Gaps in the knowledge of human platelet lysate as a cell culture supplement for cell therapy: a joint publication from the AABB and the International Society for Cell & Gene Therapy

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Cell therapeutics are emerging as a viable modality to treat challenging diseases such as cancer, organ degeneration, or (auto)immune diseases resulting in an increased demand for their large-scale, high-quality production. Currently, fetal bovine serum (FBS) is the most widely used growth factor supplement for the expansion of human cell therapy products. However, there has been a strong impetus from regulatory agencies and biomedical professionals in the field to develop methods for cell expansion that do not utilize animal products in their production process.1 Human platelet lysate (hPL) has been identified as a possible growth supplement contender—rich in growth factors and produced in most cases from expired platelets (PLTs)—that can be used to replace FBS.

What is hPL? hPL is essentially the product of lysing human PLTs typically involving freezing and thawing.2 There are several protocols that have been generated for hPL production, some of which are proprietary. The general approach is to generate large pools of hPL to limit batch-to-batch variation because PLTs are by nature one of the most heterogeneous components of blood. hPL is routinely produced from PLT-rich plasma (PRP) or expired PLTs collected via apheresis.3,4 Multiple freeze/thaw cycles cause the PLT membranes to burst and release trophogens and growth factors resulting in a lysate. Aggregates, debris, and PLT fragments are removed by centrifugation.3,4

Human PLT lysate can be derived from autologous (same donor) collections, which may minimize the risk of immunologic reactions or infections; however, most efforts have focused on preparing allogeneic pooled batches from blood donations from a large number (50-100) of different individuals. The use of hPL in standard cell culture protocols could prove to be a promising tool for further development of cell therapy products in animal protein-free systems. There are a number of challenges and unknown factors to investigate in the development of hPL as a growth factor supplement starting with its heterogeneous composition. A variety of factors can affect the composition and bioactivity of hPL including the plasma content, growth factor content, age and gender of the donor, PLT counts and/or

ABBREVIATIONS: bFGF = basic fibroblast growth factor; EGF = epidermal growth factor; FBS = fetal bovine serum; hPL = human platelet lysate; ISCT = International Society of Cell Therapy; MSC(s) = mesenchymal stromal cell(s); PC(s) = platelet concentrate(s); PDGF = platelet-derived growth factor; pHPL = pooled human platelet lysate; PRP = platelet-rich plasma; PRT(s) = pathogen reduction treatment(s); tPRP = thrombin-activated platelet releasate.

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volume, production process, heparin and/or anticoagulants, mRNAs, metabolites, blood group, storage conditions of the unit, extracellular vesicle content, and potential treatment to reduce and/or inactivate pathogens.\(^5\) AABB and International Society of Cell Therapy (ISCT) established a joint working group on hPL to identify gaps of knowledge that could eventually lead to developing and defining recommendations for standardized manufacturing and quality control (QC) of hPL. This review is the first result of this working group, summarizing identified gaps of knowledge to pave the way for necessary consensus.

**hPL AS AN ALTERNATIVE TO FBS**

Fetal bovine serum has been used for decades as a supplement in a wide range of cell culture applications in cell-based research, drug discovery, diagnostics, toxicity testing, cell therapy, in vitro fertilization, human and animal vaccine production, and biopharmaceutical manufacturing.\(^9\) FBS has been considered rather unique in its universal capacity to support the growth of multiple cell types in culture. Although widely used, there are various reasons to find suitable alternatives to the use of FBS.

**Ill-defined nature of FBS**

Fetal bovine serum adds proteins, attachment factors, growth factors, enzymes, protease inhibitors, hormones, vitamins, trace elements, and numerous others to media to help cells adhere, survive, and proliferate; however, the relevant factors contributing to promotion of cell growth still remain largely unknown.\(^5\) Replacing ill-defined serum with chemically defined medium appears beneficial, yet these media can be complicated to develop\(^10\) and specific formulation adjustments may be necessary to support the growth of different cell types. Serum-free, animal component-free, and or chemically defined media have been developed.\(^11\)

There are databases (e.g., https://fcs-free.org/fcs-database or http://www.sefrec.com/) that list different cell types, products, and resources; however, to date only a small number of protocols are available where these can be applied.

**Animal welfare**

Despite international standards set by the International Serum Industry Association,\(^12\) there is strong concern regarding the inhumane method of procuring FBS by cardiac puncture without anesthesia.\(^13\,14\)

**Economic issues, sourcing, and supply**

There is an increasing market for FBS that is dependent in large part on the beef processing industry.\(^15\,16\) Increasing demand from life science and pharmaceutical users, restricted supply due to climate changes, and reduced cattle inventories have impacted costs and have led to questionable practices in production. Cases where bovine serum albumin, water, and/or cell growth-promoting additives have been used to blend the FBS have been reported\(^15\).

**Xenogenic properties**

Xenoantigens can serve as immunogens that compromise therapeutic efficacy.\(^16\)–\(^19\) Sundin and colleagues described the induction of alloantibodies against FBS proteins in three of 12 individuals who received matched or mismatched mesenchymal stromal cells (MSCs). Similar patterns of FBS reactivity were observed in healthy subjects, indicating a degree of preimmunization against FBS, which could compromise the therapeutic efficacy of cell products.\(^20\)

**Pathogen safety**

The potential presence of extraneous agents in FBS is considered a risk to the safety of biologic medicinal products, although to the best of our knowledge no cases have been reported. Regulatory authorities clarified that “the use of adequately controlled bovine sera is allowed.” Reinhardt and colleagues\(^17\) specify that FBS must be controlled according to the European Pharmacopoeia, 7th ed, Monograph 01/2008:2262 Bovine Serum,\(^18\) and manufacturing shall involve virus testing and inactivation (e.g., irradiation at a dose higher than 30 kGy). The risk of transmitting animal spongiform encephalopathy agents, in particular, has prompted the search for alternative supplements from nonanimal origin.\(^16\)–\(^18\)

Regulatory authorities released guidelines or notes of guidance for the manufacture of human medicinal products. Initially the European Medicines Agency recommended, “When a manufacturer has a choice between the use of ruminant or non-ruminant material, the use of non-ruminant material is preferred.” If there are no alternatives to FBS available, specific risk assessment is needed to account for extraneous agents and lot-to-lot reproducibility.\(^19\)\(^,\)\(^21\)

Human PLT lysate was proposed as a xeno-free culture supplement in the 1980s to expand rabbit articular chondrocytes and cell lines from a variety of tissues and tumors.\(^22\)–\(^24\) In 2003, Lucarelli and coworkers\(^25\) while investigating the effect of PRP released by PLT gel on stromal stem cell proliferation and differentiation, observed significant growth-promoting capabilities of hPL. Doucet and coworkers\(^5\) introduced hPL as a safe substitute for FBS in MSC culture, followed by intense research in the field of MSC culture and documented by numerous publications.\(^5\)\(^,\)\(^26\) Muraglia and coworkers\(^31\) reported on various preparations of hPL for MSC and human articular cartilage, namely “(i) an heparin-free human platelet lysate (PL) devoid of serum or plasma components (v-PL) and (ii) an heparin-free human serum derived from plasma devoid of PL components (Pl-s).” Not too surprisingly, the hPL derived from plasma (as it lacks PLT factors) failed to support growth of...
both MSC and chondrocytes, but supported that of certain tumor cell lines, whereas the v-PL was fully active.27

**Effects of hPL: focus on MSCs**

Human PLT lysate has been tested on a variety of cell types as exemplified in Table 1. Here we will focus on MSCs. MSCs have stromal activity; that is, they contribute to the hematopoietic stem cell niche, they are potent immunomodulators interacting with different immune cells, and they produce and secrete various trophic factors and extracellular vesicles that likely mediate their regenerative potential.42,43 hPL can safely replace FBS for clinical-scale MSC manufacturing: Data show that hPL can support MSC proliferation, maintain colony-forming units–fibroblasts content during in vitro culture and hold chromosomal stability comparable to or in some cases better than FBS (reviewed in Astori et al.,5 Bieback,6 and Tan et al.26). MSCs cultivated in hPL show enhanced osteoblastic differentiation potential44,45 indicating that using hPL may be a superior option in MSCs cultured for bone regeneration. However, reduced, similar, or increased immunomodulatory MSC activities have been reported comparing hPL and FBS-cultivated MSCs.46-48 This functional variability emphasizes the need for studies comparing FBS and hPL (different hPL products) in MSC (or other cell types of interest) culture.

Numerous clinical trials are currently being conducted with MSCs for a variety of indications.49 MSCs isolated and expanded in FBS were tested in initial trials. Trials using MSCs isolated and cultivated in hPL have also been conducted.50 In the orthopedic setting, autologous hPL-expanded MSCs appeared to be safe as no neoplastic transformations were reported.51 Introna and colleagues52 concluded that MSC infusion is feasible and safe in patients with steroid-refractory acute graft-versus-host disease (GVHD). Specifically, no immediate or late toxic events against infusions of third-party–expanded MSCs (MSCs from a donor, independent of the patient and the hematopoietic stem cell transplant donor) were observed. Complete and

| Cell type | Effects of hPL | Reference(s) |
|-----------|----------------|--------------|
| Primary cells | | |
| Articular chondrocytes | Promotion of cell growth | Moraes et al.28 |
| Activated quiescent chondrocytes | Transient boost of inflammatory factors followed by rapid decline of these factors | Nguyen et al.29 |
| CAR NK cells | hPL derived from plasma failed to support growth of chondrocytes | Muraglia et al.31 |
| Dendritic cells | hPL was inferior to fresh-frozen plasma in supporting NK-cell proliferation | Nowakowska et al.32 |
| Endothelial cells | Promotion of cell growth | Svajger33 |
| Endothelial progenitor cells/endothelial colony-forming cells | Compared to FBS: maintained phenotype, functional characteristics like tube-like vessel formation on matrigel and metabolic state | Thiemer et al.38 |
| Corneal endothelial cells | Improved cell survival and proliferation | Ruggiu et al.39 |
| Osteoblasts | Promotion of cell growth | Hofbauer et al.37 |
| Established cell lines, human and nonhuman | Promotion of cell growth of 24 of 29 cell lines from different species: growth-promoting activity was heat stable (100°C for 15 min), even after acidification. Ion-exchange chromatography was not able to separate the mitogenic activity. Comparing two cell lines, for one of the cell lines the mitogenic activity was trypsin-sensitive, suggesting different proteins or protein structures to be relevant. PDGF could be identified as one major mitogen for one of the cell lines, but not for the other cell line. | Eastment and Sirabasku33 |
| Neuro-2a (mouse neuroblastoma), RAG (mouse renal adenocarcinoma), R2C (rat Leydig cell testicular tumor), mouse mammary tumor 060562 | Mitogenic factor(s) were nondialyzable, heat stable (56°C), partially trypsin labile, but sensitive to periodate oxidation, suggesting a glycoprotein or glycopeptide. Potentially a factor stored in alpha granules, since ADP activation did not release mitogenic factors. | Hara et al.24 |
| HaCaT | Vascular endothelial growth factor and PDGF-AA concentrations in hPL correlated with HaCaT proliferation | Baik et al.40 |
| KG-1, K562, JURKAT, HL-60, HeLa, and MCF-7 | Lower proliferation in hPL compared to FBS; however, cells lines were adapted to FBS. | Fazzina et al.41 |

ADP = adenosine diphosphate; CAR = chimeric antigen receptor.
partial clinical responses were observed in 27 of 40 patients. A different study of 13 patients treated with third-party MSCs expanded in hPL-containing medium speculated whether hPL-expanded cells exerted a reduced immunomodulatory capacity.53

In vitro data regarding effect on T- and natural killer (NK)-cell proliferation support this observation.56 It is unknown whether the cell culture supplement affects cell potency in the context of clinical settings. Nevertheless, a recent questionnaire among centers manufacturing MSCs for the treatment of GVHD reported that 77% of the centers already rely on hPL (54% used hPL produced in a blood bank and 23% used commercially available hPL) compared to 23% of centers that still use FBS.50

Human PLT lysate preparations in these studies likely differ based on the way they were manufactured. Recent data indicate that the presence of fibrinogen in hPL may impair the immunosuppressive capacity of MSCs.54

We and others have observed differences when comparing marrow and adipose tissue–derived MSCs using FBS, human serum, and different PLT preparations (PLT lysate or releasate).55–60 In our hands, MSCs from adipose tissue only grew extremely well in the first passage using hPL. MSC proliferation was reduced thereafter, even when compared to FBS. Human serum or thrombin-activated PLT releasate (tPRP), in contrast, continuously supported expansion of MSC from adipose tissue exceeding the rate induced by FBS. MSCs derived from marrow, however, expanded strongly in hPL and showed reduced proliferation in human serum and tRPR. These data indicate that MSCs from different sources might prefer different compositions of their cell culture supplement. We identified a few factors that differed in concentrations comparing hPL and tPRP using a proteomic approach. Fibrinogen, reduced during the thrombin-induced clotting in tPRP, exerted a growth-promoting effect on MSCs from marrow and adipose tissue.56 Further studies may help to identify the crucial growth factors for specific cells and how these factors can be utilized to optimize the medium formulations. A better understanding of the molecular mechanisms could inform production of a better defined medium.

**hPL PRODUCTION**

**PLT supply and criteria to consider**

Human PLT lysate can be used in an autologous or allogeneic setting. Here, we focus on allogeneic hPL. The number of cells typically needed to reach clinical doses requires more than the volume typically acquired from one blood donation or apheresis PLT collection and it is challenging to organize multiple blood donations or collections from a single donor or patient whose health is frequently impaired.28,61 Allogeneic hPL is easier to standardize, has less lot-to-lot variation, can be predictably produced, and is more economical than autologous hPL. Consequently, allo-hPL has been the supplement of choice in most published studies.

Large “off-the-shelf” pools have been prepared that differ in pool size. Donor-related variabilities cannot be completely avoided; however, variations may be decreased by pooling a substantial number of donors. A few pooled hPL (phPL) products have been manufactured using more than 50 donors but the size of pools is an issue. Recently, large pool sizes have been under scrutiny by the regulatory authorities, citing concerns of viral contamination. Representatives of the German Federal Regulatory Authority have suggested limiting the pool sizes to a maximum of 16 donors and requiring serum and/or phPL testing according to current guidelines on blood quality and safety.17,62 The European Pharmacopeia states specifically in Chapter 5.2.12 that “Because of the inherent risk of transmitting infectious agents from pooled plasma, pooled sera, or other derivatives derived from pooled human blood or plasma, consideration is given to limit the number of donations which are pooled, unless sufficient methods for inactivation/removal of viruses are applied during production, where applicable.” This prompted activity to evaluate the applicability and effects or potential quality issues of pathogen reduction treatments (PRTs) on phPL (see “PRT of hPL”).

Serologic criteria for PLT donors for hPL production must not be less strict than those required for blood product transfusion (human immunodeficiency virus [HIV], hepatitis B virus [HBV], hepatitis C virus [HCV], and Treponema pallidum hemagglutination assay negative, although additional pathogens can be tested for depending on health history and local regulations). hPL lots produced from different donors have distinct growth factor content exerting variable effects on cell proliferation.63 The age of donors appears to affect quality of hPL and its effect on cell growth. Lohmann and colleagues64 showed that the proliferation rate of MSCs was significantly higher with hPL produced from young donors (<35 years) than with hPL produced from older donors (>45 years), which was not associated with growth factor content. Moreover, hPL from old donors increased the activity of the senescence-associated enzyme β-galactosidase. To date, no studies have addressed potential differences of hPL related to donor sex or disease history, which may also impact hPL quality (Fig. 1). Such data would be needed to refine donor selection criteria to decrease hPL batch-to-batch variability. There may also be effects of blood group on hPL quality. It is suggestive that hPL from blood group O PLTs resuspended in AB plasma may be the product of choice, as potential problems with antigens and isoagglutinins on cells and in the patient are avoided. However, it has been shown in several studies that hPL derived from blood group AB PLTs supports cell proliferation without affecting cell physiology (reviewed in Burnouf et al.26). Tan and coworkers27 measured the amount of PLT cell membrane residues in hPL by assaying for the cell surface antigens CD41 and CD61, finding very low concentrations (at the picogram level). This would...
indicate that blood group AB PLTs could be used as a starting material for hPL production without compromising safety involving antigens present on PLT cell membranes.

**Manipulation and storage**

The procedure for PLT isolation including the source of the PLTs, the way they are manipulated, and the conditions under which they are stored can impact the quality of the final product. hPL can be generated from buffy coat, PRP, or apheresis-derived PLT concentrates (PCs; recently reviewed in Shih et al.7,65). The percentage of contaminated white blood cells in PCs varies depending on how the PLTs are prepared, which can affect the final cytokine content in hPL.7,66 Other variables include how PLTs are resuspended. PCs can be resuspended in plasma or in a combination of plasma and PLT storage additive solution (AS) containing sodium-potassium chloride, citrate, phosphate, and mannitol,7 which can cause variation. Sonker and Dubey67 showed that growth factor content is higher in PRP from buffy coat compared with that derived from apheresis. However, Fekete and coworkers68 did not find significant differences in growth factor content or impact on MSC proliferation between hPL produced from PCs derived either from buffy coat or from apheresis. To address this lack of consistency interlaboratory studies comparing the effects of different hPL products on various cell types using standardized readouts (e.g., promotion of cell proliferation and/or growth factor content) are recommended.

**Freshness**

Fresh, stored, and expired PLTs can be used as the starting material for hPL production; however, there is variability depending on the freshness of the PLTs. Depending on local regulations, PCs can be stored for up to 4 to 7 days. The use of PCs during or after their shelf life will depend on the respective region or country where the hPL will be produced. Because of this potential for variation, it probably would be more appropriate to report the days after isolation for each hPL preparation. PLTs used for transfusion are stored at 22°C under agitation; however, it has been shown that growth factors are not stable in plasma at 22°C,67 suggesting that 1-day-old PLTs could be a better quality product as a starting material for hPL. The data collected thus far do not indicate that hPL from expired PCs has substantial disadvantages over hPL from nonexpired PCs. Chan and colleagues69 showed that hPL prepared from 21-day-old PLTs was as stimulatory for fibroblast proliferation as hPL prepared from 2-day-old PLTs and retained total protein concentration, as well as PLT-derived growth factor (PDGF)-AA and transforming growth factor (TGF)-β content. Fekete and coworkers compared hPL prepared from PCs 2 and 6 days after donation, finding no significant differences in their growth-promoting effects on marrow-derived MSCs. Jonsdottir-Buch and coworkers70 confirmed these data and further showed that hPL made from 6-day-old PLTs was not significantly different to hPL produced from 1-day-old PLTs in their effect on MSC proliferation, differentiation potential, immunomodulation, and phenotype. Scientific studies support the use of outdated PLTs and highlight potential uses of donations otherwise unsuitable for transfusion that can serve as alternative potent and safe media supplements for cell therapeutics.71,72 It is estimated that 5% to 20% of PCs produced in transfusion centers expire.75 Utilizing this valuable resource for hPL manufacture would be an ethically and economically optimal strategy to support cell therapies manufacturing without disturbing regular transfusion dynamics at hospitals and blood banks. Studies should be designed to determine optimal conditions to make best quality PLT lysates.
Cell therapy is a growing field and the quantity of hPL that will be needed for cell-based products in the near future will be high. Therefore, it is important to have consistencies between procedures used for hPL production. The use of expired or any type of PC (independent of the type of isolation and ABO blood group) would be the desirable setting for manufacturing hPL in sufficient quantities to provide for all expected cell therapies.

**hPL production protocols and related issues**

Human PLT lysate can be generated from PCs by freezing/thawing cycles, sonication, solvent/detergent (S/D) treatment, and PLT activation with calcium or thrombin. An extensive review on the different production methods for hPL has recently been published. In addition to the use of different sources of PLTs, variability is inherent in the various protocols used to manufacture hPL reported in the literature, limiting the ability to best compare findings. The protocols used for hPL production could affect growth factor profiles and the performance of the product. For example, we showed that PLT releasate obtained by thrombin activation and hPL obtained by freezing/thawing cycles had different effects on marrow MSC proliferation. Nevertheless, even with the lack of standardized protocols, hPL has thus far proven successful for in vitro culture of different cell types. Although distinct methods have been tested (reviewed in Burnouf et al.), the most common technique for PLT lysis is freezing/thawing (ranging from one to five freeze/thaw cycles). This is a simple, fast, and nonexpensive approach to produce hPL; however, the optimal number of freeze/thaw cycles remains to be established. Too many freeze/thaw cycles can result in growth factor degradation, while an insufficient number of freeze/thaw cycles could decrease growth factor concentration due to ineffective PLT breakage. Fekete and coworkers compared one and two freezing cycles to lyse PLTs and found no significant impact on the stimulatory activity of hPL on MSC proliferation. Similarly, Laitinen and colleagues compared two and five freezing/thawing cycles, finding that the cell population doubling time was shorter when two cycles were used. These data indicate that one to two cycles might be sufficient to release growth factors without compromising their stability. The reported temperature used for freeze/thaw cycles ranges from −196°C to −20°C for freezing and 4°C/37°C for thawing. Mojica-Henshaw and colleagues compared different freezing/thawing settings finding that the highest growth factor levels were obtained when liquid nitrogen freezing at −196°C and thawing at 4°C was performed.

Platelets can be lysed in the solution in which they are stored (e.g., plasma, PLT AS, or a combination of both) or can be centrifuged and resuspended in medium, plasma, phosphate buffered saline or saline solution. The percentage of plasma in the final product appears to be important as plasma also contains growth factors that can supplement those obtained from PLTs. At least one study has demonstrated that hPL supplemented with plasma is more efficient than unsupplemented hPL in cell expansion.

The addition of heparin to cell culture medium containing hPL is required to avoid clotting; however, heparin is derived from porcine tissue (which is a non-xeno-free product) and some cases of hypersensitivity to heparin have been reported. Impairment of cell proliferation at high heparin concentrations and possible interference with migration and homing of bone marrow-derived mononuclear cells to wounds has also been described. Fibrinogen depletion procedures have been introduced in the production of hPL. The use of fibrinogen-depleted hPL (= serum-converted hPL) would be desirable, where no heparin addition is needed. Fibrinogen depletion would also decrease the presence of unwanted clotting that is sometimes present in hPL even when heparin is added. Although fibrinogen-depleted hPL might be desirable, fibrinogen depletion procedures (using calcium or thrombin) can decrease the concentration of growth factors and deplete coagulation factors and adhesive proteins such as fibronectin. Mojica-Henshaw and colleagues showed that whereas serum-converted hPL was effective for supporting MSC proliferation, non-fibrinogen-depleted hPL resulted in higher MSC yields. There were no differences in the concentrations of growth factors such as PDGF-AA, PDGF-BB, vascular endothelial growth factor, epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF) between hPL-plasma and hPL-serum. Effects on residual particulates (i.e., decrease in numbers, size, and granularity) were observed in both hPL-plasma and hPL-serum. Whether or not this can serve as a measure of quality needs to be determined. Systematic comparisons of these treatments would be helpful for application to different cell types. Synthetic heparin analogues have been developed, which could possibly replace porcine heparin for a totally xenofree hPL supplement without the need to deplete fibrinogen.

Once hPL is produced, important questions arise. At which temperature (−20, −80, or +4°C) and for how long can hPL be stored? Attempting to answer these questions, Rauch and colleagues showed that hPL could be stored at −20°C for at least 5 months while maintaining a stable concentration of EGF. More recently it has been shown that serum-converted hPL solutions are stable at −80°C for 2 years. Mojica-Henshaw and coworkers showed that hPL could be maintained for 4 weeks at 4°C without significant loss of activity.

The final concentration of hPL in cell culture media varies between studies and cell types, ranging from 1% to 60%, with 5% to 10% being the most common concentration used. This percentage will depend on the starting PLT concentration, which varies between 1 million PLTs/mL to 15,000 million PLTs/mL in studies.

Standardization of hPL production is currently a challenge (Fig. 1). Growth factor content significantly differs due to the use of diverse protocols and additives such as plasma...
utilized for hPL production. Systematic and collaborative studies in which different hPL products (generated by distinct procedures) are used in parallel to test cell growth of different cell types, and where important features of the cells are measured, are needed to shed light on the field and foster the change from FBS to hPL in clinical and basic research. Most of the studies published thus far have been performed on MSCs, but other cell types of potential utility for cell therapy and basic research, such as keratinocytes, lymphocytes, neural stem cells, and pluripotent stem cells, among others, should be tested exchanging FBS with hPL (Fig. 1).

**QC and standards for release**

Clinical application of hPL requires standardized protocols according to good manufacturing practice guidelines and **Pharmacopoeia** standards. There is a need to reach an international consensus on quality criteria for hPL products for cell therapy. The "International Forum on GMP-grade human platelet lysate for cell propagation" provides good insight into the huge variability in production and quality testing. The **European Pharmacopoeia** general chapter on “Raw materials of biological origin for the production of cell-based and gene therapy medicinal products for human use” (Chapter 5.2.12) sheds some light in this direction. It states that only carefully evaluated donors adequately tested for infectious transmissible agents according to EU and/or national regulation should be used. A risk assessment for viral safety and spongiform encephalopathies should also be performed according to **European Pharmacopoeia** Chapters 5.1.7 and 5.2.8, respectively. With respect to quality of the final product, identity, purity, and biologic activity should be tested.

An identity test must reflect the uniqueness of the material and distinguish it from other similar products. Some tests that can be used for hPL identity are appearance, pH, total protein, albumin content, and concentration of specific growth factors. Growth factor ranges usually found in hPL have been previously reviewed. Because it is still not known which growth factors are required for cell growth of a specific cell type, measurement of the PDGF family of factors to measure PLT lysis can be a reasonable gauge.

Purity should be assessed by sterility, endotoxin, mycoplasma, and viral testing. Activity can be tested by assaying the cell growth–promoting effect of the specific batch on the cell type of use.

The QC tests mentioned here can be adapted to each manufacturing process and potential usage of the product. Quality release criteria should be different depending on the PLT source as quality standards for PLT donations are different between developed and nondeveloped countries.

**PRT OF hPL**

Substantial improvements in pathogen detection technologies such as highly sensitive nucleic acid testing have decreased blood products’ transmission risks of infectious diseases like HIV, HBV, or HCV. International travel, global migration, and possibly also climate changes may increasingly introduce “emerging” pathogens such as *Plasmodium*, Dengue virus, or Zika virus into blood donor populations with a previous low exposure rate. Regions with high prevalence of such pathogens may face extraordinary challenges in manufacturing safe blood products, when the respective testing technologies are not available. Even the most sophisticated pathogen detection method has its limit, especially with respect to yet unidentified microorganisms or viruses. Donor bacteremia or inoculation of skin microorganisms during venipuncture can cause contamination of blood products.

To address these issues, technologies for PRT have been developed that can effectively decrease the potential viral and bacterial content of blood products. The **European Pharmacopoeia** for pooled phPL for the production of cell-based or gene therapy medicinal products. To date the following PRT treatments exist for PLT products: amotosalen + ultraviolet (UV)A light, riboflavin + UVB light, UVC light, and S/D treatment, and most recently introduced, gamma irradiation. The technologies using UV light target pathogen nucleic acids effectively impairing their replication. Upon illumination with UVA light amotosalen hydrochloric acid covalently bonds and crosslinks nucleic acids whereas riboflavin intercalates with DNA and, under UVB illumination, induces irreversible核酸 strand breaks. UV light directly interacts with nucleic acids generating cyclobutane pyrimidine and pyrimidine-pyrimidone dimers that block nucleic acid transcription. These UV light-based PRTs have been shown to effectively reduce the loads of enveloped and non-enveloped viruses, bacteria, and parasites in PCs. Notably, remaining amotosalen and photo by-products must be removed; however, there is no need to remove riboflavin (vitamin B2) or its by-products after PRT. In contrast, S/D treatment destroys lipid membranes; therefore, it is only applicable for the inactivation of enveloped viruses.

Solvent/detergent treatment leads to the release of PDGF-AB, EGF, and TGF-β1 from apheresis PCs. S/D-treated hPL can skew the immune system to an immunosuppressive phenotype (shown in a mouse asthma model that detected the release of immunomodulatory factors from PLTs).

It is reasonable to question whether UV light–based PRTs too do not only impact the biology of pathogens but also affect PLTs and their cargo. Amotosalen + UVA light treatment has been shown to change the PLT transcriptome. A recent study comparing PRT effects on apheresis PCs found that amotosalen + UVA light, but not riboflavin + UVB light, decreased PLT microRNAs and mRNAs and that this decrease was associated with PLT...
activation. Effects of PRT on the PLT proteome are plentiful, for example, altering proteins related to the regulation of vesicle trafficking, oxidative stress, or PLT metabolism. A systematic review of eight studies reported on 58 PLT proteins affected by amotosalen + UVA light, 67 proteins affected by riboflavin + UVB light, and 48 proteins affected by UVC light.

The absorption rates of UVA light (wavelength, 320-400 nm) and UVB light (wavelength, 265-370 nm) to DNA and/or RNA and proteins are relatively similar, while the absorption rate of short wavelength UVC light (wavelength, 190-290 nm) is much higher for DNA and/or RNA compared to proteins. This suggests that, compared to amotosalen + UVA light or riboflavin + UVB light, the potential maximal effect of UVC light may have a lesser effect on pathogen proteins. Application of high dosage (15-35 kGy) of gamma irradiation on hPL resulted in efficient inactivation of HIV, HAV, bovine viral diarrhea virus, pseudorabies virus, and porcine parvovirus.

Effects of PRT on PLT lysate as medium supplement

Pathogen reduction treatment has been found to have some effects on growth factor content in hPL such as decrease of TGF-β1 after UVC treatment; however, analyzed factors to date remain unchanged or are found to be increased as shown for the presence of bFGF upon amotosalen + UVA light treatment. Neither amotosalen + UVA light treatment nor UVC light treatment of PCs affected the proliferation of MSC cultured in medium with hPL that was manufactured from these PCs. Importantly, MSCs cultured with PRT-hPL (UVC light or S/D treatment) kept their superior proliferation capacities compared to MSC grown with FBS.

Analyses of surface marker expression and assessment of the in vitro differentiation potential are part of the MSC characterization process. The use of hPL produced from UVC light-treated, amotosalen + UVA light-treated, or S/D-treated PCs did not change the surface marker expression profile of the MSCs. In these studies the tested markers were limited; therefore, possible effects on other structures on the MSC surface cannot be ruled out completely at this time.

Reported effects of PRT-hPL on MSC in vitro functions are inconsistent. Most studies did not observe an impact of UVC-PRT-hPL or amotosalen + UVA-PRT-hPL on MSC adipogenic, osteogenic, or chondrogenic differentiation in vitro and MSC potential to suppress the proliferation of immune cells in vitro, as well as on their colony formation capacity, karyotype, and viability. In contrast, one study that analyzed MSC from two donors reported effects of amotosalen + UVA-PRT-hPL on expression of genes involved in osteogenesis and chondrogenesis in MSCs under in vitro differentiation. In the same study, MSCs cultured with amotosalen + UVA-PRT-hPL more strongly suppressed immune cell proliferation in vitro. As recently shown, gamma-irradiated PRT-hPL contained less coagulation factors, but the gamma irradiation did not alter the content of various growth factors (except for bFGF) in hPL or its supportive effects for MSC culture and function.

PRT in the hPL manufacturing process

In current hPL production, PRT is often applied on intact PCs before PLT lysis. What if PRT could be applied on the hPL product itself? PRT can affect the biology of intact PLTs. It is reasonable to assume that the effects of PRT applied after PLT lysis may, at least in part, differ to those of prelysis PRT as the photoactive substances and/or the UV light are not interacting with functioning cells or PLTs but with their released proteins and metabolites. Further studies are needed to better understand PRT effects on hPL to identify the optimal PRT technology for the respective hPL application, and to implement PRT at the appropriate step of the hPL manufacturing process.

CONCLUSIONS

Human PLT lysate has been accepted as a suitable alternative to FBS (e.g., in clinical-scale manufacturing of MSCs). As hPL production expands and efforts to establish it as a growth supplement for cell therapy products grow, multiple challenges are likely to arise (Fig. 1). A central question yet to be clarified is what is the optimal process for production of hPL? The product varies from vendor to vendor and each has its own proprietary process. It may very well turn out that a particular vendor’s hPL product is optimal for expansion of one cell type and one clinical application but not the other. The uniformity of hPL product is critical and dependent on optimal process of production. The requirements of the hPL product may need to be cell product and application dependent and may even require personalization to individual patients whose cells are being expanded. It is also of importance to consider the mode of expansion of the cell product. Most hPL studies have been conducted on cells that are being manually expanded in cell factories or culture dishes. The field of cell therapy production is moving toward using bioreactor-based systems for large-scale expansion to cut costs and labor. It has yet to be determined if hPL can be used in some of these bioreactor systems in commercial use but HPL is an option worthy of further investigation.

Manufacturers of cellular therapies are moving toward better defining cell therapy products. The hPL product may contribute to and alter characteristics of the cell product being produced, which can subsequently impact the potency, therapeutic benefit of the cells, and ultimately patient outcomes. The potency and functionality of the cells
produced with a specific hPL product, including PRT-treated hPL products, is a key challenge and an area for further investigation. It is clear that PRT is essential for the production of a safe product for clinical use. Does PRT-treated hPL have an altered capacity to support cell expansion and maintain cell potency?

There are challenges with sourcing of starting material for hPL production. Can the industry meet the demand for hPL? Should it be widely adopted as a larger number of commercial cell products come to market and the demand increases? Can hPL be applied adequately not only for cell-based therapies but also for biotechnological processes (e.g., vaccine production or even research)? hPL producers cannot compete for blood donors who are donating PLTs for patient PLT transfusions; therefore, the use of outdated PLTs for hPL manufacture is favored to fresh PLTs especially with their demonstrated equivalent properties.

How hPL needs to be sourced and produced and how this process will impact product potency, product functionality, and patient outcomes will be important to explore. There are convincing data that human or species-specific PLT lysate may be an excellent alternative to FBS; however, there are a number of open questions regarding hPL manufacturing and QC (Table 2). Supply shortages, manufacturing issues, limited understanding of quality variables with respect to function, and not least, safety are the utmost critical issues to be addressed.

The diverse manufacturing protocols offer opportunities to identify critical quality variables and how they are affected in manufacturing. To date, however, there is not enough information, especially comparative data, available, which would allow for drawing recommendations for standardized manufacturing and QC. This article presents the first results of the AABB-ISCT Joint Working Group and the Human Platelet Lysate Project Team identifying gaps of knowledge and drafting concepts to overcome these issues to develop rationales for harmonization and standardization of hPL as cell culture supplement.

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**CONFLICT OF INTEREST**

BFM is author of a patent application for the use different media based on hPL (n° application Spanish Patent Office: P201730713). The authors have no other commercial, proprietary, or financial interest in the products or companies described in this article.

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