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Lights and shadows concerning platelet products for musculoskeletal regeneration

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1. ABSTRACT

Various types of platelet (PLT) products, such as Platelet Rich Plasma (PRP) and Platelet Gel (PG), derived from autologous peripheral blood, have been used for tissue repair. The good clinical outcomes, due mainly to their safety and Growth Factor (GF) content, have led to a wide use of PLT products in many fields of medicine. However, until now the existing literature adds controversies to the use of PLT concentrates. When talking about PLTs and their products, a great number of variables have to be considered. These variables are mainly related to PRP preparation methods, the type of activators, intra- and inter-species variability, types of pathology to be treated, the ways and times of administration and the association of PRP or PG with other treatments. This review considers and discusses these causes of variability with particular attention to orthopaedic implications. The possibility of improving the knowledge on variables affecting therapeutic efficacy will surely help in addressing the best combination of factors implied in the different steps of PLT concentrate preparation and use.

2. INTRODUCTION

Various types of platelet (PLT) products, such as Platelet Rich Plasma (PRP) and Platelet Gel (PG), derived from autologous peripheral blood, have been used for tissue repair and are of increasing interest in many fields of medicine and surgery. PRP is defined as an autologous PLT preparation, generated by differential centrifugation, and suspended in a small volume of plasma. PG is produced by mixing autologous PRP with a gelling activator. Historically, PLT products were introduced clinically in the late 1980's as topical adjuvant therapy to treat chronic leg ulcers (1,2). PRP and PG, are under investigation or already used in clinical practice to improve the healing of a wide range of tissues (3-9). In orthopaedics, much effort has been made to treat several pathologies, such as bone defects and nonunions, bone fractures, lateral epicondylitis, joint arthroplasty loosening, tendon trauma and tendinosis (10-15). Furthermore, novel approaches in tissue engineering-based therapies are testing PRP and PG in combination with scaffolds and cells (16-20).

Table 1. Main factors implicated in PRP and PG efficacy

	Major variables
PRP and PG-linked factors	• Platelet number, GF, cell type and other molecules content • PRP preparation • PG activator • Inter-, intra-species variability
Target tissue-linked factors	• Type of pathology
Other factors	• Mode of delivery • Time of delivery • Combined biomaterials

The rationale of the use of PRP and PG for the treatment of so many different tissues is that PLTs provide a reservoir of GFs and cytokines, which may govern and regulate the tissue healing process, which is quite similar in all kinds of tissues. In fact, the response to an injury is coordinated and regulated by mediators and cellular events that are shared by most tissues during the early, inflammatory, reparative and remodelling phases. PLTs participate predominantly in the early and inflammation phases and, by degranulating, they produce a great number of GFs that initiate and maintain the healing process. The positive effects of PLT concentrates, such as PRP and PG, on tissue healing might be attributed to the high content and secretion of GFs, when considering the concept that "if a few are good, then a lot may be better" (21). Nevertheless, the existing data on the PRP and PG use in pre-clinical and clinical studies are controversial. In the field of bone research, a low regenerative potential (22-33) or improvement (34-40) was found when PRP and PG were added to various biomaterials. Other examples derive from spinal fusion where PRP and PG have been used clinically since 1999: Carreon *et al,* observed that PG failed to enhance the fusion rate when added to autografts in 76 patients (41); conversely, Lowery *et al*, reviewing 19 patients, found PRP to be a biological enhancer of lumbar fusion, suggesting that the local application of GFs seems to promote early maturation of bony fusion and gives good fusion results even when used at levels higher than L5-S1 (42). Finally, also the use of PRP in chronic ulcers therapy is still under debate, although PLT derivatives have been under study in that field for a long time. Senet *et al,* proposed PRP as adjuvant therapy for chronic venous leg ulcers and conducted a well-designed randomized clinical trial, but found no significant effect of PRP, although there was a very strict control of patients, number of PLTs and GF content both in the PLT concentrates and in the wound fluids (43). On the other hand, a recent clinical study conducted by Anitua *et al,* suggested that the topical application of PRP, depleted of most leukocytes, might be more effective than standard therapy in helping a chronic ulcer to heal (44).

As recently shown by Griffin and colleagues, only few clinically relevant articles, investigating the use of PRP on bone healing promotion, have been published and among these, only one article was a randomized controlled trial (45).

How can we explain that the use of PLT derivatives produces such different clinical results in the same field of application? Several factors have led to results that are difficult to interpret and compare. Variables affecting PLT number, GF, other molecules and cell type content, and tissue response to PRP and PG have to be considered. These variables, schematically shown in Table 1, mainly depend on the PRP preparation and conservation methods, type of activators, intra- and inter-species variability, type of pathology to be treated, and finally the ways and times of administration and the combination of PRP with other treatments. This review considers and discusses these variability factors with particular attention to orthopaedic implications.

3. PLT NUMBER, GFs, OTHER MOLECULES AND CELL TYPE CONTENT

PLTs are well known to be involved in haemostasis: at site of injury PLTs adhere, aggregate to form a clot and prevent blood loss, then they release molecules that promote tissue repair by stimulating specific target cells responsible for both neoangiogenesis and inflammation. GF content and release from PLTs is the main rationale for the clinical use of PRP and PG, but, as stated by Borzini *et al*, the role of several other PLT-derived molecules in the tissue healing processes should not be underestimated (46). PLTs, when activated, secrete many GFs contained in the alpha granules involved in tissue healing: platelet-derived growth factor (PDGF-AA, -BB, -AB and -C isoforms), transforming growth factor-beta1 and -beta2 (TGF-beta1, TGF-beta2), insulin-like growth factor (IGF), epidermal growth factor (EGF), epithelial cell growth factor (ECGF), hepatocyte growth factor (HGF), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), and platelet factor 4 (PF-4). Alpha granules are also a source of cytokines and chemokines, such as RANTES, interleukin 1-beta (IL-1beta, IL-8, macrophage inflammatory protein 1-alpha (MIP-1alpha, and monocyte chemotactic protein 3, MCP-3). All these factors are involved to varying degrees in stimulating cell chemotaxis, proliferation and maturation, modulating inflammatory molecules and attracting leukocytes. Moreover, the combined action of all these GFs is complex and redundant; GFs may also interact with each other, leading to different multiple signaling pathways and each GF may exert a different effect on a specific tissue (47).

Studies from our laboratory show that not only anabolic growth factors but also IL-1beta levels increase in PRP and PG after $CaCl_2$ activation (unpublished data) (Table 2).

Alpha granules contain also adhesive proteins: fibrinogen, fibronectin, vitronectin, osteonectin and thrombospondin-1 responsible for the adhesion of PLT to injured vessels and thrombus growth. It has recently been reported that megakaryocytes and PLTs contain Bone Morphogenetic Protein-2, -4 and -6 (BMP-2, BMP4, BMP-6) which is an intriguing finding since BMPs are required for the initiation and adequate maintenance of bone fracture

Table 2. Platelet number, TGF-beta 1, PDGF-AB, IL-1beta amounts in plasma, PRP and $CaCl_2$ activated PRP. Experimental evaluation in sheep

	Plasma	**PRP**	**Activated PRP**
Platelet number (x 10^3/ml)	281±56	874±87	-
TGF-beta1 (ng/ml)	53±7	113±28	453±110
PDGF-AB (ng/ml)	62±14	87±11	232±85
IL-1beta (mg/ml)	1.6±0.4	3.3±0.4	9.7±1.5

Table 3. The comparison of some of the centrifuge-based methods used for PRP preparation

Reference	**Number of patients**	**Centrifuge speed/minutes**	**Platelet count (10000/microliter)**	
			BLOOD	PRP
34	10	80 g/15; 1000 g/7	19.7±1.7	47.1±4.0
26	39	Not specified	23.4	133
27	15	5000g/7; 300g/5	Not measured	50
33	88	5600 RPM; 2400 RPM	23.2	78.5
35	3	200 g/10; 200 g/10	22.4	68.7
36	16	1200 g/6; 4400 g/6	30±5	100±25

healing and to provide PRP with osteoinductive properties (45,47-49). Moreover, PLTs store antibacterial and fungicidal proteins to prevent infections, proteases, such as matrix metalloprotease-4 (MMP-4), coagulation factors and other membrane glycoproteins, such as CD40L, which is a strong activator of inflammation by inducing the synthesis of integrins, interleukins and chemokines (50). PLTs contain also dense granules that store and release, upon activation, ADP, ATP, calcium ions, histamine, serotonin, dopamine, catecholamins, and thromboxan. Finally, PLTs contain lisosomal granules, which can secrete acid hydrolases, cathepsin D and E, elastases and lisozima (43,51).

All these molecules are able to exert many functions in the body, but in the studies regarding the use of PLT releasates, their contents and possible activities on tissue healing have not been investigated.

Another aspect, quite often ignored, is cellularity. Not only PLTs but also leukocytes, monocytes, macrophages, and mast cells are contained and secreted by PRP upon activation.

4. PRP PREPARATION METHODS

PRP can be obtained by two main methods: a laboratory centrifuge; or a density-gradient cell separator. In the first method, the whole blood, mixed with citrate-phosphate-dextrose to prevent coagulation, is centrifuged two times: the first centrifuge separates erythrocytes from plasma, which still contains white cells and PLTs. Then the plasma is further centrifuged to separate the upper-phase PLT poor plasma from the lower-phase PRP. It is a very simple and cost-effective method and takes about 30 minutes. Despite the feasibility of this method, the literature reports great differences in terms of centrifuge speed and timing. As schematically represented in Table 3, the choice of centrifugation speed and time varies markedly and, most of all, the PRP produced can vary in terms of PLTs and GF concentration. Centrifugation spin is an important factor for PRP properties because the mechanical forces activate PLTs in an early release of their alpha-granules (52,53). Obviously, the centrifugation process must be sterile and the PLT separation should take place without lysing or damaging them so that they can no longer actively secrete their GFs (54). Probably, being a hand-made process, the final products, obtained by the laboratory centrifuge method, are greatly dependent on the operator and are not reliably reproducible (55).

The second method to prepare PRP involves the use of one-step separators: they separate PLTs from erythrocytes and plasma by exploiting their different cell density. These devices are standardized and automated, they are a closed circuit, so by reducing the necessity to manipulate the intermediate products, they are also safe with a lower risk of contamination.

Table 4 shows some of the most commonly used devices. It can be noted that the times needed to separate PRP are quite similar, ranging between 16 and 33 minutes but there are notable differences in the PLT concentration and yield in PRP depending on the system used (56). Furthermore, it has been demonstrated that each system exerts different strengths and biomechanical stresses on PLTs, hence influencing the growth factor recovery and availability at the end of the process (57).

The PRP preparation method is a source of variability itself and also affects the number of PLTs yielded, the amount of GFs and other proteins and molecules, and finally the different cell types delivered with PRP. Some authors also highlighted the importance of instruments or containers because glass initiates the coagulation cascade or diminishes the PLT count because of PLT adhesion to silica (52).

With regards to the PLT concentration, a PRP concentrate should approximate 400% (4x) of the peripheral blood PLT count, while anything less than this concentration is PRP diluted with PLT Poor Plasma (58).

Table 4. Some of the cell-density gradient blood separators commercially available with manufacturer-declared time of platelet recovery, platelet concentration and yield

Device	Recovery Time	Platelet Concentration	Platelet yield (%)	Website
Biomet GPS™	27 mins	3.2 x	70	http://www.biomet.com
Cell Saver Based Systems	20 mins	4-6x	75	http://www.haemonetics.com
Sorin Angel	25 mins	4.3x	76	http://www.soringroup-usa.com
Harvest® SmartPrep2 BMAC™	16 mins	4.0x	72±10	http://www.harvesttech.com
Depuy Symphony II	16 mins	4.0x	72±10	http://www.depuyspine.com
Medtronic Magellan™	33 mins	5.1x	70	http://www.medtronic.com/
3i PCCSII®	20 mins	3.2x	61±9	http://www.biomet3i.com
Vivostat® PRF	23 mins	1.5-4x	65	http://www.vivostat.com
Plateltex®	20 mins	1-2x	79±7	http://www.plateltex.com

On this basis, clinicians should choose the PRP preparation method which might ensure maximal PLT concentration. However, this kind of choice might also be incorrect; in fact, the above-mentioned theory of "if a few are good, then a lot may be better" seems not to be appropriate to PLT concentrates, as demonstrated by Weibrich and colleagues in rabbits. They found that PRP exerts beneficial effects on bone regeneration only when PLT concentration varies within a limited range (2-6x the concentration of PLTs in whole blood, i.e. 50.3-172.9 x 10^4/microliter PRP). The use of lower PLT concentration (0.5-1.5x, i.e. 16.4-37.3 x 10^4/microliter PRP) did not increase bone regeneration, the use of highly concentrated PLT preparations (9-11x concentration, i.e. 184.5-320.0 x 10^4/microliter PRP) appeared to have a paradoxically inhibitory effect. The authors ascribed these sub-optimal effects to inhibitory and cytotoxic effects of GFs at high (59).

Small variations in GF concentrations can exert very different effects. A bi-phasic or bi-directional pattern is reported for many cytokines and, among them, for TGF-beta1 that, when released by PLT degranulation, facilitates the resolution of inflammation and promotes tissue repair. Conversely, excessive TGF-beta1 within a lesion has been associated with unresolved inflammation and fibrotic events (41). Moreover, TGF-beta is able to autoinduce its own synthesis (60,61).

Knowledge of the GF level in PRP samples is necessary to ensure a reliable and reproducible use of PRP for clinical treatment, since the regenerative potency of PRP undoubtedly depends on its content and GF level (62). Many authors have detected important variations in GF concentrations among individuals even if they have very similar PLT baseline counts (63-65). Therefore, it seems that GF content in patients may also be influenced by additional unknown biological factors.

TGF-beta and PDGF concentrations of 52 ng/ml and 30 ng/ml, respectively, have been reported in whole blood (7). Their amount in PLT concentrates varies from about 40 ng/ml (both TGF-beta and PDGF) to about 200 ng/ml of TGF-beta and 170 ng/ml of PDGF so that, 10-25 times more PDGF and TGF-beta than those at a baseline level is obtained (7,11,66). Moreover, Kalen *et al,* showed that the release of PDGF and TGF-beta from PLT concentrates is pH-dependent: acidic environment, such as that found in the initial hematoma stage of the healing process, leads to a more sustained GF release than neutral pH conditions (67). To understand the importance of this variability in GFs concentration better, it has to be remembered that these molecules have a great biological potency. As an example, there are approximately 0.06 ng of PDGF per one million PLTs, or, in other terms, $6x10^{-17}$ g of PDGF, or about 1200 molecules of PDGF in every individual PLT (68). Last but not least, PDGF in some studies seems to have a negative effect on bone formation (69,70).

As explained above, the PRP preparation method affects not only the PLT and GF contents, but also the cell types and concentration sequestered with PLT. A natural blood clot contains 95% red blood cells, 5% PLTs, less than 1% white blood cells and large amounts of fibrin strands. A PRP blood clot contains 4% red blood cells, 95% PLTs and 1% white blood cells (71). Thus PRP is not only a concentrate of PLTs; Roussy *et al,* measured the concentrations of all cell types present in both whole blood and PRP. They found that the concentrations of lymphocytes and monocytes were increased by 5.2- and 3.5-fold, respectively, in PRP compared with those in whole blood, neutrophyls almost remained the same, and red blood cells were reduced by 6.7-fold in PRP (69). The presence of other cell types, besides PLTs, is of controversial relevance in the lesion site treated with PRP.

The buffy coat, which is sequestered in the PRP preparation process, also contains concentrated leukocytes. Some authors believe that the presence of leukocytes adds an anti bacterial component to the PRP (10,72), while others, considering neutrophyls to be an important source of MMP-8, MMP-9 and other proteases, consider their presence to be destructive for GFs and, releasing reactive oxygen, deleterious for cell survival (73,74). Interestingly, Frechette *et al*, assaying the GF content in PG from five donors over a six-day incubation period, found a great increase in IL-1beta at day 6 (65). This increase was greater than the total IL-1beta levels measured after the PRP lysis, suggesting a *de novo* synthesis. In fact, it is recognized that PLTs do not contain DNA, but hybridization studies have suggested that PLTs contain as many as 1500 different mRNA, including the one encoding for IL-1 beta (75). Since PRP contain many different cell types, the authors proposed that IL-1beta *de novo* synthesis has to be related to leukocytes and monocytes contained in PRP (65,76,77).

Table 5. Comparative platelet counts of several experimental animals (90)

Species	Platelets/microliter
Human	150000-400000
Calf	175000-620000
Horse	90000-350000
Pig	100000-400000
Sheep	180000-475000
Rabbit	205000-750000
Guinea-pig	260000-740000

In the scope of GF concentration, data on the detectable amount of GFs showed that pre-analytical sample preparation techniques as well as PLT and white cell content influence the measurable levels of GFs (78).

5. PRP ACTIVATION METHODS

After concentrating PLTs through centrifugation, a subsequent polymerization of the fibrinogen, present in the concentrates to form a three-dimensional semisolid fibrin gel, is sometimes necessary. Currently, common methods of PRP gelling use calcium chloride and/or thrombin to initiate PRP clot formation (79). The use of bovine thrombin has been associated with the development of antibodies to clotting factors V and XI and thrombin, resulting in the risk of potentially life-threatening coagulopathies (80,81). Autologous thrombin is often used; thrombin concentrations used in clinical practice to prepare PRP vary between 100 and 200 units/ml (52), while PLT aggregation is at a maximum in the range of 0.5 to 4 units/ml (82). Thrombin is a strong inducer of PLT activation leading to GF release but this does not seem to be true to the same extent for all PRP containing GFs. As examples of two extreme cases, EGF was found to be present in low concentrations in non-activated PRP and increased from 4.7 to 11 fold only after the addition of Ca and thrombin, whereas IGF-1 was found to be significantly high in both non-activated and activated PRP (65). Ranly *et al,* observed that PRP without thrombin activation contained about 20 ng/ml of latent TGF-beta and no active TGF-beta1. Following activation with thrombin, PRP had about 35 ng/ml of the latent form and about 3 ng/ml of the active form (70). The use of calcium chloride alone as a clot activator was introduced by Dugrillon *et al,* to obviate immunological and disease-risk transmission problems associated with the use of exogenous bovine thrombin (53). It has been reported that calcium chloride is able to promote the formation of native thrombin directly "*in situ*", mimicking the physiological clotting process and enabling a more sustained release of GFs (73,74).

To date little is known about the calcium and thrombin concentrations needed to trigger optimal PLT degranulation and tissue repair but it was shown that the activation of PRP with different concentrations of Ca and thrombin regulates GF release and endothelial cell division *in vitro* (69).

Batroxobin, which is the fibrinogen activating enzyme, is also able to induce fibrin polymerization and formation of three-dimensional platelet-embedding fibrin mesh. While PLTs treated with trombin become activated and release their growth factors quickly, PLTs treated with batroxobin do not become activated but passively entrapped within the fibrin network and their growth factors release slowly. Due to these differences, it is expected that batroxobin-induced PRP might provide a longer in loco availability of trophic factors and might be a favourable gelling agent for selected clinical conditions (83).

Freeze-thaw procedures are a common method for releasing GFs (64,84). In fact, PLTs can be stored for as long as 21 days and retain their proliferative activity (85) even if there are risks for bacterial proliferation and accumulation of pyrogenic cytokines. Cryopreservation (-80 or -196° C) reduces the potential for bacterial proliferation but shortens the PLT lifespan, by inducing irreversible activation, commonly known as cold lesion (86).

Some biomaterials have been proposed as PRP activators. Chou and colleagues found that chitosan, a cationic polysaccharide, enhanced rabbit and human PTL adhesion and aggregation, accompanied by a significant increase in intracellular calcium levels and GFs (87,88).

Finally Zhang and colleagues described the application of a nanosecond-pulsed electric field as a novel PLT clot activator. They found that when PRP was pulsed with one 300 ns pulse with an electric field of 30kV/cm, PLTs aggregated and a platelet gel was produced (89).

6. INTER- AND INTRA-SPECIES VARIABILITY

PRP effectiveness has been investigated by adopting both *in vitro* and *in vivo* studies.Although, animal models provide important knowledge for the development of effective clinical treatments of diseases, care should be taken regarding the inter- and intra-species variability especially in terms of PLT count and dimension. As shown in Table 5 (90), each animal species presents a very different PLT number range; moreover, within the same species, i.e. calf and rabbit, the range is notably large. Thus, when animal models are adopted in experimental procedures to test PRP efficacy, the knowledge of the PLT baseline count is fundamental for each animal. Additional variability is added by some species-specific peculiarity; in the calf, PLTs appear as rosettes of prominent-purple granules, the individual thrombocyte is small, but giant forms, up to the size of a red blood cell, may be seen. The PLTs in horse's blood vary in shape from the more common oval form to elongated sperm-like structures and giant forms equal to red cell size may be observed (90). These factors have to be considered in interpreting results extrapolated from animal models. As an example, Mooren and colleagues did not find any enhancement to bone healing in goats when PRP was used in critical size bone defects (91). The main reason, used to explain the results, was that they could not define the baseline PLT values of each animal. This was due to the fact that, in goats, red blood cells are very small and therefore almost indistinguishable from PLTs when counting by an analyst,

using a microscope (91). Finally, the concentration of GFs is species dependent, being highest in human PRP followed by goat and rat PRP (92,93).

7. TYPE OF TISSUE AND LESION SITE

There is increasing awareness that the microenvironment at the lesion site strongly influences the success of any therapy based on the local application. The exact changes in the tissue microenvironment outside the cells, that plays a role in mesenchymal stem cell differentiation and tissue-specific differentiated cell protein expression, should be taken into consideration.

Changes in the degree of vascularization of the tissue, such as in the oxygen tension influence the healing response to biological stimulators (94).

High levels of proinflammatory cytokines, catabolic enzymes, anti-angiogenic agents and cell apoptosis were found in skin, bone, cartilage and tendon chronic or degenerative diseases (43,95-98).

Kalen *et al,* showed that the release of PDGF and TGF-beta from PLT concentrates is pH-dependent. An acidic environment, such as that found in the initial hematoma stage of the healing process, leads to a more sustained GF release than neutral pH conditions (67).

8. MODE AND DELIVERY TIME

The underlying practical difficulty with designing a multiple GF treatment is to predict the proper time-dependent ratios for optimal healing (86). There is general agreement on the fact that GFs may be administered in appropriate doses and application timing.

Tissue healing is a very complex process only partially understood that involves the coordinated and temporal presence of cells and signaling molecules. Chen *et al,* studied the gene expression and protein production of GFs at different time points up to 3 weeks after surgery in the flexor digitorum profundis tendon of chickens (99). Gene expression and production of TGF-beta were high but basic-FGF (bFGF) and PDGF-B were low or minimally expressed (99). During the spontaneous healing of a rabbit rotator cuff tear, the bFGF level was high at 7-9 days, IGF-I at day 5, PDGF at 7-14 days and TGF-beta was present in mild concentrations throughout the 28 days of healing after trauma (100).

Unfortunately, there is a lack of studies on the temporal expression of cells and molecules during the spontaneous healing of tissues, to improve our information on the physiological expression of GFs at different stages of the healing. Obviously, the selection of single or repetitive treatments is strictly dependent on the clinical topic (i.e. osseointegration *vs* chronic soft tissue injuries) (46), however, the importance of timing the application of recombinant GFs and PLT derived GFs has been addressed by many researchers and clinicians involved in tissue healing. As for the appropriate dose, timing and length of intervention are areas requiring further investigations (101). When administering PDGF-BB at various dosages and times in a rat tendon injury, it was observed that a late supplementation after the inflammatory stage (day 7 after injury) was better than an earlier time (day 3 after injury) in restoring the mechanical properties of the tendon (102). The dosage of 1000 ng/wound of PDGF resulted in better healing in comparison with lower dosages of 10 and 100 ng (102). In a medial collateral ligament injury model in rats, a significant drop in effectiveness of PDGF was observed when it was administered more than 24 hours after injury and the lack of difference between 5.0 micrograms and 1.0 microgram also suggested a plateau effect (103).

Obviously, this information derives from animal models and even if in humans the healing mechanism is probably not the same, dynamic and temporal studies on this topic are needed despite being difficult to realize in human beings.

9. COMBINED BIOMATERIALS

PRP and PG are often used in combination with biologic and synthetic scaffolds by retaining and releasing the GFs at the site of implantation, by accelerating the initial healing around implants, and by increasing the osteointegration potential of the biomaterial and prosthesis surface. In comparison with other approaches based on the development of osteoinductive-osteogenic biomaterials towards bone regeneration, such as surface modifications, recombinant GFs and other compounds or drugs, PRP is probably safer, cheaper, easier and faster to be transferred to the clinical situation. The combination of PRP and PG with biomaterials has been tested with regards to both tissue defect healing and implant osteointegration enhancement. PRP and PG modify biomaterial properties and the biomaterials seem to modify PRP and PG, or at least the evidence of their effectiveness. In a recent study it was observed that the resorption of calcium deficient hydroxyapatite (HA) was increased by the addition of allogenic PRP, mesenchymal stem cells and both. GFs and mesenchymal stem cells can attract other progenitor cells on osteoclasts and therefore initiate bone remodelling (104).

Regarding bone defect healing, PRP or PG were usually added to bone derivatives, ceramics and metals.

No advantages on the combination of HA and tricalcium phosphate (TCP) and PRP or PG were reported in experimental studies in the early phase of bone healing (26).

In our experience with the addition of mesenchymal stem cells to PRP-HA it is possible to enhance significantly bone formation (105), and also the addition of cells to freeze-dried allogenic bone combined with PRP has a synergic effect on the healing of critical size defects in rabbits (106).

Regarding biological biomaterials, Wiltfang *et al,* found enhancement of autogenous bone healing in critical-

sized defects of minipigs with PRP at 2 weeks, PRP did not enhance the bone healing when combined with xenogenic bone substitutes, degradable TCP, bovine bone matrix and bovine collagen (107).

PRP and PG have also been tested to enhance implant osseointegration. Some authors report the lack of beneficial effects when PG was added to endosseous implants, both in cortical and trabecular bone (59,108,109). A mechanical trauma due to high pressure after gel application in press-fit implant surgery has been hypothesized (109). Better results have been obtained from other authors using PRP but, also in this case, the effect depended on the implant chemical surface (23,34,37,38,109,110).

Sanchez *et al,* studied the regenerative influence of PRP, added to xenogenic bone grafts, on peri-implant osteointegration in a dog model. They found that the addition of PRP led to a low regenerative potential in this animal model (111). Similarly, Jensen *et al,* compared processed morselized bone allograft with fresh-frozen bone graft with and without the addition of PRP around non-cemented titanium implants. They did not find any improvement of bone formation or implant fixation by adding PRP, also owing to high biological variation among animals (23). The same authors showed that PLT concentrates, prepared using a commercialized kit, increased fixation of non-HA-coated implants in a similar animal model (112).

PRP added to beta-TCP ceramic slightly increased new bone formation by 8-10% in patients submitted to sinus floor elevation, did not affect biomaterial degradation (24). Other studies did not show significant healing improvement on the osteointegration rate, de novo bone formation and degradation of bone substitute using autologous bone in comparison with fluorohydroxyapatite with PRP (25).

Kim *et al,* found that the use of PRP in combination with dentin-plaster of Paris improved the osteointegration rate around titanium dental implants (38), while Sanchez *et al,* did not find a beneficial effect of PRP when used in combination with xenogeneic bone graft in peri-implant dental defects (22).

The reasons for these findings are not completely understood although some hypotheses have been advanced: the presence of collagen on experimental biomaterials induces *in vitro* the activation of PLTs and GF release (32); the specific surface area of rough and porous materials modulates PRP absorption and causes increased degranulation and release of the GFs from the PLTs (92); a fibrous tissue and a foreign body giant cell reaction, which was observed when adding PRP to synthetic biomaterials, might be due to the presence of TGF, which also support fibroblast chemotaxis (24). Finally, some authors have suggested the need to supply cells (i.e. with bone chips) in order to support the effect of PRP when combined with synthetic or acellular biomaterials (113).

10. CONCLUSIONS

The use of autologous PLT concentrate to deliver GFs locally is an ideal vehicle to provide the biological environment of the growing cells with a physiological combination of all factors that are needed to initiate the healing process and to create a positive feedback cycle that sustains itself throughout the healing process. Another advantage of using PLT concentrates is that they can also act as a binding medium for the bone grafts or biomaterials, making them easier to handle and place into the graft site. However, the most significant benefit of using the PLT concentrate is its being endogenously derived, and easily available. There are no issues about immunogenicity or transmission of infection. The process is also considerably cost effective compared with use of purified or recombinant growth factors, and may also be more physiologically sound to provide a combination of all factors in the PLTs rather than single factors (42). PRP and PG can be considered as a matrix graft, often referred to as an autologous tissue graft. PRP matrix is defined as a “tissue graft incorporating autologous growth factors and/or autologous undifferentiated cells in a cellular matrix where design depends on the receptor site and tissue of regeneration” (114).

However, until now the existing literature adds controversies to the use of PLT concentrates. This is obviously an expected problem because of the biological nature of PLT concentrates but the possibility of improving the knowledge on variables affecting its therapeutic efficacy could surely help in finding the best combination of factors in the different steps of preparation and use.

11. ACNOWLEDGEMENT

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12. REFERENCES

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Abbreviations: PLT: platelet; PRP: platelet rich plasma; PG: platelet gel; GF: growth factor; PDGF: platelet derived growth factor; TGF: transforming growth factor; IGF: insulin like growth factor; EGF: epidermal growth factor; ECGF: epithelial cell growth factor; HGF: hepatocyte growth factor; PDAF: platelet derived angiogenesis factor; VEGF: vascular endothelial growth factor; PF: platelet factor; IL: interleukin; MIP: macrophage inflammatory protein; MCP: monocyte chemotactic protein; CaCl2: calcium chloride; BMP: bone morphogenetic protein; MMP: matrix metalloprotease; ADP: adenosine diphosphate; ATP: adenosine triphosphate; DNA: deoxyribonucleic acid; FGF: fibroblast growth factor; HA: hydroxyapatite; TCP: tricalcium phosphate.

Key Words: Platelet Rich Plasma, Platelet Gel, Musculoskeletal Tissue, Healing, Growth Factors, Review

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