Partnership between platelet-rich plasma and mesenchymal stem cells: *in vitro* experience

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Summary

We aim to identify current *in vitro* research exploring platelet-rich plasma (PRP) effects in human Mesenchymal Stem Cells (MSCs) that may encourage or limit the clinical application of MSCs along with PRP. After a systematic search, we identified 57 *in vitro* studies, focused on optimization of MSC manufacturing, and expanding knowledge about how PRP modifies MSCs behavior for translational purposes. Influences of PRP on proliferation, migration, stemness, preservation of MSC immune-modulatory properties and appearance of senescence phenotype have been explored. Overall PRP stimulates MSC proliferation, preserves MSCs multipotency and does not interfere with any lineage differentiation. PRP (as platelet lysate or releasate) preserves the immune-privileged potential of MSCs and may delay the appearance of the senescent phenotype. Currently there are few data linking precise molecules and biological mechanisms. Various gaps of knowledge need to be addressed in order to obtain enough useful information for translational purposes.

KEY WORDS: cell culture, in vitro*, mesenchymal stem cells (MSC), platelet-rich plasma (PRP), regenerative medicine, tissue engineering.*

Introduction

The use of mesenchymal stem cells (MSCs) for tissue healing and Regenerative Medicine research has been extended in the last decade. Three accepted MSCs capabilities are centrally involved in their regenerative properties. First, their potential to differentiate into tissue specific cells, second their ability to influence the fate of other cell types through paracrine signaling and third their immune-modulatory potential through secretion of prostaglandins such as PGE2[1]. It was Caplan[2] in 1991 who proposed the MSC definition, currently adopted by the International Society for Cellular Therapy (ISCT), based on three main criteria: first, MSC adhesion to plastic; second, their expression of a set of membrane molecules (CD73, CD90, CD105), together with a lack of expression of HLA-DR and absence of hematopoietic and endothelial markers CD11b, CD14, CD34, CD31, and CD45; and, third their ability to differentiate along adipogenic, osteogenic, and chondrogenic pathways[3, 4].

Current research in MSCs aims not only to the development of cell therapies in regenerative medicine, but to provide experimental models that can inform about molecular mechanisms such as inflammation, angiogenesis and apoptosis among others. For these purposes and because of their accessibility, MSCs are most commonly isolated from the bone marrow (BMSC), or the adipose tissue (ADSC), and then grown in 2D or 3D conditions.

In vivo MSCs activities are regulated by the molecular microenvironments that modulate their anabolic status. Thus, signaling factors in the microenvironment instruct MSCs to remain quiescent, proliferate, migrate, and/or differentiate. In this context, autologous formulations derived from own patient's blood, named platelet rich plasma technologies are being developed to be used alone or in association with cells for regenerative medicine purposes. The foundation of PRP use is the release of a pool of signaling factors that will create a pro-healing environment in the injured site.

The first description of Platelet Rich Plasma when it was introduced as a MeSH (Medical Subject Headings) term in 2007 is: "a preparation consisting of PLATELETS concentrated in a limited volume of PLASMA. This is used in various surgical tissue regeneration procedures where the GROWTH FACTORS in the platelets enhance wound healing and regeneration". Growth factors (GFs) from PRP have their source in alpha granules from platelets (50-80 per platelet). However, recent proteomic studies have revealed the complexity of platelet secretome, and indexed not only GFs but a vast array of molecules including cytokines and chemokines, adhesive proteins, enzymes, fibrinolytic and antifibrinolytic proteins. This molecular pool is released upon platelet

activation that is the interaction of molecules such as collagen, thrombin, platelet-activating factor, serotonin, calcium, magnesium, thromboxane A2, and adenosine di-phosphate with platelet receptors. Also, mechanical disruption of platelets causes their activation and the subsequent release of their content. When platelets are activated, there is an initial burst of GFs that is lately stabilized and maintained in a sustained release.

The aim of the present systematic review is to evaluate the relevance of current preclinical research towards the development of combination products (biological therapies) that may encourage or limit the clinical application of MSCs along with PRP. Therefore, we will discuss MSCs effects in proliferation, differentiation, migration and immune modulation and report on the controversial results obtained by different authors due to the heterogeneity of PRP formulations, and the different conditions used in MSCs cultures.

Methods

A systematic literature search[5] was performed from January 2007 to October 2013 in Pubmed, Web of Knowledge and Google Scholar. As keywords, we have used Platelet Rich Plasma AND/OR Platelet Lysate AND/OR Platelet Releasate AND Mesenchymal Stem Cells.

Inclusion criteria

The search was limited to "humans" and English language. Articles presenting bone as a MeSH major topic were excluded. Only original journal and classical articles (not reviews or clinical trials) were included.

Extraction of data

Title and abstracts were screened by two researchers independently, and the full text of the selected articles was read by both reviewers. The following data were extracted: cell source, platelet count, leukocyte content, whether the study was performed with the PRP from single or pooled donors, the biological mechanism that was evaluated, i.e. proliferation, differentiation, cell morphology or immunomodulation.

We classified PRPs according to the categorization system made by Delong JM et al[6]. Briefly, 1x platelet count above baseline or less is considered low platelet content, 1x-4x is a moderate platelet concentration, 4x-6x is a high content and 6x or more is super high content. Finally studies were sorted in two groups based on their main intended goal: 1) research studies (2D or 3D studies) and 2) cell production studies. To accomplish this goal, these authors perform long term proliferation studies and calculate cell population doublings through cell passages. They also check MSCs viability at high passages by testing phenotype, differentiation capacity and even chromosomal stability and senescence markers.

Results

Study selection

Using the above described search algorithm, we found 277 articles of which 143 articles had been performed with human cells, and were written in English. After excluding reviews and articles pertaining to dental and bone biology, and adding hand search relevant articles, we included 57 articles as relevant to research in biological mechanisms, of which 22 articles perform large scale MSC production. Twelve articles perform studies on Adipose Stem Cells (ADSCs)[7-18] (Tab. 1). 36 articles study PRP biological effect in BMSCs[19-55] (Tab. 2), and 8 others on several stem cell types including ADSCs, BMSCs and Umbilical Cord MSCs (UCMSCs)[56-63] (Tab. 3).

PRP formulations

The different procedures for PRP preparation produce different compositions that may account for result variability. Most often the two step centrifugation is used to separate blood components, and to concentrate platelets (and optionally leukocytes) in the final PRP product. Alternatively, one centrifugation step generates a PRP product with a moderate/low concentration of platelets depending on centrifugal force and time. Overall, we found that in "PRP+MSC" research there is a slightly higher use of PRPs with high or super high concentrations of platelets (Fig. 1).

Leukocyte content in PRP

Leukocyte content influences the molecular composition in PRP. However, most of the *in vitro* studies don't even mention leukocyte content in their final product. Leukocyte content in PRP samples is merely described by two authors[21,62].

PRP activation methods

About 85% of the studies perform freeze/thaw cycles to cause mechanical disruption of platelet membranes and release alpha granule content. When those steps are performed, they proceed to filtration through 0.22 um filters and addition of heparin (2U/ml) to the lysates[35,40,57]. This procedure is the most commonly found in the papers studied, but there are some authors which prefer performing chemical activation instead of thermic/mechanical activation and use of thrombin and/or calcium chloride[7,17,34,41,45,49]. In general, bovine thrombin is used except for Vogl[24] who obtains human thrombin from blood donations. Thrombin and/or $CaCl_2$ trigger clot formation, and after incubation the liquid extruded from the clot is collected. This PRP fraction is called PRP releasate or supernatant.

Table 1. Cell culture studies performed with Adipose Derived Stem Cells and Platelet Rich Plasma.

ADIPOSE DERIVED STEM CELLS (ADSCs)							
		BIOLOGICAL EFFECTS (PRP vs FBS)					
					Stemness		
Author, year, [reference]	Platelet count Pool or single donor	Proliferation	Migration	Immune modulation	Chondr.	Adipo.	Osteo.
Van Pham 2013 [7]	Super high (6x) Single donor	Enhanced	-	-	Enhanced	Similar	Similar
Hildner 2013 [8]*	- -	Enhanced	-	-	Enhanced	-	-
Chen L. 2012 [9]#	Super high (6x) Single donor	Enhanced	-	-	-	-	Enhanced
Cervelli 2012 [10]	- Pool	Enhanced	-	-	-	Enhanced with insulin	-
Sell 2011 [11]#	High (3.82x) Pool	Enhanced	Enhanced		-	-	-
Shih 2011 [12]*	- Single donor	Enhanced	-	-	Enhanced	Similar	Similar
Cholewa 2011 [13]*	Super high (6.2x) Pool	Enhanced	-	-	-	Similar	Similar
Chieregato 2011 [14]*	Moderate (2x) -	Enhanced	-	-	Similar	Enhanced	Enhanced
Castegnaro 2011 [15]*	Moderate (2.74x) Pool	Enhanced	-	Enhanced	-	Similar	Similar
Souza Blande 2009 [16]*	Moderate (3.64x) Single donor	Enhanced	-	-	-	Similar	Similar
Kakudo 2008 [17]	Super High (7.9x) Single donor	Enhanced	-	-	-	-	-
Kocaoember 2007 [18]*	Super high (10x) Pool	Enhanced	-	-	-	Similar	Similar

* Articles performing long term expansion studies; # Articles performing 3D studies.
Abbreviations: ADSCs, Adipose Derived Stem Cells; PRP, Platelet Rich plasma; FBS, Fetal bovine serum; Chondr, Chondrogenic; Adipo., Adipogenic; Osteo., Osteogenic.

MSC+PRP: assessment of biological effects (Tabs. 1-3)

Proliferation: PRP and MSCs large expansion in CMOs

The goal of these studies was to establish the best conditions for large-scale expansion of MSCs in cell manufacturing facilities in terms of safety, cost and time. The main goal was to substitute with PRP the xenogenic component of cell expansion, i.e. FBS (fetal bovine serum). 23 articles out of the 57 selected for this review perform long term expansion studies, and are indicated with * in Table 1, 2 and 3. These studies primarily calculate cell population doublings through cell passages and population doubling times. They also check MSCs viability at high passages by testing CFU-f (colony-forming unit-fibroblast), phenotype, differentiation capacity and even chromosomal stability and senescence markers. In all the articles, PRP increases the number of cell population doublings, and decreases the time necessary for the population to duplicate. Of note, methodological differences inter studies, including initial cell seeding concentration, MSC passage number conditions, renewal of the medium, and time of the experiment difficult comparisons.

Importantly, PRP delays the appearance of the senescence phenotype[12,26,42,60], and protects from chromosomal instability longer than FBS, which has traditionally been used in MSC laboratory cultures.

Tissue engineering

In order to assess MSCs+PRP application in tissue engineering, 3D proliferation studies in different biomaterials used for the development of tissue grafts have been performed.

These studies are displayed with "#" in Table 1 and 2. PRP can be incorporated in a gel or powdered form to the 3D scaffold, generally used for bone or cartilage tissue engineering. Other studies examine

Table 2. Cell culture studies performed with Bone Marrow derived Stem Cells and Platelet Rich Plasma.

		BONE MARROW STEM CELLS					
		BIOLOGICAL EFFECTS (PRP vs FBS)					
					Stemness		
Author, year, [reference]	**Platelet count Pool or single donor**	**Proliferation**	**Migration**	**Immune modulation**	**Chondr.**	**Adipo.**	**Osteo.**
P.H. Warnke 2013 [19]#	- Pool	Enhanced	-	-	-	Similar	Similar
Jonsdottir-Buch 2013[20]*	- Pool	Enhanced	-	Similar	Similar	Similar	Similar
Schallmoser 2013 [21]	Moderate (3.8x) Pool	Enhanced	-	-	-	-	-
Leotot 2013 [22]#	High (4x) Pool	Enhanced	-	-	-	-	Enhanced
Copland 2013 [23]	High (4x) Pool	Enhanced	-	Enhanced when fibrinogen depletion	-	-	-
Vogl 2013 [24]	Moderate (3.9x) Pool	Enhanced	-	-	-	-	similar
Mojica-Henshaw 2013 [25]	High (4.3x) Pool	Enhanced	-	-	Similar	Similar	Similar
Griffiths 2013 [26]*	- Pool	Enhanced		-	-	-	-
Gottipamula 2012 [27]*	- Pool	Enhanced	-	Decreased	Enhanced	Similar	Enhanced
Fekete 2012 [28]*	- Pool	Enhanced	-	-	Similar	Similar	Similar
Teixeira 2012 [29]#	- Single donor	Enhanced	Enhanced	-	Enhanced	Similar	Similar
Walenda 2012 [30]*#	Super high (8.4x) Pool	Enhanced	-		Similar	Similar	Similar
Ben Azouna 2012 [31]	Moderate (3.8x) Pool	-	-	-	Similar	Similar	Similar
Murphy MB et al 2012 [32]	High (4x) Single donor	Enhanced	Enhanced	-	-	-	-
Fekete 2012 [33]	High (≥4x) Pool	-	-	-	Similar	Similar	Similar
Cho 2011 [34]	High (4.65x) Single donor	Enhanced	-	-	-	-	-
Xia 2011 [35]	High (5x) Single donor	Enhanced	-	-	-	Decreased	Enhanced
Flemming 2011 [36]	- Pool	Enhanced	-	Similar	-	-	-
Abdelrazik 2011 [37]*	Super high (10x) Pool	Enhanced	-	Decreased	-	-	-
Goedecke 2011 [38]	Low (≤1x) Single donor	Enhanced	Decreased	-	-	Similar	Similar
Lange 2011 [39]	- Pool	-	-	-	-	Decreased	-
Horn 2010 [40]*	Super high (6.2x) Pool	Enhanced	-	-	-	Similar	Enhanced

to be continued

Table 2. (cont.) Cell culture studies performed with Bone Marrow derived Stem Cells and Platelet Rich Plasma.

		BONE MARROW STEM CELLS					
		BIOLOGICAL EFFECTS (PRP vs FBS)					
					Stemness		
Author, year, [reference]	Platelet count Pool or single donor	Proliferation	Migration	Immune modulation	Chondr.	Adipo.	Osteo.
Lucarelli 2010 [41]#	Moderate (2.1x) Single donor	Enhanced	-	-	-	-	-
Schallmoser 2010 [42]	Moderate (3.8x) Pool	-	-	-	-	Similar	Similar
Chevallier 2010 [43]#	High (≥4x) -	Enhanced	-	-	Similar	-	Enhanced
Mishra 2009 [44]	Super High (7x) Single donor	Enhanced	-	-	Enhanced	-	-
Huang 2009 [45]	Super high (6.6x) Single donor	Enhanced	-	-	-	-	-
Pérez-Ilzarbe 2009 [46]	Super high (6x) Pool	Enhanced	-	Enhanced	Similar	Similar	Similar
Prins 2009 [47]#	High (4x) Pool	Enhanced	-	-	Similar	Similar	Similar
Zaky 2008 [48]*	High (5.6x) Single donor	Enhanced	-	-	Enhanced	-	Similar
Kasten 2008 [49]#	High (4.2x) Pool	Enhanced	-		-	-	Similar
Carrancio 2008 [50]	- Pool	Enhanced	-	-	Similar	Similar	Similar
Schallmoser 2008 [51]*	Moderate (3.8x) Pool	Enhanced	-	-	Similar	Similar	Similar
Schalmosser 2007 [52]	Moderate (3.8x) Pool	Enhanced	-	-	-	Similar	Similar
Capelli 2007 [53]*	High (4.8x) Single donor	Enhanced	-	Similar	-	Similar	Similar
Bernardo 2007 [54]*	Super high (10x) Pool	Enhanced	-	Decreased	-	Similar	Similar
Lange 2007 [55]*	Moderate (4x) Pool	Enhanced	-	Enhanced	Similar	Similar	Similar

* Articles performing long term expansion studies; #Articles performing 3D studies
Abbreviations: BMSCs, Bone Marrow derived Stem Cells; PRP, Platelet Rich plasma; FBS, Fetal bovine serum; Chondr., Chondrogenic; Adipo., Adipogenic; Osteo., Osteogenic.

MSCs-scaffold constructs with culture medium supplemented with PRP, and observed not only enhanced cell proliferation but improved cell loading into the scaffolds[49]. PRP benefits have also been reported in terms of cell adhesion and colonization of the biomaterial scaffold[29].

MSC migration

MSC migration is investigated in few studies[11,29,32,38]all but one showed enhanced MSC migration in PRP comparing to FBS[29,32]. Moreover, Murphy[32] et al. have compared ucPRP (umbilical cord PRP), aPRP (adult PRP), aPPP and FBS effect in MSC migration and found that all forms of human plasma were much more effective in promoting cell migration than FBS.

Cell differentiation

MSCs characteristically differentiate into multiple mesoderm-derived cell types including adipocytes, chondrocytes and osteoblasts. Aiming to develop articular therapies by combining PRP and MSCs, Mishra

Table 3. Cell culture studies performed with Bone Marrow derived Stem Cells and Adipose Derived Stem Cells or Umbilical Cord Derived Mesenchymal Stem Cells (UCMSCs) and Platelet Rich Plasma.

SEVERAL STEM CELL SOURCES								
			BIOLOGICAL EFFECTS (PRP vs FBS)					
						Stemness		
Author, year, [reference]	**Platelet count Pool or single donors**	**Cell source**	**Proliferation**	**Migration**	**Immune modulation**	**Chondr.**	**Adipo.**	**Osteo.**
Hemeda 2013 [56]	Super high (6.2x) Pool	BMSCs and ADSCs	Decreased at high heparin concentrations	-	-	-	Decreased at high heparin concentrations	Decreased at high heparin concentration
Lohmann 2012 [57]	Super high (6.2x) Single donors	BMSCs and ADSCs	Enhanced	-	-	-	Similar	Decreased with old d.
Menard 2013 [3]	- Pool	BMSCs and ADSCs	-	-	Enhanced in ADSCs	-	-	-
Torensma 2013 [58]*	- Pool	BMSCs and ADSCs	-		Decreased	-	-	-
Jenhani 2011 [59]*	- Pool	UCMSCs and BMSCs	Enhanced	-	-	-	-	-
Crespo-Diaz 2011 [60]*	- Pool	BMSCs and ADSCs	Enhanced	-	-	Similar	Similar	Similar
Avanzini 2009 [61]*	Super high (10x) Pool	UCMSCs and BMSCs	-	-	-	-	-	-
Reinisch 2007 [62]	Moderate (3.8x) Pool	UCMSCs	Decreased in late passages	-	Similar	Similar	Decreased	Similar

* Articles performing long term expansion studies
Abbreviations: ADSCs, Adipose Derived Stem Cells; BMSCs, Bone Marrow derived Stem Cells; UCMSCs, Umbilical Cord Derived Mesenchymal Stem Cells**;** PRP: Platelet Rich plasma; FBS: Fetal bovine serum; Chondr.: Chondrogenic; Adipo.: Adipogenic; Osteo.: Osteogenic.

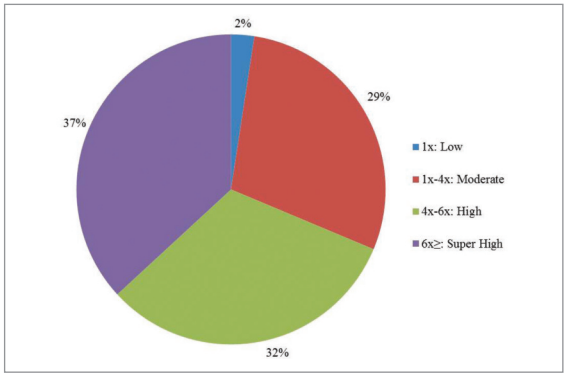

Figure 1. Sectors illustrating the use of different PRP formulations (according to De Long Classification [ref. 5]) *in vitro* studies of mesenchymal stem cells and PRP.

et al.[44] showed that buffered PRP enhanced the expression of chondrogenic markers, such as Sox9 and Aggrecan. These findings were corroborated in further studies[7,8,12,27,29,47] that showed enhanced chondrogenic differentiation, and extracellular cartilage matrix synthesis in the presence of PRP. Likewise, Shih et al.[12] also proved that chondrogenic differentiation was enhanced and maintained through cell passages in PRP cultures comparing to FCS cultures. Moreira Teixeira[29] proved that chondrogenic differentiation in dextran based scaffolds was enhanced by the addition of PRP in the form of platelet lysate (PL). Interestingly PRP did not hinder chondrogenic differentiation in any of the studies reviewed here.

Besides, PRP did not interfere with osteogenic differentiation and most authors found similar osteogenic

differentiation capacity when using one or the other supplement (PRP *versus* FBS). Albeit few studies have shown that PRP enhanced MSCs osteogenic differentiation in 2D cultures[14,27,35,40] and in 3D cultures[9,22,43]. Interestingly, Hemeda et al.[56] found reduced osteogenic differentiation when MSCs where cultured with PRP with high heparine concentrations which were used to avoid PRP clotting. Also, Lohmann et al.[23] found less osteogenic differentiation capacity in stem cells cultured with PRP obtained from old donors.

In the same trend, adipogenic differentiation was similar with PRP or FBS. Yet, Chieregato[14] observed enhanced adipogenic differentiation of ADSCs cultured with PRP, as well as Cervelli[10], but in the latter case only when PRP was combined with insulin. On the contrary, Reinisch[62] and Lange[39] observed decreased adipogenesis when ADSCs and BMSCs where cultured with PRP, respectively. As occurred in chondrogenesis and osteogenesis assays, high heparin concentrations interfered with adipogenic differentiation[56].

The fact that PRP supplementation preserves multipotency of MSCs, and does not interfere with any lineage differentiation, establishes it as a suitable substitute for FBS.

Modulation of the immune response

MSCs can modulate the immune response by suppressing the proliferation of T lymphocytes, as shown in coculture models[15]. Furthermore, MSCs can inhibit proliferation and function of other adaptive immune cells including B cells, NK cells and dendritic cells[55]. Additionally MSCs alter the cytokine secretion profile of immune cells generating an anti-inflammatory phenotype. MSCs cultured with PRP (in the form of platelet lysate or releasate) preserve their immune-privileged potential as shown by suppression of T-cell proliferation[20,36,53,55,62] and activation[46]. Interestingly, the immune potential of MSCs was enhanced when cultured with fibrinogen-depleted PRP[23]. In fact, fibrinogen compromised the ability of MSCs to up-regulate indoleamine dioxygenase that negatively correlates with T-cell proliferation[23].

Remarkably, super-high concentration of platelets (10x) impaired MSC inhibitory effect on T-cells[37,54] and NK-cells, and stimulate secretion of IL-6, IL-8 and RANTES while decreasing PGE2[37].

Discussion

As shown in this review, *in vitro* research in the field "PRP+MSC" is focused on two main areas, first optimization of the manufacturing process for mesenchymal stem cell therapy purposes, and second expanding the knowledge about how PRP modifies MSCs behavior for translational purposes, that is to say enhancement of PRP therapies, or design of the new combination products primarily based on "PRP+ MSCs".

Three central mechanisms are investigated in the *in vitro* models including: proliferation, migration, and the maintenance of the differentiation potential. In addition, the influence of PRP in the immune-modulatory properties of MSCs as well as the appearance of the senescence phenotypehas been explored.

There is no doubt that any PRP formulation (L-PRP, pure PRP, lysate, releasate) activates MSC proliferation in a controlled non-tumorigenic manner, a property that is of great value not only for cell manufacturing, but also for the clinical applications. Also, PRP is a useful tool to be incorporated in tissue engineering as it acts as a stimulator for cells to proliferate and colonize the scaffold.

Merely three studies[11,29,32] have explored the potential of PRP to induce MSC migration. The chemotactic properties of PRP on MSCs can be partially attributed to the chemokine SDF-1a (stromal derived factor 1, CXCL12) stored in the alpha granules[63,64]. This cytokine acts via CXCR-4 promoting cell migration and homing. Whether PRP enhances precursor cell migration is crucial in order to augment the efficiency of bone marrow stimulating techniques, i.e. drilling to subchondral bone as performed in knee pathology and rotator cuff[65,66]. These conditions illustrate the importance of augmenting cell migration, and the relevance of translational research in PRPs in getting the essential knowledge to design successful therapies. Thus, the need is clear to augment our knowledge, and introduce procedural modifications that may help to control the migration, proliferation and differentiation of these cells for successful healing.

Regarding differentiation, our Review shows that PRP maintains stemness and does not hinder differentiation to bone, cartilage or fat, when the appropriate factors are added to the culture media.

Cell cultures are a useful tool to design models that help to elucidate molecular mechanisms, and identify specific molecules involved therein. However, linking specific molecules with biological mechanisms is challenging, and results are not conclusive yet. For example, proliferation illustrates how difficult it is to link detailed molecules with biological mechanisms. Aiming to identify the main factors involved in proliferation, Horn et al.[40] denoted PDGF-AB and IGF as responsible for proliferation. Accordingly, Huang et al.[45] separated PRP in different fractions according to their molecular weight, and found that the fractions in which there was higher PDGF-AB and TGF-β1 content were the ones that enhanced MSC proliferation the most. The contribution of TGF-β1 to proliferation was confirmed by Cho HS et al.[34]. Notwithstanding, Lohmann et al.[57] reported a lack of correlation between the concentrations of PDGF-AB, bFGF, TGF-β1 and IGF-1,and proliferation. Fekete et al.[28] found that proliferation decreased when inhibition of PDGF-BB and b-FGF was performed. Moreover, the strongest inhibition was observed when combinations of anti-bFGF and anti-PDGF-BB or anti-bFGF + anti-TGFβ + anti-PDG-BB were used. On the contrary, when different combinations of recombinant PDGF-BB, bFGF and TGF-β were added to platelet poor

plasma, cell proliferation wasn't promoted[14]. This is in accordance with similar experiments performed in tenocytes[67]. This paradox can be explained assuming that the proliferative potential stems from molecular redundancy, and a delicate balance between multiple pro- and anti-proliferative molecules[68].

Likewise, increasing PRP percentage in cultures does not necessarily increase proliferation; 10% PRP seemed to be the optimal[19,21,36,57], and increasing up to 30% PRP didn't enhance proliferation but lowered it compared to FBS.

These results are relevant for cell manufacturing organizations where large-scale expansion of MSCs is performed according to Good Manufacturing Practice (GMP) procedures. To date, conventional MSC culture systems involve the use of about 10% of FBS (fetal bovine serum) in culture medium, but recently there is an emerging interest to avoid its use, due to the risk of xenogeneic immune reactions provoked by FBS antigens[18,57]. Also, it has been reported that FBS can be implicated in prion transmission, and its therapeutic use is not recommended by European legislation[69]. Furthermore, it is complicated to use FBS in a controlled, reproducible manner, because of its complex composition, with varying efficacy. Human platelet rich plasma (PRP), in the form of platelet lysates (HPL) or releasates, have emerged as possible substitutes for FBS because of their autologous, non-immunogenic features, and adequate physiological composition. Indeed, currently PRPs are commercialized for cell expansion purposes and even lysates resulting from expired platelet bags obtained from blood banks seem to be adequate as substitutes of FBS and provide the same good results as freshly isolated PRPs[20].

In the present review, we have identified several sources of variability in PRP technology that may influence the efficiency of large-scale expansion, and also the above described biological mechanisms. First, the formulation i.e. platelet and leukocyte count in a given volume of plasma, second, the activation procedure, and lastly the inter-individual variability. The composition of PRP depends on the preparation methodology, i.e. single *versus* double spinning[70,71]. Final platelet concentration ranges from 1x above baseline[35] to 12x[18]. The most commonly used platelet concentration *in vitro* ranges between 3x and 6x. It is known that there is a variation in platelet content produced not only by preparation methods, but influenced by inter-and intra-donor variations. The latter include factors related to patient state, i.e. hydration status, inflammation, lipemia, diet or circadian rythms[72].

To avoid inter-donor variations in PRPs and optimize reproducibility, several author use pooled PRP from at least ten donors[55]. Alternatively, other authors[27,36] pool PRPs from donors of the three blood groups, whereas others pool blood from the O group in order to avoid the presence of blood group determinants and isoagglutinins[21]. In this review, 30% of the articles use single donor´s PRP whereas 70% use pooled PRPs.

The age of donors is a crucial parameter to be considered because it significantly affects PRP biology. For instance, MSCs proliferation is higher with PRP from young donors while MSCs cultured with PRP from elder donors can have diminished proliferation, present a senescence phenotype[34,57], and hindered differentiation capabilities to specific lineages[57].

In clinical applications, L-PRPs often contain concentration of leukocytes above peripheral blood. Although they contribute to increase the pool of GFs, they also release active cytokines that are primarily catabolic or inflammatory, e.g. IL1β, TNF-α, IFN-γ[73]. Moreover leukocytes release degrading enzymes, mainly lysozymes and elastases that compromise the stability of GFs as well as gelatinases and metalloproteases, catabolic for collagen proteins.

Clinicians have to be careful when making sense of laboratory results, because cells *in vitro* do not mimic correctly the *in vivo* environment, and can only provide partial explanations of PRP biology. In fact, the *in vivo* conditions involve the impact of PRP on several cell types present in the host tissues; also relevant is the dynamic influence of PRP in the innate immune response, and in precursor cell migration. These are few among the simultaneously occurring events that cannot be mimicked in cell culture models. But despite important limitations of *in vitro* studies, we still can learn some lessons. Any formulation of PRP stimulates MSC activation, but currently there is no link with a single molecule and proliferation or migration or differentiation. The molecular pool released from PRPs is very complex, and experimental design for *in vitro* experiments to achieve meaningful results is challenging. Many gaps of knowledge need to be addressed *in vitro* in order to achieve enough information useful for translational purposes.

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Conflict of interests

None reported.

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