Stem Cell Research & Therapy

RESEARCH

Open Access

# Human platelet lysate standardization across three independent European blood establishments

Willem Delabie[1], Gabriele Boretti[2,3], Stephanie A. Groot[4], Davina Ardanary[4], Olafur Sigurjónsson[2,3], Thomas R. L. Klei[4], Philippe Vandekerckhove[5,6,7] and Hendrik B. Feys[1,5,8*]

## Abstract

Human platelet lysate (hPL) is a clinically safe alternative to fetal bovine serum (FBS). However, variability in blood donation practices, platelet concentrate preparation methods, storage and hPL manufacturing complicates standardization across jurisdictions. This study aimed to establish a first multinational hPL manufacturing standardization across three European blood centers to test feasibility and variability. A single batch of hPL production sets was distributed to the participating centers. There, hPL was produced following a single standard operating protocol but starting from each center's unique platelet concentrates. Each center prepared four 'national' hPL batches and four 'international' batches. Researchers conducted blinded quality and variation analyses to ensure unbiased results. All hPL batches exhibited comparable total protein levels, pH, ionic strength, and lactate content. Analysis of twelve growth factors showed minor variations across batches. Endothelial cell outgrowth and wound closure were slower in hPL than FBS but remained consistent across batches. Mesenchymal stem cell (MSC) doubling was significantly faster in hPL than in FBS, with MSC phenotype consistency confirmed via flow cytometry. Differentiation into adipogenic and osteogenic tissue was successful in all hPL samples. The inter-institutional variation across all national batches was higher for all critical outcome parameters compared to the variation in the four international batches. These findings confirm the feasibility of manufacturing standardized hPL across borders and show lower variability when doing so. This supports further efforts to stabilize hPL supply and advance cytotherapy standardization in Europe.

**Keywords** Human platelet lysate, Mesenchymal stem cells, Standardization, Tissue culture, Culture media, Manufacturing

*Correspondence:
Hendrik B. Feys
Hendrik.feys@rodekruis.be
[1] Transfusion Research Center, Belgian Red Cross Flanders, Ottergemsesteenweg 413, 9000 Ghent, Belgium
[2] The Blood Bank, Landspitali University Hospital, Reykjavik, Iceland
[3] Department of Engineering, Reykjavik University, Reykjavik, Iceland
[4] Department of Product and Process Development, Sanquin, Amsterdam, The Netherlands
[5] Blood Services, Belgian Red Cross Flanders, Mechelen, Belgium
[6] Department of Public Health and Primary Care, Leuven Institute for Healthcare Policy, KU Leuven, Leuven, Belgium
[7] Centre for Evidence-Based Health Care, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa
[8] Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

## Introduction

Human platelet lysates (hPL) have gained significant attention as a promising alternative to fetal bovine serum (FBS) in cell culture and regenerative medicine [1]. The use of FBS raises ethical, safety and practical concerns like animal welfare, zoonoses, immunogenicity, batch variability and supply limitations [2–6]. Derived from human platelets, hPL is rich in growth factors, cytokines, and other bioactive molecules that promote cell proliferation, differentiation, and tissue repair. These advantages make hPL an attractive supplement for mesenchymal stromal cell (MSC) expansion and other cell therapy applications, reducing the risks associated with xenogeneic components. Despite the growing interest in hPL, the field faces challenges of standardization, batch-to-batch variability, and regulatory compliance [7, 8].

One of the primary issues for widespread adoption of hPL is the lack of a standardized protocol for hPL preparation, storage, and quality control. Platelet lysis methods [9] and downstream processing steps like centrifugation, filtration and pooling potentially lead to inconsistencies in the composition and biological activity of hPL products [7, 10]. In addition, differences related to the primary platelet concentrate like the number of donors per concentrate, the inclusion of pathogen inactivation and the use of additive solutions may further contribute to variability. In theory, the hPL manufacturing method can be standardized internationally, as can its quality control testing. Standardization of the primary platelet concentrate is much more challenging because competent authorities have made historical choices to particularly match the needs per country, or per region with a strong focus on optimal transfusion medicine. For instance, the need for pathogen inactivation and additive solutions is different per region depending on the prevalence of pathogens and the incidence of transfusion complications, respectively. It thus makes sense to attempt to reduce variability by pooling of hPL units from different sources of primary platelet concentrates or even international pooling of nationally sourced hPL batches to very large volume stocks, even though hard evidence on the added value is lacking [11].

Several single center efforts towards standardization have been made [12–15] and guidelines have been proposed by regulatory agencies and research consortia [7, 16]. However, a practical analysis of multicenter hPL standardization is lacking. In this study we provide data on a first effort to standardize hPL across three unrelated and different European blood establishments keeping intact the intrinsic variability of their respective platelet concentrates but harmonizing hPL manufacturing. Intralaboratory as well as interlaboratory batches were prepared in a blinded manner and analyzed for practical outcomes like manufacturing reproducibility, and biochemical outcomes related to cell growth and differentiation.

## Materials and methods

### Study design

Each of the three participating blood institutions received 24 single-step hPL production sets [17] from the central lab in Belgium (Fig. 1). Using these sets, each institution produced 24 individual hPL products from 24 separate platelet concentrates (PC). Human platelet lysate is produced in these sets by sterile transfer of any PC to the production set using sterile welding. Next a 3-h incubation allows platelet growth factor release and

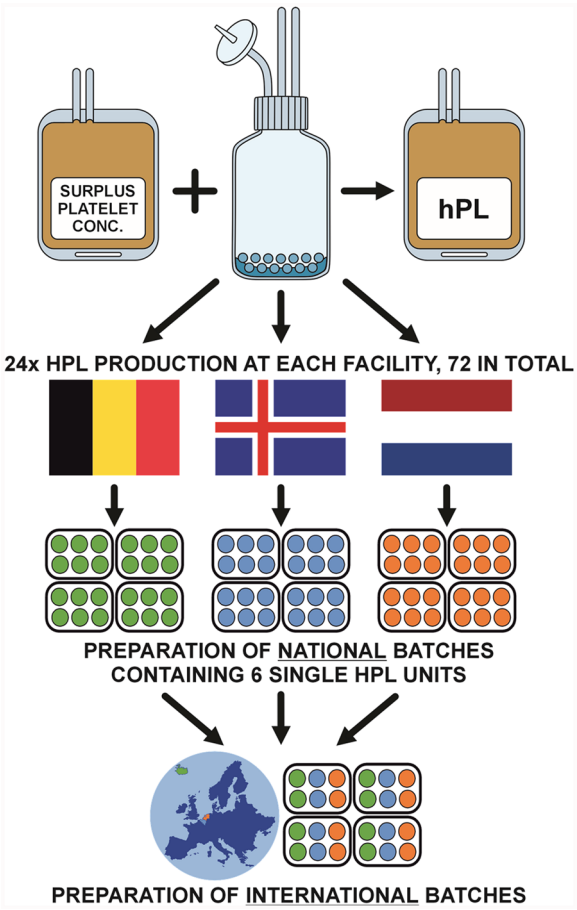


**Fig. 1** Study design. Each blood institution prepared 24 hPL products derived from 24 platelet concentrates using one hPL production set per concentrate. hPL was shipped to the central lab where 4 national 'batches' were prepared by pooling of 6 individual hPL products per country, symbolized by unicolored spheres per country (*middle*). In addition, the central lab prepared 4 international 'batches', consisting of 2 individual hPL products per country (*bottom*). From these 'batches', blinded samples were prepared and sent back to the participating centers for analysis

clot contraction caused by $Ca^{2+}$ ions and glass beads, respectively. This product was then stored at −80 °C for minimally two days. After overnight thawing at room temperature, the products were ready for transfer of the growth factor rich supernatant using sterile welding to an hPL collection bag. As previously described by Delabie et al., this method yields a transparent, functional hPL that supports expansion of primary and continuous cell lines [17]. From each single hPL product 45 mL was aliquoted in pre-labeled test tubes supplied by the central lab to the participating centers. The tubes were then transported cryopreserved on dry ice to the central lab.

At the central lab, six single hPL 45 mL aliquots from each blood institution were randomly selected and pooled to create a 'national batch.' This process was repeated four times, resulting in four national batches per participating institution (n = 4). Additionally, two individual hPL aliquots from each institution were randomly selected and pooled in groups of six to form an 'international batch,' which was also prepared four times. The donor composition of each batch varied based on the number of buffy coats used for PC preparation. Belgian, Icelandic, Dutch, and international batches consisted of 36, 48, 30, and 38 donors, respectively.

Subsequently, 1 mL and 10 mL aliquots of each batch were prepared in coded tubes to ensure that all researchers, including those preparing the batches, remained blinded. Only one individual at the central lab who was not involved in the study, had access to the code. These coded samples were then cryopreserved and shipped back to the participating blood institutions for laboratory experiments and quality control. Coded experimental data were sent back to the central lab for statistical analysis, after which the results were unblinded.

#### Preparation of platelet concentrates

All three blood establishments prepared PC using the buffy coat (BC) method as previously described [18]. ABO blood group matched buffy coats from single donors were pooled and supplemented with platelet additive solution (PAS)-E. Belgian, Dutch and Icelandic pooled PC consisted of 6, 5 and 8 individual donor buffy coats respectively. PAS used was T-PAS+ (Terumo BCT, Lakewood, CO) in Belgian and Dutch PC and SSP+ (Macopharma, Mouvaux, France) in Icelandic PC [19]. Pooled BCs were centrifuged and the buoyant platelet suspension was taken off by automated separation while passing over a leukocyte reduction filter. Belgian and Icelandic PC were treated with the amotosalen and ultraviolet light pathogen inactivation method (Cerus Corporation, Concord, CA) [20]. Dutch PC were checked for bacterial contamination using the BacT/ALERT system (Biomérieux, Marcy-l'Étoile, France) on day 1 and day 8 post donation. The PC were stored in a temperature controlled environment (22 °C) with continuous agitation. All PC were checked for swirling and were used for hPL production before day 10 post donation. All donations were from voluntary non-remunerated donors who gave informed consent for all experiments performed on the use of the donation or of fractions of the donation for scientific research purposes. Experiments adhered to local legislation. Belgian donor material used was registered in the biobank with accession number (BB190034) and the study was approved by the University Hospital Institutional Review board of UZ Leuven (S62549).

#### Biophysical and biochemical analysis

To record hPL turbidity, light transmittance was measured in using a spectrophotometer (NanoDrop OneC, Thermo Fisher Scientific, Waltham, MA). Transmittance was calculated using the formula $T = 10^{(2-A680nm)}$, where T is transmittance and $A_{680nm}$ is the compound of light absorbance and light scattering of an undiluted sample measured at a wavelength of 680 nm and a light path length of 1 cm. Osmolality and concentrations of glucose, lactate and $Ca^{2+}$ were measured in raw hPL as well as in complete culture media using a point-of-care blood gas analyzer (RAPIDPoint 500, Siemens, Munich, Germany). pH at 20–24 °C was determined by glass electrode (HI 5221, Hanna Instruments, Woonsocket, RI).

#### Growth factor, fibrinogen and total protein content

All hPL batches were measured on the Luminex analyzer MAGPIX (DiaSorin, Saluggia, Italy), using three different 96-well plate assays. A first panel was the MILLIPLEX Human Cytokine/Chemokine/Growth Factor Panel A Magnetic Bead Panel (Millipore, Sigma-Aldrich) 7 PLEX containing Epidermal Growth Factor (EGF), Fibroblast growth factor 2 (FGF2), soluble CD40 Ligand (sCD40L), Interleukin (IL)−1α, IL-6, Tumor Necrosis Factor (TNF)-α and Vascular Endothelial Growth Factor (VEGF)-A. hPL used in this kit was 50 µL per well, which is double the amount stated in the protocol. Second, a MILLIPLEX Human Cytokine/Chemokine/Growth Factor Panel A Magnetic Bead Panel (Millipore, Sigma-Aldrich) was used containing Platelet Derived Growth Factor (PDGF)-AA and PDGF-AB/BB and for which hPL was diluted 1/20. Lastly, also the MILLIPLEX Transforming Growth Factor (TGF-β) Magnetic Bead Kit (Millipore, Sigma-Aldrich) was used containing TGF-β1,2,3 for which hPL was diluted 1/10.

hPL was added to the 96-well plates followed by the beads and incubated with agitation on a plate shaker overnight (16 h) at 4 °C. A handheld magnet was used to hold the beads into the plate when the well content was removed. After incubation, wells were washed 3

times with buffer. Detection antibody was added and the plate was incubated with agitation for 1 h at room temperature (19–23 °C). After incubation, streptavidin conjugated with phycoerythrin was added. After 30 min incubation at room temperature, well content was removed and washed 3 times. The wells were filled with Drive Fluid and analyzed. Median Fluorescent Intensity (MFI) median-data were used to calculate the analyte concentrations in the hPL samples. An in-house calculation program Log-it (made by: Ed Nieuwenhuys, Sanquin) was used for the calculation of the end results. Outcome data are given as signal per concentration of calibration sample and are non-linear. The excel algorithm performs non-linear logistic regression to allow interpolation of the concentration in the test samples.

Fibrinogen was measured with the STartMax analyzer (Stago, Asnières-sur-Seine, France) with Liquid Fib, g/L, Clauss method (Stago). This is a quantitative, clot-based, functional assay. Total protein was measured at an external lab (OLVG Lab BV, Amsterdam, The Netherlands). All hPL was measured with the Total Protein gen. 2 assay (Cobas, Roche, Basel, Switzerland), on the COBAS8000-c502 (Roche) which is a colorimetric assay with $Cu^{2+}$ ions in alkaline solution that forms a purple-colored biuret complex. The color intensity is directly proportional to the protein concentration which can be determined spectrophotometrically at a wavelength of 546 nm.

### HUVEC culture

Cell culture assays were performed using human umbilical vein endothelial cells (HUVEC, PromoCell, Heidelberg, Germany). Basal medium was Endothelial Cell Growth Medium 2 (PromoCell) and all media was supplemented with SupplementMix (PromoCell), 100 U/mL penicillin (Sigma-Aldrich, Saint-Louis, MO), 100 µg/mL streptomycin (Sigma-Aldrich) and 4 mM L-Glutamine (Sigma-Aldrich). The flasks were coated with fibronectin to adhere the cells. Cells were incubated at 37 °C and 5% $CO_2$.

HUVEC proliferation was measured in 24-well plates (Thermo Fisher Scientific). hPL was added to Endothelial Cell Growth Medium at 10% and 2% (v/v). The positive control used the same basal medium supplemented with 10% (v/v) FBS (Cell Applications, Inc., San Diego, CA). The negative control was plain basal medium without added supplement. Proliferation was measured on day 1, 2, 3 and 4 after seeding of the cells by staining with 0.5% (w/v) Crystal Violet. Attached cells were lysed with 2% (w/v) Sodium Dodecyl Sulphate after which absorbance was measured at 590 nm.

### Scratch assay

HUVEC were seeded in 24-well plates in Endothelial Cell Growth Medium 2 (PromoCell) supplemented with SupplementMix (PromoCell), grown to confluence and "scratched" at the center using a pipette tip. Following this, hPL was diluted in serum-free basal medium at 10% and 2% (v/v). Positive control was the same basal medium supplemented with 10% (v/v) FBS and negative control plain basal medium without supplement. All growth media were supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin. Cell migration was photographed every 30 min using bright field phase contrast microscopy (ZEISS Axio Observer Z1) at 100× magnification (10× objective and 10× ocular lens). The percentage of scratched area filled by migrated cells was then calculated using ImageJ software (plugin: wound healing size tool) at the endpoint of 24 h. Parameters of the tool include variance filter radius and threshold in order to optimize noise reduction without over-smoothing.

## MSC differentiation

Human adipose-derived mesenchymal stem cells (MSC, Thermo Fisher Scientific) were cultured in a standard growth medium consisting of Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) supplemented with 10% Fetal Bovine Serum (FBS) and 1% (vol/vol) penicillin–streptomycin (100 U/ml). Cells were cultured at a seeding density of 5,000 cells/$cm^2$ in regular 75 $cm^2$ tissue culture-treated flasks. Cells were used at passage 5 to assess the effects of the different batches of hPL on cell viability and differentiation potential. Seventeen media formulations were prepared, each containing a unique serum type: the control medium included 10% FBS, while each experimental medium contained one of the sixteen hPL batches supplemented at 10% in DMEM/F-12. All complete media contained 1% (vol/vol) penicillin–streptomycin (100 U/ml) and was filtered through 0.2 µm filters (Whatman, Cytiva). Media were stored at 4 °C for up to one week.

Osteogenic, and adipogenic differentiation assays were conducted to evaluate the differentiation potential of MSCs cultured with different hPL batches. For each differentiation lineage, two time points were assessed: day 0 (T0) and at endpoint (TE; 14 days for adipogenic differentiation and 28 days for osteogenic differentiation).

MSCs at passage 5 were seeded in 24-well plates at a density of 5,000 cells/$cm^2$. Twelve wells were assigned per hPL batch, with six wells designated for each differentiation type (three wells for T0 and three wells for TE). Cells were cultured in their respective media until they reached 80% confluence. At that point, half of the wells were fixed with 4% paraformaldehyde (for T0), and differentiation

was induced in the remaining wells using the StemPro Differentiation Kit (Thermo Fisher Scientific).

Upon reaching the endpoint, cells were fixed with 4% paraformaldehyde and stained for histological analysis. Osteogenic differentiation was assessed using Von Kossa staining by 30 min incubation with 5% (m/V) silver nitrate (Sigma-Aldrich), and adipogenic differentiation by 10 min incubation with 0.3% (m/V) Oil Red O (Sigma-Aldrich). Stained wells were visualized qualitatively under a bright field microscope (Olympus, Tokio, Japan) at 40× magnification (4× objective and 10× ocular lens), and images were taken using Infinity Analyze software (Teledyne, Waterloo, Canada).

## MSC expansion

hPL was diluted at 10% (vol/vol) in DMEM/F-12. Human MSC Screened FBS (Cytiva, Marlborough, MA) was included as a positive control and was also diluted at 10% (vol/vol) in DMEM/F-12. Prior to use, all complete media were supplemented with 1% (vol/vol) penicillin–streptomycin (100 U/ml, Thermo Fisher Scientific) and subsequently sterile filtered (0.2 µm). Media were aliquoted in single-use doses and stored at 2–8 °C for 2 weeks at most. Upon use the aliquot was warmed to 37 °C and remainder material was discarded. A single clone of human adipose-derived MSC (Lonza, Basel, Switzerland) was cultured in regular 25 $cm^2$ tissue culture-treated flasks at a seeding density of 4,000 cells/$cm^2$. Culture medium was changed every 2–3 days and cell dissociation was performed at 80–90% confluence using trypsin-ethylenediamine tetraacetic acid (EDTA, Thermo Fisher Scientific). MSCs were kept in culture for a maximum of 42 days as per protocol. Cumulative population doublings (cPD) were calculated according to the following formula where $cPD_0$ is the cPD at previous passage, $N_0$ is the amount of cells at seeding and $N_1$ is the amount of cells at the end of the passage: $cPD = cPD_0 + 3.322 * (\log(N_1) - (\log(N_0))$ [21].

### Flow cytometric phenotyping

At seeding and at cPD8 (during long term expansion), flow cytometric phenotyping of MSCs was performed according to the International Society for Cellular Therapy (ISCT) guidelines using an acoustic focusing flow cytometer (Attune, Life Technologies, Carlsbad, CA) [22]. Cells were suspended at $10^6$ per mL in phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, pH7.4). Antibodies used to characterize positive markers were anti-CD90 fluorescein isothiocyanate (FITC, BD Biosciences, Franklin Lakes, NJ), anti-CD105 Super Bright 436 (SB436, Thermo Fisher Scientific), anti-CD73 allophycocyanin (APC, BD Biosciences) and anti-CD13 phycoerythrin-cyanine7 (PE-Cy7). Exclusion markers were anti-CD34, anti-CD11b, anti-CD19, anti-CD45 and anti-HLA-DR, all labeled with PE (BD Bioscience). Cells were diluted to $2.5 \times 10^5$ per mL in PBS before analysis in the flow cytometer. Gating was done by including 0.5% positive events of fluorescence minus one (FMO) controls for positive markers, and for negative markers by including 0.5% of positive events of an isotype antibody control.

### *Statistical analysis*

All statistical analyses were performed using Prism Version 10.4.1 (GraphPad Software Inc.). Kruskal–Wallis test with Dunn's multiple comparison test was used for comparison of hPL production methods. Repeated measures one-way ANOVA for comparison of hPL relative volume yield was used with a Tukey's correction for multiple comparisons. For cell culture data, a two-way ANOVA was used with a Šidák correction. Null hypotheses were rejected when $p < 0.05$. Coefficient of variation (CV) was calculated with correction for small sample sizes and according to the following formula: $CV = [SD/\overline{x}] * [1 + \frac{1}{4n}]$ where SD is standard deviation, $\bar{x}$ is mean and n is sample size. For calculation of CV for scratch wound assays, half life was determined by one phase decay. CV categories were set up for clarification and were selected based on a directed search in the Pharmacopoeia's of the United States of America and Europe for "specifications for biotechnological/biological products" (ICH Q6B), "Good Manufacturing Practice for API's" (ICH Q7), "Uniformity of Dosage Units" (Ph. Eur. 2.9.40).

## Results

### Human platelet lysate production and biochemical properties

Prior to hPL production, the volume of the PC was measured and the platelet quality was checked by presence of swirling [23]. General differences in platelet concentrate manufacturing per blood establishment are given in Table 1. Detailed composition of all used PC and final hPL is given in Table S1. All PC were successfully converted to hPL using the hPL production sets and the standard operating procedure provided with the sets (Fig. 2A), irrespective of institution and starting product. Tubing from PC bags in the different blood establishments was compatible for sterile welding to the hPL set tubing. No issues were reported during transfer of the PC to the hPL set, nor during transfer of the intermediate hPL to the final storage container. Relative volume yield was expressed as the fraction of volume of the original PC that was retrieved as hPL in the final storage container (Table S1 and Fig. 2B). Relative volume yield was highest for Dutch hPL (84%), compared to Belgian (73%) and

**Table 1** Main variables of standard platelet concentrates prepared in each institution. (BE; Belgium, IS; Iceland, NL; the Netherlands)

| Variables | BE | IS | NL |
|---|---|---|---|
| Number of constituting buffy coats, i.e. number of donors | 6 | 8 | 5 |
| Platelet additive solution category (type name) | E (T-PAS+) | E (SSP+) | E (T-PAS+) |
| Pathogen inactivation by amotosalen photochemical treatment | Yes | Yes | No |
| Bacterial testing | No | No | Yes |
| Average volume (mL) | 307 | 187 | 286 |
| Average platelet concentration ($10^3$/µL) | 987 | 1,240 | 856 |
| Maximum storage time (days post donation) | 5 | 7 | 7 |
| Average storage time for hPL production since donation (days) | 7.8 | 7.8 | 8.0 |

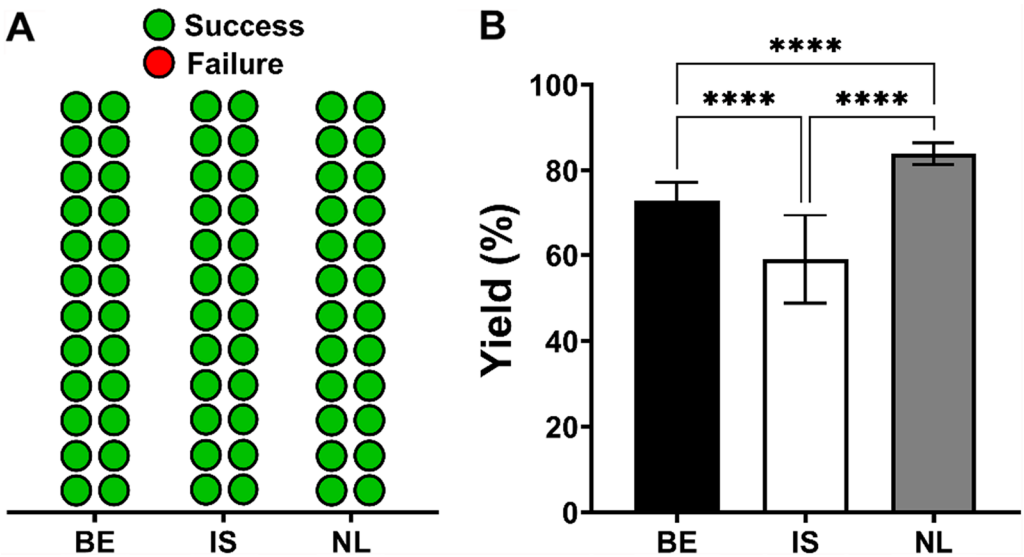


**Fig. 2** hPL production set efficiency. **A** The success rate of PC conversion to hPL is shown. **B** hPL volume yield was the ratio of hPL volume over original PC volume. Data are shown as mean with standard deviation (n = 24), ****$P < 0.0001$. Two-letter country code is used for Belgium (BE), Iceland (IS) and the Netherlands (NL)

Icelandic hPL (59%). No differences were found across blood establishments in terms of hPL transmittance (Fig. S1) as a measure for product turbidity.

pH varied between 7.0 and 7.5 between batches (Fig. 3A). No differences were found for ionized calcium (Fig. 3B) and osmolality (Fig. 3C). Glucose was higher in Dutch hPL compared to other hPL (Fig. 3D) but lactate was not different (Fig. 3E). Average phosphate concentration per batch type ranged from 14 to 19 mM with minor variation across batches (Fig. S2). Possible sources of variation during hPL production were the operator, ambient temperatures and duration of cryopreservation.

#### Growth factors, cytokines and other protein content

No significant differences were found across batches or across institutions for EGF, PDGF-AA, PDGF-AB/BB, TGF-ß2, TGF-ß3, IL-1a, IL-6, sCD40L and TNF-α (Fig. 4A, C-D, F-G, I-L). FGF2 was significantly higher in Icelandic hPL (759 pg/mL) than in Belgian hPL (512 pg/mL, $P < 0.01$) and Dutch hPL (323 pg/mL, $P < 0.0001$) (Fig. 4B). TGF-ß1 was slightly higher in Icelandic hPL (36.9 ng/mL) compared to Belgian hPL (33.6 ng/mL) (Fig. 4E). Similarly, VEGF difference was only significant ($P < 0.01$) between Icelandic (743 pg/mL) and Belgian hPL (506 pg/mL) (Fig. 4H). As a result of fibrinogen depletion during hPL production, fibrinogen always remained below the assay's limit of detection (33 µg/mL) for all hPL (Fig. S3). Total protein ranged from 17.8 to 20.5 g/L with only Icelandic hPL containing significantly higher ($P < 0.0001$) concentrations compared to other hPL (Fig. S3). Any difference found in protein content was leveled out in the international batches.

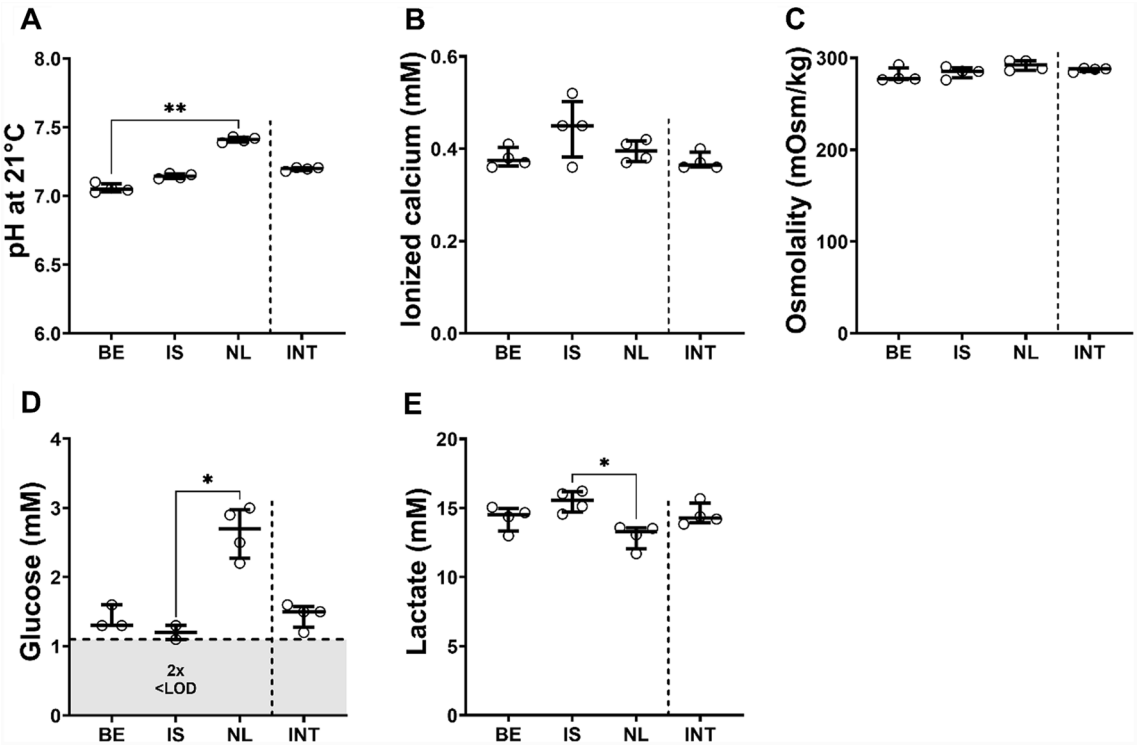


**Fig. 3** Biochemical parameters in hPL. **A** pH, **B** ionized calcium, **C** osmolality, **D** glucose concentration and **E** lactate concentration were measured in undiluted hPL. The vertical dotted line is used to separate national batches (*left*) from international ones (*right*). Individual data are shown and error bars represent median with interquartile range (n = 4), $^{*}P < 0.05$, $^{**}P < 0.01$. Two-letter country code is used for Belgium (BE), Iceland (IS) and the Netherlands (NL). The international batch is labeled INT

#### HUVEC proliferation and scratch wound assay

HUVEC successfully proliferated in the presence of hPL (Fig. 5A). A small decrease was found at day 4 for cells grown in Dutch hPL compared to Belgian and Icelandic hPL ($P < 0.01$). No proliferation was observed in basal medium without supplement. In a scratch assay, hPL was successful at closing artificial wounds in vitro when supplemented at 10% and followed up for 24 h. At 2% hPL still supported partial wound closure however the rate of closure was fastest in FBS (Fig. 5B-C).

### MSC growth kinetics, phenotype and differentiation potential

No significant differences were found between hPL batches on any moment during the five week long culture (Fig. 6A). Cumulative population doublings (cPD) of MSC cultured in presence of hPL was always higher than cPD of MSC cultured in FBS. The cell doubling time of the first five passages was faster in hPL than in FBS (Fig. 6B-C). No significant differences were found between hPL batches in initial MSC growth kinetics (Fig. 6C). Cell phenotype was measured in flow cytometry when reaching cPD8. All maintained cell surface expression of CD13 and CD73 (Fig. 6D). Cell surface expression of CD90 was decreased in all conditions. However, median fluorescence intensity of CD90 was always higher than negative control suggesting that CD90 expression was not zero (Fig. S4). This may be related to the MSC clone, the anti-CD90 antibody or its labeling. CD105 expression was reduced in hPL cultured MSC, not in FBS-cultured cells as published before [24]. Expression of hematopoietic lineage specific markers was negative in all conditions (Fig. 6E).

After MSC culture in media with hPL or FBS, adipogenic and osteogenic differentiation was successfully induced (Fig. 7). Adipogenic differentiation appeared more prominent in hPL cultured MSC compared to FBS cultured MSC. No clear differences were observed within or across hPL batches for either adipogenic (Fig. S5) or osteogenic differentiation (Fig. S6).

#### Batch-to-batch variation and coefficient of variation

The coefficient of variations (CV) was tabulated in Table 2. A mathematical correction for CV of small sample size was applied. Variation was below 15% for total protein, pH, $Ca^{2+}$, $PO_4^{3-}$, osmolality, glucose, lactate, FGF2, TGF-ß1, TGF-ß2, sCD40L, TNF-α, HUVEC expansion and MSC expansion. Intra-institution CV of

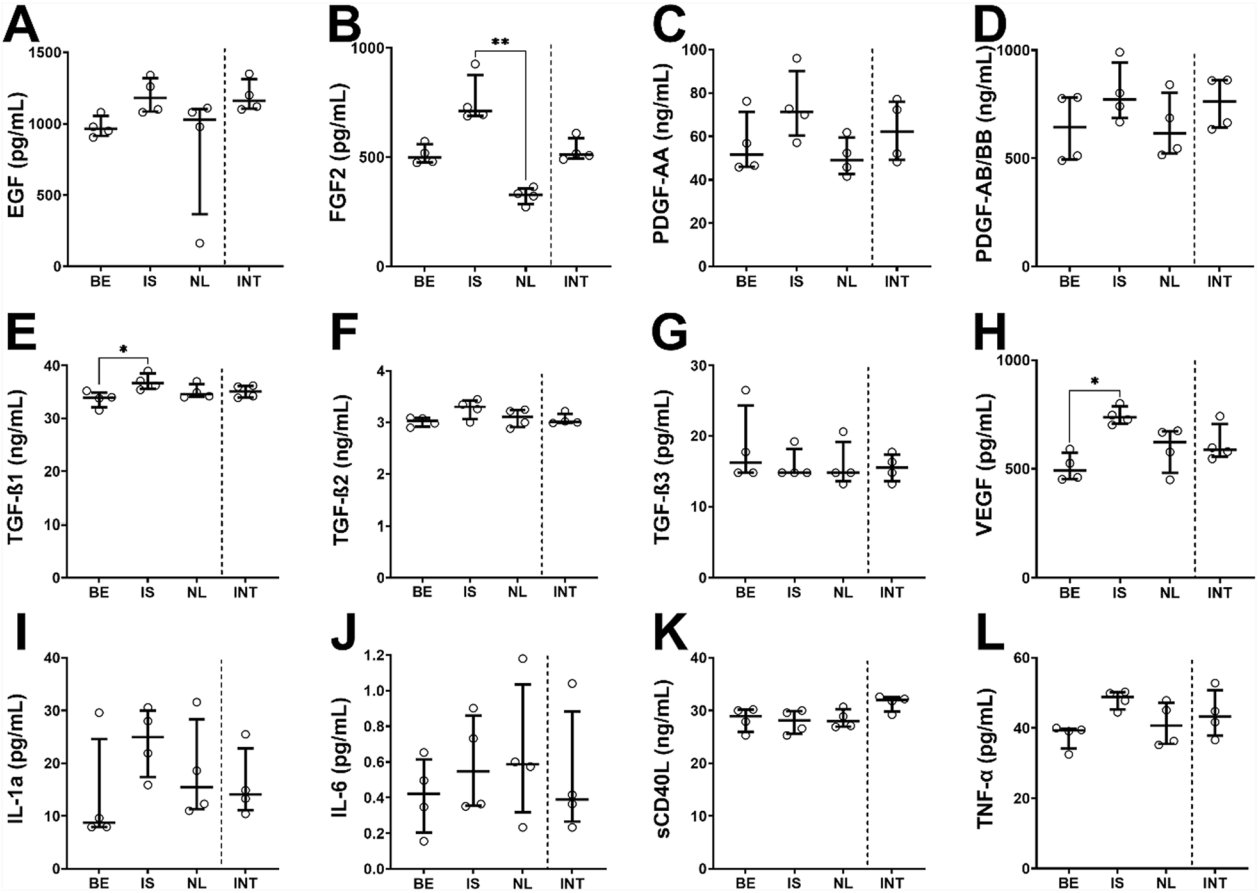


**Fig. 4** Growth factors and cytokines in hPL. A Luminex assay was used to quantify concentrations of **A** EGF, **B** FGF2, **C** PDGF-AA, **D** PDGF-AB/BB, **E** TGF-ß1, **F** TGF-ß2, **G** TGF-ß3, **H** VEGF, **I** IL-1a, **J** IL-6, **K** sCD40L and **L** TNF-α. The vertical dotted line is used to separate national batches (*left*) from international ones (*right*). Individual data are shown and error bars represent median with interquartile range (n = 4), *$P < 0.05$, **$P < 0.01$. Two-letter country code is used for Belgium (BE), Iceland (IS) and the Netherlands (NL). The international batch is labeled INT

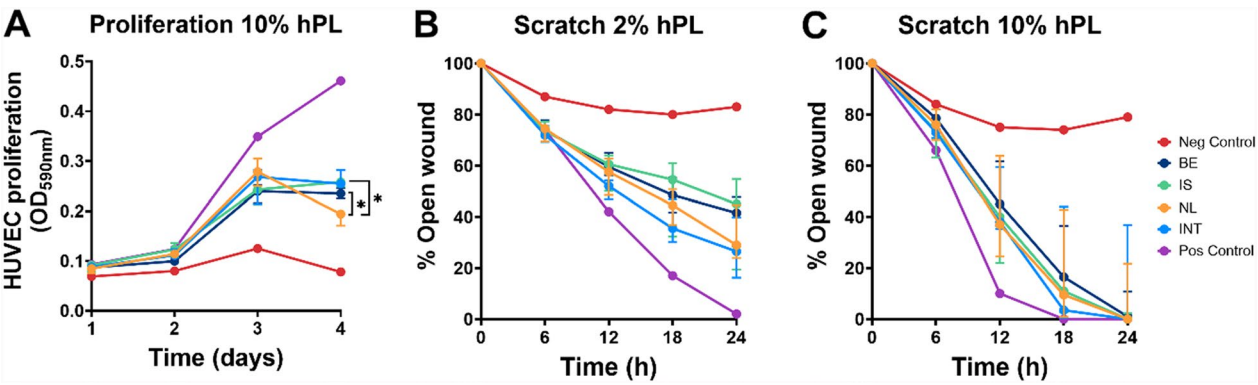


**Fig. 5** HUVEC proliferation and scratch assay. **A** All hPL batches and FBS were used for culturing HUVECs. Proliferation was tracked by measuring absorbance at 590 nm of cell staining with crystal violet on days 1, 2, 3 and 4 post seeding. Negative control was basal medium without supplement. **B** Scratch assays were performed with confluent HUVEC cultures in 2% hPL and **C** 10% hPL. The fraction of open wound is expressed as a function of time post scratch. Error bars represent median with interquartile range (n = 4), *$P < 0.05$. Two-letter country code is used for Belgium (BE), Iceland (IS) and the Netherlands (NL). The international batch is labeled INT

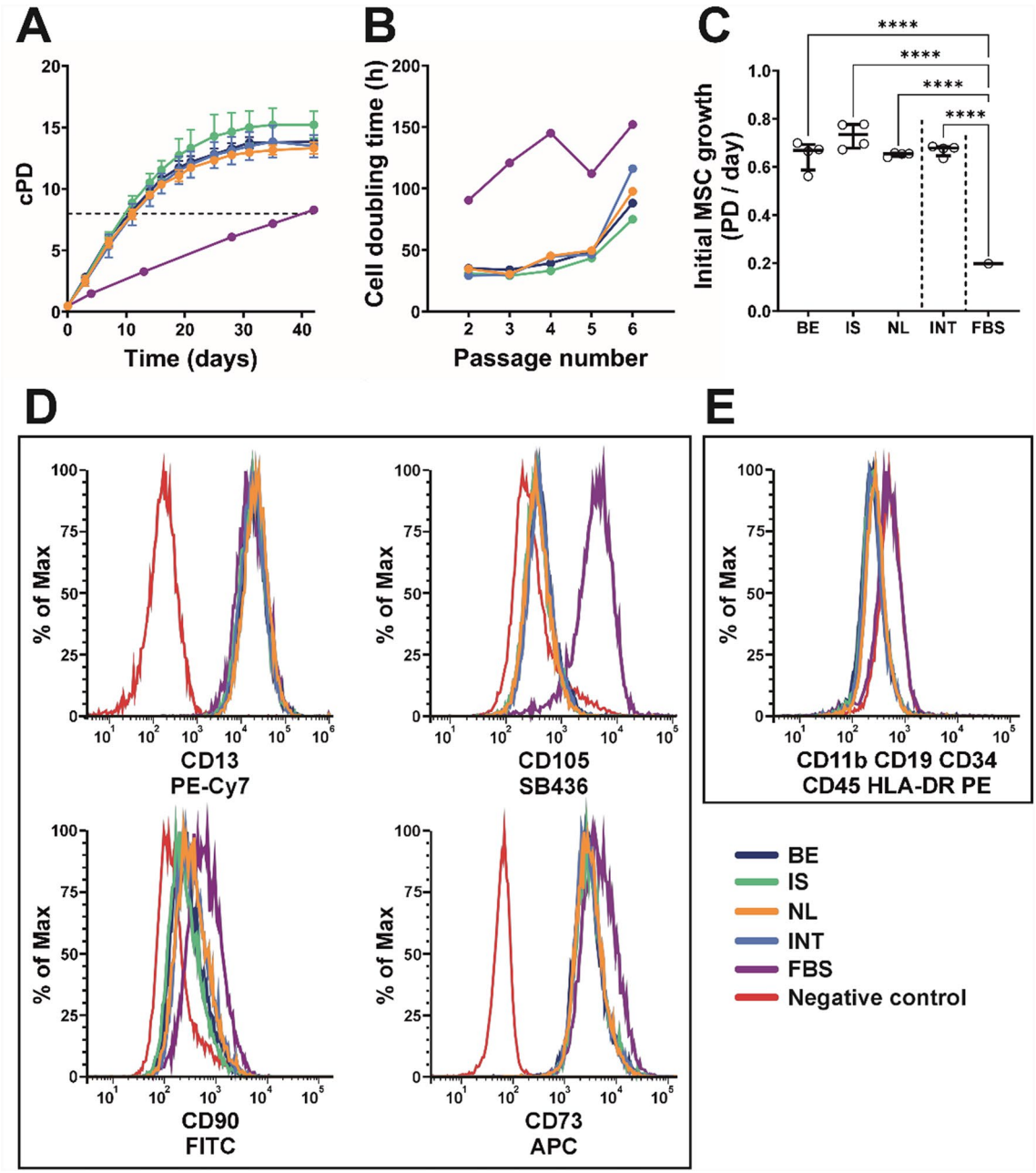


**Fig. 6** Expansion of MSC and phenotypic characterization by flow cytometry. MSCs were cultured in hPL or FBS for five consecutive weeks. **A** Cumulative population doublings were recorded. The horizontal dotted line is set at cPD8, depicting the moment that cells were phenotyped by flow cytometry. **B** Cell doubling time of first five passages and **C** the growth kinetics during the linear phase of culture in panel A. The vertical dotted lines are used to separate national batches (*left*) from international ones (*middle*) and FBS (*right*). Error bars represent median with interquartile range (n = 4), ****P < 0.0001. MSCs were phenotyped using flow cytometry at cPD8 for **D** markers CD13, CD105, CD90 and CD73. **E** MSC should be negative for markers CD11b, CD19, CD34, CD45 and HLA-DR. Two-letter country code is used for Belgium (BE), Iceland (IS) and the Netherlands (NL). The international batch is labeled INT

Belgian batches exceeded 15% in 7 of 23 tested parameters, in 6 of 23 in Icelandic batches and in 9 of 23 in Dutch batches. These differences levelled out in the international batches because the CV was above 15% in only 5 of 23 parameters. The CV of the 4 international batches was lower than the single CV calculated for all 12 national batches across institutions in 21 of 23 tested parameters.

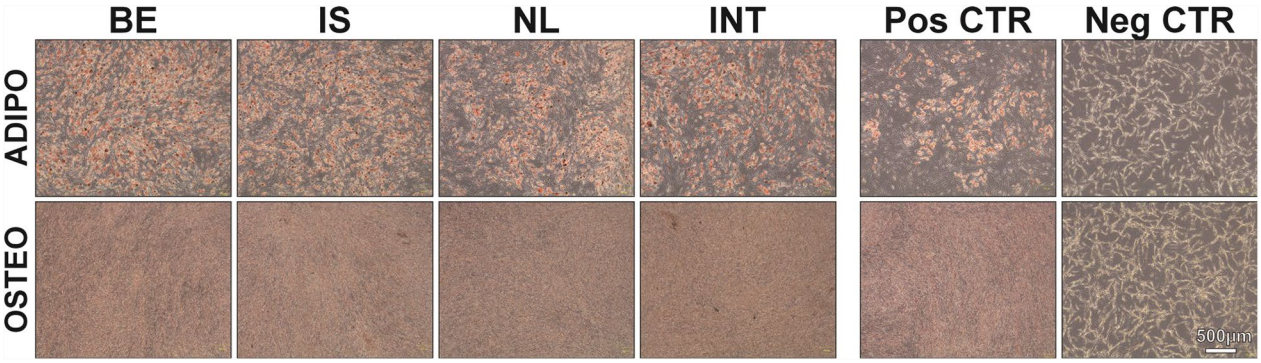


**Fig. 7** MSC differentiation potential. MSC were cultured in the presence of either hPL or FBS and subsequently differentiated in identical differentiation media. Adipogenic differentiation was analyzed using Oil Red O staining and Osteogenic differentiation by Von Kossa staining. The positive control is a biologically paired cell sampled cultured in FBS, differentiated and stained. The negative control is biologically paired cell sample that was equally stained, but not differentiated. Microscopic pictures were selected representative for each hPL batch group. Scale bar is 500 µm, total magnification 40×

**Table 2** Coefficient of variation

| | | BIOCHEMICAL | | | | | | PROTEIN CONTENT | | | | | | | | | | | | GROWTH KINETICS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | mM | mM | mOsm/kg | mM | mM | g/L | pg/mL | pg/mL | ng/mL | ng/mL | ng/mL | ng/mL | ng/mL | pg/mL | pg/mL | pg/mL | pg/mL | ng/mL | pg/mL | $OD_{490}$ on day 4 | Half life | Half life | PD/day |
| | | pH | $Ca^{2+}$ | $PO_4^{3-}$ | Osmolality | Glucose | Lactate | Total protein | EGF | FGF2 | PDGF-AA | PDGF-AB/BB | TGF-ß1 | TGF-ß2 | TGF-ß3 | VEGF | IL-1a | IL-6 | sCD40L | TNF-α | HUVEC growth | Scratch wound 10% | Scratch wound 2% | MSC growth |
| BE | 1 | 7.043 | 0.37 | 15.60 | 277.7 | 1.3 | 14.37 | 18.5 | 1080 | 519 | 56.8 | 777 | 34.0 | 3.09 | 17.7 | 525 | 9.6 | 0.155 | 30.0 | 39.1 | 0.244 | 7.5 | 13.56 | 0.67 |
| | 2 | 7.056 | 0.38 | 15.84 | 276.4 | <LOD | 15.06 | 18.2 | 978 | 572 | 76.2 | 782 | 33.8 | 2.98 | 14.8 | 451 | 29.6 | 0.653 | 30.2 | 39.5 | 0.246 | 8.4 | 7.51 | 0.70 |
| | 3 | 7.027 | 0.41 | 16.73 | 277.2 | 1.6 | 14.67 | 18.0 | 904 | 475 | 46.5 | 512 | 35.2 | 3.08 | 14.8 | 589 | 7.9 | 0.347 | 27.9 | 32.5 | 0.226 | 14.0 | 11.81 | 0.66 |
| | 4 | 7.101 | 0.36 | 14.97 | 292.8 | 1.3 | 12.99 | 18.4 | 950 | 480 | 45.8 | 490 | 31.5 | 2.90 | 26.5 | 460 | 7.9 | 0.496 | 25.3 | 40.0 | 0.225 | 9.3 | 11.17 | 0.56 |
| CV BE (%) | | 0.4% | 5.2% | 4.2% | 2.6% | 10.7% | 5.8% | 1.1% | 7.0% | 8.1% | 23.2% | 23.1% | 4.2% | 2.7% | 27.6% | 11.7% | 70.8% | 47.4% | 7.4% | 8.6% | 4.4% | 27.1% | 21.3% | 8.6% |
| IS | 1 | 7.133 | 0.45 | 15.24 | 285.3 | 1.3 | 16.23 | 20.0 | 1100 | 688 | 57.1 | 668 | 37.1 | 3.26 | 14.8 | 746 | 15.9 | 0.902 | 26.6 | 47.8 | 0.260 | 8.4 | 7.37 | 0.78 |
| | 2 | 7.155 | 0.52 | 13.83 | 290.5 | 1.1 | 15.13 | 20.7 | 1340 | 728 | 72.7 | 802 | 36.2 | 3.35 | 14.8 | 726 | 30.6 | 0.351 | 30.0 | 49.9 | 0.259 | 7.9 | 10.06 | 0.77 |
| | 3 | 7.126 | 0.45 | 10.95 | 285.8 | <LOD | 14.56 | 21.4 | 1080 | 926 | 70.0 | 741 | 35.4 | 3.45 | 14.8 | 800 | 28.0 | 0.732 | 29.6 | 44.5 | 0.246 | 8.1 | 4.85 | 0.70 |
| | 4 | 7.165 | 0.36 | 15.42 | 276.1 | <LOD | 16.03 | 20.0 | 1260 | 695 | 96.0 | 991 | 39.0 | 3.00 | 19.2 | 701 | 21.9 | 0.364 | 25.3 | 50.3 | 0.257 | 5.4 | 9.95 | 0.67 |
| CV IS (%) | | 0.2% | 13.6% | 13.7% | 1.9% | 8.9% | 4.6% | 3.0% | 9.7% | 13.6% | 20.2% | 15.9% | 3.9% | 5.4% | 12.7% | 5.2% | 25.1% | 43.0% | 7.6% | 5.1% | 2.3% | 16.8% | 28.2% | 6.8% |
| NL | 1 | 7.431 | 0.37 | 18.64 | 288.5 | 2.9 | 11.70 | 17.6 | 162 | 333 | 41.5 | 546 | 34.9 | 3.22 | 14.8 | 667 | 18.6 | 1.180 | 30.7 | 45.1 | 0.192 | 8.9 | 9.99 | 0.66 |
| | 2 | 7.388 | 0.42 | 19.53 | 297.2 | 2.2 | 13.59 | 18.2 | 1110 | 364 | 61.8 | 841 | 37.0 | 3.25 | 13.2 | 576 | 12.3 | 0.600 | 28.9 | 47.9 | 0.202 | 6.8 | 13.50 | 0.65 |
| | 3 | 7.420 | 0.41 | 18.83 | 297.0 | 3.0 | 13.07 | 17.7 | 1080 | 322 | 45.8 | 516 | 34.2 | 2.88 | 20.6 | 449 | 11.0 | 0.574 | 26.9 | 35.2 | 0.196 | 6.3 | 6.48 | 0.66 |
| | 4 | 7.403 | 0.38 | 18.30 | 286.2 | 2.5 | 13.53 | 17.8 | 978 | 272 | 52.3 | 687 | 34.0 | 3.00 | 14.8 | 674 | 31.6 | 0.233 | 27.1 | 36.3 | 0.164 | 17.6 | 16.59 | 0.64 |
| CV NL (%) | | 0.2% | 5.5% | 2.5% | 1.8% | 12.8% | 6.2% | 1.4% | 49.8% | 10.9% | 16.1% | 21.2% | 3.6% | 5.3% | 18.9% | 16.3% | 47.2% | 55.9% | 5.8% | 14.2% | 8.2% | 49.1% | 34.5% | 1.1% |
| CV X BORDER (%) | | 2.2% | 12.0% | 15.3% | 2.7% | 39.2% | 9.4% | 7.0% | 29.7% | 37.9% | 27.1% | 22.8% | 5.6% | 5.9% | 22.9% | 20.4% | 50.3% | 54.2% | 7.0% | 14.5% | 14.1% | 38.6% | 33.4% | 8.7% |
| INT | 1 | 7.180 | 0.36 | 16.23 | 288.2 | 1.2 | 15.69 | 18.4 | 1120 | 516 | 48.2 | 665 | 36.2 | 3.22 | 13.2 | 741 | 14.9 | 1.040 | 31.8 | 52.8 | 0.262 | 7.4 | 11.44 | 0.69 |
| | 2 | 7.197 | 0.40 | 17.62 | 287.8 | 1.6 | 13.84 | 18.5 | 1200 | 610 | 72.3 | 862 | 34.2 | 2.99 | 16.3 | 596 | 10.4 | 0.364 | 32.2 | 44.8 | 0.247 | 7.2 | 12.51 | 0.64 |
| | 3 | 7.206 | 0.37 | 17.92 | 288.7 | 1.5 | 14.20 | 18.7 | 1350 | 490 | 77.1 | 860 | 36.0 | 3.00 | 17.7 | 546 | 13.3 | 0.233 | 29.2 | 41.7 | 0.288 | 17.8 | 7.07 | 0.68 |
| | 4 | 7.207 | 0.36 | 15.61 | 284.3 | 1.5 | 14.38 | 18.9 | 1100 | 508 | 52.0 | 635 | 33.9 | 3.02 | 14.8 | 578 | 25.5 | 0.416 | 32.6 | 36.6 | 0.246 | 7.1 | 9.93 | 0.68 |
| CV INT (%) | | 0.2% | 4.7% | 6.0% | 0.6% | 11.0% | 5.1% | 1.1% | 8.8% | 9.3% | 21.3% | 14.9% | 3.1% | 3.3% | 11.5% | 12.9% | 37.8% | 64.5% | 4.5% | 14.2% | 6.9% | 49.3% | 21.2% | 3.1% |
| delta (INT - X BORDER) | | -2.1% | -7.3% | -9.3% | -2.1% | -28.2% | -4.3% | -5.9% | -20.9% | -28.6% | -5.8% | -7.9% | -2.5% | -2.7% | -11.4% | -7.5% | -12.5% | 10.2% | -2.5% | -0.3% | -7.2% | 10.7% | -12.2% | -5.6% |

| | |
|---|---|
| <5% | Excellent |
| 5-7% | Good |
| 7-11% | Acceptable |
| 11-15% | Poor |
| >15% | Bad |

Coefficients of variation per endpoint and per batch produced in each institution (BE; Belgium, IS; Iceland, NL; the Netherlands). The overall coefficient of variation of 12 batches produced in all institutions is labeled "CV X BORDER" and is compared to the CV of the 'international' batch (INT) by calculating the difference between both in the line "delta (INT-X BORDER)"

## Discussion

The use of hPL as an alternative to FBS in cell culture and regenerative medicine has gained significant attention due to their rich growth factor content and ethical advantages [25]. hPL derived from platelet concentrates prepared for transfusion, offers a promising supplement for promoting cell proliferation, differentiation, and tissue regeneration [26]. However, despite their potential, the field faces a critical challenge: the lack of standardization in hPL production, characterization, and application [10, 27]. Variability in donor selection, platelet concentration, preparation methods, pathogen inactivation and storage conditions has led to inconsistencies in hPL composition and performance, hindering reproducibility and comparability across studies. This lack of uniformity poses a significant barrier to the widespread adoption of

hPL in clinical and research settings. Typical standards for 'active pharmaceutical ingredient' dosing and low inter-batch variability are not easy to obtain for cell and gene therapies. Therefore, sources of variation during manufacturing of the therapeutic should be kept as low as possible. As a tissue culture supplement aiming at supporting the cytotherapy field, the need for standardized protocols and quality control measures for hPL is therefore urgent [7].

Most blood establishments must maintain a small surplus of PC to assure steady supply for transfusion. Platelets are vulnerable to expiration [28, 29] because their storage time is limited to days, not weeks. Consequently, the many blood establishments worldwide can collectively provide quite a volume of expired PC. Instead of going to waste, PC may be redirected for hPL production thereby contributing to a circular economy. In addition, when hPL is used for cell therapy purposes, the blood establishment still abides to its commitments to help patients, and to manage donor material in an optimal way. Blood establishments can contribute in broadly two ways, they can either provide the expired PC directly to an hPL manufacturer or they can make hPL themselves. The former is the least burdensome but does not allow maximal return on the intrinsic value of donated material. The latter comes with the challenge of product development and few blood establishments have the resources and time to do this, let alone navigate the regulatory specifics.

A simple, single-use consumable set was developed at Belgian Red Cross-Flanders for the conversion of PC to hPL [17]. This set is compliant to closed system manufacturing, is easy to use and owing to the data obtained in this work now also demonstrates robustness for handling and for geographic location. Blood establishments have various methods of manufacturing PC [10, 18] so it had to be tested if these different input products would fit with the original hPL manufacturing method in all 72 instances of PC to hPL conversion foreseen in the study plan. This was the case showing that different input PC do not influence the flow of hPL manufacturing. The PC from Iceland for instance had small volumes of 187 ± 6 mL compared to the ones from the Netherlands 286 ± 9 mL or Belgium 307 ± 17 and although this impacted the relative hPL volume yield significantly, it did not influence the final hPL chemical and growth factor composition or efficacy. The performance part of the study therefore demonstrates robustness and translatability across different blood establishments of this technology.

The data also show that hPL production in an individual, even small Icelandic, blood establishment is possible since translation from development to implementation and manufacturing was straightforward. In specific cases when national regulatory bodies would require hPL to originate solely from national donors *one PC for one hPL* approach can be an asset [30]. However, the best way to maximize standardization is by pooling source material that is produced at different facilities. We did just that by combining hPL from the participating centers in a pooled 'international' batch series. The data show that the composition and efficacy of this unified hPL is within the orders of magnitude reported in literature [13, 31, 32] and is low in variation. Furthermore, small differences in product composition identified between participating centers, e.g. in glucose or VEGF content, are significantly leveled out in the international batch. This implies that a model whereby blood establishments convert PC to hPL prior to pooling in a central facility is feasible to increase available volumes in a standardized manner. The biggest challenge for scaling hPL production is sufficient control over the value chain: i.e. from donor to manufacturing rights and distribution network. Currently, the value chain of hPL manufacturing often is disrupted because hPL manufacturing is not a core business of blood establishments. This increases cost for end users and risks inventory stockouts.

The primary aim of the study was to assess variability in hPL batches produced within a facility and across facilities. The data show that variation in chemical parameters like pH, ionic strength, glucose and lactate is typically < 15% within participating centers and for the international pools. The contribution to variation of machine and operator is low for these laboratory tests because blood gas analyzers are continuously calibrated and labeled CE-IVD as a point-of-care diagnostic tool. Glucose was higher in Dutch hPL. This is explained by the effect of pathogen inactivation in Belgian and Icelandic platelet concentrates, known to induce decreased glucose concentrations at expiration day [33]. The Dutch blood establishment does not have pathogen inactivation, while the Belgian and Icelandic do. The effect of amotosalen pathogen inactivation on hPL has been shown to be negligible [31, 34]. Although infrequent, the CV for certain growth factors was sometimes > 20%. Contributions to variation are probably not intrinsic to hPL because it was found for certain growth factors only. It is more likely that day to day, machine, non CE-IVD test kit and operator contributions are most prominent. There is no well-established correlation between individual growth factor concentrations and cell expansion ex vivo [27], so cPD of MSC is still the most relevant readout. The data show that for the adipose derived MSC clone that we used, variability

in tissue culture was low. This is based on both the cPD dataset and the cell surface expression of characteristic MSC markers. Initial growth kinetics too, expressed as PD/day had maximal CV of 8.6%. Most importantly, the CV of all outcomes was consistently lower in the international batch (n = 4) compared to the coefficients of variation calculated over the 12 batches produced per institute. This confirms that a pool of hPL prepared from material at different blood establishments is more standardized than the sum of the individual products produced at the individual blood establishments. Direct implications for clinical practice would require trials comparing the effect of variability in culture media on patients outcome. As this is unlikely to be a primary focus of the industry, simply striving for lowest possible variability in hPL batches is the most cost effective.

We chose to perform the study in a blinded way so that none of the researchers involved could not know what batch was being analyzed. In addition, the experimental readouts we chose were performed at the participating centers allowing these to handle the material not only during production but also during an extensive round of quality control. Growth factor multiplexing and HUVEC scratch assays were performed in the Netherlands. MSC differentiation towards adipogenic and osteogenic phenotypes was performed in Iceland. MSC culture and phenotyping by flow cytometry was performed in Belgium. Unblinding of all data was in Belgium only after executing all experiments and data analysis. This is the strength of the study. A limitation is the small number of participating centers, but now that the protocol for this type of studies is ready, we aim to widen the territorial application and test more centers. In addition, next studies will include clinically relevant endpoints like chondrocyte organoid culture.

## Conclusion

Addressing the issue of standardization in hPL presents both challenges and opportunities. On one hand, the inherent biological variability of human-derived products like hPL complicates efforts to establish universal standards. On the other hand, advancements in bioprocessing technologies, analytical methods, and regulatory frameworks offer a path toward harmonization. Collaborative efforts among researchers, industry stakeholders, and regulatory bodies are essential to define critical quality attributes, optimize production processes, and develop robust characterization tools. Progress in this area could not only enhance the reliability of hPL but also accelerate its translation into clinical applications, paving the way for safer and more effective cell-based therapies.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-025-04445-9.

Additional file 1.

**Acknowledgements**
We thank our non-remunerated, voluntary and consenting blood donors for contributing to this study. We thank the Belgian Red Cross Research Foundation for funding. None of the work was outsourced, all data were collected by the authors. The authors declare that they have not used AI-generated work in this manuscript. Belgian Red Cross-Flanders is responsible for supplying adequate quantities of safe blood products to hospitals in Flanders and Brussels on a continuous basis and is financially compensated for this activity.

**Author contributions**
WD, OES, TRLK and HBF designed the study, developed the protocols. WD, SAG, DA and GB executed experiments, assembled and provided materials, analyzed data and wrote the paper. HBF analyzed data, supervised the study, wrote the paper and provided materials. PVDK supervised the study. OES, PVDK and TRLK reviewed the paper.

**Funding**
The study was funded by the Foundation for Research and Development of the Belgian Red Cross Flanders.

**Availability of data and materials**
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval and consent to participate**
Material was obtained from consenting healthy voluntary blood donors. The project was entitled 'Adviesaanvraag voor biobank Rode Kruis Vlaanderen, Versie 3, Amendement 3' approved by the Ethics Institutional Review Board named Universitair Ziekenhuis Leuven with number S62549, dd 22/08/2023. Cells obtained from Lonza (material number PT-5006, batch number 22TL213997) and PromoCell (material number C-12208, batch number 505Z011) were isolated from donated human tissue after obtaining permission for their use in research applications by informed consent or legal authorization.

**Competing interests**
WD, PV and HBF are listed as inventors of pending patent applications for producing platelet lysates. All authors are employed by the Belgian Red Cross-Flanders.

Received: 11 April 2025 Accepted: 10 June 2025
Published online: 01 July 2025

**References**

1. Palombella S, Perucca Orfei C, Castellini G, Gianola S, Lopa S, Mastrogiacomo M, et al. Systematic review and meta-analysis on the use of human platelet lysate for mesenchymal stem cell cultures: comparison with fetal bovine serum and considerations on the production protocol. Stem Cell Res Ther. 2022;13(1):142.
2. Jochems CE, van der Valk JB, Stafleu FR, Baumans V. The use of fetal bovine serum: ethical or scientific problem? Altern Lab Anim ATLA. 2002;30(2):219–27.
3. van der Valk J, Brunner D, De Smet K, Fex Svenningsen A, Honegger P, Knudsen LE, et al. Optimization of chemically defined cell culture media–replacing fetal bovine serum in mammalian in vitro methods. Toxicol Vitro Int J Publ Assoc BIBRA. 2010;24(4):1053–63.

4. Erickson GA, Bolin SR, Landgraf JG. Viral contamination of fetal bovine serum used for tissue culture: risks and concerns. Dev Biol Stand. 1991;75:173–5.
5. Sakamoto N, Tsuji K, Muul LM, Lawler AM, Petricoin EF, Candotti F, et al. Bovine apolipoprotein B-100 is a dominant immunogen in therapeutic cell populations cultured in fetal calf serum in mice and humans. Blood. 2007;110(2):501–8.
6. Brindley DA, Davie NL, Culme-Seymour EJ, Mason C, Smith DW, Rowley JA. Peak serum: implications of serum supply for cell therapy manufacturing. Regen Med. 2012;7(1):7–13.
7. De Korte D, Delabie W, Feys HB, Klei T, Larsen R, Sigurjónsson Ó, et al. Towards standardized human platelet lysate production in Europe: an initiative of the European blood alliance. Vox sanguinis. 2023.
8. Hemeda H, Giebel B, Wagner W. Evaluation of human platelet lysate versus fetal bovine serum for culture of mesenchymal stromal cells. Cytotherapy. 2014;16(2):170–80.
9. Yeh WT, Yu EY, Lu YH, Livkisa D, Burnouf T, Lundy DJ. Bioprocessing of human platelet concentrates to generate lysates and extracellular vesicles for therapeutic applications. MethodsX. 2024;13:102822.
10. Strunk D, Lozano M, Marks DC, Loh YS, Gstraunthaler G, Schennach H, et al. International Forum on GMP-grade human platelet lysate for cell propagation. Vox Sang. 2018;113(1):e1–25.
11. Hesler M, Kohl Y, Wagner S, von Briesen H. Non-pooled human platelet lysate: a potential serum alternative for in vitro cell culture. Altern Lab Anim ATLA. 2019;47(3–4):116–27.
12. Schallmoser K, Strunk D. Preparation of pooled human platelet lysate (pHPL) as an efficient supplement for animal serum-free human stem cell cultures. J Vis Exp. 2009;32:e1523.
13. Viau S, Lagrange A, Chabrand L, Lorant J, Charrier M, Rouger K, et al. A highly standardized and characterized human platelet lysate for efficient and reproducible expansion of human bone marrow mesenchymal stromal cells. Cytotherapy. 2019;21(7):738–54.
14. Pierce J, Benedetti E, Preslar A, Jacobson P, Jin P, Stroncek DF, et al. Comparative analyses of industrial-scale human platelet lysate preparations. Transfusion. 2017;57(12):2858–69.
15. Bianchetti A, Chinello C, Guindani M, Braga S, Neva A, Verardi R, et al. A blood bank standardized production of human platelet lysate for mesenchymal stromal cell expansion: proteomic characterization and biological effects. Front Cell Dev Biol. 2021;9:650490.
16. Henschler R, Gabriel C, Schallmoser K, Burnouf T, Koh MBC. Human platelet lysate current standards and future developments. Transfusion. 2019;59(4):1407–13.
17. Delabie W, De Bleser D, Vandewalle V, Vandekerckhove P, Compernolle V, Feys HB. Single step method for high yield human platelet lysate production. Transfusion. 2022.
18. Hardwick J. Blood processing. ISBT Sci Ser. 2008;3(2):148–76.
19. Van Aelst B, Bens K, Sabot B, Vertongen S, Feys HB, Compernolle V, et al. Platelet additive solutions SSP+ and T-PAS+ Are interchangeable for platelet concentrate storage despite differences in composition and plasticizer. Transf Med Hemother Off Organ Deutsch Ges Transf Immunhamatol. 2024;51(6):424–9.
20. Lin L, Cook DN, Wiesehahn GP, Alfonso R, Behrman B, Cimino GD, et al. Photochemical inactivation of viruses and bacteria in platelet concentrates by use of a novel psoralen and long-wavelength ultraviolet light. Transfusion. 1997;37(4):423–35.
21. Petrenko Y, Vackova I, Kekulova K, Chudickova M, Koci Z, Turnovcova K, et al. A Comparative analysis of multipotent mesenchymal stromal cells derived from different sources, with a focus on neuroregenerative potential. Sci Rep. 2020;10(1):4290.
22. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. Cytotherapy. 2006;8(4):315–7.
23. Bertolini F, Murphy S. Biomedical excellence for safer transfusion working party of the international society of blood T. A multicenter inspection of the swirling phenomenon in platelet concentrates prepared in routine practice. Transfusion. 1996;36(2):128–32.
24. Gottipamula S, Muttigi MS, Kolkundkar U, Seetharam RN. Serum-free media for the production of human mesenchymal stromal cells: a review. Cell Prolif. 2013;46(6):608–27.
25. Doucet C, Ernou I, Zhang Y, Llense JR, Begot L, Holy X, et al. Platelet lysates promote mesenchymal stem cell expansion: a safety substitute for animal serum in cell-based therapy applications. J Cell Physiol. 2005;205(2):228–36.
26. Bieback K. Platelet lysate as replacement for fetal bovine serum in mesenchymal stromal cell cultures. Transf Med hemother Off Organ Deutsch Ges Transf Immunhamatol. 2013;40(5):326–35.
27. Bieback K, Fernandez-Munoz B, Pati S, Schafer R. 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 of cell therapy. Cytotherapy. 2019.
28. Flint AW, McQuilten ZK, Irwin G, Rushford K, Haysom HE, Wood EM. Is platelet expiring out of date? A Systematic Review Transfus Med Rev. 2020;34(1):42–50.
29. Veihola M, Aroviita P, Linna M, Sintonen H, Kekomaki R. Variation of platelet production and discard rates in 17 blood centers representing 10 European countries from 2000 to 2002. Transfusion. 2006;46(6):991–5.
30. Oeller M, Laner-Plamberger S, Krisch L, Rohde E, Strunk D, Schallmoser K. Human platelet lysate for good manufacturing practice-compliant cell production. Int J Mol Sci. 2021;22(10).
31. Christensen C, Jonsdottir-Buch SM, Sigurjonsson OE. Effects of amotosalen treatment on human platelet lysate bioactivity: a proof-of-concept study. PLoS ONE. 2020;15(4):e0220163.
32. Kinzebach S, Dietz L, Kluter H, Thierse HJ, Bieback K. Functional and differential proteomic analyses to identify platelet derived factors affecting ex vivo expansion of mesenchymal stromal cells. BMC Cell Biol. 2013;14:48.
33. Feys HB, Devloo R, Sabot B, De Pourcq K, Coene J, Compernolle V. High platelet content can increase storage lesion rates following Intercept pathogen inactivation primarily in platelet concentrates prepared by apheresis. Vox Sang. 2017;112(8):751–8.
34. Delabie W, De Bleser D, Vandewalle V, De Prest ML, Vandekerckhove P, Compernolle V, et al. The impact of amotosalen photochemical pathogen inactivation on human platelet lysate. Curr Stem Cell Res Ther. 2025;20(2):218–27.


## Publisher's Note