Special Report

Regenerative Medicine

Contributions for classification of platelet rich plasma – proposal of a new classification: MARSPILL

Platelet-rich plasma (PRP) has emerged as a significant therapy used in medical conditions with heterogeneous results. There are some important classifications to try to standardize the PRP procedure. The aim of this report is to describe PRP contents studying celular and molecular components, and also propose a new classification for PRP. The main focus is on mononuclear cells, which comprise progenitor cells and monocytes. In addition, there are important variables related to PRP application incorporated in this study, which are the harvest method, activation, red blood cells, number of spins, image guidance, leukocytes number and light activation. The other focus is the discussion about progenitor cells presence on peripherial blood which are interesting due to neovasculogenesis and proliferation. The function of monocytes (in tissue-macrophages) are discussed here and also its plasticity, a potential property for regenerative medicine treatments.

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Platelet-rich plasma (PRP) can be considered as a form of autologous nonimmunogenic therapy, which contains a high concentration of growth factors (GFs) and cytokines. It plays important actions in various stages of regeneration and tissue repair [1,2].

According to the literature, PRP activation bursts the release of platelet α-granules, which are rich in proteins and GFs, such as PDGF, TGF-β, IGF, VEGF and EGF. All these molecules are important in different stages of tissue regeneration. They act as regulatory agents, stimulating chemotaxis and cellular differentiation and proliferation [2–5].

PRP has been widely investigated and used in medicine (orthopedics [6,7], dermatology [8] and plastic surgery), odontology [9] and veterinary medicine [10,11] due to its properties and simplicity to obtain the product. It is obtained with the use of commercial kits or not automated techniques (*in house*) that results in different types of PRP. Despite the increasing number of studies and some classifications published, there is no consensus regarding the classification used for different types of PRP. These procedures are obtained via machine or in house. As a consequence, different terminologies may be observed for the same type of PRP and vice-versa [12].

Given the numerous classifications presented, the purpose of this report is to describe the main types of PRP in the literature. And also, from the critical analysis of these publications, to propose a terminology based on the main parameters used during the preparation of PRP. Thus, some variables, such as automated method (machine) or not, spin cycles number, activation form, presence or absence of cells, fibrin and concentration of different factors and cytokines that compose PRP, must not only be well defined, but also be easily identified.

Future Medicine part of fsg

Classifications of plasma rich in platelets: literature review

A priori, platelet concentrate was used in the treatment and prevention of bleeding [13]. Later, blood products such as fibrin glue and PRP were also suggested to improve and accelerate the process of wound healing, in both medical and odontology fields. Since then, there have been numerous publications related to different PRP preparation techniques and compositions [12].

In general, the preparation techniques described in the literature have in common collecting peripheral blood mixed with anticoagulant, followed by centrifugation [14]. From this point, different parameters were adopted, which differ in time and centrifugal force, plasma fractions collected and type and concentration of agonist used in PRP activation. The combination of these parameters results in different compositions of PRP [12]. The use of different preparation methods and consequently PRP types refers to different terminologies and abbreviations. The actual classifications are basically based on the type of activation, platelet concentration, growth factors and the presence or absence of leukocytes or fibrin (Table 1) [15].

Anitua [16] described the method for obtaining plasma rich in growth factors (PRGF) from the harvest of peripheral blood from healthy individuals, and it was centrifuged at 160 ×*g* for 6 min. The authors used the intermediate fraction termed PRGF. They described their positive effect on bone regeneration in patients undergoing dental extraction [16].

Later, other articles have adopted the gel terms or matrix to name the PRPs activated by agonists (calcium chloride, autologous or bovine thrombin), classifying them as rich autologous platelet-rich clots (APRC), platelet-leukocyte gel, autologous platelet gel and platelet-rich fibrin (PRF) [17–20].

Dohan Ehrenfest *et al.* [12] classified different PRP types described in the literature, and distributed in four families according to the presence or absence of leukocytes and fibrin. According to these authors, the PRPs described until that moment in the literature could be termed as pure platelet-rich plasma (P-PRP called PRGF by Anitua [16]), leucocyte- and platelet-rich plasma, pure platelet-rich fibrin (P-PRF), leucocyte- and platelet-rich fibrin. It is important to note that the preparations with fibrin use tubes without anticoagulant in order to promote clot formation and the fibrin mesh [12].

More recently, Delong *et al.* [21] established a PRP classification system called platelet, activation, white blood cells (PAW), which is based on the following parameters: absolute number of platelets; adopted activation form; and presence or absence of white cells. In this classification, the authors identified four different levels of platelet concentration as follows: P1 (≤baseline), P2 (>baseline–750,000 cells/μl), P3 (>750,000–1,250,000 cells/μl) and P4 (>1,250,000 cells/μl). Other points considered refer to the use or not of platelet activators, presence of white blood cells and neutrophils (above or below baseline present in whole blood). According to the authors, the precise determination of cellular components, and the use and type of activator adopted, are important informations when comparing studies with PRP [21].

Likewise, Mishra *et al.* (2012) [22] established a classification system based on the platelet concentration and the presence or absence of white blood cells in the PRP. However, the authors classified PRP in four different types, determined by the increased (type I and II) or decreased (type III and IV) of the white cell number in relation to the whole blood platelet concentration. They still considered the concentration of platelets five-times above (A) or below (B) baseline. Another parameter used in this classification relates to the use of agonists to activate PRP (type II and IV) or not (type I and III) [22].

Mautner *et al.* (2015) described some variable that, in the author's opinion, none of the previously published PRP classifications present all the characteristics that may influence PRP activity and efficacy. In this context, the authors evidenced that is important to describe the platelet count (absolute number/μl), leukocyte content (as positive or negative) and, when presented, described the percentage of neutrophils, red blood cells content (as positive or negative), and activation (yes or no for exogenous activation) – Platelet, leukocyte, red blood cells and activation (PLRA) classification [23].

Magalon *et al.* (2016) published another classification based on four components: (1) dose of injected platelets, (2) efficiency of the production, (3) the purity of PRP obtained, and (4) activation process. In this classification, it is necessary to determine the complete cell count for whole blood and PRP, as well as the volume of collected blood and injected PRP. The dose is calculated by multiplying the number of platelets in PRP by the obtained volume of PRP, classifying from A (>5 billion of platelets) to D (<1 billion of platelets). The efficiency corresponds to the percentage of platelet recovery from whole blood, ranging from A (>90%) to D (<30%). The purity corresponds to the percentage of platelets and cells, such as leukocytes and red blood cells, varying from A (>90% platelets in relation of other cell types) to D (<30%). Activation is related to exogenous activation. The Magalon classification called Dose of platelet, efficiency, purity and activation (DEPA). [24].

Certainly, these different classifications and, especially the lack of communication between the terms used by the authors to refer the PRP in the

Table 1. Classifications reported in the literature to denote the platelet-rich plasma prepared by different methods, considering the following parameters: type of study.

Classification	Study	Type of:		Main parameters					Ref.
	First author (year)	Study	Preparation	CF (g)	T (min)	A (conc.)	Plt	WBC	
Plasma rich in growth factors	Anitua (1999)	C	H	160	6	$CaCl_2$ (10%)	ND	ND	[16]
Autologous platelet-rich clots	Anitua *et al.* (2005)	E	H	400	8	$CaCl_2$ (22.8 mM)	D	ND	[17]
Platelet–leukocyte gel	Everts *et al.* (2008)	L	–	–	–	–	–	–	[18]
Autologous platelet gel	Bielecki *et al.* (2007)	E	H	3200	12	$CaCl_2$:BT (10%:25%)	D	D	[19]
Platelet-rich fibrin matrix	Sanchez *et al.* (2007)	C	H	460	8	$CaCl_2$ (22.8 mM)	D	D	[20]
Pure platelet-rich plasma Leukocyte and platelet-rich plasma Pure platelet-rich fibrin Leukocyte and platelet-rich fibrin	Dohan Ehrenfest *et al.* (2009)	R	–	–	–	–	–	–	[12]
Platelet, activation, white blood cells	Delong *et al.* (2012)	R	–	–	–	–	–	–	[21]
Platelet-rich plasma (Types 1–4)	Mishra *et al.* (2012)	R	–	–	–	–	–	–	[22]
Platelet, leukocyte, red blood cells and activation	Mautner *et al.* (2015)	R	–	–	–	–	–	–	[23]
Dose of platelet, efficiency, purity and activation	Magalon *et al.* (2016)	R	–	–	–	–	–	–	[24]

For the type of Study; R: revision, L: Letter, C: Search Clinical and E: Experimental. For the type of Preparation; M: machine and H: in house. The main parameters assessed were centrifuge force (CF) versus time (T), use of agonists (A), Calcium Chloride ($CaCl_2$) or bovine thrombin (BT), concentration of platelets (Plt) and white blood cells (WBC).
D: Dosed; ND: Not Dosed.

literature and therapeutic applications, contributed to the emergence of different terminologies. Besides, it should be noted that the cellular and molecular components present in PRP have not been stated and fully analyzed in the current ratings, which compromise its reproducibility and might provide a better understanding regarding the biological effectiveness.

PRP consists mainly of cellular and molecular components. The major cellular components are platelets and peripheral blood mononuclear cells (PBMCs). Nami *et al.* verified the influence in tissue repair of the interaction of PRP gel and PBMC in co-culture [25]. In our previous study, we reported that the factors of great importance in PRP are the platelets and the PBMC. PRP preparations that use buffy-coat layer will present a great number of mononuclear cells. It plays important roles in the secretion of proteins and it shows a great relevance that cytokines have in tissue repair [26].

Molecular components correspond to GFs and a diverse family of immunomodulatory proteins [26–29]. Both cellular and molecular components are widely described in the literature and will be presented in this report concisely.

Cellular fraction

By definition, PRP presents high concentration of platelets, which are the main cellular components [30]. Platelets are cytoplasmic anucleated fragments derived from megakaryocytes produced in bone marrow and are also present in the spleen (30%) and peripheral blood (70%) [30,31]. In particular, during PRP preparation, platelets can be activated with the use of agonists or even during the centrifugation procedure. Under these conditions, the platelet α-granules release low molecular weight proteins, such as GFs and cytokines [1]. The elements released by platelet degranulation phase are described in molecular components item in this revision.

The other cellular components present in PRP correspond to the peripheral (PBMCs), which include

monocytes, natural killer cells and polymorphonuclear cells, which are the neutrophils [31].

Neutrophils, which are also called peripheral blood polymorphonuclear cells, play an important role in the immune system, and are the first line of defense cells to be recruited to the site of infection or tissue regeneration. In addition, there are in lower concentration in PRP, other granulocytes (basophils and eosinophils), which present the ability to produce the GFs, such as TGF-β, VEGF and PDGF. These GFs along with others released by platelets, play an important role in angiogenesis and vasculogenesis [32]. The neutrophils are activated by bacterial products, cytokines and GFs, the latter two products are found at high-levels in PRP [32].

Monocytes are derived from myeloid precursor cells present in bone marrow, and they act as a chemotatic agent necessary to cellular tissue regeneration process [33,34]. Macrophage Type M2 offers a healing function, while Type M1 promotes the host defense. After injury, M2 can switch into M1, and this change is modulated by the molecules ornithine (M2) and nitric oxide (M1), both of which are derived from arginine [18]. The presentation of antigens to lymphocytes amplifies the signal to M1 or M2 responses [35].

Lymphocytes are divided into three main types: B lymphocytes, T lymphocytes and T helper, whose functions are to produce antibodies, activate other cells and lyses foreign organisms, respectively. They are also considered part of lymphoid family natural killer cells, defined as nonspecific cytotoxic cells capable of lysing infected cells without prior sensitization [36].

With regard to macrophages, they still perform an important function of secreting bone morphogenetic proteins, IL-1β, TGF-β, PDGF and IGF, in areas of infection or injury in different tissues in the body, promoting the necessary recruitment and proliferation of osteoblasts, stem cells and progenitor cells [37].

Among other limited peripheral blood cells recruited by macrophages, and therefore present in PRP, there are hematopoietic stem cells (HSCs), hematopoietic progenitor cells and endothelial progenitor cells. These cells present auto renewal ability, pluripotency, neovasculogenesis function and maintenance of vascular integrity, respectively [38–40].

The number of HSCs in peripheral blood is 0.06% of the total nucleated cells in healthy individuals [41]. They are responsible for hematopoiesis and give rise to hematopoietic progenitor cells, which subsequently are divided into two main types of progenitor cells committed to the myeloid and lymphoid lineage. Even concentrated in small number in circulation, HSCs possess a high proliferation index by assigning to each cell the ability to produce about 1×10^3 mature blood cells per day [40,41].

Mesenchymal stem cells are also found in peripheral circulation. According to the literature, these cells are derived from bone marrow and their presence in the blood is likely due to the occurrence of lesions or inflammatory processes [38]. There is no evidence regarding the process of mobilization of bone marrow mesenchymal stem cells into peripheral blood. However, as the injuries occur in different parts of the body, it is assumed that they are recruited to these sites through mobile signals in order to compose the niche cells involved in the regeneration process and/or tissue repair [38].

Other populations of stem cells, such as very small embryonic-like (VSEL) and multilineage – differentiating enduring stress (Muse) cells, can be found in peripheral blood [42,43]. VSEL can be mobilized from the bone marrow to peripheral blood under pathological situations, such as stroke or heart attack. Under these conditions, concentrated circulating blood VSELs play an important role in renewal of stem cells injured tissues [43]. Similarly, Muse cells can be found in bone marrow and migrate into connective tissue of various organs when damaged via bloodstream. At these sites, Muse cells possess the ability to differentiate into cells that are compatible with the injured tissues [42].

Molecular components

The molecular components of PRP are composed of peptides and proteins released by platelet α-granules. They play an important role in recruitment and signaling of immune cells, progenitor cells and bone marrow cells for tissue regeneration. Among these molecules, there are GFs, cytokines and chemokines to help signalization [17].

PDGF, TGF-β, IGF, VEGF and EGF present the ability to regulate the migration and cell proliferation, and also play a major important role in the initial healing process. These GFs present mitogenic action that induces DNA synthesis and mitosis, cell growth inhibitory or controlling the end of the proliferative process [1,5]. PDGF regulates the migration of macrophages, attracting them to the site of injury, and also plays a role in chemotaxis and angiogenesis. TGF-β stimulates the proliferation of osteoblasts, and regulates collagen deposition in bone healing and scarring of tissue. IGF binds to insulin receptors and stimulates proliferation of different cell types. VEGF acts on angiogenesis and endothelial cell proliferation. EGF acts on endothelial cells and fibroblasts and stimulates angiogenesis, cellular proliferation and synthesis of collagen and epithelial tissue. In general, these molecules act through its binding to transmembrane receptors present on target cells, resulting in proliferation or inhibition of cellular growth [17].

Cytokines comprise a large and diverse family of immunomodulatory proteins, such as IL-1, IL-4, IL-6, IL-10, interferons, fibrin, fibronectin, and vitronectin. These molecules send various stimulatory, modulatory or inhibitory signals to different cells of the immune system, acting on cell itself (autocrine), in nearby cells (paracrine) and at distance (endocrine) [44–48]. IL-1 and IL-6 present a generally proinflammatory effect. The major source of IL-1 is from monocytes, macrophages and B and T lymphocytes [45]. Its primary activities include stimulation of CD4+ cells, proliferation and activation of B lymphocytes, neutrophils, monocytes and macrophages, in addition to phagocytic and chemotactic effects. IL-6 is produced by B and T lymphocytes and primarily monocytes [46]. Cytokines are mediators of acute inflammation and specific immune responses [48]. Moreover, IL-4 and IL-10 present anti-inflammatory activity. IL-4 is synthesized by Th2 cells and its main functions are to determine the profile of the immune response and to increase MHC-II38 synthesis. IL-10 is produced by $CD8^+$ cells, B lymphocytes, mast cells and monocytes, and its main function is to inhibit the synthesis of other cytokines [44].

Interferons (IFN-α and IFN-β) are produced by monocytes, macrophages and fibroblasts and its main function is to prevent infections spread [44]. Fibrin proteins, fibronectin and vitronectin act on cell–cell interaction as adhesion molecules, and cell migration. They play an important role in regenerative processes of connective, epithelial and bone tissue. In bone metabolism, although these mechanisms are not yet well known, it assumes that cytokines are able to stimulate or inhibit the formation and function of osteoblasts, osteoclasts and precursor cells [17,49].

Regarding chemokines present in the PRP, Stromal cell-derived factor-1 (SDF-1) is able to promote, when available in high concentration, cell–cell adhesion, and it triggers an early inflammatory response with up regulation of various inflammatory chemokines. According to the literature, SDF-1 also operates in stem cell homing and stimulates their migration to damaged areas. These events result in cell signaling in inflammatory phases as well as the migration of essential cells for regenerative process [50].

Another component present in plasma is the exosomes. They are nanovesicles secreted by PBMC with bioactive lipid bilayer. Its functions vary according to their origin and type of expressed protein [51]. In general, exosomes are derived from B lymphocytes that present MHC complex molecules, and they are able to present antigens to $CD4^+$ T lymphocytes. Since, those are released by antigen-presenting cells (APC), and dendritic cells are involved in sensitizing T cells in addition to the immunostimulatory function, exosomes may carry molecules between cells promoting intracellular communication and act on inflammatory processes.

New proposal for PRP classification: MARSPILL

Based on previous studies developed by this research group in hematology and orthopedics fields, we propose the adoption of a new classification of PRP. It refers to different parameters used during its preparation, and focusing especially on PBMCs.

According to the literature, the PBMCs pool available in PRP present the ability to phagocytose, antigens to activate the synthesis of lymphocytes, and also send signal and recruit cells to the areas of tissue injury [31–34]. When evaluated together, PBMCs can modulate regenerative process in areas of tissue injury, and, given this reason, its presence and concentration must be properly quantified and reported in PRP classification. Due to the biological efficacy of the leukocytes discussed above, especially PBMCs, in local tissue regeneration, it is proposed that their concentration drives this new PRP classification proposal, as one of the most important components in this product.

In this sense, this classification is focused on the differentiation of platelet-rich plasma and rich mononuclear cells or platelet-rich plasma and poor mononuclear cells, related to the use of leukocytes, but special attention was given to the mononuclear cell population. This classification will also consider other parameters used during the preparation and application of PRP, among them: whether it was prepared automated manner (machine: M) or handmade (H); spin number (Sp1 or Sp2); red blood cells (RBCs; Rich: RBC-R, Poor: RBC-P); platelet concentration (PL: 2–3; PL: 4–6; PL: 6–8 and PL: 8–10 folds baseline); leukocyte rich (Lc-R) or poor (Lc-P) and the range; activated (A+) or not (A-); light activated (L+) or not (L-). We also included the use (G+) or non-use (G-) of imaging guidance. Regarding red blood cells, the cut-off proposed for classifying it as poor is a reduction about 15-fold baseline, as previously published by our group [52]. Given that the presence of leukocytes might has an impact on clinical results due to their immune and antibacterial properties, we consider rich-leukocyte PRP if the concentration of leukocytes is higher than baseline value. And a poor-leukocyte PRP would be when the concentration in the final product is lower than baseline value.

This new classification is called MARSPILL: M: Method; A: activation, R: red blood cells, S: spin, P: platelets, I: image guidance, L: leukocytes, L: light activation, as described in Table 2.

Table 2. MARSPILL classification.

Letter	Relates to	Type
M	Method	Handmade (H) Machine (M)
A	Activation	Activated (A+) Not activated (A-)
R	Red blood cells	Rich (RBC-R) Poor (RBC-P)
S	Spin	One spin (Sp1) Two spins (Sp2)
P	Platelet number (folds basal)	PL 2–3 PL 6–8 PL 4–6 PL 8–10
I	Image guided	Guided (G+) Not guided (G-)
L	Leukocyte concentration	Rich (Lc-R) Poor (Lc-P)
L	Light activation	Activated (A+) Not activated (A-)

Lc: Leukocyte concentration; PL: Platelet concentration; RBC: Red blood cell.

Image guidance has been used by authors in order to identify the correct site of application [53], and it has been associated with limitation of some studies due to its nonuse [54]. In this way, Abate *et al.* published a chapter, which evidenced that, especially for hip, it is difficult to compare studies due to a large number of variables, such as blind or ultrasound-controlled injection, demonstrating the importance of a procedure with image guidance for application [55].

Recently in the literature, there has been an increase in publications about photoactivation applied in PRP. This incrementing interest is due to some promising results evidencing changes in cytokine profile in peripheral blood, such as an increase in anti-inflammatory cytokines, and a decrease in proinflammatory cytokines (IL-2 and 6) [56]. There are few studies exploring photo-activation of PRP. The majority of them are case series, showing an improvement in scores with the treatment using photo-activated PRP. Light activation was usually made after blood collection and preparation. Freitag and Bernard (2013) published a case series with osteoarthritis (OA). The study included one patient graded IV Kellgren-Lawrence and III for lateral compartments. The follow-up was 18 months and the photo-activation of leucocyte- and platelet-rich plasma was made for 10 min. It was verified an improvement in WOMAC score and numeric pain scale of the patient. As conclusion, the authors mentioned that photo-activated PRP may have a role in nonsurgical managements of knee OA. This is a promising development for the patients who remain symptomatic despite maximal conservative management and who are unsuitable for surgical intervention. However, larger studies are necessary to confirm this hypothesis [57]. Paterson *et al.* (2016) published a randomized controlled trial using photo-activated PRP comparing with hyaluronic acid. In this study, it was included 33 patients with 12 weeks follow-up. The conclusion is that its preliminary results of improvement in the patients showed feasibility and safety of photo-activated PRP necessary to inform a larger clinical trial in people with knee OA [58].

In this context, there is a necessity for studies of light activation of PRP that can be important and should be added in this classification.

The use of hematologic counter becomes necessary for the application of this classification system, in order to adequately quantify the cellular elements

Box 1. Example of a platelet-rich plasma production of clinical study through MARSPILL classification.

Standardized PRP according the new classification

PRP-RMC (platelet-rich plasma, rich in mononuclear cells)

M $_{(H)}$, **A** $_{(A-)}$, **R** $_{(RBC-P)}$, **S** $_{(Sp2)}$, **P** $_{(PL[4–6])}$, **I** $_{(G+)}$, **L** $_{(Lc-R[2–3])}$, **L** $_{(A-)}$

M: Method, A: Activation, R: Red blood cells, S: Spin, P: Platelet number, I: Image guided, L: Leukocyte concentration and L: Light activation. A-: Not activated; G+: Guided; H: Handmade; Lc-R: Leukocyte rich; PL: Platelets; RBC-R: Red blood cell rich; Sp2: Two spins.

present in platelet-rich plasma and poor mononuclear cells or PRP-RMC.

Our group has used this new classification (Box 1) in research and clinical practice for patients who present knee OA. The method consisted of preparation of previously standardized PRP-RMC. Peripheral blood was collected in vacuum tubes (BD Vacutainer-ACD 8.5 ml) containing ACD anticoagulant (citric acid, sodium citrate, dextrose), and these tubes were centrifuged at 300 ×*g* for 5 min. Then, the plasma was collected with the buffy coat and centrifuged a second time at 700 ×*g* for 17 min.

Approximately 80% of plasma was removed, and 20% of the remaining plasma, which corresponds to PRP-RMC, was transferred to new tubes, and an aliquot was analyzed in a hematology counter (Cell-Dyn, Abbott Diagnostics) in order to properly quantify the cell components. Finally, the PRP-RMC obtained was applied to the patient with ultrasound assistance.

Conclusion

To finalize, it is important to note that the attempt to standardize PRP products has been registered in the literature by other authors [12,21–22]. In the literature, due to many preparation methods and types of PRP, there is no nomenclature standardization and, consequently, different terminologies may be observed for the same type of PRP. And it may lead to a heterogeneity of results. In this sense, this report aimed to propose the use of a standard method of PRP classification, which will endorse the parameters used in its preparation as well as its composition. In our report, we believe we have reviewed and adjusted the current classifications, incorporating variables that we believe are of great value in PRP products. This new classification focuses on mononuclear cells, which, in our opinion, are as important as platelet content due to the fact the comprise progenitor cells and monocytes as key elements. Progenitor cells are interesting due to the neovasculogenesis and proliferation. And monocytes, when become macrophages, present plasticity to change the behavior to M1 or M2, according to the microenvironment. This property has great potential in regenerative medicine. Together, we believe that the proposed MARSPILL is an easily recalled and useful method for the classification of PRP.

Future perspective

Cell therapy has gained a great deal of attention which is clear given its increase in number of publications in the recent years. The global market of regenerative medicine is growing rapidly and is expected to reach 17 trillion yen globally by 2030 [59]. The use of PRP, which has applications in several fields of medicine, such as aesthetics, orthopedics, odontology and wound healing, is still under investigation and promising results can be found in some studies, especially in orthopedics [6,26]. On the other hand, it is difficult to compare the studies due to the heterogeneity of PRP products. In this way, this new classification proposal tries to standardize the technique of PRP preparation. PRP shows great potential, and there is therefore, a necessity of well-designed studies to evaluate its effectiveness alone or in combination with agonists as scaffolds and other cell products in order to improve life quality of the patients.

Executive summary

Classifications of plasma rich in platelets: literature review

- The use of different preparation methods, and consequently different platelet-rich plasma types, refers to different terminologies and abbreviations, and the actual classifications are based only on the type of activation, platelet concentration, growth factors and the presence or absence of leukocytes or fibrin.

Cellular fraction

- Macrophages present plasticity to change the behavior to M1 or M2, a potential property in the Regenerative Medicine, and still perform an important function of secreting bone morphogenetic proteins, IL-1β, FGF, TGF-β, PDGF and IGF, promoting the necessary recruitment and proliferation of osteoblasts, stem cells and progenitor cells.
- Stem and progenitor cells (hematopoietic stem cells, mesenchymal stem cells and endothelial progenitor cells) present in platelet rich plasma, are recruited to these sites through mobile signals in order to compose the niche cells involved in the regeneration process and/or tissue repair.

Molecular fraction

- PDGF, TGF-β, IGF, VEGF and EGF present the ability to regulate the migration and cell proliferation, and also play a major important role in the initial healing process.
- IL-1, IL-4, IL-6 and IL-10, interferons, fibrin, fibronectin and vitronectin send various stimulatory, modulatory or inhibitory signals to different cells of the immune system, acting on the cell itself (autocrine), in nearby cells (paracrine) and at distance (endocrine).

New proposal for classification platelet-rich plasma: MARSPILL

- This classification is focused on: platelet-rich plasma and rich mononuclear cells or platelet-rich plasma and poor mononuclear cells.

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