Research Article

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Regenerative Medicine

Modeling biological and economic uncertainty on cell therapy manufacturing: the choice of culture media supplementation

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Aim: To evaluate the cost-effectiveness of autologous cell therapy manufacturing in xeno-free conditions. **Materials & methods:** Published data on the isolation and expansion of mesenchymal stem/stromal cells introduced donor, multipassage and culture media variability on cell yields and process times on adherent culture flasks to drive cost simulation of a scale-out campaign of 1000 doses of 75 million cells each in a 400 square meter Good Manufacturing Practices facility. **Results & conclusion:** Passage numbers in the expansion step are strongly associated with isolation cell yield and drive cost increases per donor of $1970 and 2802 for fetal bovine serum and human platelet lysate. Human platelet lysate decreases passage numbers and process costs in 94.5 and 97% of donors through lower facility and labor costs. Cost savings are maintained with full equipment depreciation and higher numbers of cells per dose, highlighting the number of cells per passage step as the key cost driver.

First draft submitted: 23 March 2018; Accepted for publication: 8 November 2018; Published online: 29 November 2018

Keywords: autologous mesenchymal stem/stromal cells manufacture • bone marrow • decision support tools • donor variability • early health technology assessment • human platelet lysate • process transfer • scale-out • stochastic bioeconomics modeling • xeno-free culture

Mesenchymal stem/stromal cells (MSCs) have been researched as attractive therapeutic medical products due to their immunosuppressive and anti-inflammatory properties for a multitude of clinical indications, with the most common applications being related to the regeneration of damaged tissues, autoimmune and neurodegenerative diseases [1–9]. There are fifteen approved products (either as cell therapies or combination products/devices) in specific countries as of June 2018 [9–11] and 666 clinical trials registered with MSCs as interventions (clinicaltrials.gov, accessed 1 June 2018). While they have been administered routinely in the form of cells in clinical trials [8–12], MSCs can be used to produce exosomes with secreted factors, which have therapeutic potential as a cell-free therapy [13]. These cells can be found in diverse sources in the human body, including the bone marrow, adipose tissue, umbilical cord blood or matrix, among others, and are isolated by adherence to plastic surfaces [14,15]. Clinically relevant numbers are on the order of 1 million cells/kg [8,12,16] or higher [17,18]; however, MSCs isolated directly from the native sources are found in low numbers. Typical bone marrow aspirate volumes for clinical trials range from 10 to 50 ml [18–20], and can be isolated by density gradient centrifugation or through plating directly the whole bone marrow aspirate [21–23], yielding mononuclear cells in the range of 10^6–10^7 cells/ml [21,24], from which approximately one in 10^4–10^5 are MSCs [23,25]. Consequently, the cells have to undergo several generations of replications *in vitro* in order for clinically relevant cell numbers to be achieved, typically in flat 2D flasks or cell stacks supporting adherent cell culture [23,26]. Finally, the cell products need to be harvested and purified from their expansion technology (i.e, plastic tissue culture flasks or sacks for tissue culture) and culture media to ensure proper formulation for either a fresh product

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to be administered shortly after manufacturing or a cryopreserved product for future administration, in compliance with the defined quality controls for the relevant clinical application [9,26,27]. However, the bioprocessing of MSCs is a labor-, time- and money-consuming task due to the need for a facility complying with Good Manufacturing Practices (GMP), highly costly clinical-grade reagents, long process times, labor-intensive tasks, impractical scaling up of production and high costs of quality control [28–32].

The choice of culture medium and consequent supplementation are among the most critical decisions in MSCs bioprocessing for clinical applications, both from the cost and the product quality points of view. Typically, MSCs are cultured in defined basal media, such as Dulbecco's modified Eagle medium (DMEM) or Minimum Essential Media (α-MEM), and supplemented with fetal bovine serum (FBS) for the supply of multiple growth factors and cytokines [9,12]. As of 2014, approximately 80% of clinical trials were performed with MSCs expanded in media with FBS supplementation [12,33]. The use of animal origin serum has several disadvantages, such as the lack of chemical composition definition, high batch-to-batch variability, safety issues with the transmission of prion or viral diseases, possible host immune response, supply chain limitations and animal welfare concerns [9,12,33–35]. Additionally, directives by the European Medicines Agency and the US FDA recommend the replacement of the use of animal origin reagents whenever possible [35]. Under these considerations, the interest in supplementation with human origin products, such as human serum and human platelet lysate (hPL) has grown. The hPL is today commonly used as an alternative to FBS in MSCs culture, showing improved proliferation ability in MSCs from different sources, relative to FBS [27,36–39]. However, depending on the MSCs origin and the desired clinical application, cells cultured with hPL may show decreased immunomodulatory properties and a higher likelihood of lineage commitment in comparison with FBS [9,23,40]. While hPL is viewed as safer than FBS, its current price is higher than animal-based supplementation and concerns remain in terms of batch-to-batch variability, possible coagulation and risk of human pathogen transmission [9,23,27,33,34].

Computational decision support tools have been developed to evaluate alternative bioprocess configurations and impact of several manufacturing parameters on cell therapy process, including different types of stem cells. The work in this field has focused on the utilization of either commercial software packages or in-house built code to determine, for stem cell-based therapies or research applications, the least expensive upstream and downstream processing strategies for a given demand in terms of doses/lot, as well as optimizing the production for compatibility with the maximum allowed reimbursement price [29,41–52]. Some of these approaches include a combination of experimental and cost model data [42,48,52]. Regarding the evolution of the process toward a xeno-free culture, previous work focused on automated allogeneic MSCs manufacturing [44] has evaluated the use of commercially available chemical defined xeno-free culture media.

Building upon these contributions, the present work reports a full bioprocessing model from cell isolation to release, considering biological variability driven by the culture medium, intrinsic donor characteristics and multipassage expansion. This work evaluates the relevance and impact of stochasticity in the manufacturing costs. The model includes the process unit operations, namely isolation, expansion, downstream processing and release testing of the cells. For this particular case study, the effect of selecting either FBS or hPL on an autologous culture manufacturing process will be simulated. The biological parameters for model calibration will be estimated from experimental data obtained from the literature and the impact of cost variability will be introduced through several stochastic parameters. The simulation results will provide a statistical distribution of the total manufacturing costs per dose while identifying factors that affect the cost-effectiveness of the process, when changing to a xeno-free-based culture, considering differences in price of the supplements, growth rate and cell morphology, and compliance with quality attributes at the quality control level for a possible autologous MSC-based product.

Methods

Model overview

The discrete event simulation model was adapted from the tool described in [29] to determine the impact of donor, multipassage and culture media associated variability on the manufacturing cost structure of autologous mesenchymal stem/stromal cell-based therapies. The tool was implemented in Python, comprising a database of resources and experimentally derived inputs.

The model is designed such that bone marrow aspirates queue for incubators and once an incubator and operator are available, the density gradient centrifugation process to isolate mononuclear cells (MNCs) starts, yielding a number of MNCs to be seeded into an appropriate adherent cell culture flask. The model selects for the optimal number and area of these flasks to seed cells with an appropriate initial density [30] (Supplementary Tables 1–3). The

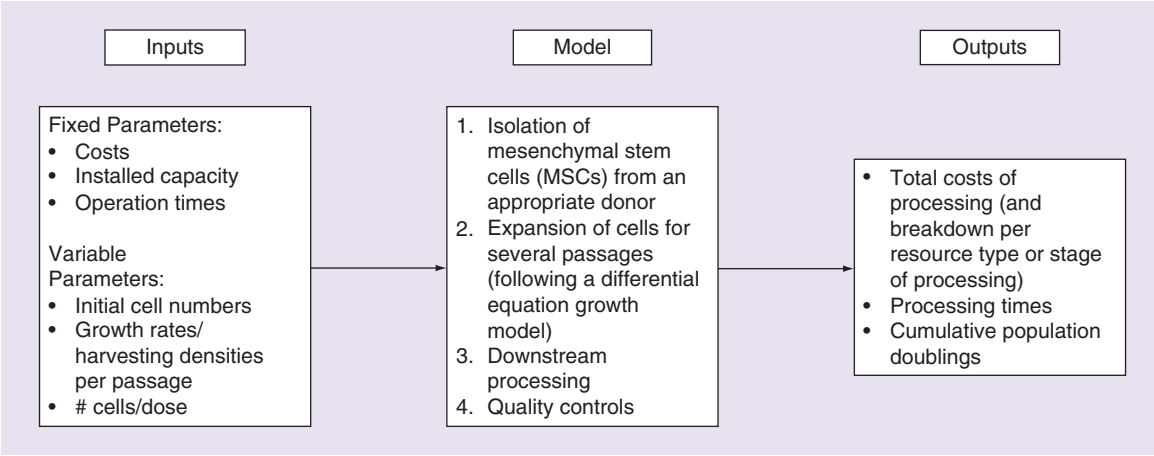

Figure 1. Inputs and outputs of the model.
MSC: Mesenchymal stem cells.

flasks undergo simulated media changes until reaching confluence, as specified by confluence times and yields of MSCs per MNCs seeded [21,22]. After obtaining the initial MSCs population, expansion starts in order to multiply the cell number until the target number of cells is reached. The same process for optimal flask selection occurs and cells are incubated until reaching the appropriate harvesting density and confluence time. Cells are removed from the flasks using a harvesting agent and reseeded in new flasks to mimic the passaging process until the target number is reached. Afterward, cells are separated from the culture medium, undergo cell wash and concentration, and are then cryopreserved. Finally, release testing of the batch is performed.

Biological variability is input in the model at the level of the number of MNCs per bone marrow aspirate volume [21], the yield of MSCs per MNCs seeded [22] and the multipassage harvesting densities at confluence [53]. The published experimental data were used to derive probabilistic distributions (Supplementary Tables 4 & 5) from which these parameters are sampled for each donor. These parameters will drive a Monte Carlo simulation method for stochastic simulation. The different resulting initial MSCs population and the number of MSCs per area at confluence will impact the number and area of culture flasks used for isolation and expansion that are calculated accordingly as a model variable. As a result, appropriate reagent volumes are inputted into the database and the different number of cells per passage will drive expansion processes occurring with varying numbers of passages, driving a range of process times that will impact the operational costs related to the facility and labor as well.

Throughout the simulated process, the operation times, occupancy times of equipment and the amount of single-use expansion technologies and reagents used are stored into appropriate databases to allow for the final calculation of the manufacturing costs per donor (Figure 1). The cost structure has capital costs (CAPEX) components, related to the depreciation of facility materials and equipment, and fixed and variable operational costs (OPEX). The fixed costs are related to maintenance and qualification of the facility. The variable costs are directly dependent of the volume of production of cells, comprising all the expenditure in reagents (culture medium, isolation reagents, cryopreservation medium and harvesting solution), single-use expansion technologies, labor and quality controls. The total manufacturing costs are calculated on a per dose basis within the same batch. The costs associated with doses that fail the quality controls are distributed equally by the accepted batches.

Case study definition

The case study baseline scenario encompasses the simulation of a process transfer from an animal-based culture to a xeno-free culture in a GMP facility manufacturing setting for a prospective autologous mesenchymal stem/stromal cell-based therapy. Clinical trial information for the only approved autologous MSCs based product (Hearticellgram) reports doses of 1 million cells/kg, yielding 72 +/− 0.9 million cells per patient [20]. A therapeutic dose of 75 million cells per donor will be simulated accordingly. We assume a scale-out manufacturing scheme, where several donors are processed in parallel while there is equipment availability. The study will be parameterized using a study reporting the multipassage expansion of bone marrow mesenchymal stem/stromal cells (BM-MSCs) with DMEM + 10% FBS and with DMEM + 10% hPL using several donors (Table 1) [36,54]. To estimate growth rates and isolation

Table 1. Growth rates (mean & 95% CI) sampled from 1000 simulations.

Passage number	Growth rate, FBS (day^{-1})	Growth rate, hPL (day^{-1})
1	0.18 (0.16–0.21)	0.27 (0.26–0.29)
2	0.19 (0.17–0.21)	0.30 (0.29–0.31)
3	0.20 (0.17–0.24)	0.29 (0.27–0.30)
4	0.18 (0.17–0.19)	0.18 (0.11–0.24)
5	0.18 (0.17–0.19)	0.18 (0.11–0.24)

CI: Confidence interval; FBS: Fetal bovine serum; hPL: Human platelet lysate.

yields, we used studies related to the yield of MSCs per seeded MNCs obtained from the isolation of bone marrow aspirates using the Ficoll-Paque density method [20,21]. Simulations will consider up to five passages and a seeding density of 5000 cells/cm^2. To account for donor, multipassage and culture media variability on isolation yields and expansion growth rates, 1000 donors will be simulated and parameterized through sampling from the aforementioned probabilistic distributions. In the baseline scenario, the isolation yields and growth rates of BM-MSCs with either FBS or hPL supplementation will be derived from the experimental studies directly and the current market prices of the two supplements will be used for comparison, with hPL being considered twice as expensive per volume as FBS [54,55]. Probabilistic distributions of the total costs per dose and numbers of passages will be derived, reflecting the different numbers of steps required to reach the demand per dose and how the different variable factors affect the costs of manufacturing. Due to batch-to-batch variability verified in different hPL products and consequent possible impact on the biological parameters, key model drivers (isolation yield, isolation time, multipassage harvesting density, price of hPL, labor rate, equipment depreciation and number of cells per dose) were varied to determine the robustness of the cost-effectiveness of the process transfer.

Results & discussion

Impact of culture media supplement on isolation & expansion manufacturing cost variability & processing times

The biological variability inputs from the culture with hPL versus FBS stem from two major contributions: higher ratio of isolated MSCs per number of seeded MNCs and faster isolation times [22], and higher numbers of cells at confluence and faster growth rates through several passages in planar expansion [34,53]. From the sampled donors, we created a distribution of the number of MNCs per donor with an average number per donor of 68.8 million cells (95% CI: 30.8–106.7; Figure 2A). For the simulation of the isolation of MSCs from 10 ml of bone marrow aspirate using the Ficoll-Paque density gradient method, the FBS group yielded, on average, 6.0 million P0 MSCs (95% CI: 2.2–14.5), while the hPL group yielded an average of 9.54 million P0 MSCs (95% CI: 3.32–21.7; Figure 2B). A total of 89% of the simulated donors were computed to have an increase in the yield of P0 MSCs. On average, the MSC yield per donor with hPL was 1.72-times higher than with FBS (95% CI: 0.77–3.34). Given the different number of cells obtained after isolation (i.e, at P0) is expected that the sequential passages of MSCs to achieve the final dose of 75 million cells per patient will start with more cells with hPL than with FBS for most of the donors (Figure 2C).

For hPL-based cell culture, fewer passages are needed to reach the final target number of cells relative to FBS-based cell culture. This is a consequence of the higher numbers of cells at the end of each passage, combined with the higher initial numbers of MSCs for expansion. For isolation and cultures with hPL, the 75 million cells are obtained between one and four passages, with 79% of donors reaching the final dose in two passages; while for isolation and cultures with FBS reaching the same number of cells took two or more passages, with 61.5% of donors reaching the final cell number after four passages (Figure 3A). By analyzing the difference in the number of passages necessary to achieve the final cell demand, the majority of the donors reached the final cell number with hPL in two passages less than with FBS (54.6%). Only 5.5% of the donors did not experience any difference in the number of passages required (Figure 3B). The number of passages required to expand the cells to the final dose is strongly negatively correlated with the initial number of cells at isolation, in particular for FBS cultures (R^2 = -0.86 for FBS vs R^2 = -0.68 for hPL; Figures 3C & D). However, the harvesting densities at passage one and the number of passages required to expand the cells in both culture media supplements did not show any meaningful correlation (Figures 3E & F), suggesting that the initial cells after isolation are a key factor to drive faster processing in the xeno-free culture.

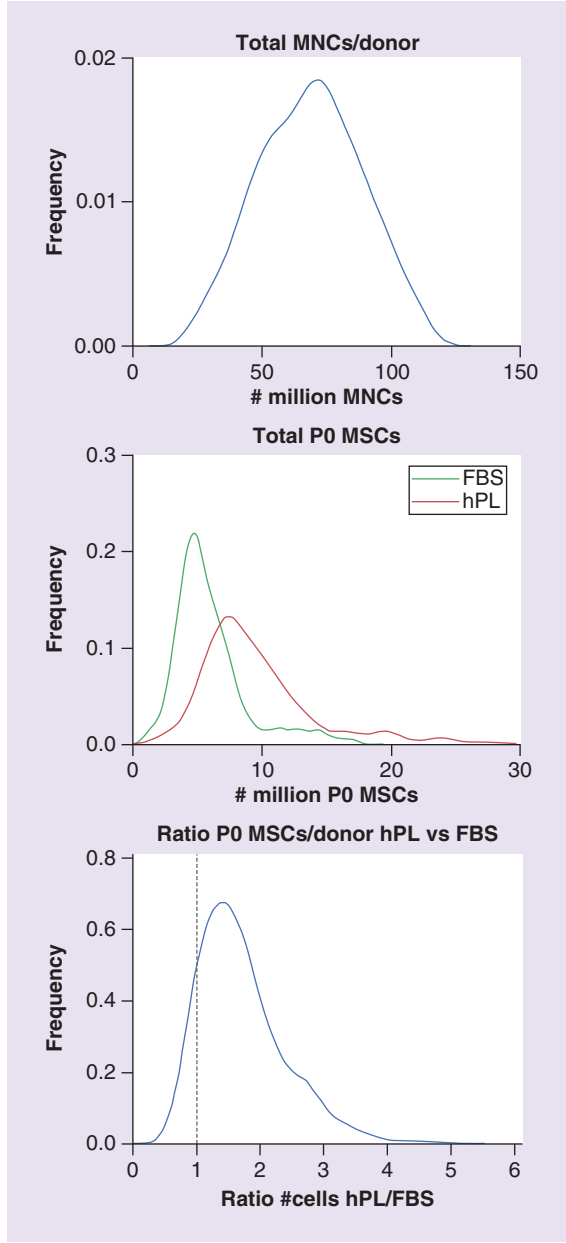

Figure 2. Probability density functions related to the isolation of bone marrow mesenchymal stem/stromal cells with the Ficoll-Paque density gradient method. (A) A total of 10 ml of bone marrow aspirate yielded average numbers of MNCs of 68.8 million cells (95% CI: 30.8–106.7). The distribution was derived from experimental data. **(B)** Bone marrow aspirates isolated with hPL generate more initial mesenchymal stem/stromal cells per donor than with FBS. The distribution of the number of cells per donor with hPL is broader due to the higher ratio of MSCs per MNCs seeded after isolation compared with FBS. Ratios of MSCs per initial MNCs seeded were derived from **(C)**. The vast majority of donors shows increased MSC yield (as assessed by hPL/FBS ratios higher than 1) when adherent cell fraction isolation is performed with hPL, with an average fold increase of 1.71 relatively to isolation with FBS. Total of 1000 donors were simulated.
FBS: Fetal bovine serum; hPL: Human platelet lysate; MNC: Mononuclear cell; MSC: Mesenchymal stem/stromal cell.

The integration of the biological aspects and variability of cell culture in the model allows the translation of their impact on manufacturing decisions by calculation of variable process costs. The calculations show that the stochastic nature of cell isolation and expansion rates and its impact on the processing times will lead to considerable differences in the total costs of the process (Figure 4A). Bioprocessing of autologous BM-MSCs using FBS as a culture medium supplement has an average total cost per donor of $24883 (95% CI: $21365–27421), while the same process with hPL as a supplement has an average cost of $20947 (95% CI: $18033–23423). Out of all donors, 97.1% of donors experience savings in total process costs when processed with hPL supplementation. On average, processing costs of the same donor with hPL are reduced by 0.84-fold relatively to culture with FBS (95% CI: 0.77–1.00; Figure 4B). This cost reduction is connected to the lower number of passages required to expand the cells (Figure 4C). There is a very strong correlation between the total number of passages required and the total costs (R^2 = 0.99 for FBS; R^2 = 0.93 for hPL), with an average increase in the total costs with an additional passage of $1970 for FBS and of $2802 for hPL. The breakdown of the ratio of the costs per donor with hPL relative to FBS shows that, if no passages are saved, there is virtually no reduction in the final process costs (mean: 1.00; 95% CI: 0.95–1.01; Figure 4D). The reduction in costs is strongly associated with the number of passages saved per

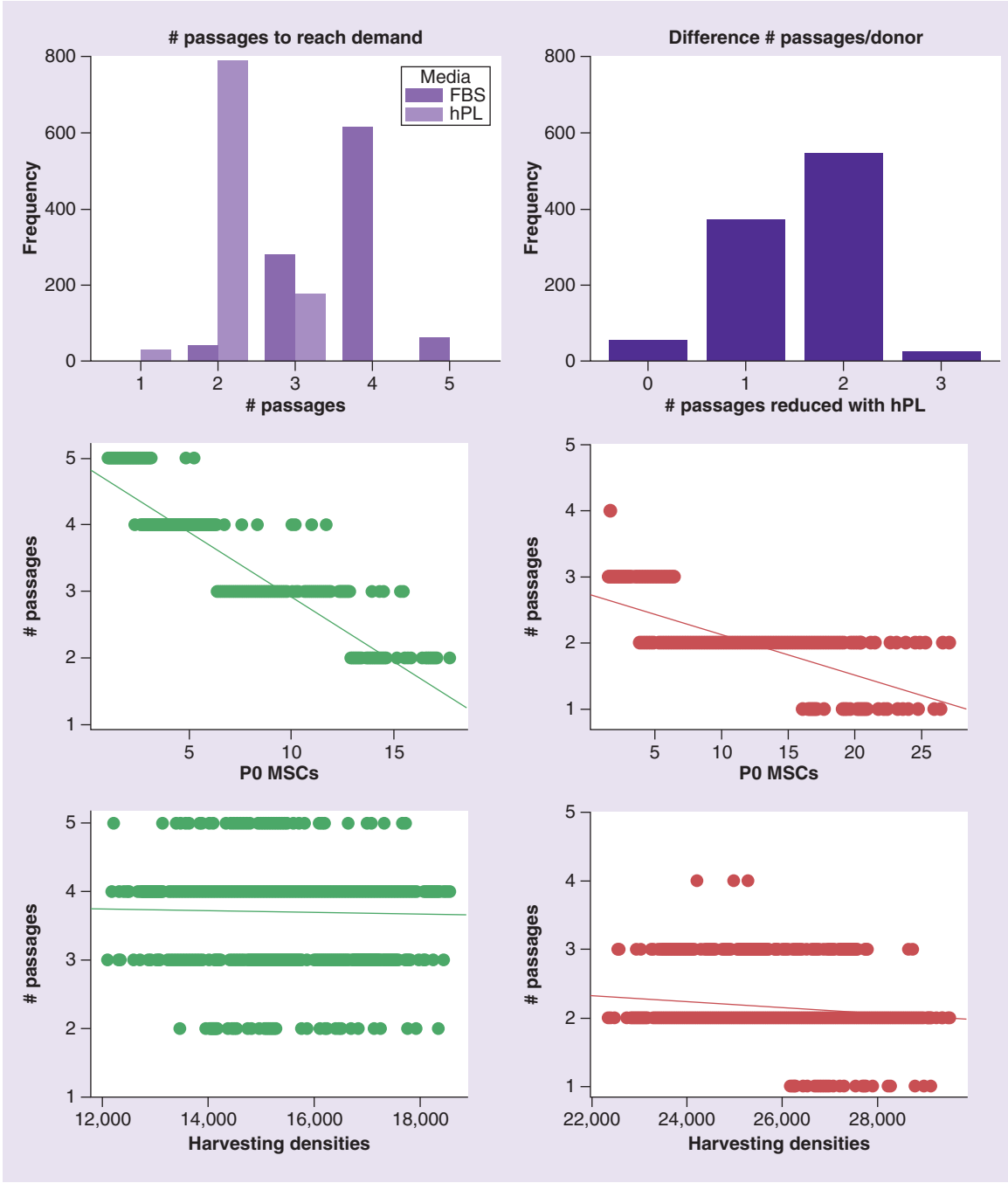

Figure 3. Human platelet lysate reduces the number of passages required to expand the cells to the final dose. **(A)** Passage numbers required per donor to expand the cells to the dose of 75 million cells with FBS (green) or hPL (red). **(B)** Number of passages per donor saved with the process change to hPL. **(C, D)** The number of MSCs out of isolation is strongly negatively correlated with the number of passages required to expand the cells to the final dose, in particular in the FBS case (green). For hPL, the correlation is less strong (red). **(E, F)** The final number of passages is not correlated with the harvesting densities per donor at the first passage post isolation in either FBS (green) or hPL (red).
FBS: Fetal bovine serum; hPL: Human platelet lysate; MSC: Mesenchymal stem/stromal cell.

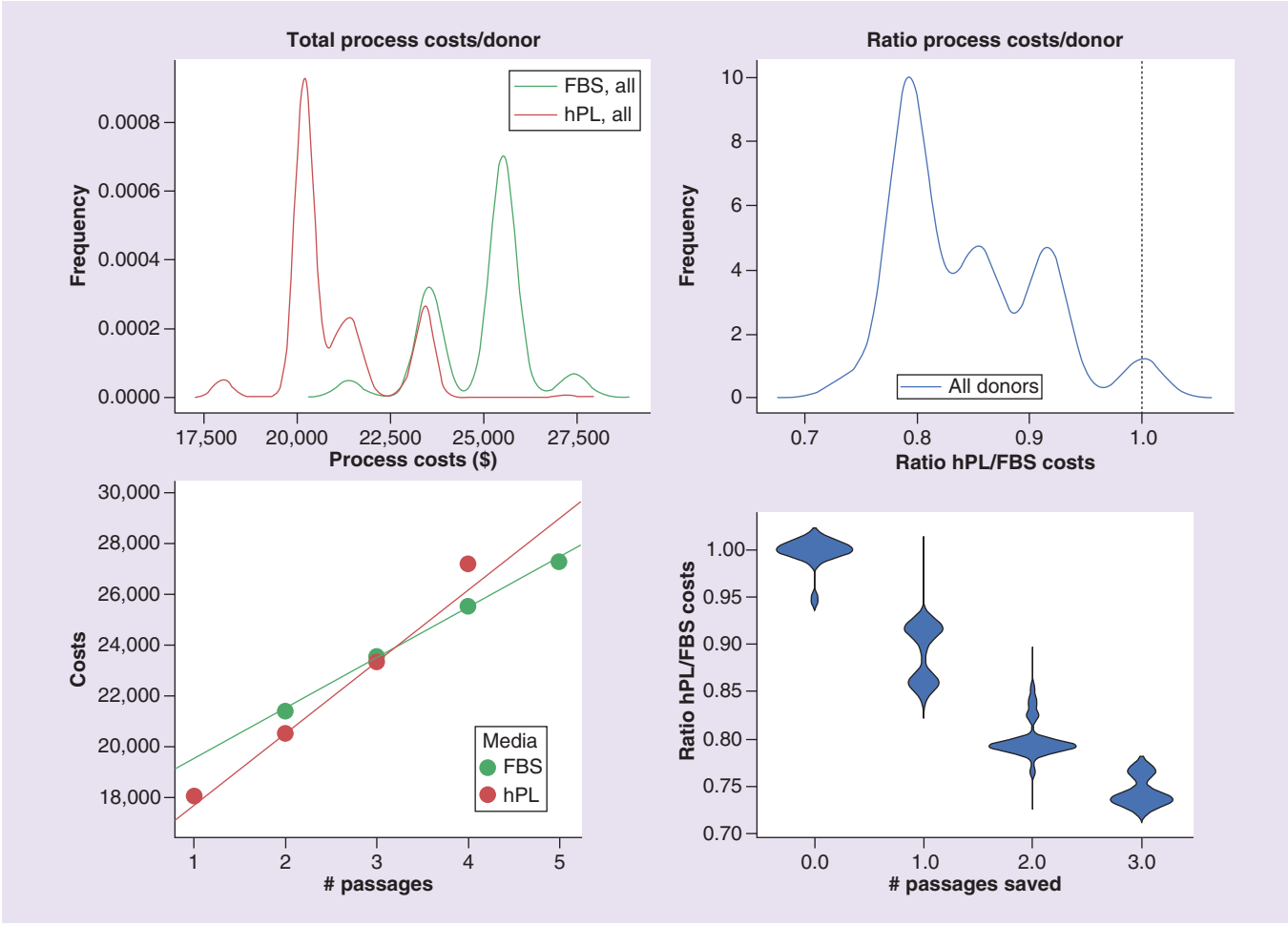

Figure 4. Human platelet lysate is a cost-saving alternative in comparison with fetal bovine serum for bioprocessing of autologous bone marrow mesenchymal stem/stromal cells therapies. (A) Frequency distribution of the processing costs of 1000 donors with FBS (green) or hPL (red) until reaching the final dose of 75 million cells per donor. **(B)** Ratio of hPL versus FBS processing costs per donor. The hPL reduces the processing costs of 97% of the donors. **(C)** Both FBS and hPL show a strong relationship between the number of passages for processing per donor and the total manufacturing costs, with a steeper increase for hPL cultures. Points represent the mean of the distributions per passage and per culture media. **(D)** The cost savings per donor when transferring the culture for supplementation with hPL are dependent on the number of passages saved. For donors with no passages saved, there are no cost savings while the cost savings become more relevant with an increasing number of passages saved for up to 30% of the FBS value.
FBS: Fetal bovine serum; hPL: Human platelet lysate.

donor, with the most common case of two passages saved per donor occurring in manufacturing cells with hPL with 20% relative cost savings compared with FBS (mean ratio: 0.8; 95% CI: 0.77–0.85).

Due to the expensive quality controls that have to be performed for every dose, the main fraction of operational costs is occupied by the quality controls in each donor. The ratio of total costs ranges from approximately 0.41 (95% CI: 0.37–0.47) for FBS to 0.48 (95% CI: 0.43–0.55) for hPL (Figure 5A). This shift toward a higher relative contribution of quality controls for the cost structure in the xeno-free cultures is related to lower facility and labor costs. Facility and labor costs make up for 27 and 16% of costs, on average, for cultures with hPL, rendering them important cost drivers. This is actually a specificity of an autologous therapy, and therefore a limitation of our model imposed by the selected case study. In absolute terms, donors cultured with hPL have average facility and equipment depreciation and operational costs of $5692, which is a significant reduction from the average $8016 facility costs for FBS (Figure 5B). A similar pattern is seen for the labor costs, with a reduction from the average labor costs of $4596 for donors cultured with FBS to $3264 labor costs for hPL. However, on the other hand, the consumables costs related to the vessels used for cell expansion, culture media and other reagents, are very similar on average between the two different culture supplements ($1984 for FBS vs $1913 for hPL). By analyzing the

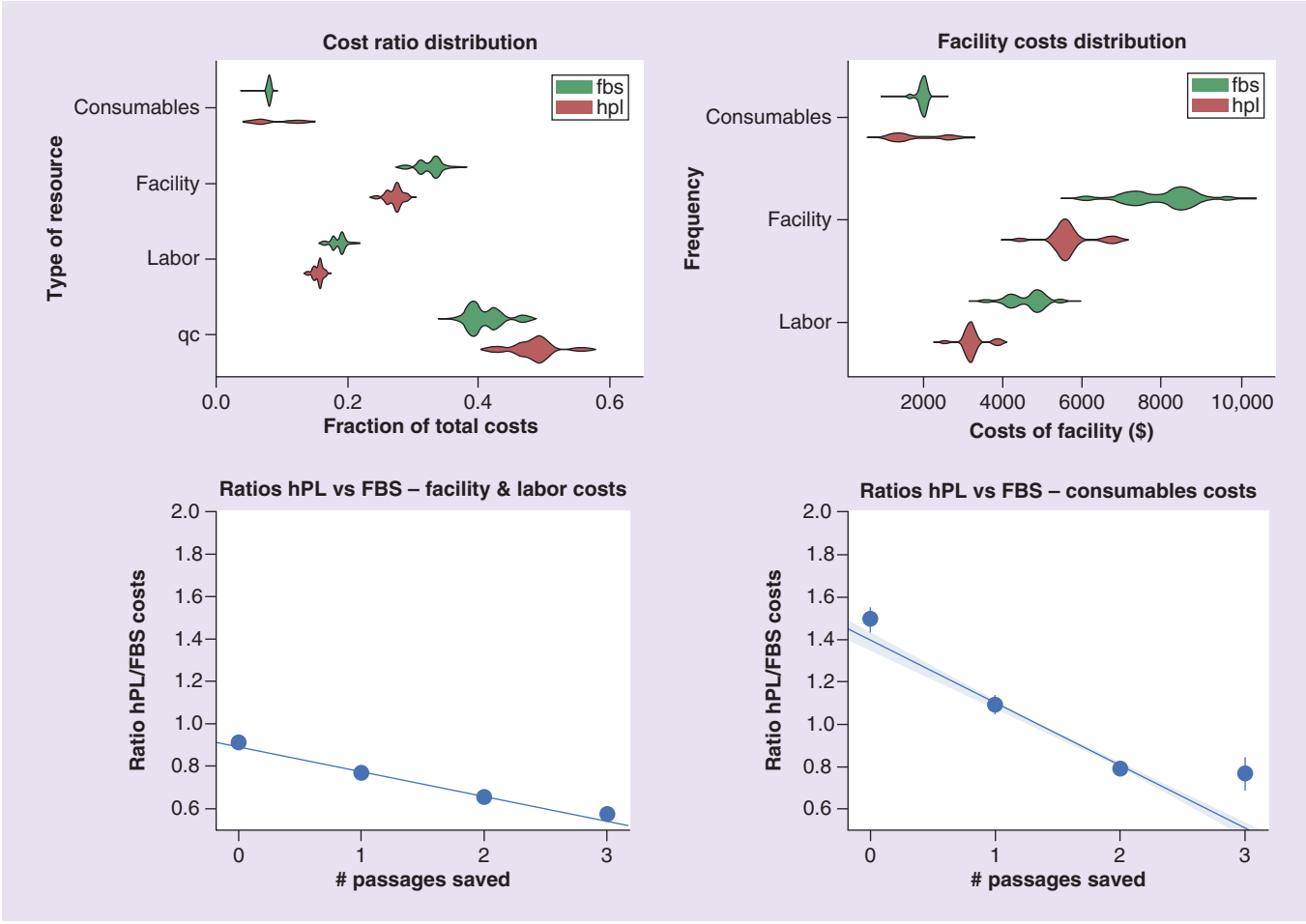

Figure 5. The cost savings emparted by human platelet lysate are focused on the facility and labor costs. (A) Violin plots of the distribution of total costs ratios per operational resource: consumables (culture flasks, culture media and other reagents), facility and equipment depreciation and operational costs, labor costs and quality control costs. **(B)** Total cost values of the variable resources. Facility and labor costs are higher in FBS cultures due to higher number of passages and operational times. **(C)** Linear correlation between the ratio of hPL versus FBS facility and labor costs per number of passage saved per donor when switching to hPL. For all donors, hPL enables cost savings in facility and labor operational costs due to shorter isolation times and passages saved. **(D)** Linear correlation between the ratio of hPL versus FBS consumables costs per number of passages saved per donor. The hPL starts becoming a cost-saving option when two or more passages are avoided by switching to hPL. For zero or one passages saved, the higher culture medium costs and the higher areas and costs of the culture vessels necessary to process a higher number of initial cells overpower the savings in the amount of consumables for averting passages. Total of 1000 donors were simulated.
FBS: Fetal bovine serum; hPL: Human platelet lysate.

cost savings per donor divided by the number of passages saved by the culture with hPL, facility and labor costs are always lower in the hPL culture, with a very strong linear association (R^2 = -0.97; Figure 5C). Regardless, the number of passages saved, due to the fact that the isolation time was considered to be 3 days shorter, is in agreement with the experimental data for model calibration. The cost savings become higher with the number of passages averted, since each additional passage means, under this dataset, six additional days of operation. However, for consumable costs, there is not such a clear linear relationship between the ratios and the number of passages (R^2 = -0.55; Figure 5D). When no passages or one passage is avoided, on average, the consumables costs are higher for hPL than for FBS, due to the more expensive culture medium and the use of higher areas and more expensive expansion flasks. On average, only after two passages saved does hPL start to be cost-saving, with an apparent stabilization of the ratio at approximately 80% of consumable costs with FBS.

Cost-effectiveness of process transfer is maintained with higher cell numbers per dose and with depreciated equipment

The variability in the costs per manufactured dose derived from the donor and multipassage cell numbers at confluence showcased two relevant operational questions associated with autologous therapies manufacturing: the additional costs of manufacturing when more passages are required to reach the number of cells per dose and the different process times, causing batch timing issues [36].

A large portion of these additional costs comes from the operational costs of higher processing times. A conservative assumption for the baseline scenario was made such as the facility and equipment were not yet fully depreciated. Here, an additional scenario in which manufacturing in a facility with fully depreciated equipment was considered. In this case, benefits on overall cost through the use of hPL are more modest. Still, labor costs, among others, will be sensitive to processing times (and passages) reduced by the use of hPL. In the absence of depreciation costs, the impact of the supplement cost on the overall cost structure increases. The depreciated equipment process configuration decreased the average total processing costs per donor to $19,402 (95% CI: $17,367–21,070) for FBS and $17,195 (95% CI: $15,192–19,052) for hPL. With depreciated equipment, the percentage of donors for which the manufacturing is more expensive with hPL rose from 5 to 8% (Figure 6A & B).

Autologous therapies may use higher cell numbers per dose (from 2 to 5 million cells/kg) than the numbers considered in the baseline case study [17,56]. Two additional scenarios that imply an increase in facility scale were assessed: manufacture of MSCs with doses of 150 million and 300 million cells (Figures 6C–F). For these numbers of cells, the costs of processing have a considerable increase to values above $30,000 per donor. While for the 150 million cells/dose assumption, there is not a relevant impact on the number of donors for which hPL processing is more expensive (1–3%, undepreciated vs depreciated), for the higher dose of 300 million cells/dose there is a considerable number of donors with more expensive hPL based processing, starting at 16% for the nondepreciated case and reaching 24% for the depreciated case. This increase is related to the higher numbers of passages required to produce the cells, pointing toward a higher relative contribution of culture media in the process. With depreciated equipment, the relative facility contribution is decreased, decreasing the cost-effectiveness of process transfer. Therefore, when doses have high cell numbers, at the current price ratio between hPL and FBS, it is critical to control the relative proliferative advantages of hPL to ensure robust cost-effectiveness.

Multipassage harvesting density is the key cost driver for xeno-free process cost–effectiveness

To bring additional insights on the decision rationale brought by the model, sensitivity analyses were conducted to vary some of the inputs used.

- Isolation and expansion of cells: importantly, the model calculates costs taking into account the biological features of the cells; therefore, sensitivity analysis for the effects of isolation and expansion yields were analyzed as a function of the number of cells obtained per surface area, as a consequence of the cell size at confluence [53]. Additionally, the time of isolation was also varied.

- Media supplement costs: the effect of media supplement on overall costs is important, as a result of different processing times (and passages) and consequent changes in facilities and labor costs. While the media costs represent a small fraction of the overall cost, it is still an important parameter to address due to possible market fluctuations.
- The contribution of labor was further assessed considering a lower labor pay rate since labor is one of the main cost drivers, and is more pronounced in hPL cultures due to lower passage numbers than in FBS.

A 25% increase or decrease in these factors was assumed to evaluate the impact on the costs per donor of processing with hPL relatively to the baseline (Figure 7).

The hPL promotes the growth of MSCs of smaller size and therefore a higher number of cells are isolated when subconfluence is reached in P0 [53]. Despite the fact that the number of passages that cells undergo is highly correlated to the number of P0 MSCs after isolation, a decrease in 25% of this factor did not show a significant impact in costs, with an increase of 5% (-4–18 %) with depreciation and 2% (-7–14%) with fully depreciated equipment. The rationale for this is related to the fact that, as long as the proliferative potential is maintained, the number of cells required would be reached in the same number of passages. The main impact of the reduced isolation yield is on the type of flask selected for passage 1, pointing toward lower areas and cheaper consumable

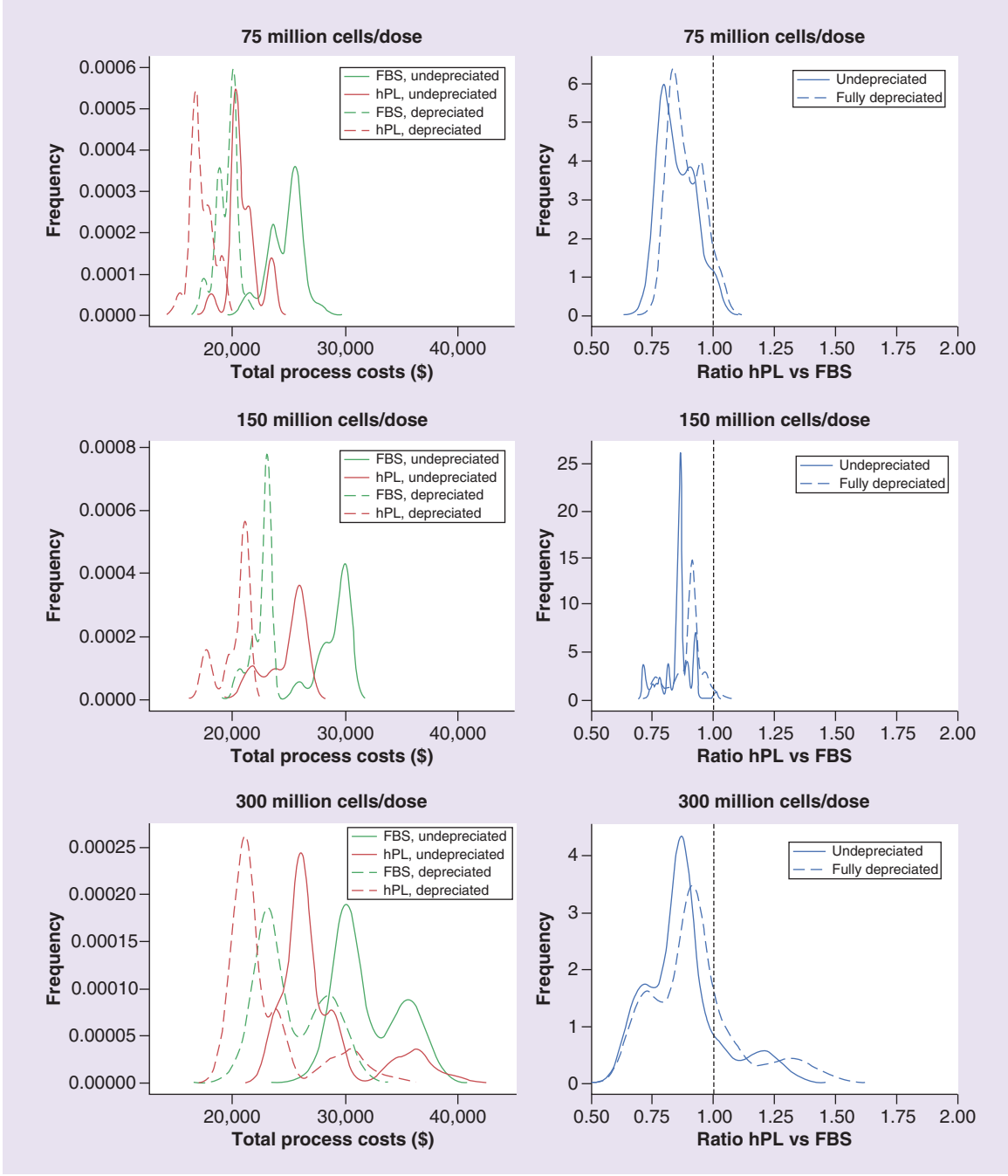

Figure 6. Cost–effectiveness of process transfer to human platelet lysate holds for depreciated equipment and different numbers of cells per dose. (A, C & E) Frequency distributions of the costs of processing 75 **(A),** 150 **(C)** and 300 **(E)** million cells/donor for FBS (green) or hPL (red), with undepreciated (solid) or fully depreciated (dashed) equipment and facility. While the relative differences between the two culture supplements are reduced when equipment and facility are fully depreciated, the majority of the donors have lower processing costs when processed with hPL **(B, D & F)**. Cost–effectiveness is less evident for the higher dose and depreciated equipment, pinpointing the relevance of maintenance of higher proliferative abilities with hPL.
FBS: Fetal bovine serum; hPL: Human platelet lysate.

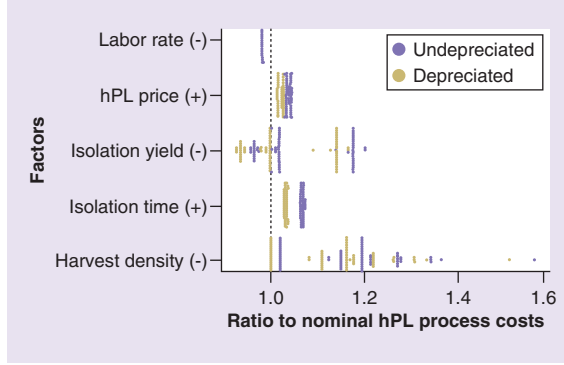

Figure 7. The multipassage harvest density is the most relevant parameter for cost–effectiveness of the process transfer to a xeno-free culture. Sensitivity analysis to the total process costs of 1000 doses of hPL. Key process cost drivers were increased (+) or decreased (–) by 25% for both nondepreciated (purple) and depreciated (yellow) facility and equipment manufacturing scenarios. The ratios of the new hPL process costs per donor with the varied parameter relatively to the nominal parameter case showcase harvesting density as the key process cost driver, while costs are robust to isolation time, supplement price and labor rate variations. The role of isolation yield is mixed and depends on the multipassage proliferative abilities of each donor. Points represent individual donors.
hPL: Human platelet lysate.

costs in this first expansion step. Interestingly, the impact of an increase of the isolation time by 25% has a more homogeneous effect on the cost structure than the yield, but both average to similar values.

The factor with the most relevant impact on the cost structure is the multipassage harvest density, where a decrease of 25% in the number of cells at confluence per area yields increases in the processing costs of 18% (95% CI: 2–35%) with undepreciated equipment and 15% with fully depreciated equipment (95% CI: 1–32%). This factor, in combination with the number of MSCs at P0, reduced the need for additional passages to obtain the desired number of cells for a clinical dose (Figure 2). Cells that undergo fewer passages are more likely to retain their multipotency and therapeutic potential [57]. The underlying assumption taken in this model is supported, for bone marrow MSCs, by several studies in the literature stating that growth with hPL yields smaller cells than with FBS, making this supplement more attractive from a proliferative point of view in adherent technologies, since more cells are yielded per area [28,58–60]. Regardless of the changes in the parameters performed within the considered range of 25% of the nominal value, all donors still reach the final cell number and thus there are no changes in batch failure rates. Note that batch failures are driven from cells failing the potency and/or sterility testing.

In the baseline scenario for the autologous process considered, culture medium costs are a small fraction of the total manufacturing costs and it is not expected that fluctuations in the ratio of hPL and FBS prices per volume affect the cost–effectiveness of the xeno-free process transfer for autologous therapies. In fact, an increase of the price per volume of hPL by 25% had a minimal (2–4%) effect on the costs of processing per donor, assuming that all biological factors are kept constant. Therefore, for low doses, the process is robust to fluctuations in the price of hPL as long as the supplement batches retain the quality attributes for improved proliferation. Due to the relatively low cell numbers and the fact that only one dose is required per donor, the culture medium volumes used are low in comparison with allogeneic therapies, aimed at a scale economy. In an allogeneic process, the media costs are generally the most relevant cost contributor per dose [29,50] and the impact of the price of the supplement would be probably more relevant. In future work, the impact of hPL versus FBS as a supplement for allogeneic mesenchymal stem/stromal cell therapies should be the object of analysis.

A decrease in labor rate has a minimal impact of 2% on costs. This analysis points to the relevance of the selection of hPL batches with strong proliferative abilities while retaining the quality attributes required to comply with quality controls and ensure process comparability with FBS. Under this autologous process, the variability in the ability to generate smaller cells and more numbers per passage comparatively to FBS would be more detrimental to the cost-effectiveness of the process transfer to hPL than fluctuations in price. This is a direct result of decreasing processing times when using hPL and thus of costs with facility and labor.

Considerations for model-driven cost-effective process transfer

The results of this modeling study support the cost–effectiveness of the use of hPL as a xeno-free culture media supplement for manufacturing autologous MSCs based therapies. The cost–effectiveness comes as a result of the superior proliferative performance in isolation and expansion of MSCs cultured with hPL comparatively to FBS, yielding considerable cost savings. However, additional considerations for the establishment of a xeno-free process need to be considered. All of the analyses were conducted assuming comparability between the two culture media supplements in terms of quality controls. Quality controls for release testing of MSCs are related to safety, identity, purity and potency [61,62]. While some studies claim that hPL does not modify the

immunomodulatory characteristics and the multipotency of MSCs [28,58,60,63], others show that there is a decrease in the immunomodulatory activity and surface marker modifications in BM-MSCs cultured with hPL [9,40,64].

Decisions concerning manufacture process should be taken relatively early on the development of a cell-based therapy, preferable before a Phase III clinical trial, as effects on changes in cell potency and safety with process specificity may be a concern [50]. Therefore, in addition to cost, the decision of the manufacturing process to be selected should also consider the therapeutic action of the obtained cells. Furthermore, when a donor fails the quality controls in an autologous therapy, it means that the patient will not be treated. Then, before any process transfer is considered for an autologous therapy, quality comparability has to be guaranteed. For that reason, sensitivity analysis to batch failure rates was not performed. However, in an allogeneic setting, the failure of a quality control will have less impact on treating the patients and more so on the cost distribution. Eventual future studies on xeno-free process transfer for allogeneic therapies should include this comparison. Multicriteria decision models for autologous therapies would allow addressing the quality attributes of the process and the final product in a quantitative manner [49,51].

While this analysis was performed with BM-MSCs, which involve a particularly tedious and expensive collection method, other types of MSCs with less invasive collection methods, such as adipose stem cells or umbilical cord matrix/Wharton's Jelly MSCs (UCM-MSCs/WJ-MSCs), have been isolated and expanded with hPL with improvement in the proliferative potential with lower cell size while retaining potency attributes [37,38,65–67]. As a xeno-free alternative, hPL has the caveats of being dependent on donor samples, high batch to batch variability, poor definition of its components and the possible transmission of viruses. The sterility of hPL could be improved with pathogen inactivation methods without detriment to cell potency, while supplement variability could be reduced with pooling of samples from different donors [58,59,65]. In order to minimize the negative impact of these caveats, chemically defined serum-free/xeno-free media have gained relevance for MSCs processing with comparable proliferation potential to serum containing media [40,65,68,69]. However, the yield of MSCs after isolation is not yet comparable [70] and this factor might impair the cost–effectiveness of a fully serum-free/xeno-free process transfer. The application of the methodology described in this work could provide technological and economical cues to improve the cost–effectiveness of bioprocessing of less invasive MSCs sources under chemically defined media.

The fact that, in autologous therapies, cells from each donor have different proliferative and morphological attributes, results in variable processing times. Implementation of a manufacturing process with donor-dependent process times, where expansion of cells from different donors may start to be processed at the same time, can create process and supply chain management issues that may entail additional operational costs [36]. These issues are particularly noticeable under the current paradigm for the administration of fresh cell products for infusion. Therefore, the creation of off-the-shelf stem cell therapies that come from cryobanks is gaining momentum with the first off-the-shelf MSCs based therapy approved in Europe. We followed an optimistic view for cryopreservation of manufactured doses to mitigate the batch timing issues associated with a manufacturing campaign under the scale-out system, such as the one approved. However, the negative impact of cryopreservation on cell quality attributes after thawing limits this approach [71]. For fresh products, the simulation of the proliferative abilities per donor, after prescreening, would contribute to optimization of resource utilization, for example, selecting batches with similar processing times for parallel manufacturing.

Conclusion

The costs of bioprocessing of MSC therapies are very high in comparison with those of small drug molecules and biologics. Previous work points out the contributions to the total cost of the maintenance of expensive GMP-complying facilities and equipment, high costs of culture media and other reagents, labor-intensive tasks and expensive quality controls [29,30,72].

In the case of autologous cell therapies, relatively low cell numbers are required. In that sense, these therapies are amenable to parallel, scale-out processing. Still, quality control costs are very high, since they are required for each prepared dose that the patient requires [73,74]. In addition to these hurdles, biological variability in the number of BM-MSCs after isolation and in the number of cells at confluence per donor render additional uncertainty to the costs of manufacturing. A new bioprocess and bioeconomics model for MSCs manufacturing simulation embracing the biological variability was developed and used for a specific case study. The case study consists of evaluating the impact, on the total process costs of autologous BM-MSCs therapies of replacing FBS by hPL, as an example of a xeno-free alternative, in culture medium supplementation. The use of hPL promotes the isolation of a higher number of cells after their isolation from bone marrow and after multipassage growth, which translate to

the need of lower passages to achieve the targeted therapeutic dose of cells per patient required. The decrease in the number of passages in turn results in cost savings of 16%, on average, per donor compared with supplementation with FBS. The impact of additional passages required to achieve the therapeutic dose creates a multimodal cost distribution with average additional costs per passage per donor of $1970 for FBS and $2802 on average for hPL. The findings of biological variability in process times and resource consumption and, finally, in process costs per donor, will have an impact on the profit margins once a fixed price for therapy reimbursement is set. However, the supplementation with hPL reduces this variability in comparison with FBS, with the advantage of rendering a robust process cost wise to fluctuations in hPL supplement price, as long as the higher proliferative potential and the generation of more cells per passage at confluence, due to smaller cell size, are maintained and combined with comparable potency and sterility attributes. The modeling methodology, integrating biological variability can help to better plan for the impact of process changes before the start of a clinical trial to design more robust processes, both biologically and economically.

Translational perspectives

Bioprocess economics decisional tools, like the one described here, are powerful tools to assess the impact of changes in a technology and how process decisions should be made to ensure profitability, while quality attributes and regulatory demands are met. With regulatory agencies encouraging a shift from animal-based methods to xeno-free cultures, the economic model presented allows the determination of the full costs of MSCs bioprocessing, assuming a xeno-free supplement and encourages steps to reduce variability in the efficacy of the supplements for autologous based cell culture, since the donor's own cells are the products. It highlights how the need for additional unit operations increases the costs of manufacturing significantly and how determining the worst-case scenarios might help in planning for possible profitability under a set reimbursement price. Cost-efficient processes that retain quality attributes make it more likely that stem cell therapies will reach the clinic. Further work should involve the use of this tool with different types of stem cells and under allogeneic, 3D configurations and incorporate health economics modeling for a specific clinical indication. This model is an open source tool to be made available in the future to stimulate discussion of the utility of early health technology assessment tools for process design in costly therapies that embody stem cells.

Supplementary data

To view the supplementary data, please find files Supplemental material 1 and Supplemental material 2 enclosed [75–78]. To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/rme-2018-0034

Financial & competing interests disclosure

The authors would like to acknowledge support by Institute for Bioengineering and Biosciences (FCT grant number UID/BIO/04565/2013, POR Lisboa 2020 grant number 007317), Programa Operacional Regional de Lisboa 2020 (POR Lisboa 2020) project PRECISE (Project number 16394) and Fundação para a Ciência e Tecnologia (FCT) (grant number PD/BD/105868/2014). On two separate studies involving different research hypotheses, we collaborated with co-authors from Compass Biomedical and presented posters at two scientific conferences. Compass Biomedical reimbursed C Bandeiras for the conference registration fees. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Summary points

Evaluation of culture media for mesenchymal stem cells

- The current gold standard for mesenchymal stem/stromal cells (MSCs) expansion for clinical trials is fetal bovine serum (FBS), associated with ethical concerns and possible viral contamination. Animal-free alternatives are encouraged by regulatory agencies. Human platelet lysate (hPL) has been one of the most attractive options due to the high proliferative potential.

Decision support tool

- Open source development for future distribution under a free license to the community.
- Total manufacturing costs of autologous MSCs bioprocessing from the isolation step from bone marrow, expansion, downstream processing and release testing were evaluated as a means to compare the cost-effectiveness of using hPL supplementation.
- Stochasticity was included from literature data on the isolation efficiency of bone marrow mesenchymal stem/stromal cells and the multipassage growth rates and harvesting densities at the expansion steps.
- Doses of 75 million cells per donor were simulated for 1000 donors and the costs of manufacturing, including facility and equipment, labor, reagents, expansion technologies and release testing, were specified for each donor.

Biological variability is key to determine process costs

- Assuming hPL is twice as expensive as FBS, hPL expansion is more favorable economically due to its superior growth characteristics, allowing relative cost savings of 16% in average per donor.
- The need for additional passages for donors that show weaker growth characteristics has an impact of over $1970 for FBS and $2802 in average for hPL in additional costs of manufacturing per donor, mostly due to the increased equipment and labor operational costs from additional days of culture.
- A sensitivity analysis determined that the most critical parameter for maintenance of cost-effectiveness of the process transfer to a xeno-free culture is the harvesting yield, related to the ability to generate more cells per unit area at confluence per passage.
- It is crucial that comparability is maintained in the culture media supplement substitution to improve or maintain the release testing success rates before any transfer of the process is considered, since a batch failure in an autologous therapy means that the patient will not be treated.

Conclusion

- The hPL is a cost-effective alternative to FBS for bone marrow mesenchymal stem/stromal cells bioprocessing.
- The control of batch to batch variability is fundamental to ensure that the proliferative advantages of hPL are retained across the different donor characteristics.
- The developed tool can be used for process planning and early health technology assessment of autologous MSCs therapies with added detail gained from biological variability specifications.

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