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Review

Human platelet lysate: Replacing fetal bovine serum as a gold standard for human cell propagation?

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ABSTRACT

The essential physiological role of platelets in wound healing and tissue repair builds the rationale for the use of human platelet derivatives in regenerative medicine. Abundant growth factors and cytokines stored in platelet granules can be naturally released by thrombin activation and clotting or artificially by freeze/thaw-mediated platelet lysis, sonication or chemical treatment. Human platelet lysate prepared by the various release strategies has been established as a suitable alternative to fetal bovine serum as culture medium supplement, enabling efficient propagation of human cells under animal serum-free conditions for a multiplicity of applications in advanced somatic cell therapy and tissue engineering. The rapidly increasing number of studies using platelet derived products for inducing human cell proliferation and differentiation has also uncovered a considerable variability of human platelet lysate preparations which limits comparability of results. The main variations discussed herein encompass aspects of donor selection, preparation of the starting material, the possibility for pooling in plasma or additive solution, the implementation of pathogen inactivation and consideration of ABO blood groups, all of which can influence applicability. This review outlines the current knowledge about human platelet lysate as a powerful additive for human cell propagation and highlights its role as a prevailing supplement for human cell culture capable to replace animal serum in a growing spectrum of applications.

1. The regenerative potential of platelets

Platelets play an essential role not only in primary hemostasis but also in wound healing and tissue regeneration. Due to their short lifespan of 8–10 days in the healthy organism, about 15–40 $\times$ 10^9 platelets have to be produced daily from megakaryocytes to maintain a normal blood count of 1.5–4.5 $\times$ 10^5/μL [1]. Meaningful knowledge about platelet biology and pathophysiology has been gained during the last two centuries, reaching back to 1865 and 1882, when Schultze and Bizzozero described this 'third group of corpuscular particles' in the blood [2]. Initiated by Roskam's report in 1922, the content of blood derived substances within platelets was associated with a passive 'sponge-like' basic function rather than with an active synthesis and storage capacity, inducing the coagulation cascade at endothelial damage [3]. Notably, the interaction between platelets and endothelial cells supporting the integrity of capillary endothelium has been known since the 1960s (for review [4,5]). Initial studies by Folkman and coworkers [6] used autologous platelet-rich plasma (PRP) supplemented medium to 'nurture' microvascular endothelial cells to improve preservation of vascular integrity in organs perfused for transplantation. By the 1980s, human platelet lysate (HPL), prepared by repeated freeze/thaw cycles and sonication from fresh blood or outdated platelet concentrates, was found to support proliferation of established cell lines and primary fibroblasts [7,8]. Subsequent in depth analysis of the multiplicity of platelet derived

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growth factors indicated a role for platelets in vascular regeneration and wound healing [9].

Besides coagulation factors, platelets store a series of potent bioactive mediators primarily in their α-granules [10] (for review [11–13]), including various chemokines [14] and growth factors, such as platelet derived growth factor isoforms (PDGF-AA, -AB and -BB), transforming growth factor-β (TGF-β), insulin-like growth factor-1 (IGF-1), brain derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF or FGF-2), hepatocyte growth factor (HGF), connective tissue growth factor (CTGF) and bone morphogenetic protein 2, -4 and -6 (BMP-2, -4, -6) [13,15] as shown in Fig. 1.

As a consequence of activation, platelets release their granule content. Pro-inflammatory cytokines and chemokines contribute to wound healing (Fig. 2) by attracting macrophages and granulocytes to remove damaged cells and tissue as well as by aiding defense against bacterial contamination [15,16]. The stimulatory and inhibitory effects of these mediators cause migration and proliferation of fibroblasts, smooth muscle cells and endothelial cells, resulting in vessel remodeling and tissue repair [13]. The mixture of growth factors in HPL was found to be sufficient to promote endothelial cell survival and vasculogenesis *in vivo* in the absence of pericytes [17]. The multifaceted role of platelets in modulating innate and adaptive immune responses has been elaborately reviewed recently [14]. The complexity of the α-granules' content has also been described [18], postulating distinct subpopulations storing either pro-angiogenic or anti-angiogenic mediators released by specific protease-activated receptor agonists. A more stochastic pattern of co-distributed α-granule proteins has subsequently been reported [19]. Stromal cell derived factor (SDF)-1 supplied by activated platelets, has recently been shown to be responsible for alveolar regeneration in mice after pneumonectomy [20]. Serotonin (5-hydroxytryptamine, 5-HT), primarily known as a neurotransmitter, is another potent mitogen stored in dense granules and capable to support liver regeneration [21,22].

Due to tissue and vessel injury, activated platelets aggregate and degranulate to initiate primary hemostasis. The release of growth factors consecutively leads to inflammation, fibroblast and smooth muscle cell activation as well as collagen synthesis and angiogenesis, resulting in epithelialization and tissue regeneration (for review [13,16]).

Described for the first time as 'platelet dust' by Wolf in 1967 [23], activated platelets release not only proteins but also cellular microvesicles as plasma membrane derived microparticles and exosomes (for review [24,25]). Notably, their molecular cargo can

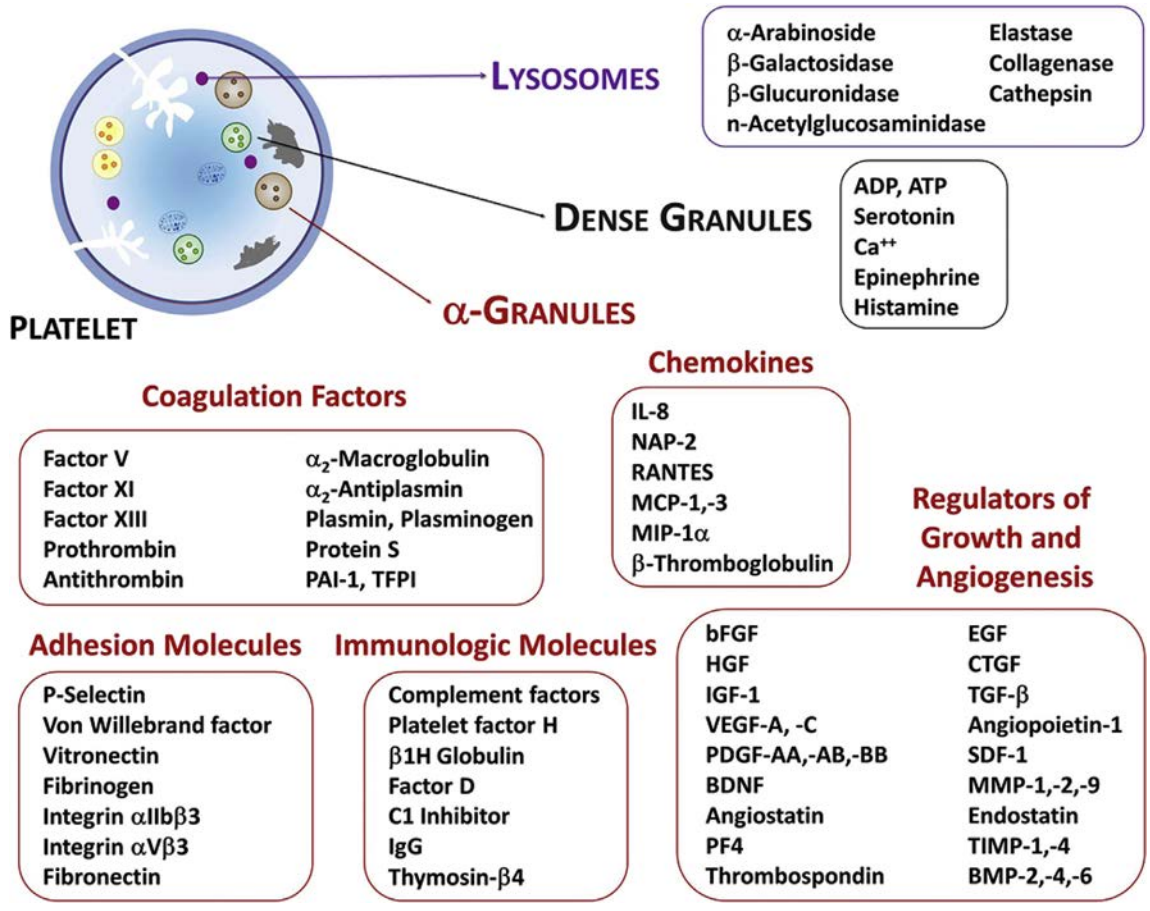


Fig. 1. Platelet granule cargo. The various types of platelet granules store a plethora of potent substances including lysosomal enzymes, coagulation factors, immunologic and adhesion molecules, chemokines and growth factors for hemostasis, host defense, angiogenesis and tissue repair [10–15]. *Abbreviations*: **ADP**, adenosine diphosphate; **ATP**, adenosine triphosphate; **BDNF**, brain derived neurotrophic factor; **bFGF**, basic fibroblast growth factor; **BMP**, bone morphogenetic protein; **C**, complement; **CTGF**, connective tissue growth factor; **EGF**, epidermal growth factor; **HGF**, hepatocyte growth factor; **IGF-1**, insulin-like growth factor-1; **IL-8**, interleukin 8 (*CXCL8*); **MCP-1**, monocyte chemotactic protein-1 (*CCL2*); **MCP-3**, monocyte chemotactic protein-3 (*CCL7*); **MIP-1α**, macrophage inflammatory protein 1α (*CCL3*); **MMP**, matrix metalloproteinase; **NAP-2**, neutrophil-activating protein-2 (*CXCL7*); **PAI-1**, plasminogen activator inhibitor-1; **PDGF**, platelet derived growth factor; **PF4**, platelet factor 4 (*CXCL4*); **RANTES**, regulated on activation, normal T cell expressed and secreted (*CCL5*); **SDF-1**, stromal cell derived factor-1; **TFPI**, tissue factor pathway inhibitor; **TGF-β**, transforming growth factor-β; **TIMP**, tissue inhibitor of metalloproteinases; **VEGF**, vascular endothelial growth factor.

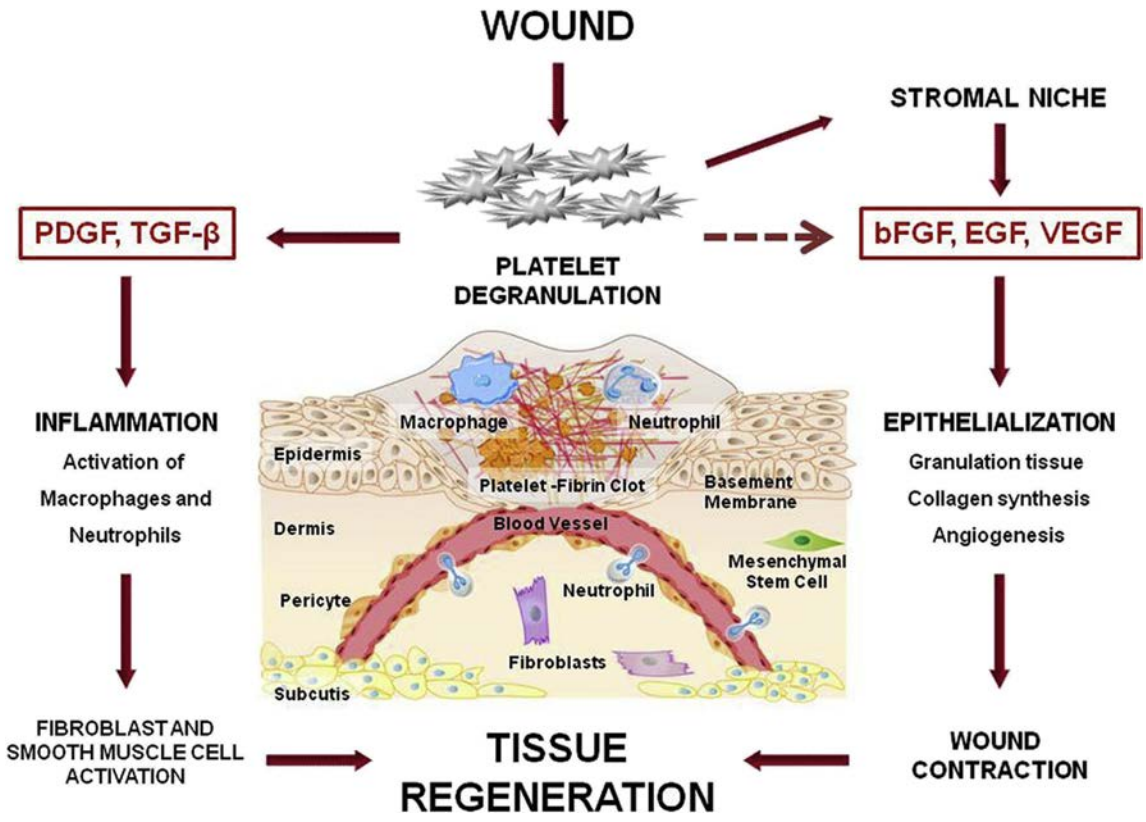


Fig. 2. Platelet derived growth factors in wound healing.

comprise growth factors, such as VEGFs, bFGF, PDGFs, TGF-β and other cytokines [26,27] suggesting not only a putative influence on angiogenesis, post-ischemic vascular and neuronal regeneration, but also a role in tumor metastasis and inflammatory arthritis [27–31]. Even though platelets are anuclear cell fragments, they also contain miRNA [32] and mRNA, presumably transferred from megakaryocytes during pro-platelet formation, and protein neosynthesis is feasible due to signal-dependent pre-mRNA splicing and translation of mRNA [33–35].

1.1. Platelets for in vivo application

The clinical use of platelets, either from whole blood or apheresis products, is well established in the prophylaxis and treatment of bleeding due to thrombocytopenia [13]. The potential of blood as a liquid tissue has also been therapeutically utilized for surgical hemostasis and tissue adherence as fibrin sealants since the beginning of the last century ([36] and for review [37]). The development of fibrin biomaterials enriched with platelets, now termed 'platelet-fibrinogen-thrombin mixtures', 'platelet fibrin glue' or 'gelatin platelet – gel foam', dates back to the late 1970s [38]. This led to the development of PRP and platelet gel for local use, e.g. for bone grafts in oral and maxillofacial surgery [39,40]. Current tissue engineering strategies focus on more sophisticated three-dimensional naturally or artificially assembled transplantable tissue constructs, which combine HPL derived growth factors and cell-instructive materials [41–45].

Within a few years, the amount of scientific reports dealing with clinical applications of mainly autologous PRP with varying and divergent success is constantly increasing [46–54], with different preparation modes still under discussion [55–59]. Current attempts to establish a classification of PRP, depending on platelet concentration factor, residual level of leukocytes, and mode of platelet activation during preparation have thus far not proven successful, primarily due to a lack of standardized protocols, diversity in medical devices used to isolate the PRP, as well as the variability and often incomplete or unavailable details of methodology in the literature [38,60,61]. At present, numerous clinical studies are registered testing PRP and its derivatives in maxillofacial, orthopedic and plastic surgery and in the treatment of burns, scars, chronic ulcers and wounds, osteoarthritis, repair of tendons and ligaments (169 studies found for the search term 'platelet-rich plasma' by July 21, 2015 [62]). Variability in patient characterization, limited number of patients and diversity of methodology results in restricted comparability of the trials and largely disable meta-analysis. Completion of currently ongoing prospective randomized phase three and four trials has been expected to provide first answers regarding efficiency of selected platelet derivatives in regenerative medicine [46].

1.2. Platelets for in vitro cell propagation

So far the standard medium supplement and source of growth factors for cell culture and tissue engineering has been fetal bovine serum (FBS). Regenerative cells like mesenchymal stem/progenitor cells (MSC) occur in low frequency in tissues and generally have to be propagated to achieve a suitable dose for clinical application. Since the initial report by Lazarus and colleagues in 1995 [63], MSC have mostly been cultured in FBS for clinical trials (for review [64]). Recognizing the risks of xeno-immunization against bovine antigens, transmission of pathogens, ethical issues associated with crude methods of FBS collection, and potential limits of availability [65–70], precipitated the urgent need for suitable human alternatives for the manufacture of clinical cell therapeutic products

[70,71]. This has become more important in the context of a fast growing field of regenerative medicine and advanced cell therapy where the use of FBS for propagation of clinically applied cell products and in medicinal products is discouraged and should be avoided, if possible, in accordance with current international guidelines and regulatory frameworks [72–74].

In parallel with the spread of *in vivo* use of platelet derived preparations, the use of platelet lysates for *in vitro* cell proliferation was initiated in the 1980s. After lysis of outdated platelet concentrates or PRP, the lysates were efficiently used for *in vitro* culture of fibroblasts, endothelial cells or tumor cell lines [7–9,75,76]. Since 2005, initiated by a study of Doucet et al. [77] describing the preparation and efficient use of human platelet lysate (HPL) for expanding MSC, a series of reports have shown that both allogeneic and autologous HPL are superior to FBS for stimulation of cell proliferation [9,42,77–112] (see also Tables 1A and 1B). This highly efficient stimulation of cell proliferation enabled large-scale expansion of MSC for clinical use in one single culture period of 11–16 days without further passage, while maintaining genomic stability [113]. There is, to our knowledge, only one published study directly comparing genomic stability of FBS- and HPL-cultured MSC applied for the treatment of GvHD and accidentally irradiated patients, revealing culture independent but instead donor dependent recurring chromosomal alterations of MSC at passage 2 [114].

Tables 1A and 1B contain a comprehensive collection of scientific publications reporting the use of allogeneic and autologous HPL for culture of various human cell types from the 1980s until 2015.

Similar to the unclear situation observed for *in vivo* PRP use, there is still no uniform procedure for both the starting material (Fig. 3) and the mode of preparation of HPL (Fig. 4). The quality of the final product and the effects on cell biology and function varies substantially. The majority of studies with allogeneic HPL used apheresis platelet concentrates (n = 30), compared to 17 studies starting from buffy coat derived platelet concentrates, 3 studies used both and 17 reported results with undefined platelet concentrates. The material was used either fresh or expired, irradiated or pathogen inactivated. The platelets were suspended mainly in plasma, but also additive solution, medium, phosphate-buffered saline (PBS) or saline. As far as indicated, the pooling included from two up to 52 blood donations. Platelet concentration varied between 1×10^6/mL and 15×10^9/mL. The release of platelet content was mainly induced by repeated freeze/thaw cycles (−20 °C to −196 °C freezing and 37 °C thawing), by direct platelet activation using calcium chloride ($CaCl_2$) or thrombin, by sonication at different frequencies or solvent/detergent (S/D) treatment. Due to incomplete fibrinogen depletion, the addition of anticoagulant heparin to the cell culture is usually required before adding HPL to avoid clotting. Broad variations were observed for centrifugation conditions, additional filtration steps and the final concentration in the medium from 1% to 60%. Despite substantial variations in the type of HPL, most reports concluded that HPL is an efficient substitute for FBS. A comparison of HPL and FBS considering MSC quality is summarized in Table 2 [9,42,77–112].

2. Variables in platelet isolation and HPL manufacturing

2.1. Types and production methods of blood derivatives used as FBS substitute

Several human blood fractions including plasma, serum and platelet concentrates subjected to either freeze/thaw cycles or serum-converted by addition of thrombin or calcium have been evaluated as FBS substitute for *ex vivo* expansion of MSC and other cells [71,146–149].

I. Plasma:

Plasma prepared by centrifugation from anti-coagulated blood has rarely been used as supplement, although it has been recognized to contain numerous nutrients and factors like growth hormones and IGF-1 that can be useful for cell proliferation and differentiation (see also Fig. 5) [146]. Platelet-poor plasma was found to be sufficient for outgrowth of initial colonies from bone marrow (BM) derived MSC but the addition of platelet lysate was essential to ensure cell proliferation [103].

II. Serum:

Serum is typically prepared by physiological clotting of whole blood after blood drawing in the absence of anticoagulants. After centrifugation and filtration serum can be stored frozen in aliquots of appropriate volume [71,101,148,149]. Endogenous thrombin generation induces platelet degranulation and release of growth factors. Owing to the natural platelet count in the starting whole blood, the content of growth factors in serum samples has been shown to be 30%–50% lower compared to HPL derived from platelet concentrates (see below) [71].

III. Platelet concentrates:

The preparation of pooled allogeneic platelets from apheresis or buffy coat (BC) derived platelet concentrates with a minimum platelet content of more than 2×10^{11}/unit in Europe [150] and more than 3×10^{11}/unit in the United States of America (USA) [151] is routinely performed at blood banks for the transfusion of thrombocytopenic patients. This allows large scale, cost-effective and standardized manufacturing of an allogeneic 'off-the-shelf' product of HPL for cell culture. Human platelet concentrates are obtained by three main procedures licensed for clinical products in regulated countries (Fig. 3). All types have been used for HPL production and proven effective for expanding MSC. The mode of preparation may possibly affect biochemical and functional properties of HPL, although this has not been conclusively studied yet. The preparation process of platelet concentrates as a source of HPL has been reviewed recently [147] and technical details can be obtained from the Guide of the Council of Europe [150]. Briefly, these concentrates can be prepared either from anticoagulated whole blood following (a) the buffy coat method (largely used in Europe) or (b) the PRP method (mostly used in the USA and Asia), or (c) they are obtained by plateletpheresis technology (used worldwide, but more particularly in high-income countries). For the buffy coat method whole blood is centrifuged and four buffy coat units plus one plasma unit from four donors are pooled. After a second centrifugation step the platelet concentrate is transferred via a leukocyte depletion filter into the storage bag. For the PRP method, 4 or 5 platelet concentrates from whole blood are pooled under aseptic conditions to obtain a therapeutic unit of approximately 200 mL. Apheresis platelet donations of approximately 200–300 mL are produced from single donors (Fig. 3). All platelet concentrates can be stored for up to 5–7 days at 22 ± 2 °C under agitation. When not used for transfusion until the end of storage, they are regarded as "expired" (or outdated). In some blood centers, they are frozen and stored for further manufacturing into HPL (for review [152]). Whole blood, or platelets from whole blood, can be subjected to pre-storage leukocyte depletion by filtration to reduce residual leukocytes to less than 5×10^6/unit (USA [151]) or less than 1×10^6/unit (Europe [150]). The apheresis procedure, by its design, allows for the manufacture of platelet concentrates that are less contaminated with leukocytes. The platelet concentrate can be maintained in plasma or in a mixture of plasma and platelet

Table 1A
Use of allogeneic human platelet lysate (HPL) for the culture of various human cell types. Selected publications from 1980 to 2015 and details of the preparation technique and composition of HPL, types and sources of analyzed cells, culture conditions, comparison with FBS culture and analysis of growth factors and cytokines are listed if reported. Studies are sorted for starting material of Aph-PC, BC-PC and non-defined PC and different methods of lysis.

Reference	Starting material	ABO blood groups	Solution	Pooling	Platelet counts $\times 10^9$/mL (mean)	1st Centrifugation	Platelet lysis		Filtration	Fib depletion	2nd Centrifugation	Expanded cells	Supplementation	HPL superior to FBS	Growth factor analysis
							Freeze/ Thaw	Others							
Apheresis platelet concentrates															
[77]	Aph-PC	–	Plasma	10 PC	1	–	−80 °C	–	–	–	900× *g*	BM-MSC	5%	Yes	Yes
[115]	Aph-PC	–	Plasma	10 PC	1	–	Yes	–	–	–	900× *g*	BM-MSC	8%	n.t.	n.t.
[78]	Aph-PC	–	Plasma	10 PC	5×10^{11}/unit	–	−80 °C	–	–	–	900× *g*	BM-MSC	1–5%	Yes	Yes
[116]	Aph-PC	–	Culture medium	–	2	Yes	−80 °C	–	–	Indirect	3000× *g*	Keratinocyte cell line HaCaT, Scratch wound healing	20%	n.t.	n.t.
[117]	Aph-PC	–	Culture medium	–	4	4600× *g*	−196 °C	–	–	Indirect	16,000× *g*	MSC (bone, BM, pulp, AT)	5–10%	No	Yes
[79]	Exp Aph-PC	–	Plasma	Yes	–	–	−80 °C	–	–	–	900× *g*	BM-MSC	10%	Yes	n.t.
[118]	Aph-PC	–	Plasma	10 PC	5×10^{11}/unit	–	−80 °C	–	–	–	900× *g*	UCB-MSC BM-MSC	5%	n.t.	n.t.
[119]	Exp Aph-PC	–	Plasma	–	–	–	−80 °C	–	–	–	900× *g*	BM-MSC	5%	no	n.t.
[120]	Aph-PC	–	Plasma	–	2	–	−80 °C	–	–	–	3000× *g*	Skin fibroblasts	1–100%	n.t.	n.t.
[121]	Aph-PC	–	Plasma	10 PC	5×10^{11}/unit	–	−80 °C	–	–	–	900× *g*	Clinical BM-MSC	5%	n.t.	n.t.
[80]	Aph-PC	–	Plasma	–	>1	–	−80 °C	–	–	–	1400× *g*	BM-MSC	5%	Yes	n.t.
[122]	Aph-PC	–	Culture medium	–	4	4600× *g*	yes	–	–	Indirect	16,000× *g*	Cryopreservation of hepatocytes	10–20%	n.t.	n.t.
[81]	Aph-PC	–	Plasma	12 PC	2–3	–	−80 °C	–	–	–	900× *g*	Bone derived MSC	10%	Yes	Cellular CK
[123]	Aph-PC	–	Plasma	–	–	–	−80 °C	–	Yes	–	900× *g*	BM-MSC	7.5%	No	Yes
[82]	Exp Aph-PC	–	Plasma	8–12 don	–.	–	Yes	–	–	–	–	BM-MSC AT-MSC	1–5%	Yes	Proteomics
[83]	Aph-PC	–	Plasma	10 PC	–	–	−80 °C	–	–	–	1400× *g*	BM-MSC UCB-MSC	5–10%	Yes	CK
[68]	Exp Aph-PC	–	Saline	–	15	6000× *g*	−20 °C	–	–	Indirect	8000× *g*	Immortalized cell lines (kidney)	5%	n.t.	Yes
[84]	Aph-PC	–	Plasma	10 don	–	–	−30 °C	–	–	–	1400× *g*	BM-MSC	5–10%	Yes	Yes
[85]	Exp Aph-PC male	–	Plasma	–	1.0–2.1	–	−80 °C	–	Yes	–	2500× *g*	BM-MSC AT-MSC	10%	Yes	Yes
[124]	Exp Aph-PC	–	Plasma	Yes	–	–	−80 °C	–	–	–	900× *g*	BM-MSC	5%	n.t.	n.t.
[125]	Aph-PC	–	Plasma	–	10	–	−80 °C	–	–	–	900× *g*	BM-MSC Chorionic villi MSC Amniotic fluid MSC	5%	No	Yes
[126]	Aph-PC	Similar	Plasma	–	–	–	−80 °C	–	–	–	900× *g*	Venous myofibroblasts	5%	n.t.	n.t.
[127]	Aph-PC	–	Plasma	2 don	1	–	−20 °C	Heat inactiv	–	–	5000× *g*	BM-MSC	10%	n.t.	n.t.
[86]	Aph-PC	–	Plasma	–	0.1	–	−20 °C	Heat inactiv	Yes	–	5000× *g*	BM-MSC	5–10%	Yes	n.t.
[87]	Exp Aph-PC	–	Plasma	5–6 PC	1.1	–	−80 °C or −196 °C	$CaCl_2$ or not activated	–	Yes –	4000× *g*	Commercial human MSC	10%	Yes	Yes
[88]	Exp Aph-PC	–	Plasma	5 don	1	–	−80 °C	$CaCl_2$	Yes	Yes	4000× *g*	BM-MSC	10%	Yes	Yes
[89]	Aph-PC	–	Plasma	4–6 don	–	–	– −80 °C	Sonication	Yes	–	1600× *g*	BM-MSC	10%	Yes	PDGF AB
[128]	Aph-PC	–	PBS	3 PC	–	2000× *g*	−20 °C	Sonication	–	Indirect	–	BM-MSC, EPC	5–8%	n.t.	Yes
[129]	Aph-PC	–	Plasma	–	–	–	–	S/D, 23 mM CaCl	–	Yes	–	Human embryonic kidney fibroblasts	10%	no	Yes
[130]	Aph-PC	–	Plasma	–	1.0–1.3	–	–	S/D	Yes	–	–	AT-MSC	10%	No	Yes

(*continued on next page*)

Table 1A (*continued*)

Reference	Starting material	ABO blood groups	Solution	Pooling	Platelet counts $\times 10^9$/mL (mean)	1st Centrifugation	Platelet lysis		Filtration	Fib depletion	2nd Centrifugation	Expanded cells	Supplementation	HPL superior to FBS	Growth factor analysis
							Freeze/ Thaw	Others							
Buffy coat derived platelet concentrates															
[90]	BC-PC	O/AB	Plasma	10–13 PC	0.95	–	−30 °C	–	–	–	4000× *g*	BM-MSC	10%	Yes	Yes
[91]	BC-PC	O/AB	Plasma	10–13 PC	0.95	–	−30 °C	–	–	–	4000× *g*	UCB-MSC	10%	Yes	n.t.
[92]	BC-PC	–	Plasma	7–10 PC	1.2–1.9	200× *g*	−80 °C	–	–	–	900× *g* or 8000× *g*	BM-MSC	5%	Yes	n.t.
[131]	BC-PC	O/AB	Plasma	10–13 PC	0.95	–	−30 °C	–	–	–	4000× *g*	PB-ECFC	10%	n.t.	n.t.
[93]	BC-PC	O/AB	Plasma	10–12 PC	–	–	−80 °C	–	–	–	4000× *g*	Dental pulp SC	10%	Yes	n.t.
[94]	BC-PC, leuko-depleted	–	Plasma	–	1	–	−80 °C	–	–	–	2000× *g*	Adipose derived stromal cells	5%	Yes	n.t.
[132]	BC-PC	O/AB	Plasma	10–13 PC	0.95	–	−30 °C	–	–	–	4000× *g*	MSC (BM, amnion, placenta), UC-ECFC	10%	n.t.	n.t.
[133]	BC-PC	AB (plasma)	Plasma	2–13 PC	0.1	3200× *g*	−20 °C	–	–	–	3200× *g*	BM-MSC	10%	No	n.t.
	Exp BC-PC	–	Add Sol Plasma	15 PC	15		−70 °C						3%		
[95]	Exp BC-PC	–	Plasma	Yes	–	–	−80 °C	–	Yes	–	4975× *g*	BM-MSC	10%	Yes	n.t.
[96]	BC-PC	Identical	Saline	6 PCs	3.34	–	−80 °C	–	–	Indirect	5348× *g*	AT-MSC, fibroblasts	5%	Yes	Yes
			Plasma		3.58					–					
[97]	BC-PC	O/AB	Plasma	10 PCs	–	–	−40 °C	–	–	–	4000× *g*	Adipose derived stem cells	10%	Yes	VEGF
[98]	BC-PC path inactiv	–	Add Sol	12 don	–	–	−80 °C	–	–	Indirect	–	Mediastinal AT-MSC	20%	Yes	Yes
[99]	BC-PC path inactiv	–	Add Sol	12 don	1	–	−80 °C	–	–	–	4000 rpm	BM-MSC	10%	Yes	Yes
[134]	Exp BC-PC or path inactiv		Add Sol	16 don	–	–	−80 °C	–	–	–	4975× *g*	BM-MSC	10%	n.t.	n.t.
[100]	BC-PRP, pellet	–	Plasma	10–20 don	10	–	−196 °C	Lyophilization Irradiation	–	Indirect	19,000× *g*	HeLa Cells, BM MSC, skin fibrobl, osteobl, chondrocytes	5%	Yes	n.t.
[101]	BC-PC	AB	Plasma	–	2–3	–	−196 °C	–	Yes	Yes	1500× *g*	AT-MSC	10%	Yes	Yes
							–	Thrombin/TRAP	Yes	–	3000× *g*				
[102]	BC-PC	AB (plasma)	Plasma	2 PCs	–	–	−30 °C	–	–	–	2000× *g*	AT-MSC, BM-MSC	2.5–10%	Yes	Yes
							–	Thrombin		Yes					
Buffy coat-derived and apheresis platelet concentrates															
[103]	BC-PC, Exp Aph-PC	AB+	Plasma	–	2–4.2 × 10^{11}/ unit	–	−80 °C	–	Yes	–	2600× *g*	BM-MSC	10%	Yes	Yes
[135]	Exp BC-PC irradiated, Exp Aph-PC	–	Saline	3 PCs	–	–	−30 °C	–	Yes	–	4000× *g*	BM-MSC	10%	No	Yes
			Plasma	–	>1		−80 °C				yes				
[136]	BC-PC, Exp Aph-PC	–	Plasma	–	>1	–	−80 °C	–	Yes	–	4000× *g*	BM-MSC AT-MSC	8% 2%	n.t.	n.t.
Non-defined platelet concentrates															
[104]	PRP	–	Plasma	–	1.2	–	−40 °C	–	–	–	3000 rpm	BM-MSC	5%	Yes	n.t.
[105]	Whole blood derived	–	Plasma	–	0.9	–	−80 °C	–	Yes	–	3313× *g*	AT-MSC	10%	Yes	n.t.
[106]	PC	–	Plasma	5 PCs	2–4.2 × 10^{11}/ unit	–	−80 °C	–	Yes	–	2600× *g*	AT-MSC	1–20%	Yes	n.t.
[137]	Pooled PC	–	Plasma	Yes	–	200× *g*	−80 °C	–	–	–	–	BM-MSC	5%	No	n.t.
[107]	PC	O/AB	Plasma	30 don	–	–	Yes	–	–	–	4000× *g*	BM-MSC	5–20%	Yes	n.t.
[138]	PRP	–	Saline	5 don	1.3	1500× *g*	−80 °C	–	–	Indirect	–	Endothelial cells, monocytes, fibroblasts	0–20%	n.t.	Yes

[42]	PRP	–	Saline	10 don	1	–	−80 °C	–	–	Indirect	900× *g*	Human dental pulp SC	1–10%	Yes	Yes
[139]	PRP		Plasma	20 units	1	–	−80 °C	–	Yes	–	4000× *g*	BM- and AT-MSC	n.a.	n.t.	Yes
[8]	PRP	–	PBS	–	17	700× *g*	Yes	Sonication	Yes	Indirect	10,000× *g*	Skin fibroblasts	n.a.	n.t.	n.t.
[140]	Exp PC	–	Plasma	–	–	3000× *g* after thawing	Yes	Sonication, $CaCl_2$	Yes	Yes	Dialysis	Pancreatic stellate cells	3%	n.t.	n.t.
[108]	Exp PC	–	Plasma	10 PC	–	–	−80 °C	–	–	– Yes	3000× *g*	Differentiation of PSC	10%	Yes	n.t.
[7]	Exp PC or fresh PRP	–	PBS	–	–	Yes	–	$CaCl_2$ Sonication	–	Indirect	100,000× *g*	Tumor cell lines	n.a.	n.t.	n.t.
[9]	Exp PC	–	PBS	–	–	20,000× *g*	–	Sonication	–	Indirect	100,000× *g* dialysis, fractionation	EC, fibroblasts	fractions	Yes	n.t.
[141]	Citrate PRP	–	Plasma	–	1.32	3200 rpm	–	$CaCl_2$ + Thrombin	–	Yes	10,000 rpm	Adipose derived SC, dermal fibroblasts	1–20%	n.t.	Yes
[109]	PRP (Medtronic)	–	Plasma	–	0.001	–	–	–	–	–	–	Commercial human MSC	1–20%	Yes	n.t.
[142]	PRP gel sup	–	Plasma	10 don	–	1100× *g*	–	Cascade Esforax – Ca^{++}		Yes	–	Adipose derived stem cells	20–50%	n.t.	n.t.
[110]	PRP (SmartPReP2)	–	Plasma	–	1.0–1.2	–	–	Thrombin	–	Yes	600× *g*	BM-MSC	1–5%	Yes	Yes

Abbreviations: Add Sol, Additive Solution; Aph, apheresis; AT, adipose tissue; BC, buffy coat; BM, bone marrow; CK, cytokines; don, donors; ECFC, endothelial colony forming cells; EPC, endothelial progenitor cells; Exp., expired; FBS, fetal bovine serum; Fib, fibrinogen; MSC, mesenchymal stem/progenitor cells; n.t., not tested; path inactiv, pathogen inactivated; PB, peripheral blood; PBS, phosphate buffered saline; PC, platelet concentrate; PDGF, platelet derived growth factor; PRP, platelet-rich plasma; PSC, pluripotent stem cells; SC, stem cells; TRAP; thrombin receptor-activating peptide; UCB, umbilical cord blood; VEGF, vascular endothelial growth factor.

additive solution, containing sodium/potassium chloride, citrate, phosphate, and mannitol [153,154]. This may be important as decreased plasma proteins potentially influence cell growth (Fig. 5), as it has been reported that expansion of BM-MSC was less efficient in a medium supplemented with platelet lysates only [103]. The total platelet count in a therapeutic unit dedicated for adult patients ranges from $3–6 \times 10^{11}$ [155]. The residual leukocyte content depends on leukocyte depletion during production and storage in solely plasma or residual 30%–40% plasma plus additive solution.

2.2. Processing of platelet concentrates into HPL

The methods used to induce the release of growth factors and other bioactive molecules from platelets can be classified into four general categories:

I. Repeated freeze/thaw cycles:

Implementation of one or more freeze/thaw cycles is the most common and simple, but efficient and economic manner to induce platelet lysis in the bag (Fig. 4). Typically, the platelet concentrate is shock-frozen at −30 °C or −80 °C and thawed at 37 °C to fragment platelets [77,90]. The number of cycles so far described in the literature varies from one to five (see references of Table 1). A systematic analysis determining the optimal number and precise conditions of freeze/thaw cycles is still pending.

II. Direct platelet activation:

Another procedure involves addition of calcium salt solution (usually $CaCl_2$) to induce endogenous thrombin generation, fibrin formation and platelet degranulation [87,88,108,111,129,140–142,156,157]. Also direct activation by human or recombinant thrombin has been performed [101,102,105,110,112,141,144], but these extraneous substances can complicate regulatory approval of HPL for cell therapy.

III. Sonication:

Sonication alone or in combination with additional freeze/thaw cycles has also been used as a rapid, efficient and cost effective method to release the platelet cargo for experimental purposes since more than 30 years [7–9]. Sonication for up to 30 min at a frequency of 20 kHz was found to be efficient to release the platelet granule content [89,140].

IV. Solvent/detergent (S/D) treatment:

S/D treatment achieves the double goal of platelet lysis and inactivation of lipid-enveloped viruses (Table 1 and Fig. 4). These procedures have been reviewed recently in detail [147]. In most procedures, the HPL is subjected to centrifugation for removing cell debris and fibrin clots and is kept frozen until use. For processes that do not remove fibrinogen, heparin as an anticoagulant – ideally without stabilizer – has to be added to the medium, typically to a final concentration of 0.6–2 IU/mL, to avoid subsequent coagulation in the medium during cell culture. Higher heparin concentration has been claimed to impair cellular proliferation [113,158]. For designing a “fully humanized” cell propagation system future strategies are required, that either replace the porcine heparin by a human recombinant equivalent or rely on the use of fibrinogen-depleted HPL serum [88].

All these preparation modes result in HPL products that are capable of supporting expansion of MSC from various origins [12,147,159]. Variations in the capacity to expand and differentiate

Table 1B
Use of autologous human platelet lysate (HPL) for the culture of various human cell types. Publications from 2008 to 2015 and details on preparation technique and composition of HPL, types and sources of analyzed cells, culture conditions, and comparison with FBS culture are listed as far as reported.

Reference	Starting material	Solution	Platelet counts × 10^9/mL (mean)	1st Centrifugation	Platelet lysis		Fib depletion	Expanded cells	Supplementation	HPL superior to FBS
					Freeze/ Thaw	Others				
[143]	PRP	Plasma	1	1000× *g*	Yes	–	–	BM-MSC for degenerative joint	5–20%	n.t.
[144]	PRP	Plasma	1.24	–	–	Thrombin	Yes	AT SC expanded in FBS, neurogenic differentiation with activated PRP	10%	n.t.
[111]	New-PRP Pro Kit	Plasma	–	1000× *g*	–	$CaCl_2$	–	AT-MSC	10–20%	Yes
[112]	PRP, RegenKit	Plasma	0.24	–	–	Autol thrombin or non activated	Yes No	AT-MSC	5–60%	Yes
[145]	PRP	Plasma	–	1000× *g*	−20 °C	–	–	BM-MSC	10–20%	n.t.

Abbreviations: AT, adipose tissue; Autol, autologous; BM, bone marrow; FBS, fetal bovine serum; Fib, fibrinogen; MSC, mesenchymal stem/progenitor cells; n.t., not tested; PRP, platelet-rich plasma; SC, stem cells.

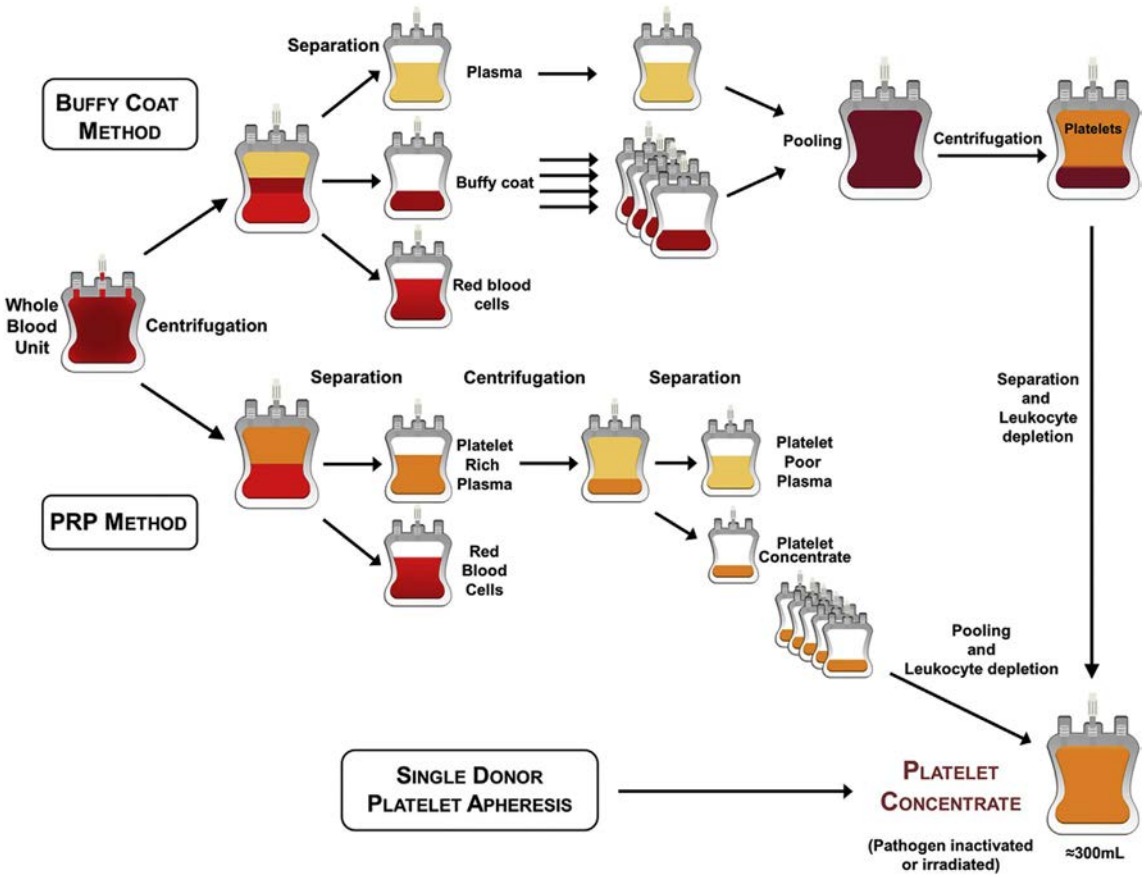


Fig. 3. Different modalities of platelet concentrate preparation. Whole blood derived platelet concentrates are prepared either by the buffy coat method pooling four buffy coats and one plasma unit and a centrifugation step, or by the platelet-rich plasma (PRP) method pooling four to six PRP units. Leukocytes are depleted by filtration. Single-donor platelet concentrates are produced by apheresis. Further processing may include pathogen inactivation or gamma irradiation.

MSC have been reported, but a systematic comparison to favor one technique is lacking [71,101]. Divergent technical sequences can have significant impact on protein content and composition, particularly when thrombin or $CaCl_2$ addition is performed, as this depletes coagulation factors like fibrinogen or von Willebrand factor, and adhesive proteins like fibronectin. How variations of protein content and composition of HPL mechanistically influence cell expansion, differentiation and immunomodulatory effects, certainly deserves further investigation [160]. Although a freeze/thaw cycle has been found not to alter the biological activity of PDGF in the rat fibroblast cell line 3Y1 proliferation model [161], it has recently been confirmed that growth factors are not stable in plasma, arguing for rapid freezing [162]. The biological impact of temperature and duration of liquid storage or repeated freeze/thaw steps of HPL preparations on growth factor and nutrient functionality as well as MSC expansion has not been sufficiently evaluated. In two recent reports there was evidence that repeated freezing and thawing has rather negative effects on growth factor content [87,133].

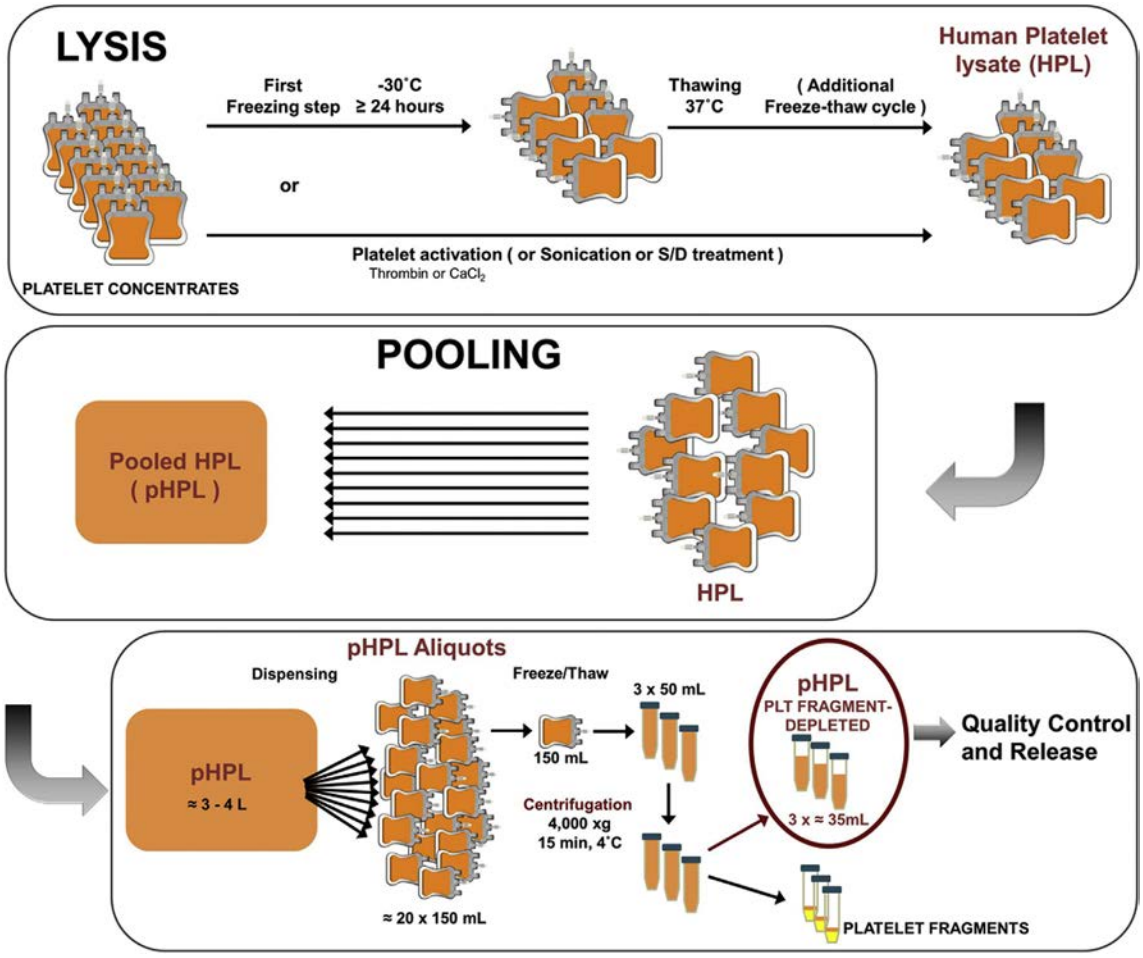


Fig. 4. Preparation of allogeneic pooled human platelet lysate (pHPL) from platelet concentrates. Fresh or expired platelet concentrates can be lysed by several freeze/thaw steps, by platelet activation induced by addition of thrombin or $CaCl_2$, by sonication or by solvent/detergent (S/D) treatment. By mixing the lysates a large pool of HPL (pHPL) is manufactured and further aliquoted. Platelet fragments are depleted by centrifugation. After quality control and release suitable pHPL aliquots can be stored at −30 to −80 °C until use, to supplement culture medium.

Table 2
Comparative capacity of HPL vs. FBS to support *ex vivo* propagation of MSC.

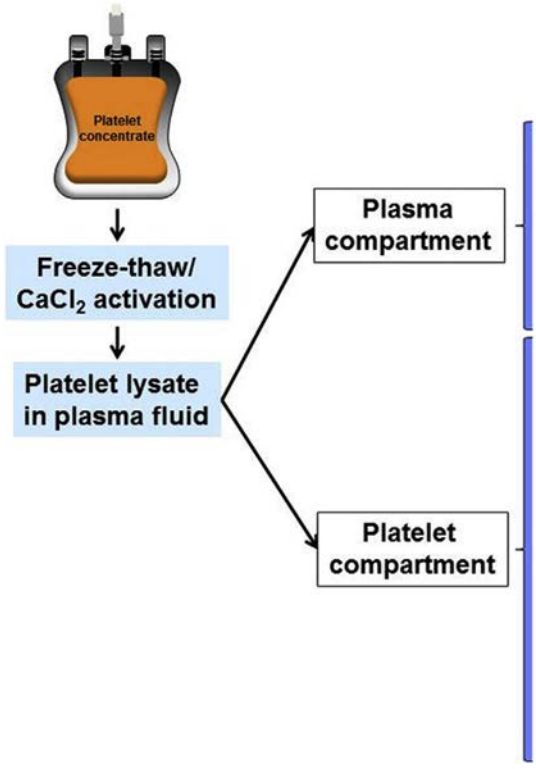
Criteria for MSC quality	HPL vs. FBS
Proliferation behavior	Improved
Population doubling time	Decreased
Clonogenicity	Maintained
CFU-F size	Increased
Characteristic immunophenotype	Maintained
In vitro trilineage differentiation capacity	Maintained
In vitro T cell immunosuppression	Maintained
In vivo tumorigenicity	Not observed

3. Platelet donor selection

3.1. Autologous versus allogeneic HPL

In contrast to allogeneic HPL, which can be processed as a pooled product by large scale manufacturing, preparation methods for autologous or single-donation HPL should be simple for ease of implementation in medical devices under aseptic conditions. In a few reports autologous human serum or HPL (see also Table 1B) has been used for *ex vivo* expansion of human MSC for transplantation [111,112,143,145,147,148]. Autologous HPL is usually obtained from whole blood samples after centrifugation and isolation of PRP, with one optional additional centrifugation step to concentrate the platelet fraction. Like in the allogeneic production, platelet lysis is induced either by freeze/thaw cycles or by addition of $CaCl_2$ [111] or thrombin [112,144] to the PRP to induce fibrinogen conversion into a fibrin clot, platelet activation, and growth factor release. Further procedures of centrifugation, storage and use in cell culture are the same as for allogeneic HPL. The *ex vivo* propagation of BM-MSC using autologous HPL was described for the treatment of knee cartilage defects [143] and for conditions affecting other peripheral joints or intervertebral discs [145]. Autologous PRP was also shown to promote the proliferation of adipose tissue-derived MSC (AT-MSC), which, when treated with neural inductive conditioned medium, revealed stronger neurogenic differentiation *in vitro*. This indicates a possible application of autologous PRP with AT-MSC in nerve regeneration [144]. Recently, the use of non-activated autologous PRP was effective to promote cell proliferation without changing the AT-MSC phenotype, differentiation capacity, and chromosome stability. The majority of the platelets co-cultured with MSC remained viable for 10 days of culture. Interestingly, in this study PRP activated by autologous thrombin provided lower AT-MSC expansion rate [112].

The advantages of autologous over allogeneic HPL include avoiding any possible risks of contamination by donor derived



Mean Protein Content	
Total protein mg/mL	55-65
Albumin mg/mL	35-40
Immunoglobulin G mg/mL	8-12
Fibrinogen mg/mL*	1.5-3
IGF-1 ng/mL	50-200
PDGF-AB ng/mL	50-300
PDGF-BB ng/mL	10-30
PDGF-AA ng/mL	1-10
TGF-β1 ng/mL	50-300
TGF-β2 ng/mL	~ 0.5
BDNF ng/mL	10-50
VEGF ng/mL	5-10
EGF ng/mL	0.5 - 10
HGF ng/mL	0.1-2
b-FGF ng/mL	1-5

Fig. 5. Typical protein and growth factor content of human platelet lysate (HPL) prepared from a platelet concentrate obtained stabilized in plasma. * The fibrinogen content in HPL prepared by calcium chloride ($CaCl_2$ activation) is <0.1 mg/mL).

plasma-borne viruses or prions and immune reactions associated with the internalization of allogeneic proteins by MSC. In practice, however, the volume of autologous HPL may be insufficient for clinical cell doses, especially when the donor is not eligible for multiple blood donations [146]. The use of autologous HPL may therefore be limited to applications when small numbers of MSC are needed [163,164] or when the therapeutic strategy calls for small number of cell culture passages to limit any risks of cellular modifications [164]. Autologous HPL is also an option as a supplement for cell storage [145]. Further limitations of autologous HPL include a lack of standardization due to variations in individual platelet count and growth factor composition that can be influenced by patient's age and overall biological condition. Previous studies have suggested that a single preparation of 10^8 human MSC grown in FBS would contain approximately 7–30 mg of FBS proteins [165]. Development of antibodies against bovine proteins in patients receiving allogeneic MSC expanded in FBS has been described [166]. To our knowledge, the risk of immune reactions due to internalization of proteins from allogeneic HPL has not been observed so far.

Further studies are needed to clarify the specific role of autologous HPL for cell therapeutics, as 'off-the-shelf' allogeneic preparations are now available. The capacity of allogeneic HPL to be used for MSC expansion has long been demonstrated and the advantages include availability independent of patient's health status [77]. When pooled at sufficient scale, 3.0–3.5 L of HPL can be obtained by mixing the platelet concentrates from up to 50 donors [90]. Pooling, however, increases the risks of contamination with human blood-borne pathogens, a risk that can be greatly reduced by the current donor screening and testing strategies applied in licensed blood centers, as well as by pathogen inactivation. Table 3 summarizes the respective advantages and limits of autologous HPL and allogeneic HPL.

3.2. Selection of allogeneic PRP donors for HPL production

For allogeneic HPL preparation platelet concentrates should be produced according to national and international regulatory requirements for donor qualification, testing and processing of blood components [167]. In many countries, the preparation of platelet concentrates falls under the responsibility of licensed blood centers conform with the principles of good manufacturing practice (GMP) [168]. Consideration of pathogen inactivation is of particular importance when platelet concentrates are pooled to prepare HPL.

3.2.1. Impact of donor platelet count, age and gender

MSC have been efficiently expanded in medium supplemented with 5%–15% HPL for over 20 population doublings (indicating a one million-fold amplification of the starting cell number). This was higher than what achieved with equal amounts of FBS [90]. There were variations in the capacity of individual HPL prepared following the technically comparable procedures indicating the need to standardize donor selection [103]. While the mode of preparation of the platelet concentrate and the HPL influences protein and growth factor profile, donor characteristics such as age, sex, and platelet count influenced growth factor content in HPL as well as capacity to support MSC expansion [169]. Sera from old, but not young, female donors significantly decreased osteoblastic gene expression in MSC despite lack of correlation with platelet count or concentration of PDGF-AB, TGF-β1, bFGF, or IGF-1 [85]. These age-associated effects have not been observed in other studies evaluating supplementation with serum on the expansion of human skin fibroblast [170] and expansion and differentiation capacity of human myoblasts [171]. The precise mechanism by which donor age-related factors affect cell expansion and differentiation is not clear [85]. While studies are needed to confirm the impact of donors' age on cell behavior, individual donor effects may be less crucial and

Table 3
Comparative relative advantages and limits of pooled allogeneic and autologous HPL.

HPL	Allogeneic	Autologous
Advantages		
Large scale manufacturing possible	Yes	No
Cost effectiveness	Yes	No
Automation possible	Yes	Yes
High standardization	Yes	No
Unlimited 'off-the shelf' availability	Yes	No
Disadvantages		
Possible alloimmunization	Yes	No
Infectious risk	Yes	No[a]
Individual variations in growth factor content	Low from batch to batch	Yes
Low sample volumes	No	Yes

[a] Contamination during *ex vivo* processing needs to be avoided.

would be leveled out by the pooling required in larger scale manufacture of allogeneic HPL [69,71,172].

3.2.2. Blood group selection

As the influence of blood group antigens and isoagglutinins on *in vitro* MSC biology is still not proven, some groups have initially suggested to prepare HPL by matching whole blood derived platelets of blood group O (four buffy coat units) lacking A and B antigens with one plasma unit of blood group AB lacking anti-A and anti-B isoagglutinins [69,90,93,97,107,132]. Others have used platelet concentrates of blood group AB [101–103], of 'similar' [126] or 'identical blood group and rhesus factor' [96]. However, the majority of publications do not elaborate on this detail (Table 1A). In 2011, mRNA for blood group AB antigens could not be detected by RT-PCR and surface antigen expression as well as adsorption of soluble AB antigens from AB serum supplemented medium on BM-MSC was excluded by flow cytometry [173]. A more recent study analyzing the possible impact of ABO antigens on the outcome of MSC therapy [174] mainly confirmed these data, showing only a slight adsorption of AB antigens on BM-MSC after 3 h of incubation with 100% AB plasma, but not with 10%. Of course, HPL has also been used for expansion of other human cell types, as e.g. endothelial progenitor cells [9,128,131,132,138,175], known to express AB antigens [176]. Until now there are no data in the literature about the interference of anti-A and anti-B isoagglutinins and soluble AB antigens derived from the medium supplement with cultured endothelial progenitor cells. Nevertheless, just in a few studies using HPL for endothelial cell culture the HPL was prepared from blood group O buffy coat units and AB plasma [131,132,175]. Human heterologous blood group AB serum was published to provide significantly higher proliferative effects on AT-MSC, with retention of the differentiation capacity and marker expression over long-term culture [101].

3.2.3. Donor screening and testing for infectious markers

Safety aspects encompass epidemiological control of the population where the blood is collected, exclusion of donors at high risk, and continuous donor testing for known pathogens [177]. A validated traceability system should ensure the link between donations and HPL batches and vice-versa, with capacity for backtracking in case of suspected safety issues with the donors or the HPL batches. Donors should be adequately informed about the use of their donations and transfusion associated risks for recipients, in particular regarding transmission of infectious agents. The mandatory testing performed on each individual donation includes the detection of human immunodeficiency virus (HIV-1/HIV-2) antibodies, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) antibodies. Confirmed reactive donations must not be used for HPL production. Samples of the donations, pooled or separate, are also recommended to be tested by genomic assays (nucleic acid testing; NAT) for hepatitis B virus (HBV), HCV, HIV, to eliminate serologically negative donations that may already be positive by NAT. Some countries have implemented additional testing for blood components, to exclude donations positive for treponema pallidum, hepatitis B core antibodies, human T cell leukemia virus (HTLV) I/II, hepatitis E virus (HEV) and/or west nile virus [167]. In well-regulated legislations, the residual viral infectious risk from viruses tested by blood centers is very low [178–180]. NAT for hepatitis A virus (HAV) and parvovirus B19 is currently done for the industrial manufacturing of plasma products [177]. In summary, the selection and testing criteria of whole blood or platelet donors for HPL preparation should not differ from regular blood donations if HPL is used for manufacturing cell therapeutics.

3.2.4. Impact of pathogen inactivation

Despite donor testing, allogeneic HPL, like any other blood product, bears the risk of transmission of human blood-borne pathogens as viruses, bacteria, fungi and prions. Pathogen inactivation in the source material or during the manufacturing process of HPL can reduce the pathogen transmission risk [98,99,134,181]. Production of clinical grade Langerhans' islets (for treating type I diabetes), MSC propagation for treating graft versus host disease (GvHD) grade III or IV, as well as expanding T cells for immune therapy against malignant melanoma and BM-MSC for experimental purposes has been achieved using psoralen/UV treated human serum [99,182–184]. Pathogen inactivated HPL was found to support the growth and expansion of various human cell lines [185].

Another approach designed for large pool applications used solvent/detergent (S/D) treatment that has been introduced for plasma products in the mid-1980s for inactivating lipid-enveloped viruses [186]. The process has been adapted to apheresis or whole blood derived pooled HPL to induce release of platelet growth factors [187]. This S/D-treated HPL, made from buffy coat or apheresis platelet concentrates, was efficient at 5%–10% supplementation for expanding AT-MSC [130], BM- and cord blood (CB-) MSC [188] using standard evaluation criteria. More efficient chondrogenic differentiation of AT-MSC compared to cells grown with FBS supplementation was noted [130].

4. HPL applicability

4.1. Protein and growth factor content in HPL

HPL, like FBS, is a complex protein mixture. Fig. 5 presents an

overview of the protein content and growth factor composition of HPL. Most HPL preparations currently used for *ex vivo* expansion of MSC contain both the plasma and platelet proteomes. The total protein content of HPL typically exceeds 50–55 mg/mL, comprising albumin (35–40 mg/L), immunoglobulin G (8–12 mg/mL), immunoglobulin A (1–2 mg/mL), and immunoglobulin M (0.5–1.5 mg/mL) as major protein components [189,190]. Fibrinogen is present at approximately 1.0–3 mg/mL in HPL obtained by a freeze-thaw or S/D processes [189], but in much lower quantity (<0.1 mg/mL) when serum-converted due to consumption of coagulation factors [59,191].

FBS is rather ill-defined, with limited information on growth factors and cytokine content. FBS composition is complex and includes low and high molecular weight proteins and lipids, and other nutrients required for cell growth [192,193]. The mean protein content is 38 g/L (ranging from 32 to 70 mg/mL), including a mean of 23 mg/mL of albumin (20–36 mg/mL), 310 mg/L (120–630 mg/L) of cholesterol, and 10 μU/mL (6–14 μU/mL) of insulin, substantial differences are found among batches [194]. One particular feature of FBS is low immunoglobulin content [192]. FBS lots with low IgG content (<5 μg/mL) are now being commercialized for some research applications.

Two main differences in HPL protein content compared to FBS, in addition to the human versus bovine origin, are the higher content of immunoglobulins and the possible presence of fibrinogen and other coagulation factors, when HPL is produced without thrombin activation. Other plasma proteins including protease inhibitors (such as alpha 1-antitrypsin or alpha 2-macroglobulin) or carriers (such as transferrin, haptoglobin, and ceruloplasmin) are present in HPL [191]. Several studies have reported the content of key growth factors in HPL used for regenerative medicine or cell expansion. Most (IGF1; PDGF-AA, -BB, -AB; TGF-β1; BDNF, EGF, VEGF, HGF, bFGF) are present in a range of 0.1–300 ng/mL. TGF-β1, PDGF-AB, IGF1, and BDNF are typically present in higher quantity than other growth factors [59,71,85,90,129,147,157,187,191,195,196].

Many other cytokines and chemokines (see Figs. 1 and 5) are present, at pg/mL to ng/mL levels as revealed by semi-quantitative cytokine array profiling [10,103] or quantitative luminex technology [135]. Proteomic analyses by two-dimensional electrophoresis and mass spectroscopy [197] will likely be instrumental to further unveil the composition of HPL, being useful to its characterization and standardization. Finally, HPL, like FBS [192], contains minerals such as sodium, potassium, magnesium, iron, as well as glucose and vitamins that can influence cell growth, differentiation and function [190].

4.2. Use of HPL for propagation of cells other than MSC

In addition to its application for the expansion of MSC, HPL was shown to be an efficient supplement of growth media used for *ex vivo* expansion of other cell types. HPL can promote *in vitro* growth of human gingival fibroblasts [191,198–200], peridontal ligament cells [191], meniscal fibrochondrocytes [201], chondrocytes [202,203], osteocytes and myocytes [204], tenocytes [204–206], endothelial cells [17,131,175,207,208], annulus fibrosus cells [209], corneal endothelial cells [196] and corneal epithelial cells [210], indicating potential applicability in various fields of regenerative therapies. HPL was also used to replace FBS as supplement for *in vitro* growth of various animal and human immortalized cell lines [185,191,193].

4.3. Industrial scale HPL products

With the need for pooled allogeneic HPL for cell therapy applications and direct clinical use in regenerative medicine, industrial large pool HPL is currently entering the market with products that display the entire spectrum of methodological variations. In the future, HPL could be manufactured from large pools of frozen platelet concentrates following regulations and production principles similar to those already in place for industrial plasma fractionation. Industrial HPL may include state-of-the-art manufacturing including pathogen inactivation of the starting material [167,211,212], and/or after pooling (S/D treatment, pasteurization or nanofiltration) [177,213]. Preparations may be enriched for a particular set of growth factors for specific approaches (as for clinical applications in soft tissue healing, bone regeneration, joint repair or neuroregeneration) [59]. Appropriate standardization will be a major challenge.

4.4. Supply of HPL

The use of HPL as a supplement for the expansion of cells for transplantation raises the question whether the supply will be sufficient considering the expected increasing needs for cell therapy. Available data support the notion that platelet concentrates obtained from both whole blood and apheresis can be used for cell expansion. In addition, platelet concentrates that cannot be transfused after reaching the five to seven day storage limit can be frozen, stored and used as growth medium supplement. This indicates that there is currently a substantial volume of platelet concentrate available for HPL preparation. It is considered that approximately 15%–20% of whole blood donations that are driven by the need for red blood cells are currently used to prepare platelet concentrates and that 5%–20% of those expire and can serve as raw material for HPL. Considering that an estimated 100 million whole blood donations are collected each year in the world [214], these figures suggest a potential for approximately 100,000–250,000 L of HPL per year from outdated platelet concentrates. Compared to the estimated volume of 500,000 L of FBS produced in 2003 [68,192], pHPL availability may be sufficient for human cell research and clinical applications. HPL volume could increase gradually, as demand raises, by preparing more platelet concentrates from whole blood donations and by considering dedicated apheresis collection of platelet concentrates for the production of HPL. The processing of such volumes, at small scale by blood centers or at industrial scale by commercial providers should be technically achievable especially when compared to the plasma fractionation industry [215]. To date, a number of companies has established commercial distribution of 'ready to use' HPL in the USA and Europe. These products may facilitate the implementation of HPL in cell culture protocols for research groups without access to platelet products.

4.5. Towards standardization of HPL production and use

Data available demonstrate that HPL is an efficient human blood derived material for the supplementation of growth media for *ex vivo* expansion of cells for transplantation, most particularly MSC. HPL allows to compensate some of the known disadvantages of FBS including the risk of xenogeneic infection and immune reactions and also contributes to addressing possible FBS supply limitations, batch-to-batch quality inconsistency and ethically debatable production methods. As described above, HPL used as 5%–10% supplement can generally support cell growth and expansion of several cell types better than 10% FBS [9,42,77–112]. However, there is a need to reach international consensus on production, quality, and safety criteria of both platelet concentrates and PRP used as raw materials and resulting HPL used as GMP-compliant supplements for *ex vivo* expansion of therapeutic cells for transplantation. As the use of HPL in cell therapy procedures is still emerging, it is quite conceivable that specialized human

platelet derivatives will be developed in the near future both for specific therapeutic applications as for *ex vivo* cell expansion, thereby opening far greater potential applications than what FBS can provide.

In this article we have reviewed the current knowledge about preparation of HPL and its efficient use in cell culture as alternative for FBS. Key questions still need to be addressed systematically, ideally in advance of future studies, to clarify specifications of this fascinating new human blood product:

- What are the most important components/growth factors of HPL for specific cell therapy applications?
- What is the optimal platelet concentration and lysis method?
- Which role does the age of the donor play for potency and applicability of autologous HPL?
- Do health status and disease history effect autologous HPL quality and functionality?
- Do we have to consider ABO blood groups and isoagglutinins?
- Is the depletion of leukocytes and fibrinogen advantageous or necessary?
- What are the functional effects of serum-converted HPL in addition to easing use and avoiding porcine heparin addition during cell propagation?

5. Conclusions

HPL produced under controlled and standardized conditions has been demonstrated to be an efficient serum supplement for the clinical grade expansion of various cells including MSC, ECFC and various other cells and cell lines. Increasing evidence indicates that HPL has the potential to become the gold standard supplement for human cell propagation for tissue engineering and regenerative purposes.

Conflict of interest

The authors declare that no conflict of interest has to be disclosed.

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