Review

Effect of Photobiomodulation on Platelet-Rich Plasma: Review Series on New Tools in Regenerative Medicine

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Short title: **Photobiomodulation and Platelet-Rich Plasma**

Abstract

Objective: platelet-rich plasma is one of the blood-derived autologous biological products, that has now become a therapeutic tool. Though its properties have not been fully elucidated yet, the ease of its sample obtention, product processing, and patient application, along with the good results obtained, has extended its application to many medical specialties such as orthopedics, sports, and aesthetic medicine, or gynecology. Lately, photobiomodulation has been presented as an effective PRP activator, resembling what occurs on mesenchymal cells that have been widely studied. This article aims to give a modern view on PRP and its activation through photobiomodulation.
Methods: A review series was carried out in PubMed, Cochrane, and Scopus to find articles about studies done on humans on PRP and photobiomodulation.
Results: a total of five studies with small samples were found. In all of them, the activation with photobiomodulation had positive results.
Conclusion: photobiomodulation showed great potential for PRP activation. However, more studies must be carried out to establish the appropriate protocols with which all potential clinical benefits can be obtained.

Keywords

Photobiomodulation, photoactivation, platelet-rich plasma, platelet, infrared, near-infrared

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Introduction

In the last decade, blood-derived autologous biological products have become useful therapeutic tools for treating several conditions. Below are some medical specialties in which the application of these products is most developed: 1) wound healing, and regenerative medicine, especially for diabetic foot ulcers[1]; 2) orthopedic and sports medicine, where they are used to alleviate pain caused by conditions such as tendinitis, arthritis, ligament sprains, tears, or intervertebral disc degeneration[2-4]; 3) gynecology, where they are used to treat cervical ectopy, vulvar dystrophy, reconstructive surgery for vulvar cancer in urogenital disorders, genital prolapse or urinary incontinence[3]; 4) dermatology and aesthetic medicine, which use them for hair restoration, skin rejuvenation, acne scars, dermal augmentation, and striae distensae, among other conditions[3,5]; 5) cardiac surgery[6]. These types of autologous therapeutic strategies include platelet concentrates (PC), mainly represented by platelet rich-plasma (PRP) and platelet-rich fibrin (PRF), which combine bioactive components derived from plasma/platelets like the cytokines, chemokines, growth factors (GF), and enzymes[7].

PRP is obtained by processing blood as a supra-physiological platelet concentration included in a small volume of plasma, which can be used in the liquid phase or in gel form[8]. Its rationale is based on improving the healing process by increasing the concentration of the platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), transforming growth factor ß (TGF-ß), vascular endothelial growth factor (VEGF), interleukins, hormones, and several hundred other proteins released by platelets[9-11].

Despite the large diversity of protocols for PRP preparation, all of them involve a common sequence: 1) extraction of peripheral blood; 2) centrifugation of the sample; 3) concentration of the platelets; 4) platelet stimulation. Multiple variations can be identified in each of these phases, mainly: 1) volume of drawn blood; 2) type of anticoagulant used; 3) centrifugation parameters; 4) extraction and sample collection materials; 5) the type of platelet-activating agents[12]. All of this results in a highly heterogeneous biological potential[13].

PRP Collection Procedures

There are two basic protocols: those designed based on plasma and those based on the leucocyte layer[8]. Those based on plasma retrieve from 300,000 to 500,000 platelets/µL, employing lower revolutions and less centrifugation time. Alternatively, protocols based on the leucocyte layer, which use buffy-coat systems, apply higher revolution centrifugation cycles for more minutes. The product obtained through these systems is leucocyte-rich PRP (L-PRP). It is characterized by a high concentration of platelets (500,000 to 1,500,000 platelets/µL) and the variable presence of leucocytes and erythrocytes[14].

The efficiency of PRP collection procedures could be improved by adjusting some of the process variables and by standardizing protocols[13,15]. Giusti et al. demonstrated that the optimal platelet concentration for angiogenesis induction in human endothelial cells was 1,500,000 platelets/µL; however, excessively high concentrations may inhibit the angiogenic process[16]. In addition, other factors could also influence the final product and should be taken into consideration, such as age, gender, circadian rhythm, or the pharmacological regime[17]. Moreover, it has been shown that its effectiveness can be increased if PRP is combined with other procedures, such as microneedles, dermal fillers, autologous fat grafting, or laser therapies[3]. The effects of PRP photoactivation are currently being studied through light irradiation: photomodulation or photobiomodulation (PBM). The activated PRP results in a product called photoactivated platelet-rich plasma (PA-PRP), of which there are still limited published trials[18].

PRP Chemical Activation Process

The activation of platelets during the preparation of PRP produces the release of the bioactive molecules stored in α–granules and stimulates the development of the matrix through fibrinogen cleavage[19].

The formation of clots traps the GFs generated, allowing bioactive molecules to be released and confined in the injured area. Exogenous or endogenous factors may induce activation. The most common exogenous factors include thrombin, calcium chloride, calcium chloride a thrombin mixture, and calcium gluconate[13,14,20]. Endogenous activation consists of platelet exposure to native collagen or another coagulation factor, such as ADP, thrombospondin, or a platelet-activating factor. Both processes spontaneously induce the formation of clots at the site of the injury, where platelets act[20].

Another exogenous factor that has been suggested as a PRP activator is PBM. In 2020, Irmak et al.[9] described platelet photoactivation in vitro by applying a polychromatic light source in the near-infrared range region and comparing platelets at rest with calcium chloride-activated PRP. This study showed that photoactivation of PRP induced a release of PDGF, FGF, and TGF-beta, which is significantly more significant and more prolonged than that obtained with calcium chloride-mediated activation.

Proposed Mechanism of Action of PBM

Scientific research of PBM started about 50 years ago[21] and, although it is now a promising procedure for treating several diseases, both its intimate mechanism and the wavelengths responsible for triggering its effects remain uncertain[22]. In general, PBM refers to non-invasive, non-toxic phototherapy in the range of 600 to 1000 nm[23]. Its biological effect is attributed to 1) the absorption of light by a photoreceptor of the respiratory chain that would induce mitochondrial activation[23], and to 2) the photons absorbed by mitochondria, which would produce an increase in adenosine triphosphate (ATP)[24]. Another proposed hypothesis associates PBM

with ion channels as the latter would be sensitive to light, thus allowing calcium to enter the cell[25].
Laser light has been shown to stimulate several biological processes, such as cell growth and proliferation[26]. In particular, infrared irradiation affects mammals' bioenergetic balance and mitochondrial biogenesis[27], and can stimulate them[28]. Isolated mitochondrial irradiation induces changes in mitochondrial transcription and translation, increasing the cascade reactions and the number of certain components of the respiratory chain, such as cytochromes, cytochrome oxidase, and flavin dehydrogenase[29]. Lock et al. 2019 suggested that mitochondrial stimulation by PBM could be due to the absorption of light at the metal centers of some molecules of the respiratory chain, which could lead to the stimulation of the cytoplasm and the mitochondrial enzymes[30].
PBM increases complexes I, II, III, IV, and succinate dehydrogenase activity in the electron transfer chain. It has been observed that, following irradiation, some enzymes, such as NADH dehydrogenase or cytochrome C oxidase (CCO), and substrates such as adenine nucleotides, show a significant change in their biochemical properties[31]. Most photostimulation effects could be explained by the absorption of light by cytochrome C oxidase (COX), also known as complex IV, the enzyme that limits the speed of terminal phosphorylation in the mitochondrial respiratory chain9, which seems to be the main photoaceptor. This is supported by 1) increased oxygen consumption during low-level light irradiation, since most of a cell's oxygen consumption is produced at complex IV, in the mitochondria, and 2) the fact that sodium azide (NaN3), a COX inhibitor, cancels out this beneficial effect.
The absorption of photons by COX results in the acceleration of electron transfer reactions and ATP production[26,27]. In addition to increasing ATP and AMPc, PBM also increases the level of nitric oxide (NO), whether from the release of metal complexes into COX (which has two hemes and two copper centers) or due to the regulation of COX activity as a nitrite reductase[32].
There are several devices used for PBM: 1) helium-neon gas lasers (He-Ne), gallium arsenide (GaAs), neodymium-doped yttrium-aluminum garnet (Nd: YAG), aluminum gallium arsenide (GaAlAs), aluminum gallium indium phosphide (InGaAlP), carbon dioxide (CO2); 2) light-emitting diode (LED) matrices; and 3) visible light[23].
It has been well established that the biostimulating effects of the laser depend on parameters such as wavelength, the density of energy, power, frequency, and the duration of the irradiation[33]. Achieving adequate energy dosage to produce the beneficial effects of PBM is a complex task since it not only depends on the parameters of the light applied but also on the correct setting of the source emitting the light energy, the technical application, or the intervals between sessions. Different wavelengths exert different effects on the cells. In stem cells derived from human adipose tissue, for example, it has been observed that red light (660 nm) and near-infrared (810 nm) light stimulate cell proliferation, while blue light (415 nm) and green light (540 nm) inhibit it[34]. To date, the beneficial effects of PBM have been verified both in vivo and in vitro, and in several conditions and physiological processes, such as wound healing, hypoxic injury, and brain degeneration[35], where a decrease in inflammation or stimulation of injury repair have been observed[36]. However, there is still no consensus regarding the molecular, cellular, or tissular mechanisms of action of PBM[37].

PRP Photoactivation

Studies published on PBM and PRP are still scarce. However, many studies on mesenchymal cells have led to promising results, and that could be highly useful to interpret PBM-PRP interactions[38]. Wavelengths between 600 and 1000 nm produce changes in the viability, proliferation, and/or migration of MSCs, predicting an excellent regeneration potential. However, drawing sound conclusions from PBM research on MSCs is no easy task since the multiple devices used and the large variability of parameters applied have caused treatment protocols of varying results[34].
Mandle et al. 2011[39] assessed the viability of platelets and white blood cells, platelet activation, and the release of growth factors and cytokines in the PA-PRP of seven subjects. Cell viability was high in all samples, but the authors concluded that PBM did not activate the platelets. The authors indicate that the treatment effect with this device could be related to white blood cell activation and that experiments are therefore necessary with the PRP produced from whole blood which needs to be compared with paired leucocyte blood samples.
In 2020, Gülseen et al.[40] conducted a study with Gel-MA/PRP hydrogels to assess GF release following the regular application of polychromatic light. They noted that the application of light increased elasticity and decreased the hydrogel radiation rate. The regular application of light resulted in controlled and sustainable GF release, regardless of the number of platelets or GF concentrations, and the protection of PRP bioactivity with high mechanical properties.
In 2021, Ghidini et al.[41] concluded that only a defined amount of energy (fluence 5 J/cm^2 delivered in two minutes and 10 J/cm^2 in four minutes) is most effective to induce cell proliferation and calcium deposition in the presence of platelet-rich plasma.
Considering that platelets also have mitochondria, it could be argued that the effects observed in MSCs following PBM might, to a certain extent, also be observed in platelets: the conditioning of PRP with PBM could provide benefits, such as enhanced regenerative properties[38]. The published studies on PRP conditioning with PBM on humans before application to the patient are summarized below.

In Vivo Research on PRP PBM

A total of five articles were found about the conditioning of human PRP with PBM in vivo: Freitag et al. 2012 and 2013[18,42], Paterson et al. 2016[43], Mohiuddin et al. 2018[44], and Irmak et al. 20209. The parameters and procedures for PRP collection and PBM can be seen in *Table 1 (click here to see Table 1)*.

In 2012 and 2013, Freitag et al.[18] published two clinical cases of two men: a 38-year old with pain in his left knee and a 50-year old with osteoarthritis in the left knee[42]. Both were injected intra-articular PA-PRP. Conditioning was performed at 600-1200 nm. Results showed that the Numerical Pain Rating Scale (NPRS) score improved at 7 weeks and was maintained at 0 until week 15. The Western Ontario and McMaster Universities Arthritis (WOMAC) Index was normalized in both patients between visits 7 and 12, and the patient suffered instability symptoms with entirely resolved osteoarthritis at 15 weeks. In 2016, Paterson et al.[43], conducted a randomized, double-blind study in 19 patients with knee osteoarthritis, Kellgren-Lawrence 2-3. Results of the Osteoarthritis Outcome Score (KOOS) and the Knee Quality of Life 26-item questionnaire (KQoL-26) were measured at 4 and 12 weeks following the PA-PRP injection (n=10). They were compared with a control group (n=9) that received intra-articular hyaluronic acid (HA) injections. The results obtained did not have sufficient statistical power to assess efficacy. Still, preliminary data provided some evidence that the application of PA-PRP in patients with knee arthrosis improved: 1) self-assessed pain, 2) KOOS and KQoL-26 sub-scales, and 3) lower-limb functional capacity tests.
In 2020, Irmak et al.[9], conducted a study in healthy males between 20 and 26 years of age, assessing the level of ATP following the application of polychromatic light to PRP for 10 minutes, after incubation in α-MEM in a CO2 incubator at 37°C for 24 h (*Table 1*). The study consisted of 2 parts: the first showed a significant increase in ATP following PBM; in the second, platelets were also stimulated with PAC, and there was a significant increase in ATP at 24 h. Mohiuddin et al.[44], in 2018, conducted a study in 232 patients between 40 and 70 years old with knee arthrosis. Patients were treated with PA-PRP (*Table 1*), and, to measure their level of effectiveness, total WOMAC scores were measured at the baseline visit and after treatment. At the end of treatment, 12 months following the PA-PRP injection, the total score was significantly reduced (p = 0.00).

Current Barriers of the Effective Implementation of PBM

Cellular conditioning procedures that could help optimize PRP results (and other autologous materials) are well documented. However, this type of protocol requires implementing several successive steps, some of which are not supported by the necessary scientific evidence or are still being developed.

Technical Issues

When deciding the level of effectiveness of PBM for PRP or any other tissue, the following must be considered: 1) control of the energy-emitting source; 2) an understanding of what will happen with the media through which the light will travel; and 3) an understanding of the scope of the obstacles entailed by the media and containers where the PRP will be housed for the application of PBM.
The characteristics of the light energy applied should be accurately known: wavelength, power, and focal distance, among other items. On the other hand, understanding how dispersion, scattering, and other optical phenomena occurring every time light travels through a medium will entail an additional challenge. Finally, it will be essential to study the behavior and properties of PRP as a liquid medium where the target cells will be found to ensure accurate dosage. All these are mainly physical problems.
In general, experimental PBM protocols conducted in PRP or other liquid media-embedded cells placed the target structures in Petri dishes, to which some light-emitting energy source (LEDs or lasers) was applied. These types of setups could have led to some interesting conclusions regarding potential cell responses to light, but in no way could they be used to build therapeutic protocols since they failed to comply with the most basic rule of experimental science: reproducibility. It is necessary to develop a technology or model that will guarantee control of the emitting source and the optical phenomena occurring from the emission of light energy until it impacts the target cell.
In recent years, promising efforts have been undertaken to control the flaws of these types of setups. However, these designs failed since they did not consider the other steps required to obtain an adequate PBM. For example, the study conducted by Mandle et al. in 2011[39], where they applied energy in reproducible conditions but failed to control the container where the target cells were held, lighting the PRP in 10 ml syringes. It cannot be guaranteed that these cells would have received the intended dose since the optical characteristics of the syringe are inadequate and, a setup such as this can cancel out the influence of internal movements or flows of the liquid where the cells are embedded. This is a very sensitive matter, as it means that some of the target cells may have received a higher dosage than planned, while other cells may not have received any energy at all. It becomes apparent that failing to control one or several of these factors may seriously compromise the outcomes and conclusions of any trial. It is necessary to develop a technology or model that consider and control the medium housing the target cells to receive exactly the intended dose since this is the basis for any medical therapeutic procedure.

Biological Issues

The intimate interrelation mechanism between light and the PRP must be understood and agreed upon. Understanding the platelet response changes to the variations in the setup of the source of energy applied will be crucial for the PBM-PRP relationship to change its status from “physical stimulus” to that of a “therapeutic procedure.” Lastly, and perhaps most importantly, we must determine the clinical impact of these changes on cellular responses. In vivo studies in humans are done to understand said clinical impact and the physiological changes that occur after the light energy is absorbed by the PRP target chromophores.

Conclusions

As a renowned therapy with extraordinary regenerative potential, PBM has already embarked upon its transformation journey and will stop being an up-

and-coming tool to become a crucial element in the medical armamentarium. This transformation entails awareness and understanding of all the steps and elements involved through sound evidence. These framework steps can be summarized as follows: 1) Platelets are active quasi-cellular elements, which are fully capable of responding to PBM; 2) Platelet responses are complex, susceptible to modulation, and have extraordinary potential, which can lead to all kinds of clinical improvements; 3) The technology for the useful and effective application of PBM must guarantee the proper cellular stimulus and an accurate dosage; and 4) A larger corpus of evidence must be built, to adequately support and endorse all the steps. This transformation will only be possible to answer the following questions concerning any PBM procedure: 1) What cells should be stimulated? 2) How should they be stimulated to obtain the intended response? And 3) With what clinical goal? All our PBM-related R&D efforts must follow this direction.

Conflict of Interest

The authors have no conflicts of interest or financial ties to disclose.

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