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Patient-specific hiPSC bioprocessing for drug screening: Bioprocess economics and optimisation

Michael Jenkins[a], James Bilsland[b], Timothy E. Allsopp[b], Sa V. Ho[c], Suzanne S. Farid[a,*]

[a] Department of Biochemical Engineering, University College London, Gordon Street, London WC1H 0AH, United Kingdom
[b] Neurosciences & Pain Research Unit, Pfizer, The Portway Building, Granta Park, Great Abington, Cambridge CB21 6GS, United Kingdom
[c] Biotherapeutic Pharmaceutical Sciences, Pfizer, 1 Burtt Road, Andover, MA, USA

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ABSTRACT

This paper describes a decisional tool that is designed to identify cost-effective process designs for drug screening products derived from human induced pluripotent stem cells (hiPSC). The decisional tool comprises a bioprocess economics model linked to a search algorithm to assess the financial impact of manual and automated bioprocessing strategies that use 2D-planar tissue culture technologies. The tool was applied to a case study that examines the production of patient-specific iPSC-derived neurons for drug screening. The production strategies were compared across three analytical drug screening methods, each requiring cell production at a distinct scale (manual patch-clamp analysis, high throughput screening and plate-based pharmacology), as well as different annual cell line utilization requirements ('throughputs') (between 10 and 100 lines) so as to represent different industry scenarios. The tool determined the critical cell line throughput where the most cost-effective production strategy switched from the manual to automated workflow. The key process economics driver was the number of iPSC expansion stages required. Stochastic modelling of the bioprocess illustrated that the automated was more robust than the manual workflow in the scenarios investigated. The tool predicted the level of performance improvements required in iPSC expansion and differentiation as well as reductions in indirect costs and media costs so as to achieve an acceptable cost of goods (COG).

1. Introduction

The advent of hiPSC technology [1] has provided an opportunity to revolutionise modern medicine. Aside from their potential use in the cell therapy sector as clinical grade raw material to produce treatments for a variety of disorders with unmet clinical needs, hiPSCs also offer a more near term application as a tool with which current drug discovery, phenotypic screens, and safety testing programmes might be qualitatively improved [2–5]. This article investigates the production of patient-specific hiPS-cell lines, namely a cell line which is derived from a single patient in order to capture their individual genotype and phenotype. Manufacture of patient-specific cell lines will require scale-out of the process, whereby the manufacturing scale or lot size is kept constant and replicated for each cell line. In contrast, manufacture of non patient-specific cell lines can benefit from scale-up, whereby the manufacturing scale or lot size is increased when larger demands of a cell line are required (see Fig. 1a). Non patient-specific cell lines are more likely to be used for purposes that are independent of the specific genotype or phenotype of a cell.

In drug development, many pre-clinical testing platforms based on animal species prove to be of limited predictive value due to fundamental biochemical, physiological and genomic variations from humans [6–8]. hiPSC-differentiated somatic cells offer an alternative, humanised platform for pre-clinical efficacy and toxicity studies for novel therapeutics in development [9]. They also afford a predictive platform at the preclinical to clinical interface in, for example, safety vigilance of novel therapeutics in development, pinpointing drug responders from non-responders and stratifying patients into treatment groups in patient cohorts. Furthermore, the ability of patient-specific hiPSC-derived cells to model genetic and epigenetic variations of a broad spectrum population may also augment phase I/II clinical trials via the demonstration of a drug's safety

Abbreviations: COG, cost of goods; CTS, CompacT SelecT™ automated cell culture machine; FCI, fixed capital investment; hPSC, human pluripotent stem cell; HTS, high throughput screening; iPSC, induced pluripotent stem cell; PCA, patch-clamp analysis; NCE, novel chemical entity; PBP, plate-based pharmacological analysis; PSC, pluripotent stem cell.

* Corresponding author.
E-mail address: s.farid@ucl.ac.uk (S.S. Farid).

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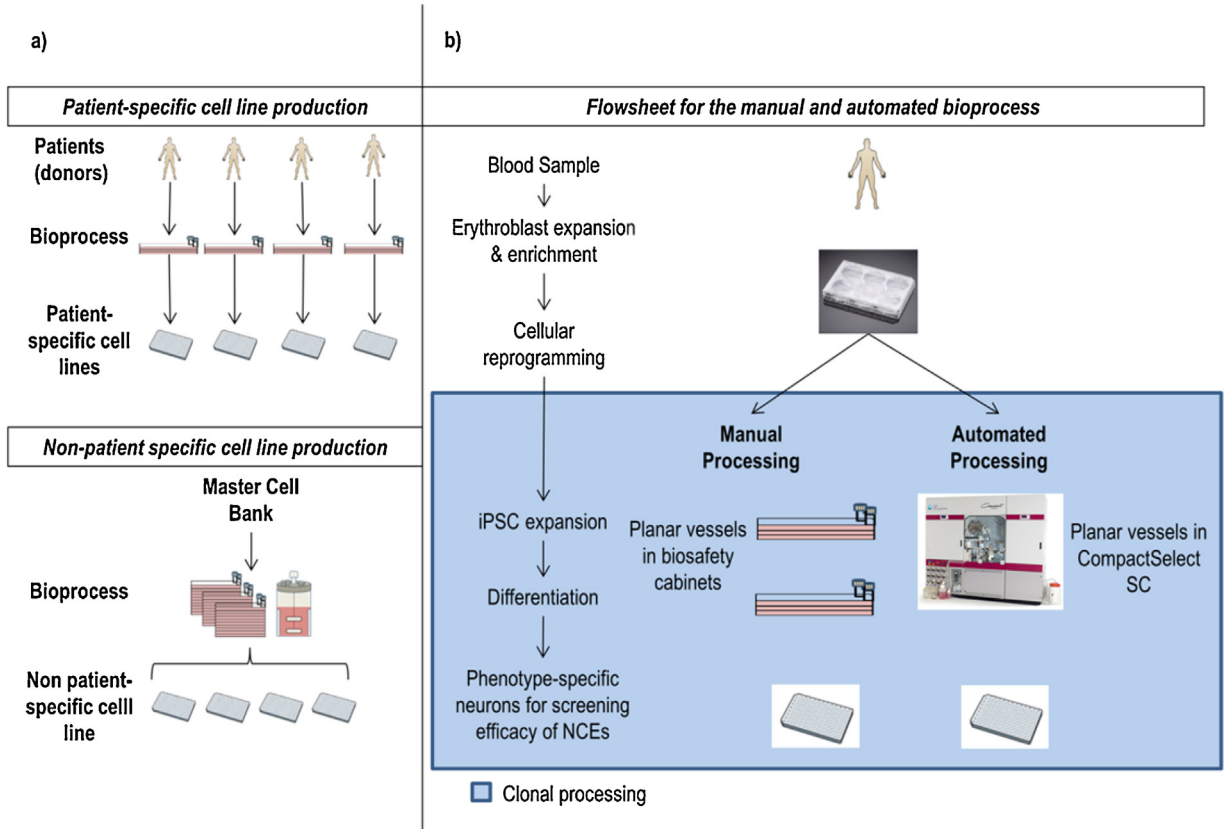

Fig. 1. (a) Outline of process techniques for patient-specific cell lines and non-specific cell lines. The bioprocess for a single patient-specific cell line is scaled-out to achieve a higher throughput. Non-specific bioprocesses are scaled-up to achieve a higher throughput. (b) An overview of the bioprocess strategies considered within this case study. Clonal processing is employed following cellular reprogramming, therefore the technology used to process each donor sample remains constant until this point, regardless of the scale of the bioprocess.

and efficacy within a target population in vitro. Patient-specific hiPSC-derived cells may be of particular use in the assessment of NCEs where the degree of efficacy observed within a cohort may depend upon a specific geno- or phenotype [10]. In this manner they have the potential to lower the time, costs, and risks associated with committing a drug to clinical trials. The cost of bringing a new drug to market is currently estimated to be US$1.2bn—US$1.7bn. When juxtaposed with a clinical attrition rate that can be close to 90% (phase I to approval) [11], such an expensive and complex development cycle has caused drug developers to display caution when committing candidate therapies to clinical trials [12,13].

hiPSC-derived cell types have faced challenges in their implementation owing partly to key issues associated with their production at large scale. Many current iPSC manufacturing protocols are based upon planar, 2-D culture vessels due to their affordability and simplicity [14]. However, lack of scalability owing to limitations in surface area to volume ratio and the inability to conduct online process monitoring are major drawbacks to many 2-D culture systems, as is their labour-intensive nature, which limits their throughput and applicability to larger scale processes [15,16]. Non patient-specific cell processing may benefit from recent iPSC bioprocessing advances, such as single-use bioreactors (SUBs) and microcarriers, which offer both greater potential for scale-up and an enhanced degree of containment in comparison to planar vessels [17–22]. However, patient-specific bioprocesses are not amenable to scale-up and will likely depend upon planar culture technologies, which may be useful when producing cell populations numbering in the low millions [23,24]. Automation systems designed to accommodate planar culture vessels, such as the CompacT SelecT^{TM} (CTS) (Sartorius, Royston, UK), have the potential to reduce the labour requirements, possible points of contamination, and to improve the reliability associated with autologous hiPSC bioprocessing [25–27]. Such systems could be implemented to help achieve large-scale manufacture of patient-specific stem cell products.

The biopharmaceutical sector has benefitted from the use of decisional tools that are able to evaluate alternative process designs in silico in order to achieve cost-effective process design and equipment selection [28–32]. As a nascent field, hiPSC processing is faced with a lack of consensus as to optimal process designs and scale-up/out strategies. Decisional tools similar to those applied to the biopharmaceutical sector can therefore prove useful for identifying key economic drivers and technical innovations required to bridge the gaps constraining widespread application of hiPSC-derived cells. There are only a limited number of studies investigating the impact of process design on manufacturing COG within the stem cell sector. Previous analyses have provided estimates of the current limitations [15,33] and relative cost of PSC processing technologies, including the use of commercially available flow-sheeting software (SuperPro Designer, Intelligen Inc., NJ, USA) to evaluate the economic potential of large-scale iPSC-derived cell bioprocess designs [34]. For other cell types, for example mesenchymal stromal cells for therapy, others have illustrated how a decisional tool can be developed to determine the scales at which microcarriers in SUBs becomes preferable to planar processing platforms during the expansion phase [16].

In this paper, an integrated decisional tool that combines both bioprocess economic modelling and optimisation of the manufacture of patient-specific iPSC-derived neurons for use as a tool in the screening of NCEs is described. The bioprocess economics model and integrated brute-force search algorithm are designed to iden-

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tify the process design that minimises the COG. The use of specified automation equipment such as the CTS for iPSC cell bioprocessing and its impact upon the overall COG is also evaluated as an alternative to existing, manual bioprocessing options (Fig. 1b).

2. Tool description

A decisional tool was developed to evaluate the COG associated with different bioprocess strategies and equipment sizing of technologies to be used during the manufacture of patient-specific iPSC-derived cell lines. It comprises a bioprocess economics model, an information database, and an integrated brute-force search algorithm. The tool was constructed in and is implemented via Microsoft Excel (Microsoft Corporation, WA, USA) and the Visual Basic for Applications tool (VBA) (Microsoft Corporation). A brute-force search algorithm generates possible process configurations, from a database of specified process technologies, to be evaluated by the bioprocess economics model. Microsoft Excel was used because of its dual functionality in being able to act as a database for model inputs and outputs in addition to a tool by which to construct a bioprocess economics model. VBA's simple integration with MS Excel makes it ideal for efficiently linking the database and bioprocess economics model to the optimisation algorithm.

2.1. Deterministic bioprocess economics model

The bioprocess economics model evaluates the COG per cell line for different bioprocess configurations. A critical feature of patient-specific cell bioprocesses is that they dictate the adoption of a scale-out, rather than scale-up, approach to process design and development as throughput increases. Therefore, separate processing of individual donor products is assumed throughout the model, i.e. separate culture vessels are used for cells from separate donors.

A key parameter when evaluating equipment sizing strategies is the type of technology to be used within each unit operation and the number of units required to process the required number of cells from each donor sample. For a given cell output, P (cells per cell line), the number of units ($u_{\mathrm{con},i,j}$) required of a particular technology, j, for a given unit operation, i, is calculated as;

$$u_{\mathrm{con},i,j} = \frac{P_i}{d_{h,i} \times a_j} \tag{1}$$

where a_j is the available surface area per unit for each technology and $d_{h,i}$ represents the harvest density (cells/cm^2) for a given unit operation.

Determining the number of units for a given technology allows the bioprocess economics model to draw on information stored in the database so as to evaluate the COG/cell line via a cascade of equations. Thus, the bioprocess economics model is able to calculate the total COG/cell line:

$$\frac{\mathrm{COG}}{\mathrm{Cell\ line}} = \left(\sum_i \left[C^{\mathrm{ann}}_{\mathrm{mat},i} + C^{\mathrm{ann}}_{\mathrm{lab},i} \right] \right) + \frac{C^{\mathrm{ann}}_{\mathrm{indirect},}}{D_{\mathrm{ann}}} \tag{2}$$

where $C_{\mathrm{mat},i}$ and $C_{\mathrm{lab},i}$ are the costs of raw materials and labour, or the direct costs, associated with each individual unit operation within the bioprocess. D_{ann} is the annual throughput (cell lines produced per year). Direct costs attributed to a specific unit operation are calculated separately for each individual unit operation, whereas indirect costs are calculated on a whole bioprocess basis because fixed equipment may be shared between multiple unit operations.

Table 1
Methods and feeding regimes for bioprocess unit operations.

Unit operation	Method	Feeding regime
Erythroblast expansion & enrichment	Culture-based expansion and purification of erythroblasts	Daily media replacement
Cellular reprogramming	Sendai virus transduction of Yamanaka factors	Media replacement every two days during transduction Daily media replacement during iPSC generation period
iPSC expansion	Monolayer 10% of viable cells banked to safeguard against process failure during differentiation	Daily media replacement
Differentiation	Monolayer, small molecule based	Twice weekly media replacement

Table 2
Labour tasks and their duration.

Labour task	Time required (h) (t_i)
Media preparation	1
Cell harvest	0.75
Cell seeding	0.5
Media replacement	0.5
Viral transduction	1.5
CompacT SelecT setup	2
CompacT SelecT termination	2
Culture check (manual)	0.5
Culture check (automated)	0.25

Material costs per unit operation, $C^{\mathrm{ann}}_{\mathrm{mat},i}$, are calculated as a function of the utilisation of chemicals (e.g. media) and consumables (e.g. plastic culture vessels):

$$C^{\mathrm{ann}}_{\mathrm{mat},i} = u_{i,j} \left[V_{\mathrm{med},j} \times a_{i,j} \times C_{\mathrm{med},i} \right] + C_j \tag{3}$$

where $V_{\mathrm{med},j}$ is the media utilisation (mL/cm^2), $C_{\mathrm{med},i}$ the cost of media used within a particular unit operation i and C_j the price per unit associated with a given technology j.

Assumptions regarding media replacement regimes, which are taken into account by the deterministic model, can be found in Table 1.

Labour costs are calculated as a function of the time required to carry out tasks specific to each unit operation of the iPSC bioprocess (t_i). It is assumed that for each technology type an operator could handle a given number of units, ω_j, within this time period. Therefore:

$$C^{\mathrm{ann}}_{\mathrm{lab},i} = \left(\frac{u_{ij}}{\omega_j} \right) \times t_i \times w \tag{4}$$

where w represents the hourly cost of labour. Individual labour tasks and their durations can be found in Table 2.

2.1.1. Indirect costs

Fixed capital investment costs are calculated using an adjusted Lang factor developed at UCL for estimating capital investment costs associated with disposable facilities [32]. The Lang factor method assigns a proportionality constant to the total equipment purchase cost in order to provide a facility capital investment (FCI) estimate [35]. The Lang factor accounts for items that contribute to the cost to build the facility such as pipework, instrumentation, building works, construction, commissioning and validation.

$$\mathrm{FCI} = F_{\mathrm{lang}} \times C_{\mathrm{fixed}} \tag{5}$$

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where C_{fixed} represents the cost of any fixed equipment within the bioprocess facility. The fixed equipment costs are calculated thus:

$$C_{fixed} = \sum_{i=1}^{n} \left(\lceil \frac{u_{i,x}}{\alpha_{inc,i}} \rceil \times C_{inc} + \lceil \frac{u_{i,x}}{\alpha_{bsc,i}} \rceil \times C_{bsc} + \lceil \frac{u_{i,x}}{\alpha_{CTS,i}} \rceil \times C_{CTS} \right) \quad (6)$$

where α_{inc}, α_{bsc} and α_{CTS} are the capacities of incubators, biosafety cabinets and CTS (when automated processing is employed), respectively, with regards to a particular technology, *i*, during the bioprocess. C_{inc}, C_{bsc} and C_{CTSt} refer to the cost of individual units of fixed equipment used within the bioprocess. It was assumed that biosafety cabinets could only be used by one operator at a time.

In this study the main annual indirect cost considered was the depreciation, calculated by dividing fixed capital investment costs by the depreciation period, t_{dep}, representing the lifespan of fixed equipment:

$$C_{indirect}^{ann} = \frac{C_{fixed}}{t_{dep}} \quad (7)$$

Within unit operations such as iPSC expansion, where multiple expansion stages are required, different technologies may be preferable for each subsequent stage. Therefore, each expansion stage was treated as a de facto unit operation and logic constraints were put in place such that technologies in preliminary expansion stages were smaller, or equal in size to candidate technologies in subsequent ones (i.e. $a_{i,j-1} \leq a_{i,j}$). This is in order to abide by standard processing protocols, whereby the size of the equipment used in the expansion stages of stem cell culture is traditionally scaled up as cell populations continue to grow.

Additionally, process designs returning fixed equipment utilisation values of greater than 95% were discarded from results. This is to avoid utilisation of such equipment exceeding capacity in the event where additional vessels might be required owing to variations in processing parameters.

2.2. Brute-force search algorithm

The brute-force algorithm has been designed to determine optimal equipment sizing for each unit operation within the process via a procedure which examines all possible bioprocess designs according to process technologies housed in the database.

The bioprocess economics model described above is able to calculate the COG per cell line for a specified process configuration using technologies of a given size. A brute-force search algorithm develops all of the available process designs for the bioprocess economics model to quantify on the basis of COG per cell line. This bioprocess economics model then generates COG data for the array of alternative process designs. The output data is then screened in order to identify the bioprocess design that minimises COG. Fig. 2 illustrates how the optimisation algorithm works alongside the bioprocess economics model to achieve cost-effective process design.

2.3. Stochastic modelling

The deterministic COG provided by the tool described in the previous section allowed identification of the optimal bioprocess design and equipment sizing within each unit operation of the bioprocess. However, deterministic analyses cannot offer an evaluation of the robustness of iPSC bioprocessing strategies. Achieving process reproducibility is a significant challenge within stem cell bioprocessing, particularly when manual processing strategies are employed [14,25,26]. Furthermore, accounting for donor-to-donor variability also presents a unique challenge. The Monte Carlo simulation method was applied to the decisional tool to provide stochastic modelling of the iPSC bioprocess in order to quantify the robustness and reproducibility of automated and manual iPSC bioprocess strategies. Adjusting input parameters also allowed the model to capture variations in COG that arise as a result of donor-to-donor variability within key process parameters.

Previously published reports have suggested that manual bioprocesses result in greater variability than those which are automated [26,37]. Hence, narrower distributions have been assigned for the automated iPSC bioprocess to reflect this (input probability distributions are shown in Table 6). Probability ranges have been corroborated with industry experts to ensure they are representative.

The Monte Carlo simulation method was used to carry out probability-based economic assessment of the manual and automated bioprocess. During the Monte Carlo simulation, the process flow-sheet was held constant. The culture vessels tested within the Monte Carlo analysis were those selected as the most cost-effective by the brute-force search algorithm. In order to ensure that the target cell population size was achieved for each donor cell-line, the direct resources and fixed equipment required were re-calculated during each Monte Carlo simulation to account for the variations in process parameters as a result of the assigned input distributions. Future improvements to screening methods may allow one to identify single-nuclear polymorphisms (SNPs) in the original donor sample that correlate with bioprocess performance. In future, this method could allow the likely performance of a donor's iPSCs to be ascertained prior to the bioprocess. Thus, prospective re-sizing of the bioprocess may be a feasible option; therefore, the number of culture vessels has been left as a variable in the Monte Carlo analysis.

3. Case study setup

3.1. Tool application

A representative case study was developed in partnership with Pfizer's Neuroscience and Pain Research Unit (Great Abington, Cambridge, UK) that focused on optimising the production of patient-specific iPSC-derived cells for drug screening. Specifically, it addressed the generation of iPSC-derived neurons to supply patient drug responder versus non-responder, "trial in a dish" screening platforms with which to augment clinical trials for NCEs. It is estimated that cell lines from 50 separate donors could be required to run such a screening programme. To investigate the effect of scale-out on the COG associated with the bioprocess, throughputs of 10, 50 & 100 cell lines per year were investigated by this study.

There are a variety of analytical methods by which to carry out drug screening. Currently, the resting or active status of the membrane potential in functional iPSC-derived neurons are analysed by patch-clamp analysis (PCA), a manual technique which requires a small sample population of iPSC-derived neurons (10^5). Whilst this is a data-rich analytical method, it samples only a small number of cells and is therefore a poor representation of whole cell populations [36]. Powerful, automated analytical techniques such as high throughput screening (HTS) and plate-based pharmacological analysis (PBP) are also available methods by which to screen cells' reactions to NCEs. These techniques require larger sized cell populations (2×10^6 and 10^7 cell, respectively), but offer high throughput analytical platforms. The level of scale-up required of the bioprocess required to manufacture each individual cell line is dependent on the analytical technique used for drug screening, along with characteristics of different analytical techniques (Fig. 3).

Two different manufacturing strategies were considered during this case study; manual bioprocess techniques were evaluated alongside an automated iPSC bioprocess, whereby iPSC expansion and differentiation were carried out using the CompacT SelecT SC

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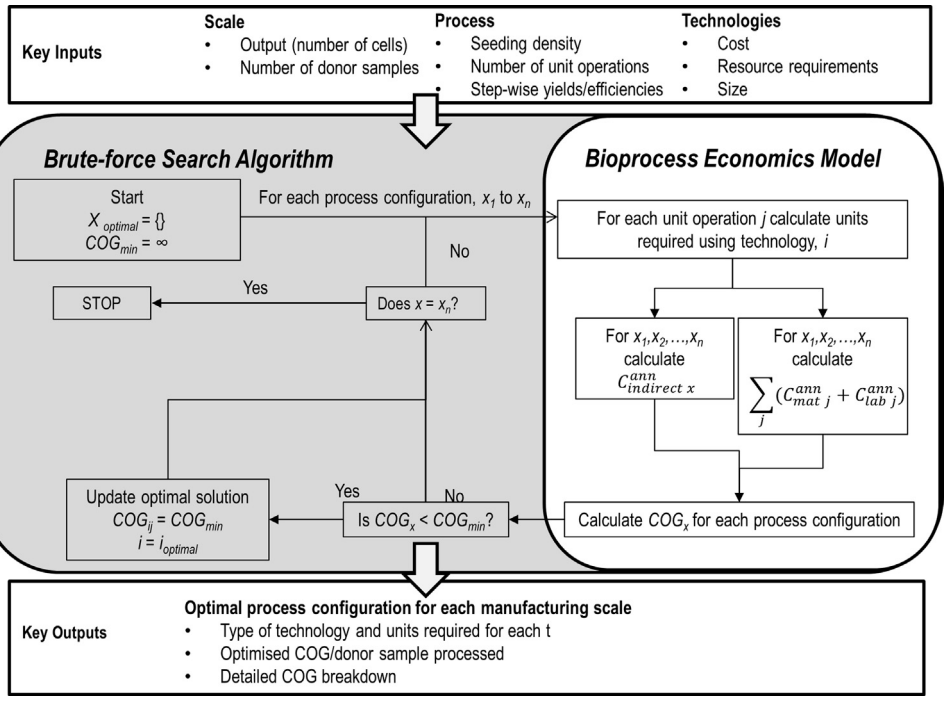

Fig. 2. Tool schematic showing evaluative network used to identify the optimal bioprocess design, $x_{optimal}$.

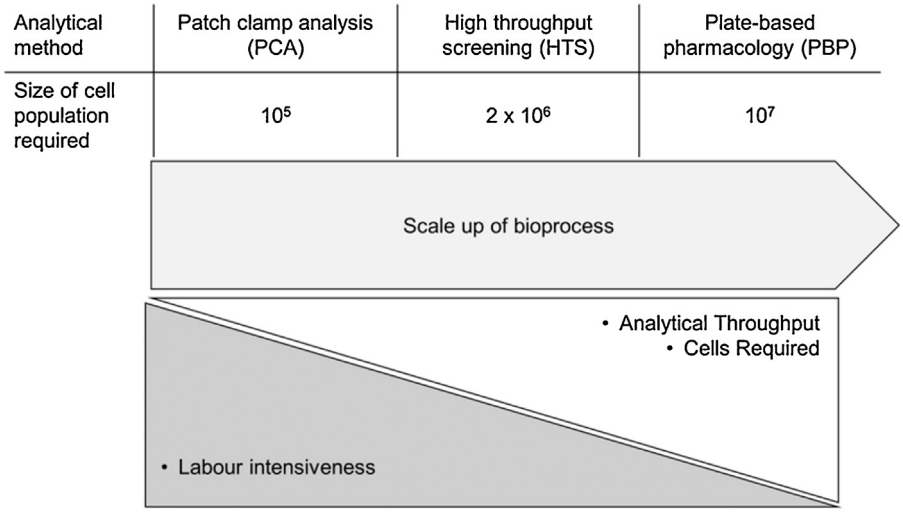

Fig. 3. Cell population sizes of iPSC-derived neurons required to satisfy the demands of different analytical drug screening techniques. The required population size dictates the scale of the bioprocess required to produce cells for each analytical method. The lower panels illustrate graphically key characteristics of each analytical technique.

(Sartorius, Royston, UK). The decisional tool was applied primarily in order to select the most cost-effective process design in both instances. However, process reproducibility in stem cell bioprocessing is a significant challenge. As such, stochastic modelling was also employed to ascertain the robustness of both manufacturing strategies using the Monte Carlo simulation method.

3.2. Process overview

The process flowsheet used for the case study (Fig. 1) employs processing of individual clones following the reprogramming stage; as such, the preliminary unit operations are of a constant scale regardless of the final number of iPSC-derived neurons produced from each cell line. Therefore, the main focus of this case study comprises the iPSC expansion and differentiation unit operations. Further details regarding the methods used and feeding regimes assumed for each unit operation can be found in Table 1. Media changes are assumed to involve a full exchange of media within a process vessel.

The planar technologies considered within the case study included well plates (WP) as well as standard and compact multi-layer versions of T-flasks (T, cT, 3-F, 5-F) and stacked vessels (L-2, L-5, cL-12). Their key input parameters are stored within the tool's database (Table 3). This data was compiled using vendor websites and consultation with industry experts so as to properly capture the characteristics of each candidate technology. Key process and cost assumptions are summarised (Tables 4 and 5) and where possible, these values have been compiled via a thorough review of available literature on planar bioprocessing of iPSCs and their derivatives. All assumptions listed in Tables 4 and 5 were then corroborated with

Table 3
Process technologies tested by the tool and their key performance parameters.

Technology type (i)	Surface area (cm^2/unit) (a_i)	Cost (£/unit) ($C_{con\ i}$)	Media requirements (mL/unit) ($V_{req\ i}$)	Max units per operator ($\omega_{op\ i}$)	Incubator capacity (double stack) ($\delta_{inc\ i}$)	Requires BSC cabinet (Y/N)	CompacT SelecT capacity
T-25	25	£1.54	7	4	100	Y	–
T-75	75	£2.21	18.75	4	100	Y	90
T-175	175	£4.95	50	4	100	Y	90
T-225	225	£5.27	56.25	4	100	Y	–
6-WP[a]	9.5	£2.10	2	24	600	Y	–
24-WP[a]	1.9	£2.66	0.5	48	2400	Y	–
L-2[b]	1272	£55.08	315	2	60	Y	–
L-5[b]	3180	£180.49	787.5	2	24	Y	–
cL-12[c]	6000	£287.69	1300	2	24	Y	–
3-F[d]	525	£10.20	150	–	–	N	90
5-F[e]	875	£17.00	250	–	–	N	90
cT[f]	1720	£63.58	500	–	–	N	90

[a] Well plate.
[b] Multilayer planar vessels e.g. CellSTACK (Corning).
[c] Compact multilayer vessel e.g. 12-layer HYPERStack (Corning).
[d] 3-layer T-flasks e.g. Triple Flask (Nunc) or Falcon Multi-Flask (Corning).
[e] 5-layer T-flasks e.g. Falcon Multi-Flask (Corning).
[f] Compact multilayer flask e.g. 10-layer HYPERFlask (Corning).

Table 4
Key process assumptions.

Unit operation (j)	Seeding density ($cells/cm^2$)	Harvest density ($cells/cm^2$) ($d_{h,i}$)	Yield/harvest yield/efficiency (%)	Fold expansion
Erythroblast enrichment	1.5×10^5	3.2×10^5	25 (Yield)	N/A
Erythroblast expansion	1.5×10^5	3.2×10^5	95 (harvest yield)	2
Reprogramming (transduction)	2.6×10^5	2.6×10^5	95 (harvest yield)	N/A
Reprogramming (generation)	10^4	10^4	0.5 (efficiency)	N/A
iPSC expansion	4.2×10^4	3×10^5	90 (per passage)	7
Differentiation	3×10^5	3×10^5	35 (efficiency)	N/A

Table 5
Key process cost parameters.

Cost Parameter		Value[a]
Media		
	Differentiation media	£0.55–£0.67/mL
	Expansion medium	£0.43/mL
	Reprogramming vector	£664/donor
Labour		£60/h
Fixed equipment		
	Incubator (Large)	£11,890
	Incubator (Small)	£5,175
	CompacT SelecT	£550,000
	Biosafety cabinet	£11,390
Depreciation period		10 Years
Lang factor (manual bioprocess strategy)		23.7
Lang factor (automated bioprocess strategy)		16

[a] Price ranges are provided where more than one type of media is used within a unit operation.

industry experts to ensure they were representative. While sensible inputs and assumptions were sought, the primary aim of the paper was to demonstrate the application of the proposed methodology to provide visibility of the cost structure and the most significant process economics drivers for iPSC generation for drug screening applications. Hence, the actual inputs and answers should not be seen as definitive but an illustration of how to approach such an assessment. The risk of batch failure was also considered. The manual bioprocess strategy was assumed to exhibit a higher probability of batch failure (4%) compared to the automated strategy (2%) given the greater human intervention and degree of open processing in the manual strategy. Failure rates were assumed to capture the worst-case scenario, i.e. failure occurs during the final stages of differentiation. Therefore, the direct costs of iPSC expansion and differentiation for additional donor samples required due to process failure were added to the final COG figure within the deterministic model to account for additional processing as a result of the batch failure rate. Only iPSC expansion and differentiation were assumed to be repeated following a failed lot because of the cell banking procedure in place.

4. Results and discussion

The decisional tool was used to assess the cost-effectiveness of alternative bioprocess designs across a range of different scales of production. A deterministic model was developed in order to carry out COG comparisons between different process designs and sensitivity analyses were used to further investigate economic drivers associated with the iPSC bioprocess. The tool was then adapted for stochastic modelling in order to evaluate the robustness under uncertainty of automated and manual bioprocessing strategies using the Monte Carlo simulation method.

4.1. Deterministic cost modelling

4.1.1. Optimal bioprocess designs across different scales and throughputs

The optimal combination of process technologies for the final iPSC expansion stages and differentiation operation, for both the manual and automated bioprocess are depicted in Fig. 4. The number of iPSC-derived neurons produced per cell line is representative of the size of cell populations needed to satisfy the demands of PCA, HTS and PBP analysis (moving vertically from the top to the bottom

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a)

		Cell lines produced per year	
Analytical Method [required output (iPSC-neurons/cell line)]	Unit Operation	50	100
Patch Clamp Analysis [10^5]	Expansion (final stage)	6-WP [4]	6-WP [4]
	Differentiation	T-25 [1]	T-25 [1]
High Throughput Screening [2 x 10^6]	Expansion (final stage)	T-75 [4]	T-75 [4]
	Differentiation	T-175 [1]	T-175 [1]
Plate-Based Pharmacological Assay [10^7]	Expansion (final stage)	L-2 [2]	L-2 [2]
	Differentiation	T-225 [4]	T-225 [4]

b)

		Cell lines produced per year	
Analytical Method [required output (iPSC-neurons/cell line)]	Unit Operation	50	100
Patch Clamp Analysis [10^5]	Expansion (final stage)	T-75 [4]	T-75 [4]
	Differentiation	T-75 [1]	T-75 [1]
High Throughput Screening [2 x 10^6]	Expansion (final stage)	T-75 [4]	T-75 [4]
	Differentiation	T-175 [1]	T-175 [1]
Plate-Based Pharmacological Assay [10^7]	Expansion (final stage)	T-175 [25]	3-F [11]
	Differentiation	5-F [1]	5-F [1]

Fig. 4. Matrices showing the optimal bioprocess configuration for the final stage of expansion and differentiation for (a) manual bioprocess strategies and (b) automated bioprocess strategies across the range of scale investigated in this study. Lighter shaded cells indicate cheaper COG per cell line relative to other bioprocess designs shown in the matrices, where the lightest shade indicates the cheapest COG per cell line output value.

of the matrices). The cell population outputs per cell line required for each of these analytical methods are shown in Fig. 3, and also in Fig. 4. Annual throughputs of 10, 50 and 100 cell populations have been shown to depict the effects of scale-out on optimal bioprocess design. The bioprocess throughput, in terms of cell lines produced per year, increases horizontally from left to right across each matrix. The matrices show the optimal technology size and the number of units required (in square brackets) per cell line for each scale tested.

Depicting optimal technology sizing for manual bioprocessing shows that as the size of cell populations produced increases, there is a trend towards technologies with a larger surface area (Fig. 4a). Larger technologies, which require fewer vessels, are selected as the optimal bioprocess design. For example 2×2-layer CellSTACK vessels (L-2) are preferred for the final expansion stage when producing 10^7 cells per cell line (for PBP), as opposed to $4 \times$ T-75 flasks if 2×10^6 cells per cell line were to be produced (for HTS analysis). Larger vessels are preferred when larger cell populations are produced when a manual bioprocess strategy is employed as depicted by the direct cost breakdown for optimal technologies for the manual bioprocess at different production scales at a throughput of 50 donor samples per year for the final expansion stage and differentiation (Fig. 5). The utilisation of available surface area in smaller technologies is higher, thus they make more efficient use of media. Material expenditures can be reduced if smaller cell populations are produced (such as those required for PCA and HTS analysis) if smaller technologies are used. This is exhibited by a 39% reduction in direct costs if T-75 flasks are used as opposed to L-2 vessels for the final iPSC expansion stage at the HTS scale (Fig. 5a). However, when satisfying the demands of PBP, the additional labour costs incurred by the use of large numbers of units of smaller vessels far outweigh the incremental reduction in material costs that such technologies offer within the manual bioprocess. The COG per cell line for the differentiation can be reduced by 76% if T-225 flasks are employed rather than T-25 flasks (Fig. 5b). This is due to the reduction in the resultant labour costs associated with the use of T-225s. Darker shades within each area of the matrices (Fig. 4) are illustrative of higher COG/cell line. Thus, the matrix also demonstrates the economies of scale that can be achieved when the number of cell lines produced per year increases. This is particularly true with regards to automated processing (Fig. 4b), where a greater range in the COG between different annual donor sample throughputs can be observed than for the manual bioprocessing strategy.

A COG breakdown for the different equipment sizing configurations available for use in the final stage of expansion and differentiation for the automated bioprocess strategy, whereby 10^7 iPSC-derived neurons are produced per cell line at a throughput of

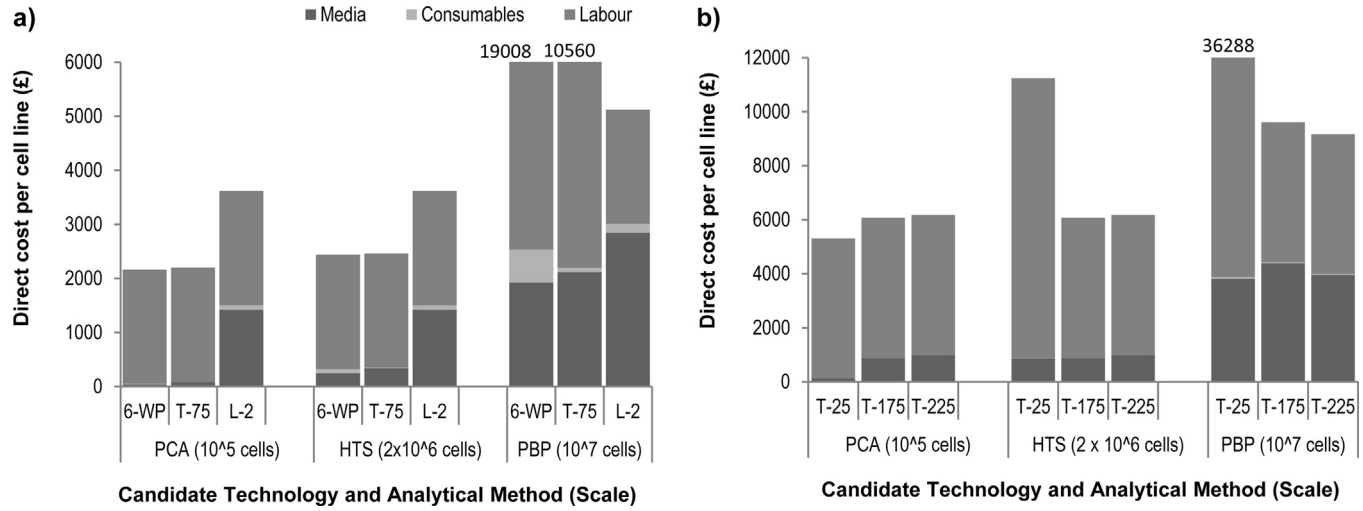

Fig. 5. Direct cost breakdown for the optimal technologies at scales satisfying the demands of the three analytical drug screening techniques (PCA, HTS & PBP) at a throughput of 50 cell lines/yr for (a) the final stage of expansion and (b) differentiation. Required iPSC-derived neuron outputs per donor are shown in brackets on the *x*-axis. Maximum values on the *y*-axis have been assigned to maintain scales whereby data can be clearly seen. Therefore for scales satisfying demands of PBP, direct costs beyond these values are labelled above the relevant columns.

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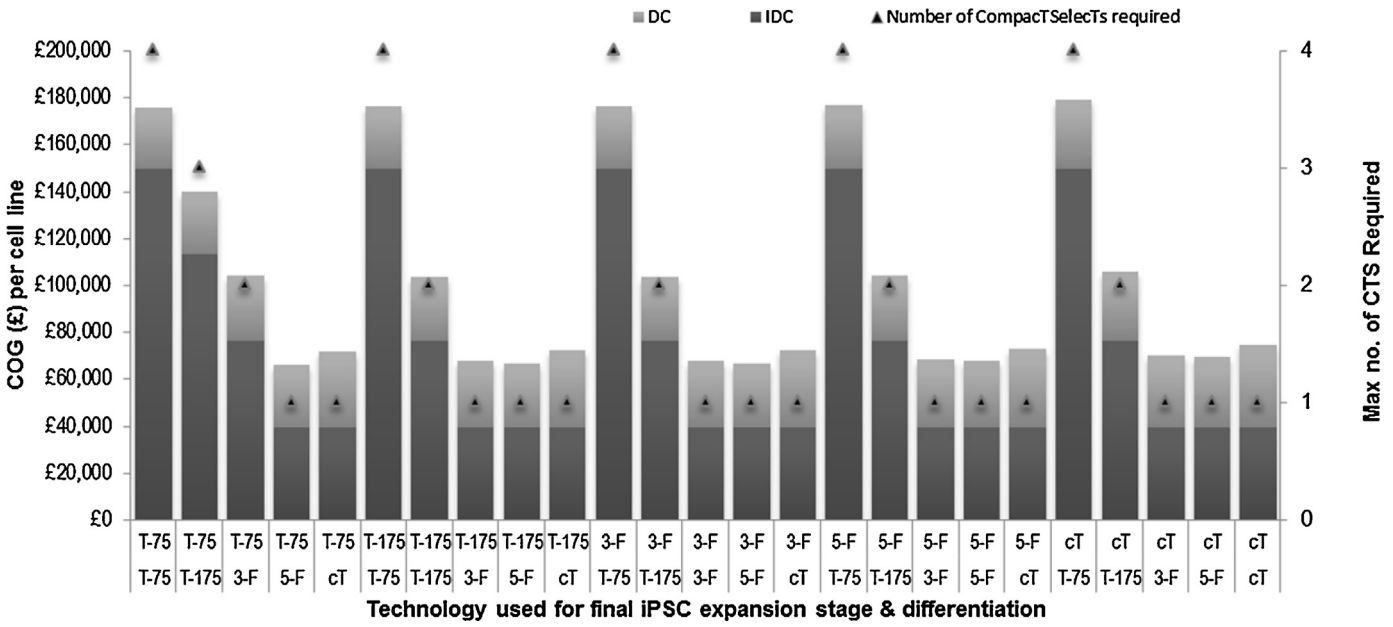

Fig. 6. COG breakdown for possible equipment sizing configurations for an automated process strategy for the final iPSC expansion stage and differentiation unit operations at a throughput of 50 cell lines/yr, whereby populations of 10^7 cells are produced to satisfy the demands of PBP analysis. CTS utilisation refers to percentage utilisation of CompacT SelecT automated processing equipment, i.e. 367% will require 4 CompacT SelecT units. DC = direct costs. IDC = indirect costs.

50 cell lines per year is shown in Fig. 6. The use of smaller vessels, such as T-75s, necessitates the use of multiple CTS machines due to the number of vessels required for such technologies, which corroborates results described earlier (Fig. 4b), in that larger technologies are preferred for this throughput in the optimal technology matrix. This is particularly true of vessels used during differentiation, a lengthy process that occupies equipment capacity for long periods of time. The optimal process configuration, where the technologies selected were 11 × T-75 flasks for the final iPSC expansion stage and 1 × 5-F (5-layer T-flask) for differentiation, results in a maximum requirement of 1CTS machine. However, were T-75s and T-175s to be used for the respective process steps above (as is optimal for producing cells for HTS analysis), then 4CTS machines would be required to produce cell populations satisfying the demands of PBP analysis. The latter of these options results in only an incremental direct costs reduction (~1%), whereas the resultant indirect costs are 375% higher than the optimal process design. Larger technologies, which can house cell populations in a smaller number of vessels, are preferred as they do not have as significant an impact on automated processing equipment utilisation. Owing to the automated nature of this processing strategy, the use of multiple vessels does not impact significantly upon labour costs, unlike manual processing, where it is important to minimise the number of units required in order to drive down labour costs.

Analysis of COG breakdowns for manual and automated bioprocesses when the demands of HTS and PBP analysis at throughputs of 50 and 100 cell lines per year are satisfied shows that automated processing can significantly reduce labour costs associated with stem cell bioprocesses (Fig. 7). This is particularly due to additional ancillary tasks associated with manual bioprocess strategies, such as media changes and cell seeding and harvesting. Material costs do not fluctuate greatly between manual and automated processing. However, savings on the cost of labour are outweighed by the additional indirect costs (a function of fixed capital investment required) for the automated bioprocess when 50 cell lines are produced. A 10% COG per cell line reduction is offered by manual processing when cells are produced for PBP analysis. At a throughput of 100 cell lines per year, the additional indirect costs associated with automated bioprocess strategies are spread across enough cell lines to provide significant COG reductions (19% at PBP scale) against the manual bioprocess - the cost of which is heavily weighted by labour costs. This is supported further by percentage COG breakdowns for the manual and automated bioprocesses when cell lines are produced to satisfy the demands of PBP analysis. At higher annual throughputs, direct costs make up a higher percentage of the COG breakdown. For the automated bioprocess, direct costs account for 41% of COG (70% for manual processing) when 50 cell lines are produced per year, compared to 58% (72% for manual processing) if 100 cell lines are produced annually. This analysis provides evidence that automation of patient-specific iPSC bioprocesses can provide significant COG reductions at scales sufficient to warrant the additional capital expenditure. Indirect costs dominate the COG breakdown for the automated bioprocess (Fig. 6) hence minimisation of the number of pieces of automation equipment must be achieved in order to curtail COGs.

There is no significant reduction in either the labour costs or material costs for both the manual and the automated bioprocess at throughputs of 50 and 100 cell lines per year (Fig. 7). However, when the process is scaled out, smaller COG figures are realised as a result of reductions in the indirect costs per cell line. This suggests that economies of scale can only be achieved for patient-specific hiPSC bioprocesses requiring scale-out through shared use of fixed equipment. Bioprocess scheduling and equipment sizing in order to maximise use of fixed equipment and minimise the required size of cleanrooms must therefore be considered during process design in this area.

It can be concluded that a threshold throughput exists where automated bioprocesses offer a COG reduction compared to a manual bioprocesses. This is in accordance with previous studies in stem cell bioprocess design [16]. When assessing the COG per cell line for both the automated and manual bioprocess at throughputs ranging from 50 to 200 cell lines, the point at which the automated bioprocess becomes more cost-effective than the manual bioprocess is at a throughput of 65 cell lines (Fig. 8). The spike on the line representing COG per cell line for automated bioprocesses also depicts the throughput at which it is necessary to purchase additional automated processing equipment (a throughput of 110 cell lines). The switchpoint identified between automated and manual processing

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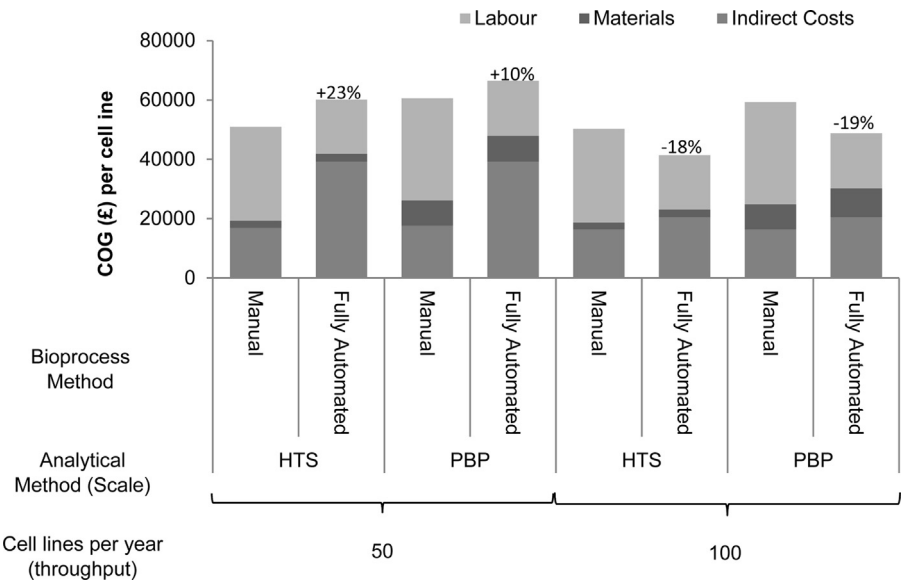

Fig. 7. COG breakdown for manual and automated bioprocess strategies at throughputs of 50 and 100 cell lines, whereby populations of 10^7 cells are produced in order to satisfy the demands of PBP. Percentage changes in COG/cell line caused by implementing an automated bioprocess strategy are shown above columns representing automated bioprocess COG breakdowns.

Table 6
Probability distributions assigned to key bioprocess performance parameters in the stochastic Monte Carlo analysis of the manual and automated bioprocess strategies.

Parameter	Probability distribution type	Manual bioprocess probability distribution profile (min, most likely, max)	Automated bioprocess probability distribution profile (min, most Likely, max)
iPSC expansion harvest yield	Triangular	75%, 90%, 95%	85%, 90%, 95%
iPSC expansion harvest density	Triangular	2.5×10^5, 3×10^5, 3.5×10^5	2.7×10^5, 3×10^5, 3.3×10^5
iPSC expansion fold	Triangular	6.3, 7, 7.7	6.6, 7, 7.4
Differentiation efficiency	Triangular	20%, 35%, 45%	30%, 35%, 40%
Differentiation harvest yield	Triangular	80%, 90%, 95%	85%, 90%, 95%
Differentiation seeding density	Triangular	0.8×10^4, 1×10^5, 1.1×10^5	0.9×10^5, 1×10^5, 1.1×10^5

Note: most likely values in the distribution profiles are taken from the base case scenario from the deterministic bioprocess economics model.

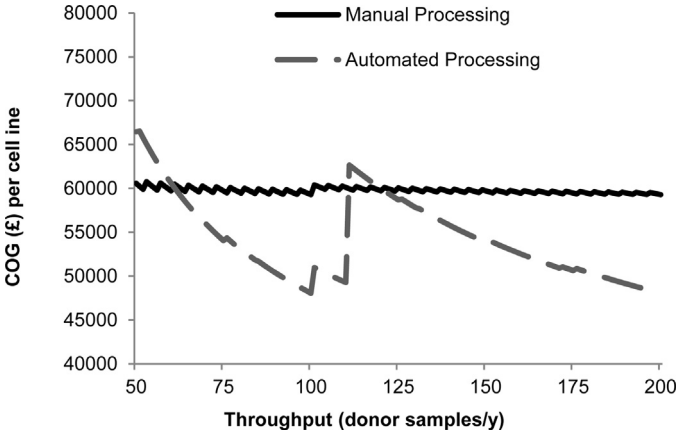

Fig. 8. COG per cell line at annual throughputs ranging from 50 to 200 cell lines of 107 iPSC-derived neurons for manual and automated processing.

are specific to the modelling assumptions made in this particular case study.

The assumed cost of automation equipment is based upon the current purchase cost for a functional CTS machine. At the time of writing, this was the only piece of automation equipment that could fully support this bioprocess. In the future, other pieces of equipment may become available and competition may drive down the cost of automation equipment. The effect of the price of automation equipment price on bioprocess COG can be seen in the Supplementary information accompanying this paper (Fig. S1).

4.1.2. Sensitivity analysis

In order to further identify key economic drivers associated with the iPSC-derived bioprocess, and to provide further understanding of where process development and optimisation resources might be best focused, a sensitivity analysis was carried out. Several process and cost parameters were varied to reflect their best and worst case values. The tool was used to test the resultant change upon the COG per cell line values that these variations. The Tornado charts (Fig. 9) illustrate the effect on COG that variations in key parameters caused for (a) the manual bioprocess and (b) the automated bioprocess at a throughput of 50 cell lines per year (where cell populations that satisfy the demands of PBP are produced). The number of iPSC expansion stages required was the greatest economic driver for both the manual and automated bioprocess strategies (Fig. 9). Variations in the number of iPSC expansion stages can occur due to fluctuations in key performance parameters. The impact of this parameter upon COG can be significant, as each additional iPSC expansion stage necessitates additional direct resources and fixed

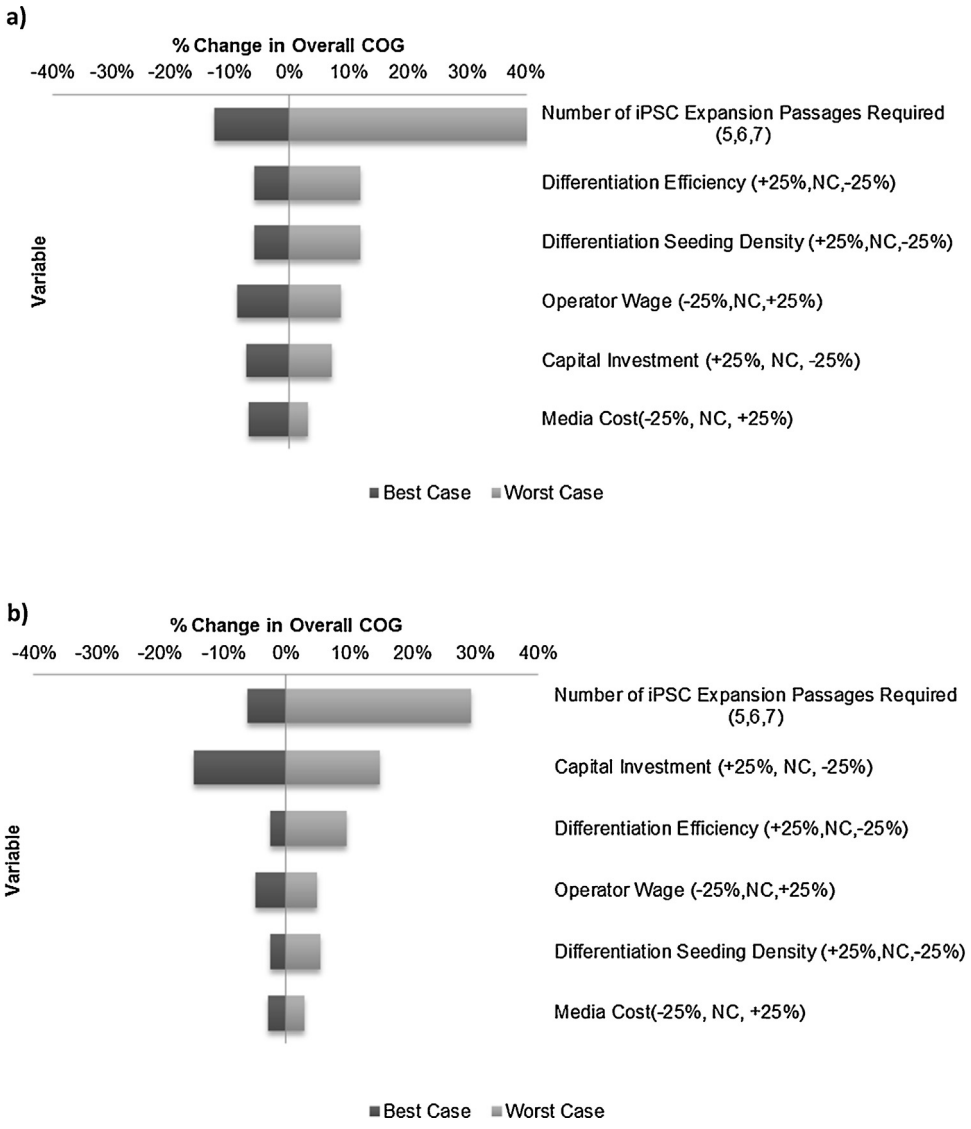

Fig. 9. Tornado plots showing the effect of variations in key parameters on COG for (a) the manual bioprocess strategy and (b) the automated bioprocess strategy at a throughput of 50 cell lines/ year. Best case and Worst case values are shown in brackets on the *y*-axis labels.

Table 7
Key statistical parameters of the COG per cell line values for the manual and automated bioprocess strategies from the stochastic Monte Carlo analysis.

	50 cell lines per year		100 cell lines per year	
	Manual bioprocess	Automated bioprocess	Manual bioprocess	Automated bioprocess
Mean (μ)	£64,841	£66,655	£65,405	£48,601
Standard deviation	£8481	£3025	£9493	£3221
$P(\text{COG} < \mu_{\text{automated}})$	0.81	0.93	0	0.89

equipment capacity. Thus, a change in the number of iPSC expansion stages required within a bioprocess will significantly affect COG per cell line.

Labour costs are also a significant cost driver for the manual bioprocess, resulting in an 18% difference in COG between the best and worst case scenarios. The fact that labour costs have been identified as a key process economic driver is perhaps unsurprising; this parameter dominates the COG breakdown at high cell line throughputs (see Fig. 7a). Similarly, the secondary cost driver for the automated bioprocess is FCI costs, which are the largest contributor to the COG breakdown for this process strategy (Fig. 7b).

4.2. Stochastic modelling

The mean COG/cell line value for the automated bioprocess is higher than that of the manual bioprocess when producing 50 cell lines per year, whereas at 100 cell lines per year, this value is higher for the manual bioprocess, as described by the stochastic analysis (Table 7). These figures are consistent with the deterministic model results, which also show the same trend (Fig. 7).

The results of the Monte Carlo analysis (Table 7 and Fig. 10a and b) show that the standard deviation and range of COG values for the automated bioprocess strategy at throughputs of both 50 and 100 cell lines per year (£3,025 (GBP) and £3,221 respectively) are significantly lower than those of the manual bioprocess strategy

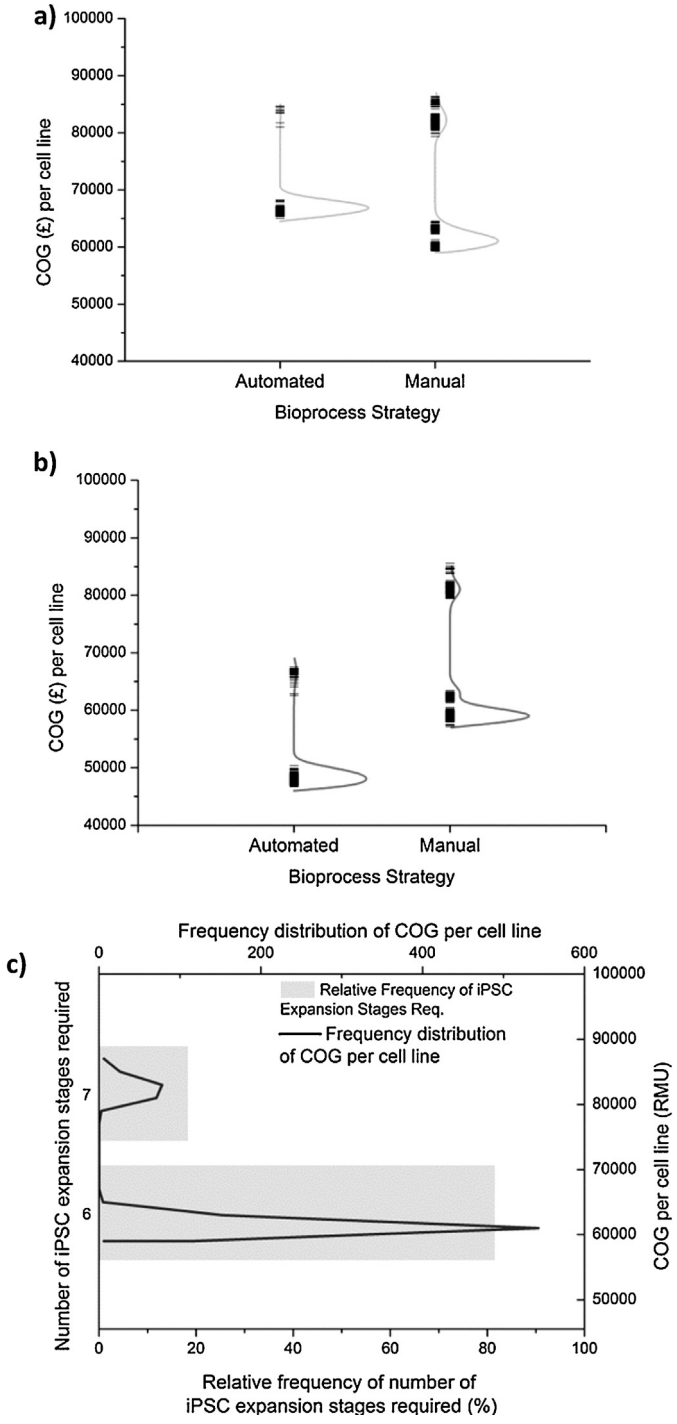

Fig. 10. Frequency distributions for the COG per cell line outputs for the automated and manual bioprocess strategies under uncertainty when producing (a) 50 cell lines per year and (b) 100 cell lines per year. (c) Relative frequency of the different number of iPSC expansion stages for the manual bioprocess strategy for 50 cell lines per year superimposed onto the COG per cell line frequency distribution. Dashed lines depict individual COG per cell line outputs and continuous curves represent the frequency distributions of the COG per cell line outputs.

(£8,481 and £9,493 at 50 and 100 cell lines per year, respectively). This suggests that the automated bioprocess is more robust from a bioprocess economics standpoint than the manual bioprocess. The frequency density curves and COG distributions (Fig. 10a and b) follow a distinct pattern. They are bimodal with a positive skew and hence the majority of COG values are concentrated at the lower end of the distribution and are followed by a long tail with a smaller peak at the upper end of the distribution with higher COG values. This is particularly true of the frequency distribution curves of the manual bioprocess, where the peaks at the upper end of the distribution where high COG values occur are larger compared to those for the automated bioprocess. One explanation of the shape of the frequency distributions can be illustrated in Fig. 10c, which shows the relative frequency at which the number of iPSC expansion passages required for the manual bioprocess (at a throughput of 50 cell lines per year) rises from six (as in the base case) to seven based on process variability modelled in Monte Carlo analysis. The frequency distribution of the COG per cell line for the manual bioprocess from Fig. 10a is also displayed on Fig. 10c. This shows that the minor peak with high COG values that appears in Fig. 10a and b is due to the requirement of an additional stage of iPSC expansion owing to process variations modelled during the Monte Carlo simulation. Fig. 10c also emphasises the importance of minimising the number of iPSC expansion stages as a strategy for minimisation of COG.

4.3. Can an acceptable COG for in-house manufacture of patient-specific hiPSC derived cell lines be achieved?

List prices from vendors for a vial containing 10^6 non patient-specific hPSC-derived cells currently lie in the region of US$ 1000–2000. This study shows that, unlike non patient-specific stem cell bioprocesses, where both direct and indirect cost savings can be achieved on a per million cells basis via appropriate scale-up strategies [16] economies of scale can only be achieved in patient-specific cell lines through shared use of fixed equipment. Direct costs per cell line do not fluctuate significantly, regardless of throughput (Fig. 7). The market price of patient-specific cell lines may therefore be assumed to be higher than their non-specific counterparts; not only because of the added expenditure as a result of scale-out processing, but also the added analytical value provided by such products in responder versus non-responder studies and potential personalised medicine regimes (see Section 1). This is reflected in the fact that patient-specific hPSC-derived cell lines are marketed for ~US$50k (~£35k) for 10^7 cells, including the additional costs of iPSC derivation and genetic engineering.

This case study reflects an in-house hiPSC-derived cell line manufacturing regime. According to the stochastic modelling above, the minimum COG per cell line that can be achieved is ~£45,000. This is significantly higher than current market prices for such products. In order for in-house production of cell lines for drug screening to be worthwhile, an acceptable COG per cell line must be below current market prices. A scenario analysis was designed to identify the process improvements required to reduce COG per cell line to below £35,000. Within the scenario analysis, reductions in the indirect costs and media costs have been assumed. A one-off new-build facility has been assumed for this case study in order to capture the fixed equipment costs associated with manufacturing patient-specific cell lines. In reality, such an in-house facility would likely be used for multiple research activities. To reflect this, indirect costs of 60% and 75% of the base case value have been investigated in this analysis. Established Big Pharma companies might also make use of existing experience in pharmaceutical reagent manufacturing in order to produce media components and small molecules required for certain unit operations in-house. This would allow some media components to be produced at cost price, rather than purchasing them at vendor list prices (as assumed in base case results). COG per cell line at both base case media costs and a 25% reduction in media costs have been modelled in this analysis. The process parameters varied in this analysis were the differentiation efficiency and iPSC expansion fold per stage. These parameters were chosen for two reasons; primarily they both impact upon the number of iPSC expansion stages required. This was identified as a key process economics driver in the sensitivity analysis (Fig. 9). Secondly, advances

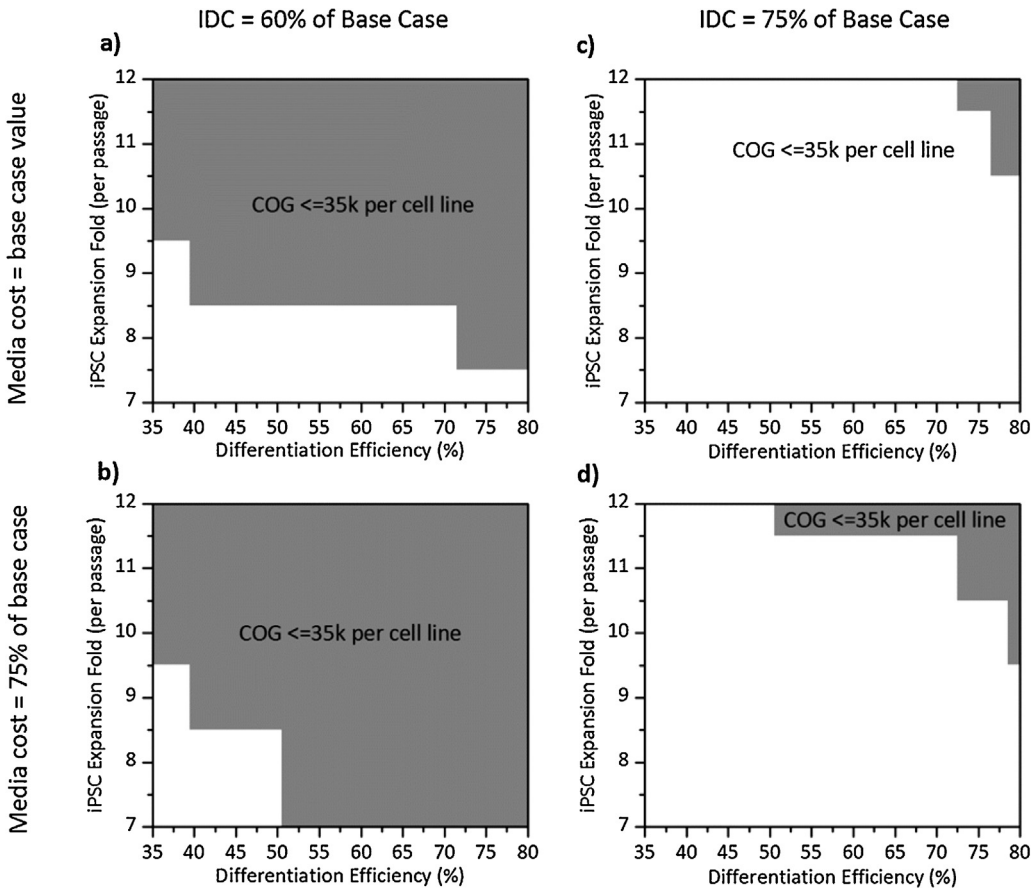

Fig. 11. Contour plots showing windows of operation where COG ≦ £35k/ cell line when (a) media cost = base scenario and IDC = 60% of base case values (b) media cost = 75% of the base scenario and IDC = 60% of base case values (c) media costs = base case value and IDC = 75% of base case values (d) media costs = 75% of base case value and IDC = 75% of base case. Shaded areas on the plots show windows of operation whereby COG ≦ £35,000/cell line, white areas of the plots represent windows of operation whereby COG > £35 k/ cell line. IDC = indirect costs.

in media composition and morphogen delivery systems during iPSC expansion and differentiation are areas of concentrated research. Innovations in these areas have proven that improvements in these parameters are achievable on the base case values assumed in this case study [21,22,38–41].

The in-house scenario considered in this project was for a single drug screening project application and hence the indirect costs were only spread across this activity. In future it is possible to envisage several drug screening applications where the investment is offset across several projects similar to how a vendor may achieve economies of scale. Hence the impact of lowering the indirect costs was explored. If indirect costs are 60% of base case value, there is a large window of operation whereby a COG ≦ £35k per cell line, even when media costs remain at the base case value (Fig. 11a). Indeed, if the achievable iPSC expansion fold can be increased from 7 to 10, then it would be acceptable for differentiation efficiency to remain at 35%. If media costs could be reduced by 25%, then a differentiation efficiency of 50% (up from 35% in the base case scenario) would be required for COG ≦ £35k per cell line, even if the iPSC expansion fold could not be improved. The windows of operation shrink significantly at both base case and reduced media cost values when indirect costs increase from 60% to 75% of the base case values (Fig. 11c and d). In this instance, improvements in both differentiation efficiency and iPSC expansion to 76% and 11%, respectively, would be required. Less dramatic improvements would be necessary if media costs could be reduced by 25%. In this instance differentiation efficiencies as low as 52% and iPSC expansion folds of 10 could allow COG ≦ £35k per cell line to be achieved. The maximum indirect cost (% base case) whilst media costs remain at base case value whereby a COG ≦ £35k per cell line is still feasible was found to be 77%. When media costs are 75% of the base case values, this value increases to 81%. Both of these instances would require improvements to produce a 12-fold expansion during iPSC culture and a differentiation efficiency of 79%. Conversion of hiPSCs to neurons at efficiencies beyond 80% have been reported [41] as have iPSC expansion folds of up to 11.3 over 7 days in planar conditions, or up to 28 in bioreactor conditions [22,42] Therefore, realising the windows of operation discussed above is not infeasible. In the event that fixed capital investment (or indirect costs) can be reduced by 25% or more there are realistic scenarios whereby in-house manufacture of patient-specific, iPSC-derived cell lines could be worthwhile for companies wishing to carry out responder versus non-responder studies as part of drug development regimes. Lower indirect cost values could be achieved by either retrospective fitting of an existing research facility, or by diversifying the functionality of a new-build facility. The scenarios analysed above also illustrate how changes to the bioprocess in terms of the key process parameters might impact upon COG and shows how the findings of this study might be applied to bioprocesses other than the one that is the focus of this work.

The scenarios examined here determine an acceptable COG to be less than current market prices for patient-specific, hiPSC-derived cell lines. This is because this case study examines production of such cell lines for use in in-house studies. Were the cell lines discussed in this case study to be manufactured for sale, the acceptable COG figure would be far lower in order to take into account COG as

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a percentage of sales. Smith (2010) determines this value to be in the range of 40–60% for autologous products. It is possible to conclude that acceptable COG for patient-specific hiPSC-derived cell lines designed for sale is in the region of £14,000–£21,000 per cell line (whereby 10^7 cells are produced for each line). It is assumed that a process producing cell lines for commercial sale would be of a far greater scale than the bioprocess examined in this study (i.e. 100 cell lines per year). Identification of process improvements required to reduce the COG derived in this study to such values is therefore beyond the scope of this paper. However, future work will focus upon large-scale bioprocess design strategies in order to maintain a supply chain of hPSC-derived cell lines to a number of clients.

5. Conclusion

A decisional tool is presented that consists of a bioprocess economics model with an integrated brute-force search algorithm. The tool has been applied to an industrial case study, in which patient-specific iPSC-derived neurons are produced for use in responder versus non-responder studies as part of the development of NCEs to be used in personalised medicine regimes. Via the use of this tool, optimal equipment sizing regimes were identified for both a manual and an automated hPSC bioprocess. Furthermore, it can be concluded that whilst the most cost-effective option of the two process strategies tested is dependent on the throughput of the process, the use of automation equipment was found to result in a more robust bioprocess in terms of COG values when tested under uncertainty. Indirect cost reductions are required in order to achieve acceptable manufacturing COG. This might be done by retrospectively fitting existing facilities, or diversifying functionality of a new-build facility in order to offset some of the required fixed capital investment to other projects. Were an existing full-scale process to be adapted from a manual to an automated bioprocess it would be wise to take into account the cost and time required to train staff to use automated equipment. Furthermore, the logistics and costs of updating facility infrastructure to cope with automated processing should be considered in the case of retro-fitting an existing facility. This work modelled a relatively small scale bioprocess in commercial terms. However, the outputs can be of use in aiding decision making early on in bioprocess design for patient-specific cell line production at a variety of scales. Expanding the decisional tool presented here to account for larger scale bioprocesses, including the production of cells for autologous and allogeneic cell therapies will be the focus of future work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bej.2015.09.024.

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