Platelet-rich plasma (PRP): history of the platelets' concentrates and current applications in medicine

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Abstract

Over the years, regenerative medicine has been improved thanks to new therapies and new innovative clinical protocols. The purpose of this work was to evaluate the efficacy of Platelet-rich Plasma by retracing the history of the evolution of the preparation technique, enhancing the role of platelets in tissue healing, as well as its use in various sectors of medicine. At the same time, through a critical review of recent innovations in the field of bone regeneration, was paid attention to new clinical protocols obtained from second generation platelet concentrates.

Key words: Platelet-rich Plasma, Preparation Technique, Platelets, Regenerative Medicine, Tissue Healing, PRF, PRGF.

Introduction

Pathologies affecting the bone tissue have always been of great interest both for the high incidence with which they occur and for the difficulty of having a complete healing and therefore a full functional recovery of the structures involved. The therapeutic protocols currently in use in the treatment of these diseases are far from a real returned ad integrum. Current research is therefore converging towards the study of therapies that aim to stimulate endogenous repair and the formation of a tissue with morpho-functional characteristics similar to those of healthy tissue. Research has increasingly allowed the implementation of non-surgical treatments. For this reason, scientists have focused their research on the enhancement of some biological substances active on alternative healing processes to autologous bone transplantation, which for many years was considered the gold standard treatment[1] . The biotechnologies used to recreate bone tissue and optimize the biological substrate can be identified in: bone morphogenic proteins (rhBMP), autologous growth factors contained in platelet-enriched plasma (PRP), mesenchymal stem cells and scaffolds; these can be used alone and / or in combination [2]. Platelet Rich Plasma (PRP) is a blood product and presents as an autologous concentrate of platelets suspended in a small volume of plasma[3]. The PRP has an autologous origin [4] and exploits the biological properties of platelets[5]. Platelets play a fundamental role in haemostasis and have pro-inflammatory, regulatory and regenerative properties and release growth factors (GFs), chemokines and other regulatory molecules[6]. Research now shows that platelets also release many bioactive proteins responsible for the attraction of macrophages, mesenchymal stem cells and osteoblasts that not only promote the removal of degenerated and necrotic tissues, but also improve tissue regeneration and healing[7-8]. Numerous experimental and clinical studies carried out in recent years have made it possible to thoroughly investigate the effectiveness of the platelet concentrate and the possibility of using it frequently and on a daily basis as a therapeutic support for various diseases of both the oral cavity and bone tissue in general[9]. This is because the use of PRP has advantages: the supraphysiological concentration of growth factors which appears to be able to increase the speed of wound repair, reduce inflammation associated with trauma and minimize the production of scar tissue [10].

History of the Use of PRP

The use of lyophilized fibrin, bank or autologous, dates back to the seventies, in particular in orthopedic surgery where it was useful in grafts for the effect of conglobation of the particulate bone tissue and for a better neo-osteogenesis, then attributed to a generic effect osteoconductor of fibrin. Tayapongsak et al[11] who first adopted autologous fibrin in maxillofacial surgery (1994), adhered to this explanation. In 1995, Slater et a [12] found that adding platelets to an osteoblast culture in vitro accelerated its development. In the field of oral and maxillofacial surgery, the PRP technique has been the subject of numerous publications[13]. In 1997, on the other hand, Whitman et al[14] studied the efficacy of "Platelet Gel" in accelerating the wound healing process[15].
The use of PRP begins with the publications of Robert E. Marx who in 1998 demonstrated the beneficial effects of an amplification of PDGF and TGF-β on bone regeneration. Described as a quick, safe, inexpensive and easy-to-obtain product, PRP is the subject of ever-increasing clinical interest[16-17].

Marx et al[16] in their work dated 1998, compared, in 88 cases in which mandibular restriction occurred, the regenerative and maturative results obtained by doing a regenerative with ground bone taken from the iliac crest with those deriving from bone grafting iliac creast added with PRP. The results obtained showed that in the cases treated with bone graft added with PRP there was a faster bone regeneration and maturation and, consequently, a better quality and quantity of bone trabeculae with the higher density of the regenerated bone tissue. they were also evaluated through histological examinations and histomorphometric data showed a greater extension of the bone trabeculae in cases with bone and PRP compared to that found in samples with only bone grafts. Another confirmation came from immunohistochemical examinations: by treating the samples with monoclonal antibodies for the growth factors PRGF and TGF-R, their presence was detected in the platelets of the PRP and in numerous cells of the bone tissue taken for grafting [16] .
The first studies were carried out in the field of maxillofacial surgery, however the most sensational results were obtained first on experiments on the jaw of goats and then on the vertebral column in humans. Fennis et al[17] reports in its article dated 2002 how the use of PRP improves healing in physiological and timing terms; in fact, in 28 goats mandibular defects were created, 14 goats were treated with only bone graft while the remaining with PRP added with particulate bone. The latter showed excellent healing in a short time. Lowery et al[18] demonstrates a percentage of positive results of 30% of the use of PRP in lumbar spinal fusions. PRP was used with hydroxyapatite. The results obtained in the 39 treated patients confirmed the excellent properties: chemotactic and mitogenic effect on mesenchymal stem cells and osteoblasts.
Even more recent studies confirm the efficacy of PRP in vertebral and non-vertebral surgery. In fact, from a systematic review on PUBMED, in 2020, considered 274 patients undergoing meniscus reconstruction, it stated a lower failure rate in subjects treated with PRP than in those cases treated without PRP[19]. By recreating critical size defects in rabbits, Leng et al[20] believes that PRP can compensate for the porous DBM deficiency. In fact, the radiographic evaluation and the histological analysis revealed a greater formation of new bone after implantation in the DBM group added with PRP at 6 and 12 weeks compared to the DBM group without PRP.

PRP in Muscle Tissue Repair

Recent studies are trying to identify strategies aimed at improving endogenous muscle repair potential. The administration of bone marrow-derived mesenchymal stromal cells (BM-MSC) together with the effect of PRP represents a very promising strategy. Although there are clinical results, they are very controversial for therapeutic application in skeletal muscle injuries[21]. Vallone et al[22,] in their study, evaluated the viability, survival, proliferation and myogenic differentiation induced by PRP on C2C12 and BM-MSC myoblasts and at the same time also the effect of PRP in combination with BM-MSC in the induce myogenic differentiation. Assays of incorporation of MTS and EdU, expression of Ki67 and signaling of Akt and Notch-1 have decreed how the treatment with PRP increased the survival, viability and proliferation of myoblasts. The increase of all parameters survival, proliferation and differentiation also occurred in C2C12 / BM-MSC co-cultures in the presence of PRP compared to treatment with PRP alone[22].
However, the use of PRP in long bones seems to be somewhat controversial. In a randomized clinical study carried out in 2007, a lower healing capacity was shown in subjects treated with PRP, about 60% of the testers, both against BMP-7 and autograft transplant[23].

PRP in Plastic Surgery

In recent years, the use of treatment in cosmetic surgery has also grown considerably. The use of platelet concentrate for non-transfusional use, in particular in regenerative medicine or dermatology, is an internationally established practice. The PRP is, in fact, indicated for these applications as a facilitator in the biorevitalization treatment. Numerous clinical studies have been conducted to evaluate its application in the field of aesthetic medicine in particular for hair loss conditions, skin rejuvenation, scarring and dispigmentation conditions[24].
A meta-analysis and randomized clinical trials aimed at quantifying hair density in subjects with androgenic alopecia show a hair density of 0.58 with a 95% confidence interval in subjects treated with PRP compared to placebo[25].

PRP in Uro-Gynecological Disorders

A search of the literature, carried out in 2021, also revealed an excellent use with excellent results in the treatment of uro-gynecological disorders[26]: PRP could be used in alternative protocols for the management of vaginal atrophy, in some subjects it also led to an improvement in the symptoms of stress urinary incontinence[27].

PRP in Gynecology

The use of PRP in gynecology and in particular in assisted fertilization techniques is also spreading to address both the problem of thin endometrium and to improve implantation rates in women with previous failures. Important scientific works report interesting success rates in patients treated with PRP and with previous repeated embryonic implantation failures, due to insufficient endometrial growth. In a recent publication, dated July 2022, the net increase in implantation rates in participating women undergoing PRP treatment is confirmed, but no efficacy on abortion rates[28]. Another study conducted by Tremellen et al[29] considered 20 female patients under the age of 45, with severe decreases in ovarian reserves. Treatment with PRP showed an increase in the number of embryos generated but an insignificant increase in the number of oocytes generated. The results are to be considered excellent compared to the results obtained in untreated women.

Evolution of Technique

The mode of action of the PRP exploits the role of action of platelets. The preparation techniques of the product, therefore, must keep the platelets unaltered and intact[30].
It is during the inflammatory phase that platelets play

different roles: antimicrobial effect, induces the coagulation cascade and retroactive the clot, releases growth factors and cytokines. The secretion of growth factors is stimulated by the coagulation cascade, therefore PRP must be obtained from blood treated with anticoagulant, so that the platelets remain viable long enough to allow treatment[31].
PRP treated with anticoagulant lasts for about eight hours. The preparation of the PRP must take place in such a way as to effectively separate the platelets from the erythrocytes and concentrate them without damaging the platelets themselves. The method chosen is important since the growth factors contained within the alpha granules are activated when the granules themselves merge with the platelet membrane[31-32].
According to Marx's protocol, the one described above and the one used to date, the PRP is prepared from autologous blood starting from a centrifugation which, using the different density gradients, can collect and concentrate platelets during surgery. The collection of PRP is done in 20-30 minutes through a gradient density cell separator (Medtronics). This cell separator draws 400 to 450 mL of autologous whole blood through a central venous catheter placed during surgery. With a spin speed of 5600 RPM, whole blood is drawn at a rate of 50ml / min. Citrate phosphate dextrose (CPD) is then added to the centrifugate in a ratio of 1 ml of CPD to 5 ml of blood to achieve anticoagulation. The PRP is then activated with calcium chloride and bovine thrombin in order to have a platelet gel then added with both spongy and cortical autogenous bone and with synthetic bone matrix[32].
In fact, literature data have shown that when calcium and thrombin are added to PRP, platelets are activated and can thus release the content of their granules a, which include both PDGF and TGF-ß[33-34].
Over the years have been examined different methods for the preparation of PRP, starting in 1994 when Tayapongsak et al[35] produced an autologous fibrin adhesive, from a whole blood unit with its plasma fraction, to be used in the following 2 -3 weeks.
In 1999, Anitua studied an open cycle technique of single centrifugation for the preparation of PRP in order to obtain a platelet concentration 2-3 times higher than the classic one: it used a protocol characterized by a 160 g centrifugation for 6 minutes , subsequently changed to 270 g for 7 minutes[36]. The author suggests the use of calcium chloride to activate the product with the possibility of accelerating the gelling process by also associating autologous bone[36].
In 2000, Sacchi et al[37] Described a double centrifugation technique, respectively at 180 rpm and 580 rpm for a duration of 20 minutes each in open cycle and for outpatient use, involving an enrichment of the PRP of 3.57 times compared to the basal concentration. subsequently to be activated with calcium chloride and Botropase.
Landesberg et al[38], in 2005, proposed an alternative protocol for the preparation of PRP: compares samples with the addition of the classic bovine thrombin used as anticoagulant to the thrombin receptor agonist peptide-6 (TRAP) samples. Thrombin caused rapid clotting of the PRP with clot formation within 3.25 minutes. Adding TRAP at 100 µmol / L took 9.25 minutes for the clot to solidify completely. The author himself therefore proposes the use of TRAP as a valid alternative to bovine thrombin.
In 2012 Inchingolo et al[15] shows the effectiveness of a method that involves the use of PRP as graft material in bone regeneration before dental implant rehabilitation, in liquid form in order to soak sponges of fibrin and implant wires[15].
However, in a recent study there is talk of lyophilized PRP. Such platelet concentrate in titanium scaffolds most strongly promoted cell viability and osteogenic differentiation of BMSCs[39].

PRP vs PRF

PRP represents a source of bioactive growth factors, however optimal preparation, activation, as well as quantification of the various growth factors present is a controversial and still unclear topic[40].
Recently, it has attracted widespread attention for its regenerative potential in soft tissues, however, the influence on bone healing and dental tissue regeneration is still unclear[41]. In fact, the literature reports the use of fibrin as an osteonic material, to be used alone or to be compacted with bone grafts: the importance of growth aids in the process of tissue repair, cell proliferation and chemotaxis is emphasized[42]. The protocol for the realization of the PRF is a single centrifugation of the tubes without coagulant. The absence of the coagulant allows the activation of coagulation and therefore the formation of a fibrin clot is expected that contains all the platelets and leukocytes. While PRP is considered a plasma derivative with two consecutive centrifuges and can be cryopreserved, PRF is an autologous scar that cannot be preserved[43].
Several studies have analyzed how osteoid tissue is present in the PRF graft area for 4 months and the absence of anticoagulant means that the platelets are fully activated and used for a long-term effect[44].
The fibrin network that forms after the PRF protocol is three-dimensionally more homogeneous than the one that forms with the PRP protocol; in fact, in the PRP protocol, the addition of bovine thrombin and calcium chloride determines a more rapid polymerization of fibrin with a three-dimensional structure that is less elastin and more disordered[45]. The absence of manipulations during the PRF preparatory phase and a front of a greater number of intrinsic growth factors makes the PRF protocol safer than the old PRP preparation protocols[46].

PRP vs PRGF

The evolution of platelet concentrates is aimed at promoting the regeneration process. Today we talk about PRGF, plasma rich in growth factors, growth factors that initiate the migration of undifferentiated stem cells towards the site and to induce their differentiation as well as their growth[47-48] .
For the realization of the product only one centrifugation of 8 minutes is foreseen. From centrifugation there will be two fractions. Fraction 2 (F2) is defined as the 2 mL of plasma just above the Buffy coat and Fraction 1 (F1) is the plasma column above F2. Adding 10% calcium chloride to F1 can form a fibrin barrier membrane to accelerate soft tissue healing and adding to F2 can form a fibrin clot. F2 can also be mixed with bone graft materials in order to accelerate the healing processes and resorption of degradable bone graft materials.
A study from 2022 confirms its use in in vitro regenerative medicine. In this study, were evaluated the chromo-

somal stability of the gingival and fibroblasts at the same time alveolar osteoblasts after long-term culture. Cultured cells were expanded with PRGF or fetal bovine serum (FBS) as a culture medium supplement. The results showed a higher cell proliferation rate in PRGF-treated cells. Analysis of the CGH array (Genomic Hybridization Assay) to consider chromosomal stability did not reveal genetic instability. The autologous PRGF technology, according to Anitua et al., Preserves the genomic stability of the cells and represents a valid alternative to the FBS culture medium and therefore a good supplement for cell therapy[49].

Conclusions

The mechanisms of bone formation and repair have been rapidly investigated and elucidated over the past decade. Although many problems still remain open, it is clear for now what the gold standard is for tissue restitution ad integrum but it is only clear that numerous cytokines and GFs as well as polypeptides play an essential role in these processes. . The GFs and BMPs regulate the proliferation and differentiation of mesenchymal cells and play a role of primary importance in the remodeling, regeneration and healing phases of both tissues. A prospect that has become interesting in the last decade for the treatment and repair of some traumatic pathologies is the use of autologous platelet concentrate (PRP). Platelets are very important in tissue healing as they have pro-inflammatory, regulatory and regenerative properties. The advantage obtained from the use of PRP could be due to the secretion by the platelets, contained in it, of growth factors in high concentrations which seem to be able to increase the speed of wound repair and reduce the inflammation associated with the trauma. To date, PRP is used in surgery, in dentistry and maxillofacial surgery, in plastic surgery, in orthopedic surgery, for the treatment of urogenital pathologies and a useful use also to stimulate ovarian fertilization. However, the use of PRP shows disadvantages, first of all the handling of the concentrate through the use of calcium chloride for freezing. The PRF could be considered a valid substitute for the PRP; also the use of PRGF with stem cells can open new perspectives to regenerative therapies and give way to new studies in the health sector.

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