

Platelet-derived bio-products: classification update, applications, concerns and new perspectives

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Abstract

Platelet derived bio-products in the form of platelet rich plasma, plasma rich in growth factors, or plasma-free platelet releasates, are being studied worldwide with the aim of proving their efficacy in tissue regeneration within many different clinical areas, such as traumatology, maxillofacial surgery, ophthalmology, dermatology and otorhinolaryngology, amongst others. The current lack of consensus in the preparation method and application form, or in the quality assessment of each bio-product, precludes adequate interpretation of the relevance of reported clinical outcomes, and while many clinicians are very positive about them, as many are sceptic. Relevant aspects of these products are considered to propose a classification nomenclature, which would aid at the comprehensive comparison of clinical outcomes of bio-products of the same characteristics. Finally, the uses of platelet-derived bio-products in *in vitro* culture (for cell therapy purposes) as a substitute of animal-origin sera, and other future perspectives of applications of platelet-derived bio-products are discussed.

Background: an overview of platelet biology and key functional aspects

Platelets are circulating anucleate blood components (2-4 μm in diameter) and key players in maintaining the body hemostasis (1). Platelets have a limited life-span in the circulation (around 7-12 days), and therefore, the right balance between platelet production (approximately 10^{11} platelets daily) and clearance must be tightly regulated (2). In addition to their key role in hemostasis, many other functions have been attributed to platelets, such as immunomodulation or lymph and blood vessel separation during development (3, 4), and they have been identified as participants in various pathological processes (beyond bleeding or thrombosis), such as inflammation and cancer metastasis (5, 6). Therefore, platelets are considered metaphorically as double-edged swords, and international efforts and research are directed to the better understanding of platelet physiology in health and disease.

Platelets are capable of detecting endothelial damage through their receptors, as certain substrates get exposed or accumulate locally, such is the case of subendothelial collagen and von Willebrand factor (vWF), present in plasma and released as well by injured endothelial cells. After recognizing the vascular damage, platelets get activated and adhere at the site of injury (1, 7). This activation induces signaling pathways leading to cytoskeletal rearrangements (filopodia and lamellipodia formation) and integrin activation (favoring platelet-platelet interaction and aggregation) and secretion of their granular cargo (also called platelet releasate or secretome). The platelet secretome contains a multitude of growth factors, chemokines, cytokines, and immunomodulation factors that are involved in the key stages of wound healing and tissue regeneration, including cell migration, differentiation, proliferation and neovascularization (8, 9). While platelets react basically in the same manner, it is the physiological context and the initial signal that will condition the outcome, *i.e.* wound healing, or concomitant to a subjacent pathological situation, immunothrombosis (10).

While blood components, including platelets, have been used in transfusion medicine, it is the specific healing and regenerative property of platelets and platelet cargo that has been used as rationale for the development of platelet-based bio-products as adjuvant therapy in many areas of advanced cell therapy and regenerative medicine (11). The focus of the current manuscript is to highlight the development status of platelet-derived bio-products, their clinical and research applications, the lack of consensus on preparation methods and application forms and to provide novel future perspectives in the field.

Blood-derived bio-products in regenerative medicine

1. Not quite yet platelet rich plasma: fibrin and platelet-rich fibrin

Among the fibrinogen-based biomaterials, fibrin sealant (also called fibrin glue) is amongst the best-known fibrinogen-based biomaterials. This product mimics the last step of the coagulation cascade through the activation of fibrinogen by the biologically active alpha thrombin, leading to the formation of a semisolid fibrin clot. Its network architecture provides the required scaffold to support tissues or materials, while retaining its hemostatic and healing properties (12). It is widely used as a biodegradable tissue adhesive or sealant to control bleeding and promote tissue regeneration in many surgical interventions (13). To date, no other biological or synthetic adhesive material has posed as useful in terms of tissue biocompatibility, lack of toxicity, and clinical benefits. However, thrombin of bovine origin to induce the clot formation should be avoided, as it has been reported an elevated risk to develop inhibitors to bovine thrombin and co-immunization to human factor V (14), a complication that may pose danger in patients undergoing cardiovascular surgery, especially when they require a second intervention.

A variant of this, platelet-rich fibrin (PRF) is made from a volume of autologous whole blood collected without an anticoagulant and immediately centrifuged. Activation of the coagulation cascade during centrifugation leads to the formation of a fibrin clot containing live platelets and white cells. While the fibrin clot behaves as a physiological resorbable membrane or scaffold, the live cells contained in it will be gradually released at the site of injury to promote tissue regeneration, through cell-cell interactions or by releasing growth factors and cytokines (15). Depending on the centrifugation force, different compositions of PRF (at the cellular level) have been described (16). One of the main differences between fibrin adhesives and PRF is attributable to the clotting mode. PRF has the characteristic of polymerizing naturally and slowly during centrifugation without the addition of exogenous thrombin. This mode of polymerization will considerably influence the mechanical and biologic properties of the final fibrin matrix. (17) On the other hand, fibrin glue is an inert bio-material devoid of live cells (and their cargo content). Whether live cells may eventually be responsible of adverse events such as local inflammatory responses, hence needs to be carefully studied application-wise (18).

2. Platelet Rich Plasma bio-products

The concept of platelet concentrates or platelet-rich plasma (PRP) was born in the 1970s, when hematologists described the plasma fraction after differential centrifugation, which contains a supraphysiological platelet concentration (higher than 150.000-

350.000/ μ L), and was initially used as a transfusion product to treat patients with thrombocytopenia (19). The therapeutic use of PRP in regenerative medicine constitutes a relatively new approach with clinical benefits in a wide range of medical fields (20, 21). However, despite the popularity and increasing demand of PRP-based therapies, there are some aspects, including the real efficacy of the therapy application-wise, that need to be agreed upon. The main concern is the lack of consensus on PRP preparation that results in PRPs with different composition related to blood cells (platelets, leukocytes, and red blood cells), plasma, or fibrinogen that makes difficult the evaluation and comparison of clinical results amongst different clinical trials and applications (22, 23). These variation has led to the fact that PRP bio-products respond to many names, depending on the preparation method and authorship, as for example, platelet-enriched plasma, platelet-rich concentrate, platelet concentrate, leukocyte-rich PRP, platelet-rich fibrin, plasma rich in growth factors, platelet-rich fibrin matrix, autologous concentrated plasma, platelet gel, pure PRP, platelet releasate, etc, which does not conciliate but rather makes it even more complicated to assess their properties and find a consensus. Efforts to homogenize the production method variable are necessary to evaluate the efficiency of the product (24).

3. Serum or plasma containing platelet factors

The use of Serum Eye Drops (SED) for the treatment of ocular disorders has become increasingly popular in recent years. As it occurs with PRP, the protocols to produce SED are poorly standardized. In general, SED are prepared from peripheral blood serum (autologous or allogenic) or umbilical cord blood (allogenic). The product works as a tear substitute capable of lubricating the ocular surface, and also containing a mixture of growth factors, interleukins, vitamins and nutrients that enhance epithelial wound healing, but do not contain platelet derived growth factors, and should not be mistakenly categorized as a platelet rich plasma product. Nowadays, SED is widely used for the treatment of extreme dry eye, persistent corneal epithelial defect, corneal ulcer, ocular surface burn, recurrent corneal erosion and limbal stem-cell deficiency when conventional drops do not work (25, 26).

Plasma rich in growth factors (PRGF), is a bio-product of plasma containing the releasate of activated platelets. The first preparation steps are common to PRP preparation. After platelet concentration by centrifugation, platelet activation and fibrin formation are induced by adding calcium chloride, collagen or thrombin. The clot is allowed to retract at 37°C for about 2 hours (which may also vary depending on protocol, or commercial kit used). The released supernatant is rich in growth factors and can be diluted with sodium

chloride to be used as eye drops (27). However, further development of platelet releasates, either in plasma or plasma-free solutions, is broadening its applications, beyond ophthalmology (28-31).

Clinical uses of platelet-derived bio-products and concerns: does it work?

The effect of PRP on tissue regeneration has been supported by *in vitro* and *in vivo* studies that suggest a positive impact on the proliferation, differentiation and migration of several cell types. Its clinical use initially developed in the areas of dental and maxillofacial surgery. PRP and platelet-based biomaterials were found to accelerate endosseous wound healing in oral surgery (32-34). Using these products combined with autologous and allogeneic bone grafts provides better grafting results than autogeneous bone alone (35-37).

The regenerative effects of PRP on bone, cartilage, skin, tendon and muscle have also attracted interest in other medical fields such as traumatology and orthopaedics, ophthalmology, plastic surgery and sports and aesthetic medicine. The PRP in traumatology has been investigated in diverse pathologies (tendinopathies, arthropathies, bone grafts) with different degrees of effectiveness. Although *in vitro* studies and initial clinical results were promising (38, 39), there are currently few quality clinical studies favourable to the PRP reinforcement of some frequent surgical interventions, such as rotator cuff or meniscal repair, Achilles tendon reconstruction or repair, and cartilage regeneration, and the use of PRP in traumatology is still a matter of debate (40-46). PRP applications to musculoskeletal pathologies, including osteoarthritis, are also a wide field of development, opening new possibilities (47).

The main uses of PRP in ophthalmology are dry eye, graft-versus-host disease, persistent epithelial defects, corneal ulcers or perforations, burns and post-LASIK syndrome (27, 48-50). The regenerative potential of platelet-rich products has also been tested in tympanic perforation (51, 52), skin lesions (53) -such as skin ulcers of multifactorial etiology: diabetic foot (54-56), pressure ulcers (57, 58), venous ulcers (59) and even leprosy ulcers (60)-, vitiligo (61) and androgenetic alopecia (62). In the area of gynecology there are reports of the use of PRP to aid wound healing and recovery in caesarean delivery, vulvovaginal atrophy, benign cervical ectopy and it has also been applied in reproductive medicine and erectile dysfunction (63-69).

As mentioned before, a lack of standardization is observed at the level of product preparation, characteristics and application form, which entails a great difficulty in

comparing studies and drawing conclusions of real effectiveness or adverse events. Cases of adverse events as a result of the application of autologous PRP or PRGF are present in the literature, with minor or mild events reported in ophthalmology, generally related to inflammation or intolerance (70, 71). However, in this regard, there are not well-designed prospective studies that would address this aspect in a rigorous manner, including all clinical applications. The use of L-PRP (containing leukocytes) or fresh PRP may predispose to this type of reactions because living cells (not only white or red blood cells, but also platelets themselves) might exacerbate basal inflammation at the site of application, considering the novel functions of platelets beyond haemostasis (72-75). Predisposing factors of patients (*i.e.* diabetes or other autoimmune diseases) could favour inflammation and severe adverse reactions as well, as it has been documented (76).

Another different type of anticipated adverse event is related to the function and concentration of platelet-derived growth factors themselves. The presence of VEGF and PDGF and other growth factors in PRP is the reason for the contraindication of using these products in patients with a history of neoplasia in the area of infiltration (77, 78). Furthermore, the availability of certain growth factors seems conditioned by the activation or not of platelets to release their content, and authors suggest necessary to study the implications of growth factor levels on the efficacy of PRP products (79).

Autologous vs allogenic

The use of autologous vs allogenic PRP products is largely conditioned by the country-regulatory dispositions. As an example, in Spain it is considered a medicine for autologous human use (INFORME/V1/23052013), while in Italy, the allogenic non-transfusional use of blood components is allowed (80). On one hand, the use of autologous products has a positive balance on the benefit-risk assessment, but on the other hand, the production and use of allogenic PRP from well characterized donors or a pool of donors would achieve more consistent and reliable therapeutic results through the production of more homogeneous bio-products. For some patients, the use of allogenic PRP could represent an attractive option, when they suffer from a potential condition that might affect platelet function (*i.e.* autoimmune diseases, cancer or thrombocytopenia) or certain infection diseases (*i.e.* hepatitis B and C viruses, syphilis or human immunodeficiency virus (HIV)) precluding the use of autologous products as therapeutic source (81).

To date, the allogenic use of PRP products is positioned as a viable alternative mainly in traumatology and ophthalmology. Recently, Smrke *et al.* described the use of allogenic

platelet gel in combination with autologous bone graft to treat long bone defects (82). On the other hand, the use of allogeneic SED to treat ocular disorders is already a reality in several hospitals and clinics (83, 84).

In sports medicine, the use of allogeneic PRP opens a challenging debate. In 2010, the World Anti-Doping Agency (WADA) included intramuscular infiltrations of autologous PRP in the list of prohibited products based on the possible synergistic effect of growth factors on muscle cells that can potentially induce the increase of muscle mass acting as an ergogenic substance (85). A year after, WADA excluded PRP from their list due to lack of evidence beyond the expected therapeutic effect and the use of PRP by all routes of administration has been allowed back. Noteworthy, purified or recombinant growth factors (*i.e.* FGF, HGF, IGF-1, PDGF or VEGF) are currently prohibited (86). However, the use of allogeneic PRP opens the door to future considerations in the context of anti-doping regulations given that allogeneic PRP has not been studied as an ergogenic substance (81).

Classification of PRP products

As clinical technology products in development, the above-mentioned blood-products are prone to high variability that leads to different product types and compositions, due to a lack of consensus in the preparation method/application form or product characteristics (purity, content, quality) with severe impact on their potential clinical efficacy (39). For example, just reviewing the literature on the use of PRP as intra-articular injury therapy, only around 5% of the reports specify the type of PRP used in terms of preparation and product characteristics (39). In the last few years, several efforts have been done by the scientific community to address the product variability problem by proposing new classifications of platelet bio-products. In 2009, Dohan Ehrenfest *et al.* proposed the first classification of platelet concentrates based on the combination of two main variables, *i.e.* leukocyte and fibrin content. As a result, four main categories were proposed: pure PRP (P-PRP), leucocyte-rich PRP (L-PRP), pure PRF (P-PRF) and leucocyte-rich PRF (L-PRF) (87). In each category, the concentrate can be produced by different processes (preparation kits and centrifuges), either in a fully automatized setup (closed-system) or by manual protocols. In 2016, Magalon *et al.* proposed a more comprehensive classification, the DEPA (Dose, Efficiency, Purity, Activation) classification based on four different parameters: (A) the dose of injected platelets (which ranges from less than 1 billion -low- to more than 5 billion -high-), (B) the efficiency of the production (which considers the recovery of platelets from the starting material), (C)

the purity of the PRP obtained (which considers presence of RBC or WBC in the preparation) and (D) the activation process (which considers whether the platelets have been activated to release factors, or thrombin has been applied to provide with a fibrin mesh). The calculation of these parameters is only possible if complete quality control is assessed in whole blood and in the associated PRP (final product) (88). Still, this classification does not consider whether cells are being injected live or not when the PRP is not activated. This situation has been recently acknowledged for the first time at the International Society on Thrombosis and Haemostasis (ISTH) in the Scientific Standardization Committee (SSC) meeting in 2018. The Platelet Physiology SSC formed a working team of experts with the aim of producing a series of consensus recommendations for standardizing the use of platelets in regenerative medicine (89). The ISTH classification is the most comprehensive to date (Table 1) (23, 88).

	Dohan Ehrenfest (87)	DEPA (88)	ISTH (89)
Coagulated or not (PRP or Fibrin Rich)	Yes (PRP vs Fibrin Rich)	No	Yes
Preparation Method	No	No	Yes
WBC content	Yes (Leukocyte poor vs rich)	Yes (Purity)	Yes
RBC content	No	Yes (Purity)	Yes
Platelet number	No	Yes (Dose)	Yes
Platelet enrichment (PRP vs Whole Blood)	No	Yes (Efficiency)	No
Activation (Thrombin, CaCl ₂ ...)	No	Yes (Activation)	Yes
Frozen/Thawed	No	No	Yes

Table 1. Summary of aspects covered by proposed PRP-product classifications. PRP-product classification, as proposed by previous communications consider several aspects. The ISTH classification proposal is the most comprehensive, considering sample collection conditions (anticoagulated blood or not), the purity (red blood cells -RBC- or white blood cells -WBC-content), the number of platelets in the product, whether platelets have been activated or not, or whether the PRP product has been frozen/thawed prior use.

Based on this, we propose the variables to consider when describing PRP products and a tentative nomenclature system, with the aim of making possible the comparison of results amongst different groups. We also consider important to recommend certain product quality assessment and considerations. Importantly, the platelet, white blood cell (WBC) and red blood cell (RBC) counts should be performed on the product (before further processing – *i.e.* activated or ruptured -). The ideal PRP product would also abide to the quality thresholds set for leukoreduced platelet or plasma products (*i.e.* < 10⁶

WBC/unit), with no RBC contamination (24). Additionally, we give a lot of emphasis to whether the PRP product is applied fresh, activated or frozen/thawed, based on the relevance that it poses to the appearance of adverse effects (Table 2 and Figure 1).

Subscript	Description
F	Fresh product Contains live/intact platelets
A	Platelets have been stimulated to release their cargo. It should be stated whether it has been activated by thrombin, collagen, CaCl ₂ , etc
FT”N” Sn	Platelet rupture has been induced, and platelet content is released. It should be stated whether platelets have been ruptured by freezing/thaw (FT) cycles (“N” is the number of cycles) or by sonication (Sn)

Superscript	Description
NoD	When an extra centrifugation is applied to remove cell debris, after activation, or mechanical rupture, it should be indicated.
Aph/WB	Apheresis or Whole Blood donation

Prefix	Description
L	Contains WBC above threshold for pure PRP
R	Contains RBC contamination
Fn	Fibrin Rich
PF	Plasma Free

Table 2. Proposed matrix for new nomenclature classification of PRP products obtained.

Where PRP is the core ID, the Subscript refers to the application method, the Prefix refers to the characteristics and quality of the product, and the Superscript might be used to refer to other processing procedures prior application. For example, Leucocyte rich PRP lysed by 2 cycles of freeze-thaw eliminating cell debris by centrifugation, obtained from whole blood donation should now be called L-PRP_{FT2}^{WB,NoD}. The matrix could be extended per demand of new variables.

Platelet-derived factors in tissue engineering and advanced therapies

To date, the most widely used animal serum supplement in cell culture is fetal bovine serum (FBS), which stimulates cellular proliferation, differentiation and survival of many cell types (90). However, whenever a therapeutic approach requires cell culture, the use of animal-derived biomaterials associates with different types of risks that should be avoided in order to abide good manufacture procedures (GMP) requisites. In particular, a major concern is the immunogenicity associated to xenogeneic proteins, as for example N-glycolylneuraminic acid, known to be incorporated in human embryonic stem cells and consequently leading to the activation of the host’s immunity causing unwanted immune responses in patients receiving cell-based therapies (91). Another major concern is the possibility of disease transmission, through prions, bacteriae, or viruses

present in the animal material. To address this issue, the European Medicine Agency (EMA) published a guideline with the purpose of minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01) (92). This guideline recommended the use of material of non-animal origin, especially with regard to bio-products derived from transmissible spongiform encephalopathy-relevant animal species. Hence, there is an increased interest among the health authorities, industry and scientific community in general, to replace FBS by human-derived alternatives in terms to ensure safe and animal product-free conditions for biomedical tissue engineering, stem cell technology, and cell-based therapies (90, 93, 94).

Over the last few years, various human alternatives have been tested for their use as culture supplement to sustain proliferation and survival of cells *in vitro* (and *ex vivo*). In this sense, several research groups have investigated the feasibility of using human platelet lysates (hPLs) as an alternative source of growth factors and other bioactive molecules for their use in clinical applications (95). Several studies show that hPL is a viable alternative to FBS for the culture of many cell types used for clinical cell-therapies, such as mesenchymal stem cells (MSCs) or T cells for adoptive immunotherapy, that require *ex vivo* expansion prior to infusion (96-98). However, the method of platelet activation (agonist/receptor pathway, CaCl_2) or disruption (freeze-thaw cycles or sonication), the source material (apheresis concentrates, outdated platelet pools or PRP) as well as the medium in which the platelets are re-suspended (plasma, platelet additive solution or saline buffer) modified the composition of the bio-product and contribute to batch-to-batch variation, making it difficult to compare clinical outcomes (99). On the other hand, the plasma components of hPL normally require the addition of anticoagulants such as heparins to prevent clotting formation (100). Furthermore, commercially available heparins are usually from porcine origin, which represents a handicap in the development of a totally xeno-free culture system. Additionally, usage of heparin *in vitro* has been shown to negatively affect proliferation, differentiation and migration of MSC, because heparins are molecules that bind growth factors inhibiting their biological function and impairing cell expansion (101-103). Thus, the ideal scenario would be the use of plasma-free supplements, such as human platelet lysates or secretomes (HPSs), in which washed platelets are resuspended in a saline buffer (*i.e.* HEPES/Glucose buffer) before inducing their lysis or release of their granular cargo avoiding the use of anticoagulants and plasma-protein sequestering effects in cell culture. However, few studies have evaluated the use of HPS as a substitute of FBS for clinical-scale cell expansion. In this sense, S. Kazemnejad and coworkers show that the

use of HPS is an efficient and safe substitute for FBS in culture media for the expansion of human bone marrow-derived MSCs and promote their differentiation into hepatocytes (104). Further analysis of the precise composition of platelet-derived bio-products in terms of relevant growth factors, attachment factors, microRNAs and exosomes as well as dosage requires optimization to well-defined culture conditions (105).

Future perspectives in platelet applications

The harnessing of the biological functions of platelets to develop novel therapeutic strategies has been widely explored in the last few years. In this section we will focus on the most important advances in the field, opening up new methodologies and applications of platelets and their derived bio-products.

Platelets as carriers: “Beware of platelets bearing gifts”

Platelets are proposed to be used as “Trojan Horses” for loading drugs or biological therapies because of their biocompatibility and the possibility to target specific locations. Chemotherapy is widely used as first-line treatment in many tumor types. However, chemotherapy can induce various side effects on normal cells due to their cytotoxicity and non-specific targeting in the body. Development of novel drug delivery systems is one of the main goals in the management of cancer. In this sense, emerging evidence has shown that platelets have the capability to recognize and interact with tumor cells, and the cross-talk between platelets and tumor cells can be used to design novel therapeutic strategies (106, 107). It has been recently shown that, in a mouse model of lymphoma, a hematological malignance, doxorubicin (DOX) loaded platelets facilitated intracellular drug accumulation in tumor cells through “tumor cell-induced platelet aggregation”, which improved the anti-tumor activity of DOX due to the targeting of tumor cells for drug delivery (108). In the case of solid tumors, surgery is considered as the main therapeutic option by clinicians. However, there is growing evidence supporting the notion that invasive surgery may increase the risk of metastases and accelerate the growth of residual tumor cells (109). One of the most promising therapeutic options to reduce local and distal tumor relapses could be the use of platelets as carriers, considering their ability to recognize and get activated at the site of injury after surgery. Supporting this idea, Zhen Gu and collaborators generated PD-1-expressing platelets for their use as post-surgery consolidation treatment. The PD-1-expressing platelets could accumulate specifically within the tumor surgical wound and enhance the anti-tumor immune response, allowing the elimination of residual tumor cells (110).

Platelet-derived microparticles

Platelet-derived microparticles (PMPs or platelet “dust”) are the most abundant cell-derived microparticles in the blood circulation, constituting approximately 70-90% of all circulating microparticles. PMPs are produced by platelets in response to activation and can be classified depending on size and composition, amount of growth factors, chemokines, RNA, and cell-to-cell communication messengers (111). While the physiological significance of this “platelet dust” may have been unexplored for many years, recent work suggests that PMPs may play an important role in the transport and delivery of bioactive molecules and signals that are implicated in several physiological and pathological conditions (112).

It is known that PMPs play a key role in thrombosis and hemostasis, and are also involved in a variety of coagulation and bleeding disorders. It is the case of Scott syndrome patients, which have a defect in the production of PMPs that associates with bleeding complications (113, 114). Recently, microparticles have also attracted interest as potential early diagnostic markers of autoimmune and cardiovascular diseases. It has been proposed that PMPs may contribute to the pathogenesis of arterial thrombotic disease and several studies have suggested that circulating microparticles provide a potential prognostic marker in these patients (115). In addition, PMPs have also emerged as important players in the exacerbation of inflammation in autoimmune diseases. In rheumatoid arthritis, PMPs contain high levels of IL-1 α and IL-1 β , which are the cytokines responsible for the production of IL-6 and IL-8 by synoviocytes, contributing to the inflammatory condition (116).

PMPs are also produced under storage conditions in blood-derived transfusion products (117). Their presence in platelet concentrates is associated with transfusion refractoriness and transfusion-related acute lung injury (TRALI) caused by inflammatory mediators, such as CD40L (CD154), P-selectin and mitochondrial DNA (of platelet origin), that constitute a strong pro-inflammatory stimulus (118-121). It is because of that, that screening and characterizing the microparticle content in platelet concentrates constitutes a new quality improvement initiative for hospital blood banks in order to optimize the use of this limited blood product (122, 123).

Increasing evidence support the role of PMPs in angiogenesis and cancer progression. Numerous studies have shown that PMPs promoted the proliferation, survival and migration of endothelial cells. This effect was mediated by the action of VEGF and other growth factors contained in PMPs. It is well known that some tumor cells have the capacity to activate platelets and induce platelet aggregation with the consequent

accumulation of PMPs in the tumor microenvironment. Recent studies show that PMP levels are better predictors of metastasis than other plasma biomarkers and highly correlate with aggressive tumors and a poor clinical outcome, with emphasis on the MP-associated Tissue Factor expression (124-126). Considering their nature and diverse known functions, PMPs could be used as diagnostic (and/or prognostic) biomarkers for diseases (*i.e.* cancer, autoimmune diseases, bleeding disorders) and potentially used as delivery system for therapeutics.

Advanced delivery systems for the use of the platelet secretome in regenerative medicine

As summarized before, the platelet secretome contains a milieu of bioactive factors with short effective half-life, low stability, and susceptible for rapid inactivation by enzymes at physiological conditions. To address these limitations, delivery systems that allow loading and release of these proteins at specific locations for effective tissue regeneration have been developed (127). Lipid-based nanocarriers are typically composed of naturally-derived phospholipids that mimic the properties of biological membranes, the best known are liposomes. Liposomes can protect the activity of biomolecules against environmental conditions (*i.e.* temperature and pH) (128). Hence, the use of liposomes for the encapsulation of platelet cargo has several advantages, such as, biocompatibility, low immunogenicity, protection of the growth factors against enzymatic degradation, long-term bioavailability in addition to the easy surface modification for selective targeted delivery (cell/tissue specificity). Moreover, the combination of biodegradable scaffolds (*i.e.* calcium phosphates, Poly Lactic-co-Glycolic Acid) with biopolymers such as hyaluronic acid (HA) and gelatin for the encapsulation of PRP have shown promising results for enhanced bone regeneration in *in vitro* studies (129).

A novel promising delivery system is RegenoGel™, a bio-product designed by Procore Company that links high molecular weight HA with fibrinogen. RegenoGel™ combined the viscoelastic properties of HA with the regenerative and wound healing activity of fibrinogen. This combination renders a bio-material especially indicated for mild to severe osteoarthritis where there is enough residual joint cartilage capable of regeneration. RegenoGel has shown significant pain relief and enhanced quality of life of these patients. Furthermore, it can be used as a carrier for microRNA or ADAMTs (A Disintegrin and Metallo Proteinase with Thrombospondin Motifs) inhibitors, which means that it could be coupled to other molecules, custom-made (130, 131).

Pursuing the universality in transfusion medicine

The primary role of platelets is to maintain the body hemostasis thus, platelet transfusion during massive hemorrhage is of vital importance to treat bleeding complications derived from trauma, surgery or platelet-related disorders such as thrombocytopenia. Only in the USA, over 2 million of platelet transfusions are performed annually; however, donor-derived platelet concentrates represent a scarce resource due to limited availability and have certain disadvantages related to antigen matching, high risk of bacterial contamination, short half-life (5-7 days at room temperature) and specific storage/portability requirements. Despite the efforts to optimize the use of cryopreserved platelets (132), there is still increased clinical interest in searching universal alternatives that can render efficient hemostasis.

Refractoriness to platelet transfusion caused by alloimmunization against human leukocyte antigen (HLA) class I constitutes a severe clinical complication with associated risk of bleeding and reduced survival in thrombocytopenic patients. The use of leukocyte-reduced blood products has decreased the incidence of refractoriness to platelet transfusion but is still a problem of high clinical relevance. To date, the concept of generating HLA-universal platelets (lacking HLA antigens on the cell surface) from human-induced pluripotent cells (hiPSC) or primary progenitors using different tools (*i.e.* RNA interference or gene editing by CRISPR/Cas9) has been explored as a promising therapeutic approach to prevent platelet refractoriness. Numerous studies have shown that silencing HLA expression prevents an allogeneic immune response *in vitro* and *in vivo*. The reduction of HLA expression was shown to be sufficient to inhibit an allogeneic T-cell response and even prevent natural killer cytotoxicity (133, 134). However, manufacturing the clinically required number of platelets for transfusion purposes remains unattainable due to the low platelet release from hiPSC-derived megakaryocytes in addition to their excessive production cost (135, 136).

Haima Therapeutics, a biotechnology company led by Anirban Sen Gupta, has designed SynthoPlate™, a novel biocompatible surface-engineered liposomal vesicle (~150nm diameter) with Fg-mimetic peptides that recognized vWF, collagen and fibrinogen. Sen Gupta and coworkers showed that SynthoPlate™ is able to control bleeding by amplifying the natural clotting mechanisms *in vivo*. Specifically, it can amplify recruitment and aggregation of donor active platelets at the bleeding site in thrombocytopenic mice, at similar levels to wild-type mice as well as during the 'golden hour' following traumatic hemorrhagic injury in a pig model (137, 138). This novel biomaterial can be sterilized and stored as lyophilized powder for long periods of time, a great advantage for their use in remote places or at war conflict locations.

Artificial Intelligence (AI) in the management of blood derived products

Blood-derived products are a scarce resource that represent a vital treatment for many patients. In these sense, Hospitals and Blood Banks are evaluating the possibility of using artificial neural networks (ANN) to predict the transfusion requirements in order to reduce costs and to make more efficient the use of blood-related products (139). Recently, the National Health Service of UK has launched a project to develop a machine learning based planning solution to improve the management and to help in decision making of the platelets supply. These will enable the National Health System to better manage their complex blood supply chain as well as leading to improve clinical outcomes for patients who require a platelet transfusion (140). This type of actions would also be very relevant, with the notion that unused platelet concentrates could be redistributed to produce platelet-lysates, platelet-secretomes, drops, etc, either for culture purposes or therapeutic allogeneic use (141).

Final remarks/conclusions

While there is a tremendous expansion of the use of platelet-derived bio-products for various purposes in the clinic, the clinicians in favour equal the sceptic ones. The use of different nomenclatures and the lack of information in reported works make it difficult not only to obtain data on effectiveness, but also on such an important aspect as safety. Most products, especially PRP products, are used (in some countries) in an autologous manner, which minimizes the risk of infectious disease transmission. However, patients with impaired platelet function or contraindications for self-donation may benefit from the use of allogeneic PRP. So far, there are few adverse effects reported from the therapeutic use of PRP. It is difficult to compile the bibliography in this regard, precisely because of the variability of products and the indistinct use of certain terms to refer to fresh or frozen/thawed products. In many publications, the type of product used, whether it was leuko-depleted or not, or the method that was used to prepare it, was not specified. To our knowledge, the products that have generated inflammatory reactions are either products with leukocytes or products used fresh, or both, because the living cells (including platelets, considering their immunomodulatory functions beyond hemostasis) might exacerbate local inflammatory responses. We strongly encourage researchers and clinicians to fully describe the type of product they use in their future communications, as PRP applications continue to expand from their first uses in maxillofacial surgery and traumatology to other fields such as ophthalmology, plastic surgery, dermatology, aesthetic medicine and gynecology, amongst many others. The expansion of

applications, in the clinic and in the lab, as well as the development of technologies to facilitate targeted therapy, adds up to the complex matrix of platelet-derived bio-products and their possibilities in regenerative medicine, which requires at the same time, rigorous characterization.

Conflict of Interest

The authors declare no conflict of interest.

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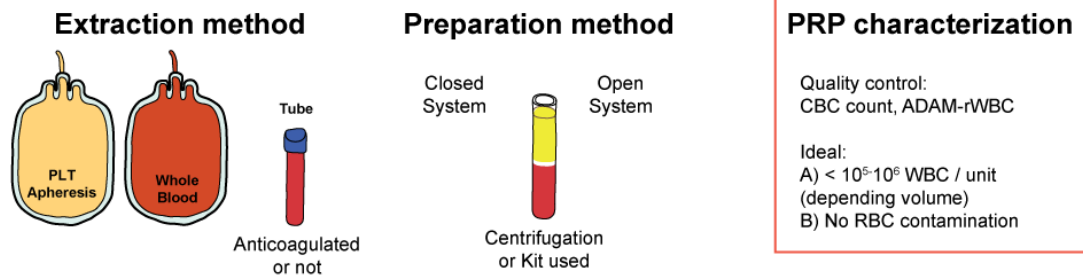
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Application and further processing of PRP method

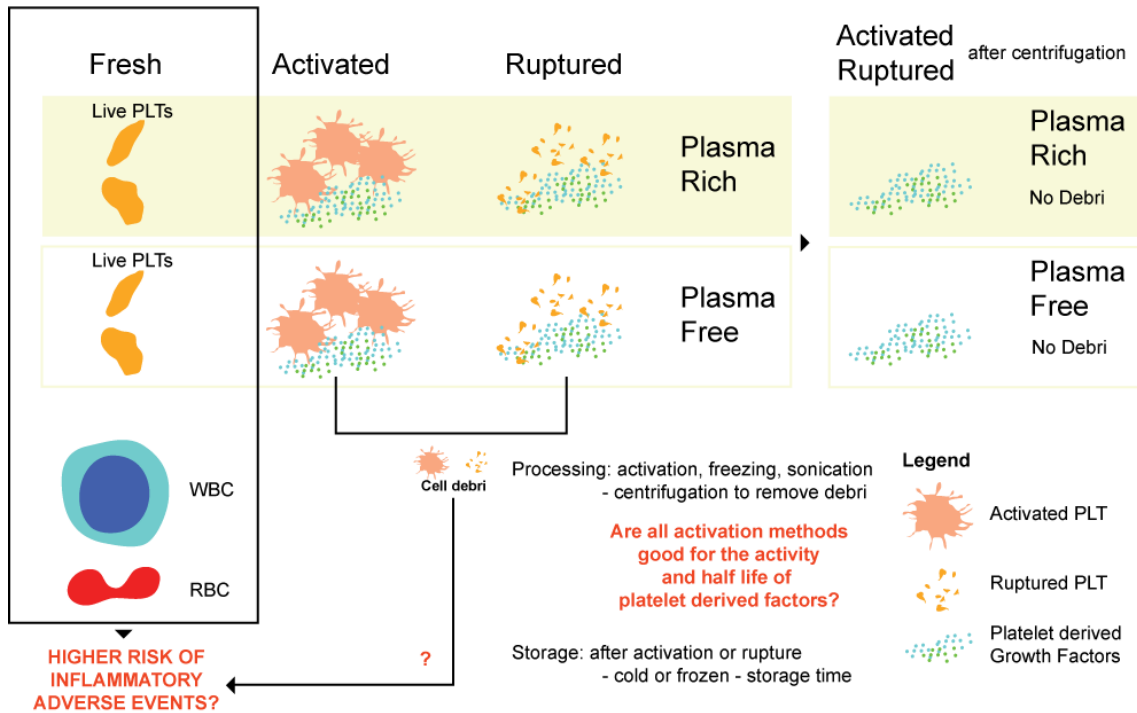


Figure 1: Scheme depicting relevant variables to take into account when describing PRP products. The components associated with adverse events are also highlighted (live platelets, WBC -white blood cells-, and RBC -red blood cells-), which are undoubtedly dependent on the patient subjacent condition or inflammatory state, and might not always pose risk for adverse events. Even cell debris might have a certain contribution to adverse events. The activation method (thrombin, re-calcification, etc, opens the question “are all activation methods appropriate for the conservation of the activity and half life of platelet derived factors?”.