UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

CARLOS HENRIQUE BERTONI REIS

Evaluation of the use of heterologous fibrin biopolymer and hydroxyapatite/tricalcium phosphate synthetic ceramic, associated or not with photobiomodulation therapy, in the repair of bone defects

Avaliação do uso do biopolímero heterólogo de fibrina e cerâmica sintética de hidroxiapatita/fosfato tricálcico, associado ou não à terapia por fotobiomodulação, no reparo de defeitos ósseos

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Orientador: Prof. Dr. Rogério Leone Buchaim

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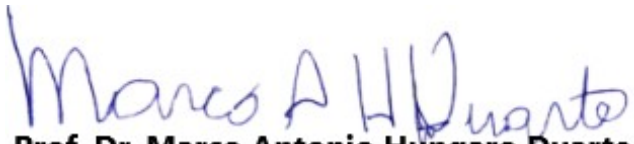

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ERRATA

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ABSTRACT

Evaluation of the use of heterologous fibrin biopolymer and hydroxyapatite/tricalcium phosphate synthetic ceramic, associated or not with photobiomodulation therapy, in the repair of bone defects

Extensive bone loss resulting from fractures or tumor resection poses a challenge for tissue bioengineering areas, in the search for morphological and functional recomposition in a shorter period. The joint use of low-level laser (currently called photobiomodulation therapy - PBM) and bioproducts provides new horizons for tissue repair with a greater chance of success, such as, for example, biocomplexes consisting of fibrin sealant and particulate biomaterials. Objectives: In article 1, the systematic review aimed to evaluate the relationship between PBM and the use of fibrin compounds, referring to the results of previous studies published in PubMed/MEDLINE, Scopus and Web of Science databases and, in article 2, to evaluate the grafting of hydroxyapatite/tricalcium phosphate (BCP) ceramic biomaterial (B) together with the heterologous fibrin biopolymer (FB) and with photobiomodulation (PBM) in the repair process of bone defects. Materials and methods: In article 1, the descriptors “fibrin AND low-level laser therapy” and “fibrin AND photobiomodulation” were used, without restriction on publication time. In article 2, fifty-six rats were randomly divided into four groups of seven animals each: the biomaterial group (G1/B), the biomaterial plus FB group (G2/BFB); the biomaterial plus PBM group (G3/B + PBM), and the biomaterial plus FB plus PBM group (G4/BFB + PBM). After anesthesia, a critical defect was performed in the center of the rats’ parietal bones, then filled and treated according to their respective groups. The rats were euthanized at 14 and 42 postoperative days. Results: In article 1, the bibliographic search found 44 articles in PubMed/MEDLINE, of which 26 were excluded due to duplicity or being outside the eligibility criteria. We also found 40 articles in Web of Science and selected 1 article, 152 articles in Scopus and no article selected, totaling 19 articles for qualitative analysis. The fibrin type most used in combination with PBM was fibrin sealant, mainly heterologous, followed by PRF or L-PRF. In PBM, the gallium-aluminum-arsenide (GaAlAs) laser prevailed, with a wavelength of 830 nm, followed by 810 nm. Among the preclinical studies, the most researched association

of fibrin and PBM was the use of fibrin sealants in bone or nerve injuries; in clinical studies, the association of PBM with medication-related treatments osteonecrosis of the jaw (MRONJ). In article 2, in the comparison between the groups, in the two experimental periods (14 and 42 days), in relation to the percentage of formation of new bone tissue, a significant difference was found between all groups (G1/B (5.42 ± 1.12; 21.49 ± 4.74), G2/BFB (5.00 ± 0.94; 21.77 ± 2.83), G3/B + PBM (12.65 ± 1.78; 29.29 ± 2.93), and G4/BFB + PBM (12.65 ± 2.32; 31.38 ± 2.89)). Conclusion: The literature consulted on PBM, associated with fibrin compounds, scores positive results in several areas of tissue bioengineering, mainly in the recovery of extensive bone loss and peripheral nerve injuries. The reproducibility of research in this area presents problems, due to the numerous protocols that are used and not always fully described in scientific articles. The interaction of the biocomplex composed of Hydroxyapatite/Tricalcium Phosphate Ceramic and Fibrin Biopolymer was potentially effective in the reconstruction of critical bone defects in the calvaria of rats, because the combined use generated perspectives of faster regeneration than when biomaterials and biopharmaceuticals are used separately.

Keywords: Fibrin Tissue Adhesive. Biocompatible Materials. Durapatite. Low-Level Light Therapy. Ceramics.

RESUMO

As extensas perdas ósseas, decorrentes de fraturas ou ressecção de tumores, ocasionam um desafio para as áreas de bioengenharia tecidual, na busca da recomposição morfológica e funcional, em menor espaço de tempo. O uso conjunto do laser de baixa potência (atualmente denominada terapia de fotobiomodulação - FBM) e bioprodutos fornece novos horizontes para reparação tecidual com maior chance de sucesso como, por exemplo, os biocomplexos constituídos por selante de fibrina e biomateriais particulados. Objetivos: No artigo 1, a revisão sistemática teve como objetivo avaliar a relação entre FBM e o uso de compostos de fibrina, referindo-se aos resultados de estudos anteriores publicados nas bases de dados PubMed/MEDLINE, Scopus e Web of Science e, no artigo 2, avaliar o enxerto do biomaterial cerâmico de hidroxiapatita/fosfato tricálcico (BCP) (B) juntamente com o biopolímero heterólogo de fibrina (FB) e com fotobiomodulação (PBM) no processo de reparação de defeitos ósseos. Materiais e métodos: No artigo 1, foram utilizados os descritores “fibrin AND low-level laser therapy” e “fibrin AND photobiomodulation”, sem restrição de tempo de publicação. No artigo 2, cinquenta e seis ratos foram divididos aleatoriamente em quatro grupos de sete animais cada: grupo biomaterial (G1/B), grupo biomaterial + FB (G2/BFB); grupo biomaterial + PBM (G3/B + PBM) e o grupo biomaterial + FB + PBM (G4/BFB + PBM). Após a anestesia, um defeito crítico foi feito no centro dos ossos parietais, preenchido e tratado de acordo com seus respectivos grupos. Os ratos foram eutanasiados aos 14 e 42 dias de pós-operatório. Resultados: No artigo 1, A busca bibliográfica encontrou 44 artigos no PubMed/MEDLINE, dos quais 26 foram excluídos por duplicidade ou por estarem fora dos critérios de elegibilidade. Também encontramos 40 artigos na Web of Science e selecionamos 1 artigo, 152 artigos na Scopus e nenhum artigo selecionado, totalizando 19 artigos para análise qualitativa. O tipo de fibrina mais utilizado em combinação com FBM foi o selante de fibrina, principalmente heterólogo, seguido de PRF ou L-PRF. No FBM, prevaleceu o laser de arseneto de gálio-alumínio, com comprimento de onda de 830 nm e 810 nm. Entre os pré-clínicos, a associação de fibrina e FBM mais estudada foi o uso de selantes de fibrina em lesões ósseas ou nervosas; em estudos clínicos, a associação de FBM com tratamentos medicamentosos relacionados à osteonecrose

da mandíbula. No artigo 2, na comparação entre os grupos, nos dois períodos experimentais (14 e 42 dias), em relação ao percentual de formação de tecido ósseo novo, foi encontrada diferença significativa entre todos os grupos (G1/B (5,42 ± 1,12; 21,49 ± 4,74), G2/BFB (5,00 ± 0,94; 21,77 ± 2,83), G3/B + PBM (12,65 ± 1,78; 29,29 ± 2,93) e G4/BFB + PBM (12,65 ± 2,32; 31,38 ± 2,89)). Conclusão: A literatura consultada sobre FBM, associada a compostos de fibrina, apresenta resultados positivos em diversas áreas da bioengenharia tecidual, principalmente na recuperação de extensas perdas ósseas e lesões de nervos periféricos. A reprodutibilidade das pesquisas nessa área apresenta problemas, devido aos inúmeros protocolos que são utilizados e nem sempre totalmente descritos em artigos científicos. A interação do biocomplexo composto por Cerâmica de Hidroxiapatita/Fosfato Tricálcico e Biopolímero de Fibrina foi potencialmente eficaz na reconstrução de defeitos ósseos críticos na calvária de ratos, pois o uso combinado gerou perspectivas de regeneração mais rápida do que quando biomateriais e biofármacos são utilizados separadamente.

Palavras-chave: Adesivo tecidual de fibrina. Materiais biocompatíveis. Hidroxiapatita. Terapia por luz de baixa intensidade. Cerâmicas.

SUMMARY

1 Introduction

1 **INTRODUCTION**

In clinical practice, routinely and habitually, there is a need for the use of biomaterials that replace biological bone and favor the repair of injuries, especially in cases of trauma, surgery and diseases, in order to recover aesthetics, function and the psychosocial health of patients (GUSKUMA *et al.*, 2010; XU *et al.*, 2018; ZAFAR *et al.*, 2020).

Tissue regeneration, such as the repair of bone loss, is the main objective of most therapies in medicine, especially in orthopedics (SCHINDELER *et al.*, 2018) and dentistry (BRESSAN *et al.*, 2011), with great use in the areas of periodontics (PIETRUSZKA *et al.*, 2021), oral and maxillofacial surgery (FERNANDEZ DE GRADO *et al.*, 2018) and implantology (SAKKAS *et al.*, 2017).

Preclinical studies, based on a translational science that aims to link the bench to the bedside, are an important scientific basis for the clinical use of bone substitute materials (FERREIRA *et al.*, 2017; REIS *et al.*, 2022). The grafting material considered the “gold standard” in the area of bone reconstruction is autogenous bone, which is taken from the patient himself, because it contains a high rate of compatibility and low immune rejection, which makes it very favorable (DOS SANTOS *et al.*, 2020).

However, it has disadvantages, such as the difficulty in obtaining the amount needed for use, two surgical areas in the same patient (BUCHAIM *et al.*, 2013; CUNHA *et al.*, 2015), in addition to a higher degree of morbidity (CUNHA *et al.*, 2021; SOHN; OH, 2019). Due to this fact and the advancement of science, other materials were created that reach results similar to the same (DELLA COLETTA *et al.*, 2021; IATECOLA *et al.*, 2013; TALLARICO *et al.*, 2022).

When there is an injury or some bone trauma, damage occurs to the blood vessels of the periosteum, endosteum and the surrounding soft tissues. This damage can cause bleeding at the site, which in a physiological defense, the body allows for vasoconstriction, along with platelet formation and clotting (CASSARO *et al.*, 2019). In the clotting process, a hemostatic clot will be produced where there is aggregation of platelets, cells and serum proteins, making it more stable in relation to the lesion (GAROLA *et al.*, 2021; PIETRUSZKA *et al.*, 2021).

This is known as a hematoma, which stabilizes the lesion and induces granulation tissue formation and bone remodeling (DE FREITAS DUTRA JÚNIOR *et al.*, 2022). Therefore, it is known that the beginning of each regeneration requires the

formation of clots, with an important role of the fibrin mesh, in order to start the bone healing process (KHURSHID *et al.*, 2022; PRIGLINGER *et al.*, 2018; ROSSO *et al.*, 2017).

With the efficiency of fibrin derivatives being proven in tissue bioengineering experiments, there has been an increase in the use of these composite materials with some blood components, such as fibrin sealant (BUCHAIM *et al.*, 2019; LE GUÉHENNEC *et al.*, 2004). It has been used, due to its positive interaction with other biomaterials, in the formation of a biocomplex, with the purpose of associating scaffolds, facilitating the insertion, permanence and repair of the injured site (BUCHAIM *et al.*, 2022; BUCHAIM; BUCHAIM, 2022).

The new fibrin sealant produced by CEVAP (Center for the Study of Venoms and Venomous Animals), at Universidade Estadual Paulista (UNESP, Botucatu, São Paulo, Brazil), composed of substances derived from snake venom (gyroxine) and buffalo blood (fibrinogen), has been used in several regenerative studies of venous ulcers (ABBADE *et al.*, 2021; STATE *et al.*, 2015), tendons (DE FREITAS DUTRA JÚNIOR *et al.*, 2022), bone (DE OLIVEIRA GONÇALVES *et al.*, 2016), nerve (BUCHAIM *et al.*, 2017; ROSSO *et al.*, 2020) and others. It is safer because it does not pass infections, is biocompatible and has a low production cost. Considering all the properties described for this bioproduct, which go beyond the adhesive properties of a sealant, it became known as fibrin biopolymer (MASSIMINO *et al.*, 2020; VENANTE *et al.*, 2021).

Although the medical and dental field has several mechanisms that help in accelerating bone regeneration, it was discovered that the use of low-level laser (currently called photobiomodulation therapy - PBM) also influences the tissue, stimulating the proliferation of cells mainly osteoblasts, vascular budding, reduction in pain and tissue inflammation, therefore increasing bone neoformation. These factors help to accelerate tissue regeneration, producing very satisfactory results (ALVES *et al.*, 2020; ESCUDERO *et al.*, 2019; GONÇALVES *et al.*, 2021; POMINI *et al.*, 2019; ZEIN; SELTING; BENEDICENTI, 2017).

Therefore, it is evident that intervention techniques in the bone regeneration process play an important and generally effective result. In view of this, there is a need for research, such as this one, aiming to review and evaluate their interaction and integration in this neoformation process, such as the combined use of regenerative therapies, biomaterials and bioproducts with laser photobiomodulation.

2 Articles

2 ARTICLES

The two articles that make up this doctoral thesis are formatted following the specific instructions for submission in each journal.

- Article 1: Application of Fibrin Associated with Photobiomodulation as a Promising Strategy to Improve Regeneration in Tissue Engineering: A Systematic Review. (Published on journal Polymers).

- Article 2: Effects of a Biocomplex Formed by Two Scaffold Biomaterials, Hydroxyapatite/Tricalcium Phosphate Ceramic and Fibrin Biopolymer, with Photobiomodulation, on Bone Repair. (Published on journal Polymers).

2.1 Article 1:

Application of Fibrin Associated with Photobiomodulation as a Promising Strategy to Improve Regeneration in Tissue Engineering: A Systematic Review.

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polymers

MDPI

Review

Application of Fibrin Associated with Photobiomodulation as a Promising Strategy to Improve Regeneration in Tissue Engineering: A Systematic Review

Carlos Henrique Bertoni Reis [1,2], Daniela Vieira Buchaim [3,4], Adriana de Cássia Ortiz [2], Simone Ortiz Moura Fideles [2], Jefferson Aparecido Dias [3,5], Maria Angelica Miglino [6], Daniel de Bortoli Teixeira [3,7], Eliana de Souza Bastos Mazuqueli Pereira [3], Marcelo Rodrigues da Cunha [8] and Rogerio Leone Buchaim [2,6,*]

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Abstract: Fibrin, derived from proteins involved in blood clotting (fibrinogen and thrombin), is a biopolymer with different applications in the health area since it has hemostasis, biocompatible and three-dimensional physical structure properties, and can be used as scaffolds in tissue regeneration or drug delivery system for cells and/or growth factors. Fibrin alone or together with other biomaterials, has been indicated for use as a biological support to promote the regeneration of stem cells, bone, peripheral nerves, and other injured tissues. In its diversity of forms of application and constitution, there are platelet-rich fibrin (PRF), Leukocyte- and platelet-rich fibrin (L-PRF), fibrin glue or fibrin sealant, and hydrogels. In order to increase fibrin properties, adjuvant therapies can be combined to favor tissue repair, such as photobiomodulation (PBM), by low-level laser therapy (LLLT) or LEDs (Light Emitting Diode). Therefore, this systematic review aimed to evaluate the relationship between PBM and the use of fibrin compounds, referring to the results of previous studies published in PubMed/MEDLINE, Scopus and Web of Science databases. The descriptors "fibrin AND low-level laser therapy" and "fibrin AND photobiomodulation" were used, without restriction on publication time. The bibliographic search found 44 articles in PubMed/MEDLINE, of which 26 were excluded due to duplicity or being outside the eligibility criteria. We also found 40

articles in Web of Science and selected 1 article, 152 articles in Scopus and no article selected, totaling 19 articles for qualitative analysis. The fibrin type most used in combination with PBM was fibrin sealant, mainly heterologous, followed by PRF or L-PRF. In PBM, the gallium-aluminum-arsenide (GaAlAs) laser prevailed, with a wavelength of 830 nm, followed by 810 nm. Among the preclinical studies, the most researched association of fibrin and PBM was the use of fibrin sealants in bone or nerve injuries; in clinical studies, the association of PBM with medication-related treatments osteonecrosis of the jaw (MRONJ). Therefore, there is scientific evidence of the contribution of PBM on fibrin composites, constituting a supporting therapy that acts by stimulating cell activity, angiogenesis, osteoblast activation, axonal growth, anti-inflammatory and anti-edema action, increased collagen synthesis and its maturation, as well as biomolecules.

Keywords: tissue regeneration; fibrin; scaffolds; fibrin glue; fibrin sealant; platelet-rich fibrin; photobiomodulation; review; low-level laser therapy

1. Introduction

The word fibrin, in etymology, derives from the Latin 'fibre' (fiber) and –in (chemical substance). It can be defined as a protein formed in blood plasma from the action of thrombin on fibrinogen, being the main component of blood clots (that is, fibrin aggregating produces clots). Wound healing depends entirely on the initial mechanisms of tissue homeostasis. When an injury occurs, the first tissue to respond is blood, as bleeding is a potentially serious risk to the body. There is a cascade of molecular and cellular reactions that lead to the sealing of the vascular lesion with an aggregate of platelets, which stop the hemorrhage by forming a tampon in the injured tissue, triggering the next steps of tissue regeneration. Stable blood clot, containing cross-linked and polymerized fibrin, is essential to prevent bleeding and lead to wound repair after vascular injury [1,2].

Fibrin is a viscoelastic polymer and its mechanical and structural properties as a fibrin scaffold determine its effectiveness in hemostasis and in the development and outcome of thrombotic complications. Fibrin polymerization comprises a series of consecutive reactions, each affecting the final structure of the 3D porous network. Structural features in the fibrin molecule determine the physical properties of clots, and it is important for the blood clot to support arterial flow, clot contraction by platelets, and other dynamic forces [3,4].

The three-dimensional structure of fibrin allows for a series of cellular interactions and provides a temporary matrix in which cells can proliferate, organize, and perform their functions, especially at injured or inflamed sites. Thus, fibrin has been used with the aim of accelerating healing and regeneration in several surgical procedures, especially in medicine in the areas of orthopedics [5,6], neurology [7–9], and plastic surgery [10,11], as well as in dentistry in the areas of periodontics [12,13], implantology [14,15], and oral and maxillofacial surgery [16,17].

One of the ways to use fibrin in tissue regeneration is platelet-rich fibrin (PRF) which, unlike platelet-rich plasma (PRP), PRF has a high concentration of fibrin and white blood cells, not platelets. PRP and PRF have the same ability to accelerate the healing of soft and hard tissues by increasing the concentration of growth factors, but PRF acts to release growth factors over a longer period, providing longer lasting benefits, as well as stimulating a faster healing process than PRP [18]. PRF increases the concentration of these factors, among which we can exemplify the platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), factors which help to accelerate neovascularization and cell differentiation [18,19].

Studies also evaluate "fibrin glues" that can be called fibrin adhesive, fibrin sealant or fibrin biopolymer in tissue regeneration [20–23]. Human fibrin glue is manufactured

using two components, one of which is a concentrate of clotting proteins (fibrinogen, fibronectin and Factor XIII) and the other is thrombin, both lyophilized. The first component is reconstituted with an aprotinin solution that inhibits tissue fibrinolysis. Thrombin is mixed with calcium chloride, thus being a grouping of substances participating in hemostasis and wound repair, giving the product properties such as hemostatic action, sealant and biological stimulation, which favor the formation of new tissue matrix [24,25]. In Brazil, a group of researchers from the Center for the Study of Venoms and Venomous Animals (CEVAP/UNESP Botucatu) developed and has been using in several studies, a fibrin sealant without the presence of derivatives from human blood, being totally heterologous, which has components derived from snake venom and fibrinogen from buffalo blood. This sealant, due to its diversity of use, is currently called fibrin biopolymer [8,26].

However, in view of the search for a rapid morphological and functional recovery of the injured tissues, more than one type of therapy can be combined (in this case, a set of therapies complementary to the treatment). One of them is the low-level laser (LLLT), with tissue stimulation properties through red or infrared light with the ability to modulate the repair process, reducing pain, increasing tissue vascularization, promoting an increase in the production of mitochondrial ATP, and a series of biostimulatory effects, which led to the current name of photobiomodulation (PBM) therapy [27,28].

The combined use of fibrin glue with photobiomodulation has shown promising results in the repair of peripheral nerve injuries, being effective in the neurorrhaphy procedure, as well as providing a better quality of axonal regeneration to the interior of the distal stump [29]. In addition, this associated form of therapeutic use has demonstrated the ability to assist in the repair process of bone defects, stimulating angiogenesis and osteoblast proliferation, contributing to the formation of new bone in shorter postoperative periods and in greater volume [30].

However, there are still gaps in explaining the mechanisms of PBM therapy and its effects in combination therapies with fibrin. Therefore, this systematic review was designed from the PICO strategy (P: problem; I: intervention; C: control; O: outcome) [31,32], in order to analyze the relationship between PBM therapy and the use of fibrin compounds, such as PRF and fibrin sealants.

2. Materials and Methods

This systematic review was developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist, as well as other similar research [32–34]. For this, PubMed/MEDLINE, Scopus (Elsevier) and Web of Science databases were searched, with a specific search period (1 January 2002–30 April 2022), using the keywords: "fibrin AND low-level laser therapy" and "fibrin AND photobiomodulation".

With the crossing of keywords, a detailed analysis of the results was carried out, being important in the selection the title and the abstract. From there, the manuscripts were separated into included and excluded according to the eligibility criteria. The authors carried out this process impartially and independently.

- Eligibility Criteria:

  The inclusion criteria were:

  - Therapeutic use of fibrin and PBM therapy as complementary therapy;
  - Studies in humans;
  - Studies in animals;
  - In vivo studies;
  - Case reports;
  - Publications only in English and that allowed full access to the text;
  - Each article included must present data on the PBM protocol.

- The exclusion criteria were:

  - Articles that were duplicated;
  - When the title had no connection to the objective;

- Did not use fibrin;
- Did not use photobiomodulation;
- Used high power laser;
- Other languages (except English);
- When access to the full text was not obtained;
- Incomplete data on the type of fibrin used.
- Letters to the editor;
- Review papers;
- Commentaries;
- Unpublished abstracts;
- Dissertations or theses from repositories

Initially, the manuscripts with the title and abstract connected to the topic of the search were verified, with the terms: fibrin and PBM therapy, and then we evaluated and restricted the articles only to the focus of the question in this review. Methodology, the results obtained, and the importance of these results were important to list the selected manuscripts. The selected articles on the topic were carefully read. In addition, two independent reviewers participated in the selection phases, ensuring that the inclusion and exclusion criteria were carefully followed, with the clear objective of minimizing bias.

Data related to the subject of this review were selected and extracted from the manuscripts by independent reviewers, taking into account the characteristics of the individual studies that contributed to their outcome as well as their aggregated results, without the objective of performing a meta-analysis.

The selection scheme, according to the PRISMA flow diagram [32,35,36], is shown in Figure 1.

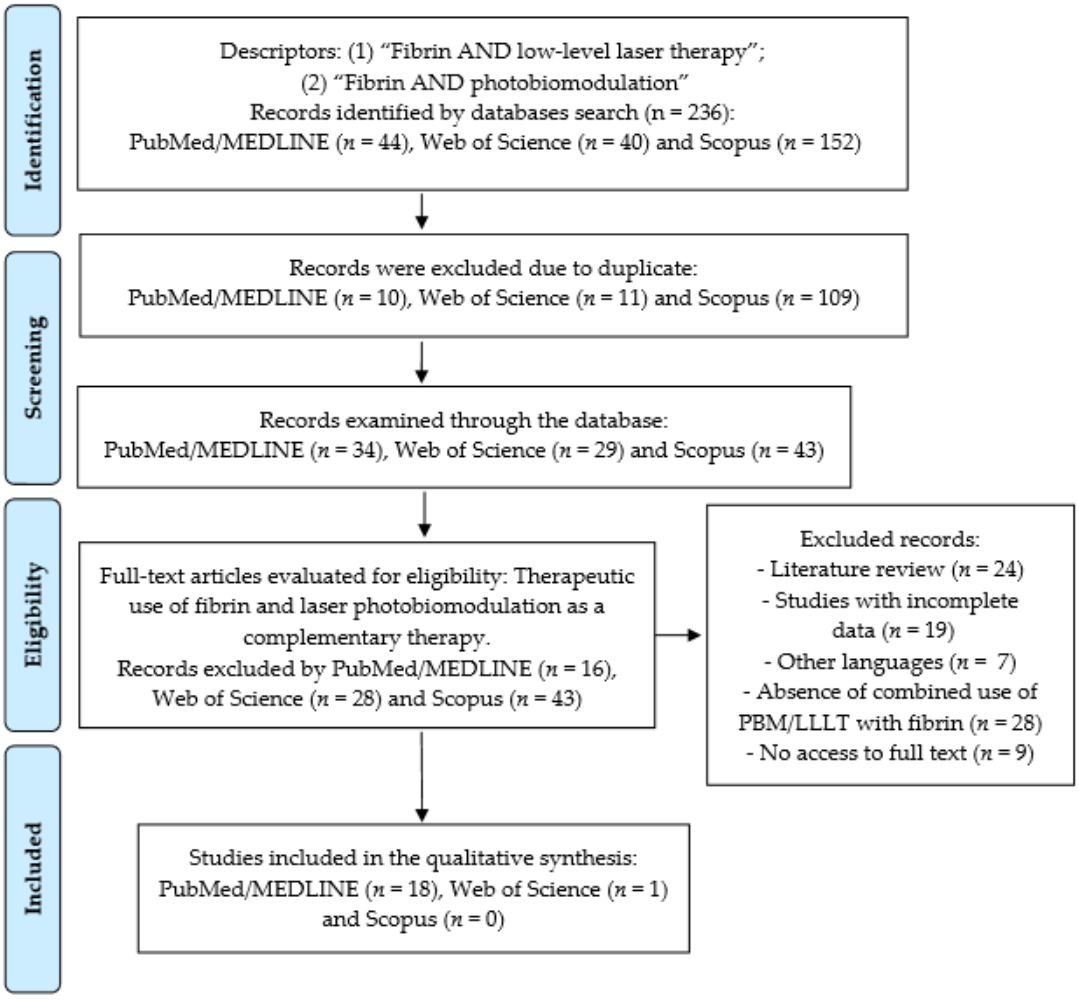

Figure 1. Flow diagram showing study selection.

3. Results

The bibliography search found 44 articles in the PubMed/MEDLINE database, of which 26 were excluded since they were duplicates or due to inclusion/exclusion criteria. We also found 40 articles in Web of Science and selected 1 article, 152 articles in Scopus and no article selected, totaling 19 articles for qualitative analysis.

From the studies selected for a detailed description, we can see that, due to their physicochemical characteristics, fibrin compounds are widely used in several areas that mainly involve medicine and regenerative dentistry. In this way, three selected studies were selected in which the researchers used hydrogels or 3D fibrin, 3 with L-PRF, 10 with fibrin sealants (or also called glue, adhesive or biopolymers) and 3 with autologous PRF (Figure 2).

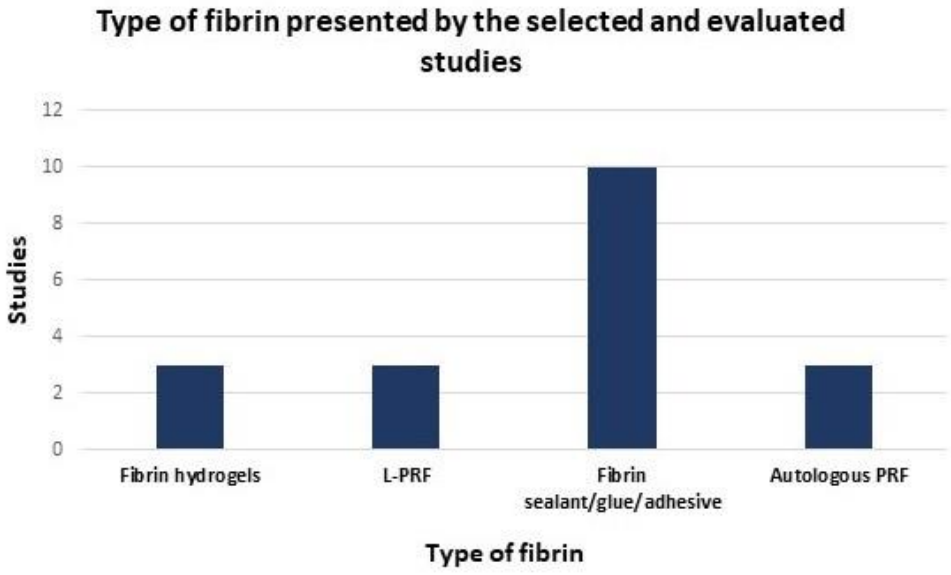

Figure 2. Configurations of fibrin preparations used in tissue regenerative processes. Three studies were used hydrogels or 3D fibrin, 3 with L-PRF, 10 with fibrin sealants (or also called glue, adhesive or biopolymers), and 3 with autologous PRF.

Regarding the results of photobiomodulation, we found (according to the eligibility criteria) three studies that used the red LED (Light Emitting Diode - original apparatus LDM-07 or Repuls Lichtmedizintechnik GmbH, Vienna, Austria), 1 infrared LED (original apparatus LDM-07), 1 GaAs (Gallium-Arsenide) laser (Fisioline; Lumix® C.P.S. Dental Multidiodic laser, Verduno, Cuneo, Italy), 10 GaAlAs (Gallium-Aluminum-Arsenide) laser (Laserpulse IBRAMED®, Amparo, Brazil), 2 ND: YAG (neodymium-doped: yttrium aluminium garnet) laser (Fotona, Ljubljana, Slovenia), 1 InGaAlP (Indium-Gallium-Aluminum-Phosphide) laser (MMOptics®, São Carlos, Brazil) and two studies did not identify the type of laser used (Figure 3).

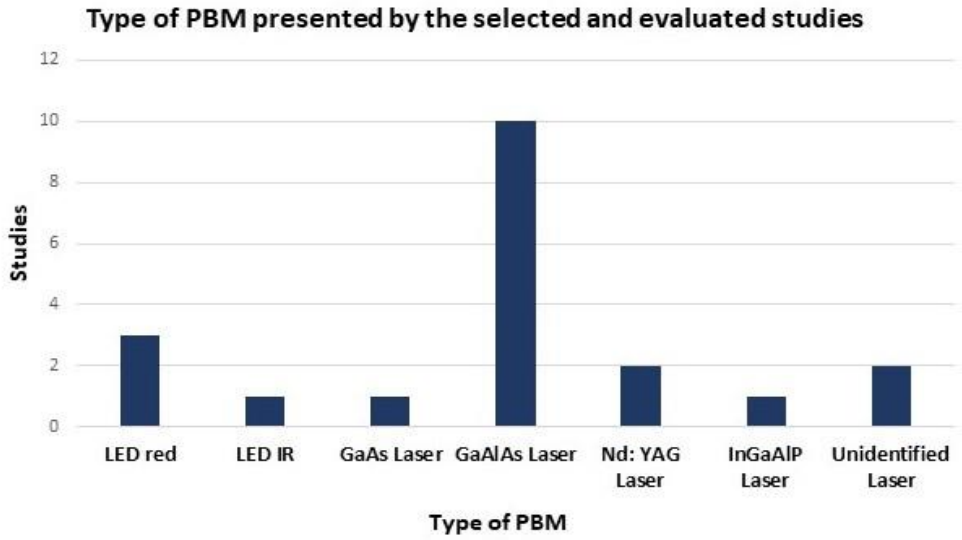

Figure 3. Type of photobiomodulation presented by the selected and evaluated studies. Gallium-Aluminum-Arsenide (GaAlAs) laser that presented greater use in the selected studies in tissue regenerative processes (10 studies). Two studies did not specify the type of PBM used. One study used different types of PBM, therefore considered separately in the data in the figure.

In the photobiomodulation protocols of the selected studies, when the wavelengths were analyzed, the most used was 830 nm, in nine studies. Then, 810 nm in three studies; 475 nm, 516 nm, 635 nm, and 1064 nm in two studies each; 633 nm, 650 nm, 660 nm, 840 nm, 910 nm with one study each; and one study did not disclose the wavelength used (Figure 4).

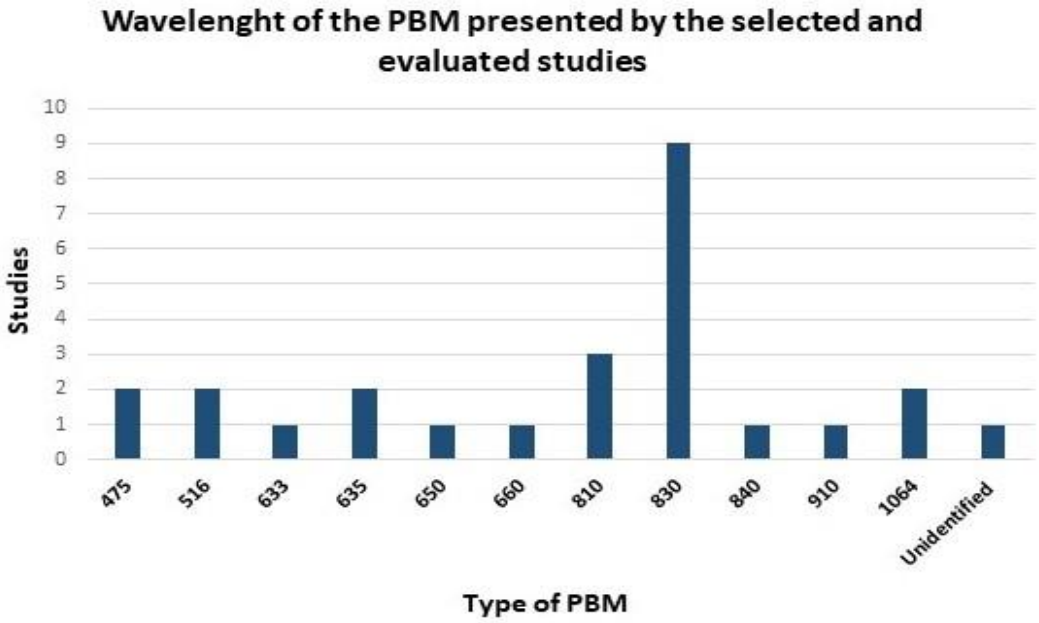

Figure 4. Protocols of PBM. Wavelength (nm) used by the studies included in Table 1. 830 nm that presented greater use in the selected studies in tissue regenerative processes (nine studies). One study did not present the wavelength used. Studies that used different wavelengths were considered separately in the data in the figure.

The articles selected to compose this review are presented in Table 1.

Table 1. Articles that were selected for detailed analysis, following the eligibility criteria.

Reference (Database)	Type of Laser/LED (Manufacturer)	Wavelength (nm) and Output Power (mW)	Power Density (mW/cm²)	Energy Density (J/cm²)	Objective	Fibrin	Intervention	Outcome/Results	Conclusions
Bikmulina et al., 2020 [37] (PubMed)	LED light red and infrared (IR) (Original apparatus LDM-07)	Red: 633 IR: 840 and Red: 160 ± 20 IR: 320 ± 40	Red: 1.8 ± 0.2 IR: 3.6 ± 0.4	Red and IR: 2.2 ± 0.2	Evaluation of PBM therapy for cell stimulation in hydrogels	Mesenchymal stromal cells (MSCs) obtained from human gingiva mucosa were encapsulated in fibrin (hydrogels)	A single exposure was made to low-intensity light, both red and infrared. After three days of culture, the physiological activity and viability of the cells were verified	The authors observed a dependence on cell viability in relation to the concentration of gel-forming proteins and the thickness of the hydrogels	Infrared light can be indicated for stimulation of MSCs proliferation and metabolism, in hydrogels with thicknesses of up to 3 mm
Tenore et al., 2020 [38] (PubMed)	Red and Infrared Gallium-Arsenide laser (GaAs) (Fisioline; Lumix® C.P.S. Dental Multidiodic laser)	Three wavelengths: 650, 810, 910 and G1: total power of 600 mW; G3 total power of 1100 mW	-/-	-/-	To evaluate the effect of three different protocols on the healing outcome in patients with established medication-related osteonecrosis of the jaw (MRONJ)	Leukocyte- and platelet-rich fibrin (L-PRF)	G1 was treated with antibiotic therapy, surgery, L-PRF and PBM; G2 with antibiotic therapy and surgery; G3 with antibiotic and PBM	There was no significant association between MRONJ results and location, stage, duration of drug treatment, diabetes, smoking, corticosteroid therapy, underlying disease, sex, and chemotherapy history at three and six months	The combination of antibiotic therapy, L-PRF, surgery and PBM can effectively contribute to the treatment of MRONJ
Buchaim et al., 2015 [29]	Gallium-Aluminum-Arsenide	830 and 30	-/-	4	To analyze whether the fibrin adhesive allows,	Fibrin glue derived from snake venom	Experimental Group (EG; *n* = 12 rats), sural nerve graft was	There was sprouting of axons from the vagus nerve into the	LLLT potentiates nerve regeneration and fibrin glue

(PubMed)	(GaAlAs) (Laserpulse IBRAMED ®, Amparo, Brazil)				through end-to-side neurorrhaphy, the collateral growth of axons without an epineural window of the vagus nerve into a sural nerve graft and whether laser therapy contributes to the regeneration process		coapted to the vagus nerve with fibrin glue; and experimental group laser (EGL; *n* = 12 rats), EG + LLLT and control group (CG; *n* = 8 rats), the intact sural nerve was collected	autologous graft in the EG and EGL, and in the CG all of the dimensions measured were better, with a significant difference in relation to the EG and EGL, except for the area and thickness of the myelin sheath, which showed a significant difference only in relation to the EG	provided conditions for axonal regeneration in peripheral nerve injuries
de Oliveira Gonçalves et al., 2016 [39] (PubMed)	GaAlAs (Laserpulse IBRAMED ®, Amparo, Brazil)	830 and 30	258.6	6	To evaluate the effects of LLLT on an autogenous bone graft integration process stabilized with a new heterologous fibrin sealant (NHFS)	Heterologous fibrin sealant	Autogenous bone graft from rat calvaria, removed from the right parietal bone, with a 5 mm osteotomy, was adhered on the left side with fibrin sealant; groups: autogenous Fibrin graft (AFG) and autogenous fibrin graft laser (AFGL), with the same procedures as the AFG, plus LLLT	The bone regeneration process was not complete, with new bone tissue partially integrating the graft into the recipient bed, with some areas of connective tissue. Morphometrically, minor interfaces occurred in the AFGL group, with significant differences in all analyzed periods	LLLT stimulated bone neoformation and improved the process of integration of autogenous bone graft

Buchaim et al., 2017 [40] (PubMed)	GaAlAs (Laserpulse IBRAMED ®, Amparo, Brazil)	830 and 30	258.6	6.2	To analyze the efficacy of LLLT on quantitative, qualitative and functional aspects in the facial nerve regeneration	NHFS derived from snake venom	Suture experimental (SEG) and fibrin experimental (FEG) groups, the buccal branch of the facial nerve was sectioned, end-to-end epineural suture on the right side, and a NHFS on the left side; laser suture experimental (LSEG) and laser fibrin experimental (LFEG) groups, the same procedures as SEG and FEG with the addition of LLLT; control group (CG), facial nerve intact	LLLT resulted in a significant increase in the density and number of new axons. The LSEG and LFEG presented better scores in functional analysis in comparison with the SEG and FEG	Both repair techniques were effective in promoting axonal growth and LLLT improved these results, in addition to accelerating the functional recovery of whiskers
Rohringer et al., 2017 [41] (PubMed)	LED lamps were provided by Repuls Lichtmediz intechnik GmbH, Vienna, Austria	Pulsed LED light of either 475 nm (blue), 516 nm (green), 635 nm (red) or remained unstimulated (control)	Peak irradiance intensity of 80 mW/cm² on all LED devices; average irradiance intensity of 40 mW/cm²	Dose 24 J/cm² (daily)	To compare the effects of PBM using light-emitting diodes (LED) with different wavelengths on endothelial cells in vitro	3D fibrin matrices and fibrin gels	Migration and proliferation tests were performed in 2D and 3D. 3D fibrin gel co-culture model with human umbilical vein endothelial cells (HUVEC) and adipose-derived stem cells (ASC) was used to analyze early vasculogenic effects, continuous stimulation	Stimulation with green and red LED light increased 3D migration and proliferation of HUVEC. HUVEC also had greater potential for 2D migration with green light stimulation. Blue light was ineffective	Green light, in several parameters, has been shown to be more potent in stimulating endothelial cell migration and proliferation than red light

						of LLLT, after one week of culture		
Priglinger et al., 2018 [42] (PubMed)	LED lamps were provided by Repuls Lichtmediz intechnik GmbH, Vienna, Austria	Pulsed LED light 475 nm (blue), 516 nm (green), 635 nm (red)	All LED devices had a peak irradiance intensity of 80 mW/cm^2	Fluence of 24 J/cm^2	To analyze the effects of green, blue and red light (RL) emitted by LEDs directly on freshly isolated SVF and analyzed cell phenotype, cell number, viability, ATP content, LDH cytotoxicity and proliferation, but also osteogenic, adipogenic and pro-angiogenic differentiation in vitro	3D fibrin matrices	Pulsed blue (475 nm), green (516 nm) and RL (635 nm) from LEDs applied on freshly isolated Stromal Vascular Fraction (SVF)	LLLT increased, compared to untreated cells, the colony-forming unit fibroblast assay with RL. The frequency of colony forming cells was not affected. LLLT with green light and RL resulted in a better potential to form vascular tubes by SVF compared to untreated cells when grown in 3D fibrin matrices	LLLT has beneficial effects in relation to SVF cell proliferation and vascularization potential. LLLT may represent a good method for clinical practice in activating SVF cells
Pomini et al., 2019 [43] (PubMed)	GaAlAs (Laserpulse IBRAMED ®, Amparo, Brazil)	830 and 30	258.6	6	In rat calvaria (critical size defect—CSD), to evaluate the scaffold formed by a fibrin sealant (FS) plus xenograft associated with PBM therapy	Tisseel Lyo® (Baxter Healthcare Ltd., Norfolk, UK)	CSD in calvaria, 36 rats: 4 groups: BC (n = 8), defect with blood clot; FSB (n = 10), FS and xenograft; BC^{PBMT} (n = 8), blood clot and PBM; FSB^{PBMT} (n = 10), FS, xenograft, and PBM	Bone neoformation was observed in all groups, limited to the defect margins. In the FSB group, new bone increased between periods (4.3 ± 0.46 to 6.01 ± 0.32), but with lower volume when compared to the FSB^{PBMT} (5.6 ± 0.45 to 10.64 ± 0.97)	The biocomplex formed by the xenograft plus FS associated with the PBM therapy had a positive effect on the new bone formation
Hemaid et al., 2019 [44]	Diode Laser Gallium-	810 and 100	-/-	46.8	To observe and compare the combined use of	Autologous platelet-rich fibrin (PRF)	Sixteen defects in rabbits divided in four groups: laser irradiated	NanoHA-Graft + PRF + L showed significantly higher	The best form of treatment was the combined use of

(PubMed)	Aluminum -Arsenide (GaAlAs)				LLLT (810 nm), PRF and NanoHA in the healing of induced intraosseous periodontal defects		control (CL); Control non-treated (C); PRF + NanoHA graft treated group and laser irradiated (NanoHA-Graft + PRF + L)	bone density in relation to the other groups	LLLT + PRF + NanoHA as it presented the best results in the formation of new bone
Sahin et al., 2020 [45] (PubMed)	Nd: YAG laser (Fotona, Ljubljana Slovenia)	1064 and 1250	-/-	-/-	To analyze the surgical procedures used to prevent the development of MRONJ after dentoalveolar surgery in patients who received bisphosphonates	Leukocyte and platelet-rich fibrin (L-PRF)	Sixty-three surgeries were performed on forty-four patients taking bisphosphonate. Procedures: performed dentoalveolar surgical; antibiotics; fill the socket with L-PRF; LLLT (Nd: YAG laser)	There were no intercurrences until cure. Complete mucosal healing occurred in all patients within one month with no long-term failures	The surgical protocol demonstrates promising results for the protection of MRONJ after performing dentoalveolar surgeries
Thalaimalai et al., 2020 [46] (PubMed)	Diode laser	810 and 500	-/-	-/-	To evaluate the combined effect of LLLT and PRF, in site modulated intra-bony defects, which were accessed using a simplified papilla preservation flap (SPPF), on the periodontal disease	Autologous platelet-rich fibrin	Thirty patients with intra-bony defects (2 groups, n = 15 each). There was SPPF access at test group (TG) sites and defects received intramedullary penetration (IMP) after debridement, followed by LLLT and PRF grafting. In the control group (CG), the defects were accessed with SPPF and grafted only with PRF	TG showed a clinically relevant increase in mean probing pocket depth reduction, clinical attachment level gain, and radiographic bone fill compared to the CG, six months post-intervention	Together, LLLT with PRF caused an improvement in clinical and radiographic results within modulated intraosseous defects
Della Coletta et	GaAlAs (Laserpulse	830 and 30	258.6	6.2	To evaluate the effects of PBM	Fibrin biopolymer (FB)	Thirty Wistar rats: BMG, defects filled	There was more evident bone growth	PBM has been shown to be effective

al., 2021 [47] (PubMed)	IBRAMED ®, Amparo, Brazil)				therapy on the guided bone regeneration process (GBR) in defects in the calvaria of rats filled with biphasic calcium phosphate (BCP) associated with fibrin		with biomaterial and covered by membrane; BFMG, biomaterial and fibrin biopolymer (FB) covered by membrane; and BFMLG, biomaterial and FB covered by membrane and biostimulated with PBM	in the BFMLG, in addition to a progressive increase in new bone tissue in all groups, with a significant difference in the BFMLG, whose group presented greater bone neoformation in the periods of 14 and 42 days, followed by BFMG and BMG	in improving and accelerating the GBR process when associated with BCP and FB
Sahin et al., 2021 [48] (PubMed)	Nd: YAG laser (Fotona, Ljubljana Slovenia)	1064 and 1250	-/-	-/-	To analyze the surgical technique described in the treatment of advanced stages of MRONJ patients	Autologous L-PRF concentrate	Twnty-one patients affected by Stage 2-3 MRONJ were treated with ultrasonic piezoelectric for bone surgery, with necrotic bone removing, L-PRF and LLLT	Two patients, who were Stage 3, had delayed healing at 1 month after surgery. Complete mucosal healing occurred in all patients in the third month	The surgical protocol shows promising results for surgical management of advanced stages of MRONJ patients
de Freitas Dutra Júnior et al., 2021 [49] PubMed	Indium-Gallium-Aluminum-Phosphide laser (InGaAlP) (MMOptics ®, São Carlos, Brazil)	660 and 40	1000	6	To verify, in tendon injuries, the action of the new heterologous fibrin biopolymer (HFB) associated or not with PBM	Heterologous fibrin biopolymer	Partial transection calcaneus tendon (PTCT) was performed in 84 rats divided into 4 groups: control (CG); HFB; PBM; HFB + PBM. HFB was applied immediately after PTCT, while PBM started 24 h after injury and continued every 24 h for 7, 14 and 21 days.	It can be noted that the reduction of edema was effective in the treatment groups when compared to the CG. In the periods of 14 and 21 days, PBM had a better repair process compared to GC	The HFB and PBM treatments, associated or isolated, promoted a reduction in the edema volume, favoring the repair process. HFB alone contributed more in promoting the tendon repair process

Buchaim et al., 2022 [50] (PubMed)	GaAlAs (Laserpulse IBRAMED ®, Amparo, Brazil)	830 and 30	258.6	6.2	To analyze the effects of PBM on CSD filled with xenogenic bone substitute associated with HFB	Heterologous fibrin biopolymer (HFB)	CSD in 36 Wistar rats, four groups: BC and BC-PBM (controls) with defects filled by a clot (without or with PBM); XS and XS-PBM, filled with biocomplex Bio-Oss® + HFB. PBM was applied transoperatively and continued three times a week	BC-PBM and XS-PBM had a higher density of the bone neoformation in relation to the groups without PBM. Significant vascular proliferation and new bone deposition around the XS particles were observed in the animals which biocomplex (XS and XS-PBM)	PBM allowed an improvement in none neoformation, with a more organized deposition of collagen fibers. Biocomplex favored the permanence and insertion of the particulate biomaterial in bone defect
Rosso et al., 2017 [51] (PubMed)	GaAlAs (Laserpulse IBRAMED ®, Amparo, Brazil)	830 and 30	260	6.2	To evaluate the action of PBM on lesions of the facial nerve repaired with the end-to-side technique or coaptation with a NHFS	New Heterologous Fibrin Sealant	Thirty-two rats, five groups: control (CG); experimental suture (ESG) and experimental fibrin (EFG) groups, end-to-side sutured to the zygomatic branch on the right side of the face or NHFS on the left side; experimental suture laser (ESLG) and experimental fibrin laser (EFLG) groups, with PBM	There was a significant difference in the fiber nerve area between the EFG and the EFLG. There was also faster functional recovery of the whisker movement in the ESLG and EFLG, where PBM was used, with results closer to the CG	Photobiomodulation with LLLT accelerated functional and morphological nerve repair, in both techniques

Rosso et al., 2020 [52] (PubMed)	GaAlAs (Laserpulse IBRAMED ®, Amparo, Brazil)	830 and 30	258.6	6	To evaluate the action of PBM on rat tibial defect filled with biomaterial of the lyophilized bovine bone matrix (BM) associated or not with HFB	Heterologous fibrin biopolymer (HFB)	Thirty rats, three groups. A noncritical bone defect of 2 mm was produced. Four Groups: (1) BM + PBMT; (2) BM + HFB; (3): BM + HFB + PBM. In Groups 1 and 3 the animals were submitted to intraoperative PBM and every 48 h until the period of euthanasia	Statistical difference in bone neoformation between Groups 3 and 2 (26.4% ± 1.03% and 20.0% ± 1.87%, respectively) at 14 days and 42 days (38.2% ± 1.59% and 31.6% ± 1.33%, respectively). In 42 days there was presence of new bone with mature characteristics	The combined use of PBM with HFB and BM contributed to the process of reconstruction of non-critical bone defects
Buchaim et al., 2016 [53] (PubMed)	GaAlAs (Laserpulse IBRAMED ®, Amparo, Brazil)	830 and 30	258.6	6	To evaluate the effects of LLLT in the repair of the buccal branch of the facial nerve with two techniques: coaptation with HFS and end-to-end epineural suture	Heterologous fibrin sealant (HFS)	Forty-two rats, five groups: (1) control (CG), facial nerve (buccal branch) was collected without lesion; (2) experimental suture (EGS) and experimental fibrin (EGF) groups: end-to-end suture on the right side and HFS on the left side; (3) experimental suture laser (EGSL) and experimental fibrin laser (EGFL): plus LLLT	Axonal growth occurred in the distal stump of the facial nerve in all groups. The morphological aspect was similar to the GC fibers, with the majority of myelinated fibers. In the last period of the experiment, the EGSL presented the best results, being closer to the CG, in all measurements performed, except in the axon area	Laser therapy showed better results in facial nerve regeneration, being an effective technique to stimulate the repair process of peripheral nerve injuries

Doan et al., 2020 [54] (Scopus)	MLS laser (ASA laser, Vicenza, Italy)	-/-	-/-	1.27	Two clinical cases with piezoelectric surgery (PES), concentrated growth factors (CGF) and PBM, used in the search to increase the formation of new blood vessels and tissue repair after maxillary sinus lift surgeries with dental implants	Autologous concentrated growth factors (CGF)	The lateral sinus windows were created using PES. The implants were inserted in the same surgery and wrapped with CGF. A laser treatment of PBM was performed at the site, applied in the apical, buccal, lingual, coronal, mesial and distal regions of the surgical wound	Vascular budding and wound closure was observed after the first day. New bone formation was detected in the enlarged maxillary sinuses next to the implants, through radiographs and cone-beam computed tomography	PBM, PES, and CGF promoted the formation of new vessels, favored the approximation of the edges, closing the wound and reducing edema and bleeding. In addition, there was less postoperative pain, less use of analgesics and speech impairment, without trismus

4. Discussion

This systematic review aimed to analyze published research on the association of photobiomodulation therapy, through the use of LLLT or LED, with fibrin scaffolds. The focus was on its use in tissue regeneration, mainly fibrin in the form of PRF and fibrin sealants (glues or adhesives) in order to verify the possible beneficial effects of PBM in three-dimensional fibrin scaffolds.

The initial description of fibrin comes from the classic coagulation cascade, proposed in 1964 by Macfarlane [55] and Davie and Ratnoff [56], documented in several articles. This model referred to as the "cascade" has been proposed to explain the physiology of blood clotting, whereby clotting occurs through sequential proteolytic activation of proenzymes by plasma proteases, resulting in the formation of thrombin, which then breaks down the fibrinogen molecule into fibrin monomers [57]. The fibrin network formed in the clot presents a particularly homogeneous and three-dimensional organization [58]. Furthermore, a progressive polymerization mode means increased incorporation of circulating cytokines in the fibrin meshes (intrinsic cytokines), providing an increase in the lifespan of these cytokines. Thus, cytokines are kept in situ for a convenient period when the scar cells begin to remodel the matrix, at which time they need to be stimulated to participate in the reconstruction of the injured site [59,60].

Due to its characteristics and properties, fibrin has been used in several areas, one of which is tissue regeneration in medical and dental procedures. Among the forms presented, in this review, three studies were selected for qualitative analysis that used hydrogels or 3D fibrin, 3 with L-PRF, 10 with fibrin sealants and 3 with autologous PRF (Figure 2). The production of autologous platelet concentrates (APCs) occurs by centrifuging the patient's own blood, injecting isolated plasma, which is rich in growth factors. In tissue regeneration, two generations of APCs have been used: PRP, which are first generation, produced by double-spin centrifugation of blood; and PRF, the second generation, produced by single-spin centrifugation and has the fibrin matrix network intact. The effectiveness of platelet concentrates in promoting wound healing and tissue regeneration is at the center of recent academic discussion [61].

In a preclinical study, using LLT, PRF and Nano-HA nanohydroxyapatite graft (Fisiograft®, Ghimas, Italy) as variables, Hemaid et al., (2019) observed that the use of PRF + NanoHA mix results in an increase in bone fill and density regarding the radiographic outcomes in induced periodontal intrabony defects in rabbits, and LLLT may improve the results [44]. To prepare the PRF, five-milliliter blood samples were collected from each rabbit and then centrifuged at 30,000 RPM for 15 min. The PRF was separated into two pieces; one was used as a membrane and the other was cut into pieces to be added with Fisiograft® plus Nano-HA.

However, the study by Doan et al., (2020) the clinical applicability of the combination of autologous concentrated growth factors (CGF) and photobiomodulation (PBM) was made. Lateral sinus windows were created using piezoelectric surgery (PES) and the dental implants were concurrently fixated and wrapped with autologous fibrin (AF) rich CGF. Wound sites PBM treatment using a multiwave locked system laser. Bovine demineralized freeze-dried bone (Bio-Oss®, Chatswood, Australia) and hydroxyapatite and calcium triphosphate (Genoss®, Seoul, Korea) were incorporated into CGF for

grafting. The application of AF offers benefits such as being a safe procedure, easy to perform and low cost [54] (Figure 5).

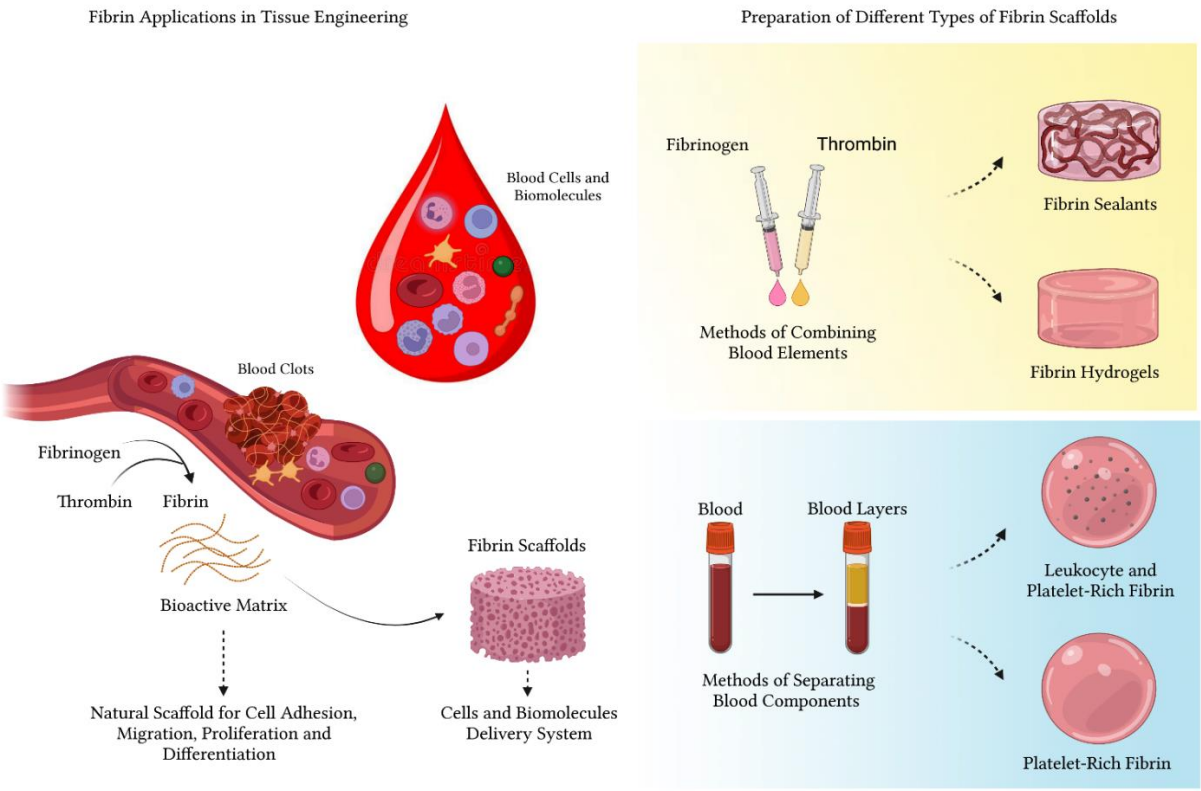

Figure 5. Schematic overview of fibrin applications in tissue regeneration. Fibrin is a plasma protein formed by the action of thrombin on fibrinogen, and constitutes a natural component of the blood coagulation cascade. The three-dimensional structure of the fibrin matrix serves as a natural scaffold that favors cell adhesion, migration, proliferation and differentiation, in addition to favoring the interaction with biomolecules and growth factors. Thus, fibrin has been used to promote tissue regeneration in various segments of medicine, in the form of sealants, hydrogels, PRF or L-PRF.

Three studies used L-PRF, developed in 2001 in France by Dr. Joseph Choukroun [62], during the production technique an attempt was made to accumulate platelets and release cytokines in a fibrin clot. This technique does not require anticoagulants, bovine thrombin or any other gelling agent, unlike other platelet concentrates; it is simply centrifuged natural blood without additives [58,63]. When using L-PRF, there are different methods and protocols in its production. In a pre-clinical study, in critical defects in the calvaria of rats, two methods of obtaining the concentrate were analyzed, by means of high (L-PRF) or low speed (A-PRF) centrifugation. The L-PRF and A-PRF groups had significantly higher bone volume and newly formed bone area than the control group (clot only) and reduced bone porosity values, but with no significant difference between them in the histomorphometric and microtomographic analysis. Therefore, L-PRF and A-PRF potentiated the healing of critical defects, and high and low-speed centrifugation protocols did not produce PRF matrices with different biological impacts on the amount of new bone formation [64].

Leukocyte and platelet-rich fibrin (L-PRF) also have been used widely for bone tissue engineering. L-PRF has the potential to, in cases of bone loss, collaborate in osteogenic differentiation, increase osteoblast

proliferation, tissue neovascularization and lower risk of local contamination [65,66]. The three studies in Table 1 that used L-PRF were combinations with PBM for the treatment of jaw osteonecrosis, all with good and promising results for use in the treatment of this type of bone disease [38,45,48]. Among the growth factors stored in platelets, which are essential for the tissue repair, are PDGF. Also present are VEGF-A, transforming growth factor-beta (TGF-β1), FGF–2, epidermal growth factor (EGF), hepatocyte growth factor (HGF), and insulin-like growth factor–1 (IGF–1) [67]. It should be taken into account the fact that L-PRF does not use the inclusion of anticoagulant and activating agents ($CaCl_2$) to obtain the platelet concentrate. The inclusion of these agents and activators, in addition to hard-centrifugation (≥210 g), can affect the amount and quality of platelet recovery and growth factor release, which can significantly influence healing behavior compared to natural fibrin clotting [68].

Three studies were used hydrogels or 3D fibrin [37,41,42], associated with PBM, being incorporated into the fibrin matrix endothelial cells [41], stromal vascular fraction (SVF) and mesenchymal stromal cells (MSCs) isolated from human gingival mucosa [37]. These studies agree that photobiomodulation combined with fibrin enhances the improvement of results, collaborating in cell and vascular proliferation.

Fibrin sealants were most commonly used in combination with PBM, in ten studies [29,39,40,43,47,49–53]. One of the studies used the fibrin sealant derived from human plasma (Tisseel Lyo® (Baxter Healthcare Ltd., Norfolk, UK) [43] and the others a heterologous fibrin sealant (HFS). This bioproduct (HFS) is composed of a thrombin-like enzyme purified from the venom of *Crotalus durissus terrificus* snake and a cryoprecipitate rich in fibrinogen extracted from *Bubalus bubalis* buffaloes (produced by CEVAP/UNESP—Center for the Study of Venoms and Venomous Animals, Botucatu, Brazil). HFS has several advantages in its use, such as a fast production process, low cost, potential to act as a scaffold for stem cells [69–71] and biomaterials [50,72], and as a new drug delivery system [73]. Its indications are in medical, veterinary and dental practice, due to the possibility of personalized formulation and replacement of conventional sutures. Considering all of the properties described for this bioproduct, which go beyond the adhesive capacity, the name "sealant" was reconsidered, and it has recently been called "fibrin biopolymer" [74,75].

In order to improve the tissue repair process, studies in the area of regenerative science seek the association of different therapies to accelerate and improve morphological recomposition and faster functional recovery. Among these conjunctions, light-based therapies, such as the use of low-power lasers and LEDs, have expanded their use in clinical and pre-clinical practices. The laser consists of a pure and well-defined color, while the LED can display different shades of colors at once. Therefore, the laser is a monochromatic light (only a well-determined color) and the LED is a polychromatic light, being able to present all of the shades of a specific color. Currently called photobiomodulation (PBM), consists of the application of light (Laser or LED) with therapeutic effect for tissue modulation (activation or inhibition). It has important potentialities such as angiogenesis and neovascularization [76], increase in collagen production [77], increase in muscle regeneration and decrease in its atrophy [78], favors nerve regeneration [9,79], increases cartilage production [80], and decreases inflammation, edema and pain [81] (Figure 6).

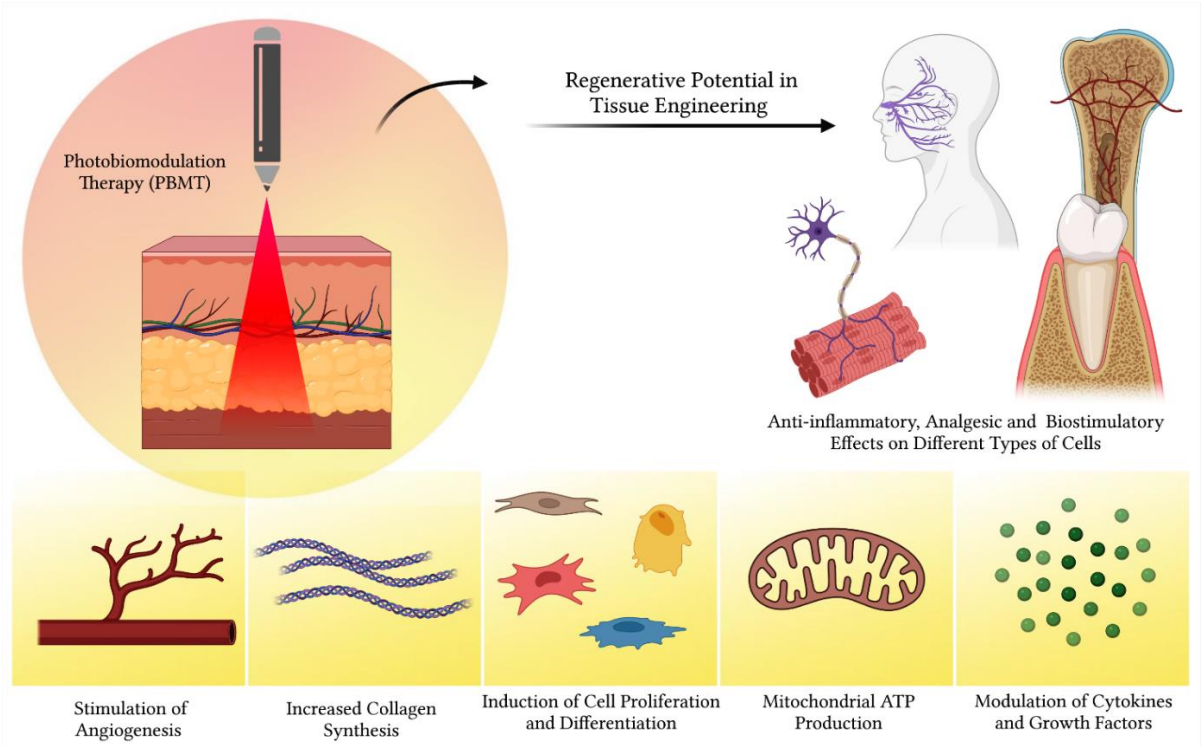

Figure 6. Schematic overview of beneficial properties of photobiomodulation therapy in regenerative medicine. The application of laser therapy favors angiogenesis, collagen synthesis, mitochondrial ATP production, cytokines and growth factors synthesis, in addition to inducing cell proliferation and differentiation. Additionally, photobiomodulation therapy has anti-inflammatory, analgesic and biostimulating effects, acting mainly in the initial stages of tissue healing.

In the studies selected for Table 1, according to the eligibility criteria, three used the red LED, 1 infrared LED, 1 GaAs laser, 10 GaAlAs laser, 2 ND: YAG laser, 1 InGaAlP laser and two studies did not identify the type of laser used (Figure 3). Handler et al. (2021) carried out a study to investigate the effects of photobiomodulation at wavelengths of 660 nm (Aluminium-gallium-indium-phosphide laser, AlGaInP) and 830 nm (Arsenide-Gallium-Aluminum laser, AsGaAl) at different numbers of application points on the healing of open wounds in mice. Photobiomodulation with total energy of 3.6 J was applied at 1, 4, 5 and 9 points for 14 days. When comparing the photobiomodulation wavelength, the 830 nm (AsGaAl) groups were more effective, and the groups irradiated at 5 points stand out, which showed improvement in macroscopic analysis and epidermis thickness, increased number of vessels and lower number of fibroblasts on the 14th day after the skin lesion [82].

Regarding the wavelength, the most used was 830 nm, in nine studies. Then 810 nm in three studies; 475 nm, 516 nm, 635 nm, and 1064 nm in two studies each; 633 nm, 650 nm, 660 nm, 840 nm, and 910 nm with one study each; and one study did not disclose the wavelength used (Figure 4). A study conducted by Ma et al. (2018), to determine the effect of low-level laser therapy (LLLT) on diabetic wound healing and confirm its effect on the activity of healthy human fibroblasts, used PBM with an 830 nm (IR) wavelength, 635 nm (Red) and 635 nm + 830 nm (FX) with the same fluency of 60 J/cm^2. Irradiation in the FX and IR groups showed a significant increase in fibroblast proliferation and collagen synthesis compared to the control and RED groups. However, there was no significant difference in

collagen synthesis and fibroblast proliferation between the FX group and the IR group. These data allowed the authors to conclude that healthy human fibroblasts showed better cell proliferation and collagen synthesis when irradiated at the wavelength of 635 nm + 830 nm or 830 nm [83].

The use of LED photobiomodulation is more recent than laser therapy. Current research advances in the evaluation of the separate or combined use of the two therapies in tissue repair. Doses ranging from 0.1 to 10 J/cm² and wavelengths from 405 to 1000 nm promote therapeutic benefits in tissue regeneration. Ranges of light energy sources, from lasers to LEDs, have been used and have specific advantages and limitations. There is no consensus on standardized treatment parameters such as wavelengths, therapeutic outcomes and doses, which limits direct comparison and clinical protocol recommendation [84–90].

The use of combined therapies that involve the use of fibrin associated with photobiomodulation therapy has shown to be a promising strategy to favor the regeneration of injured tissues with better quality and less time. When fibrin is applied to the lesion site, it forms a bioactive matrix in the microenvironment that exerts a hemostatic effect, in addition to favoring interactions between cells and biomolecules (Figure 7). These effects, added to those of PBM, constitute a supporting therapy that acts by stimulating cell activity, angiogenesis, and the synthesis of collagen and biomolecules [49,91–100].

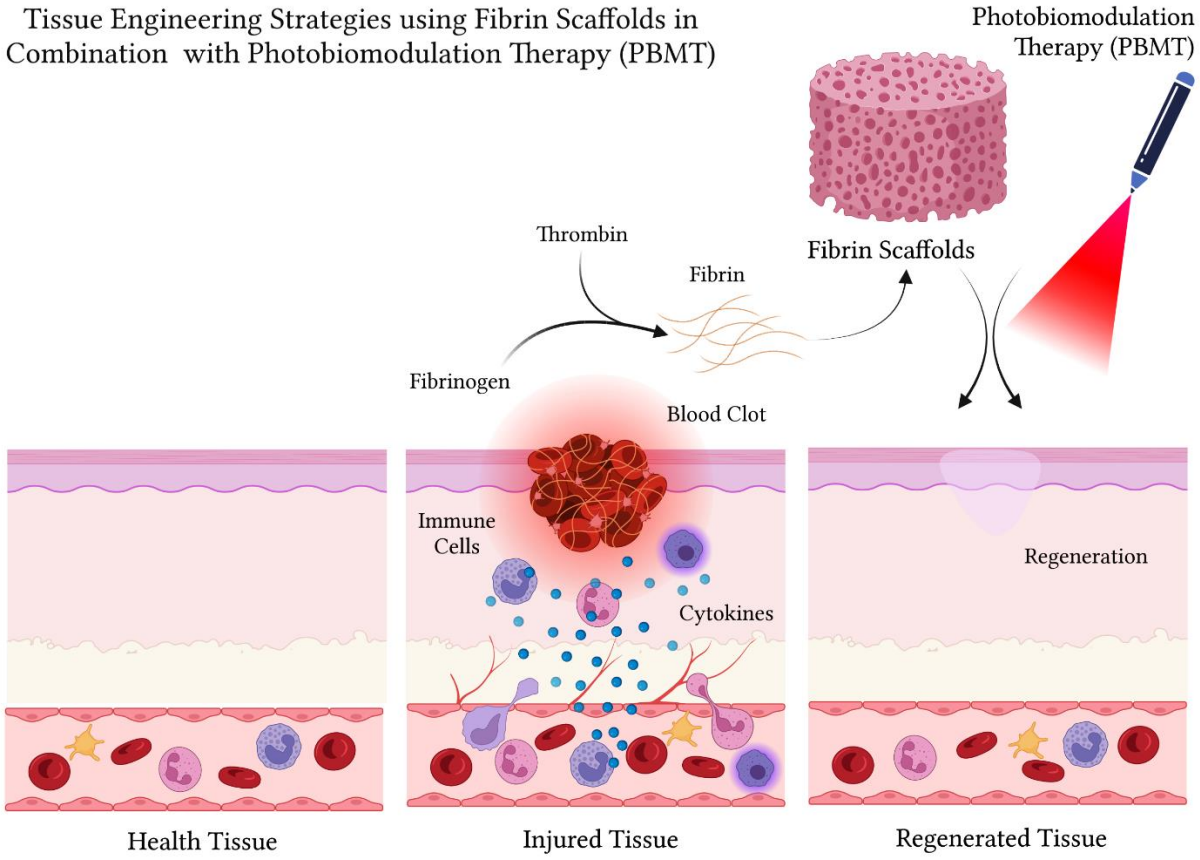

Figure 7. The application of fibrin combined with photobiomodulation therapy constitutes a promising strategy to favor regeneration in tissue engineering. Fibrin applied to the injury site forms a bioactive matrix that exerts a hemostatic effect, in addition to favoring interactions between cells and biomolecules. Photobiomodulation constitutes a coadjuvant therapy that acts by stimulating cell activity, angiogenesis and

the synthesis of collagen and biomolecules. Thus, the application of fibrin associated with photobiomodulation therapy may have a beneficial effect, accelerating tissue healing.

In this review, among preclinical studies, the most researched association of fibrin and photobiomodulation was the use of fibrin sealants in bone or nerve injuries. In clinical studies, the association of PBM with medication-related treatments osteonecrosis of the jaw (MRONJ). All experimental protocols concluded that the association is effective; promoting a more effective repair of lesions, in a shorter period of time and with effectiveness that can reinforce the indication of its use. In peripheral nerves, PBM therapy accelerated morphological and functional nerve repair. In bone tissue [51], PBM allowed for an improvement in the formation of new bone, with a more organized deposition of collagen fibers in the defect area [50]; and in osteonecrosis of the jaw, PBM may effectively contribute to MRONJ management [38,45,101].

In this way, we can see that few studies used the association fibrin + PBM, but given the good results, the technique is promising, with the potential to collaborate in tissue repair. The difficulty in comparing the different types of PBM can be considered as a limitation, due to the different protocols reported in the experiments. Therefore, protocols with favorable results are generally standardized and reused by the same researchers in an attempt to reduce this limitation. In addition, the scarcity of randomized clinical trials in the scope of this review can also be considered a limitation.

5. Conclusions

This review was designed and carried out with the objective of analyzing studies, both clinical and pre-clinical, that used the association of photobiomodulation and fibrin. This association occurs with the purpose of tissue regeneration, in the search for its possible beneficial effects on morphophysiological and functional rehabilitation. The fibrin matrix, with its three-dimensionality, is a natural scaffold, which enables events that favor the repair of injured tissues, which is desired in tissue engineering procedures, through adhesion, migration, proliferation, and cell differentiation, in addition to contributing to the interaction with biomolecules and local tissue growth factors.

In the findings of this study, it can be shown that PBM contributed to improve tissue regeneration that used fibrin composites as scaffolds, constituting an important adjuvant therapy that acts by stimulating cell activity, angiogenesis, osteoblastic activation, axonal growth, anti-inflammatory and anti-edema action, increased collagen synthesis and its maturation, as well as biomolecules. More studies should be carried out in order to seek standardization in PBM protocols, in the same way that new fibrin concentrates will be developed with the same objective of recovering injured organs and tissues.

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References

1. Vilar, R.; Fish, R.J.; Casini, A.; Neerman-Arbez, M. Fibrin(ogen) in human disease: Both friend and foe. *Haematologica* **2020**, *105*, 284–296. https://doi.org/10.3324/haematol.2019.236901.
2. Jahani-Sherafat, S.; Mokmeli, S.; Rostami-Nejad, M.; Razzaghi, Z.; Tavirani, M.R.; Razzaghi, M. The Effectiveness of Photobiomudulation Therapy (Pbmt) in Covid-19 Infection. *J. Lasers Med. Sci.* **2020**, *11*, S23–S29. https://doi.org/10.34172/JLMS.2020.S4.
3. Litvinov, R.I., Pieters, M., de Lange-Loots, Z., Weisel, J.W. Fibrinogen and Fibrin. In *Macromolecular Protein Complexes III: Structure and Function. Subcellular Biochemistry*; Springer: Berlin/Heidelberg, Germany, 2021; pp. 471–501.
4. Reddy, M.S.B.; Ponnamma, D.; Choudhary, R.; Sadasivuni, K.K. A comparative review of natural and synthetic biopolymer composite scaffolds. *Polymers* **2021**, *13*, 1105. https://doi.org/10.3390/polym13071105.
5. Wang, M.; Gao, W. Fixation of platelet-rich plasma and fibrin gels on knee cartilage defects after microfracture with arthroscopy. *Int. Orthop.* **2022,** *474*. https://doi.org/10.1007/s00264-022-05377-2.
6. Grecu, A.F.; Reclaru, L.; Ardelean, L.C.; Nica, O.; Ciucă, E.M.; Ciurea, M.E. Platelet-rich fibrin and its emerging therapeutic benefits for musculoskeletal injury treatment. *Medicine* **2019**, *55*, 141. https://doi.org/10.3390/medicina55050141.
7. Vasilikos, I.; Beck, J.; Ghanaati, S.; Grauvogel, J.; Nisyrios, T.; Grapatsas, K.; Hubbe, U. Integrity of dural closure after autologous platelet rich fibrin augmentation: An in vitro study. *Acta Neurochir.* **2020**, *162*, 737–743. https://doi.org/10.1007/s00701-020-04254-4.
8. Buchaim, D.V.; Cassaro, C.V.; Shindo, J.V.T.C.; Coletta, B.B. Della; Pomini, K.T.; De Oliveira Rosso, M.P.; Campos, L.M.G.; Ferreira, R.S.; Barraviera, B.; Buchaim, R.L. Unique heterologous fibrin biopolymer with hemostatic, adhesive, sealant, scaffold and drug delivery properties: A systematic review. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2019**, *25*, 1–15. https://doi.org/10.1590/1678-9199-jvatitd-2019-0038.
9. Rosso, M.P. de O.; Buchaim, D.V.; Kawano, N.; Furlanette, G.; Pomini, K.T.; Buchaim, R.L. Photobiomodulation therapy (PBMT) in peripheral nerve regeneration: A systematic review. *Bioengineering* **2018**, *5*, 4. https://doi.org/10.3390/bioengineering5020044.
10. Gentile, P.; Calabrese, C.; De Angelis, B.; Dionisi, L.; Pizzicannella, J.; Kothari, A.; De Fazio, D.; Garcovich, S. Impact of the different preparation methods to obtain autologous non-activated platelet-rich plasma (A-PRP) and activated platelet-rich plasma (AA-PRP) in plastic surgery: Wound healing and hair regrowth evaluation. *Int. J. Mol. Sci.* **2020**, *21*, 431. https://doi.org/10.3390/ijms21020431.
11. Bressan, E.; Favero, V.; Gardin, C.; Ferroni, L.; Iacobellis, L.; Favero, L.; Vindigni, V.; Berengo, M.; Sivolella, S.; Zavan, B. Biopolymers for Hard and Soft Engineered Tissues: Application in Odontoiatric and Plastic Surgery Field. *Polymers* **2011**, *3*, 509–526. https://doi.org/10.3390/polym3010509.
12. Pepelassi, E.; Deligianni, M. The Adjunctive Use of Leucocyte-and Platelet-Rich Fibrin in Periodontal Endosseous and Furcation Defects: A Systematic Review and Meta-Analysis. *Materials* **2022**, *15*, 2088. https://doi.org/10.3390/ma15062088.
13. Caramês, J.M.M.; Vieira, F.A.; Caramês, G.B.; Pinto, A.C.; Francisco, H.C.O.; Marques, D.N. da S. Guided Bone Regeneration in the Edentulous Atrophic Maxilla Using Deproteinized Bovine Bone Mineral (DBBM) Combined with Platelet-Rich Fibrin (PRF)—A Prospective Study. *J. Clin. Med.* **2022**, *11*, 894. https://doi.org/10.3390/jcm11030894.
14. Jung, M.H.; Lee, J.H.; Wadhwa, P.; Jiang, H.B.; Jang, H.S.; Lee, E.S. Bone regeneration in peri-implant defect using autogenous tooth biomaterial enriched with platelet-rich fibrin in animal model. *Appl. Sci.* **2020**, *10*, 1939. https://doi.org/10.3390/app10061939.
15. Tallarico, M.; Xhanari, E.; Lumbau, A.M.I.; Alushi, A.; Ieria, I.; Fiorillo, L.; Famà, F.; Meto, A.; Baldoni, E.; Meloni, S.M.; et al. Histological and histomorphometric evaluation of post-extractive sites filled with a new bone substitute with or without autologous plate concentrates: One-year randomized controlled trial. *Materials* **2022**, *15*, 254. https://doi.org/10.3390/ma15010254.
16. Li, Q.; Reed, D.A.; Min, L.; Gopinathan, G.; Li, S.; Dangaria, S.J.; Li, L.; Geng, Y.; Galang, M.T.; Gajendrareddy, P.; et al. Lyophilized Platelet-Rich Fibrin (PRF) promotes craniofacial bone regeneration through Runx2. *Int. J. Mol. Sci.* **2014**, *15*, 8509–8525. https://doi.org/10.3390/ijms15058509.
17. Zumarán, C.C.; Parra, M.V.; Olate, S.A.; Fernández, E.G.; Muñoz, F.T.; Haidar, Z.S. The 3 R's for platelet-rich fibrin: A "super" tri-dimensional biomaterial for contemporary naturally-guided oro-maxillo-facial soft and

hard tissue repair, reconstruction and regeneration. *Materials* **2018**, *11*, 1239. https://doi.org/10.3390/ma11081293.
18. Almeida Barros Mourão, C.F. De; Valiense, H.; Melo, E.R.; Freitas Mourão, N.B.M.; Maia, M.D.C. Obtenção da fibrina rica em plaquetas injetável (I-PRF) e sua polimerização com enxerto ósseo: Nota técnica. *Rev. Col. Bras. Cir.* **2015**, *42*, 421–423. https://doi.org/10.1590/0100-69912015006013.
19. Pietruszka, P.; Chruścicka, I.; Duś-Ilnicka, I.; Paradowska-Stolarz, A. Prp and prf—subgroups and divisions when used in dentistry. *J. Pers. Med.* **2021**, *11*, 944. https://doi.org/10.3390/jpm11100944.
20. Ikumi, A.; Gingery, A.; Toyoshima, Y.; Zhao, C.; Moran, S.L.; Livia, C.; Rolland, T.; Peterson, T.; Sabbah, M.S.; Boroumand, S.; et al. Administration of Purified Exosome Product in a Rat Sciatic Serve Reverse Autograft Model. *Plast. Reconstr. Surg*. **2021,** *148*, 200e–211e. https://doi.org/10.1097/PRS.0000000000008202.
21. Ardjomandi, N.; Duttenhoefer, F.; Xavier, S.; Oshima, T.; Kuenz, A.; Sauerbier, S. In vivo comparison of hard tissue regeneration with ovine mesenchymal stem cells processed with either the FICOLL method or the BMAC method. *J. Craniomaxillofac. Surg.* **2015**, *43*, 1177–1183. https://doi.org/10.1016/j.jcms.2015.05.020.
22. Mittermayr, R.; Branski, L.; Moritz, M.; Jeschke, M.G.; Herndon, D.N.; Traber, D.; Schense, J.; Gampfer, J.; Goppelt, A.; Redl, H. Fibrin biomatrix-conjugated platelet-derived growth factor AB accelerates wound healing in severe thermal injury. *J. Tissue Eng Regen Med.* **2016**, *10*, E275–E285. https://doi.org/10.1002/term.1749.
23. Hidd, S.M.C.M.; Tim, C.R.; Dutra, E.F. Jr.; Maia Filho, A.L.M.; Assis, L.; Ferreira, R.S., Jr.; Barraviera, B.; Silva, J.F.; Amaral, M.M. Fibrin biopolymer sealant and aquatic exercise association for calcaneal tendon repair. *Acta Cir. Bras*. **2021**, *36*, e360407. https://doi.org/10.1590/ACB360407.
24. Canonico, S. The use of human fibrin glue in the surgical operations. *Acta Biomed.* **2003**, *74*, 21–25.
25. Su, Y.Y.; Lin, Y.S.; Yang, L.Y.; Pan, Y. Bin; Huang, Y.T.; Weng, C.H.; Wu, K.Y.; Wang, C.J. Use of human fibrin glue (Tisseel) versus suture during transvaginal natural orifice ovarian cystectomy of benign and non-endometriotic ovarian tumor: A retrospective comparative study. *BMC Surg.* **2021**, *21*, 1–8. https://doi.org/10.1186/s12893-021-01061-1.
26. Ferreira, R.S.; de Barros, L.C.; Abbade, L.P.F.; Barraviera, S.R.C.S.; Silvares, M.R.C.; de Pontes, L.G.; dos Santos, L.D.; Barraviera, B. Heterologous fibrin sealant derived from snake venom: From bench to bedside—An overview. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2017**, *23*, 1–12. https://doi.org/10.1186/s40409-017-0109-8.
27. Anders, J.J.; Lanzafame, R.J.; Arany, P.R. Low-level light/laser therapy versus photobiomodulation therapy. *Photomed. Laser Surg.* **2015**, *33*, 183–184. https://doi.org/10.1089/pho.2015.9848.
28. Hamblin, M.R. Photobiomodulation or low-level laser therapy. *J. Biophotonics* **2016**, *9*, 1122–1124. https://doi.org/10.1002/jbio.201670113.
29. Buchaim, R.L.; Andreo, J.C.; Barraviera, B.; Ferreira Junior, R.S.; Buchaim, D.V.; Rosa Junior, G.M.; De Oliveira, A.L.R.; De Castro Rodrigues, A. Effect of low-level laser therapy (LLLT) on peripheral nerve regeneration using fibrin glue derived from snake venom. *Injury* **2015**, *46*, 655–660. https://doi.org/10.1016/j.injury.2015.01.031.
30. Iatecola, A.; Barraviera, B.; Ferreira, R.S. Jr.; dos Santos, G.R.; Neves, J.I.; da Cunha, M.R. Use of a new fibrin sealant and laser irradiation in the repair of skull defects in rats. *Braz. Dent. J.* **2013**, *24*, 456–461. https://doi.org/10.1590/0103-6440201302265.
31. Santos, C.M. da C.; Pimenta, C.A. de M.; Nobre, M.R.C. The PICO strategy for the research question construction and evidence search. *Rev. Lat. Am. Enfermagem* **2007**, *15*, 508–511. https://doi.org/10.1590/s0104-11692007000300023.
32. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; Altman, D.; Antes, G.; Atkins, D.; Barbour, V.; Barrowman, N.; Berlin, J.A.; et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med.* **2009**, *6*, e1000097. https://doi.org/10.1371/journal.pmed.1000097.
33. Gentile, P.; Garcovich, S. Systematic review—the potential implications of different platelet-rich plasma (Prp) concentrations in regenerative medicine for tissue repair. *Int. J. Mol. Sci.* **2020**, *21*, 5702. https://doi.org/10.3390/ijms21165702.
34. Rosso, M.P. de O.; Buchaim, D.V.; Pomini, K.T.; Coletta, B.B. Della; Reis, C.H.B.; Pilon, J.P.G.; Júnior, G.D.; Buchaim, R.L. Photobiomodulation therapy (PBMT) applied in bone reconstructive surgery using bovine bone grafts: A systematic review. *Materials* **2019**, *12*, 4051. https://doi.org/10.3390/ma1224051.
35. Ortiz, A.d.C.; Fideles, S.O.M.; Pomini, K.T.; Bellini, M.Z.; Pereira, E.S.B.M.; Reis, C.H.B.; Pilon, J.P.G.; de Marchi, M.Â.; Trazzi, B.F.d.M.; da Silva, W.S. et al. Potential of Fibrin Glue and Mesenchymal Stem Cells (MSCs) to Regenerate Nerve Injuries: A Systematic Review. *Cells* **2022**, *11*, 221. https://doi.org/10.3390/cells11020221.
36. Ortiz, A.d.C.; Fideles, S.O.M.; Pomini, K.T.; Reis, C.H.B.; Bueno, C.R.d.S.; Pereira, E.d.S.B.M.; Rossi, J.d.O.; Novais, P.C.; Pilon, J.P.G.; Rosa Junior, G.M. et al. E. Effects of Therapy with Fibrin Glue combined with Mesenchymal Stem Cells (MSCs) on Bone Regeneration: A Systematic Review. *Cells* **2021**, *10*, 2323. https://doi.org/10.3390/cells10092323.

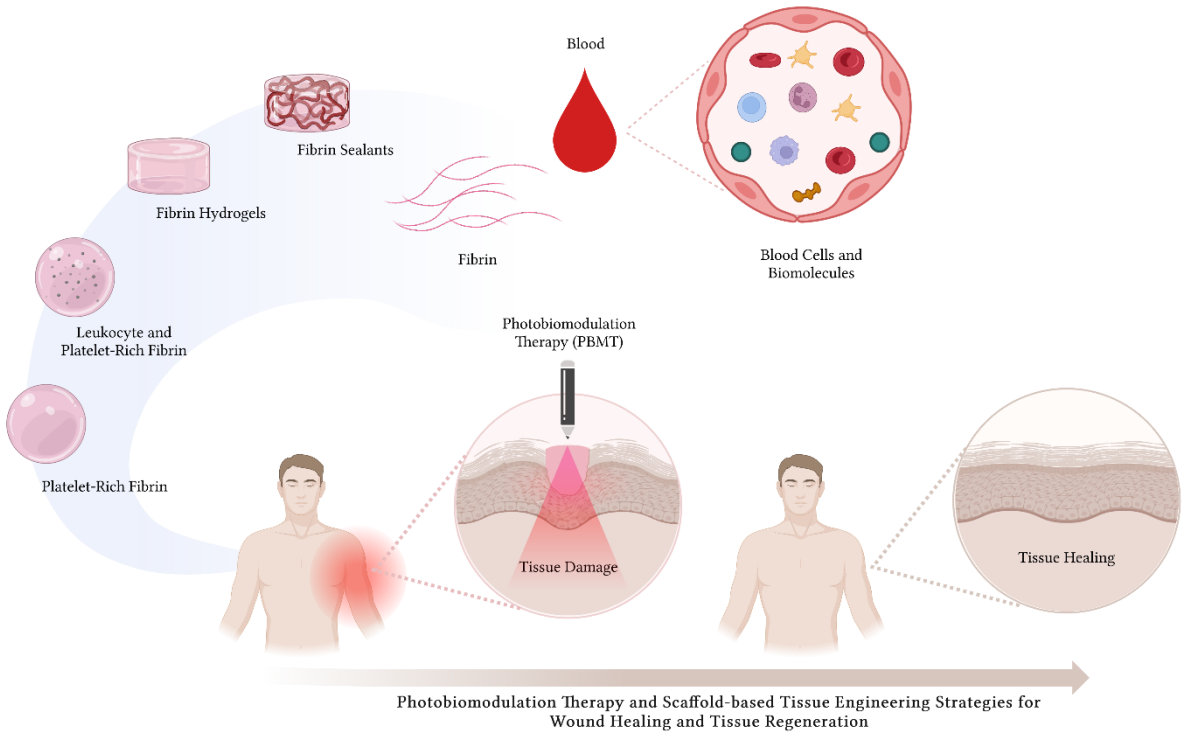
37. Bikmulina, P.Y.; Kosheleva, N.V.; Shpichka, A.I.; Efremov, Y.M.; Yusupov, V.I.; Timashev, P.S.; Rochev, Y.A. Beyond 2D: Effects of photobiomodulation in 3D tissue-like systems. *J. Biomed. Opt.* **2020**, *25*, 1. https://doi.org/10.1117/1.jbo.25.4.048001.
38. Tenore, G.; Zimbalatti, A.; Rocchetti, F.; Graniero, F.; Gaglioti, D.; Mohsen, A.; Caputo, M.; Lollobrigida, M.; Lamazza, L.; De Biase, A.; et al. Management of medication-related osteonecrosis of the jaw (MRONJ) using leukocyte-and platelet-rich fibrin (l-PRF) and photobiomodulation: A retrospective study. *J. Clin. Med.* **2020**, *9*,3505. https://doi.org/10.3390/jcm9113505.
39. de Oliveira Gonçalves, J.B.; Buchaim, D.V.; de Souza Bueno, C.R.; Pomini, K.T.; Barraviera, B.; Júnior, R.S.F.; Andreo, J.C.; de Castro Rodrigues, A.; Cestari, T.M.; Buchaim, R.L. Effects of low-level laser therapy on autogenous bone graft stabilized with a new heterologous fibrin sealant. *J. Photochem. Photobiol. B Biol.* **2016**, *162*, 663–668. https://doi.org/10.1016/j.jphotobiol.2016.07.023.
40. Buchaim, D.V.; Andreo, J.C.; Ferreira Junior, R.S.; Barraviera, B.; De Castro Rodrigues, A.; De Cássia MacEdo, M.; Rosa Junior, G.M.; Shinohara, A.L.; German, I.J.S.; Pomini, K.T.; et al. Efficacy of Laser Photobiomodulation on Morphological and Functional Repair of the Facial Nerve. *Photomed. Laser Surg.* **2017**, *35*, 442–449. https://doi.org/10.1089/pho.2016.4204.
41. Rohringer, S.; Holnthoner, W.; Chaudary, S.; Slezak, P.; Priglinger, E.; Strassl, M.; Pill, K.; Mühleder, S.; Redl, H.; Dungel, P. The impact of wavelengths of LED light-therapy on endothelial cells. *Sci. Rep.* **2017**, *7*, 11061. https://doi.org/10.1038/s41598-017-11061-y.
42. Priglinger, E.; Maier, J.; Chaudary, S.; Lindner, C.; Wurzer, C.; Rieger, S.; Redl, H.; Wolbank, S.; Dungel, P. Photobiomodulation of freshly isolated human adipose tissue-derived stromal vascular fraction cells by pulsed light-emitting diodes for direct clinical application. *J. Tissue Eng. Regen. Med.* **2018**, *12*, 1352–1362. https://doi.org/10.1002/term.2665.
43. Pomini, K.T.; Buchaim, D.V.; Andreo, J.C.; Rosso, M.P.; Della Coletta, B.B.; German, Í.J.S.; Biguetti, A.C.C.; Shinohara, A.L.; Rosa Júnior, G.M.; Shindo, J.V.T.C.; et al. Fibrin sealant derived from human plasma as a scaffold for bone grafts associated with photobiomodulation therapy. *Int. J. Mol. Sci.* **2019**, *20*, 1761. https://doi.org/10.3390/ijms20071761.
44. Hemaid, S.; Saafan, A.; Hosny, M.; Wimmer, G. Enhancement of healing of periodontal intrabony defects using 810 nm diode laser and different advanced treatment modalities: A blind experimental study. *Open Access Maced. J. Med. Sci.* **2019**, *7*, 1847–1853. https://doi.org/10.3889/oamjms.2019.484.
45. Şahin, O.; Tatar, B.; Ekmekcioğlu, C.; Aliyev, T.; Odabaşi, O. Prevention of medication related osteonecrosis of the jaw after dentoalveolar surgery: An institution's experience. *J. Clin. Exp. Dent.* **2020**, *12*, e771–e776. https://doi.org/10.4317/jced.56837.
46. Thalaimalai, D.B.R.; Victor, D.J.; Prakash, P.S.G.; Subramaniam, S.; Cholan, P.K. Effect of Low-Level Laser Therapy and Platelet-Rich Fibrin on the Treatment of Intra-bony Defects. *J. Lasers Med. Sci.* **2020**, *11*, 456–463. https://doi.org/10.34172/JLMS.2020.71.
47. Della Coletta, B.B.; Jacob, T.B.; Moreira, L.A. de C.; Pomini, K.T.; Buchaim, D.V.; Eleutério, R.G.; Pereira, E. de S.B.M.; Roque, D.D.; Rosso, M.P. de O.; Shindo, J.V.T.C.; et al. Photobiomodulation Therapy on the Guided Bone Regeneration Process in Defects Filled by Biphasic Calcium Phosphate Associated with Fibrin Biopolymer. *Molecules* **2021**, *26*, 847. https://doi.org/10.3390/molecules26040847.
48. Şahin, O.; Akan, E.; Tatar, B.; Ekmekcioğlu, C.; Ünal, N.; Odabaşı, O. Combined approach to treatment of advanced stages of medication-related osteonecrosis of the jaw patients. *Braz. J. Otorhinolaryngol.* **2021**, *88*, 613–620. https://doi.org/10.1016/j.bjorl.2021.04.004.
49. de Freitas Dutra Júnior, E.; Hidd, S.M.C.M.; Amaral, M.M.; Filho, A.L.M.M.; Assis, L.; Ferreira, R.S.; Barraviera, B.; Martignago, C.C.S.; Figueredo-Silva, J.; de Oliveira, R.A.; et al. Treatment of partial injury of the calcaneus tendon with heterologous fibrin biopolymer and/or photobiomodulation in rats. *Lasers Med. Sci.* **2022**, *37*, 971–981. https://doi.org/10.1007/s10103-021-03341-x.
50. Buchaim, D.V.; Andreo, J.C.; Pomini, K.T.; Barraviera, B.; Ferreira, R.S.; Duarte, M.A.H.; Alcalde, M.P.; Reis, C.H.B.; de Bortoli Teixeira, D.; de Souza Bueno, C.R.; et al. A biocomplex to repair experimental critical size defects associated with photobiomodulation therapy. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2022**, *28*, 1–14. https://doi.org/10.1590/1678-9199-JVATITD-2021-0056.
51. Rosso, M.P. de O.; Rosa Júnior, G.M.; Buchaim, D.V.; German, I.J.S.; Pomini, K.T.; de Souza, R.G.; Pereira, M.; Favaretto Júnior, I.A.; Bueno, C.R. de S.; Gonçalves, J.B. de O.; et al. Stimulation of morphofunctional repair of the facial nerve with photobiomodulation, using the end-to-side technique or a new heterologous fibrin sealant. *J. Photochem. Photobiol. B Biol.* **2017**, *175*, 20–28. https://doi.org/10.1016/j.jphotobiol.2017.08.023.
52. de Oliveira Rosso, M.P.; Oyadomari, A.T.; Pomini, K.T.; Coletta, B.B. Della; Shindo, J.V.T.C.; Júnior, R.S.F.; Barraviera, B.; Cassaro, C.V.; Buchaim, D.V.; Teixeira, D. de B.; et al. Photobiomodulation therapy associated with heterologous fibrin biopolymer and bovine bone matrix helps to reconstruct long bones. *Biomolecules* **2020**, *10*, 383. https://doi.org/10.3390/biom10030383.
53. Buchaim, D.V.; Rodrigues, A.C.; Buchaim, R.L.; Barraviera, B.; Junior, R.S.F.; Junior, G.M.R.; Bueno, C.R.S.; Roque, D.D.; Dias, D.V.; Dare, L.R.; et al. The new heterologous fibrin sealant in combination with low-level

laser therapy (LLLT) in the repair of the buccal branch of the facial nerve. *Lasers Med. Sci.* **2016**, *31*, 965–972. https://doi.org/10.1007/s10103-016-1939-2.
54. Doan, N.V.; Huynh, T.Q.; Tran, S.; Wang, G.; Hamlet, S.; Dau, V.; Dao, D.; Nguyen, N.T.; Nguyen, H.T.; Doan, J.; et al. Multidisciplinary approach to maximize angiogenesis and wound healing using piezoelectric surgery, concentrated growth factors and photobiomodulation for dental implant placement surgery involving lateral wall sinus lift: Two case reports. *Vasc. Cell* **2020**, *12*, 2. https://doi.org/10.24238/13221-12-1-186.
55. Macfarlane, R.G. An Enzyme Cascade in the Blood Clotting Mechanism, and its Function as a Biochemical Amplifier. *Nature* **1964**, *202*, 498–499.
56. Davie, E.W.; Ratnoff, O.D. Waterfall sequence for intrinsec blood clotting. *Science* **1964**, *145*, 1310–1312.
57. Ferreira, C.N.; Sousa, M.O.; Dusse, L.M.S.; Carvalho, M.G. A cell-based model of coagulation and its implications. *Rev. Bras. Hematol. Hemoter.* **2010**, *32*, 416–421. https://doi.org/10.1590/S1516-84842010000500016.
58. Dohan, D.M.; Choukroun, J.; Diss, A.; Dohan, S.L.; Dohan, A.J.J.; Mouhyi, J.; Gogly, B. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol Endodontology* **2006**, *101*, e45–e50. https://doi.org/10.1016/j.tripleo.2005.07.009.
59. Van Hinsbergh, V.W.M.; Collen, A.; Koolwijk, P. Role of fibrin matrix in angiogenesis. *Ann. N. Y. Acad. Sci.* **2001**, *936*, 426–437. https://doi.org/10.1111/j.1749-6632.2001.tb03526.x.
60. Rodrigues, G.; Fabris, V.; Mallmann, F.; Rech, C.A.; Carvalho, R.V.; Ruschel, G.H. Fibrinas Ricas em Plaquetas, Uma Alternativa para Regeneração Tecidual: Revisão de Literatura. *J. Oral Investig.* **2015**, *4*, 57–62. https://doi.org/10.18256/2238-510x/j.oralinvestigations.v4n2p57-62.
61. Khurshid, Z.; Asiri;, F.Y.I.; Najeeb;, S.; Ratnayake, J. The Impact of Autologous Platelet Concentrates on the Periapical Tissues and Root Development of Replanted Teeth: A Systematic Review. *Materials* **2022**, *15*, 2776.
62. Choukroun, J.; Adda, F.; Schoeffler, C.; Vervelle, A. An opportunity in perio-implantology: The PRF. *Implantodontie* **2001**, *42*, 55–62.
63. Sahu, K.; Jadhav, S.; Khan, S.S. nabi; Singh, N.; Khan, M.; Agarwal, A. Choukroun's platelet-rich fibrin (L-PRF): A benevolence to surgical and reconstructive dentistry. *Int. J. Oral Care Res.* **2020**, *8*, 45. https://doi.org/10.4103/injo.injo_26_20.
64. Moitrel, L.; Souza, D. De Sávio, F.; Ávila, C. De Vicente, R.M.; Guerra, G.; Reis, D.; Denardi, J.; Malu, N.; Henrique, P.; et al. Comparison of the effects of platelet concentrates produced by high and low-speed centrifugation protocols on the healing of critical-size defects in rat calvaria: A microtomographic and histomorphometric study low-speed centrifugation protocols on the h. *Platelets* **2022**, *00*, 1–10. https://doi.org/10.1080/09537104.2022.2071851.
65. Maaruf, N.A.; Jusoh, N. Angiogenic and Osteogenic Properties of Fibrin in Bone Tissue Engineering. *Mal. J. Med. Health Sci.* **2022**, *18*, 85–94.
66. do Lago, E.S.; Ferreira, S.; Garcia, I.R.; Okamoto, R.; Mariano, R.C. Improvement of bone repair with l-PRF and bovine bone in calvaria of rats. histometric and immunohistochemical study. *Clin. Oral Investig.* **2020**, *24*, 1637–1650. https://doi.org/10.1007/s00784-019-03018-4.
67. Crisci, A.; Marotta, G.; Licito, A.; Serra, E.; Benincasa, G.; Crisci, M. Use of Leukocyte Platelet (L-PRF) Rich Fibrin in Diabetic Foot Ulcer with Osteomyelitis (Three Clinical Cases Report). *Diseases* **2018**, *6*, 30. https://doi.org/10.3390/diseases6020030.
68. Fernández-Medina, T.; Vaquette, C.; Ivanovski, S. Systematic comparison of the effect of four clinical-grade platelet rich hemoderivatives on osteoblast behaviour. *Int. J. Mol. Sci.* **2019**, *20*, 6243. https://doi.org/10.3390/ijms20246243.
69. Orsi, P.R.; Landim-Alvarenga, F.C.; Justulin, L.A.; Kaneno, R.; De Assis Golim, M.; Dos Santos, D.C.; Creste, C.F.Z.; Oba, E.; Maia, L.; Barraviera, B.; et al. A unique heterologous fibrin sealant (HFS) as a candidate biological scaffold for mesenchymal stem cells in osteoporotic rats. *Stem Cell Res. Ther.* **2017**, *8*, 654. https://doi.org/10.1186/s13287-017-0654-7.
70. Cassaro, C.V.; Justulin, L.A.; De Lima, P.R.; De Assis Golim, M.; Biscola, N.P.; De Castro, M.V.; De Oliveira, A.L.R.; Doiche, D.P.; Pereira, E.J.; Ferreira, R.S.; et al. Fibrin biopolymer as scaffold candidate to treat bone defects in rats. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2019**, *25*, 1–17. https://doi.org/10.1590/1678-9199-jvatitd-2019-0027.
71. Mozafari, R.; Kyrylenko, S.; Castro, M.V.; Ferreira, R.S.; Barraviera, B.; Oliveira, A.L.R. Combination of heterologous fibrin sealant and bioengineered human embryonic stem cells to improve regeneration following autogenous sciatic nerve grafting repair. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2018**, *24*, 1–16. https://doi.org/10.1186/s40409-018-0147-x.
72. Reis, C.H.B.; Buchaim, R.L.; Pomini, K.T.; Hamzé, A.L.; Zattiti, I.V.; Duarte, M.A.H.; Alcalde, M.P.; Barraviera, B.; Ferreira Júnior, R.S.; Pontes, F.M.L.; et al. Effects of a Biocomplex Formed by Two Scaffold Biomaterials, Hydroxyapatite/Tricalcium Phosphate Ceramic and Fibrin Biopolymer, with Photobiomodulation, on Bone Repair. *Polymers* **2022**, *14*, 2075. https://doi.org/10.3390/polym14102075.
73. Venante, H.S.; Chappuis-Chocano, A.P.; Marcillo-Toala, O.O.; Da Silva, R.A.; Da Costa, R.M.B.; Pordeus, M.D.; Barraviera, B.; Junior, R.S.F.; Lara, V.S.; Neppelenbroek, K.H.; et al. Fibrin biopolymer incorporated with

antimicrobial agents: A proposal for coating denture bases. *Materials* **2021**, *14*, 1618. https://doi.org/10.3390/ma14071618.
74. Buchaim, R.L.; Goissis, G.; Andreo, J.C.; Roque, D.D.; Roque, J.S.; Buchaim, D.V.; Rodrigues, A.d.C. Biocompatibility of anionic collagen matrices and its influence on the orientation of cellular growth. *Braz. Dent. Sci.* **2007**, *10*, 12–20. https://doi.org/10.14295/bds.2007.v10i3.272
75. Buchaim, R.L.; Buchaim, D.V. Laser therapy together with a fibrin biopolymer improves nerve and bone tissue regeneration. *SciELO Perspect*, 6 June 2022, P2022. https://pressreleases.scielo.org/en/2022/06/06/laser-therapy-together-with-a-fibrin-biopolymer-improves-nerve-and-bone-tissue-regeneration/
76. Simões, T.M.S.; Fernandes Neto, J. de A.; de Oliveira, T.K.B.; Nonaka, C.F.W.; Catão, M.H.C. de V. Photobiomodulation of red and green lights in the repair process of third-degree skin burns. *Lasers Med. Sci.* **2020**, *35*, 51–61. https://doi.org/10.1007/s10103-019-02776-7.
77. Souza, C.; Jayme, C.C.; Rezende, N.; Tedesco, A.C. Synergistic effect of photobiomodulation and phthalocyanine photosensitizer on fibroblast signaling responses in an in vitro three-dimensional microenvironment. *J. Photochem. Photobiol. B Biol.* **2021**, *222*, 112256. https://doi.org/10.1016/j.jphotobiol.2021.112256.
78. Mandelbaum-Livnat, M.M.; Almog, M.; Nissan, M.; Loeb, E.; Shapira, Y.; Rochkind, S. Photobiomodulation triple treatment in peripheral nerve injury: Nerve and muscle response. *Photomed. Laser Surg.* **2016**, *34*, 638–645. https://doi.org/10.1089/pho.2016.4095.
79. Della Santa, G.M.L.; Ferreira, M.C.; Machado, T.P.G.; Oliveira, M.X.; Santos, A.P. Effects of Photobiomodulation Therapy (LED 630 nm) on Muscle and Nerve Histomorphometry after Axonotmesis. *Photochem. Photobiol.* **2021**, *97*, 1116–1122. https://doi.org/10.1111/php.13415.
80. Tim, C.R.; Martignago, C.C.S.; Assis, L.; Neves, L.M.; Andrade, A.L.; Silva, N.C.; Parizotto, N.; Pinto, K.Z.; Rennó, A.C. Effects of photobiomodulation therapy in chondrocyte response by in vitro experiments and experimental model of osteoarthritis in the knee of rats. *Lasers Med. Sci.* **2022**, *37*, 1677–1686. https://doi.org/10.1007/s10103-021-03417-8.
81. Gonçalves, A.B.; Bovo, J.L.; Goines, B.S.; Pigoso, A.A.; Felonato, M.; Esquisatto, M.A.M.; de Jesus Lopes Filho, G.; do Bomfim, F.R.C. Photobiomodulation (λ = 808nm) and Platelet-Rich Plasma (PRP) for the Treatment of Acute Rheumatoid Arthritis in Wistar Rats. *J. Lasers Med. Sci.* **2021**, *12*, e60. https://doi.org/10.34172/jlms.2021.60.
82. Hendler, K.G.; Canever, J.B.; de Souza, L.G.; das Neves, L.M.S.; de Cássia Registro Fonseca, M.; Kuriki, H.U.; da Silva Aguiar Junior, A.; Barbosa, R.I.; Marcolino, A.M. Comparison of photobiomodulation in the treatment of skin injury with an open wound in mice. *Lasers Med. Sci.* **2021**, *36*, 1845–1854. https://doi.org/10.1007/s10103-020-03216-7.
83. Ma, H.; Yang, J.-P.; Tan, R.K.; Lee, H.-W.; Han, S.-K. Effect of Low-Level Laser Therapy on Proliferation and Collagen Synthesis of Human Fibroblasts in Vitro. *J. Wound Manag. Res.* **2018**, *14*, 1–6. https://doi.org/10.22467/jwmr.2018.00283.
84. Mosca, R.C.; Ong, A.A.; Albasha, O.; Bass, K.; Arany, P. Photobiomodulation Therapy for Wound Care: A Potent, Noninvasive, Photoceutical Approach. *Adv. Ski. Wound Care* **2019**, *32*, 157–167.
85. Escudero, J.S.B.; Perez, M.G.B.; de Oliveira Rosso, M.P.; Buchaim, D.V.; Pomini, K.T.; Campos, L.M.G.; Audi, M.; Buchaim, R.L. Photobiomodulation therapy (PBMT) in bone repair: A systematic review. *Injury* **2019**, *50*, 1853–1867. https://doi.org/10.1016/j.injury.2019.09.031.
86. Nica, D.F.; Riviș, M.; Roi, C.I.; Todea, C.D.; Duma, V.F.; Sinescu, C. Complementarity of photo-biomodulation, surgical treatment, and antibiotherapy for medication-related osteonecrosis of the jaws (Mronj). *Medicine* **2021**, *57*, 145. https://doi.org/10.3390/medicina57020145.
87. Mosca, R.C.; Santos, S.N.; Nogueira, G.E.C.; Pereira, D.L.; Costa, F.C.; Pereira, J.X.; Zeituni, C.A.; Arany, P.R. The Efficacy of Photobiomodulation Therapy in Improving Tissue Resilience and Healing of Radiation Skin Damage. *Photonics* **2022**, *9*, 10. https://doi.org/10.3390/photonics9010010.
88. Luca, R.E.; Giuliani, A.; Mănescu, A.; Heredea, R.; Hoinoiu, B.; Constantin, G.D.; Duma, V.-F.; Todea, C.D. Osteogenic Potential of Bovine Bone Graft in Combination with Laser Photobiomodulation: An Ex Vivo Demonstrative Study in Wistar Rats by Cross-Linked Studies Based on Synchrotron Microtomography and Histology. *Int. J. Mol. Sci.* **2020**, *21*, 778.
89. Borges, R.M.M.; Cardoso, D.S.; Flores, B.C.; da Luz, R.D.; Machado, C.R.; Cerveira, G.P.; Daitx, R.B.; Dohnert, M.B. Effects of different photobiomodulation dosimetries on temporomandibular dysfunction: A randomized, double-blind, placebo-controlled clinical trial. *Lasers Med. Sci.* **2018**, *33*, 1859–1866. https://doi.org/10.1007/s10103-018-2533-6.
90. Pinheiro, A.L.; Gerbi, M.E. Photoengineering of bone repair processes. *Photomed Laser Surg*. **2006**, *24*, 169–178. https://doi.org/10.1089/pho.2006.24.169.
91. Colombo, E.; Signore, A.; Aicardi, S.; Zekiy, A.; Utyuzh, A.; Benedicenti, S.; Amaroli, A. Experimental and Clinical Applications of Red and Near-Infrared Photobiomodulation on Endothelial Dysfunction: A Review. *Biomedicines* **2021**, *9*, 274. https://doi.org/10.3390/biomedicines9030274.

92. Mendes, C.; Dos Santos Haupenthal, D.P.; Zaccaron, R.P.; de Bem Silveira, G.; Corrêa, M.E.A.B.; de Roch Casagrande, L.; de Sousa Mariano, S.; de Souza Silva, J.I.; de Andrade, T.A.M.; Feuser, P.E.; et al. Effects of the Association between Photobiomodulation and Hyaluronic Acid Linked Gold Nanoparticles in Wound Healing. *ACS Biomater. Sci. Eng.* **2020**, *6*, 5132–5144. https://doi.org/10.1021/acsbiomaterials.0c00294.
93. Tripodi, N.; Corcoran, D.; Antonello, P.; Balic, N.; Caddy, D.; Knight, A.; Meehan, C.; Sidiroglou, F.; Fraser, S.; Kiatos, D.; et al; The effects of photobiomodulation on human dermal fibroblasts in vitro: A systematic review. *J. Photochem. Photobiol. B.* **2021**, *214*, 112100. https://doi.org/10.1016/j.jphotobiol.2020.112100.
94. Cios, A.; Ciepielak, M.; Szymański, Ł.; Lewicka, A.; Cierniak, S.; Stankiewicz, W.; Mendrycka, M.; Lewicki, S. Effect of Different Wavelengths of Laser Irradiation on the Skin Cells. *Int. J. Mol. Sci.* **2021**, *22*, 2437. https://doi.org/10.3390/ijms22052437.
95. Ailioaie, L.M.; Litscher, G. Curcumin and Photobiomodulation in Chronic Viral Hepatitis and Hepatocellular Carcinoma. *Int. J. Mol. Sci.* **2020**, *21*, 7150. https://doi.org/10.3390/ijms21197150.
96. Kumar Rajendran, N.; George, B.P.; Chandran, R.; Tynga, I.M.; Houreld, N.; Abrahamse, H. The Influence of Light on Reactive Oxygen Species and NF-κB in Disease Progression. *Antioxidants* **2019**, *8*, 640. https://doi.org/10.3390/antiox8120640.
97. Spannbauer, A.; Mester-Tonczar, J.; Traxler, D.; Kastner, N.; Zlabinger, K.; Hašimbegović, E.; Riesenhuber, M.; Pavo, N.; Goliasch, G.; Gyöngyösi, M. Large Animal Models of Cell-Free Cardiac Regeneration. *Biomolecules* **2020**, *10*, 1392. https://doi.org/10.3390/biom10101392.
98. Inchingolo, F.; Hazballa, D.; Inchingolo, A.D.; Malcangi, G.; Marinelli, G.; Mancini, A.; Maggiore, M.E.; Bordea, I.R.; Scarano, A.; Farronato, M.; et al. Innovative Concepts and Recent Breakthrough for Engineered Graft and Constructs for Bone Regeneration: A Literature Systematic Review. *Materials* **2022**, *15*, 1120. https://doi.org/10.3390/ma15031120.
99. Chaudary, S.; Karner, L.; Weidinger, A.; Meixner, B.; Rieger, S.; Metzger, M.; Zipperle, J.; Dungel, P. In vitro effects of 635 nm photobiomodulation under hypoxia/reoxygenation culture conditions. *J. Photochem. Photobiol. B.* **2020**, *209*, 111935. https://doi.org/10.1016/j.jphotobiol.2020.111935.
100. Dos Santos Ferreira, F.; Cadoná, F.C.; Aurélio, A.R.; de Oliveira Martins, T.N.; Pivetta, H.M.F. Photobiomodulation-blue and red LED: Protection or cellular toxicity? In vitro study with human fibroblasts. *Lasers Med. Sci.* **2022**, *37*, 523–530. https://doi.org/10.1007/s10103-021-03290-5.
101. Robijns, J.; Censabella, S.; Bulens, P.; Maes, A.; Mebis, J. The use of low-level light therapy in supportive care for patients with breast cancer: Review of the literature. *Lasers Med. Sci.* **2017**, *32*, 229–242. https://doi.org/10.1007/s10103-016-2056-y.

Graphical Abstract



2.2 Article 2:

Effects of a Biocomplex Formed by Two Scaffold Biomaterials, Hydroxyapatite/Tricalcium Phosphate Ceramic and Fibrin Biopolymer, with Photobiomodulation, on Bone Repair.

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Article

Effects of a Biocomplex Formed by Two Scaffold Biomaterials, Hydroxyapatite/Tricalcium Phosphate Ceramic and Fibrin Biopolymer, with Photobiomodulation, on Bone Repair

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Abstract: There are several treatment methods available for bone repair, although the effectiveness becomes limited in cases of large defects. The objective of this pre-clinical protocol was to evaluate the grafting of hydroxyapatite/tricalcium phosphate (BCP) ceramic biomaterial (B; QualyBone BCP®, QualyLive, Amadora, Portugal) together with the heterologous fibrin biopolymer (FB; CEVAP/UNESP Botucatu, Brazil) and with photobiomodulation (PBM; Laserpulse®, Ibramed, Amparo, Brazil) in the repair process of bone defects. Fifty-six rats were randomly divided into four groups of seven animals each: the biomaterial group (G1/B), the biomaterial plus FB group (G2/BFB); the biomaterial plus PBM group (G3/B + PBM), and the biomaterial plus FB plus PBM group (G4/BFB + PBM). After anesthesia, a critical defect was performed in the center of the rats' parietal bones, then filled and treated according to their respective groups. The rats were euthanized at 14 and 42 postoperative days. Histomorphologically, at 42 days, the G4/BFB + PBM group showed a more advanced maturation transition, with more organized and mature bone areas forming concentric lamellae. A birefringence analysis of collagen fibers also showed a more advanced degree of maturation for the G4/BFB + PBM group. In the comparison between the groups, in the two experimental periods (14 and 42 days), in relation to the percentage of formation of new bone tissue, a significant difference was found between all groups (G1/B (5.42 ± 1.12; 21.49 ± 4.74), G2/BFB (5.00 ± 0.94; 21.77 ± 2.83), G3/B + PBM (12.65 ± 1.78; 29.29 ± 2.93), and G4/BFB + PBM (12.65 ± 2.32; 31.38 ± 2.89)). It was concluded that the use of PBM with low-level laser therapy (LLLT) positively interfered in the repair process of bone defects previously filled with the biocomplex formed by the heterologous fibrin biopolymer associated with the synthetic ceramic of hydroxyapatite and tricalcium phosphate.

Keywords: bone regeneration; bone repair; biomaterials; fibrin tissue adhesive; fibrin; low-level laser therapy; photobiomodulation

1. Introduction

Tissue bioengineering is developing strategic research seeking new therapeutic re-sources that can be applied in the bone regeneration process, such as stem cell differentiation, graft materials, and the use of membranes. Associated with these resources, alternative therapies are sought [1,2], aiming at the rapid formation of structurally intact bone for the surrounding skeleton [3,4].

The autologous bone graft, due to its characteristics and properties inherent to the re-generation process, is considered the gold standard [5,6]. However, therapy with autoge-nous grafts has, on the other hand, some negative points such as postoperative complications and increased surgical time [7]. In this scenario, and in the face of critical bone defects, the use of biomaterials has gained space in pre-clinical research [8,9]. An ideal bone substitute must present important characteristics such as the release of growth factors that enhance bone neoformation, promote a framework, and favor a microenvironment that enhances tissue growth [10].

Within the diversity of biomaterials indicated for the surgery of dental and orthopedic grafts, for guided bone regeneration and filling dental alveoli, the compounds of hydroxyapatite and tricalcium phosphate are used because they are generally of null cytotoxicity, biocompatible, low cost and easy to produce, and osteoconductive, and, at the insertion site, these biomaterials present a good level of vascularization and bone formation [11–14]. The biomaterial hydroxyapatite/tricalcium phosphate (QualyBone BCP®, QualyLive, Amadora, Portugal) is a synthetic ceramic containing 75% hydroxyapatite and 25% tricalcium phosphate. It has a macroporosity that facilitates the proliferation of bone cells and neovascularization in empty spaces. This ceramic is already commercially available and used clinically in the reconstructive surgery of bone lesions [15].

However, the joint use of particulate biomaterials with other biological scaffolds can also be performed. This association favors the insertion and permanence of the material in the graft receptor site, in addition to allowing better functional mechanisms of tissue regeneration [16]. Thus, some bioproducts can be indicated for this purpose, among them, sealants or fibrin adhesives. These are used in surgery as hemostatic agents and inducers of the healing process [17]. Being identified as active biological scaffolds, they play an important role as a structure and/or anchor for cell fixation and growth [18–21].

The biological principles of fibrin patches mimic the end of the coagulation cascade, which normally occurs in the human body [18,22,23]. The heterologous fibrin sealant (HFS) derived from snake venom was developed by the Center for the Study of Venoms and Venomous Animals (CEVAP/UNESP, Botucatu, São Paulo, Brazil). HFS was initially used as a fibrin glue for repairing nerve injuries and healing chronic venous ulcers [24,25]. It is biocompatible and has hemostatic, sealant, and adhesive properties. It currently has a variety of clinical applications as it is able to act as a scaffold for stem cells [26,27] and as a drug delivery system [28]. Considering all the properties of this bioproduct, the name “sealant” was reconsidered, and it became known as “heterologous fibrin biopolymer” [29–31].

In this context, alternatives are being developed and explored with the objective of minimizing bone regeneration time and reducing the chance of possible complications resulting from the deficient consolidation process. Among them, photobiomodulation therapy (PBM) stood out for its satisfactory effects on metabolism and bone repair. This was due to its great osteogenic potential as it is a therapy that acts positively in the process of stimulating bone repair [32,33].

The photobiostimulatory effects of laser are directly related to cellular responses. When applied, their activities are accelerated, resulting in increased mitochondrial respiration and ATP synthesis. In addition, it optimizes protein synthesis, migration, and cell proliferation, and reduces the inflammatory response, decreasing edema and providing an efficient healing and bone regeneration process [34–37]. Finally, PBM therapy is a relatively low-cost, non-invasive treatment method. Despite all these advantages, there are controversies regarding the best parameters to be used to obtain an effective result in the extensive bone repair process filled with biomaterials [38].

Given the knowledge already acquired and the experiments that seek to standardize protocols and evaluate the effects of PBM therapy on the bone repair process [39], the objective of this study was to assess whether

PBM, through the use of low-level laser therapy (LLLT), interferes in the repair process of bone defects filled with BCP biomaterial associated with the heterologous fibrin sealant produced by CEVAP as a scaffold, thus trying to standardize an ideal experimental protocol to be used in the bone regeneration process and aiming at future clinical trials and contributing to the scientific-technological advance of translational science.

2. Materials and Methods

2.1. Experimental Design

Fifty-six male Wistar rats (*Rattus norvegicus*), aged 12 weeks and with an average weight of 250 g, supplied by the Central Animal Facility of the University of Marília (UNIMAR, Marília, São Paulo, Brazil) were used. The animals were kept in suitable environments under a 12 h light/dark cycle with controlled temperature (23 ± 1 °C) and received balanced animal feed (Labina® Purina, São Paulo, Brazil). A maximum of four animals per box were kept, and after surgery, they were allocated individually. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Ethics Committee on the Use of Animals (CEUA), University of Marília (Protocol 011/2019; 3 June 2019).

In addition, this experimental study was performed according to the ARRIVE (Animal Research: Report of in vivo Experiments) guidelines and was based on the principles of NC3Rs (National Center for Replacement, Refinement, and Reduction of Research Ani-mals). Throughout the experiment, the animals were monitored for the expression of pain, apathy, and symptoms of depression, aggression, and overexcitement, characteristics that vary in their usual behavior. Changes in gait, posture, and facial expression were also observed. Unusual behaviors such as excessive consumption of water and food, as well as possible clinical symptoms, were investigated [40].

The animals were randomly divided into four groups (n = 7 each), without predetermined inclusion or exclusion criteria, and the euthanasia periods were defined between 14 and 42 days. The groups were distributed as follows: biomaterial group (G1/B), biomaterial plus fibrin biopolymer group (G2/BFB); biomaterial plus photobiomodulation group (G3/B + PBM); and biomaterial plus fibrin bipolymer plus photobiomodulation group (G4/BFB + PBM) (Figure 1).

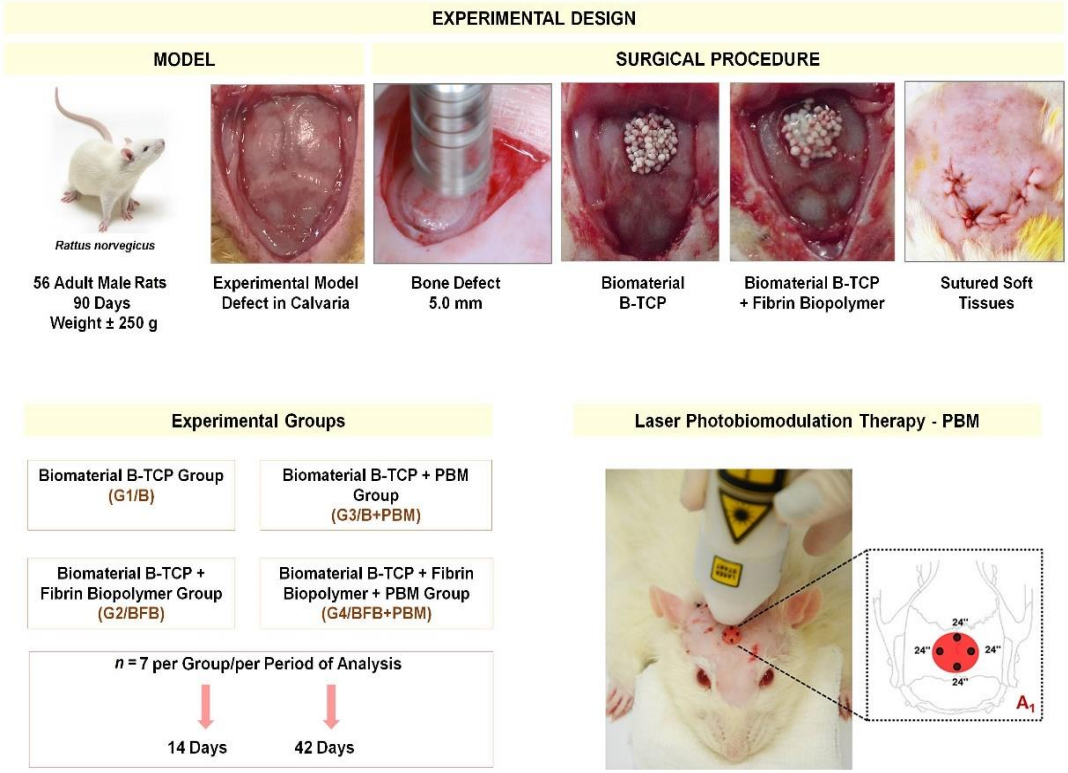

Figure 1. Experimental design. Animal model and inclusion criteria for 56 Wistar male rats (*Rattus norvegicus*): adults of 90 days old, weight of approximately ± 250 g; experimental model of bone defect in calvaria, exposure of parietal bones; surgical procedure: fabrication of a 5 mm diameter bone defect with a trephine drill; defect filled with biomaterial BCP (G1/B and G3/B + PBM); defect filled with biomaterial BCP and heterologous fibrin biopolymer (G2/BFB and G4/BFB + PBM); underlying soft tissue repositioned and sutured. A_1: illustration of post-immediate photobiomodulation (PBM) therapy, followed by 3x per week until the corresponding euthanasia period for the G3/B + PBM and G4/BFB + PBM groups. Experimental periods were 14 and 42 days, with 7 animals/group/period.

2.2. Sample Characterization–Biomaterial (BCP)

QualyBone BCP® (QualyLive, Amadora, Portugal) is marketed with the European Union certification seal (CE-Conformité Européenne), which indicates its compliance with the health, safety and environmental protection standards defined for the region (Certificate ES19/86908.02). Recently, it received authorization for commercialization in Brazil by the ANVISA-Brazilian Health Regulatory Agency under No. 81634410004. The product is sterilized in its double wrapping by gamma radiation at the minimum dose of 25 kGy.

Sample morphology hydroxyapatite/tricalcium phosphate biomaterial (QualyBone BCP® particles, QualyLive, Amadora, Portugal) was analyzed with a Field Emission Gun-Scanning Electron Microscope (FEG-SEM, Inspect S50, FEI, Hillsboro, USA), which was operated at an accelerating voltage of 5 kV. For the mapping, energy dispersive spectroscopy (EDS) was used (INCA x-act detector, Oxford Instruments, Great Britain), coupled to a scanning electron microscope (Figures 2 and 3).

The BCP crystal structures were investigated by analyzing the X-ray diffraction (XRD) patterns of Cu Kα radiation recorded by a diffractometer (Miniflex 600, Rigaku, Japan) in 2θ using a step size of 0.04°

and an X-ray source operating at 40 KV and 15 mA with Cu-Kα radiation (Figure 4).

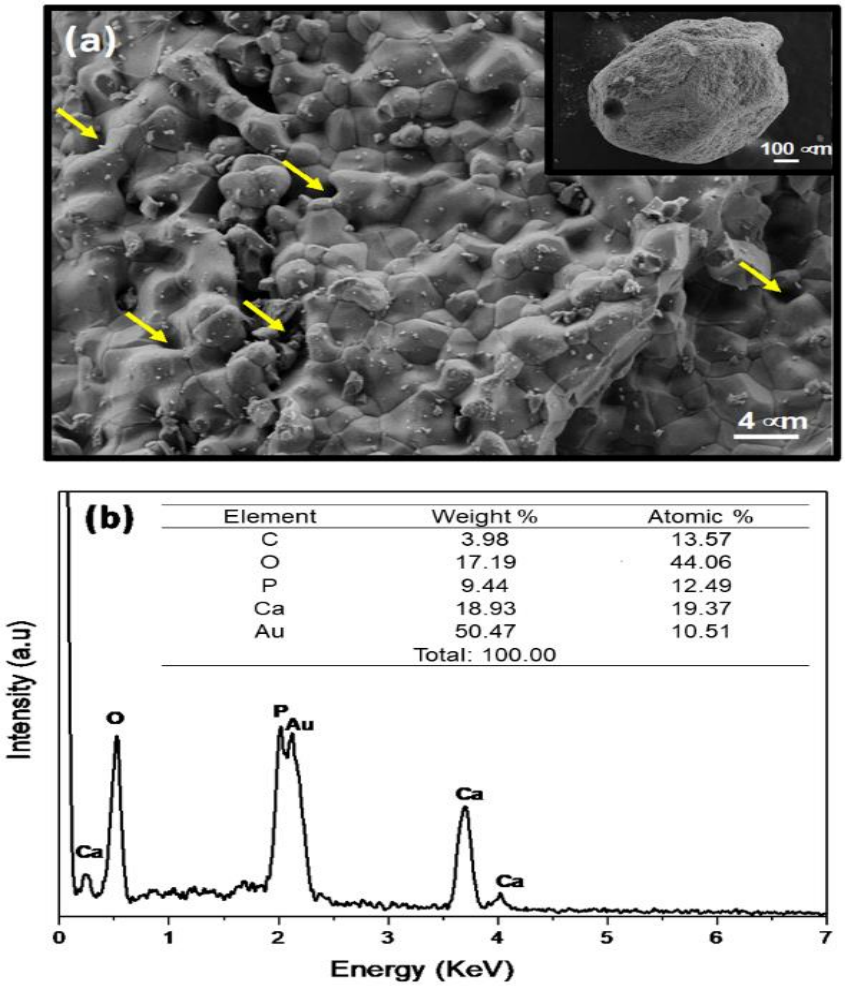

Figure 2. (**a**) FEG-SEM micrographs obtained for the BCP sample (yellow arrows shows porous structure). The inset reveals the aspect almost spherical particles. (**b**) EDS spectrum of the BCP sample.

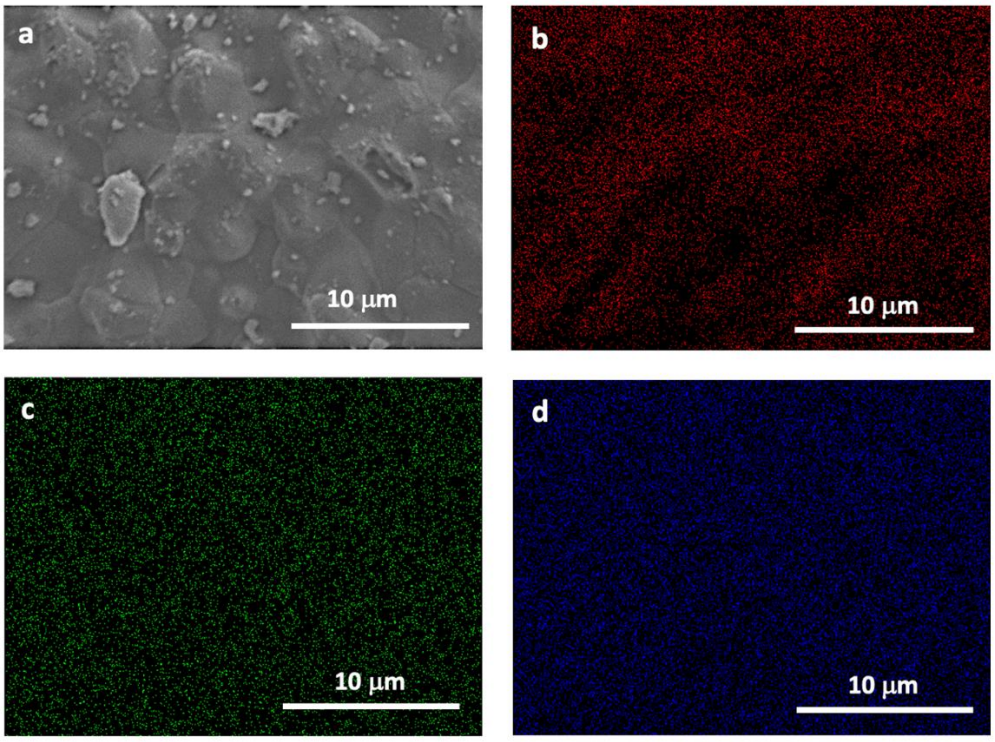

Figure 3. (**a**) SEM image and EDS mapping showing the (**b**) oxygen, (**c**) calcium, and (**d**) phosphor distribution of the BCP sample.

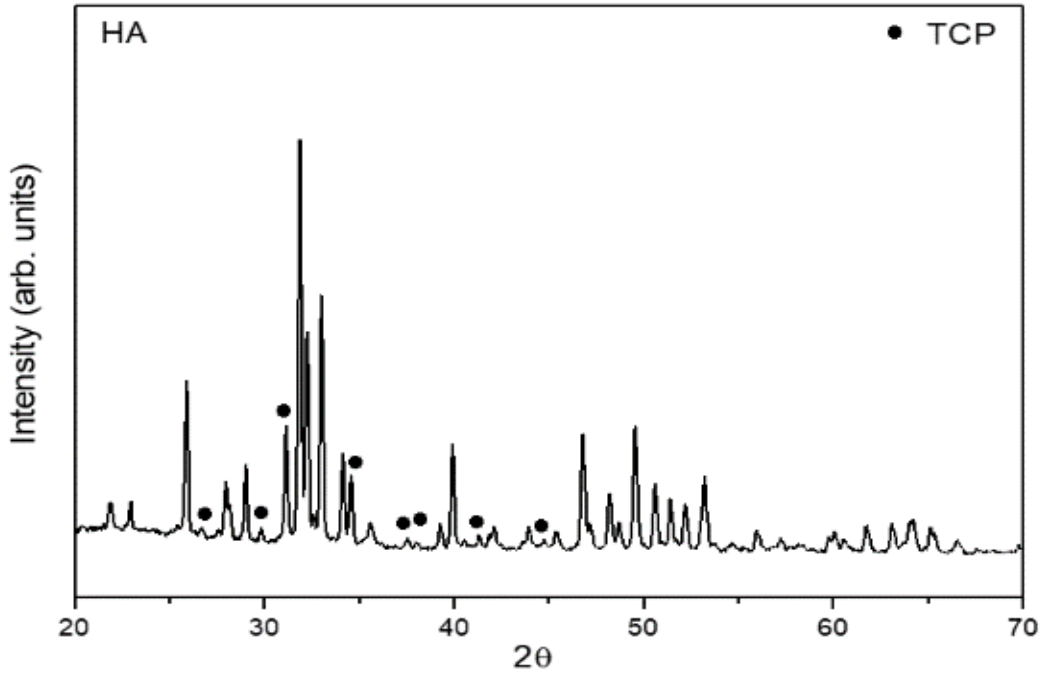

Figure 4. X- ray diffraction patterns of the BCP samples (75% hydroxyapatite–25% TCP).

2.3. Heterologous Fibrin Biopolymer (FB)

FB derived from snake venom was kindly provided by the Center for the Study of Venoms and Venomous Animals at UNESP (CEVAP/UNESP, Botucatu, Brazil). The biopolymer is composed of three fractions separated and homogenized before its application, totaling 40 μL. The

first component is fraction 1, which is composed of the thrombin-like enzyme (10 μL) added to calcium chloride diluent (10 μL). Fraction 2 is composed of fibrinogen extracted from buffalo blood (20 μL). For application in the G2/BFB and G4/BFB + PBM groups, the biopolymer components were deposited in microtubes, initially mixing fraction 1 with the diluent, adding the biomaterial, and then placing fraction 2, forming a biocomplex similar to a gelatinous substance [17,41].

2.4. Experimental Surgery

The animals were submitted to general anesthesia with an intramuscular injection of tiletamine hydrochloride and zolazepam hydrochloride (10 mg/kg-Telazol®; Fort Dodge Laboratories, Iowa, USA). Trichotomy was performed in the frontal-parietal bone region followed by antisepsis with a topical solution of 10% Polyvinyl Pyrrolidone Iodine PVPI (Povidine® Antisseptico, Vic Pharma Ind e Comércio, São Paulo, Brazil). Then, a 4 cm half-moon incision was made with a No.15 carbon steel scalpel blade (Embramax®, São Paulo, Brazil) in the integument, and the periosteum was carefully detached with the aid of the syndesmatome and folded together with the other tissues, exposing the external surface of the parietal bones (Figure 1).

A circular osteotomy of 5.0 mm in diameter was performed in the center of the parietal bones (Figure 1) with the aid of a trephine drill (Neodent®, Curitiba, Brazil) adapted to the contra-angle (Driller®, São Paulo, Brazil) attached to the electric micromotor (Driller BLM 600 Baby®, São Paulo, Brazil) at low speed (1500 rpm). Irrigation was constant and abundant using sterile saline solution (0.9% saline solution) to prevent bone necrosis by thermal action, thus obtaining a bone fragment, without spikes, in order to preserve the integrity of the dura mater and the brain.

In the animals of the groups G1/B and G3/B + PBM, the defect was filled only with the BCP biomaterial, and in the animals of the G2/BFB and G4/BFB + PBM groups, the defects were filled with the BCP biomaterial associated with the heterologous fibrin biopolymer (FB) (Figure 1). The biomaterial was weighed on an analytical balance (MicroNal® Precision Equipment, São Paulo, Brazil) to obtain a weight of approximately 0.03 mg and inserted into the defect site without exerting pressure on the brain. Tissues in the surgical area were repositioned (Figure 1), taking care that the periosteum covered the cavities, and then the integument was sutured (simple stitches) with 4–0 silk thread (Ethicon®, Johnson and Johnson Company, São Paulo, Brazil).

2.5. Photobiomodulation Protocol (PBM)

The G3/B + PBM and G4/BFB + PBM groups were submitted to laser treatment with galli-um-aluminum-arsenide (GaAlAs, Laserpulse IBRAMED®, Amparo, Brazil; registered in the ANVISA-Brazilian Health Regulatory Agency under No. 10360310030) where, in all applications, the laser beam emissions were calibrated in the device itself and previously tested to certify the dose, following the parameters described in Table 1.

Table 1. Protocol of photobiomodulation therapy.

Parameter	Unit/Description
Type of laser	GaAlAs
Output power	30 mW
Wavelength	830 nm

Power density	258.6 mW/cm²
Energy density	6.2 J/cm²
Beam area	0.116 cm²
Total power	2.9 J
Beam type	Positioned perpendicular to the skull
Emission mode	Continuous
Form of application	Four points around the surgical area
Irradiation duration	24 s per point
Total time of each application	96 s
Treatment time	Immediately after surgery and three times a week until euthanasia.

GaAlAs = gallium-aluminum-arsenide; mW = milliwatts; nm = nanometer; mW = milliwatts/centimeter²; J/cm² = joules/ centimeter²; cm² = centimeter²; J = joules.

2.6. Euthanasia and X-ray Computed Microtomography (μ-CT)

Respectively after 14 and 42 days of post-surgery, for 7 animals from each group per pe-riod, euthanasia was performed using the barbiturate (Thiopental®, Cristalia, Itapira, Brazil) dosage for rats (150 mg/kg) as follows: sodium thiopental 2.5%, per via intraperitoneal-IP, applied in the lower left abdominal quadrant of the animal (associated with a local anesthetic, lidocaine hydrochloride at a dose of 10 mg/kg). Then, the region of the defect of each animal was carefully removed with the aid of a dental conical surgical carbide bur mounted on a low rotation piece (Dabi Atlante®, Ribeirão Preto, Brazil) preserving the supraperiosteal soft tissues and fixed in 10% formalin solution in a phosphate buffer of pH 7.2 for one week for microtomographic analysis and, later, for histological processing.

The pieces were submitted to an X-ray beam scan in a computerized microtomograph SkyScan® 1174v2 (Bruker-microCT, Kontich, Belgium) of the Bauru School of Dentistry, University of São Paulo (FOB/USP, Bauru, São Paulo, Brazil). The samples were placed in tubes, positioned, and fixed in the appropriate sample holder for the equipment. Then, they were rotated 360°, with a "rotation step" of 0.5 and isotropic resolution of 19.6 μm, generating a time of 41 min and 32 s per sample.

The images of each specimen were analyzed and reconstituted with the specific software 64 Bits270013 (Bruker, Kontich, Belgium) and the NRecon® program (version.1.6.8.0, Sky-Scan, 2011, Bruker-microCT, Kontich, Belgium) in about 1000 to 1100 slices, according to the adopted anatomical parameters. The software Data Viewer® version 1.4.4 64 bit (linear measurements of the coronal, transaxial, and sagittal axes, Bruker, Kontich, Belgium) and CTvox® version 2.4.0 r868 (Bruker Micro CT, Bruker, Kontich, Belgium), were used for two-dimensional visualization.

2.7. Sample Collection and Histological Procedure

The pieces were subjected to demineralization in EDTA solution, a solution containing 4.13% tritiplex® III (Merck KGaA, Hessen, Germany) and 0.44% sodium hydroxide (Labsynth, São Paulo, Brazil) with weekly changes of the solution for a period of approximately 40 days. Subsequently, semi-serial coronal sections were performed, considering the central region of the defect with the aid of the Leica® RM2245 semi-automatic microtome (Leica Biosystems, Wetzlar, Germany). Sections 5 μm thick (six slides with four sections each) were made for hematoxylin-eosin and Masson's trichrome staining. Two evaluators previously

calibrated and blinded in relation to the groups and periods performed the constant analyses in the methodology.

2.8. Birefringence Analysis of Collagen Fibers (Picrosirius-Red Staining)

Sections stained with Picrosirius-red were evaluated under polarized light to determine the quality of the newly formed organic matrix during the experimental periods (14 and 42 days) of healing in the defects. Images were obtained from the defects using the higher resolution digital camera, Leica DFC 310FX (Leica Microsystems®, Wetzlar, Germany), connected to the confocal laser microscope, Leica DM IRBE, and the capture system LAS 4.0.0 (Leica Microsystems®, Heerbrugg, Switzerland).

2.9. Histomorphometric Analysis

In all specimens, the entire extent of the defect was considered to assess the bone repair pattern in all groups, with four semi-serial sections of the surgical bed of each defect being evaluated with an Olympus® light microscope (Olympus Corporation, Tokyo, Japan).

Quantitative image analysis was performed on a computer (Core I7 Processor; Intel Corporation, Santa Clara, CA, USA) using Carl Zeiss AxioVision (Rel. 4.8.2 White Plains, NY, USA). From the semi-serial sections obtained, two more central sections of the defect with a distance between them of 300 μm were captured. The percentage of newly formed bone tissue, biomaterial, and non-mineralized tissue was calculated.

2.10. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) to detect possible differences between groups. The ANOVA assumptions, normality of residuals, and homogeneity of variances were verified, respectively, by the Shapiro–Wilk and Bartlett tests, both at 5% probability. Subsequently, the means were compared by Tukey test at 5% probability. Within each treatment, the comparison of new bone formation, biomaterial, and non-mineralized tissue as a function of the treatment period (14 and 42 days) was evaluated using the Student's t-test at 5% probability. All analyses were conducted using the R software (R Core Team, Vienna, Austria).

3. Results and Discussion

Regarding the in vivo studies, there were no complications that needed to be reported, and there was no disease or sign that strongly motivated the removal of an animal (clinical outcome).

3.1. Sample Characterization

The morphology of the sample, observed by FEG-SEM, is shown in Figure 2. The inset in Figure 2 shows almost spherical particles obtained for BCP. FEG-SEM provides details of a constituent structure which clearly reveals that the BCP sample mostly consists of particles submicron size of order 2 μm that are homogeneous and have a uniform distribution and high degree of packing (Figure 2a). The energy dispersive spectroscopy (EDS) spectra of the elements show peaks only for the elements (oxygen-O, phosphor-P, and calcium-Ca) (Figure 2b). In addition, the gold signal has been observed. The sample was coated with gold before SEM analysis.

The distribution of the elements in the samples was evaluated using EDS image mapping of the surfaces where red points represent oxygen, blue points represent phosphor, and green points represent calcium. A good distribution of the elements, without precipitates or aggregates, was observed in both samples (Figure 3), indicating good homogeneity.

The XRD pattern showed that the BCP samples exhibited well-defined diffraction peak characteristic of hydroxyapatite (HA, major phase), which is confirmed by the no. #09-0432 JCPDS card. In addition, the XRD pattern of the BCP samples showed diffraction peaks corresponding the minor phase (tricalcium phosphate/TCP, black circle, JCPDS card no #09-0169, Figure 4).

Grafting materials and their properties can be improved, mainly in their characteristics that lead to better tissue performance and new bone formation. A new approach increasingly studied is ion substitutions in calcium phosphate bioceramics. Ressler et al. (2022) prepared porous composite scaffolds based on CaPs substituted by Sr^{2+}, Mg^{2+}, Zn^{2+}, and SeO_3^{2-} ions and chitosan by the freezing technique. The scaffolds presented a highly porous structure with very well interconnected pores, with osteogenic potential together with human mesenchymal stem cells. The findings demonstrated that ionic substitutions have a beneficial effect on cells and tissues and increased the expression of osteogenesis-related markers and increased phosphate deposits compared to scaffolds with unsubstituted CaPs [42].

3.2. Qualitative Analysis of Two-Dimensional Microtomographic Images

At 14 days, the bidimensional (transaxial and coronal) microtomographic images showed, in all experimental groups, a centripetal pattern of bone formation, evidenced by the increased gradation of bone tissue density in gray scale in the peripheral areas of the bone defect. The biomaterial particles were surrounded by immature bone trabeculae (Figure 5).

In all groups, at 42 days, there was an increase in bone growth, but without complete closure of the defect, remaining limited to the surgical edges, and with focal areas of mineralized tissue in the G3/B + PBM and G4/BFB + PBM groups. The regions of bone remodeling were observed peripherally, relative to the difference in tissue density. The central area of the wound remained filled with biomaterial particles (Figure 5).

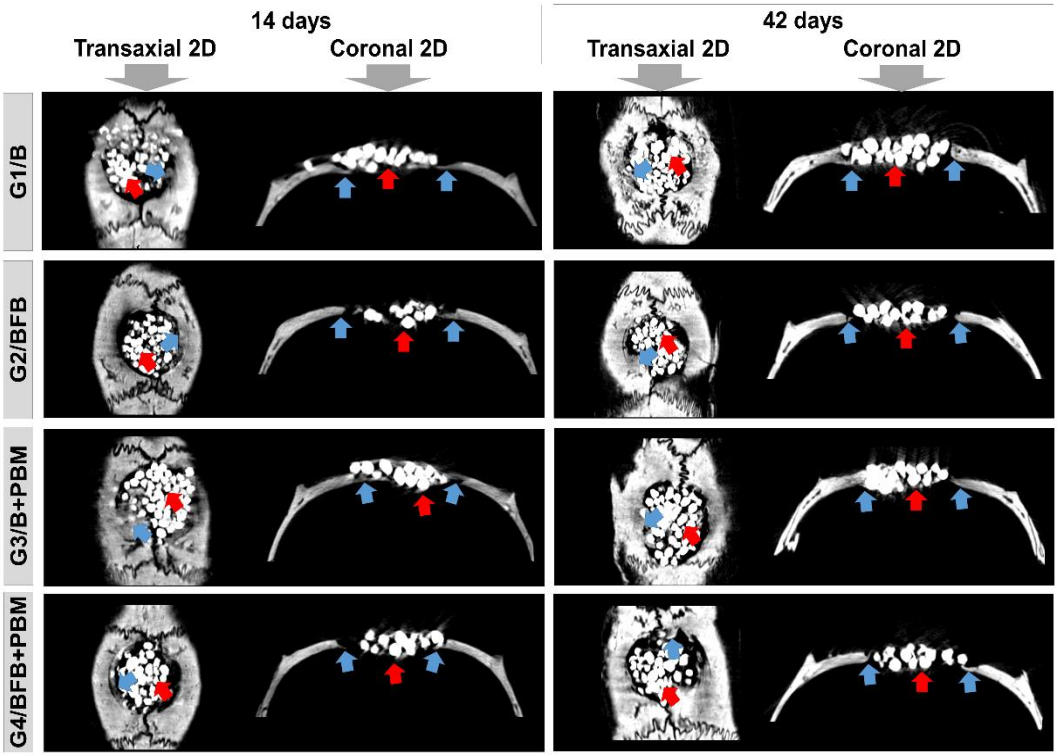

Figure 5. Two-dimensional (2D) reconstructed microtomographic images in transaxial and coronal sections of the bone defects in rat calvaria at 14 and 42 days, respectively. Defect filled with biomaterial (G1/B), biocomplex consisting of biomaterial plus heterologous fibrin biopolymer (G2/BFB), biomaterial and PBM (G3/B + PBM), and biocomplex consisting of biomaterial plus heterologous fibrin biopolymer and photobiomodulation with low-level laser therapy (G4/BFB + PBM). Bone formation (blue arrow) and biomaterial particles (red arrow).

The intertwining of bone trabeculae with the biomaterial, observed in the initial stage of tissue repair (14 days), evidences the porous characteristic of the scaffold, which is important to favor cell proliferation and migration [43]. The process of new bone formation that occurred over the 42 days was also favored by the combination of the biomaterial with the fibrin polymer. This association of biopolymers constitutes a promising strategy to regenerate bone defects since the fibrin favors the incorporation of the biomaterial at the lesion site [44].

Tissue regeneration was also improved by PBM therapy, which stimulates bone formation and favors the integration process of the biomaterial stabilized with fibrin [44,45]. The increasing bone formation observed over time in this research was also reported in studies that used similar methodologies [20,44]. These data demonstrate that PBM may have positive effects on bone tissue, improving the quality and density of newly formed bone [44,46,47].

A study carried out with a similar biocomplex, except that the biomaterial was a bone substitute established in preclinical and clinical studies (Bio-Oss® bone substitute, Geistlich Pharma AG, Wolhusen, Switzerland), with the same laser therapy protocol, showed similar results in bone neoformation increased by PBM in which the biocomplex created a favorable microenvironment for an adequate repair process as an innovative drug delivery system [44]. The phase I/II clinical trial involving the treatment of chronic venous ulcers with FB has been completed and its results were recently published [17].

3.3. Histomorphological Analysis

In this preclinical study, two postoperative periods were used for evaluation, 14 and 42 days. In the initial period (14 days), photobiomodulation plays an important role in the initial phases of the repair process, as it helps in the biological response by reducing the inflammatory process, decreasing pain, and creating conditions for accelerating the formation of new bone. In the final period (42 days), in non-critical defects in rats, the process would progress to complete repair of the surgical area. In the case of critical defects, which do not repair spontaneously until the end of the experiment, the evaluation of the formation of new bone is important to analyze the action of photobiomodulation and grafting materials in the evolution of the process, in addition to the amount of tissue non-mineralized material that remained inside the defect and the permanence of biomaterials at this site [48–52].

All the experimental groups, G1/B, G2/BFB, G3/B + PBM, and G4/BFB + PBM, exhibited peculiar characteristics at 14 days, with the area of the defect interpolated by reactive connective tissue, densely cellularized, and permeated by inflammatory cells, random arrangements of thin collagen fibers, and biomaterial particles. Bone growth described the same pattern in all defects, adjacent to peripheral regions with irregular trabecular conformation. The G4/BFB + PBM group showed a marked angiogenic response, with evident vascular sprouts (Figures 6 and 7).

At 42 days, the height of the bone remaining in the surgical region was preserved in all bone defects. In the central region, a slight invagination of overlying soft tissues was observed in the G1/B and G2/BFB groups, unlike the G3/B + PBM and G4/BFB + PBM groups. The new bone tissue showed a continuous growth, but was restricted to the edges of the defects, with mineralized bone focal areas between the biomaterial particles. The G4/BFB + PBM group exhibited a more advanced maturation transition, with more organized and mature bone areas, forming concentric lamellae, surrounded by regions of immature bone trabeculae (Figures 6 and 8).

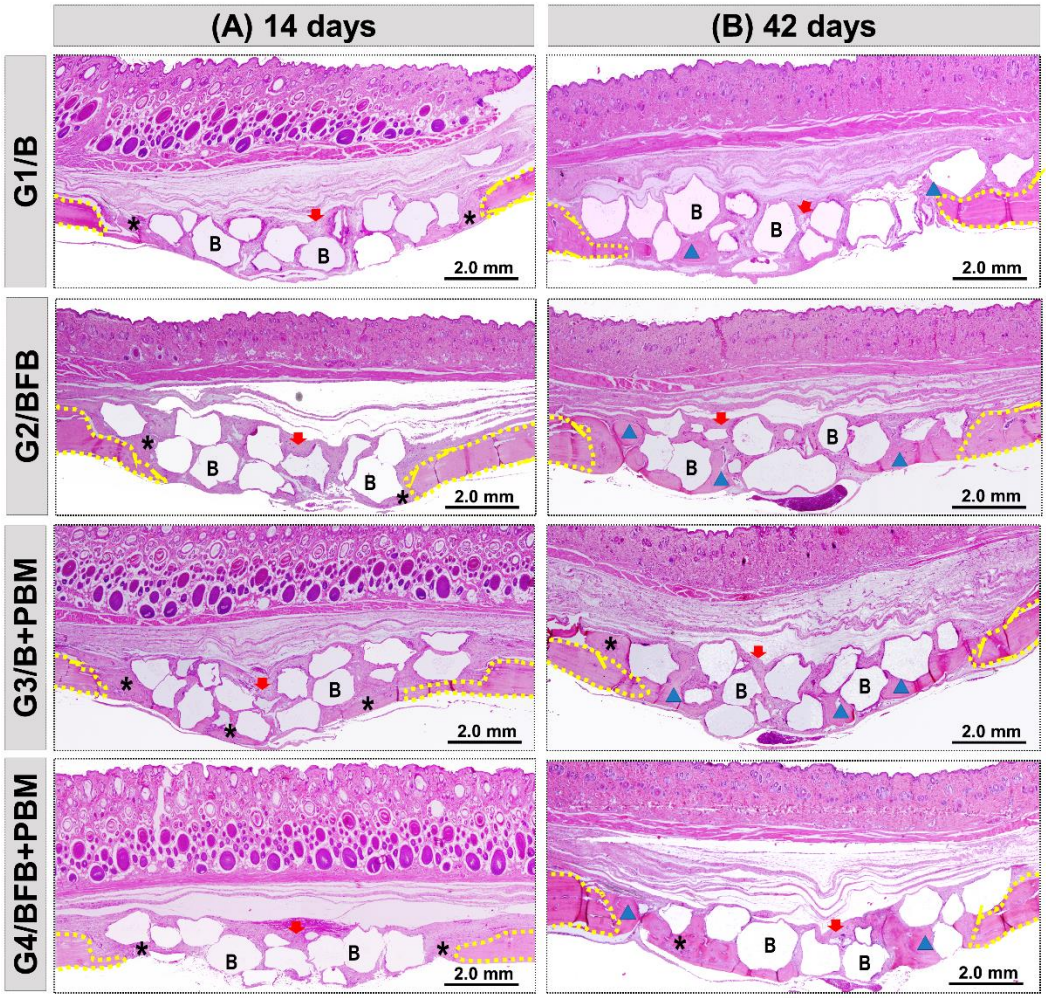

Figure 6. Panoramic histological views at 14 (**A**) and 42 (**B**) days in the cranial defects filled with biomaterial (G1/B), biocomplex consisting of biomaterial plus heterologous fibrin biopolymer (G2/BFB), biomaterial and PBM (G3/B + PBM), and biocomplex consisting of biomaterial plus heterologous fibrin biopolymer and photobiomodulation with low-level laser therapy (G4/BFB + PBM). Immature trabecular formation (asterisk) occurring at the edge of the defect (dashed line) and overlying the dura mater surface. Biomaterial particles (**B**) permeating the reaction connective tissue (red arrow). The transition from bone maturation to mineralized tissue (triangle), with primary bone areas (asterisk) and biomaterial particles in densely fibrous connective tissue (red arrow). HE; original magnification × 4; bar = 2 mm.

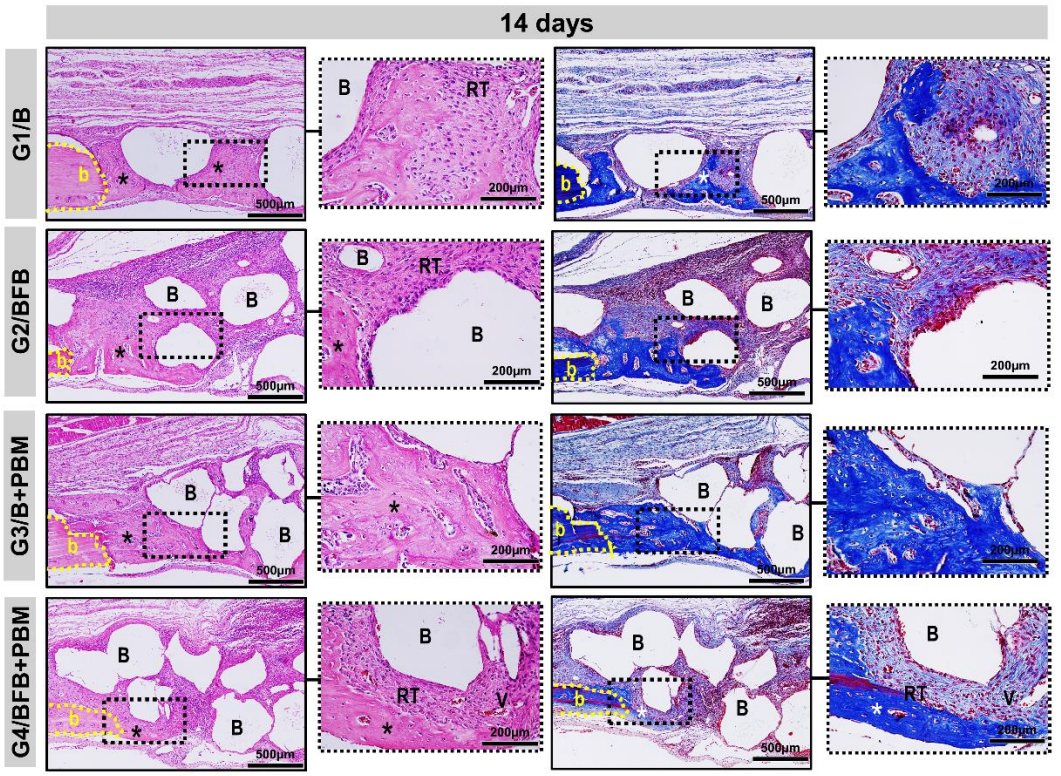

Figure 7. Details of the evolution of the bone repair process of the cranial defects at 14 days filled with biomaterial (G1/B), biocomplex consisting of biomaterial plus heterologous fibrin biopolymer (G2/BFB), biomaterial and PBM (G3/B + PBM), and biocomplex consisting of biomaterial plus heterologous fibrin biopolymer and photobiomodulation with low-level laser therapy (G4/BFB + PBM). The deposition of the osteoid matrix (asterisk) from the edges of the defect (b), particles of the biomaterial (B) interspersed with densely cellular reactive connective tissue (RT) and vascular budding (V). HE and Masson Trichrome; original magnification × 10; bar = 500 µm and insert, magnified images × 40; bar = 100 µm.

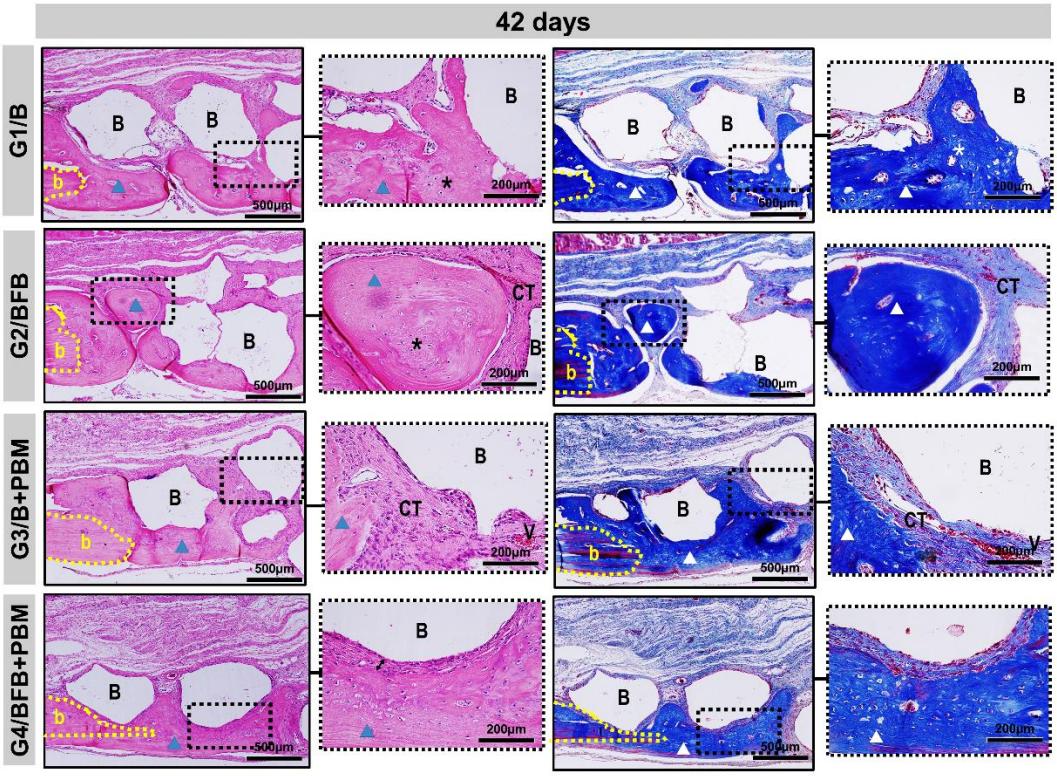

Figure 8. Details of the evolution of the bone repair process of the cranial defects at 42 days filled with biomaterial (G1/B), biocomplex consisting of biomaterial plus heterologous fibrin biopolymer (G2/BFB), biomaterial and PBM (G3/B + PBM), and biocomplex consisting of biomaterial plus heterologous fibrin biopolymer and photobiomodulation with low-level laser therapy (G4/BFB + PBM). Mature lamellar tissue (triangle) was restricted to the edge of the defect (b) and areas of immature bone trabeculae (asterisk) in the fibrous connective tissue (CT). Biomaterial particles (B) surrounded by thicker collagen fibers, with a fibrous interface between the particles and newly formed bone (arrow). HE and Masson Trichrome; original magnification x 10; bar = 500 µm and insert, images magnified × 40; bar = 100 µm.

The histological data also showed positive effects regarding the combination of treatment methods in the bone regeneration process, which was already visible at 14 postoperative days. In this period of analysis, it is possible to observe the presence of vascular sprouts in the G4/BFB + PBM group, which may be due to the effects of PBM therapy on the local microcirculation [46]. In addition to PBM, the fibrin biopolymer also contributed to stimulate angiogenic factors and neovascularization, as observed in previous studies [20,44]. The analyses performed at 42 postoperative days shows that there was an advance in bone formation in all experimental groups, but without the complete closure of the defects.

The bone neoformation occurred along the edges of the defect, considering the stimuli of the microenvironment of the adjacent bone tissue, and it was limited to them, being possible to observe particles of biomaterial not yet degraded in the central region of the defect. Although all groups showed bone growth, the newly formed bone tissue in the G4/BFB + PBM group presented a more mature histological aspect, which is in agreement with studies that obtained the formation of a more organized bone tissue in the biostimulated groups [20,44,53]. These data indicate that the association of biopolymers with PBM therapy had

additional effects, improving the histological characteristics of the newly formed bone tissue.

The composite of hydroxyapatite and tricalcium phosphate, selected for this experimental protocol, showed a biological response similar to several products marketed and used in bone loss restoration techniques, corroborating the properties conceptually necessary for an ideal biomaterial as they do not cause intense inflammatory reaction, they did not present encapsulation or rejection at the receptor site, and they allowed the osteoprogenitor cells adjacent to the bone defect to differentiate through the structure generated by such materials, which demonstrates osteoconduction [54–58].

3.4. Description Birefringence Analysis of Collagen Fibers

To evaluate the birefringence patterns of collagen fibers, which evidences the degree of bone maturation, sections stained with Picrosirius-red were observed under polarized light microscopy in the experimental periods of 14 and 42 days (Figure 9).

Qualitatively, at 14 days, bundles of collagen fibrils, both fine and disorganized (type III collagen), were observed in all groups, characterized by linear trabeculations interposing along the entire length of the wound and surrounding the particles of biomaterial, which presents a reddish-orange birefringence pattern, and in the receptor bed, greenish birefringence. In the G4/BFB + PBM group, zones of recent mineralization were observed, with collagen fibers in transition to yellowish-green birefringence more centrally, juxtaposed with the biomaterial particles (see asterisk, Figure 9A(A″)).

At 42 days, the bundles of collagen fibers with lamellar organization (collagen type I) were thicker, oriented parallel to each other, and, circumferentially, the biomaterial particles with birefringence transacting between yellow-green. In the evaluation of the histological section of the center of the defect, greenish birefringence is predominant in the G4/BFB + PBM group, and the loss of distinction between the margins of the remaining bone and the adjacent neoformed tissue gives the degree of advanced bone maturation (Figure 9B(B″)).

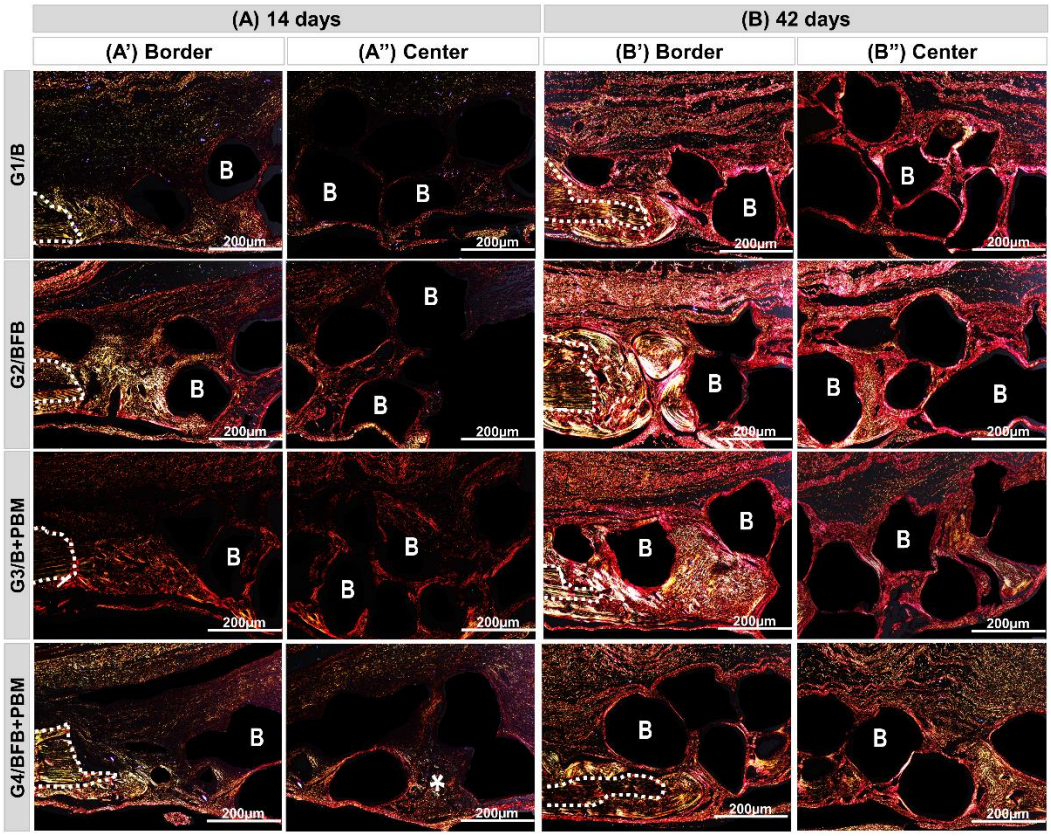

Figure 9. Histological sections of the edge (A′,A″) and center (B′,B″) of the bone defect of rat calvaria stained by Picrosirius-red under polarized light at 14 and 42 days ((**A**,**B**), respectively). Biomaterial (G1/B); biocomplex consisting of biomaterial plus heterologous fibrin biopolymer (G2/BFB); biomaterial and PBM (G3/B + PBM); and biocomplex consisting of biomaterial plus heterologous fibrin biopolymer and photobiomodulation with low-level laser therapy (G4/BFB + PBM). RGB green-yellow-red colors. Mature bone, type I collagen fibers: yellowish-green color; immature bone, type III collagen fibers: reddish color. Dashed line = edge of remaining bone; B = synthetic biomaterial particles (dark background); asterisk = collagen fibers in advanced maturation phase. Original magnification × 10, scale bar 200 µm.

The qualitative analysis and the arrangement of collagen fibers in the connective tissue formed in the defect was evaluated by Picrosirius-red staining, which allows the detection of different types of collagen [59]. At 14 days, all groups presented fine and disorganized collagen fibers (type III), but the G4/BFB + PBM group presented a more advanced stage of maturation with some zones of mineralization. At 42 days, it is possible to observe a thickening of collagen fibers which acquire a lamellar organization (type I). At this stage, G4/BFB + PBM remains the group with the most advanced pattern of tissue maturation. These findings agree with the data obtained by Della Colleta et al. (2021), and they suggest that PBM therapy can interfere with the arrangement and maturation of collagen fibers, providing thickening and parallel arrangement of fibers [4]. In addition, PBM therapy can interfere with the deposition of inorganic salts, contributing to connective tissue mineralization.

3.5. Histomorphometric Analysis

3.5.1. 14 Days

Regarding the percentage of new bone formation, that groups G3/B + PBM and G4/BFB + PBM did not show a statistically significant difference between them, but did with the G1/B and G2/BFB groups. In relation to the percentage of biomaterial, comparing all groups in the same period (14 and 42 days), no statistical difference was observed between them. Even in the same period, when comparing the percentage of non-mineralized tissue, a statistical difference was found between the G2/BFB and G4/BFB + PBM groups (Figure 10, Table 2).

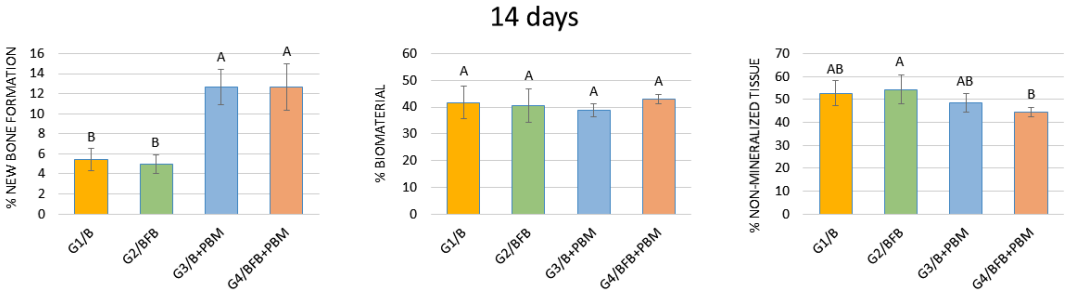

Figure 10. Percentage of new bone formation, biomaterial, and non-mineralized tissue in the experimental groups at 14 days. The different letters (A ≠ B) indicate a statistically significant difference ($p < 0.05$).

Table 2. Percentage of new bone formation in each group in the two experimental periods (14 and 42 days). G1/B defects filled with BCP; G2/BFB defects filled with BCP + fibrin biopolymer; G3/B + PBM defects filled with BCP and PBM therapy; and G4/BFB + PBM defects filled with BCP + fibrin biopolymer and PBM therapy.

Groups	G1/B	G2/BFB	G3/B + PBM	G4/BFB + PBM
14 days	5.42 ± 1.12 Bb	5.00 ± 0.94 Bb	12.65 ± 1.78 Ba	12.65 ± 2.32 Ba
42 days	21.49 ± 4.74 Ab	21.77 ± 2.83 Ab	29.29 ± 2.93 Aa	31.38 ± 2.89 Aa

Different capital letters (comparison in columns, 14 vs. 42 days, A ≠ B) indicate a statistically significant difference. Different small letters (line comparison, G1/B vs. G2/BFB vs. G3/B + PBM vs. G4/BFB + PBM in each period, 14 or 42 days, a ≠ b) indicate a statistically significant difference. Values are defined as the mean ± standard deviation. Student's test and Tukey's test, respectively, are both at $p < 0.05$.

The histomorphometric analysis that considered the percentage of new bone formation at 14 days showed a significant difference between the laser-treated groups in relation to the non-biostimulated groups. PBM therapy has a positive effect on the initial stages of tissue healing [60], influencing bone metabolism and modulating cell activity [44,61]. Studies report that the PBM therapy favors the osteogenic differentiation of pre-osteoblastic cells, increasing the expression of osteogenic markers, such as Runt-related transcription factor 2 (Runx2), osterix (OSX), and alkaline phosphatase (ALP) [36,62,63]. PBM therapy stimulates the release of growth factors and favors vascular proliferation and the synthesis of collagen and bone matrix [37]. Furthermore, it has also been reported that biostimulated cells regulate the production of inflammatory cytokines, allowing bone tissue to restore its homeostasis and function [64,65].

In this period, there was no statistical difference between all experimental groups regarding the percentage of biomaterial, which demonstrates that laser PBM therapy does not interfere with the biomaterial reabsorption process [20]. When comparing the percentage of connective tissue, a statistical difference was found between the G2/BFB

and G4/BFB + PBM groups. These results are in agreement with previous studies, which report that the combination of biomaterial, fibrin biopolymer [66], and PBM therapy promotes a decrease in the percentage of connective tissue and, consequently, favors an increase in the percentage of new bone formation, accelerating the regenerative process [20].

3.5.2. 42 Days

In the period of 42 days, when comparing the percentage of new bone tissue formation, a statistical difference was found between the G1/B and G2/BFB groups in relation to the G3/B + PBM and G4/BFB + PBM groups. Despite the percentage of biomaterial, comparing groups G1/B, G2/BFB, G3/B + PBM, and G4/BFB + PBM, no statistical difference was observed between the groups. Still, in the 42-day period, when comparing the percentage of connective tissue, a statistical difference was observed between the G4/BFB + PBM group and the G1/B and G2/BFB groups (Figure 11, Table 2).

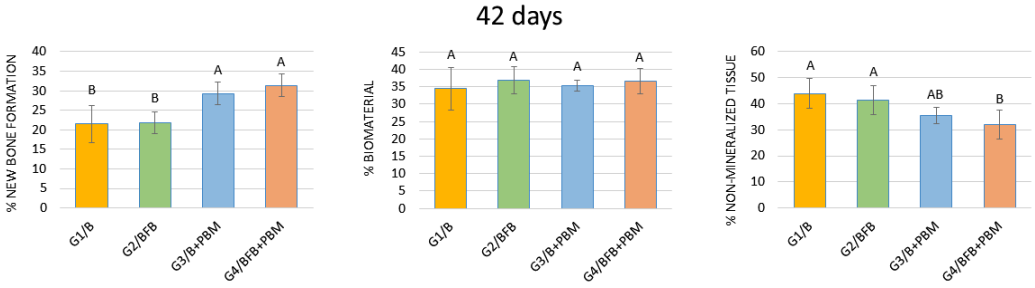

Figure 11. Percentage of new bone formation, biomaterial, and non-mineralized tissue in the experimental groups at 42 days. The different letters (A ≠ B) indicate a statistically significant difference ($p < 0.05$).

At 42 days, a significant difference in the percentage of new bone formation remains between the stimulated and unstimulated groups. These data indicate that the effects of laser PBM accelerate the deposition of mineralized bone matrix in the defect, increasing osteoblastic activity and stimulating the deposition of inorganic ions [67]. Additionally, laser therapy reduces the secretion of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and interleukin-17 (IL-17), as well as increases the production of anti-inflammatory cytokines, such as interleukin-10 (IL-10), which favors tissue regeneration [36,62,63]. In addition, studies indicate that biostimulation should occur with energy densities of between 0.05 and 10 J/cm^2, and the energy density used in the present study was 6.20 J/cm^2. This dose has been previously tested and is within the therapeutic window, considering that doses above 10 J/cm^2 have bioinhibitory effects [68,69].

Regarding the percentage of biomaterial, there was also no statistical difference between all experimental groups at 42 days. Regarding the percentage of connective tissue, at 42 days, a statistical difference was observed between G4/BFB + PBM in relation to G1/B and G2/BFB, supporting the results of previous studies which reported that biostimulation favors the collagen synthesis process, especially when associated with the bioactive properties of the biomaterial and fibrin biopolymer [20,30].

3.5.3. Comparison of Groups in the Two Trial Periods (14 vs. 42 Days)

Comparing the two periods, there was a statistically significant difference between all groups when observing the percentage of new bone formation. Regarding the percentage of biomaterial, there was a statistically significant difference between the two periods in groups G1/B, G3/B + PBM and G4/BFB + PBM. When comparing the percentage of non-mineralized tissue, there was a statistically significant difference in all groups (Figure 12; Table 2).

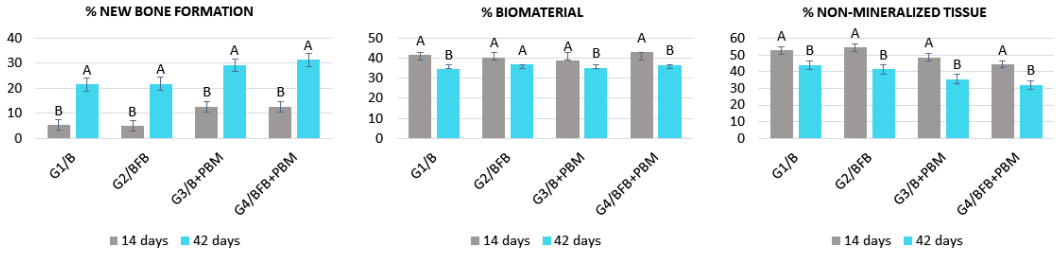

Figure 12. Percentage of new bone formation, biomaterial, and non-mineralized tissue in each experimental group in the two experimental periods (14 vs. 42 days). The different letters (A ≠ B) indicate a statistically significant difference ($p < 0.05$).

Regarding the percentages of formation of new bone tissue and connective tissue, there was a statistical difference between all experimental groups, confirming an increase in bone growth over time [53], because at 14 days, the bone regeneration process is in its initial period. At 42 days, the process of deposition and maturation of the bone matrix is more evident, with an organized microenvironment. Regarding the percentage of biomaterial, there was no statistical difference between the groups in the two analysis periods (14 vs. 42 days) except for G2/BFB due to the higher rate of resorption of the biomaterial. The other groups showed a small variation in the volumetric density of bone matrix particles [70,71].

FB provides an adequate scaffold to retain engrafted cells within the site of the lesion, changing the inflammation pattern to a Th1 cells profile [72], and it has the ability to maintain viable MSCs at bone defect sites with a modified inflammatory environment, accelerating their regeneration [73].

4. Conclusions

This experimental protocol evaluated a biomaterial composed of hydroxyapatite/tricalcium phosphate (BCP) mixed with a heterologous fibrin biopolymer (FB), together with photobiomodulation therapy (PBM). Based on the results obtained, it was demonstrated that PBM, through the use of low-level laser therapy, positively interfered in the repair process of bone defects filled with the biocomplex formed by FB plus biomaterial (BCP), accelerating the formation of new bone tissues through its biochemical and biostimulant effects. Previous studies using another biomaterial (Bio-Oss®) mixed with fibrin biopolymer showed similar results. These data reinforce the hypothesis that FB works as an adjuvant material, contributing to create a favorable environment for tissue regeneration, corroborating those results observed in the treatment of chronic venous ulcers in a clinical trial phase I/II.

Therefore, we have demonstrated its translational potential and clinical relevance for tissue bioengineering. It is possible to hypothesize that the associated use of FB with PBM works as an adjuvant in tissue regeneration. Combined use should be more fruitful, and regeneration should be faster than when used separately. These results encourage future clinical trials using this biocomplex.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Animal Use Ethics Committee (CEUA) of the University of Marília (Protocol 011/2019; June 03, 2019).

Informed Consent Statement: Not applicable

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available as they are part of a master's thesis and are not yet deposited in a public repository.

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Conflicts of Interest: QualyBone BCP was assigned for the present research by QualyBone BCP®, QualyLive, Amadora, Portugal. The heterologous fibrin biopolymer (FB) was provided by the Center for the Study of Venoms and Venomous Animals (CEVAP), Sao Paulo State University (UNESP), Botucatu, São Paulo, Brazil.

References

1. De Moraes, R.; de Guzzi Plepis, A.M.; da Conceição Amaro Martins, V.; Duarte, M.A.H.; Alcalde, M.P.; Buchaim, R.L.; Pomini, K.T.; Machado, E.G.; Munhoz, M.A.E.S.; Cunha, F.B.; et al. Suitability of the use of an elastin matrix combined with bone morphogenetic protein for the repair of cranial defects. *Am. J. Transl. Res.* **2019**, *11*, 5261–5271.
2. Munhoz, M.; Pomini, K.T.; Plepis, A.M.G.; Martins, V.; Machado, E.G.; de Moraes, R.; Cunha, F.B.; Santos, A.R., Jr.; Cardoso, G.B.C.; Duarte, M.A.R.; et al. Elastin-derived scaffolding associated or not with bone morphogenetic protein (BMP) or hydroxyapatite (HA) in the repair process of metaphyseal bone defects. *PLoS ONE* **2020**, *15*, e0231112. https://doi.org/10.1371/journal.pone.0231112.
3. Abou Neel, E.A.; Chrzanowski, W.; Salih, V.M.; Kim, H.W.; Knowles, J.C. Tissue engineering in dentistry. *J. Dent.* **2014**, *42*, 915–928. https://doi.org/10.1016/j.jdent.2014.05.008.
4. Della Coletta, B.B.; Jacob, T.B.; Moreira, L.A.C.; Pomini, K.T.; Buchaim, D.V.; Eleutério, R.G.; Pereira, E.S.B.M.; Roque, D.D.; Rosso, M.P.O.; Shindo, J.V.T.C.; et al. Photobiomodulation Therapy on the Guided Bone Regeneration Process in Defects Filled by Biphasic Calcium Phosphate Associated with Fibrin Biopolymer. *Molecules* **2021**, *26*, 847. https://doi.org/10.3390/molecules26040847.
5. Giannoudis, P.V.; Dinopoulos, H.; Tsiridis, E. Bone substitutes: An update. *Injury* **2005**, *36*, S20–S27. https://doi.org/10.1016/j.injury.2005.07.029.
6. Giannoudis, P.V.; Jones, E.; Einhorn, T.A. Fracture healing and bone repair. *Injury* **2011**, *42*, 549–550. https://doi.org/10.1016/j.injury.2011.03.037.
7. Rai, R.; Raval, R.; Khandeparker, R.V.; Chidrawar, S.K.; Khan, A.A.; Ganpat, M.S. Tissue Engineering: Step Ahead in Maxillofacial Reconstruction. *J. Int. Oral Health* **2015**, *7*, 138–142.
8. Trevisiol, C.H.; Turner, R.T.; Pfaff, J.E.; Hunter, J.C.; Menagh, P.J.; Hardin, K.; Ho, E.; Iwaniec, U.T. Impaired osteoinduction in a rat model for chronic alcohol abuse. *Bone* **2007**, *41*, 175–180. https://doi.org/10.1016/j.bone.2007.04.189.

9. Buchaim, R.L.; Andreo, J.C.; Rodrigues, A.C.; Buchaim, D.V.; Dias, D.V.; Daré, L.R.; Roque, D.D.; Roque, J.S. The action of demineralized bovine bone matrix on bone neoformation in rats submitted to experimental alcoholism. *Arq. Bras. Med. Vet. Zootec.* **2013**, *63*, 715–721. https://doi.org/10.1590/S0102-09352013000300016.
10. Cunha, F.B.; Pomini, K.T.; Plepis, A.M.G.; Martins, V.; Machado, E.G.; de Moraes, R.; Munhoz, M.A.E.S.; Machado, M.V.R.; Duarte, M.A.H.; Alcalde, M.P.; et al. In Vivo Biological Behavior of Polymer Scaffolds of Natural Origin in the Bone Repair Process. *Molecules* **2021**, *26*, 1598. https://doi.org/10.3390/molecules26061598.
11. Da Silva Brum, I.; de Carvalho, J.J.; da Silva Pires, J.L.; de Carvalho, M.A.A.; Dos Santos, L.B.F.; Elias, C.N. Nanosized hydroxyapatite and β-tricalcium phosphate composite: Physico-chemical, cytotoxicity, morphological properties and in vivo trial. *Sci. Rep.* **2019**, *9*, 19602. https://doi.org/10.1038/s41598-019-56124-4.
12. Zima, A.; Czechowska, J.; Szponder, T.; Ślósarczyk, A. In vivo behavior of biomicroconcretes based on α-tricalcium phosphate and hybrid hydroxyapatite/chitosan granules and sodium alginate. *J. Biomed. Mater. Res. A* **2020**, *108*, 1243–1255. https://doi.org/10.1002/jbm.a.36898.
13. Mohd, N.; Razali, M.; Ghazali, M.J.; Abu Kasim, N.H. 3D-Printed Hydroxyapatite and Tricalcium Phosphates-Based Scaffolds for Alveolar Bone Regeneration in Animal Models: A Scoping Review. *Materials* **2022**, *15*, 2621. https://doi.org/10.3390/ma15072621.
14. Da Silva Brum, I.; Frigo, L.; Lana Devita, R.; da Silva Pires, J.L.; Hugo Vieira de Oliveira, V.; Rosa Nascimento, A.L.; de Carvalho, J.J. Histomorphometric, Immunohistochemical, Ultrastructural Characterization of a Nano-Hydroxyapatite/Beta-Tricalcium Phosphate Composite and a Bone Xenograft in Sub-Critical Size Bone Defect in Rat Calvaria. *Materials* **2020**, *13*, 4598. https://doi.org/10.3390/ma13204598.
15. Trindade, H.F.; Batista, C.A.; Farsoun, A.; Farsoun, J.; Trindade, M.L.C. Injerto de materiales biológicos: Injertos óseos sintéticos frente a xenoinjertos I en la elevación del seno maxilar. *Quintessence* **2018**, *6*, 54–58.
16. Shi, W.; Que, Y.; Zhang, X.; Bian, L.; Yu, X.; Tang, X.; Yang, G.; Dai, Y.; Bi, S.; Lv, D.; et al. Functional tissue-engineered bone-like graft made of a fibrin scaffold and TG2 gene-modified EMSCs for bone defect repair. *NPG Asia Mater.* **2021**, *13*, 28. https://doi.org/10.1038/s41427-021-00297-w.
17. Abbade, L.P.F.; Barraviera, S.; Silvares, M.R.C.; Lima, A.; Haddad, G.R.; Gatti, M.A.N.; Medolago, N.B.; Carneiro, M.T.R.; dos Santos, L.D.; Ferreira, R.S., Jr.; et al. Treatment of Chronic Venous Ulcers With Heterologous Fibrin Sealant: A Phase I/II Clinical Trial. *Front. Immunol.* **2021**, *12*, 627541. https://doi.org/10.3389/fimmu.2021.627541.
18. Buchaim, D.V.; Cassaro, C.V.; Shindo, J.; Coletta, B.B.D.; Pomini, K.T.; Rosso, M.P.O.; Campos, L.M.G.; Ferreira, R.S., Jr.; Barraviera, B.; Buchaim, R.L. Unique heterologous fibrin biopolymer with hemostatic, adhesive, sealant, scaffold and drug delivery properties: A systematic review. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2019**, *25*, e20190038. https://doi.org/10.1590/1678-9199-jvatitd-2019-0038.
19. Machado, E.G.; Issa, J.P.; Figueiredo, F.A.; Santos, G.R.; Galdeano, E.A.; Alves, M.C.; Chacon, E.L.; Ferreira, R.S., Jr.; Barraviera, B.; da Cunha, M.R. A new heterologous fibrin sealant as scaffold to recombinant human bone morphogenetic protein-2 (rhBMP-2) and natural latex proteins for the repair of tibial bone defects. *Acta Histochem.* **2015**, *117*, 288–296. https://doi.org/10.1016/j.acthis.2015.03.006.
20. Pomini, K.T.; Buchaim, D.V.; Andreo, J.C.; Rosso, M.P.O.; Della Coletta, B.B.; German, Í.J.S.; Biguetti, A.C.C.; Shinohara, A.L.; Rosa, G.M., Jr.; Shindo, J.V.T.C.; et al. Fibrin Sealant Derived from Human Plasma as a Scaffold for Bone Grafts Associated with Photobiomodulation Therapy. *Int. J. Mol. Sci.* **2019**, *20*, 1761. https://doi.org/10.3390/ijms20071761.
21. Yamada, Y.; Boo, J.S.; Ozawa, R.; Nagasaka, T.; Okazaki, Y.; Hata, K.; Ueda, M. Bone regeneration following injection of mesenchymal stem cells and fibrin glue with a biodegradable scaffold. *J. Craniomaxillofac. Surg.* **2003**, *31*, 27–33. https://doi.org/10.1016/s1010-5182(02)00143-9.
22. Ortiz, A.C.; Fideles, S.O.M.; Pomini, K.T.; Reis, C.H.B.; Bueno, C.R.S.; Pereira, E.; Rossi, J.O.; Novais, P.C.; Pilon, J.P.G.; Rosa, G.M., Jr.; et al. Effects of Therapy with Fibrin Glue combined with Mesenchymal Stem Cells (MSCs) on Bone Regeneration: A Systematic Review. *Cells* **2021**, *10*, 2323. https://doi.org/10.3390/cells10092323.
23. Weisel, J.W. Fibrinogen and fibrin. *Adv. Protein Chem.* **2005**, *70*, 247–299. https://doi.org/10.1016/s0065-3233(05)70008-5.
24. Barros, L.C.; Ferreira, R.S., Jr.; Barraviera, S.R.; Stolf, H.O.; Thomazini-Santos, I.A.; Mendes-Giannini, M.J.; Toscano, E.; Barraviera, B. A new fibrin sealant from Crotalus durissus terrificus venom: Applications in medicine. *J. Toxicol. Environ. Health B Crit. Rev.* **2009**, *12*, 553–571. https://doi.org/10.1080/10937400903442514.
25. Gatti, M.A.N.; Vieira, L.M.; Barraviera, B.; Barraviera, S.R.C.S. Treatment of venous ulcers with fibrin sealant derived from snake venom. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2011**, *17*, 226–229. https://doi.org/10.1590/S1678-91992011000200015.
26. Gasparotto, V.P.; Landim-Alvarenga, F.C.; Oliveira, A.L.; Simões, G.F.; Lima-Neto, J.F.; Barraviera, B.; Ferreira, R.S. A new fibrin sealant as a three-dimensional scaffold candidate for mesenchymal stem cells. *Stem Cell Res. Ther.* **2014**, *5*, 78. https://doi.org/10.1186/scrt467.

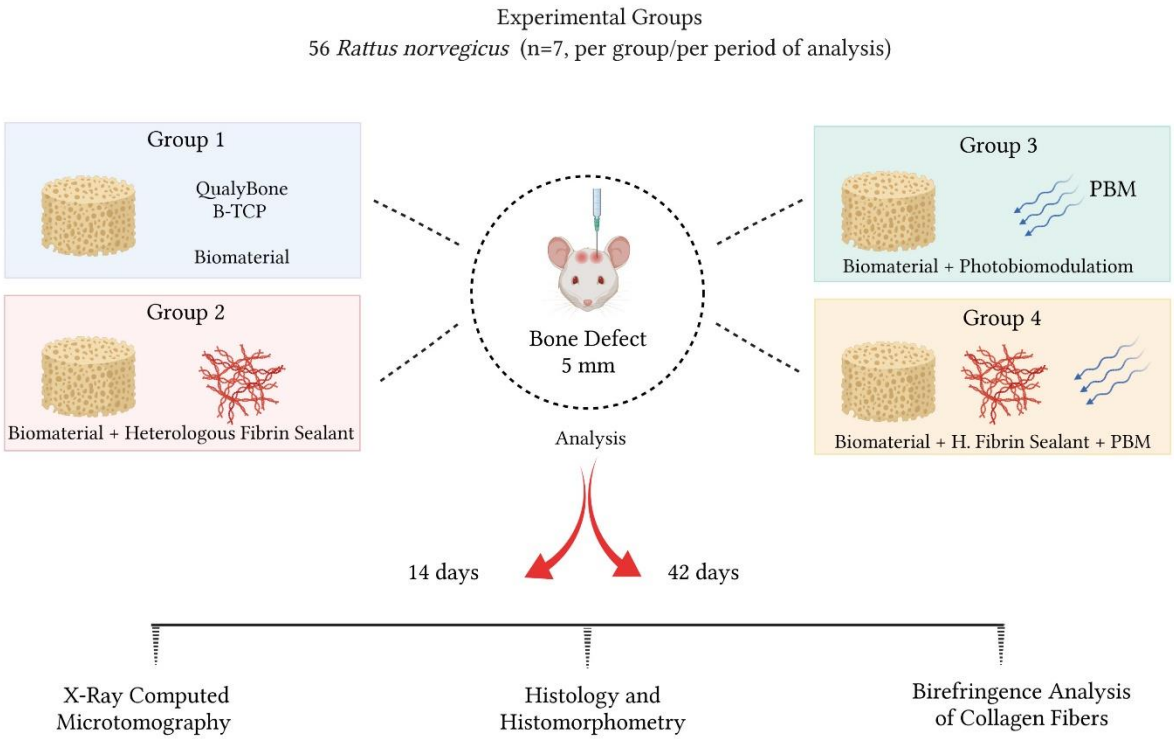
27. Orsi, P.R.; Landim-Alvarenga, F.C.; Justulin, L.A., Jr.; Kaneno, R.; de Assis Golim, M.; Dos Santos, D.C.; Creste, C.F.Z.; Oba, E.; Maia, L.; Barraviera, B.; et al. A unique heterologous fibrin sealant (HFS) as a candidate biological scaffold for mesenchymal stem cells in osteoporotic rats. *Stem Cell Res. Ther.* **2017**, *8*, 205. https://doi.org/10.1186/s13287-017-0654-7.
28. Venante, H.S.; Chappuis-Chocano, A.P.; Marcillo-Toala, O.O.; da Silva, R.A.; da Costa, R.M.B.; Pordeus, M.D.; Barraviera, B.; Ferreira, R.S., Jr.; Lara, V.S.; Neppelenbroek, K.H.; et al. Fibrin Biopolymer Incorporated with Antimicrobial Agents: A Proposal for Coating Denture Bases. *Materials* **2021**, *14*, 1618. https://doi.org/10.3390/ma14071618.
29. De Oliveira, C.T.B.; Leonel, B.C.; de Oliveira, A.C.; de Brito Paiva, M.; Ramos, J.; Barraviera, B.; Ferreira, R.S., Jr.; Shimano, A.C. Effects of fibrin sealant and bone fragments on defect regeneration performed on rat tibiae: An experimental study. *J. Mech. Behav. Biomed. Mater.* **2020**, *104*, 103662. https://doi.org/10.1016/j.jmbbm.2020.103662.
30. Iatecola, A.; Barraviera, B.; Ferreira, R.S., Jr.; dos Santos, G.R.; Neves, J.I.; da Cunha, M.R. Use of a new fibrin sealant and laser irradiation in the repair of skull defects in rats. *Braz. Dent. J.* **2013**, *24*, 456–461. https://doi.org/10.1590/0103-6440201302265.
31. Rahal, S.C.; Amaral, M.S.P.; Pai, V.D.; Barraviera, S.R.C.S.; Caporali, E.H.G.; Crocci, A.J. Effect of fibrin glue derived from snake venom on the viability of autogenous split-thickness skin graft. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2004**, *10*, 161–172. https://doi.org/10.1590/S1678-91992004000200006.
32. Bayat, M.; Virdi, A.; Jalalifirouzkouhi, R.; Rezaei, F. Comparison of effects of LLLT and LIPUS on fracture healing in animal models and patients: A systematic review. *Progr. Biophys. Mol. Biol.* **2018**, *132*, 3–22. https://doi.org/10.1016/j.pbiomolbio.2017.07.004.
33. Hanna, R.; Dalvi, S.; Amaroli, A.; De Angelis, N.; Benedicenti, S. Effects of photobiomodulation on bone defects grafted with bone substitutes: A systematic review of in vivo animal studies. *J. Biophotonics* **2021**, *14*, e202000267. https://doi.org/10.1002/jbio.202000267.
34. Inchingolo, F.; Hazballa, D.; Inchingolo, A.D.; Malcangi, G.; Marinelli, G.; Mancini, A.; Maggiore, M.E.; Bordea, I.R.; Scarano, A.; Farronato, M.; et al. Innovative Concepts and Recent Breakthrough for Engineered Graft and Constructs for Bone Regeneration: A Literature Systematic Review. *Materials* **2022**, *15*, 1120. https://doi.org/10.3390/ma15031120.
35. Magri, A.M.P.; Parisi, J.R.; de Andrade, A.L.M.; Rennó, A.C.M. Bone substitutes and photobiomodulation in bone regeneration: A systematic review in animal experimental studies. *J. Biomed. Mater. Res. A* **2021**, *109*, 1765–1775. https://doi.org/10.1002/jbm.a.37170.
36. Amaroli, A.; Agas, D.; Laus, F.; Cuteri, V.; Hanna, R.; Sabbieti, M.G.; Benedicenti, S. The Effects of Photobiomodulation of 808 nm Diode Laser Therapy at Higher Fluence on the in Vitro Osteogenic Differentiation of Bone Marrow Stromal Cells. *Front. Physiol.* **2018**, *9*, 123. https://doi.org/10.3389/fphys.2018.00123.
37. Escudero, J.S.B.; Perez, M.G.B.; de Oliveira Rosso, M.P.; Buchaim, D.V.; Pomini, K.T.; Campos, L.M.G.; Audi, M.; Buchaim, R.L. Photobiomodulation therapy (PBMT) in bone repair: A systematic review. *Injury* **2019**, *50*, 1853–1867. https://doi.org/10.1016/j.injury.2019.09.031.
38. Gerbi, M.; Miranda, J.M.; Arruda, J.A.A.; Moreno, L.M.M.; Carneiro, V.S.M.; Brasilino, N.C.; Menezes, R.F.; Brugnera, A., Jr.; Pinheiro, A.L.B. Photobiomodulation Therapy in Bone Repair Associated with Bone Morphogenetic Proteins and Guided Bone Regeneration: A Histomorphometric Study. *Photomed. Laser Surg.* **2018**, *36*, 581–588. https://doi.org/10.1089/pho.2017.4421.
39. Roddy, E.; DeBaun, M.R.; Daoud-Gray, A.; Yang, Y.P.; Gardner, M.J. Treatment of critical-sized bone defects: Clinical and tissue engineering perspectives. *Eur. J. Orthop. Surg. Traumatol.* **2018**, *28*, 351–362. https://doi.org/10.1007/s00590-017-2063-0.
40. Percie du Sert, N.; Ahluwalia, A.; Alam, S.; Avey, M.T.; Baker, M.; Browne, W.J.; Clark, A.; Cuthill, I.C.; Dirnagl, U.; Emerson, M.; et al. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.* **2020**, *18*, e3000411. https://doi.org/10.1371/journal.pbio.3000411.
41. Ferreira, R.S., Jr.; de Barros, L.C.; Abbade, L.P.F.; Barraviera, S.; Silvares, M.R.C.; de Pontes, L.G.; dos Santos, L.D.; Barraviera, B. Heterologous fibrin sealant derived from snake venom: From bench to bedside—An overview. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2017**, *23*, 21. https://doi.org/10.1186/s40409-017-0109-8.
42. Ressler, A.; Antunović, M.; Teruel-Biosca, L.; Ferrer, G.G.; Babić, S.; Urlić, I.; Ivanković, M.; Ivanković, H. Osteogenic differentiation of human mesenchymal stem cells on substituted calcium phosphate/chitosan composite scaffold. *Carbohydr. Polym.* **2022**, *277*, 118883. https://doi.org/10.1016/j.carbpol.2021.118883.
43. Gómez, S.; Vlad, M.D.; López, J.; Fernández, E. Design and properties of 3D scaffolds for bone tissue engineering. *Acta Biomater.* **2016**, *42*, 341–350. https://doi.org/10.1016/j.actbio.2016.06.032.
44. Buchaim, D.V.; Andreo, J.C.; Pomini, K.T.; Barraviera, B.; Ferreira, R.S.; Duarte, M.A.H.; Alcalde, M.P.; Reis, C.H.B.; Teixeira, D.B.; Bueno, C.R.S.; et al. A biocomplex to repair experimental critical size defects associated with photobiomodulation therapy. *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2022**, *28*, e20210056. https://doi.org/10.1590/1678-9199-jvatitd-2021-0056.

45. De Oliveira Gonçalves, J.B.; Buchaim, D.V.; de Souza Bueno, C.R.; Pomini, K.T.; Barraviera, B.; Ferreira, R.S., Jr.; Andreo, J.C.; Rodrigues, A.C.; Cestari, T.M.; Buchaim, R.L. Effects of low-level laser therapy on autogenous bone graft stabilized with a new heterologous fibrin sealant. *J. Photochem. Photobiol. B* **2016**, *162*, 663–668. https://doi.org/10.1016/j.jphotobiol.2016.07.023.
46. De Marco, A.C.; Torquato, L.C.; Gonçalves, P.R.; Ribeiro, T.C.; Nunes, C.M.; Bernardo, D.V.; Gomes, M.S.; Jardini, M.A.N.; Santamaria, M.P. The Effect of Photobiomodulation Therapy in Different Doses on Bone Repair of Critical Size Defects in Rats: A Histomorphometric Study. *J. Lasers Med. Sci.* **2021**, *12*, e53. https://doi.org/10.34172/jlms.2021.53.
47. Macedo, A.A.P.; Santos, T.D.; Cunha, J.L.S.; Matos, F.S.; Albuquerque, R.L.C., Jr.; Ribeiro, M.A.G. Effect of laser photobiomodulation associated with a bioceramic cement on the repair of bone tissue in the femur of rats. *J. Photochem. Photobiol. B* **2020**, *205*, 111813. https://doi.org/10.1016/j.jphotobiol.2020.111813.
48. De Oliveira, D.; de Oliveira Puttini, I.; Silva Gomes-Ferreira, P.H.; Palin, L.P.; Matsumoto, M.A.; Okamoto, R. Effect of intermittent teriparatide (PTH 1-34) on the alveolar healing process in orchiectomized rats. *Clin. Oral Investig.* **2019**, *23*, 2313–2322. https://doi.org/10.1007/s00784-018-2672-y.
49. Oliveira, D.; Hassumi, J.S.; Gomes-Ferreira, P.H.; Polo, T.O.; Ferreira, G.R.; Faverani, L.P.; Okamoto, R. Short term sodium alendronate administration improves the peri-implant bone quality in osteoporotic animals. *J. Appl. Oral Sci.* **2017**, *25*, 42–52. https://doi.org/10.1590/1678-77572016-0165.
50. Ramalho-Ferreira, G.; Faverani, L.P.; Momesso, G.A.C.; Luvizuto, E.R; de Oliveira Puttini, I.; Okamoto, R. Effect of antiresorptive drugs in the alveolar bone healing. A histometric and immunohistochemical study in ovariectomized rats. *Clin. Oral Investig.* **2017**, *21*, 1485–1494. https://doi.org/10.1007/s00784-016-1909-x.
51. Garcia, V.J.; Arnabat, J.; Comesaña, R.; Kasem, K.; Ustrell, J.M.; Pasetto, S.; Segura, O.P.; ManzanaresCéspedes, M.C.; Carvalho-Lobato, P. Effect of low-level laser therapy after rapid maxillary expansion: A clinical investigation. *Lasers Med. Sci.* **2016**, *31*, 1185–1194.. https://doi.org/10.1007/s10103-016-1970-3.
52. De Oliveira Puttini, I.; Gomes-Ferreira, P.H.D.S.; de Oliveira, D.; Hassumi, J.S.; Gonçalves, P.Z.; Okamoto, R. Teriparatide improves alveolar bone modelling after tooth extraction in orchiectomized rats. *Arch. Oral Biol.* **2019**, *102*, 147–154. https://doi.org/10.1016/j.archoralbio.2019.04.007.
53. Rosso, M.P.O.; Buchaim, D.V.; Kawano, N.; Furlanette, G.; Pomini, K.T.; Buchaim, R.L. Photobiomodulation Therapy (PBMT) in Peripheral Nerve Regeneration: A Systematic Review. *Bioengineering* **2018**, *5*, 44. https://doi.org/10.3390/bioengineering5020044.
54. Guimarães,K.B.; do Egito Vasconcelos, B.C.; de Assis Limeira, F., Jr.; de Sousa, F.B.; de Souza Andrade, E.S.; de Holanda Vasconcellos, R.J. Histomorphometric evaluation of calcium phosphate bone grafts on bone repair. *Braz. J. Otorhinolaryngol.* **2011**, *77*, 447–454. https://doi.org/10.1590/S1808-86942011000400007.
55. Petronis, S.; Jakusonoka, R.; Linovs, V.; Jumtins, A. Filling Bone Defects after Hip Arthroplasty Revision Using Hydroxyapatite/β-tricalcium Phosphate: A Case Report with Long-term Result. *J. Orthop. Case Rep.* **2021**, *11*, 5–9. https://doi.org/10.13107/jocr.2021.v11.i06.2234.
56. Ebrahimi, M.; Botelho, M. Biphasic calcium phosphates (BCP) of hydroxyapatite (HA) and tricalcium phosphate (TCP) as bone substitutes: Importance of physicochemical characterizations in biomaterials studies. *Data Brief* **2016**, *10*, 93–97. https://doi.org/10.1016/j.dib.2016.11.080.
57. Seifert, A.; Tylek, T.; Blum, C.; Hemmelmann, N.; Böttcher, B.; Gbureck, U.; Groll, J. Calcium phosphate-based biomaterials trigger human macrophages to release extracellular traps. *Biomaterials* **2022**, *285*, 121521. https://doi.org/10.1016/j.biomaterials.2022.121521.
58. MacMillan, A.K.; Lamberti, F.V.; Moulton, J.N.; Geilich, B.M.; Webster, T.J. Similar healthy osteoclast and osteoblast activity on nanocrystalline hydroxyapatite and nanoparticles of tri-calcium phosphate compared to natural bone. *Int. J. Nanomed.* **2014**, *9*, 5627–5637. https://doi.org/10.2147/IJN.S66852.
59. Nogueira, D.M.B.; Figadoli, A.L.F.; Alcantara, P.L.; Pomini, K.T.; Santos German, I.J.; Reis, C.H.B.; Rosa, G.M., Jr.; Rosso, M.P.O.; da Silva Santos, P.S.; Zangrando, M.S.R.; et al. Biological Behavior of Xenogenic Scaffolds in Alcohol-Induced Rats: Histomorphometric and Picrosirius Red Staining Analysis. *Polymers* **2022**, *14*, 584. https://doi.org/10.3390/polym14030584.
60. Pinheiro, A.L.; Martinez Gerbi, M.E.; de Assis Limeira, F., Jr.; Carneiro Ponzi, E.A.; Marques, A.M.; Carvalho, C.M.; Santos, R.C.; Oliveira, P.C.; Nóia, M.; Ramalho, L.M.P. Bone repair following bone grafting hydroxyapatite guided bone regeneration and infra-red laser photobiomodulation: A histological study in a rodent model. *Lasers Med. Sci.* **2009**, *24*, 234–240. https://doi.org/10.1007/s10103-008-0556-0.
61. Torquato, L.C.; Suárez, E.A.C.; Bernardo, D.V.; Pinto, I.L.R.; Mantovani, L.O.; Silva, T.I.L.; Jardini, M.A.N.; Santamaria, M.P.; de Marco, A.C. Bone repair assessment of critical size defects in rats treated with mineralized bovine bone (Bio-Oss®) and photobiomodulation therapy: A histomorphometric and immunohistochemical study. *Lasers Med. Sci.* **2021**, *36*, 1515–1525. https://doi.org/10.1007/s10103-020-03234-5.
62. Amid, R.; Kadkhodazadeh, M.; Ahsaie, M.G.; Hakakzadeh, A. Effect of low level laser therapy on proliferation and differentiation of the cells contributing in bone regeneration. *J. Lasers Med. Sci.* **2014**, *5*, 163–170.
63. Tani, A.; Chellini, F.; Giannelli, M.; Nosi, D.; Zecchi-Orlandini, S.; Sassoli, C. Red (635 nm), Near-Infrared (808 nm) and Violet-Blue (405 nm) Photobiomodulation Potentiality on Human Osteoblasts and Mesenchymal

Stromal Cells: A Morphological and Molecular In Vitro Study. *Int. J. Mol. Sci.* **2018**, *19*, 1946. https://doi.org/10.3390/ijms19071946.

64. De Freitas, L.F.; Hamblin, M.R. Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE J. Sel. Top. Quantum Electron.* **2016**, *22*, 7000417. https://doi.org/10.1109/jstqe.2016.2561201.
65. Jonasson, T.H.; Zancan, R.; de Oliveira Azevedo, L.; Fonseca, A.C.; Silva, M.C.D.; Giovanini, A.F.; Zielac, J.C.; de Araújo, M.R. Effects of low-level laser therapy and platelet concentrate on bone repair: Histological, histomorphometric, immunohistochemical, and radiographic study. *J. Craniomaxillofac. Surg.* **2017**, *45*, 1846–1853. https://doi.org/10.1016/j.jcms.2017.08.008.
66. Cunha, M.R.; Menezes, F.A.; dos Santos, G.R.; Pinto, C.L.A.; Barraviera, B.; Martins, V.C.A.; Plepis, A.M.G.; Ferreira, R.S., Jr. Hydroxyapatite and a New Fibrin Sealant Derived from Snake Venom as Scaffold to Treatment of Cranial Defects in Rats. *Mater. Res.* **2015**, *18*, 196–203. https://doi.org/10.1590/1516-1439.316014.
67. De Miranda, J.R.; Choi, I.G.G.; Moreira, M.S.; Martins, M.D.; Cortes, A.R.G.; Yoshimoto, M. Histologic Evaluation of Early Bone Regeneration Treated with Simvastatin Associated with Low-Level Laser Therapy. *J. Int. Oral Maxillofac. Implant.* **2019**, *34*, 658–664. https://doi.org/10.11607/jomi.6990.
68. AlGhamdi, K.M.; Kumar, A.; Moussa, N.A. Low-level laser therapy: A useful technique for enhancing the proliferation of various cultured cells. *Lasers Med. Sci.* **2012**, *27*, 237–249. https://doi.org/10.1007/s10103-011-0885-2.
69. Kharkwal, G.B.; Sharma, S.K.; Huang, Y.Y.; Dai, T.; Hamblin, M.R. Photodynamic therapy for infections: Clinical applications. *Lasers Surg. Med.* **2011**, *43*, 755–767. https://doi.org/10.1002/lsm.21080.
70. Guarnieri, R.; Belleggia, F.; DeVillier, P.; Testarelli, L. Histologic and Histomorphometric Analysis of Bone Regeneration with Bovine Grafting Material after 24 Months of Healing. A Case Report. *J. Funct. Biomater.* **2018**, *9*, 48. https://doi.org/10.3390/jfb9030048.
71. Buchaim, R.L.; Goissis, G.; Andreo, J.C.; Roque, D.D.; Roque, J.S.; Buchaim, D.V.; Rodrigues, A.d.C. Biocompatibility of anionic collagen matrices and its influence on the orientation of cellular growth. *Braz. Dent. Sci.* **2007**, *10*, 12–20. https://doi.org/10.14295/bds.2007.v10i3.272.
72. Spejo, A.B.; Chiarotto, G.B.; Ferreira, A.D.F.; Gomes, D.A.; Ferreira, R.S., Jr.; Barraviera, B.; Oliveira, A.L.R. Neuroprotection and immunomodulation following intraspinal axotomy of motoneurons by treatment with adult mesenchymal stem cells. *J. Neuroinflamm.* **2018**, *15*, 230. https://doi.org/10.1186/s12974-018-1268-4.
73. Creste, C.F.Z.; Orsi, P.R.; Landim-Alvarenga, F.C.; Justulin, L.A.; Golim, M.d.A.; Barraviera, B.; Ferreira, R.S., Jr. Highly Effective Fibrin Biopolymer Scaffold for Stem Cells Upgrading Bone Regeneration. *Materials* **2020**, *13*, 2747. https://doi.org/10.3390/ma13122747.

Graphical Abstract



3 Discussion

3 DISCUSSION

Fibrin has recently stood out, due to its properties and wide applicability, as an important scaffold in dentistry and regenerative medicine. The search for an efficient process of recomposition of bone defects, with restoration of the original morphology in the shortest period for functional recovery, physical methods are used and, among them, the low-level laser, whose therapy is currently defined as photobiomodulation. Therefore, the objective of this study, with two scientific works on this purpose, was to evaluate the fibrin compounds associated with photobiomodulation in the repair of bone defects.

In the first manuscript, a systematic review of the application of fibrin in tissue engineering associated with photobiomodulation was carried out and, in the second manuscript, a preclinical study in rats where a critical defect in calvaria and filling was performed with an association of two scaffolds, the heterologous fibrin biopolymer and Hydroxyapatite/Tricalcium Phosphate ceramic, with photobiomodulation therapy immediately after surgery and three times a week until euthanasia.

The biostimulatory effects on tissues, such as increased cell proliferation, with emphasis on the activation of osteoblasts in bone tissue regeneration, led to the wide applicability of low-level laser, but it should be noted that the literature demonstrates the lack of standardization in the protocols used in research, as well as how the interaction with different types of scaffold occurs (POMINI *et al.*, 2023).

In the systematic review article, during the selection of studies and their interpretation, it was noted that fibrin is a biological polymer with different indications of use with satisfactory results in the health area, due to the properties of contributing to hemostasis (FERREIRA *et al.*, 2010; SPOTNITZ, 2010), being biocompatible and having a three-dimensional framework. Therefore, we identified its wide use in regenerative science, mainly as scaffolds in tissue regeneration, but also in studies such as drug delivery (AHMAD *et al.*, 2015; RUBALSKII *et al.*, 2019; SPICER; MIKOS, 2010).

The most used fibrin for tissue regeneration, in association with photobiomodulation, is in the form of platelet-rich fibrin (PRF) or fibrin sealants. PRF is an autologous concentrate of platelets, effective in bone regeneration, helps preserve the alveolar ridge and increases osteogenesis (LIU *et al.*, 2019). Fibrin sealants or

glues are mainly composed of the collection of concentrated fibrinogen in the presence of factor XIII and assembled plasma proteins (PLUEMSAKUNTHAI *et al.*, 2013). Commercially available fibrin sealants are expensive and produced from human blood. The only completely heterologous sealant is produced by CEVAP, purified from snake venom (*Crotalus durissus terrificus*) and with buffalo fibrinogen (Bubalus bubalis) (BUCHAIM *et al.*, 2019).

In the results obtained in our studies, both in article 2 and in other previous research (BUCHAIM *et al.*, 2022; REIS *et al.*, 2022), we can observe that the fibrin biopolymer act as a biopharmaceutical that contributes to creating a favorable microenvironment for tissue regeneration, both nerve (BISCOLA *et al.*, 2017; ROSSO *et al.*, 2017) and bone (DELLA COLETTA *et al.*, 2021; IATECOLA *et al.*, 2013), corroborating the results of clinical studies (experimental phase I/II) with the aim of treating chronic venous ulcers (ABBADE *et al.*, 2021).

In addition, due to the difficulty of permanence in the receiving bed of particulate biomaterials, our group developed a biocomplex, incorporating the graft particles to the fibrin biopolymer, which molds itself to the defect, promoting greater stability, therefore, better results in relation to the formation of new bone, especially in critical defects that do not repair spontaneously (BUCHAIM *et al.*, 2022; POMINI *et al.*, 2023).

When bone lesions are not properly repaired, the sequelae can impair the individual's quality of life, one of the reasons for science's constant search for complementary methods in the tissue regeneration and engineering process (CANCEDDA; GIANNONI; MASTROGIACOMO, 2007). Synthetic biomaterials, such as the hydroxyapatite/tricalcium phosphate ceramic (QualyBone BCP® particles, QualyLive, Amadora, Portugal), used in manuscript 2 (REIS *et al.*, 2022), stood out in terms of histological characteristics, being an alternative to autologous bone graft, as it was biocompatible and collaborated as an osteoconductor in the repair of the bone defect (LI *et al.*, 2020).

The association of grafts with photobiomodulation (PBM) promoted better bone formation, both in terms of quality and volume of new bone. In the analysis of collagen by Picrosirius red staining, we observed thicker and parallel oriented collagen fiber bundles with lamellar organization (type I collagen), which is in line with previous studies (BOSSINI *et al.*, 2012; NOGUEIRA *et al.*, 2022).

PBM most used for bone repair is with infrared laser, wavelength of 808, 830 and 904 nm. Even with different wavelengths, energy densities and output power, that is, different protocols, the result has been favorable in terms of cell viability, proliferation, migration and gene expression (ESCUDERO *et al.*, 2019; SHAIKH-KADER; HOURELD, 2022). Our group has been using a protocol initially established by de Oliveira Gonçalves (DE OLIVEIRA GONÇALVES *et al.*, 2016b) and perfected according to current needs and situations. Currently, we are also testing a single application protocol, during surgery, also with good morphological and morphometric results in bone repair (POMINI *et al.*, 2023).

New analyzes with biomarkers and biomechanical functionality could deepen the findings of histomorphometry, can be considered as limiting factors of this research. As perspectives, future studies with different concentrations of HFB and specific analysis of collagen fibers may improve the evaluation of the osteogenic capacity of the association of the biopolymer.

New analyzes, such as immunohistochemistry, could improve the findings of our study, and may be considered as limiting factors of this research. As perspectives, future studies with different concentrations of fibrin biopolymer, with a reduction in the amount of fibrinogen, providing a more permeable three-dimensional mesh for the proliferation of osteoblasts, may improve the good results already obtained so far.

Furthermore, our line of research in photobiomodulation tends to expand with the use of the intravascular technique, known as ILIB (Intravascular Laser Irradiation of Blood), and also with LED (Light-Emiting Diode) clusters.

4 Conclusion

4 **CONCLUSION**

The literature consulted on PBM, associated with fibrin compounds, scores positive results in several areas of tissue bioengineering, mainly in the recovery of extensive bone loss and peripheral nerve injuries. The reproducibility of research in this area presents problems, due to the numerous protocols that are used and not always fully described in scientific articles.

The interaction of the biocomplex composed of Hydroxyapatite/Tricalcium Phosphate Ceramic and Fibrin Biopolymer was potentially effective in the reconstruction of critical bone defects in the calvaria of rats, because the combined use generated perspectives of faster regeneration than when biomaterials and biopharmaceuticals are used separately.

References

REFERENCES

ABBADE, L. P. F.; BARRAVIERA, S. R. C. S.; SILVARES, M. R. C.; LIMA, A. B. B. d. C. O.; HADDAD, G. R.; GATTI, M. A. N.; MEDOLAGO, N. B.; RIGOTTO CARNEIRO, M. T.; DOS SANTOS, L. D.; FERREIRA, R. S.; BARRAVIERA, B. Treatment of Chronic Venous Ulcers With Heterologous Fibrin Sealant: A Phase I/II Clinical Trial. **Frontiers in Immunology**, v. 12, n. February, p. 1–16, 2021.

AHMAD, E.; FATIMA, M. T.; HOQUE, M.; OWAIS, M.; SALEEMUDDIN, M. Fibrin matrices: The versatile therapeutic delivery systems. **International Journal of Biological Macromolecules**, v. 81, p. 121–136, 2015. Disponível em: <http://dx.doi.org/10.1016/j.ijbiomac.2015.07.054>.

ALVES, F. A. M.; MARQUES, M. M.; CAVALCANTI, S. C. S. X. B.; PEDRONI, A. C. F.; FERRAZ, E. P.; MINIELLO, T. G.; MOREIRA, M. S.; JERÔNIMO, T.; DEBONI, M. C. Z.; LASCALA, C. A. Photobiomodulation as adjunctive therapy for guided bone regeneration. A microCT study in osteoporotic rat model. **Journal of Photochemistry and Photobiology B: Biology**, v. 213, n. October, p. 112053, 2020. Disponível em: <https://doi.org/10.1016/j.jphotobiol.2020.112053>.

BISCOLA, N. P.; CARTAROZZI, L. P.; ULIAN-BENITEZ, S.; BARBIZAN, R.; CASTRO, M. V.; SPEJO, A. B.; FERREIRA, R. S.; BARRAVIERA, B.; OLIVEIRA, A. L. R. Multiple uses of fibrin sealant for nervous system treatment following injury and disease. **Journal of Venomous Animals and Toxins Including Tropical Diseases**, v. 23, n. 1, p. 1–11, 2017.

BOSSINI, P. S.; RENNÓ, A. C. M.; RIBEIRO, D. A.; FANGEL, R.; RIBEIRO, A. C.; LAHOZ, M. de A.; PARIZOTTO, N. A. Low level laser therapy (830nm) improves bone repair in osteoporotic rats: Similar outcomes at two different dosages. **Experimental Gerontology**, v. 47, n. 2, p. 136–142, 2012. Disponível em: <http://dx.doi.org/10.1016/j.exger.2011.11.005>.

BRESSAN, E.; FAVERO, V.; GARDIN, C.; FERRONI, L.; IACOBELLIS, L.; FAVERO, L.; VINDIGNI, V.; BERENGO, M.; SIVOLELLA, S.; ZAVAN, B. Biopolymers for Hard

and Soft Engineered Tissues: Application in Odontoiatric and Plastic Surgery Field. **Polymers**, v. 3, n. 1, p. 509–526, 2011.

BUCHAIM, D. V.; ANDREO, J. C.; FERREIRA JUNIOR, R. S.; BARRAVIERA, B.; DE CASTRO RODRIGUES, A.; DE CÁSSIA MACEDO, M.; ROSA JUNIOR, G. M.; SHINOHARA, A. L.; GERMAN, I. J. S.; POMINI, K. T.; BUCHAIM, R. L. Efficacy of Laser Photobiomodulation on Morphological and Functional Repair of the Facial Nerve. **Photomedicine and Laser Surgery**, v. 35, n. 8, 2017.

BUCHAIM, D. V.; ANDREO, J. C.; POMINI, K. T.; BARRAVIERA, B.; FERREIRA, R. S.; DUARTE, M. A. H.; ALCALDE, M. P.; REIS, C. H. B.; DE BORTOLI TEIXEIRA, D.; DE SOUZA BUENO, C. R.; DETREGIACHI, C. R. P.; ARAUJO, A. C.; BUCHAIM, R. L. A biocomplex to repair experimental critical size defects associated with photobiomodulation therapy. **Journal of Venomous Animals and Toxins Including Tropical Diseases**, v. 28, n. July 2021, p. 1–14, 2022.

BUCHAIM, D. V.; CASSARO, C. V.; SHINDO, J. V. T. C.; COLETTA, B. B. D.; POMINI, K. T.; DE OLIVEIRA ROSSO, M. P.; CAMPOS, L. M. G.; FERREIRA, R. S.; BARRAVIERA, B.; BUCHAIM, R. L. Unique heterologous fibrin biopolymer with hemostatic, adhesive, sealant, scaffold and drug delivery properties: A systematic review. **Journal of Venomous Animals and Toxins Including Tropical Diseases**, v. 25, 2019.

BUCHAIM, R. L.; ANDREO, J. C.; RODRIGUES, A. C.; BUCHAIM, D. V.; DIAS, D. V.; DARE, L. R.; ROQUE, D. D.; ROQUE, J. S. The action of demineralized bovine bone matrix on bone neoformation in rats submitted to experimental alcoholism. **Arquivo Brasileiro de Medicina Veterinaria e Zootecnia**, v. 65, n. 3, 2013.
BUCHAIM, R. L.; BUCHAIM, D. V. Laser therapy together with a fibrin biopolymer improves nerve and bone tissue regeneration. **SciELO in Perspective | Press Releases**, 2022.

CANCEDDA, R.; GIANNONI, P.; MASTROGIACOMO, M. A tissue engineering approach to bone repair in large animal models and in clinical practice. **Biomaterials**, v. 28, n. 29, p. 4240–4250, 2007.

CASSARO, C. V.; JUSTULIN, L. A.; DE LIMA, P. R.; DE ASSIS GOLIM, M.; BISCOLA, N. P.; DE CASTRO, M. V.; DE OLIVEIRA, A. L. R.; DOICHE, D. P.; PEREIRA, E. J.; FERREIRA, R. S.; BARRAVIERA, B. Fibrin biopolymer as scaffold candidate to treat bone defects in rats. **J Venom Anim Toxins Incl Trop Dis.**, v. 25, n. November, p. 1–17, 2019.

CUNHA, F. B.; POMINI, K. T.; PLEPIS, A. M. de G.; MARTINS, V. da C. A.; MACHADO, E. G.; DE MORAES, R.; MUNHOZ, M. de A. E. S.; MACHADO, M. V. R.; DUARTE, M. A. H.; ALCALDE, M. P.; BUCHAIM, D. V.; BUCHAIM, R. L.; FERNANDES, V. A. R.; PEREIRA, E. de S. B. M.; PELEGRINE, A. A.; CUNHA, M. R. da. In Vivo Biological Behavior of Polymer Scaffolds of Natural Origin in the Bone Repair Process. **Molecules (Basel, Switzerland)**, v. 26, n. 6, 2021.

CUNHA, M. R.; MENEZES, F. A.; SANTOS, G. R.; PINTO, C. A. L.; BARRAVIERA, B.; MARTINS, V. da C. A.; PLEPIS, A. M. de G.; FERREIRA JUNIOR, R. S. Hydroxyapatite and a New Fibrin Sealant Derived from Snake Venom as Scaffold to Treatment of Cranial Defects in Rats. **Mat. Res.**, v. 18, n. 1, p. 196–203, 2015.

DE FREITAS DUTRA JÚNIOR, E.; HIDD, S. M. C. M.; AMARAL, M. M.; FILHO, A. L. M. M.; ASSIS, L.; FERREIRA, R. S.; BARRAVIERA, B.; MARTIGNAGO, C. C. S.; FIGUEREDO-SILVA, J.; DE OLIVEIRA, R. A.; TIM, C. R. Treatment of partial injury of the calcaneus tendon with heterologous fibrin biopolymer and/or photobiomodulation in rats. **Lasers in Medical Science**, v. 37, n. 2, p. 971–981, 2022.

DE OLIVEIRA GONÇALVES, J. B.; BUCHAIM, D. V.; DE SOUZA BUENO, C. R.; POMINI, K. T.; BARRAVIERA, B.; JÚNIOR, R. S. F.; ANDREO, J. C.; DE CASTRO RODRIGUES, A.; CESTARI, T. M.; BUCHAIM, R. L. Effects of low-level laser therapy on autogenous bone graft stabilized with a new heterologous fibrin sealant. **Journal of Photochemistry and Photobiology B: Biology**, v. 162, p. 663–668, 2016. Disponível em: <http://dx.doi.org/10.1016/j.jphotobiol.2016.07.023>.

DELLA COLETTA, B. B.; JACOB, T. B.; DE CARVALHO MOREIRA, L. A.; POMINI, K. T.; BUCHAIM, D. V.; ELEUTÉRIO, R. G.; BASTOS MAZUQUELI PEREIRA, E. D. S.; ROQUE, D. D.; DE OLIVEIRA ROSSO, M. P.; COSIN SHINDO, J. V. T.; HÚNGARO DUARTE, M. A.; ALCALDE, M. P.; FERREIRA JÚNIOR, R. S.; BARRAVIERA, B.; APARECIDO DIAS, J.; ANDREO, J. C.; BUCHAIM, R. L. Photobiomodulation therapy on the guided bone regeneration process in defects filled by biphasic calcium phosphate associated with fibrin biopolymer. **Molecules**, v. 26, n. 4, 2021.

DOS SANTOS, D. A.; DE GUZZI PLEPIS, A. M.; DA CONCEIÇÃO AMARO MARTINS, V.; CARDOSO, G. B. C.; SANTOS, A. R.; IATECOLA, A.; ANDRADE, T. N.; MONTEIRO, F. M.; CALEGARI, A. R. A.; CHACON, E. L.; CUNHA, M. R. Effects of the combination of low-level laser therapy and anionic polymer membranes on bone repair. **Lasers in Medical Science**, v. 35, n. 4, p. 813–821, 2020.

ESCUDERO, J. S. B.; PEREZ, M. G. B.; DE OLIVEIRA ROSSO, M. P.; BUCHAIM, D. V.; POMINI, K. T.; CAMPOS, L. M. G.; AUDI, M.; BUCHAIM, R. L. Photobiomodulation therapy (PBMT) in bone repair: A systematic review. **Injury**, v. 50, n. 11, p. 1853–1867, 2019. Disponível em: <https://doi.org/10.1016/j.injury.2019.09.031>.

FERNANDEZ DE GRADO, G.; KELLER, L.; IDOUX-GILLET, Y.; WAGNER, Q.; MUSSET, A. M.; BENKIRANE-JESSEL, N.; BORNERT, F.; OFFNER, D. Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management. **Journal of Tissue Engineering**, v. 9, 2018.

FERREIRA, C. N.; SOUSA, M. O.; DUSSE, L. M. S.; CARVALHO, M. G. A cell-based model of coagulation and its implications. **Revista Brasileira de Hematologia e Hemoterapia**, v. 32, n. 5, 2010.

FERREIRA, R. S.; DE BARROS, L. C.; ABBADE, L. P. F.; BARRAVIERA, S. R. C. S.; SILVARES, M. R. C.; DE PONTES, L. G.; DOS SANTOS, L. D.; BARRAVIERA, B. Heterologous fibrin sealant derived from snake venom: From bench to bedside - an overview. **Journal of Venomous Animals and Toxins Including Tropical**

Diseases, v. 23, n. 1, p. 1–12, 2017.

GAROLA, F.; GILLIGAN, G.; PANICO, R.; LEONARDI, N.; PIEMONTE, E. Clinical management of alveolar osteitis. A systematic review. **Medicina Oral Patologia Oral y Cirugia Bucal**, v. 26, n. 6, p. e691–e702, 2021.

GONÇALVES, A. B.; BOVO, J. L.; GOINES, B. S.; PIGOSO, A. A.; FELONATO, M.; ESQUISATTO, M. A. M.; DE JESUS LOPES FILHO, G.; DO BOMFIM, F. R. C. Photobiomodulation (λ = 808nm) and Platelet-Rich Plasma (PRP) for the Treatment of Acute Rheumatoid Arthritis in Wistar Rats. **Journal of Lasers in Medical Sciences**, v. 12, n. 1, 2021.

GUSKUMA, M. H.; HOCHULI-VIEIRA, E.; PEREIRA, F. P.; RANGEL-GARCIA JUNIOR, I.; OKAMOTO, R.; OKAMOTO, T.; MAGRO FILHO, O. Bone regeneration in surgically created defects filled with autogenous bone: An epifluorescence microscopy analysis in rats. **Journal of Applied Oral Science**, v. 18, n. 4, p. 346–353, 2010.

IATECOLA, A.; BARRAVIERA, B.; JUNIOR, R. S. F.; DOS SANTOS, G. R.; NEVES, J. I.; DA CUNHA, M. R. Use of a new fibrin sealant and laser irradiation in the repair of skull defects in rats. **Brazilian Dental Journal**, v. 24, n. 5, p. 456–461, 2013.

KHURSHID, Z.; ASIRI;, F. Y. I.; NAJEEB;, S.; RATNAYAKE, J. The Impact of Autologous Platelet Concentrates on the Periapical Tissues and Root Development of Replanted Teeth: A Systematic Review. **Materials**, v. 15, p. 2776, 2022.

LE GUÉHENNEC, L.; LAYROLLE, P.; DACULSI, G.; REDL, H.; PANDIT, A.; CZERNUSZKA, J. A review of bioceramics and fibrin sealant. **Eur Cell Mater.**, v. 8, p. 1–11, 2004.

LI, X.; YUAN, Y.; LIU, L.; LEUNG, Y. S.; CHEN, Y.; GUO, Y.; CHAI, Y.; CHEN, Y. 3D printing of hydroxyapatite/tricalcium phosphate scaffold with hierarchical porous structure for bone regeneration. **Bio-Design and Manufacturing**, v. 3, n. 1, p. 15–29, 2020. Disponível em: <https://doi.org/10.1007/s42242-019-00056-5>.

LIU, Y.; SUN, X.; YU, J.; WANG, J.; ZHAI, P.; CHEN, S.; LIU, M.; ZHOU, Y. Platelet-Rich Fibrin as a Bone Graft Material in Oral and Maxillofacial Bone Regeneration: Classification and Summary for Better Application. **BioMed Research International**, v. 2019, 2019.

MASSIMINO, L. C.; DA CONCEIÇÃO AMARO MARTINS, V.; VULCANI, V. A. S.; DE OLIVEIRA, É. L.; ANDREETA, M. B.; BONAGAMBA, T. J.; KLINGBEIL, M. F. G.; MATHOR, M. B.; DE GUZZI PLEPIS, A. M. Use of collagen and auricular cartilage in bioengineering: scaffolds for tissue regeneration. **Cell and Tissue Banking**, v. 0, 2020.

NOGUEIRA, D. M. B.; FIGADOLI, A. L. de F.; ALCANTARA, P. L.; POMINI, K. T.; SANTOS GERMAN, I. J.; REIS, C. H. B.; ROSA JÚNIOR, G. M.; ROSSO, M. P. de O.; SANTOS, P. S. da S.; ZANGRANDO, M. S. R.; PEREIRA, E. de S. B. M.; DE MARCHI, M. Â.; TRAZZI, B. F. de M.; ROSSI, J. de O.; SALMERON, S.; PASTORI, C. M.; BUCHAIM, D. V.; BUCHAIM, R. L. Biological Behavior of Xenogenic Scaffolds in Alcohol-Induced Rats: Histomorphometric and Picrosirius Red Staining Analysis. **Polymers**, v. 14, n. 3, p. 1–15, 2022.

PIETRUSZKA, P.; CHRUŚCICKA, I.; DUŚ-ILNICKA, I.; PARADOWSKA-STOLARZ, A. Prp and prf—subgroups and divisions when used in dentistry. **Journal of Personalized Medicine**, v. 11, n. 10, 2021.

PLUEMSAKUNTHAI, W.; KURODA, S.; SHIMOKAWA, H.; KASUGAI, S. A basic analysis of platelet-rich fibrin: distribution and release of platelet-derived growth factor-BB. **Inflammation and Regeneration**, v. 33, n. 3, p. 164–172, 2013.

POMINI, K. T.; BUCHAIM, D. V.; ANDREO, J. C.; ROSSO, M. P. de O.; DELLA COLETTA, B. B.; GERMAN, Í. J. S.; BIGUETTI, A. C. C.; SHINOHARA, A. L.; ROSA JÚNIOR, G. M.; SHINDO, J. V. T. C.; ALCALDE, M. P.; DUARTE, M. A. H.; TEIXEIRA, D. de B.; BUCHAIM, R. L. Fibrin sealant derived from human plasma as a scaffold for bone grafts associated with photobiomodulation therapy. **International Journal of Molecular Sciences**, v. 20, n. 7, 2019.

POMINI, K. T.; BUCHAIM, D. V.; BIGHETTI, A. C. C.; HAMZÉ, A. L.; REIS, C. H. B.; DUARTE, M. A. H.; ALCALDE, M. P.; BARRAVIERA, B.; JÚNIOR, R. S. F.; DE SOUZA, A. T.; DA SILVA SANTOS, P. S.; PILON, J. P. G.; DE MARCHI, M. Â.; NOGUEIRA, D. M. B.; DE SOUZA BUENO, C. R.; SOARES, W. C.; BUCHAIM, R. L. Tissue Bioengineering with Fibrin Scaffolds and Deproteinized Bone Matrix Associated or Not with the Transoperative Laser Photobiomodulation Protocol. **Molecules**, v. 28, n. 1, 2023.

PRIGLINGER, E.; MAIER, J.; CHAUDARY, S.; LINDNER, C.; WURZER, C.; RIEGER, S.; REDL, H.; WOLBANK, S.; DUNGEL, P. Photobiomodulation of freshly isolated human adipose tissue-derived stromal vascular fraction cells by pulsed light-emitting diodes for direct clinical application. **Journal of Tissue Engineering and Regenerative Medicine**, v. 12, n. 6, p. 1352–1362, 2018.

REIS, C. H. B.; BUCHAIM, R. L.; POMINI, K. T.; HAMZÉ, A. L.; ZATTITI, I. V.; DUARTE, M. A. H.; ALCALDE, M. P.; BARRAVIERA, B.; FERREIRA JÚNIOR, R. S.; PONTES, F. M. L.; GRANDINI, C. R.; ORTIZ, A. de C.; FIDELES, S. O. M.; EUGÊNIO, R. M. de C.; ROSA JUNIOR, G. M.; TEIXEIRA, D. de B.; PEREIRA, E. de S. B. M.; PILON, J. P. G.; MIGLINO, M. A.; BUCHAIM, D. V. Effects of a Biocomplex Formed by Two Scaffold Biomaterials, Hydroxyapatite/Tricalcium Phosphate Ceramic and Fibrin Biopolymer, with Photobiomodulation, on Bone Repair. **Polymers**, v. 14, n. 10, p. 2075, 2022.

ROSSO, M. P. D. O.; OYADOMARI, A. T.; POMINI, K. T.; BOTTEON, B.; COLETTA, D.; COSIN, T.; SEABRA, R.; FERREIRA, J.; BARRAVIERA, B.; CASSARO, C. V.; BUCHAIM, D. V.; TEIXEIRA, D. D. B.; BARBALHO, S. M.; ALCALDE, M. P.; ANTONIO, M.; DUARTE, H.; ANDREO, J. C. Photobiomodulation Therapy Associated with Heterologous Fibrin Biopolymer and Bovine Bone Matrix Helps to Reconstruct Long Bones. **Biomolecules**, v. 10, n. 3, p. 1–17, 2020.

ROSSO, M. P. de O.; ROSA JÚNIOR, G. M.; BUCHAIM, D. V.; GERMAN, I. J. S.; POMINI, K. T.; DE SOUZA, R. G.; PEREIRA, M.; FAVARETTO JÚNIOR, I. A.; BUENO, C. R. de S.; GONÇALVES, J. B. de O.; FERREIRA JÚNIOR, R. S.;

BARRAVIERA, B.; ANDREO, J. C.; BUCHAIM, R. L. Stimulation of morphofunctional repair of the facial nerve with photobiomodulation, using the end-to-side technique or a new heterologous fibrin sealant. **Journal of Photochemistry and Photobiology B: Biology**, v. 175, n. August, p. 20–28, 2017a. Disponível em: <http://dx.doi.org/10.1016/j.jphotobiol.2017.08.023>.

RUBALSKII, E.; RUEMKE, S.; SALMOUKAS, C.; ALESHKIN, A.; BOCHKAREVA, S.; MODIN, E.; MASHAQI, B.; BOYLE, E. C.; BOETHIG, D.; RUBALSKY, M.; ZULKARNEEV, E.; KUEHN, C.; HAVERICH, A. Fibrin glue as a local drug-delivery system for bacteriophage PA5. **Scientific Reports**, v. 9, n. 1, p. 3–10, 2019. Disponível em: <http://dx.doi.org/10.1038/s41598-018-38318-4>.

SAKKAS, A.; WILDE, F.; HEUFELDER, M.; WINTER, K.; SCHRAMM, A. Autogenous bone grafts in oral implantology—is it still a “gold standard”? A consecutive review of 279 patients with 456 clinical procedures. **Int J Implant Dent.**, v. 3, n. 1, p. 23, 2017.
SCHINDELER, A.; MILLS, R. J.; BOBYN, J. D.; LITTLE, D. G. Preclinical models for orthopedic research and bone tissue engineering. **J Orthop Res.**, v. 36, n. 3, p. 832–840, 2018.

SHAIKH-KADER, A.; HOURELD, N. N. Photobiomodulation, Cells of Connective Tissue and Repair Processes: A Look at In Vivo and In Vitro Studies on Bone, Cartilage and Tendon Cells. **Photonics**, v. 9, n. 9, 2022.

SOHN, H. S.; OH, J. K. Review of bone graft and bone substitutes with an emphasis on fracture surgeries. **Biomater Res.**, v. 23, n. 1, p. 4–10, 2019.

SPICER, P. P.; MIKOS, A. G. Fibrin glue as a drug delivery system. **Journal of Controlled Release**, v. 148, n. 1, p. 49–55, 2010. Disponível em: <http://dx.doi.org/10.1016/j.jconrel.2010.06.025>.

SPOTNITZ, W. D. Fibrin sealant: Past, present, and future: A brief review. **World Journal of Surgery**, v. 34, n. 4, p. 632–634, 2010.

STATE, P.; REGINA, S.; SARTORI, C.; STATE, P.; SILVARES, M. R.; SATATE, P. A

new fibrin sealant derived from snake venom candidate to treat chronic venous ulcers. **Journal of the American Academy of Dermatology**, v. 72, n. 5, p. AB271, 2015.

TALLARICO, M.; XHANARI, E.; LUMBAU, A. M. I.; ALUSHI, A.; IERIA, I.; FIORILLO, L.; FAMÀ, F.; METO, A.; BALDONI, E.; MELONI, S. M.; CICCIÙ, M. Histological and histomorphometric evaluation of post-extractive sites filled with a new bone substitute with or without autologous plate concentrates: One-year randomized controlled trial. **Materials**, v. 15, n. 1, 2022.

VENANTE, H. S.; CHAPPUIS-CHOCANO, A. P.; MARCILLO-TOALA, O. O.; DA SILVA, R. A.; DA COSTA, R. M. B.; PORDEUS, M. D.; BARRAVIERA, B.; JUNIOR, R. S. F.; LARA, V. S.; NEPPELENBROEK, K. H.; HONÓRIO, H. M.; PORTO, V. C. Fibrin biopolymer incorporated with antimicrobial agents: a proposal for coating denture bases. **Materials**, v. 14, n. 7, p. 1–14, 2021.

XU, A.; ZHUANG, C.; XU, S.; HE, F.; XIE, L.; YANG, X.; GOU, Z. Optimized Bone Regeneration in Calvarial Bone Defect Based on Biodegradation-Tailoring Dual-shell Biphasic Bioactive Ceramic Microspheres. **Scientific Reports**, v. 8, n. 1, p. 1–14, 2018. Disponível em: <http://dx.doi.org/10.1038/s41598-018-21778-z>.

ZAFAR, M. S.; AMIN, F.; FAREED, M. A.; GHABBANI, H.; RIAZ, S.; KHURSHID, Z.; KUMAR, N. Biomimetic aspects of restorative dentistry biomaterials. **Biomimetics**, v. 5, n. 3, p. 1–42, 2020.

ZEIN, R.; SELTING, W.; BENEDICENTI, S. Effect of Low-Level Laser Therapy on Bone Regeneration During Osseointegration and Bone Graft. **Photomedicine and Laser Surgery**, v. 35, n. 12, p. pho.2017.4275, 2017.

Annexes

ANNEX

Annex 1: Publisher permission, open access journal

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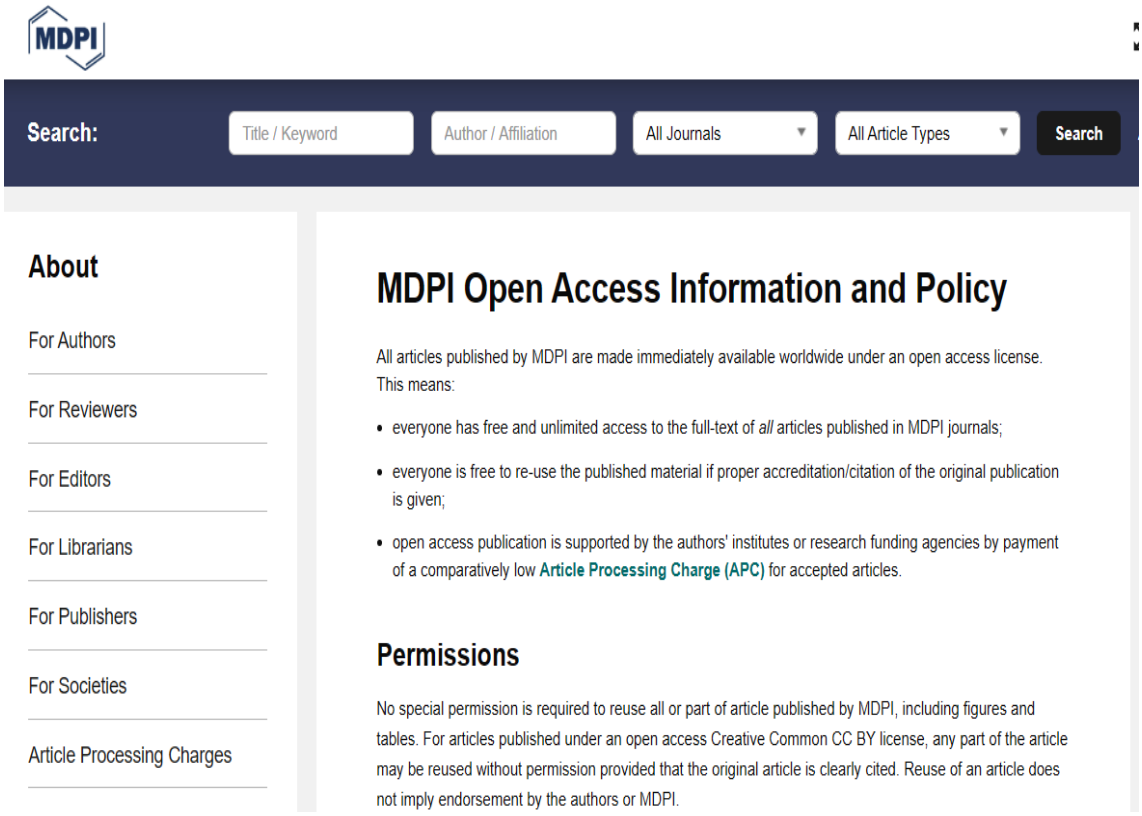

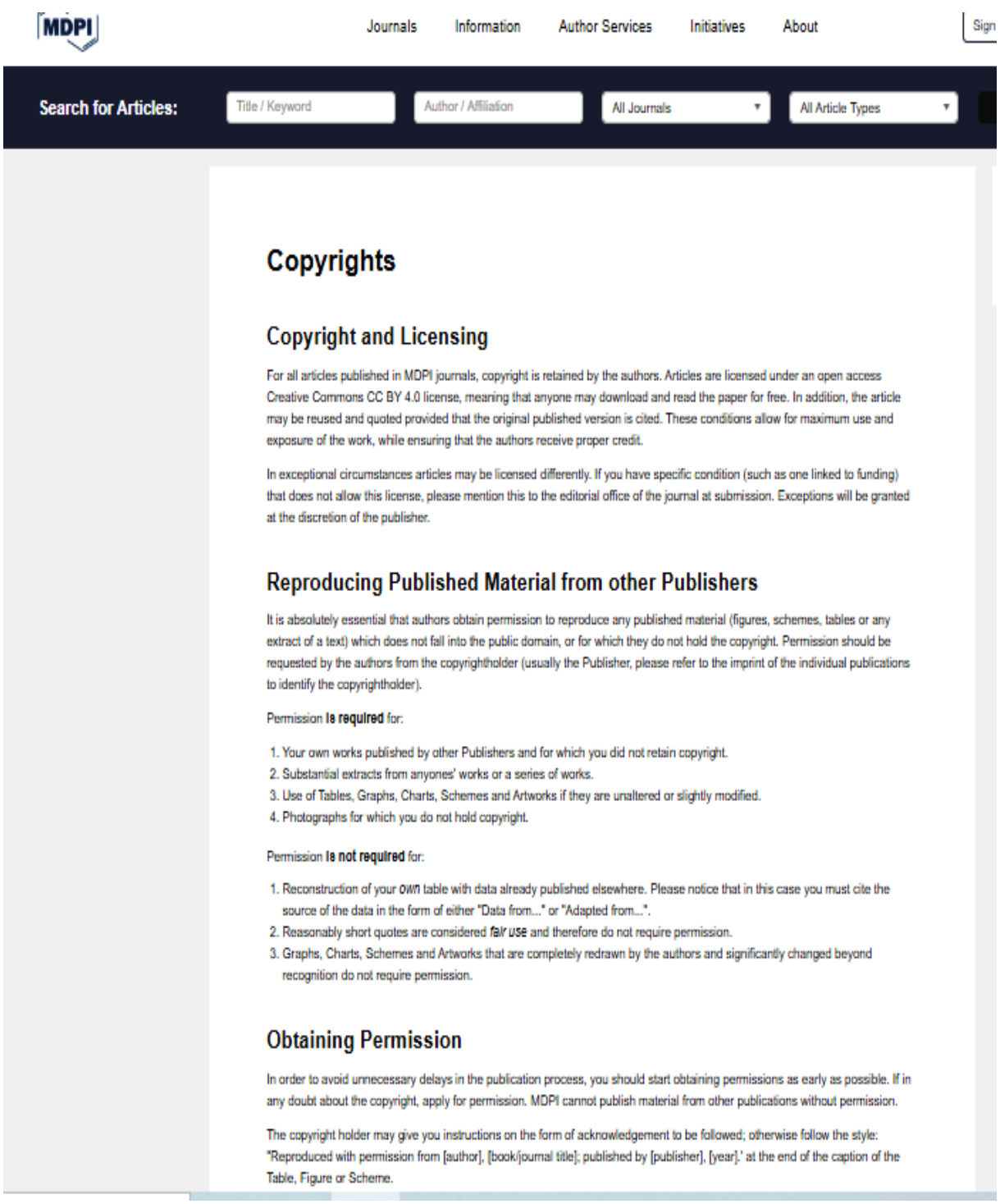

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Annex 2: Approval of Animal Ethical Committee

UNIVERSIDADE DE MARÍLIA

CEUA – *Comitê de Ética em uso Animal*

CERTIFICADO

CIAEP-01.0218.2014

Certificamos que o projeto intitulado **"EFEITOS DA TERAPIA POR FOTOBIOMODULAÇÃO NO PROCESSO DE REPARO DE DEFEITOS ÓSSEOS PREENCHIDOS POR HIDROXIAPATITA-FOSFATO TRICÁLCICO (QUALY BONE BCP) ASSOCIADO AO BIOPOLÍMERO DE FIBRINA" (Protocolo 011/2019)** que envolve produção, manutenção e /ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa cientifica encontra-se de acordo com os preceitos da lei nº 11794, de 8 de outubro de 2008, do Decreto no 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), foi aprovado pelo COMITÊ DE ÉTICA EM USO ANIMAL (CEUA) DA UNIVERSIDADE DE MARÍLIA.

Vigência do projeto	Junho a dezembro de 2020
Espécie/linhagem	Ratos Wistar
Número de animais	56
Peso / Idade	250g
Sexo	Machos

Marília, 03 de junho 2019,

Profa. Dra. Sandra Maria Barbalho
Vice Coordenadora do CEUA

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