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Comparison of culture media supplements identifies serum components in self-reported serum-free preparations

Willem Delabie[1], Vicky Vandewalle[1], Sabrina Seghers[1], Dominique De Bleser[2,3], Philippe Vandekerckhove[3,4,5] and Hendrik B. Feys[1,3,6*]

Abstract

Background Cell culture media are essential for cell expansion and many cells still depend on blood-derived supplements for optimal growth. From a regulatory perspective, these materials are ideally xeno-free, serum-free or even chemically defined. However, differences in composition and in performance are seldom clear from the terminology used in this field.

Methods Therefore, we set out to investigate the presence of serum in seven serum-free media (SFM) and link that information to the performance and cost. We used fetal bovine serum and five human platelet lysate preparations (hPL) for comparison.

Results Our data show significant differences in growth factor content between categories, but this did not correlate with mesenchymal stem cell (MSC) growth kinetics or maximal cell yield. Myeloperoxidase, glycocalicin and fibrinogen, derived from human leukocytes, platelets and plasma were detected at significant levels in 2 from 7 SFM. MSC cultured in these two SFM had a CD44-phenotype akin to hPL, essentially reclassifying them as hPL. Most SFM supported MSC expansion well, but some did not. In contract, all hPL supported MSC growth. The cost of SFM is significantly higher than hPL.

Conclusions We conclude that terminology regarding serum presence can be misleading. The cost-performance balance is best for hPL at this moment.

Keywords Serum-free media, Human platelet lysate, Cell therapy, Mesenchymal stem cells, Culture media

*Correspondence:
Hendrik B. Feys
hendrik.feys@rodekruis.be
[1]Transfusion Research Center, Belgian Red Cross Flanders, Ottergemsesteenweg 413, Ghent 9000, Belgium
[2]Transfusion Innovation Center, Belgian Red Cross Flanders, Ottergemsesteenweg 413, Ghent 9000, Belgium
[3]Blood Services, Belgian Red Cross Flanders, Motstraat 40, Mechelen 2800, Belgium
[4]Department of Public Health and Primary Care, Leuven Institute for Healthcare Policy, KULeuven, Herestraat 49, Leuven 3000, Belgium
[5]Department of Global Health, Faculty of Medicine and Health Sciences, Stellenbosch University, 49 Victoria Street, Cape Town 7600, South Africa
[6]Department of Diagnostic Sciences, Faculty of Medicine and Healthy Sciences, Ghent University, C. Heymanslaan 10, Ghent 9000, Belgium

Introduction

Tissue culture and cell expansion are foundational techniques in both basic biological research and applied biomedical sciences, including regenerative medicine, drug discovery, and biopharmaceutical production. The success of these techniques hinges on the ability to provide an optimal culture environment, which is largely determined by the composition of the culture medium. Among the critical components of culture media are supplements that provide essential nutrients, growth factors, and other signaling molecules necessary for cell survival, proliferation, and differentiation. As such, the culture medium and the actual cell culture practice often are the most expensive part of cell therapy manufacturing.

We distinguish three commonly used culture media or culture media supplements: serum-free media (SFM), human platelet lysate (hPL), and fetal bovine serum (FBS). Each of these supplements has distinct advantages and disadvantages that influence their suitability for various applications [1]. SFM can contain purified blood-derived components such as growth factors or albumin, but do not contain non-purified serum. Hence the chemical composition is known, and batch-to-batch variability is theoretically reduced. Some SFM are formulated from exactly known quantities of recombinant components, allowing for full and precise control over the culture environment and eliminating the risk of introducing undefined biological contaminants [2]. These products are termed chemically-defined media. Because of vague terminology used by manufacturers and the lack of consensus on nomenclature, it is often unclear whether SFM are always 'chemically defined'.

Human platelet lysates are derived from human platelets, often surplus platelet concentrates initially prepared for transfusion. These are rich in growth factors that support cell proliferation and tissue repair. They offer a xeno-free alternative to FBS with reduced immunogenicity and ethical concerns, making them an attractive option for clinical applications [3]. However, batch consistency and supply remain significant challenges [4]. Fetal bovine serum, the standard supplement for decades, provides a rich and complex mixture of growth factors, hormones, and extracellular matrix components that support the growth of a wide variety of cell types. Despite its efficacy, FBS is associated with considerable ethical concerns [5], including animal welfare issues, and practical challenges, such as batch-to-batch variability and the risk of contamination with bovine pathogens.

The plethora of cell culture media supplements that are available on the commercial market is hard to navigate for researchers and developers. All the more so because manufacturers prefer to keep the contents of their products hidden under the pretext of trade secrets. This paper studies the composition and the efficacy of several commercially available supplements. These are compared to several hPL preparations [6] and FBS. Understanding the trade-offs associated with each supplement is crucial for optimizing cell culture conditions and ensuring reproducibility and affordability in both research and clinical settings.

Materials and methods

Study design

We procured 13 different cell culture media or cell culture media supplements between October 2022 and December 2023. Included were 5 different hPL and 7 SFM. As a reference, two batches of HyClone MSC-screened FBS were included as a reference condition. A single batch of every test supplement was used, except for hPL1 and hPL2 that had five batches included. Catalogue numbers, commercial names and batch numbers are given in Additional file 3, Table 1. The leaflets or product inserts of every tested product are included in the Additional file 4.

Preparation of hPL gold and platinum

The in-house manufacturing of hPL2 was as previously described [6] starting from surplus platelet concentrates (PC) treated with pathogen inactivation [7, 8]. The in-house manufacturing of hPL1 used surplus buffy coats as starting material. Both platelet concentrates and buffy coats were obtained from consenting voluntary donors from the Belgian Red Cross Flanders Blood Establishment. Belgian biobank regulations were followed. All buffy coats and PC used were registered in the biobank with accession number (BB190034) and approval of the University Hospital Institutional Review board (UZ/KU Leuven, S62549).

Biophysical and biochemical analysis

To record hPL turbidity, light transmittance was measured in raw culture supplements 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 A680nm is the compound of light absorbance and light scattering of an undiluted sample measured at a wavelength of 680 nm and light path length of 1 cm. Concentrations of glucose, lactate and Ca^{2+} were measured in both raw culture supplements 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 in raw culture supplements by glass electrode (HI 5221, Hanna Instruments, Woonsocket, RI). Total protein was measured using a bicinchoninic acid (BCA) assay kit (Thermo Fisher Scientific) with a bovine serum albumin (BSA) standard of known concentration.

Growth factor concentration

Concentrations of insulin-like growth factor (IGF)-1, platelet-derived growth factor (PDGF)-AB, transforming growth factor beta 1 (TGF-ß1) and vascular endothelial growth factor (VEGF) were measured in undiluted culture supplements using a quantitative sandwich-type enzyme linked immunosorbent assay (ELISA) following the instructions of the manufacturer (R&D Systems, Minneapolis, MN). In brief, samples were serially diluted in calibrator diluent and added to a 96-well plate that was coated with monoclonal antibodies against the above mentioned antigens. For IGF-1 analysis, samples were first diluted in an acidic dissociation solution and a blue dyed buffer. For TGF-ß1 analysis, the sample was first activated by addition of 1.0 M HCl and neutralized with 1.2 M NaOH and 0.5 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Detection was with a polyclonal anti-human antibody specific for the growth factor of interest conjugated with horseradish peroxidase. Chromogenic substrate solution was tetramethylbenzidine (TMB) and hydrogen peroxide. Resulting optical densities at a wavelength of 450 nm were recorded using a multiwell plate reader (Infinite F200 Pro, Tecan).

Fibrinogen

Fibrinogen was quantified in undiluted culture supplements using ELISA (Innovative Research, Novi, MI) according to the manufacturer's instructions. Samples were added to the pre-coated 96-well plate and incubated at 20–24 °C for 30 min on a horizontal orbital microplate shaker. Polyclonal anti-human fibrinogen primary biotinylated antibody was added and incubated at 20–24 °C for 30 min. Detection of bound antibody was with horseradish peroxidase-conjugated streptavidin. Detection was as described for the growth factor's ELISA.

Myeloperoxidase activity

Culture supplements were diluted in Dulbecco's phosphate buffered saline (PBS) and transferred to a 96-well plate. The substrate solution included 15µM 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma-Aldrich, Saint-Louis, MO) and 0.03% (vol/vol) H_2O_2 (Merck, Darmstadt, Germany) in citrate buffer (100mM, pH4.2). This substrate solution was mixed in a 1:1 ratio with the diluted samples. Myeloperoxidase (MPO) acts as a catalyst for ABTS oxidation by H_2O_2 yielding a colored solution within minutes. Absorbance at 405 nm was measured every minute during 30 min to track enzyme kinetics. The slope of the linear portion was plotted and compared to the MPO standard (Abcam, Cambridge, United Kingdom).

Glycocalicin

An in-house prepared ELISA was used to quantify glycocalicin concentration in raw culture supplements. High-binding transparent 96-well plates (Greiner Bio-One) were coated with a 5 µg/mL CD42b monoclonal capture antibody (clone AK2, Invitrogen, Carlsbad, CA) in Tris-buffered saline with 0.1% (vol/vol) Tween 20 (TBS-T, Sigma-Aldrich) and incubated overnight at 4 °C. Blocking was with 1.0% (wt/vol) BSA (Sigma-Aldrich) in TBS-T at room temperature (RT) for 2 h. Purified glycocalicin and samples were serially diluted in TBS-T containing 0.5% (wt/vol) BSA and incubated at RT for 1 h. Next, biotin-labelled mouse anti-CD42b (clone VM16d, Monosan, Uden, The Netherlands) was incubated at 1 µg/mL in TBS-T with 0.5% (wt/vol) BSA for 1 h at RT. Bound antibody was developed using secondary horseradish peroxidase-conjugated streptavidin and colorimetric development with TMB.

Cell expansion

Each culture medium was prepared and used following the manufacturer's instruction. hPL and FBS were used at 10% (vol/vol) in the respective corresponding basal medium [Additional file 3], each hPL was also tested at a dilution of 5% (vol/vol). Heparin (Zen-Bio) was added at 2 U/ml to medium supplemented with hPL5. Prior to use, all complete media were supplemented with penicillin-streptomycin at 100 U/mL (Thermo Fisher Scientific) and subsequently sterile filtered (0.2 µm). In the case of SFM1, SFM3 and SFM4 culture flask precoating was required. Three different clones of human adipose-derived mesenchymal stem cells were cultured. Clones 1 and 2 were purchased from a commercial source (MSC, Lonza, Basel, Switzerland) and clone 3 was isolated in house by mechanical disruption of adipose tissue from liposuction. For culturing with clones 2 and 3 only one batch of each hPL and FBS was tested and at 10% supplementation only. Seeding was in tissue culture flasks at a density 4,000 cells/cm^2 at 37 °C and 5.0% partial CO_2 pressure. Cell dissociation was performed at 80–90% confluence using trypsin-ethylenediamine tetraacetic acid (EDTA) or using the provider's reagents. MSC were kept in culture for a maximum of 113 days or until the culture no longer surpassed 80% confluence within 14 days of culture. For clones 2 and 3 culturing was for a maximum of 35 days. Detailed culturing schedules are shown in additional file 2, figure S7 and S8. 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:: $cPD = cPD_0 + 3.322 * (\log(N_1) - (\log(N_0))$ [9]. Detailed materials and methods are given in additional file 1.

Flow cytometric phenotyping

At cPD 8 and cPD 16 cell phenotyping was performed in flow cytometry according to the International Society for Cellular Therapy (ISCT) guidelines using an acoustic focusing flow cytometer (Attune, Life Technologies, Carlsbad, CA) [10]. The MSC analysis kit (BD Biosciences, Franklin Lakes, NJ) with premixed antibody cocktails was used containing anti-CD90 fluorescein isothiocyanate (FITC), anti-CD105 peridinin-chlorophyll-protein complex-cyanine5.5 (PerCP-Cy5.5), anti-CD73 allophycocyanin (APC). Exclusion markers were anti-CD34, anti-CD11b, anti-CD19, anti-CD45 and anti-HLA-DR, all labeled with PE. A separate tube for CD44 analyses was prepared with anti-CD44 phycoerythrin (PE). Isotype antibody controls were included as a background negative reference.

Costing comparison of cell culture media

The cost of a representative MSC therapy was calculated and is described in detail in additional file 1. In brief, the amount of cell culture media and culture duration was used to calculate the cost associated with expanding MSC's to obtain 1.5 million cells per kg body weight. We included data from ten Ham et al. to refine the costing model and include the essential parameters that constitute the preparation of a MSC therapy [11].

Results

Biochemical content and appearance of supplements and complete media

The pH was between 6.0 and 8.0 in all test samples (Fig. 1A). Ionized calcium was variable and was highest in hPL1 and SFM2 at 1.4 and 1.9 mM respectively (Fig. 1B). Ionized calcium was below the detection limit of 0.2 mM in two hPL and three SFM. Glucose was also variable and highest in SFM7 at 16.0 mM (Fig. 1C). Glucose was below the detection limit of 1.1 mM in one hPL and three SFM. Lactate was > 10 mM in hPL and FBS but undetectable in four SFM (Fig. 1D). Osmolality of all supplements ranged between 235 mOsm/kg and 323 mOsm/kg (Fig. 1E). The same analysis in complete media shows that osmolality, ionized calcium, glucose, and lactate are dependent on the components present in basal medium. The contribution of the supplement is negligible (Fig. 1 and Additional file 2, figure S1). Only SFM2 and hPL1 had a turbidity > 0.5 (Additional file 2, figure S2); however, this did not impact filterability of complete media using a 0.2 µm sterile filter.

Protein content in supplements and complete media

The concentration of growth factors was at least double in hPL1 compared to all other supplements (Fig. 2 and Additional file 2, figure S3). Growth factor content in hPL2, hPL3 and hPL4 was comparable, but hPL5 consistently contained half that of the other hPL. SFM contained undetectable or very low concentrations of growth factors. Only IGF-I was significantly dosed in SFM4 and comparable to the level in hPL. TGF-β1 was found in SFM1, SFM5 and SFM6 at lower levels compared to hPL.

MPO is an enzyme present in neutrophilic granulocytes and released upon cell activation. Its presence indicates white blood cell activation and was used to identify a human blood cell origin of the media or supplements. MPO activity was highest in hPL1 which is derived from human buffy coats that are rich in white cells and served as a positive control. MPO was furthermore found at significant levels in hPL2, hPL3, hPL4, SFM2 and SFM5 (Fig. 3). Low MPO was found in hPL5, SFM3 and FBS. No MPO activity was detected in SFM1, SFM4, SFM6 and SFM7 implying these are the only media (supplements) that are not derived from leukocyte-containing source products. In a similar manner, the origin of platelets was identified by means of glycocalicin measurements. As expected, all hPL supplements contained measurable quantities of glycocalicin (Fig. 4). However, also SFM2 and SFM5 contained glycocalicin and are therefore derived from platelet-containing source products. Glycocalicin was absent in SFM1, SFM3, SFM4, SFM6 and SFM7.

Fibrinogen indicates a source of human plasma but can cause coagulation of cell culture media. Some manufacturers therefore require the use of anticoagulation, by addition of heparin to the culture medium. For this reason, hPL manufacturers often include a fibrinogen reduction step during hPL preparation. Human fibrinogen was < 5 µg/mL in hPL1, hPL2 and hPL4 which is a thousand-fold lower than normal human plasma (Additional file 2, figure S4). Fibrinogen in hPL3 was 64 µg/mL and 436 µg/mL in hPL5. Fibrinogen was also detected in SFM5 at 9.2 µg/mL and in SFM2 albeit at a very low concentration of 0.3 µg/mL.

Total protein was measured in raw supplements using a bicinchoninic acid assay (Additional file 2, figure S5) and calculated to final concentration in complete medium (Fig. 5). Protein concentration was highest in SFM4 at 14.0 g/L. hPL1, hPL3 and hPL4 contained 6 g/L which corresponds to normal plasma or to platelet concentrates suspended in plasma. hPL2 is derived from platelet concentrates in 30% (vol/vol) buffered additive solution and therefore total protein was one third of hPL1 prepared from buffy coats. Total protein concentrations in all remaining SFM were below 1.4 g/L.

MSC growth kinetics and phenotype during cell culture

For MSC clone 1, cells were expanded in vitro according to the manufacturer's instructions for a total of 113 days or until cells ceased expanding. MSC cultured in SFM1

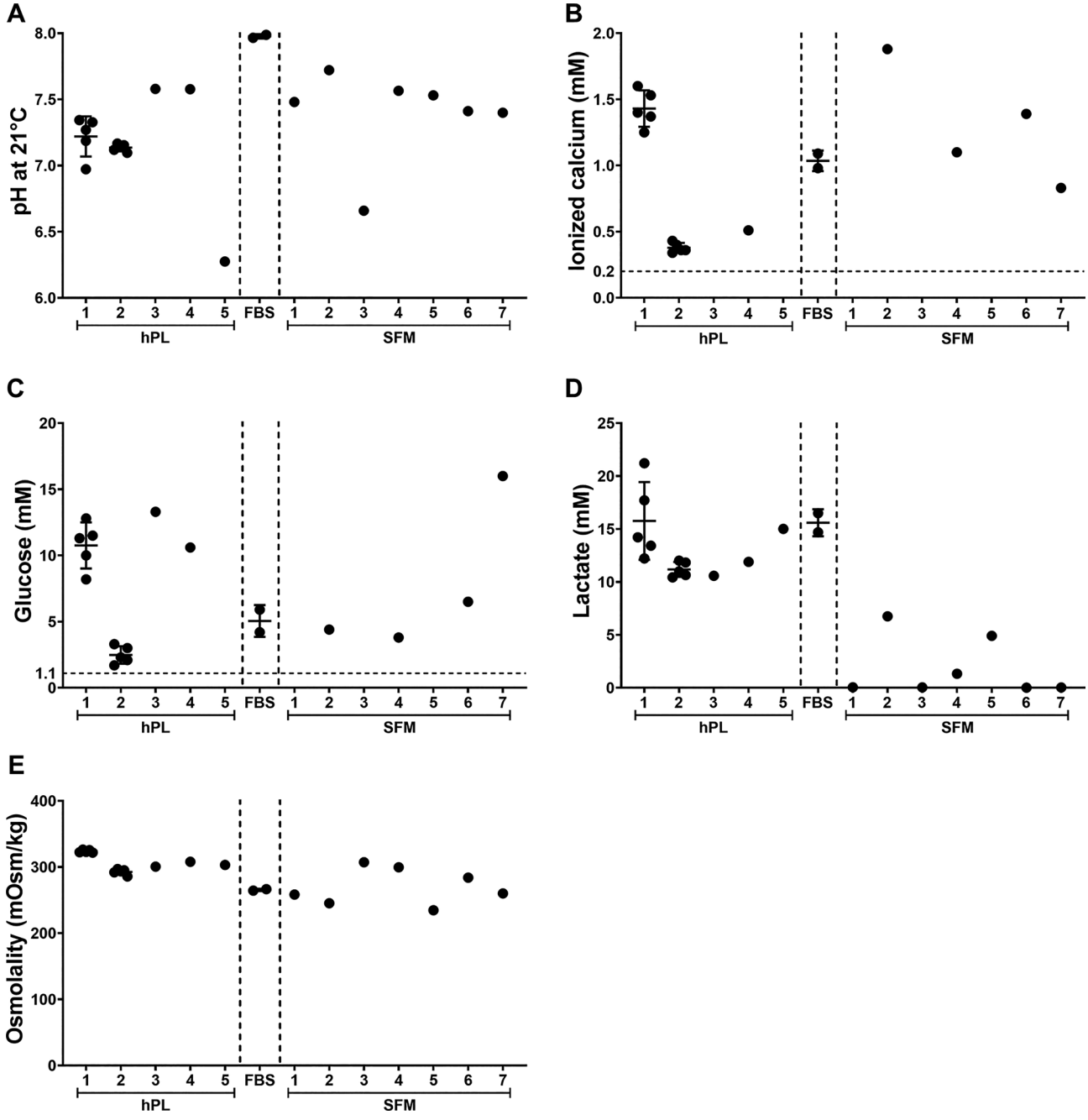

Fig. 1 (Bio)chemical parameters in supplements. (**A**) pH, (**B**) ionized calcium, (**C**) glucose concentration, (**D**) lactate concentration and (**E**) osmolality were measured in the raw supplements or in complete medium for SFM6 and SFM7. Vertical dotted lines separate the study groups. Horizontal dotted lines represent the limit of detection of the assay and was 0.2 mM for ionized calcium and 1.1 mM for glucose concentration. Individual data are shown and error bars represent mean with standard deviation

and in hPL5 (5%) ceased expansion at day 11 and 21 respectively. MSC in SFM2 expanded significantly faster than all other conditions (Fig. 6A and Interactive Fig. 6 Supplemtary material 5 was slowest in FBS. All remaining media performed broadly within the same range. For MSC clones 2 and 3, cells cultured in hPL5 did not expand. The initial growth kinetics of all clones defined as the slope over the initial cPD before the growth curve become non-linear, were independent of hPL dosing (Fig. 6B). Initial growth kinetics was faster in SFM compared to hPL and FBS, but the total number of cPD at day 21 (Fig. 6C) was only higher for SFM1, 2 and 5. Clone-to-clone variation was lowest in hPL1, SFM2, and SFM5.

Cell cultures of clone 1 were phenotyped at cPD8 and cPD16 in flow cytometry. Cells from all conditions and at both time points expressed CD90 (Fig. 7) but only MSC

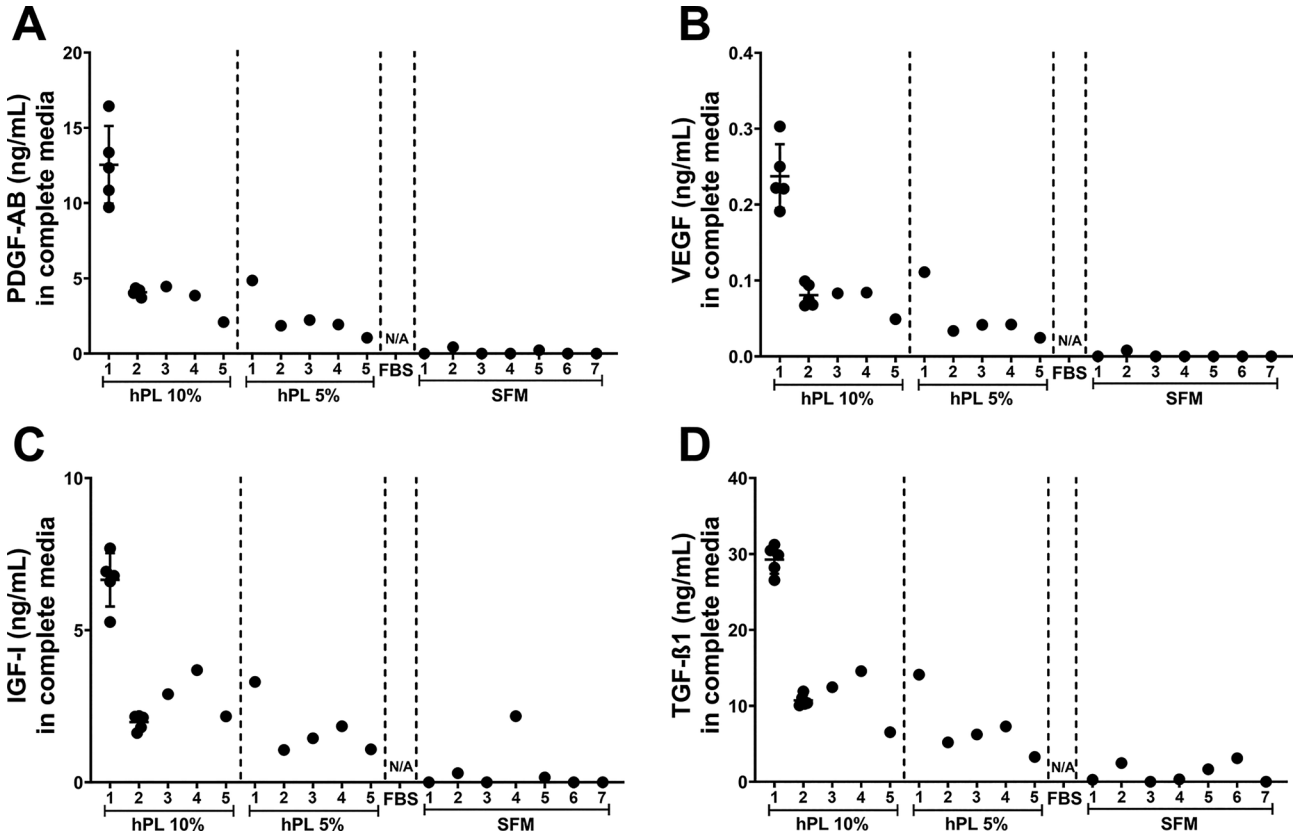

Fig. 2 Growth factor content in complete media. ELISA was used to quantify human (**A**) PDGF-AB, (**B**) VEGF, (**C**) IGF-I and (**D**) TGF-ß1 concentrations in cell culture supplements and subsequently calculated to the final concentration in complete media taking into account to the dilution of supplement in basal media. Vertical dotted lines separate the study groups. Individual data are shown and error bars represent mean with standard deviation. No data could be obtained for FBS

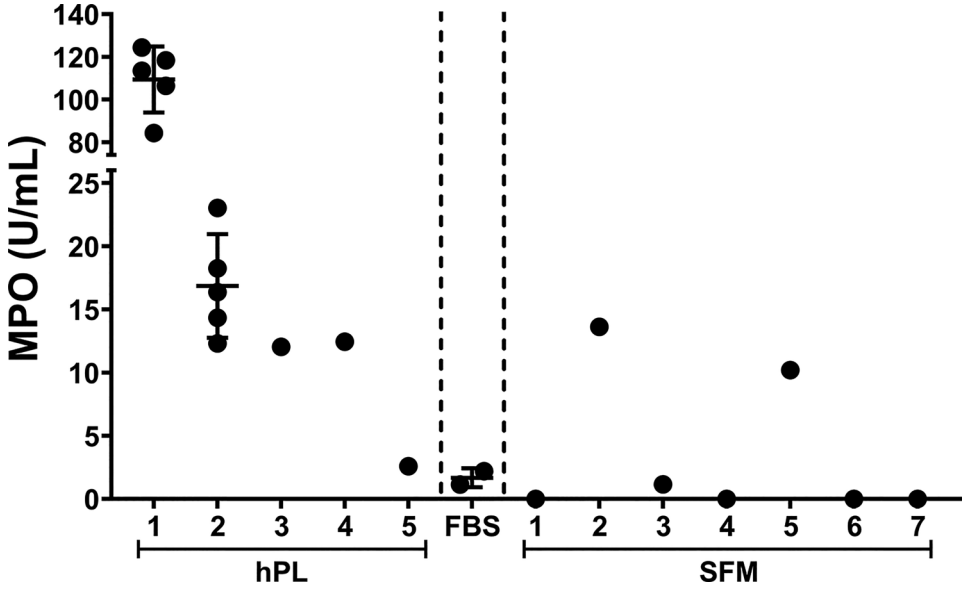

Fig. 3 Myeloperoxidase activity in supplements. ABTS was used as chromogenic substrate to determine MPO activity in cell culture supplements and in complete medium for SFM6 and SFM7. Vertical dotted lines separate the study groups. Individual data are shown and error bars represent mean with standard deviation

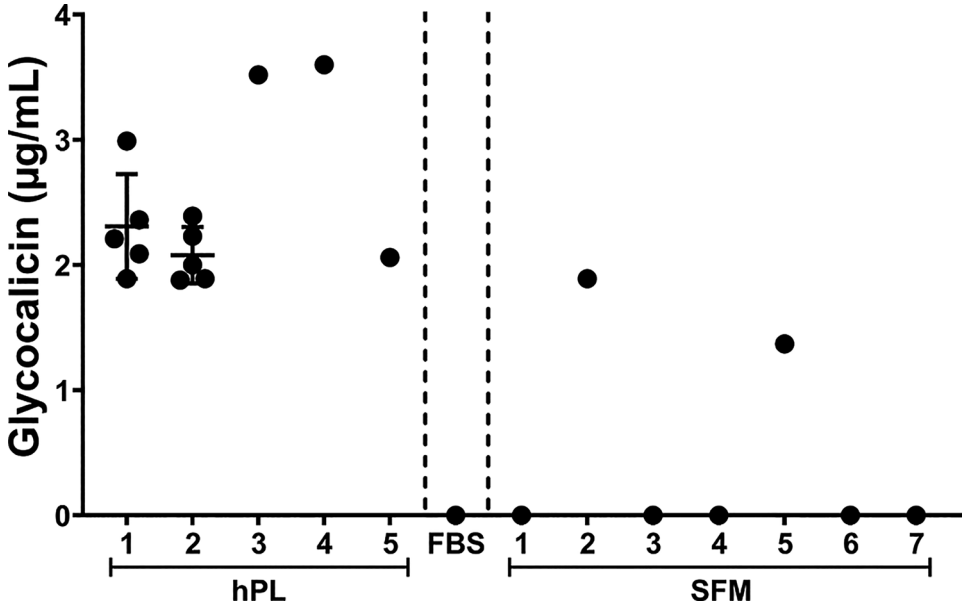

Fig. 4 Glycocalicin concentration in supplements. ELISA was used to determine glycocalicin concentrations in cell culture supplements and in complete medium for SFM6 and SFM7. Vertical dotted lines separate the study groups for clarity. Individual data are shown and error bars represent mean with standard deviation

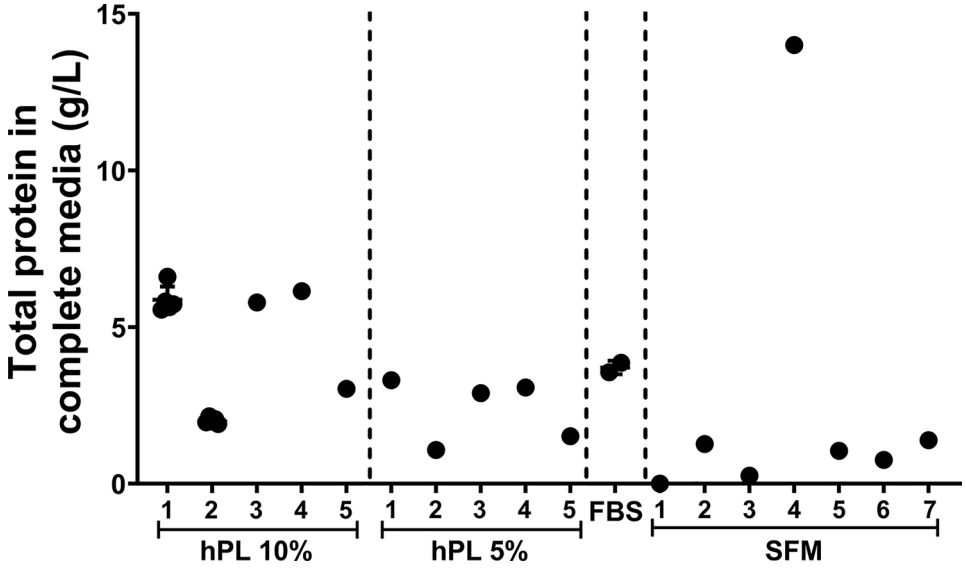

Fig. 5 Total protein in complete media. A bicinchoninic acid assay was used to determine total protein concentration in culture supplements and was subsequently calculated to the final concentration in complete media taking into account the dilution of supplement in basal media. Vertical dotted lines separate the study groups. Individual data are shown and error bars represent mean with standard deviation

in FBS maintained expression of CD105. Expression of CD105 was reduced in the absence of FBS although median fluorescence intensity was always higher than isotype control suggesting that CD105 expression was not zero (Additional file 2, figure S6). Similarly, MSC maintained expression of CD73 and CD44 although some conditions caused reduced expression at either cPD8 or cPD16.

Cost comparison of media and hypothetical cell therapies

Based on list prices from providers, the cost per milliliter of medium was calculated (Fig. 8A). This cost included growth factor supplement, basal medium and coating solution. Not included were costs of auxiliary reagents that are required for successful culture, e.g. DPBS or proprietary detachment solutions. The price was highest for SFM2 and lowest for FBS. We also modelled the cost for a single dose cell therapy using our experimentally derived

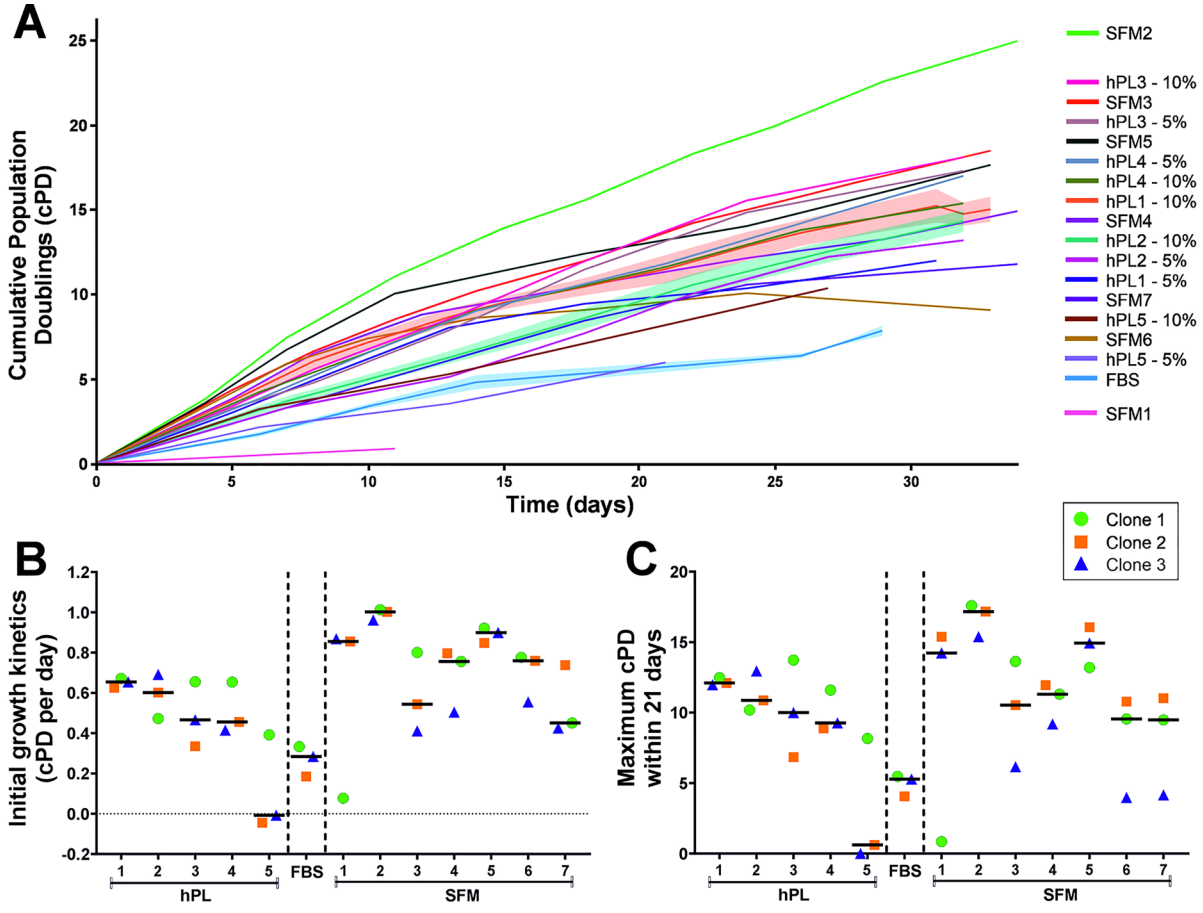

Fig. 6 Cumulative MSC doublings. (**A**) MSC were cultured in the different cell culture media and growth kinetics are represented as cumulative population doublings as a function of time. MSC clone 1 is shown as culturing was performed with more conditions (also 5% hPL) and this clone had lowest intrinsic variation. The Interactive version of the figure allows to separately open or close datasets. MSC expansion in hPL5-5% and SFM1 stopped in 21 days or less providing incomplete data. All curves represent mean values and for hPL1 10% ($n=5$), hPL2 10% ($n=5$) and FBS ($n=2$), shading represents standard deviation. (**B**) Initial growth kinetics were calculated for all three cultured clones by determining the slope of the linear portion of the growth curve in the beginning of expansion and is shown as mean ± standard deviation. (**C**) The maximal cPD reached within 21 days defined as Maximum cPD is depicted

growth kinetics and data from ten Ham et al. [11]. Cell culture duration and cost of media or media supplements weighed in on the costing model compared to staff and equipment costs (Fig. 8B). A cell therapy based on expansion in SFM4 or SFM7 would be most expensive at €83,500 and €70,000 respectively. A cell therapy prepared in FBS, in hPL1-4 at 5% or SFM5 would be least expensive, i.e. €24,500–35,500. The driving factor of cost differs per media or media supplement. Where SFM are more expensive due to a high procurement cost, the cost of FBS is caused by longer expansion times which increased bench time and staff cost. Consequently, a cell therapy based on the least expensive medium supplement (FBS) does not necessarily lead to the least expensive total production cost.

Discussion

Many different cell culture media (supplements) are available. For some of these back to back experimental comparisons have been published [12–14] summarized in a recent meta-analysis [15], but choosing a suitable supplement based only on the manufacturer's information remains a challenge. For advanced therapeutic medicinal products (ATMP), good manufacturing practices nevertheless require maximal disclosure of the content in raw materials used. Most manufacturers however operate in a grey zone between the highly regulated pharmaceutical industry and the less strict supply chain of typical research-grade materials. In addition, although chemically-defined medium (CDM) is considered the holy grail, in practice CDM or SFM are ill-defined. This is because essential growth factors require recombinant production and thus are derived from conditioned cell culture media themselves. Not to mention that primary

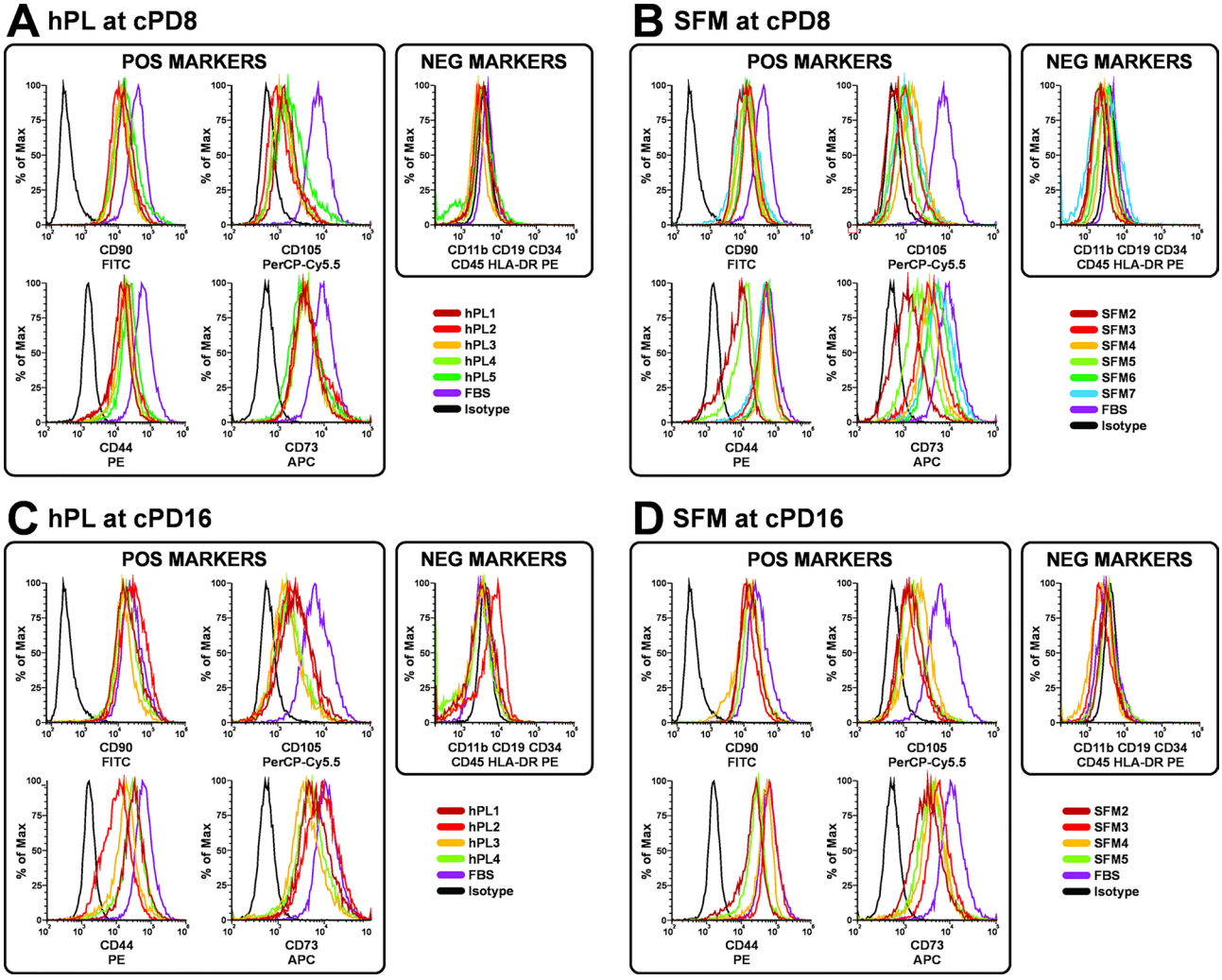

Fig. 7 MSC phenotype during culture. At cPD8 and cPD16 MSC were phenotyped using flow cytometry. (**A**) and (**C**) show data for MSC cultured in 10% hPL at cPD8 and cPD16, respectively. (**B**) and (**D**) show data for MSC cultured in SFM at cPD8 and cPD16, respectively. Cells cultured in SFM1 which did not reach cPD8. Cells in hPL5 did not reach cPD16. Cells in SFM1, SFM6 and SFM7 did not reach cPD16. Controls were MSC cultured in FBS-containing medium (purple) and pre-study-recorded MSC cultured in FBS-containing medium and stained with isotype control (black)

cells often expand poorly in the absence of blood-derived components.

Because terminologies are not regulated, vendors offer products that are attractively labeled as *xeno-free*, *serum-free*, *clinical grade*, *GMP*, *chemically defined* and alike. Therefore, in our study we set out to investigate a selection of SFM for the presence of human blood components and what this means for MSC expansion, phenotype and cell therapy cost.

We identified leukocyte- and platelet-specific molecules in SFM2 and SFM5. Although reportedly serum-free, these SFM must have been supplemented with human blood components because there is no scientific rationale to supplement a culture medium with MPO nor glycocalicin. Therefore, these molecules are present as a consequence of a preparation step that involved blood, leukocytes and/or platelets. This preparation must have involved a plasma to serum conversion given the low fibrinogen concentrations. Hence, these media are not serum-free. Cell growth kinetics and maximum cPD within 21 days were highest in SFM2 and SFM5, but these SFM need to be recategorized as hPL. On that same line, CD44 expression of MSC in SFM2 and – 5 was similar to those in hPL but different from the non-blood derived SFM3 and – 4. Differences in cell properties in response to culture media have been reported before [1, 16]. Of note, SFM2 has been recommended as a 'GMP compliant' alternative to hPL [17] but given its composition revealed in our study, this seems incorrect. Similarly, SFM5 was reported as a xeno- and serum-free alternative by Hoang et al. and as 'synthetic media' by Lensch et al. [18, 19]. Consequently, we suggest manufacturers to disclose when blood components are present so comparison

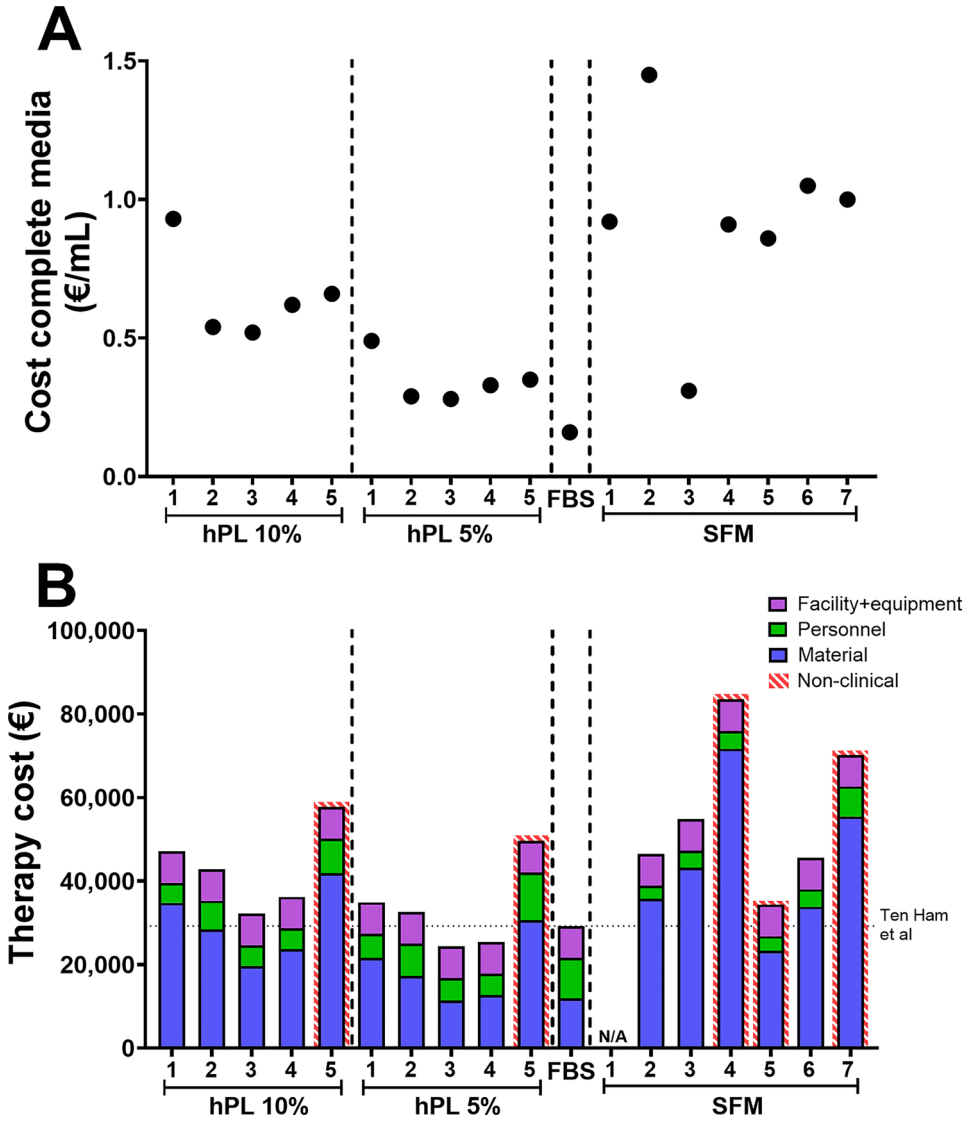

Fig. 8 Culture media and hypothetical cell therapy cost. (**A**) The cost of culture media was calculated based on list prices of cell culture supplements and basal media. Prices are expressed in euro per mL. (**B**) The total cost of a hypothetical cell therapy is shown in euro. Initial cell growth kinetics from clone 1 were used in calculations. The total yield of MSC cultured in SFM1 was too low to calculate therapy cost. Vertical dotted lines separate the study groups. The horizontal dotted line represents the reference cost of a cell therapy in FBS as calculated by ten Ham et al. Blue bars (bottom) represent material cost and is mainly determined by cost of culture media and culture duration. Green bars (middle) represent personnel cost and is dependent on culture duration. Purple bars (top) represent cost for facility and equipment and is equal for all conditions. Bars boxed in red and white striping indicate that the culture media used were marked for research use only in the leaflet. The others were 'clinical grade'

between studies can be made easy and full transparency can be obtained towards regulators and patients.

We found that complete media with SFM are considerably more expensive (~€1/mL) compared to hPL (~€0.5/mL). Often SFM require pretreatment of culture flasks with specific coating proteins. This increases both material and labor cost and may pose a higher risk for contamination. One MSC clone that we tested did not expand in SFM1 and another performed poorly in SFM6 and SFM7. Clone-to-clone variation in cell expansion was high in SFM1, 3, 6 and 7 and in hPL3 and 5. MSC phenotype in SFM at cPD8 was very similar to that in hPL except that CD73 expression was slightly higher in SFM. If this can be reproduced across biological repeats then this may imply a slightly increased CD73 ectonucleotidase activity in MSC cultured with SFM6 or SFM7 and a comparably stronger immunomodulatory effect [20]. This needs further investigation. Our data thus show that two out of three tested true SFM are good technical alternatives for blood-derived supplements, but these are 40–50% more expensive than FBS or hPL respectively, for a hypothetical cell therapy preparation.

The growth factor content is often included in scientific reports that compare media supplements but is not

necessarily related to the MSC growth kinetics. We found very high levels of growth factors in hPL1 and although this supplement had the highest growth kinetic of all hPL, SFM3, -4 and 6 were at least as supportive and had very low or undetectable growth factor levels. Therefore, either other growth factors than the ones we included in our study must relate to MSC expansion or a unique combination of growth factors is important. It could be that the ELISA's used in this study were not sensitive for potentially recombinant growth factors supplemented to the SFM.

Finally, we note that hPL can be used at 5% yielding growth kinetics and cPD at day 35 that are comparable to hPL at 10% except for hPL5, at least for our single clone of MSC used in this study. This may be different for other MSC clones or for other cell types. Lowering the fraction of supplement is an easy layup for reducing the cost of a cell therapy and deserves attention by ATMP developers. It was beyond our study design to include functional MSC assays like differentiation or immunomodulation. Future studies comparing different doses of hPL should include this. We did show for hPL1 and hPL2 that batch-to-batch variation is low for all outcome parameters tested. It was not possible to study this for other culture media because of cost, confirming that cell culture media are a significant portion of the cost of studying or developing cytotherapy products.

We conclude that manufacturers may not strictly adhere to common terminology, in particular regarding the inclusion of human blood-derived components. This is misleading and should be avoided. Our study suggests that in terms of expansion, both hPL and SFM are good alternatives to FBS but SFM are generally more expensive and manufacturers often creatively interpret SFM terminology.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-025-04561-6.

Supplementary Material 1: Additional File 1: Materials and Methods This supplementary file elaborates on the materials and methods concerning cell culture and costing analysis and offers a more detailed description for readers wishing to explore the used methods in depth. These topics were also briefly discussed in the M&M of the main file.

Supplementary Material 2: Additional File 2: Supplementary Data. This supplementary file contains figures of research data that are less crucial but further support the discussion in the manuscript.

Supplementary Material 3: Additional File 3: Supplementary Tables. This supplementary file contains tables that include information on all used catalogue and batch numbers, but also additional information on costing analysis to facilitate the reader.

Supplementary Material 4: Additional File 4: Supplementary Leaflets. This supplementary file is a merged document of the leaflets of all acquired cell culture media (supplements) at the moment of purchase. These serve as a reference for readers wishing to gather more knowledge on the products as well as to support the experimental set-up in our study.

Supplementary Material 5: Fig 6 Inteactive figure cell growth kinetic.html;

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Author contributions
WD and HBF conceptualized the study. WD performed all experiments, data analysis and wrote Materials & Methods and Results. VVW, SS and DDB provided necessary materials. SS and DDB provided consultancy on ethics and biobank regulation. PV supervised the research. HBF wrote the paper and supervised the research.

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Data availability
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 and platelet donors from the Belgian Red Cross Flanders Blood Establishment via donation procedures that follow Belgian legislation ('de bloedwetgeving'). The project was entitled 'Fundamenteel wetenschappelijk onderzoek op (rest)bloedproducten van vrijwillige bloeddonoren' approved by the Ethics Institutional Review Board named Universiteit Antwerpen Ethisch Comité with number 18/24/2089, dd 25/06/2018. All human body material was registered in a biobank registered with Federal Agency for Medicines and Health Products accession number (BB190034) approved by the University Hospital Institutional Review board of the University Hospital Leuven (UZ/KU Leuven, S62549). Cells obtained from Lonza (material number PT-5006, batch number 21TL347548) 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.

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