthetic microgranular HA + Beta-calcium triphosphate associated or not with Laser phototherapy (λ 780 nm)	TwinFlex Evolution®, MMOptics, São Carlos, São Paulo – Brazil; λ780 nm, output 70 mW, spot size 0.4 cm^2, 20 J/cm^2	Output Power: 70 mW Power Density: - Energy Density: 20 J/cm^2 session 140 J/cm^2 treatment	Irradiated every 48 hours for 15 days	40 rats (4 groups, with 2 subgroups, n = 5) Euthanasia: 15 and 30 days	Biphasic synthetic micro-granular HA + Beta-TCP (70%/30% respectively; Baumer®, São Paulo, SP, Brazil)	The qualitative analysis showed that the Laser + Biomaterial group was in a more advanced stage of repair at the end of the experimental period. It was concluded that Laser irradiation improved the repair of grafted or non-grafted bone defects.

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
De Castro et al. 2014 [20]	Evaluate, through the analysis of the intensity of the Raman spectrum, the incorporation of two types of HA, Hydroxyapatite phosphate, type B apatite carbonate and components in the repair of bone defects in animals with iron deficiency anemia or non-anemic.	LED (λ850 ± 10 nm, 150 mW, CW, Φ = 0.5 cm^2, 16 J/cm^2	Output Power: 150 mW Power Density: - Energy Density: 16 J/cm^2	Irradiation was performed every 48 hours for 15 days	40 rats (8 groups, n = 5) Euthanasia: 30 days.	GenPhos® Baumer HATCP (São Paulo, SP, Brazil)	Results demonstrated higher HA peaks, as well as a decrease in the level of organic components in healthy animals when associated with graft and LED phototherapy. On the other hand, the condition of anemia interfered with the incorporation of the graft into the bone, as the LED phototherapy only improved bone repair when the graft was not used.
Soares et al. 2014 [21]	Evaluate through intensity analysis of Raman spectra, the incorporation of two types of HA, Phosphate hydroxyapatite, type B carbonated apatite and organic components in the repair of bone defects grafted or not with HA associated or not with LED phototherapy.	(FisioLED®, MMOptics, São Carlos, São Paulo, Brazil; λ850 ± 10 nm, 150 mW, Φ ~ 0.5 cm^2, 20 J/cm^2	Output Power: 150 mW Power Density: - Energy Density: 20 J/cm^2 session 140 J/cm^2 treatment	Irradiation was performed every 48 hours for 15 days	40 rats (4 groups, with 2 subgroups, n = 5) Euthanasia: 15 and 30 days.	GenPhos®, Baumer, Mogi Mirim, SP, Brazil	It is concluded that the use of LED phototherapy associated with the biomaterial was effective in improving bone consolidation in bone defects due to the increasing deposition of HA measured by Raman spectroscopy.

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
Soares et al. 2014 [22]	Assess bone level mineralization through the analysis of the intensities of Raman spectra of both inorganic and organic in bone repair defects grafted or not with biphasic synthetic microgranular HA+calcium β-triphosphate associated or not with laser phototherapy	TwinFlex Evolution®, MMOptics, São Carlos, São Paulo – Brazil; λ780 nm, output 70 mW, spot size Φ 0.4 cm^2, 20 J/cm^2	Output Power: 70 mW Power Density: - Energy Density: 20 J/cm^2 session 140 J/cm^2 treatment	Irradiation was performed every 48 hours for 15 days	40 rats (4 groups, with 2 subgroups, n = 5) Euthanasia: 15 and 30 days.	BHiphasic synthetic microgranular HA+β-calcium triphosphate (70%/30%, respectively) GenPhos® Baumer, Mogi Mirim, SP, Brazil	It is concluded that the use of laser phototherapy associated with the biomaterial was effective in improving bone consolidation in bone defects due to the increasing deposition of calcium hydroxyapatite measured by Raman spectroscopy.
Pinheiro et al. 2017 [23]	Evaluate changes in the biochemistry of the repair process induced in filled bone defects with autologous blood clot or biomaterial associated or not with LED or laser phototherapy	a) LED phototherapy: λ 850 ± 10 nm; power 150 mW, irradiation area ~0.5 cm^2 (FisioLED®, MMOptics, São Carlos, São Paulo, Brazil). b) Laser diode: laser λ 780 nm, power 70 mW (TwinFlex Evolution®, MMOptics, São Carlos, São Paulo, Brazil)	Output Power: 150 mW Power Density: - Energy Density: 142.8 J/cm^2 Output Power: 70 mW Power Density: - Energy Density: 142.8 J/cm^2	Irradiation was performed every 48 hours for 15 days	60 rats (6 groups, with 2 subgroups, n = 5) Euthanasia: 15 and 30 days.	Biomaterial biphasic synthetic micro-granular HA + β-tricalcium phosphate (70% and 30%, respectively; GenPhos®, Baumer, Mogi Mirim, SP, Brazil)	The results indicated that the use of laser phototherapy improved the repair of bone defects grafted with the biomaterial, increasing HA phosphate deposition as marked by biochemical estimators.

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
Pinheiro et al. 2014 [24]	To evaluate the mineralization and remodeling of bone defects grafted or not with microgranular HA + Beta-TCP associated or not with two phototherapies (Laser and LED), by evaluating the proportions of the selected Raman peaks.	a) LED phototherapy: λ 850 ± 10 nm; power 150 mW, irradiation area ~0.5 cm^2 (FisioLED®, MMOptics, São Carlos, São Paulo, Brazil). b) Laser diode: laser λ 780 nm, power 70 mW (TwinFlex Evolution®, MMOptics, São Carlos, São Paulo, Brazil)	Output Power: 150 mW Power Density: - Energy Density: 142.8 J/cm^2 Output Power: 70 mW Power Density: - Energy Density: 142.8 J/cm^2	Irradiation was performed every 48 hours for 15 days	60 rats (6 groups with 2 subgroups, n = 5) Euthanasia: 15 and 30 days.	Biomaterial biphasic synthetic micro-granular HA + β-tricalcium phosphate (70 and 30%, respectively; GenPhos®, Baumer, Mogi Mirim, SP, Brazil)	Raman metrics of the selected protein matrix and phosphate and carbonate HA indicated that the use of the microgranular synthetic biphasic graft HA + Beta-TCP improved the repair of bone defects, whether or not associated with Laser or LED light, due to the increasing deposition of HA.
Pinheiro et al. 2014 [25]	Evaluate by optical microscopy and histomorphometry, the repair of fractures fixed with miniplates (IRF) treated or not with biphasic ceramic graft associated or not GBR and whether irradiated with laser	TwinFlex Evolution®, MMOptics, São Carlos, São Paulo – Brazil; λ780 nm, output 50 mW, spot area 0.5 cm^2, 16 J/cm^2	Output Power: 50 mW Power Density: - Energy Density: 4x4 J/cm^2 16 J/cm^2 =112 J/cm^2	Irradiation was performed every other day for 2 weeks	15 rabbits (5 groups, n = 3) Euthanasia: 30 days.	Particle ceramic graft (GenPhos® Baumer ®; Mogi Mirim, SP, Brazil) and demineralized bovine bone membrane (GenDerm®, Baumer®; Mogi Mirim, Brazil)	The results of the present study suggest that the association of hydroxyapatite and laser light resulted in positive and significant repair of complete tibial fractures treated with miniplates.

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
Pinheiro et al. 2014 [26]	Evaluate the level of bone mineralization, through the analysis of the intensities of both inorganic and organic Raman spectra, as well as through semiquantitative histological analysis of repair of bone defects grafted or not with synthetic microgranular HA associated or not with Laser phototherapy.	TwinFlex Evolution®, MMOptics, São Carlos, São Paulo – Brazil; λ 780 nm, output 70 mW, spot size 0.4 cm^2, 20 J/cm^2	Output Power: 70 mW Power Density: - Energy Density: 20 J/cm^2 session 140 J/cm^2 treatment	Irradiations were performed every 48 hours for 15 days.	40 rats (4 groups with 2 subgroups, n = 5) Euthanasia: 15 and 30 days.	Biphasic synthetic micro-granular HA + β-tricalcium phosphate (70 and 30%, respectively; GenPhos®, Baumer, Mogi Mirim, SP, Brazil)	It is concluded that the use of laser phototherapy associated with the microgranular synthetic biphasic graft of HA + β - calcium triphosphate was effective in improving bone consolidation in bone defects due to the increasing deposition of HA and the presence of mature trabecular bone.
Pinheiro et al. 2013 [27]	Evaluate using laser fluorescence and Raman spectroscopy, the repair of complete tibial fractures in rabbits treated by wire osteosynthesis associated or not with the use of graft biphasic ceramic associated or not with the use of GBR and irradiation. or not with laser in rabbits	TwinFlex®, MMOptics, São Carlos, São Paulo – Brazil; λ 780 nm, output 50 mW, spot size 0.5 cm^2, 16 J/cm^2	Output Power: 50 mW Power Density: - Energy Density: 4 × 4 J/cm^2 16 J/cm2	Irradiations were performed every other day during 2 weeks	15 rabbits (5 groups, n = 3) Euthanasia: 30 days.	Particle ceramic graft (GenPhos® Baumer®, Mogi Mirim, SP, Brazil) and demineralized bovine bone membrane (GenDerm®, Baumer®, Mogi Mirim, SP, Brazil)	It is concluded that the use of near-infrared - NIR laser phototherapy associated with HA and GBR grafting was effective in improving bone healing in fractured bones because of the increasing deposition of hydroxyapatite measured by Raman spectroscopy and the decrease in organic components shown by fluorescence reading.

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
Soares et al. 2014 [28]	Assess bone level mineralization, through the analysis of the intensities of Raman spectra of inorganic and organic components in the repair of clots and bone defects filled with biomaterials associated or not with laser or LED phototherapy	a) LED phototherapy: λ 850 ± 10 nm; power 150 mW, irradiation area ~0.5 cm^2 (FisioLED®, MMOptics, S ão Carlos, S ão Paulo, Brazil). b) Laser diode: laser λ 780 nm, power 70 mW (TwinFlex Evolution®, MMOptics, S ão Carlos, S ão Paulo, Brazil)	Output Power: 150 mW Output Power: 70 mW Energy Density: The energy density delivered for both devices was of 20 J/cm^2 , transcutaneously applied in four points of 5 J/cm^2	Irradiations were performed every 48 hours for 2 weeks.	60 rats (6 groups with 2 n5 subgroups) Euthanasia: 15 and 30 days.	Biphasic synthetic micro-granular HA + β-tricalcium phosphate (70 and 30%, respectively; GenPhos®, Baumer, Mogi Mirim, SP, Brazil)	The Raman intensities of the mineral and matrix components indicated that the use of laser and LED phototherapies improved the repair of bone defects grafted or not with biphasic synthetic microgranular HA + β- tricalcium phosphate.
Soares et al. 2013 [29]	To evaluate the level of bone mineralization, through the analysis of the intensities of the Raman spectra of HA in the repair of bone defects grafted or not with biphasic synthetic microgranular HA + ÿ - calcium triphosphate associated or not with LED phototherapy	LED phototherapy: λ 850 ± 10 nm; power 150 mW, irradiation area ~0.5 cm^2 (FisioLED®, MMOptics, S ão Carlos, SP, Brazil).	Output Power: 150 mW Power Density: - Energy Density: 20 J/cm^2 session 140 J/cm^2 treatment	Application at intervals of 48 hours for 15 days.	40 rats (4 groups with 2 subgroups, n = 5) Euthanasia: 15 and 30 days.	Microgranular HA + β - calcium triphosphate. GenPhos®, Baumer, Mogi Mirim, SP, Brazil	It is concluded that the use of LED light on the bone grafted with HA did not improve the treatment result, as the persistence of HA in the defect may have interfered with the Raman reading.

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
Pinheiro et al. 2013 [30]	Evaluate according to Raman spectroscopy, repair of fractures fixed with miniplates treated or not with a biphasic ceramic graft device associated or not with GBR and irradiated or not with 780 nm laser in animal model	TwinFlex®, MMOptics, São Carlos, São Paulo – Brazil; λ 780 nm, output 50 mW, spot size 0.5 cm^2, 16 J/cm^2	Output Power: 50mW Power Density: - Energy Density: 16 J/cm^2, 4 × 4 J/cm^2, 9 J)	Application every other day for 2 weeks.	15 rabbits (5 groups n = 3) Euthanasia: 30 days.	GenPhos® HATCP + Genderm® demineralized bovine bone membrane, Baumer® (Mogi Mirim, SP, Brazil)	Spectral analysis of the bone component showed an increase in hydroxyapatite levels in fractured sites, using the association of laser light with a ceramic graft
Reis CHB, et al. 2023 [31]	Evaluate PBM in the repair of bone defects filled with the biocomplex formed by fibrin biopolymer (FB) plus biomaterial	Gallium-aluminum-arsenide (GaAlAs) PBM; λ 830 nm Laserpulse®, Ibramed, Amparo, Brazil	Output Power: 30mW Power Density: 258.6 mW/cm^2 Energy Density: 6.2 J/cm^2	Immediately after surgery and three times a week until euthanasia.	56 male Wistar rats (4 groups, n = 7) 8 mm calvarial bone defect Euthanasia: 14 and 42 days after surgery	QualyBone BCP® (QualyLive, Amadora, Portugal) 75% hydroxyapatite and 25% tricalcium phosphate	LLLT positively interfered in the repair process of bone defects filled with the biocomplex formed by FB plus biomaterial (BCP)
Oliveira GJPL, et al. 2021 [32]	To evaluate the effect of different low-intensity laser therapy (LLLT) irradiation protocols on the osseointegration of implants placed in grafted areas.	GaAlAs Thera Lase, λ 808 nm, 100 mW, ϕ 0.60 mm, focal divergence 0.45 rad, DMC®, São Carlos, SP, Brazil	Output Power: 100 mW Power Density: - Energy Density: 354 J/cm^2	Seven sessions were performed – which were repeated every 48 hours for two weeks after the grafting procedure or implant placement.	84 male rats (6 groups n = 14) Bone defect in tibia 4 × 1.5mm Euthanasia: 15 and 45 days after implant placement surgery	Straumann® Bone Ceramic, Straumann AG, Basel, Switzerland	LLLT performed on implants placed in grafted areas enhances the osseointegration process.

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
de Oliveira GJPL, et al. 2020 [33]	To evaluate the osseointegration of implants placed in areas grafted with different osteoconductive bone substitutes irradiated with a low-intensity infrared laser (LLLT)	GaAlAs laser Thera Lase, λ 808 nm, 100 mW, ϕ 0.60 mm, focal divergence 0.45 rad, DMC®, São Carlos, Brazil	Output Power: 100 mW Power Density: - Energy Density: 354 J/cm²	07 LLLT sessions were performed, which were repeated every 48 hours for 13 days after the surgical procedure for bone defects filled with bone substitutes.	56 rats (4 groups of n = 14) Bone defect in tibia 4 × 1.5mm	HA/TCP: Bone defect filled with biphasic ceramic made of hydroxyapatite and β-tricalcium phosphate (Straumann® Bone Ceramic, Straumann AG, Basel, Switzerland)	The use of LLLT in areas grafted by bone substitutes before implant placement improves the osseointegration pattern.
Theodoro, LH, et al. 2018 [34]	Evaluate the bone formed after maxillary sinus floor augmentation (MSFA) by bone autograft combined with hydroxyapatite (HA) treated or not with a low-level laser (LLLT).	GaAIAs laser. λ 830 nm, 40 mW, ϕ 0.07 cm^2, BioWave® Kondortech Equipament Ltd., São Carlos, Brazil	Output Power: 40 mW Power Density: 0.57 W/cm² Energy Density: 5.32 J/point	Irradiation was performed continuously at 4 points around the maxillary sinus cavity (mesial, distal, superior and inferior) before graft placement and also at a central point over the graft.	12 patients (2 groups n = 6) Biopsy of the alveolar crest after 6 months of maxillary sinus lift with graft.	HA (SIN®, Sistema de Implante Nacional Ltd., Brazil)	LLLT did not increase new bone formation; however, it accelerated the bone remodeling process.

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
de Oliveira GJPL, et al. 2018 [35]	Evaluate the effect of low-level laser therapy (LLLT) on the healing of biomaterial graft areas (i.e., clot, deproteinized bovine bone and biphasic ceramic composed of hydroxyapatite and β-tricalcium phosphate)	GaAIAs laser, Therapy XT®, DMC Equipment, São Carlos, SP, Brazil; λ 808 nm, 100 mW, beam divergence 0.37 rad, φ 600 µm.	Output Power: 100 mW Power Density: - Energy Density: 354 J/cm^2	Seven sessions were performed, repeated every 48 hours for 13 days after surgery. The first session was applied immediately after surgery.	90 rats (2 groups n = 45; 3 subgroups n = 15) Bone defect in mandibular ramus measuring 5 × 2.5mm. Euthanasia: 30, 60, 90 days post-surgery.	HA/βTCP; Straumann® Bone Ceramic, Straumann AG, Basel, Switzerland	LLLT improved the osteoconductive potential of deproteinized bovine bone (DBB) and HA/βTCP grafts and bone formation in non-grafted areas.
Alan, H et al. 2015 [36]	Compare the effect of low-level laser therapy (LLLT) and ozone therapy on bone healing of defects grafted with nanohydroxyapatite	GaAIAs laser, CHEESE® Dental Laser System, DEN4A, λ 810 nm.	Output Power: 0.3 w Power Density: - Energy Density: 144 J/cm^2	LLLT applied immediately after the operation and was repeated 3 times a week (on alternate days) during the 4-week experimental period (totaling 12 sessions).	36 rats (2 groups n = 18, 3 subgroups n = 6) Monocortical defects in the right femur. Euthanasia after the 4th and 8th week after surgery.	Bego oss s inject, Bremen, Germany	The results show that laser and ozone therapies help in the healing of grafted bone defects. However, there was no statistically significant difference between ozone therapy and LLLT.

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
Pinheiro, ALB, et al, 2009 [37]	Histologically evaluate the effect of laser photobiomodulation (LPBM) on the repair of surgically created defects in rat femurs and filled with HA	GaAIAs Thera Lase®, λ 830 nm, 40 mW, ϕ 0.60 mm, DMC Equipamentos, São Carlos, SP, Brazil	Output Power: 40mW Power Density: - Energy Density: 112 J/cm^2	LLLT was started immediately after suturing the operative site and consisted of transcutaneous application at four points around the surgical site repeated every other day for 15 days	45 rats (4 groups, 3 subgroups) Euthanasia: 15, 21 and 30 days after surgery Defects with a 3 mm²trephine in the upper third of the lateral surface of the femur.	Gen-Phos®, Baumer S.A, Mogi Mirim, SP, Brazil	LPMB therapy may have a positive effect on the early healing of HA-filled bone defects.
Dalapria, V, et al. 2022 [38]	Evaluate the effect of photobiomodulation with LED at a wavelength of 850 nm on the bone quality of Wistar rats undergoing molar extraction with and without bone graft using hydroxyapatite biomaterial	LED λ 850 nm, 100 mW, beam area 2.8 cm^2, total energy 48 J	Output Power: 100 mW Power Density: 0.0357 W/cm^2 Energy Density: 30 J/cm^2	LED every other day (every 48 hours) for a period of 15 days	48 male Wistar rats (5 groups n 12) First lower molar extracted. Two groups had the socket filled with biomaterial immediately after extraction of the tooth. Euthanasia: 15 and 30 days	Straumann® Cerabone® Basel, Switzerland	The LED λ = 850 nm combined with Straumann's hydroxyapatite provided an improvement in bone formation, as well as a reduction in bone degradation, thus promoting an increase in bone density and volume.

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Reference	Objective	Type of Laser (Manufacturer)	Laser Specifications	Protocol	Study design	Biomaterial	Conclusions
Franco, GR, et al. 2012. [39]	Evaluate the regeneration of bone defects filled with HA and stimulated with LLLT in rats subjected to passive smoking	GaAs Bioset® Indústria de Tecnologia Eletrônica Ltda., λ 904 nm, 100 mW, Rio Claro, SP, Brazil	Output Power: 100 mW Power Density: - Energy Density: 20 J/cm^2	3 times a week for 8 weeks	20 female Wistar rats subjected to 8 months of passive smoking; 3 mm bone defect at the distal end of the epiphysis of the right femur. Euthanasia after 8 weeks.	0.5–0.75 mm of hydroxyapatite [$Ca10(PO_4)_6(OH)_2$] particles (GenPhos® HA TCP Genius, Baumer S.A., Mogi-Mirim, SP, Brazil)	Passive smoking compromised new bone formation in the defects and the LLLT protocol was not sufficient to stimulate local osteogenesis.

4. Discussion

The purpose of this review was to analyze published studies on the interaction between photobiomodulation therapy, using LLLT or LED and hydroxyapatite. The use of hydroxyapatite for bone regeneration dates to the 1980s and 1990s. Initially, it was used in maxillofacial and dental surgeries, such as bone grafts and filling bone defects [40]. Since then, its application in bone regeneration has evolved and expanded to several areas of medicine, including orthopedics and plastic surgery. It is important to note that studies and developments in this area have continued to advance since then, with hydroxyapatite being used in increasingly innovative ways [41].

Hydroxyapatite is one of the major components of bone tissue, and most of the Magnesium (Mg) ions in this tissue are bound to the hydroxyapatite surface. The lack of Mg in hydroxyapatite makes its crystals larger, offering a greater risk of fractures. The ion assists in the proliferation and differentiation of mesenchymal stem cells and contributes to angiogenesis, thus accelerating the process of new bone formation [42].

Hydroxyapatite is osteoconductive and biocompatible, but has a very low biodegradation rate [43]. Studies associate biomaterials, such as hydroxyapatite, with permeable membranes that prevent epithelial invasion before the formation of new bone, a procedure called Guided Bone Regeneration (GBR). Baumer GenPhos® HA-TCP biomaterial it was the most used hydroxyapatite in association with photobiomodulation [16–31,33,37,39]. This a biphasic ceramic (synthetic) bone graft, chemically synthesized of high purity, composed of hydroxyapatite and calcium β-triphosphate in a proportion of 70%–30%. The manufacturers report that they associated the stability of hydroxyapatite with the rapid rate of reabsorption of tricalcium phosphate, being a bone substitute with slower resorption (between 7 and 9 months). On the other hand, it allows the reconstruction of bone walls, mainly buccal (aesthetic necessity) with the maintenance of bone volume and alveolar architecture [44,45].

We can mention the use of GenPhos hydroxyapatite to repair fractures associated with the placement of miniplates. performed complete surgical fractures on the tibias of rabbits, with one of the groups having the bone fragments fixed only with miniplates. The animals that received a ceramic graft made of 0.5 mm particles (GenPhos® HATCP. Baumer®, Mogi Mirim, SP, Brazil) and covered with demineralized bovine bone membrane (GenDerm®, Baumer®; Mogi Mirim, SP, Brazil). The irradiated group received infrared laser light (wavelength 780 nm, output power 50 mW, TwinFlex®; MMOptics, São Carlos, SP, Brazil). Irradiation began immediately after surgery and was repeated transcutaneously every other day for 2 weeks. Using Raman spectrometry [30] and histological and morphometric evaluation [25], the authors identified that the group in which the fracture was treated in combination (hydroxyapatite biomaterial + LLLT) improved bone regeneration.

The periosteum has an important role in bone repair, which, together with the bone marrow, has stem cells, generally called skeletal stem/progenitor cells (SSCs), which differentiate into bone-forming osteoblasts and deposit mineralized matrix at the site of the injury. Photobiomodulation has the potential to stimulate this process [46]. In another pre-clinical experiment [17], with the same PBM protocol and graft biomaterial, complete fractures of rabbit tibias were performed and subsequently fixed with osteosynthesis, in treated or untreated groups with infrared laser (wavelength 780 nm and output power 50 mW). Histomorphometric analysis showed increased bone formation, increased collagen deposition, less resorption and inflammation when the biomaterial was associated with the laser.

Another biomaterial used was QualyBone BCP®, composed of 75% Hydroxyapatite and 25% Tricalcium Phosphate (β-TCP) and is reabsorbed between 6 and 24 months. Manufacturers report that cell adhesion is observed after 4 days of installation on the surgical bed. In this experiment, this biomaterial was used to fill critical defects in the calvaria of 56 rats. The authors observed better bone remodeling in the group in which QualyBone BCP® was associated with a fibrin compound (heterologous fibrin biopolymer) and subjected to LLLT of Gallium-Aluminum-Arsenide, with a wavelength of 830 nm and 30 mW of output power [31].

In three studies, the biomaterial BoneCeramic (Straumann®, Basel, Switzerland), formed by biphasic calcium phosphate in a homogeneous composition of 60% Hydroxyapatite (HA), was used as a durable matrix for long-term maintenance of bone volume, which prevents excess reabsorption and preserves bone volume, with 40% Beta tricalcium phosphate (β-TCP), for a rapid initial response from bone-forming cells, in addition to the β-TCP degrading more quickly and being gradually replaced by natural bone. In these studies, LLLT improved the osteoconductive potential of grafts and bone formation in defect area [32,33,35].

The role of macrophages in bone healing is explored and recent developments in biomaterials that promote bone regeneration by modulating macrophage polarization and improving the osteoimmune microenvironment are explored [47]. However, we found a study [38] that used the Cerabone biomaterial (Straumann® Cerabone® Basel, Switzerland), made up of 100% pure hydroxyapatite. The first molar of 48 rats was surgically removed and two groups had the socket filled with the biomaterial in question, and one of the groups underwent phototherapy with LED λ = 850 nm. The authors' conclusion was that the combination of LED with Straumann's hydroxyapatite resulted in improvements in bone formation, in addition to reducing bone degradation, therefore contributing to an increase in bone density and volume.

In this review, we found only a single study carried out in humans [34], which used Hydroxyapatite from the company SIN (SIN®, Sistema de Implante Nacional Ltd., Brazil), in maxillary sinus floor augmentation (MSFA) by bone autograft combined with hydroxyapatite (HA) and treated with low-level laser therapy. The authors concluded, after biopsies obtained 6 months after surgery, that the laser did not increase the amount of bone formed, but only accelerated the process of local bone remodeling.

Systemic bone diseases, such as osteoporosis, cause a reduction in bone mass and destruction of the structure, which can easily lead to fragility fractures. The association of hydroxyapatite or other biomaterials with laser therapy can help combat various systemic changes that interfere with bone remodeling [48,49]. Only one preclinical study performed monocortical defects that were filled with Bego oss nanohydroxyapatite (Bego oss inject®, Bremen, Germany), located in the right femurs of 36 rats. It was also the only study that compared the effect of LLLT with ozone therapy. Both therapies increased and accelerated bone repair, but there was no statistical difference between them [36].

Regarding the laser, the wavelengths of the LLLT devices used varied between 780 nm and 830 nm, with 780 being the most used in 12 studies. Only two studies evaluated LEDs in isolation [21,29], both with a wavelength of 850 nm. Three studies [23,24,28] compared a laser with an LED device (FisioLED® 850 nm and TwinFlex® Evolution laser 780 nm), and concluded that both improved the repair of bone defects with no statistical difference between them.

A preclinical study evaluated bone repair under altered systemic conditions, using anemic rats, grafted with GenPhos® and subjected to LED phototherapy. The results revealed elevated levels of hydroxyapatite (HA) in combination with a reduction of organic components in healthy animals when grafts and LED photobiomodulation therapy were applied. However, the presence of anemia made it difficult to incorporate the graft into the bone, as LED phototherapy only demonstrated an improvement in bone regeneration when the graft was not used [20].

In view of the studies evaluated in this review, in a general context, the effective contribution of photobiomodulation, using low-power laser or LED, isolated or combined, can be seen in the process of repairing bone defects, regardless of the hydroxyapatite used in the graft, bringing positive effects to regenerative and translational science.

5. Conclusion

In this review, we had the scope of analyzing articles that used, experimentally and clinically, the combination of grafting with hydroxyapatite and phototherapy in bone regeneration. Of the 24 articles in this review, only two used hydroxyapatite alone, as the rest used a combined biomaterial of hydroxyapatite and beta tricalcium phosphate. The gradual resorption rate of hydroxyapatite (HA) prevents excessive resorption and supports the stability of the increased bone volume. On the other hand, beta tricalcium phosphate (β-TCP) is quickly reabsorbed, which allows the regeneration of vital bone during the healing period.

Photobiomodulation therapy, whether using LED or LLLT, has demonstrated efficacy in accelerating and optimizing the bone regeneration process in grafts. However, the wide range of wavelengths used in studies indicates that there is no consensus on which wavelength would be most beneficial for bone tissue, making it necessary to carry out more studies aimed at standardization.

Use of AI tools declaration

The authors declare that they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors declare no conflicts of interest

Author Contributions:

Conceptualization, J.d.O.R. and D.V.B.; Methodology, ,J.d.O.R and G.T.R.; Formal Analysis, J.d.O.R. and D.V.B.; Investigation, J.d.O.R. and R.L.B; Data Curation, M.E.C.C.; Writing–Original Draft Preparation, J.d.O.R. and D.V.B.; Writing–Review and Editing, J.d.O.R.; D.V.B., D.V.B. and R.L.B.; Visualization, J.d.O.R.; R.L.B.; Supervision, D.V.B. All authors have read and agreed to the published version of the manuscript.

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