Biochemical Engineering Journal 137 (2018) 132–151

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Impact of allogeneic stem cell manufacturing decisions on cost of goods, process robustness and reimbursement

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ARTICLE INFO

Article history:
Received 29 November 2017
Received in revised form 23 March 2018
Accepted 22 April 2018
Available online 25 April 2018

Keywords:
Allogeneic
Mesenchymal stem/stromal cells
Robustness
Cost of goods
Reimbursement
Multi-attribute decision-making analysis

ABSTRACT

This article presents a framework to evaluate holistically the operational and economic performance of different manufacturing platforms for the expansion of allogeneic mesenchymal stromal cells (MSCs) across different commercialisation scenarios. The tool comprised models for whole bioprocess economics linked to uncertainty analysis, dynamic scheduling, brute-force optimisation and multi-attribute decision-making. The tool was used to determine the cost of goods (COG), robustness, operational ease and business feasibility of competing cell culture technologies under different scale, demand, reimbursement and dose size scenarios, and to determine the performance improvements required for commercial success. The results revealed that in low annual demand (10 billion cells/year) scenarios, multi-plate bioreactors have superior operational and economic characteristics. At larger annual demands (10 trillion cells/year), however, the tool predicts that microcarrier-based bioreactors are optimal due to their relative cost-effectiveness and operational benefits conferred by their closed and controlled characteristics that outweigh the uncertainties associated with their use. Moreover, whilst further analysis of high dose, high demand (1 billion cells/dose, 10,000 doses/year) scenarios has shown that significant improvement in the performance of cell culture processes may result in satisfactory COG, current limitations in the capacity of downstream processing (DSP) technologies may not allow full market capture.

1. Introduction

Cell therapy products have been proposed as a novel approach for treatment and possibly cure of a number of chronic indications. However, these therapeutics present significant challenges that include high COG, high process variability and scale-up restrictions [1–6]. As COG decreases with increasing manufacturing scale [7], there is an increased interest in the development and evaluation of scalable technologies for cell therapy manufacture. Methods for the evaluation of novel technologies are often based on COG and process yield. However, there are a number of less tangible issues that must also be considered when designing a manufacturing process for cell therapy products such as robustness, biological limitations, technology scalability, and ease of development. This paper aims at providing a holistic approach to evaluate the benefits of different technologies for the manufacture of adherent cells that captures economic aspects (e.g. COG, fixed capital investment, reimbursement potential) as well as operational aspects (e.g. robustness, resource requirement, ease of validation, ease of operation, ease of development).

The cell therapy market was estimated at $12 billion USD in 2016 and is estimated to grow at a CAGR of 31.1% to $61 billion USD by 2022 [8]. The global market for mesenchymal stem cells (MSCs) alone is foreseen to reach $7.5 billion USD by 2022 with the United States having the largest share of this market (34.3%) and Asia being the continent with the highest projected CAGR (14.1%) [9].

Mesenchymal stem cells also referred to as mesenchymal stromal cells were first identified by Friedenstein and colleagues [10] as post-natal cells that are capable of self-renewal and differentiation [11–14] into other cells such as cardiomyocytes, neurons and insulin producing cells. This potency of MSCs makes them suitable for the treatment of diseases such as cardiovascular diseases, spinal cord injury, Parkinson's and diabetes [15–20]. MSCs can be retrieved from multiple sources such as the bone marrow, adipose tissue, amniotic fluid, dental tissues, peripheral blood, and placenta [14,21–26]. Benefits to the use of MSCs include the fact

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https://doi.org/10.1016/j.bej.2018.04.017

Nomenclature

N^n_{unit}	Number of cell culture vessels required for the expansion stage n0
$N_{cells/batch}$	Number of cells produced per batch
y_{DSP}	DSP yield
$y_{cell\ detachment}$	Cell detachment yield
$y_{batch\ sucess}$	Batch success rate
a_t	Surface area for cell growth of a cell culture vessel
$Density_{microcarrier}$	Microcarrier's seeding density
$Utilisation_{cell\ culture\ vessel}$	% utilization a cell culture vessel
$a_{per\ gram}$	Surface area per gram of microcarrier
C_{media}	Media costs
$C_{media/ml}$	Media costs per ml
$V_{media/a}$	Volume of media required per unit surface are of cell culture vessel
$C_{trypsin}$	Trypsin costs
$C_{trypsin/ml}$	Trypsin costs/ml
$V_{trypsin/a}$	Volume of trypsin required per unit surface are of cell culture vessel
$C_{consumables}$	Consumables costs
$C_{consumable/unit}$	Consumables costs per cell culture vessel
C_{labour}	Labour costs
$C_{operator}$	Operator salary
$R_{documentation/executing}$	Ratio between the documentation operator and the executing operator
$R_{Overhead/operator}$	Ratio between labour overheads and operator salary
t_{step}	Time required per cell culture vessel to perform a particular activity
$t_{step\ max}$	Maximum time allowed for a particular activity
FCI	Fixed capital investment
$C_{equipment}$	Equipment costs
L_f	Lang factor
$C_{equipmnet/unit}$	**U**nit costs for a particular type of equipment
$Capacity_{equipment}$	Max number of cell culture vessels that the equipment can handle
C_{BSC}	Biosafety cabinet costs
$C_{BSC/unit}$	Biosafety cabinet costs per unit

that they have a low half-life [27], low immunogenicity [28–29] and immunomodulatory characteristics, making these cells suitable for the treatment of graft vs host disease (GvHD), rheumatoid arthritis and Alzheimer's among other indications [30–36]. MSCs have also paracrine and homing properties [27,37]. Given the many beneficial characteristics of MSCs, these have now been used in over 300 clinical trials across multiple indications [1,27,37–40]. To date, commercialised MSC cell therapy products include Heartcelligram-AMI® (Pharmicell, South Korea), Prochymal® (Osiris, MD, USA) and Cartistem® (Medipost, Weymouth, UK). The key information regarding these therapies and other therapies is summarised in Table 1. The COG and reimbursement potential of MSC-based products targeting some of these indications will be explored in this article. The success of the few commercially available cell therapy products has been accompanied by some high-profile failures. Reasons for these failures include high COG, failure to demonstrate sufficient clinical benefit and low market penetration due to price and competition [7,41–45].

Although allogenic cell therapy products have the potential to be more cost-effective than autologous products [38,41], as their production can be scaled-up as opposed to scaled-out [47], these products must still overcome a number of regulatory, scientific and manufacturing hurdles in order to achieve commercial success [45–48]. The limited scalability of technologies for the manufacture of anchorage-dependent cells is one of the key challenges associated with designing a process for commercial scale manufacture of MSC-based products [34,48,49]. The severity of this scalability issue depends on the indication being targeted [50]. For low dose indications ($\sim 1 \times 10^7$ cells) such as chronic low back pain [50,51], multilayer flasks may suffice for cell culture; these flasks have the adequate capacity to produce cell numbers as high as 400 billion cells per batch [53]. Multi-layer flasks employ open processing and hence require cleanrooms with high ISO qualifications [54] that can increase the initial facility investment required, which may be cost-prohibitive for some SMEs. For treatments such as GvHD or heart disease with higher dose sizes ($\sim 10^7$–10^8 cells) [28,55–58] however, larger manufacturing scales would be required. Larger manufacturing scales require the use of bioreactors with microcarriers which will allow for batch sizes as high as 1 trillion cells to be achieved [39,52], while maintaining optimal and controlled physiochemical conditions and environment growth for cell growth [59]. The use of microcarriers in suspension carries its own hurdles that include achieving consistent performance upon scale-up [60] and that the cells must be separated from the microcarriers after the cell culture step [1,53]. Cell separation is typically carried out using enzymatic intervention [61], which must be quenched rapidly to avoid damage to the cells [2]. The time between cell detachment and final formulation typically varies from less than 1 h [2,62] to 4 h [63]. Moreover, there are a number of different microcarriers available on the market which would yield different attachment efficiencies, proliferation rates and have different effects on the pluripotency of the cells [53,64,65] (Table 2). Alternative bioreactors for adherent cell expansion include the Quantum® hollow fibre (Terumo BCT, Lakewood, CA, USA) [7,53,66–69] and the Xpansion® (Pall Life Sciences, NY, USA) [7,53,70–72]. Both technologies, offer a closed and controlled environment for cell growth, however these offer lower capacity than microcarrier-based cell therapy [7]. This article will establish the economic and operational trade-offs associated with both planar and microcarrier-based cell culture.

Increasing the scale of manufacture also decreases the number of feasible candidate technologies for wash and concentration of cell therapy products [63]. One of the most promising technologies on the market for cell harvest and wash is the tangential flow filtration (TFF) system previously used in the biopharma industry for protein purification and adapted to cell therapy products by different companies [73]. The drawback associated with the use of this technology is that the TFF process may sometimes be stressful to the cells [62]. A second alternative to large scale wash and concentration of cell therapy products is the use of fluidised bed centrifuges such as kSep® (Sartorius, Göttingen, Germany) [63]; this technology is able to process batch sizes as high as 1000 L [73] with a capacity of 0.6 trillion cells per cycle [74]. Fill and cryopreservation are also a bottleneck at scale. Thousands of doses per batch can be vialled using the Crystal® PX Filling Line (Aseptic Technologies, Gembloux, Belgium) [63,73].

Cell therapy products are complex, very sensitive to environmental changes and require multiple expensive biological agents [5]. Moreover, as previously mentioned, the current cell therapy manufacturing process is highly manual, which makes an already expensive process highly variable and susceptible to operator error [3]. The chances for operator error are bound to increase with increasing batch size as more manipulations are required. Additional sources of variability are encountered in cell therapy manufacturing. These sources include: the cell source, age and health of the donor, the heterogeneity of the cell bank and reagents such as the serum batch [1–4,27,37,40]. Process variability is an important factor to be considered when selecting a manufacturing platform and this article will explore its effect on the cost-effectiveness of different technologies. Strategies to decrease donor-related variability includes donor screening.

Table 1
Current cell therapy products available on the market. Auto = autologous, Allo = allogeneic.

Product name	Product developerw	Indication	Type	Dose size	Cell type	Selling price/dose	Country
Allostem®	Allosource	Bone repair [94]	Allo [94]	6.63 K cells/ml [94]	Adipose MSC [94]	$540–$3,500 (1 ml–10ml) [94]	US
Apligraft®	Organogenisis	Chronic wounds[95]	Allo [95]	44 cm^2 [96]	Keratinocytes & Neonatal Fibroblasts [95]	$21.22 cm^2 [97]	US/Saudi Arabia
BioDfactor®	BioDlogics	Tissue repair [98]	Allo [99]	0.25 ml–1.25 ml [99]	Placenta cells [99]	–	US
BioDfence®	BioDlogics	Tissue repair [98]	Allo [99]	3 cm^2–12 cm^2 [99]	Placenta cells [99]	–	US
CardioRel®	Reliance life sciences	Myocardial infraction [100]	Auto [100]	–	MSCs [100]	–	India
Carticel®	Genzyme	Cartilage repair [101]	Auto [101]	0.6–3.3 M cells/cm^2 [101]	Chrondocytes [101]	$13,300–15,000 [102]	US/EU
Cartistem®	Medipost	Osteoarthritis [103]	Allo [104]	2.5 M cells/cm^2 [105]	UC Mesenchymal cells [105]	$20,000–$40,000 [106]	South Korea
Chrondocelect®	TiGenix	Cartilage repair [107]	Auto [107]	0.4 ml/vial (100 B cells/ml) [107]	Cartilage cells [107]	$24,000 [106]	EU (withdrawn)
Cupistem®	Anterogen	Rectal fistula [108]	Auto [109]	–	Adipose [108]	–	South Korea
Dermagraft®	Organogenisis	Chronic wounds [110]	Allo [111]	37.5 cm^2 [112]	Fetal Fibroblasts [110]	$1,406 [112]	US/ Canada
DeNovo NT®	Zimmer	Cartilage repair [113]	Allo [113]	2.5 cm^2/packet [114]	Juvenile chondrocytes [113]	$1,440/packet [113]	North America
Epicel®	Genzyme	Burns treatment [115]	Auto [115]	50 cm^2/gauze [116]	Keratinocytes [116]	$6,000–$10,000 per 1% of total body surface area [117]	US/EU
Grafix®	Osiris	Chronic wounds [118]	Allo [118]	–	Placental cells [118]	–	US
Gintuit®	Organogenesis	Mucogingival conditions [119]	Allo [119]	177 cm^2 cellular sheet with 4Mcells [119]	Keratinocytes [119]	–	US
Heartcelligram-AMI®	Pharmicell	Post-acute myocardial infraction [120]	Auto [120]	–	BM MSC [120]	$19,000 [90]	South Korea
Heartsheet®	Terumo	Heart failure [121]	Auto [121]	5 sheets [121] 3 × 0.1 ml/linear cm [122]	Skeletal myoblasts [121]	$120,000 [106]	Japan
MACI®	Genzyme	Cartilage repair [123]	Auto [123]	0.5–1 M cells/sqcm of cellular sheet [123]	Chondrocytes [123]	–	EU (suspended) US
Orcel®	Ortec	Burns [124]	Allo [124]	–	Keratinocytes, fibroblasts [124]	$27.8/$cm^2$ [125]	US
Osteoplus®	Nuvasive	Bone repair [126]	Allo [126]	50 K cells/ml [127]	MSCs [127]	$460–$5,400 (1–15 ml) [94]	US
Prochymal®	Osiris	GvHD [128]	Allo [129]	2M cells/kg [128]	BM-MSCs [129]	$20,000/dose [106]	Canada & New Zealand
Provenge®	Dendreon	Prostate cancer [130]	Auto [130]	50M cells/vial [130]	CD54 + cells [130]	$31,000/infusion (3 infusions) [131]	US
Recell®	Avita medical	Skin loss, scaring and depigmentation after burn injury [132]	Auto [132]	1 pack/320 cm^2 [132]	Skin cells [132]	£950 + VAT/pack [132]	EU, UK, Canada, Australia
ReliNethra®	Reliance life sciences	Sight loss [133]	Auto [133]	4 cm^2/graft [133]	Epithelia cells [133]	–	India
Temcell®	Mesoblast	GvHD [91]	Allo [91]	1.2–1.7B cells [91]	MSC [91]	$7,079/72 M cells [91]	Japan
Transcyte®	Organogenisis	Temporary would healing [134]	Allo [134]	–	Fibroblasts [134]	$11.75 cm^2[135]	US
Trinity/Trinity evolution®	Orthofix	Bone repair [136]	Allo [136]	>1 K cells/ml [94]	MSC [94]	$540–$5,455 for (1–15 ml) [94]	US

Table 2
Examples of microcarriers currently available on the market.

Name	Manufacturer	Type	Surface	Interaction type	Surface area (cm^2/g)	Working concentration (g/L)	Average cm^2/L
Cultispher-S	Sigma-Aldrich	Porous	Gelatin [137]	Porcine Gelatin [138]	7,500 [138]	1-3 [139, 140, 141, 142]	15,000
Cytophore 1	Pharmacia	Macroporous	Cross-linked cotton cellulose [65]	Diethylaminoethyl [65,138]	11,000 [65]	2 [65, 138]	22,000
Cytophore 2	Pharmacia	Macroporous			11,000 [65]	2 [65,138]	22,000
Cultispher-G	Sigma-Aldrich	Porous	Gelatin [137, 65]	Gelatin [65,138]	40,000 [65]	1-8.5 [140, 65, 143]	180,000
Cytodex 3	GE Healthcare	Microporous	Dextran, Gelatin [137,138]	Denatured collagen [65, 138, 144]	2,700 [65,138]	0.5-5 [145, 65, 141, 144, 142]	
Cytodex 1	GE Healthcare	Porous	Dextran, positively charged [137, 138]	Diethylaminoethyl [65, 138]	4,400 [65, 146, 144]	1.2-5 [65, 146, 143, 144]	13,860
DE53	Whatman	Non-Porous	Cellulose [65]	Diethylaminoethyl [65]	6,800 [65]	4 [65]	27,200
DE52	Whatman	Non-Porous	Cellulose [65]	Diethylaminoethyl [65]	6,800 [65]	4 [65]	27,200
QA52	Whatman	Non-Porous	Cellulose [65]	Quaternary Ammonium [65]	6,800 [65]	4 [65]	27,200
CM52	Whatman	Non-Porous	Cellulose [65]	Carboxymethyl [65]	6,800 [65]	4 [65]	27,200
Typopearl	Toshbiosciences	Non-Porous	Hydroxylated Methacrylate [65]	Tresyl ligand derivatized with Protamine sulfate [65]	42,00 [65]	1 [65]	4,200
TSKge1	Toshbiosciences	Non-Porous	Hydroxylated Methacrylate [65]	Tresyl ligand derivatized with Protamine sulfate [65]	9,000 [65]	0.2 [65]	1,800
Hillex II	Pall Solohil	Non-Porous	Dextran, surface coated [137, 138]	Modified Polystyrene, modified with cationic trimethylammonium [138]	515 [147]	10-50 [147, 138]	15,450

As seen with products such as Provenge®, in addition to the manufacturing uncertainties it is very challenging to estimate the market penetration for novel cell therapy products. Furthermore, predicting suitable selling prices for cell therapy products is also a challenge at present as some of these therapies may replace the need for transplants, which can be priced at $600,000 USD [75], or even target spinal cord injury whose treatment is currently priced at $500,000 USD to $3M USD per patient [76].

In light of such questions and uncertainties, it is evident that costs are only one aspect to be considered when constructing a feasible business plan for a novel cell therapy product, as cell therapy production requires considerably more flexibility and consideration than the traditional biopharma products.

A limited number of publications exist on the topic of process economics for MSC-based cell therapy products. These include Malik [42] who evaluated the relative cost-effectiveness of allogeneic MSC products with respect to autologous MSC products. Simaria et al. [7], Hassan et al. [63] and Pereira Chilima et al. [77] provided more detailed analyses of the COG of allogeneic MSC products with the creation of models that capture mass balancing, equipment sizing, bioprocess economics and optimisation algorithms. The tools were used to predict the optimal technologies to be employed in upstream and downstream operations across different commercialisation scenarios and identify the limits of current technologies as well as the additional development required for these to fulfil future market demands. Hassan et al. [78] extended the work to provide a tool for assessing the financial implications of switching to microcarrier-based cell culture at different stages of process development considering the total cost of development as well as the overall profitability. This was achieved by exploring the impact of different stakeholder perspectives on the optimal process change scenario. These articles have provided useful insights on COG and current limitations of technologies for cell therapy manufacture; however, they have not considered the more qualitative operational characteristics of these technologies.

Methods to combine less tangible operational features with quantitative economic features for a more holistic analysis include the use of multi-attribute decision-making (MADM), stochastic analysis and resource requirement analysis. These techniques have previously been used for protein biopharmaceuticals such as monoclonal antibodies (mAbs) in order to address questions such as the benefit of single use vs stainless steel technologies as well as the benefit of fed-batch vs perfusion cell cultures [79,80]. In cell therapy, stochastic analysis has been employed in Jenkins et al. [81] in order to evaluate the robustness of automated vs. manual platforms. These techniques have not been explored in manufacturing platform selection for large scale adherent cell therapy products.

This article builds upon the work published in Simaria et al. [7], Hassan et al. [63], and Pereira Chilima et al. [77] and presents an integrated approach to select the optimal manufacturing technology for the manufacture of MSC-products considering both economic and operational elements using an advanced decisional tool. This tool comprises a process economics model coupled with models for brute force optimization, dynamic process scheduling, robustness analysis and MADM. This article also applies multiple reimbursement strategies to hypothetical cell therapy products with industrially relevant dose sizes, and identifies scenarios where commercial feasibility will be challenging due to economic and/or operational bottlenecks. Furthermore, this study provides insights on areas within the manufacturing process, for which additional development would allow for commercial feasibility of MSC-based cell therapy products.

2. Materials and methods

2.1. Tool description

2.1.1. Overview

This tool is composed of several different components: a database, a bioprocess economics model, a brute-force optimization algorithm, a robustness analysis model and a multi-attribute analysis model (Fig. 1). When starting the simulation, the user selects the scale and demand scenarios to be evaluated. For each type of technology (e.g. multi-layer flasks, stirred tank bioreactor with microcarriers etc.), the information is retrieved from the database by the bioprocess economics model and the optimal size of the cell culture vessel is determined. The information contained in the database is also used by the bioprocess economics model to evaluate the costs associated with each manufacturing platform.

Brute-force optimization is used to run multiple scale-demand scenarios through the bioprocess economics model and store the relevant information (e.g. COG/million cells, COG/dose, FCI, number

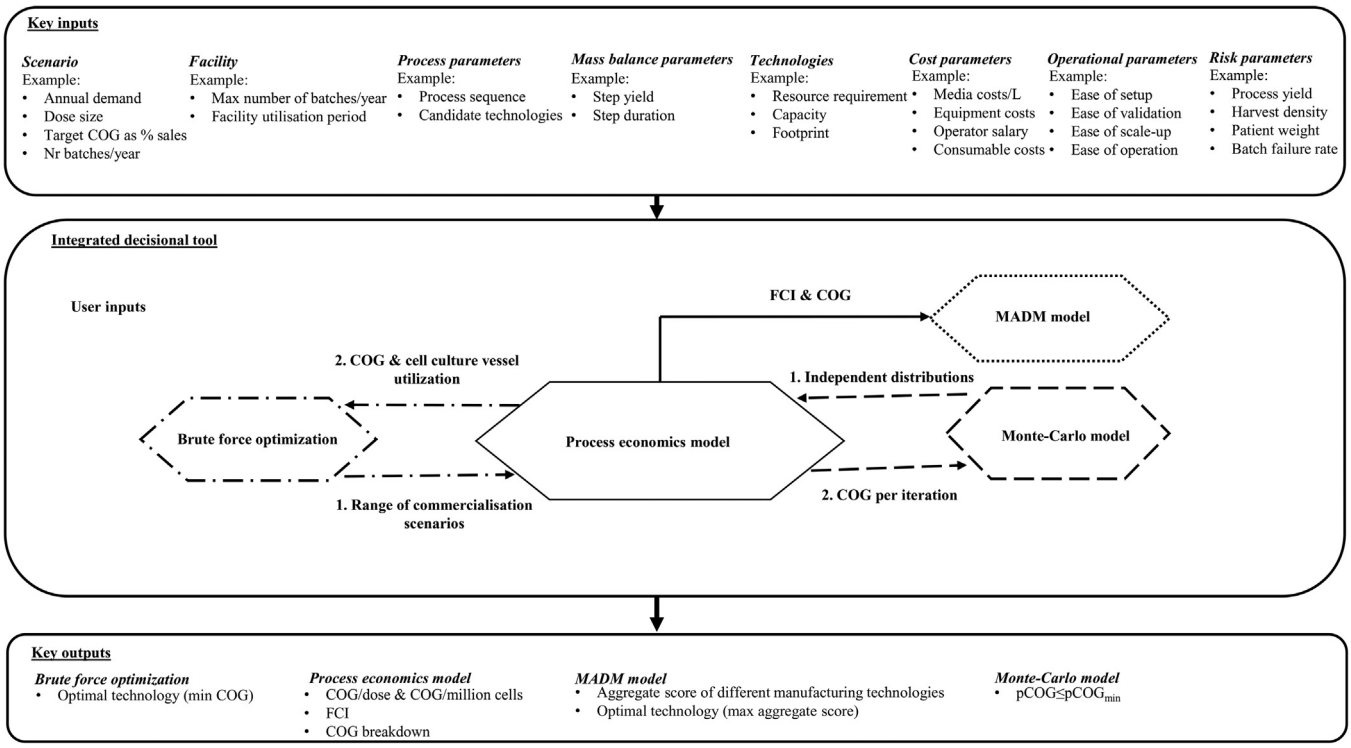

Fig. 1. Schematic representation of the integrated bioprocess economics tool. This tool encompasses a database, a process economics model coupled with brute force optimization, a multi-attribute decision making tool and a Monte Carlo simulation model.

of cell culture vessels used). During a robustness analysis, Monte Carlo simulation is used to perform a number iterations of these variables and measure the probability of different events occurring (e.g. probability of achieving target COG). Multi-attribute analysis is used to select optimal manufacturing platforms by considering both quantified operational and economic characteristics these platforms.

2.1.2. Bioprocess economics model

The process economics model described in this article was developed in Microsoft Excel (Microsoft® Corporation, Redmond, WA) as it provides a user-friendly interface and can be easily coupled with Visual Basic for Applications (VBA, Microsoft® Corporation, Redmond, WA). The economic parameters used to calculate the total COG were both direct and indirect. The direct costs included in this analysis were: media, trypsin, QC and consumables (e.g. single-use cell culture vessels). The indirect costs used in this analysis were labour and depreciation costs. The expressions used to evaluate both direct and indirect costs are summarised in Table 5. Costs that were not associated with the core manufacturing unit operations, such as waste disposal costs, were not considered in this analysis.

Additionally to the work published in Simaria et al. [7], this study also includes the downstream processing costs (DSP). These costs were computed using the same methodology described in Hassan et al. [63]. In this article however, a single technology for cell recovery and wash was used, the fluidised bed centrifuge (FBC). Final fill was modelled using an automatic fill line, the Crystal® Px system (Aseptic Technologies) and cryopreservation using a controlled rate freezer. The key economic input parameters are summarised in Tables 3 and 4.

2.1.3. Brute force optimization

This model was combined with a brute-force optimization macro built using VBA. This was used to run rapidly several scenarios through the process economic model, store the results for each technology-commercialisation scenario and, select the most cost-effective technology in each scenario.

2.1.4. Monte Carlo simulation

Monte Carlo simulations were built using @Risk® (Palisade Corporation, Newfield, NY), and coupled with the bioprocess economics model. During simulations, multiple independent triangular distributions of critical process variables such as process yield and proliferation rate were introduced to the bioprocess economics model. @Risk was then used to perform 10,000 iterations of these variables and measure the probability of different events occurring (e.g. probability of achieving target COG). This same model was employed in estimating the sensitivity of the desired metrics (e.g. COG) to different process parameters (e.g. process yield), by individually changing the value of such parameters by ±a selected percentage and measuring the impact that this change has on the COG.

2.1.5. Multi-attribute decision-making

A weighted sum method was employed to account for both the operational and economic groups of attributes of different manufacturing technologies. The different attributes were first ranked according to their importance in the cell therapy field (where a higher rank was more beneficial than a lower rank) and normalised weights (W_i) were determined relative to the sum of the rankings of all the attributes considered within either the operational or economic attribute groups:

$$W_i = r_i / \sum_{i=1}^{n} r_i \quad (10)$$

where

W_i = the normalised weight of attribute i

r_i = the importance ranking of category i

Table 3
Manufacturing platform-specific assumptions.

Category	Parameter	Multi-layer					Multi - plate				Hollow fibre	Single-use bioreactors with microcarrier[b]
		MLF 1	MLF 2	MLF 5	MLF 10	MLF 40	MPB 10	MPB 50	MPB 100	MPB 200	HFB	STR: 1 L–2,000 L
Resource capacities and requirements	Technology name	636	1,272	3,180	6,320	25,280	6,120	30,600	61,200	122,400	21,000	5,540–11,080, 000[c]
	Surface area (cm^2)	0.25	0.25	0.25	0.16	0.16	0.27	0.18	0.18	0.18	0.37	0.09
	Media volume (ml/cm^2)	60	60	24	12	16	2	2	2	2	–	–
	Incubator capacity	–	–	–	–	16	1	1	1	1	1	1
	Equipment capacity[a]	1:1	1:1	1:1	1:1	1:1	2:1	2:1	2:1	2:1	2:1	2:1
	Executing: Documentation operator ratio											
Costs	Consumable costs ($/unit)	60	73	241	507	1,265	2,310	4,412	6,778	9,820	12,000	680–10,500
	Incubator costs ($/unit))	17,835	17,835	17,835	17,835	30,000	10,000	10,000	10,000	10,000	–	–
	Equipment costs ($/unit)	–	–	–	–	425,000	59,800	59,800	59,800	59,800	150,000	88,470–575,000
Time	Start culture duration (h)	0.14	0.14	0.14	0.14	1.3	1	1	1	1	0.17	2 (for volume ≤ 200 L), 3 (for volume > 200L)
	Media exchange duration (h)	0.07	0.07	0.07	0.07	0.67	0.5	0.5	0.5	0.5	0	1 (for volume ≤ 200 L), 1.5 (for volume > 200L)
	Passage time (h)	0.21	0.21	0.21	0.21	1.97	1.5	1.5	1.5	1.5	0.37	3 (for volume ≤ 200 L), 4.5 (for volume > 200L)
	Harvest duration (h)	0.07	0.07	0.07	0.07	0.67	0.5	0.5	0.5	0.5	0.2	1 (for volume ≤ 200 L), 1.5 (for volume > 200L)
	Biosafety cabinet requirement	Y	Y	Y	Y	N	N	N	N	N	N	N

[a] Refers to any equipment required in order to use the technology excluding incubators and biosafety cabinets (eg. Controllers and manipulators).
[b] In the interest of clarity, only the maximum and minimum sizes used were listed. Other sizes considered were: 5 L, 10 L, 20 L, 50 L, 100 L, 500 L and 1,000 L.
[c] For the estimation of the surface area per cell cultures vessel, the following assumptions were made: bioreactor space efficiency = 50%, microcarrier seeding density = 4 g/L and microcarrier surface area = 2,770 cm^2/g. The media volume required for microcarrier-based cell culture was optimized such that these processes have lower media consumption.

For all operational attributes, a high score is desirable whilst for the economic cost attributes (COG and FCI) a high value is undesirable. To address this, the attribute values were standardised by converting them into a common dimensionless scale from 0 to 1 as follows:

$$S_{ij} = (s_{ij} - s_{iWorst}) / (s_{iBest} - s_{iWorst}) \quad (11)$$

where

S_{ij} = the standardised rating for technology j for attribute i
s_{iMin} = the worst outcome for attribute i
s_{iMax} = the best outcome for attribute i

The relative importance of the total weighted economic and operational ratings was varied using dimensionless combination ratios, which add up to 1 so as to create scenarios where operational attributes were more important than the financial attributes and vice-versa. The overall aggregate score of the different technologies was then calculated by using the weighted sum method as follows:

$$S_{aggrj} = (R_{C1} \times \sum_{i=1}^{n} (S_{opij} W_i)) + (R_{C2} \times \sum_{i=1}^{n} (S_{econij} W_i)) \quad (12)$$

where

S_{aggrj} = the aggregate weighted score of technology j
R_{C1} = the operational combination ratio
R_{C2} = the economic combination ratio

2.2. Case study setup

2.2.1. Case study overview

The decisional tool described in Section 2.1 was used in the case study in order to assess the economic and operational benefits of different manufacturing platforms for the production of allogeneic MSC-based cell therapy products. The bioprocess economics model simulates each day of a single product facility operating 335 days a year with two annual maintenance shutdowns, one in the summer and one in the winter. The manufacturing process modelled in this article is based on a 21 day long cell culture process with 3 expansion stages and with the final cell harvest, wash, concentration and formulation occurring on day 21(Table 3).

The annual demand for MSC-based products is likely to vary according to the indication being targeted, for example, indications such as chronic low back pain have lower dose sizes (~10^7 cells) [51,52], whilst other indications such as GvHD the dose requirement is much higher (~10^8-10^9 cells per patient) [17,55–58,82]. Given the differences in annual demands of different MSC-based cell therapy products, the tool was used to analyse the behaviour of different manufacturing technologies across manufacturing scales varying from 1 to 100 billion cells per batch and demands ranging from 10 billion to 10,000 billion cells per year. This scale limit was established in Hassan et al. [63] as the maximum number of cells which the current technologies for cell wash and concentration can process. A minimum and maximum number of batches per year that can be processed in a single facility was established through discussions with industry experts to be 10 and 100 respectively.

In Simaria et al. [7] a condition was applied where microcarrier-based cell culture was only considered in scenarios where planar platforms were infeasible due to capacity constraints, so as to accommodate the fact that planar systems are well-established technologies, and therefore preference would be given to them when possible. In the case study described in this paper, however, this assumption has been lifted in order to explore the cost-benefit of microcarrier-based cell culture at smaller manufacturing scales.

The planar technologies considered in this case study were multi-layer flasks (MLF) (e.g. Cell Factories®, CellSTACKs®), multi-plate bioreactors (MPB) (e.g. Xpansion®), hollow fibre bioreactors

Table 4
General assumptions.

Category	Parameter	Value	Unit
Scenario set up	Max number of batches	100	–
	Min nr of batches	10	–
	Lang factor	23.67	–
	Max number of FBCs/batch	1	–
	Depreciation period	7	Years
Mass balance	Seeding density	4,000	Cells/cm^2
	Harvest density	45,000	Cells/cm^2
Costs	Trypsin	30	$/L
	QC	10,000	$/batch
	Media	450	$/L
	Microcarrier costs	5	$/g
	Biosafety cabinet	17,000	$/unit
	Operator cost	120,000	$/year
Time constraints	Shift time	8	h
	Gowning, documentation and cleaning time	20%	of shift time
	Maximum time allowed for expansion process setup (day 1)	6	h
	Passage (days 7 & 14)	3	h
	Media exchange (days 4, 10 &17)	6	h
	Harvest (day 21)	3	h
	Microcarrier removal, wash and concentration (day 21)	4	h
	Vialling (day 21)	2	h

Table 5
Equations used to calculate COG.

Number	Parameter	Expression
1	Number of cell culture vessels per batch for expansion stage n	$N^n_{unit} = ((N_{cells/batch}/(y_{DSP} \times y_{cell\,detachment} \times y_{batch\,sucess}))/a_t)$
2	Cell culture vessel area for microcarrier-based cell culture	$a_{cell\,culture\,vessel} = Density_{microcarrier} \times Utilisation_{cell\,culture\,vessel} \times a_{per\,gram}$
3	Cell culture media costs across 3 expansion stages	$C_{media} = \sum_{n=1}^{n=3} C_{media/ml} \times N^n_{unit} \times V_{media/a} \times a_t \times 2$
4	Trypsin costs across 3 expansion stages	$C_{trypsin} = \sum_{n=1}^{n=3} C_{trypsin/ml} \times N^n_{unit} \times V_{trypsin/a} \times a_t$
5	Consumable costs	$C_{consumables} = \sum_{n=1}^{n=3} N^n_{unit} \times C_{consumable/unit}$
6	Labour costs	$C_{labour} = C_{operator} \times R_{documentation/executing} \times R_{overhead/operator} \times MAX_{day\,1}^{day=335} \sum_{Step\,1}^{Stepn} ((t_{step} \times N^n_{unit})/t_{step\,max})$
7	Fixed capital investment	$FCI = C_{equipment} \times L_f$
8	Equipment costs (minus biosafety cabinets)	$C_{equipment} = \sum_{equipment=1}^{equipment=n} C_{equipmnet/unit} \times MAX(N^n_{unit}/Capacity_{equipment})$
9	Biosafety cabinet costs	$C_{BSC} = C_{BSC/unit} \times MAX\left\{\sum_{day=1}^{day=335} (t_{step} \times N_{unit})/t_{step\,max}\right\}$

The definitions of the abbreviations used in the equations listed above can be found in Appendix 1.

(HFB) (e.g. Quantum®) and the 3D technology considered was single-use bioreactors with microcarriers in suspension in stirred tank reactors (STR). The key characteristics of the different technologies are outlined in Table 3. The resource requirement, COG and major cost drivers of these manufacturing platforms were identified across different scales through a detailed process economics analysis. The cost-effectiveness of microcarrier-based cell culture was further investigated by evaluating the critical process parameters which make 3D cell culture more attractive than planar technologies. The results from the process economics analysis were used in the multi-attribute decision analysis where both operational and economic attributes of different manufacturing platforms were quantified. The robustness of the different technologies was also assessed at different manufacturing scales through a Monte Carlo analysis. The process economics analysis was placed in context by evaluating the commercial feasibility of products manufactured using different technologies when current reimbursement strategies are applied. This analysis identified scenarios where successful commercialisation is unfeasible due to high COG or capacity constraints resulting in failure to meet the annual demand. These scenarios were further investigated through an optimization analysis.

2.2.2. Process overview

The manufacturing process starts with cell culture assuming that master cell banks are in place. Three cell culture stages were modelled each lasting 7 days, making a total of 21 days of cell culture. During each cell culture stage a media exchange step was performed such as to maintain nutrient concentration and hence promote cell viability. The cells were seeded at 4,000 cells per cm^2 and harvested at density of 45,000 cells per cm^2 with a doubling time of around 48 h.

The cell culture stage was followed by cell wash and concentration. Given that fluidised bed centrifuge (FBCs) are expensive (between £180,000–£500,000 [63]), and that it is impossible to manufacture 100 batches of 21 days in series within a year, the utilization of FBCs was maximised by staggering batches in parallel such that cell wash and concertation took place on different days for different batches being manufactured in parallel. The final concentration of the manufacturing process was assumed to be 10 million

Table 6
Assumptions for multi-attribute decision-making and stochastic cost analysis.

	Attribute	Rank[a]	MLF	MPB	HFB	STR
	MULTI-ATTRIBUTE DECISION-MAKING ANALYSIS					
Operational parameters	Ease development	Tr(1,2.2,3)	Tr(1,4,5)	Tr(2,3.4,5)	Tr(2,3.2,5)	Tr(1,2.4,4)
	Ease of validation	Tr(2,3.6,4)	Tr(2,3.2,5)	Tr(3,3.4,5)	Tr(1,2.8,5)	Tr(2,3,4)
	Ease of setup	Tr(1,2.8,5)	Tr(1,3.6,5)	Tr(2,3,4)	Tr(3,3.8,5)	Tr(2,3,4)
	Ease of operation	Tr(2,3.4,5)	Tr(1,2.2,4)	Tr(2,3.6,4)	Tr(1,3.6,5)	Tr(3,3.6,4)
	Ease of scale-up	Tr(1,3.8,5)	Tr(1,2.4,4)	Tr(3,3.6,5)	Tr(1,1.6,2)	Tr(1,3.2,5)
Economic parameters	COG/million[b] ($)	2	Tr(25,78,435)	Tr(21,46,99)	Tr(85,163,351)	Tr(10,37,10)
	FCI[b] ($M)	1	55.7	19.8	81	21
	ROBUSTNESS ANALYSIS					
	Cell detachment yield	–	Tr(0.85,0.9,0.95)	Tr(0.85,0.9,0.95)	Tr(0.85,0.9,0.95)	Tr(0.6,0.75,0.9)
	DSP yield	–	Tr(0.58,0.68,0.78)	Tr(0.58,0.68,0.78)	Tr(0.58,0.68,0.78)	Tr(0.512,0.612,0.712)
	Batch success rate	–	Tr(0.9,0.95,0.97)	Tr(0.93,0.95,0.98)	Tr(0.93,0.95,0.98)	Tr(0.93,0.95,0.98)
	Doubling time (h)	–	Tr(32,34,37)	Tr(33,34,35)	Tr(33,34,35)	Tr(32,34,37)

For the operational criteria, a higher score of 5 indicated the best technology in that particular criteria and a lower score of 1 indicated the worst technology.
Tr(a,b,c) refers to the triangular probability distribution where a, b, c are the minimum, most likely, and maximum values, respectively.

[a] The ranks and scores were attained from a survey distributed across industry experts in positions such as: senior pilot plant manager, business development manager, global product manager, vice president of technology and manufacturing, head of cell culture services of Eufets GmbH, Promethera, Pluristem and Pall Life Sciences. The higher the rank the better the technology. A higher value of the rank indicated a criterion of greater importance/weighting.

[b] COG and FCI are taken from a scenario with a batch size of 10 B cells per batch and a demand of 100 B cells per year.

cells per ml. These cells were then diluted in DMSO containing solution, placed in 6 ml cryovials and cryopreserved using a controlled rate freezer.

The labour requirement was evaluated by using the number of operators per team of operators. The number of operators per team varies according to the manufacturing platform used, as these require different numbers of manipulations. For example, in open processing using multi-layer flasks, a higher number of manipulations is required with respect to the use of automated bioreactors and therefore the number of operators per team is higher for these technologies.

The shift time applied here was 8 h, however, an assumption was made that only 80% of the shift time was spent in the cleanroom and the remaining 20% was used in gowning and de-gowning and documentation.

2.2.3. Key assumptions

Robustness analysis was performed using the Monte Carlo simulation model described in Section 2.1. During this analysis, the manufacturing process was fixed and different critical process parameters were varied such that the absolute COG remained constant whilst the throughput varied. Moreover an assumption was made that in scenarios where the number of cells produced exceeded the expected number of cells, these additional cells would still be commercialised and not wasted resulting in lower COG/million cells in such scenarios.

The values used for the critical process variables were attained through a series of discussions with industry experts. The rationale behind these assumptions is based on the fact that multi-layer flasks are manual systems and therefore prone to variability. This was assumed to have an impact on the cell doubling time and batch failure rate due to the limited control over the process parameters. Moreover, given that microcarrier-based cell culture is a more nascent technique in the cell therapy field, it was also assumed that the variability in the proliferation rate of cells would also be higher in these systems. The additional degree of difficulty in dissociating cells from microcarriers with respect to 2D technologies was also accounted for by allowing extra variability in cell detachment efficiency in 3D cell culturing. Table 6 summarises the minimum, maximum and most likely values of the parameters evaluated.

Multi-attribute analysis was performed in order to quantify both operational and economic parameters. The scores for operational attributes were attained by distributing a survey questionnaire across multiple industry experts on their experience in designing a process for commercial scale manufacture of cell therapy products. In total, seven interviews were carried out with experts spanning roles such as senior pilot plant manager, business development manager, global product manager, vice president of technology and manufacturing, and head of cell culture services across innovator companies, contract manufacturers and vendors. The survey asked them to rank operational categories according to their importance and to rank different cell culture technologies on those categories. The operational categories considered were ease of development, ease of validation, ease of setup, ease of operation and ease of scale-up. The actual development costs were not considered at this stage but have been explored in parallel work by Hassan et al. [78]. The scores used in this section were the average of the responses and are summarised in Table 6. The scores from both economical categories (FCI and COG/million cells) were gathered from the process economics model. Here, an assumption was made that the COG/million cells was twice as important as the FCI costs, such that the long term benefits of using a particular technology would outweigh the initial capital investment.

In this analysis, the capacity of the different manufacturing technologies for producing enough cells for different commercialisation scenarios was assessed. An assessment of the commercial feasibility of MSC-based cell products under the UK's National Institute of Health and Care Excellence (NICE) maximum reimbursement limit (approximately $40,000 per quality adjusted life) [83] was also carried out. This allowed for the identification of scenarios where commercial failure would occur due to capacity or economic constraints.

In order to recommend possible process improvements to overcome these challenges, a sensitivity analysis was performed. This analysis aimed at identifying the key factors contributing to both economic and operational bottlenecks. During the sensitivity analysis cost parameters were varied by ± 25% and operational parameters varied according to the minimum and maximum values used in the robustness analysis (Table 6). The effect of varying these factors on COG and throughput was measured and the factors with the highest impact on the economic and operational performance of MSC-based manufacturing processes were then used during the optimization analysis, where target values for these factors were recommended.

3. Results and discussion

This section summarises the key insights from the techno-economic analysis of alternative cell culture technologies for allogeneic MSC therapies across different dose-demand scenarios.

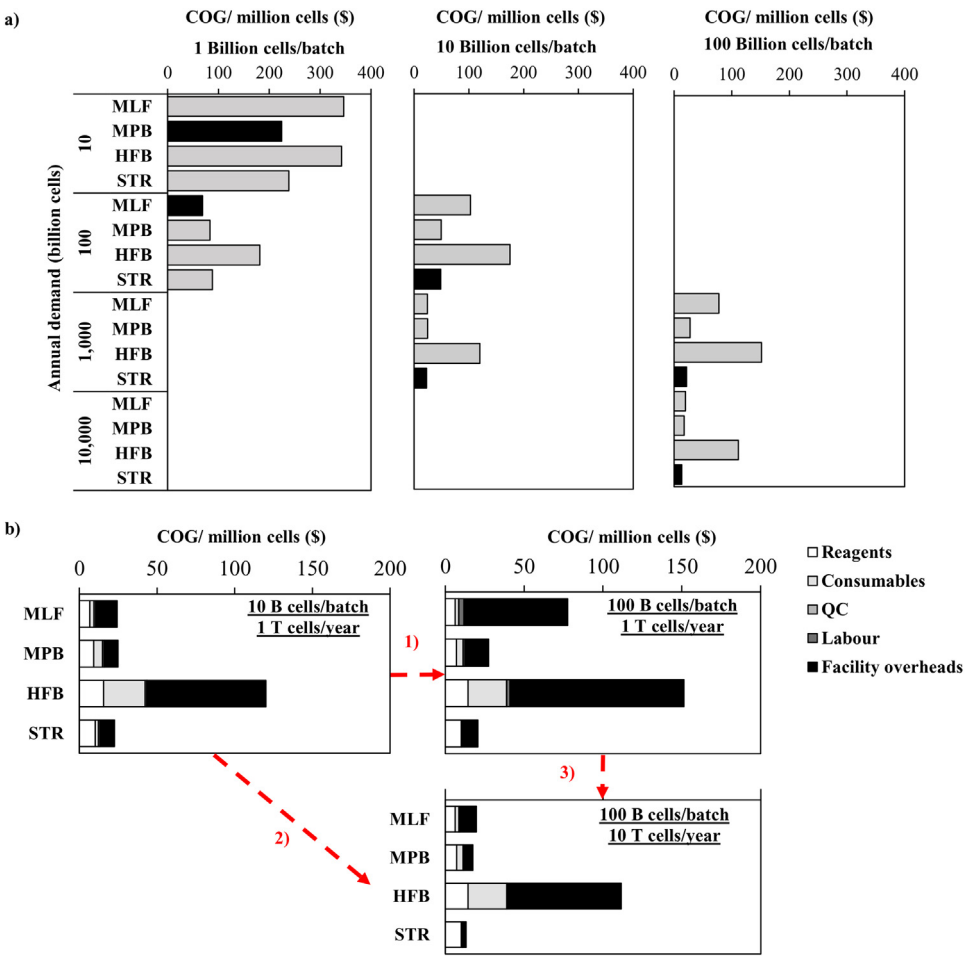

Fig. 2. a) COG per million cells across multiple demands and scales. The optimal manufacturing platform in each batch size-demand scenario is represented by a black bar b) Impact of different cost categories on COG with increasing demand and batch size. Where MLF = Multi-layer flasks, MPB = Multi-plate bioreactors, HFB = hollow fibre bioreactors and STR = single use bioreactors with microcarriers.

The analysis identifies key COG drivers, weighs up the financial and operational benefits, and determines the robustness and reimbursement potential of each technology. For scenarios where product commercialisation was deemed infeasible, the key parameters which influence both cost-effectiveness and capacity were identified and optimised.

3.1. Process economics analysis

3.1.1. Deterministic cost comparison

The relative cost-effectiveness of different manufacturing platforms is highly dependent on the scale and demand being explored [7]. This is illustrated in Fig. 2a, which shows the COG per million cells profile of the different technologies featured in this article across different annual demands and scales. At a smaller scale of 1 billion cells per batch, planar platforms are more cost-effective than 3D cell culture. When such small batch sizes are manufactured in low frequency (10 times per year) such that indirect costs dominate the overall COG, multi-plate bioreactors become the most cost-effective option due to the low equipment costs and relatively low labour requirement. When the number of batches is increased to 100, direct costs become the major cost driver, which causes the optimal technology to shift to multi-layer flasks; this is due to the fact that these have considerably lower consumable costs with respect to all other manufacturing platforms (Table 3). These scenarios can be translated into a process manufacturing 100 to 1,000 doses per year of an MSC based treatment for heart disease with a dose size of 100 million cells [17,55–58,82].

When increasing the scale to 10 billion cells per batch, reaching demands of 1 trillion cells per year (10,000 doses per year of a treatment for heart disease), the scalability of the different manufacturing platforms determines their cost-effectiveness. High scalability decreases the number of cell culture vessels used per batch, and therefore decreases the requirement for equipment and personnel. Hence, microcarrier-based cell culture becomes the most cost-effective technology at both demands, 100 billion and 1 trillion cells per year, with a very small economic advantage with respect to multi-plate bioreactors ($21/million cells vs $27/million cells). Increasing the scale further to 100 billion cells per batch reaching high demands of up to 100,000 doses of 100 million cells per year, increases the importance of scalability, making microcarrier-based cell culture significantly more cost-effective than all other technologies. Furthermore, in scenarios where the market penetration increases over time, such that the production of MSCs increases from low demands up to “blockbuster-like” quantities being manufactured annually, microcarriers offer relatively low COG across the different scales of production.

Fig. 2a portrays hollow fibre bioreactors as the least cost-effective technology across most scales and demands. These bioreactors, offer superior operational features (Table 6), however, the high consumable and equipment costs associated with this technology (Table 2) do not allow this bioreactor to be financially competitive at commercial scale.

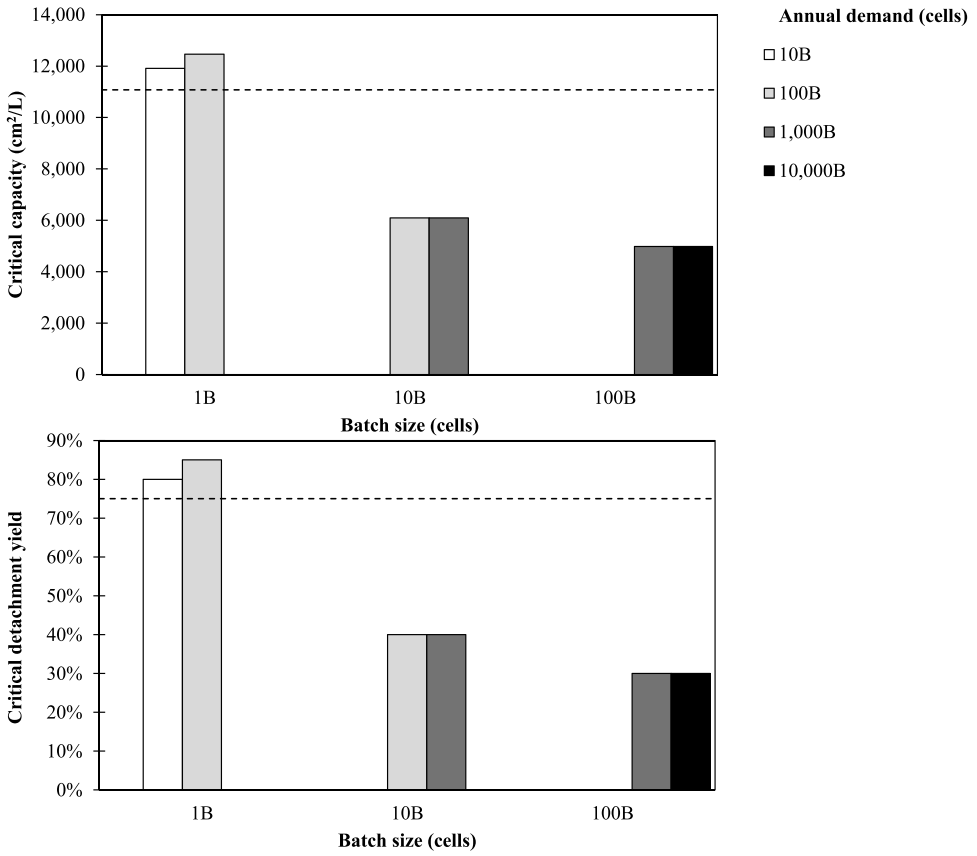

Fig. 3. Critical recovery efficiency for which single use bioreactors with microcarriers are more cost-effective than planar technologies across multiple scales and demands, for non-porous microcarriers with a surface area per litre of 11,080 cm^2 and critical surface area per litre for which single use bioreactors with microcarriers are more cost effective than planar platforms across multiple scales and demands for a recovery efficiency of 75%. Min and max number of batches per year = 10 and 100 respectively.

3.1.2. Factors affecting the cost-effectiveness of different manufacturing technologies

Understanding of the key factors affecting the COG (e.g. media, cell culture vessel, equipment etc.), is critical for effective process optimization. Since the impact of different process components on COG/million cells varies with the scale and demand selected, Fig. 2b illustrates the change in cost drivers across different scenarios.

The effect on COG/million was explored when: (1) increasing the scale of production (from, 10 billion cells/batch to 100 billion cells/batch) while keeping the same annual demand (1 trillion cells/year); (2) increasing the scale and annual demand (from 10 billion cells/batch and 1 trillion cells/year to 100 billion cells/batch and 10 trillion cells/year) while maintaining the same number of batches per year (100); (3) increasing the annual production (from 1 trillion cells/year to 10 trillion cells/year) while keeping the same batch size (100 billion cells/batch) and increasing the number of batches per year (from 10 batches/year to 100 batches/year).

1) Scaling up from 10 billion cells per batch to 100 billion cells per batch while keeping the same annual demand has a negative impact on the COG of all planar technologies. This is attributed to capacity constraints in planar technologies; scaling up means adding more cell culture vessels in parallel requiring more equipment and personnel, and therefore, increasing the depreciation and labour costs. Microcarrier-based cell culture on the other hand benefits from high capacity, therefore no increase in number of manipulations was seen. Furthermore these bioreactors can handle larger numbers of cells with no additional depreciation costs and using the same number of operators reducing changes in COG with increasing scale.
2) Increasing the annual demand by keeping the same number of batches and increasing the scale of manufacture increases the number of aseptic manipulations, however, it has cost benefits across all technologies. Although the increase in scale was previously shown to increase the labour and depreciation costs in planar technologies, these costs are now spread over a higher number of cells resulting in an overall positive impact in the COG/million cells. This positive impact is clearly seen in microcarriers where there is a dramatic drop in COG/million cells of 42% with the increase in scale causing cell culture media to be the main cost driver.
3) Increasing the annual production by increasing the number of batches per year and keeping the same batch size decreases the COG/million further for all technologies as the indirect costs are spread over more cells as seen in the previous point.

3.1.3. Critical parameters contributing to the cost effectiveness of microcarrier-based cell culture

Microcarriers in single-use bioreactors are the most cost-effective technology for the manufacture of adherent cell therapy products with high annual demands (Fig. 2a). Since different microcarriers have different surface areas and will result in different cell detachment yields [84], Fig. 3 illustrates the critical process parameters which allow for microcarrier-based cell processing to be more cost-effective than planar technologies across different scales and demands. In Fig. 3a, the efficiency in separating the cells from the microcarriers (cell detachment yield) is varied, and the critical cell detachment yield for which microcarriers are more cost-effective than planar technologies is calculated. Fig. 3a shows that at small scales of 1 billion cells per batch, where planar technologies are

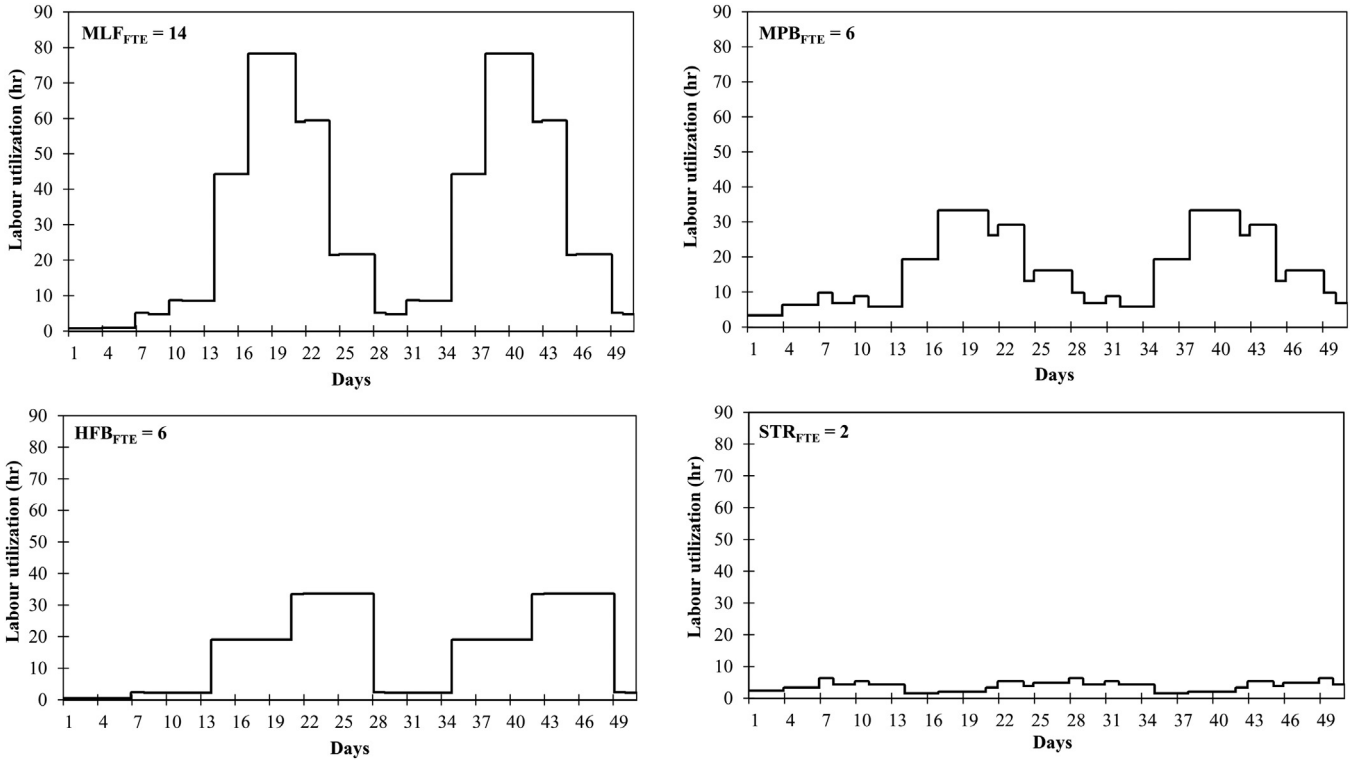

Fig. 4. Labour utilization per day for cell culture, wash and formulation throughout the first 50 days of the year, for a single product facility manufacturing 100 batches of 50 B cells and across multiple manufacturing platforms. The number of FTEs required for each manufacturing platform is represented on the top right corner of each figure. Where MLF = Multi-layer flasks, MPB = Multi-plate bioreactors, HFB = hollow fibre bioreactors and STR = single use bioreactors with microcarriers.

more cost-effective (Fig. 2a), the yield of the microcarrier-cell detachment step must be increased from 75% to up to 85% in order for these to be more cost-effective than planar technologies. Losses in detachment yield can be minimised by using thermosensitive microcarriers or even avoided with injectable or dissolvable microcarriers. When using thermosensitive mircrocarriers, the cells can be detached from the microcarriers by changing the temperature instead of using enzymes and thus this enhances the detachment yield [85]. When using injectable microcarriers, no microcarrier separation step is required [86]. The use of injectable microcarriers will pose some regulatory implications as it will change the properties of the product. Moreover, the use of injectable microcarriers may also affect the cryopreservation process. Additional strategies to enhance the detachment yield include optimizing the conditions for cell detachment (e.g. enzyme used, washing protocol, incubation time and temperature). As the scale increases, scalability plays its part and microcarriers become more cost-effective than planar platforms, hence this critical cell detachment yield drops significantly to as low as 30%. Fig. 3 also shows that the manufacturing scale has a higher impact on the relative cost-effectiveness of microcarrier-based cell culture than annual demand.

Fig. 3b explores the critical surface area per litre for which microcarriers are more cost-effective than planar technologies. Typical values for surface area per litre can vary from the 100′s cm/L to the 10,000′s cm^2/L (Table 2) depending on the microcarrier of choice and its seeding density. This figure shows that at low scales of 1 billion cells per batch, the surface area per litre must be increased in order for microcarriers to be more cost-effective than planar technologies. Different strategies can be applied in order to increase the surface area per litre of cell culture. These include increasing the concentration of microcarriers in cell culture and switching from a non-porous to a porous microcarrier. If decantation is used to separate the cells from the microcarriers, adding more microcarriers to the bioreactor may increase the overall process time if the cells settled around the beads are to be recovered through consecutive dilution. Moreover, if fluidised bed centrifugation is used for the separation of microcarriers from the solution as was assumed in this article, a higher seeding concentration of microcarriers would fill the chambers of the FBC more rapidly requiring a higher number of cycles, which also has a negative impact on the processing time. Furthermore, switching to a porous microcarrier may alter the performance of the process as cells may grow differently depending on the microcarrier surface and structure. As the scale increases, Fig. 3 shows that the flexibility in surface area per litre also increases for the reasons previously mentioned.

3.2. Operational characteristics of manufacturing platforms for mesenchymal stem cell culture

3.2.1. Labour requirement

One of the key differences across the alternative technologies used is the labour requirement. This parameter is related to the number of manual operations required in the manufacturing process. Fig. 4 shows the number of man hours required to manufacture 100 batches of 50 billion cells. For greater resolution, the figure only shows the first 50 days of the year. The different batches are staggered one day apart such that harvest occurs on different days for each batch as previously described in Section 2.2. The manufacturing process from cell culture to formulation occurs in 21 days. In Fig. 4, seven batches are being manufactured in parallel in staggered mode, and, the highest labour utilization occurs from day 14 to 24. On the 14th day the passage to the last expansion stage (the stage with the highest number of cell culture vessels per batch) is carried out. The media exchange at the last expansion stage is carried out on day 17 and harvest on day 21 (Table 4). As 7 batches are being staggered, labour utilization is increased from day 14 to day 16 as the final passage is being carried out on 3 batches, from day 17 to 20 the other 4 batches are passaged, however, media exchange of

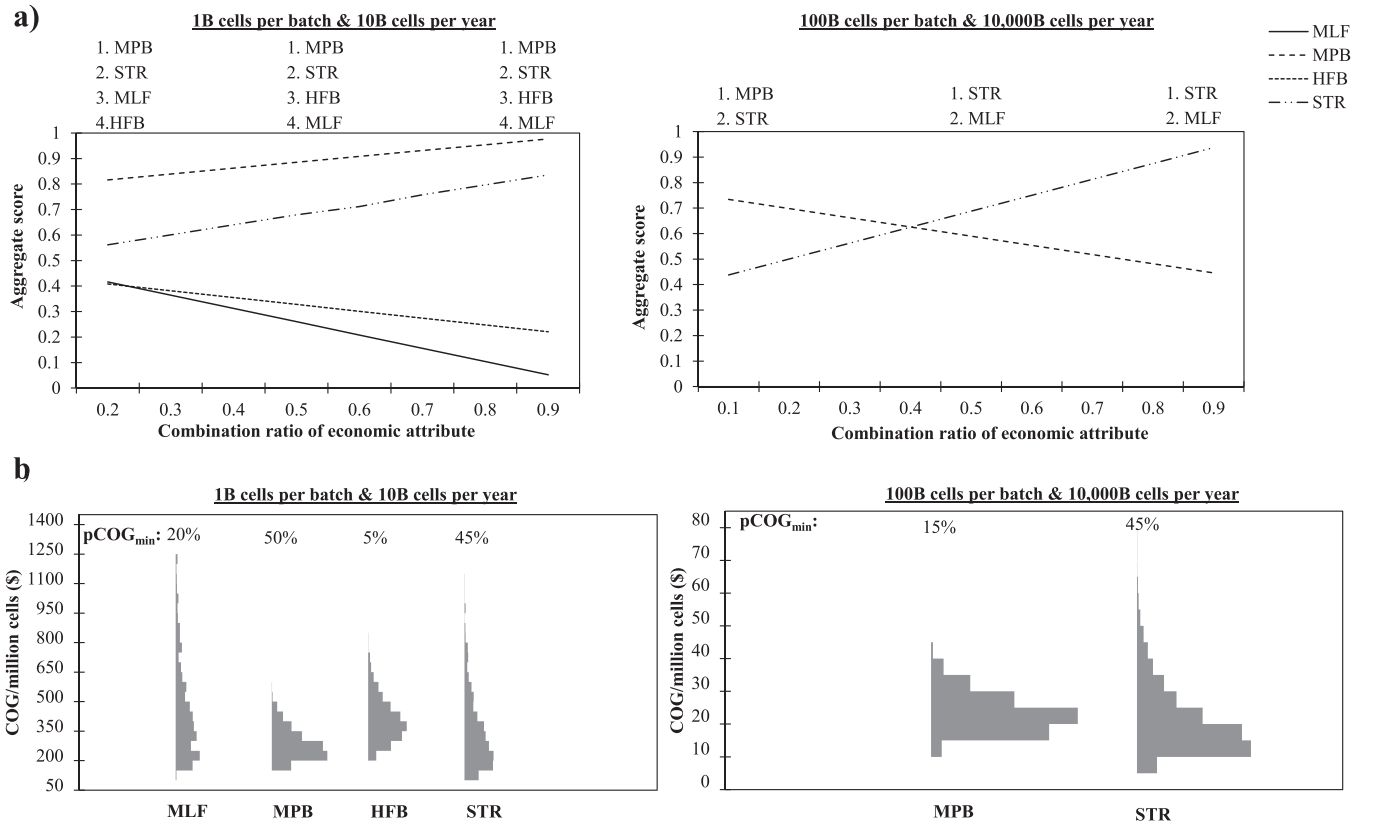

Fig. 5. a) Sensitivity plots showing the economic attribute versus the operational attribute across different commercialisation scenarios. The y-axis represents the aggregate score between both attributes, where the optimal technology has the highest score. The x-axis, represents the weight of the economic attribute with respect to the operational attribute, where towards the left-hand-side the operational attribute is more important, at the centre, where the weight of the economic attribute is 0.5, both attributes have the same importance and on the right-hand-side the economic attribute has the highest importance. b) COG per million cells distribution across two different commercialisation scenarios. Where MLF = Multi-layer flasks, MPB = Multi-plate bioreactors, HFB = hollow fibre bioreactors and STR = single use bioreactors with microcarriers.

the first batch occurs on day 17 explaining the dramatic increase in labour utilization. On day 21, all batches have been passaged but the first batch is being harvested and the last media exchange of batch 5 is initiated, explaining the slight drop in labour utilisation. Finally on day 24, media exchange in the last expansion stage was concluded for all 7 batches, and the only operation taking place is the harvest of batch 4 and therefore the number of man hours required drops further and the cycle is repeated for the next set of 7 batches.

Fig. 4 shows clearly that multilayer flasks have relatively high labour requirement. A similar trend in labour requirement would be expected when using hollow fibre bioreactors due to the fact that these also have limited capacity in comparison with multi-plate bioreactors and single-use bioreactors with microcarriers (Table 3); however, the fact that hollow fibre bioreactors are automated systems causes its labour requirement to be reduced. Microcarriers in stirred tank bioreactors have high capacity and therefore only a single bioreactor is used per batch reducing the number of manipulations required and hence reducing the number of operators needed.

3.2.2. Multi-attribute decision-making

Additional to resource requirement, operational benefits of technologies for adherent cell expansion also include: ease of development, ease of validation, ease of setup and ease of operation. The relative scores of the different manufacturing technologies in each of these categories are summarised in Table 6. The category which was voted to have the highest importance when selecting a manufacturing technology was ease of scale-up. Table 6 also shows that processes using multi-layer flasks are relatively easy to validate and develop but difficult to operate and scale-up. Multi-plate bioreactor-based processes are relatively easy to validate, are automated and easy to scale-up. These systems are however considered relatively difficult to setup with respect to hollow fibre bioreactors and multilayer flasks. Hollow fibre bioreactors are relatively easy to setup and provide a high degree of automation, however, these bioreactors pose challenges during scale-up. Microcarrier-based cell culture is relatively easy to scale-up and it is a highly automated platform; the challenges associated with this platform include setting-up the bioreactor and developing the microcarrier-based process.

Fig. 5a shows the weighted sum of the operational and economic attributes of the technologies featured in this article across different commercialisation scenarios. This figure highlights that at small scale-small demand combinations, for all values of the economic combination ratio, multi-plate bioreactors have the highest rank with the highest aggregate score. This is due to the fact that these bioreactors have the highest overall operational score, and that these are also the most cost-effective technology at 1 billion cells per batch and 10 billion cells per year as seen in Fig. 2.

In the same scenario, the second optimal technology for all combination ratios of economic features versus operational features are single use bioreactors with microcarriers, and, although these have a slightly lower operational score with respect to multi-layer flasks, they are far more cost-effective than these. This trend becomes more evident as the combination ratio of the economic attribute increases, resulting in a greater difference between the aggregate scores of single use bioreactors with microcariers and multi-layer flasks. Fig. 2 illustrated that in small scale-small demand scenarios, the decision between microcarriers and multi-plate bioreactors

Table 7
Statistical data on COG/million cells and multi-attribute decision making analysis for the competing technologies for low and high demand scenarios.

	Batch size: 1B cells per batch Demand: 10 B cells per year				Batch size: 100 B cells per batch Demand: 10,000 B cells per year	
	MLF	MPB	HFB	STR	MPB	STR
COG/million cells ($)						
$p(COG \leq COG_{optimal})$ (%)	20	50	5	45	15	45
Mean	347	224	342	238	17	13
Standard deviation	162	63	97	111	5	6
p-value	N/A	2E-23	7E-19	4E-39	N/A	9E-119
Aggregate score						
p(Aggregate score ≥ 0.5) (%)	25	80	50	70	25	80
Mean	0.26	0.89	0.32	0.68	0.59	0.69
Standard deviation	0.16	0.13	0.12	0.13	0.15	0.15
p-value	N/A	1E-155	2E-58	4E-63	N/A	2E-85

Note: The p-values were attained using a 2-tailed homoscedastic t-test with an alpha value of 0.05. A p-value below 0.05 indicates a significant difference between distributions. p-values were derived using each of the technologies as the baseline for statistical significance testing; in all cases p-values below 0.05 were obtained and this table shows the p-values using one of the planar technologies as the baseline case as an illustration.

is challenging as there is only a 10% difference in COG between the two systems. Fig. 5a helps discriminate further between these manufacturing platforms by reconciling economic and operational benefits with each. In this scenario, the multi-plate bioreactors have the highest overall aggregate score and can hence be considered the optimal technology.

On the other hand, when the annual demand is increased to 10,000 billion cells in Fig. 5a, the technology rankings change. At this higher scale, there are now only two manufacturing platforms competing against each other, as the others lack the capacity to fulfil such high scales. As previously mentioned, multi-plate bioreactors have superior operational features with respect to microcarrier-based cell culture, however, when considering the financial attributes, the COG per million for these manufacturing platforms is now $17.4 and $13.3 respectively resulting in a 30% difference. This causes the trends seen in Fig. 5a where, on the left hand side, where operational features are prioritized, multi-plate bioreactors rank first. This ranking slowly changes, and, in the middle, when both economic and operational characteristics have the same importance, microcarrier systems take over as the optimal manufacturing technology remaining in first place as the priority is shifted towards the economic benefits. Fig. 5a shows that despite the superior operational features of multi-plate bioreactors, scalability is the most important parameter in large scale manufacture of adherent cell therapy products (as shown in Table 6) and therefore microcarrier systems are the best technology to be used in such scenarios.

3.2.3. Robustness analysis

Process variability is another key operational parameter which may affect the potential for commercial success of cell therapy processes, as failure to meet the demand will increase the COG/million cells. Fig. 5b shows clearly the impact of the higher variability of processes employing microcarriers and multi-layer flasks through wider COG distributions. Despite this fact, and although the deterministic analysis portrays multi-plate bioreactors as the most cost-effective technology in small scale scenarios, microcarriers still have a similar probability of achieving the optimal COG/million cells (lowest COG/million cells). This is due to the fact that the variability of the different parameters is both positive and negative and the difference in COG between multi-plate bioreactors and stirred tank bioreactors with microcarriers is small. Increasing the scale and demand decreases the probability of multi-plate bioreactors achieving the minimal (optimal) COG/million cells and emphasises that, despite the uncertainties surrounding microcarrier systems, these are the optimal platform to be used for large scale production of MSC-based cell therapy products.

Table 7 confirms that although microcarriers have higher variability they still have the highest probability of achieving the optimal COG/million cells at higher scales. The table also indicates that at smaller manufacturing scales multi-plate bioreactors have a slightly higher probability of being the technology with the highest economic and operational aggregate score with respect to microcarrier-based platforms, and as the scale increases, this trend is altered as microcarriers have the highest aggregate score. All distributions were found to be significantly different from one another as indicated by all p-values being smaller than 0.05; an illustration of these p-values is highlighted in Table 7 compared to the baseline planar technology at low and high demands.

3.3. Performance targets for successful commercialisation analysis

3.3.1. Reimbursement analysis

The reimbursement strategy to be applied to a product is a useful parameter to establish manufacturing COG targets and may vary according to the indication, efficacy of the treatment and country of commercialisation. Fig. 6**a** shows the minimum selling price for which the manufacturing COG is 40% or 15% of sales across multiple commercialisation scenarios. COG as 40% sales has been established to be the higher end of COG as % sales in allogeneic cell therapy products, and 15% is a typical COG as % sales of small molecules [87]. Fig. 6a shows that for a drug with low patient demand (100 patients per year), achieving COG as 40% sales with a selling price of $40,000/dose will be challenging. Fig. 6a also shows that even at high annual demands (10,000 patients/year) products with high sizes (1 billion cells) will struggle to achieve COG as 15% sales as the minimum COG as % sales achievable in such scenarios is 33%. Moreover, Fig. 6a also identifies annual demands that are unachievable due to the lack of capacity of current manufacturing technologies; this is seen in high-dose high-demand scenarios. For example, with the specifications applied to this case study for a size of 1 billion cells it is challenging to achieve 100,000 doses/year as the maximum number of doses achievable is 60,000.

Fig. 6b shows the effect of process variability in the reimbursement strategy of cell therapy products for industry relevant indications by evaluating the range in selling price required for COG to be 15% sales. The indications considered were chronic discogenic lumbar back pain, congestive heart and (GvHD) with dose sizes of 10 million, 100 million and 1 billion cells per dose respectively. The reimbursement assumed for each different indication were $1,200 (costs of caudal epidural injections, [88]), $40,000 (average myocardial regeneration reimbursement, [46]) and $107,000 (extracorporal photopherisis for GvHD, [89]) respectively.

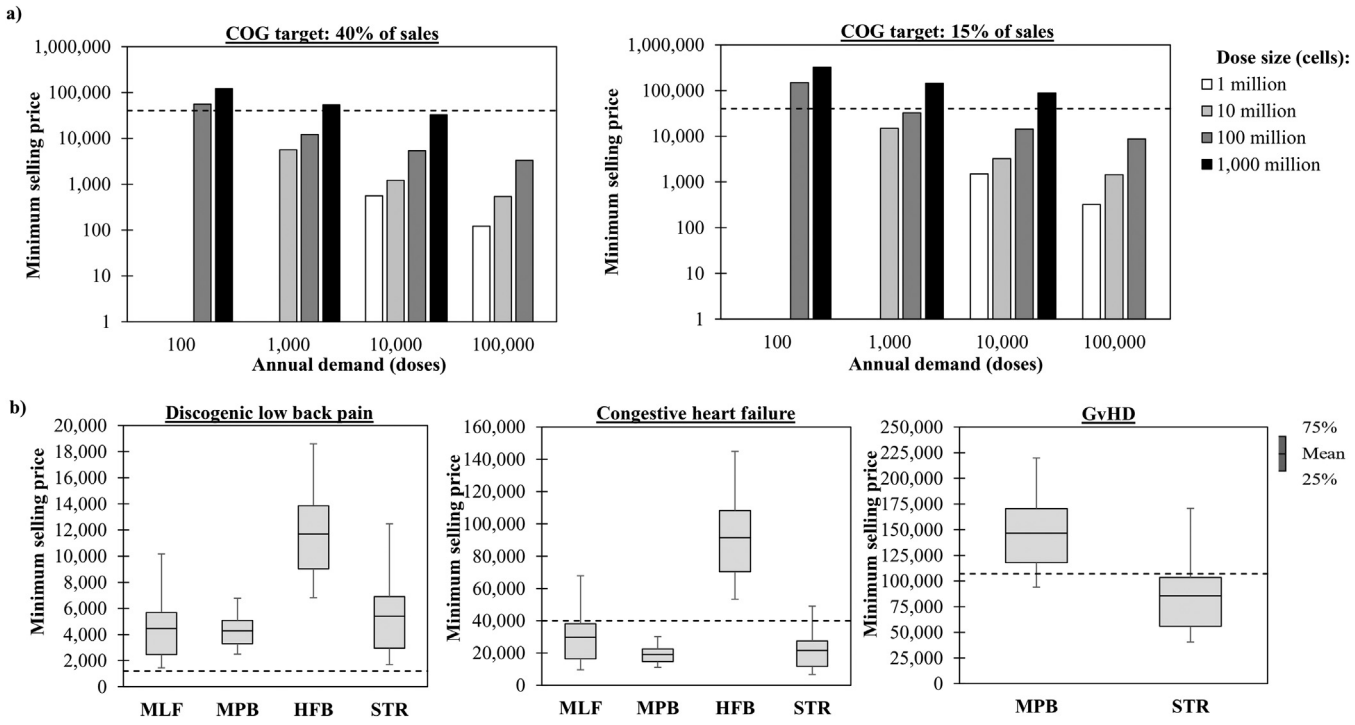

Fig. 6. a) Minimum selling price across multiple dose size and demands for which COG is 40% and 15% of sales when the minimum COG/dose across the different manufacturing platforms is applied. The dashed line represents the current reimbursement from the NICE. b) Minimum selling price distribution for which COG is 15% of sales. For an annual demand of 10,000 patients per year and for indications with different dose sizes and reimbursement strategies. The dashed line on each graph represents the typical reimbursement applied to these indications. Where MLF = Multi-layer flasks, MPB =Multi-plate bioreactors, HFB = hollow fibre bioreactors and STR = single use bioreactors with microcarriers.

Fig. 6b shows that for an MSC-based cell therapy product with a dose size of 10 million cells per patient, COG as 15% sales could be achieved under the current NICE reimbursement per QALY with any technology. However, when the reimbursement applied to chronic discogenic lumbar back pain is used in this scenario, an MSC-based cell therapy product would struggle to be competitive with current treatments, as these are more cost-effective. For an indication requiring a higher dose size of 100 million cells such as congestive heart failure, most manufacturing platforms would satisfy the COG as % sales target under the current reimbursement for congestive heart failure which coincides with the current NICE reimbursement. Higher reimbursements may be considered as some of these treatments may replace the requirement for a heart transplant which are priced up to $500,000 [75]. Furthermore, comparing the NICE reimbursement with the reimbursement applied in Heartcelligram-AMI® (Pharmicell, South Korea) an autologous MSC-based cell therapy product for post-myocardial infraction which is priced at $19,000 per dose [90], it is clear that current processes for the manufacture of MSC-based products shown in Fig. 6b will struggle even more to reach commercial success.

A further increase in dose size to 1,000 million cells will narrow down the choices in technology availability for MSC cell culture, as only multi-plate bioreactors and microcarrier systems are able to satisfy such high scales of production. Moreover, none of these platforms would be able to achieve satisfactory COG as % sales under current NICE reimbursement. Furthermore, even when the typical reimbursement with similar dose size is applied (GvHD), multi-plate bioreactors are not able to meet the target COG as % sales and microcarrier systems would struggle to do the same. When applying the reimbursement applied to Temcel® (Mesoblast, Australia) an allogeneic MSC-based product targeted at GvHD with a dose size of 1.2–1.7 billion cells resulting in a selling price of $117,983–$167,143 per dose ($7,079 per 72 million cells) [91], it is clear that even at this higher selling price, both manufacturing platforms will struggle to meet the target COG as % of sales.

3.3.2. Sensitivity analysis

In order to understand the direction in which the development effort should be focused, so as to address both the gross margin limitations, and the capacity constraints mentioned in Fig. 6, a sensitivity analysis was performed. The manufacturing platform used in this analysis is the single use bioreactor with microcarriers, as this is the most cost-effective technology to be used in a high demand scenario such as this (Fig. 2). Fig. 7 indicates that the factors that have the greatest impact on COG at high dose size-high annual demand scenarios are mostly operational since these will ultimately influence the cell quantities which are produced per batch. The factor with the highest impact on the COG is the cell doubling time, this is attributed to the fact that this is a crucial factor in determining the number of cells required from the beginning of the process to meet a particular batch size due to the model set up. A higher cell doubling time would mean a slower process, and hence more cells would be required at the beginning of the cell culture. This would increase the size of the cell culture vessels required during the initial cell culture stages since more cells are being loaded into them, increasing the resource requirement and hence the COG.

The nature of this scenario explains the asymmetry seen in the impact of operational parameters on COG in Fig. 7. At the last expansion stage of the chosen scenario, a single use bioreactor of 1,000 L is being utilised at 60% capacity. Given that the full capacity of this bioreactor is not being utilised, when the DSP or cell detachment yield are decreased to 75% of their initial value, there is no significant change in COG since the same bioreactor can accommodate the additional cells required to make up for the lower yields. When the opposite occurs, and the DSP yield and cell detachment yield are adjusted to 125% of their original value, the number of cells

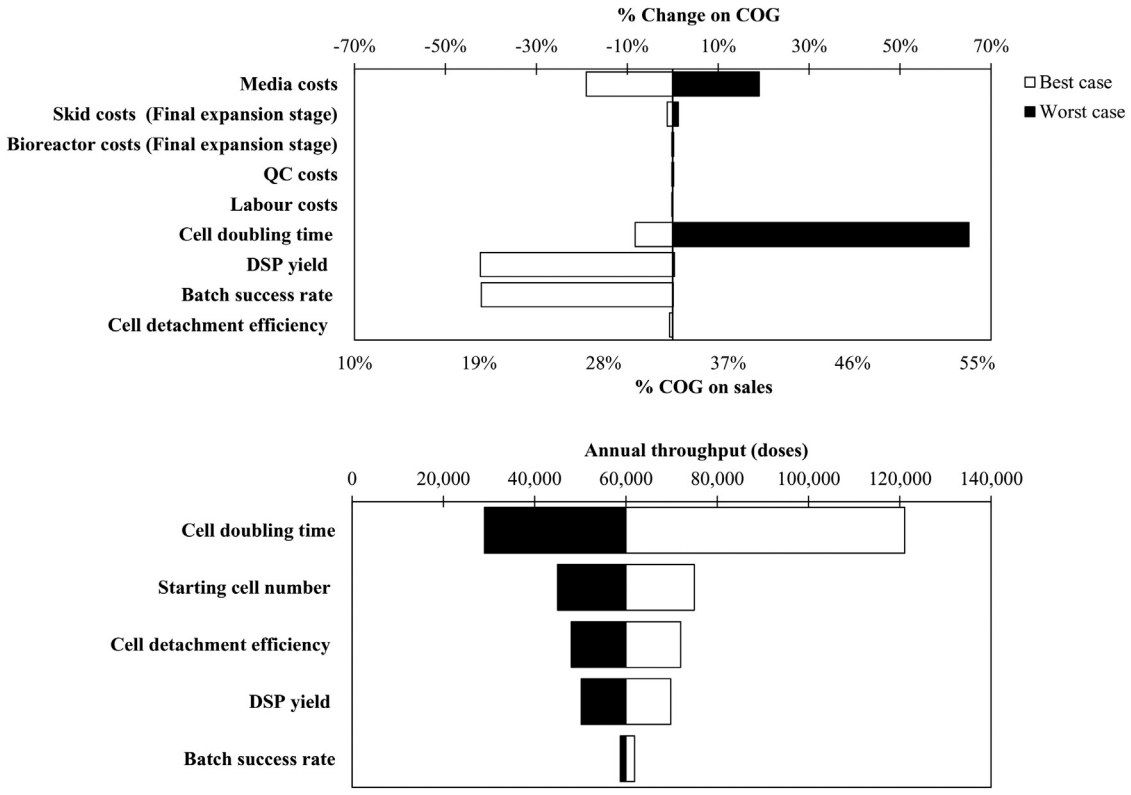

Fig. 7. Sensitivity analysis showing the impact of varying both process parameters and cost parameters by ± 25% with the exception of the batch success rate (varied by ± 5% since the base case is 95%). This figure shows the effect of process and economic parameters on the COG and throughput of an MSC product with dose size of 1 B cells and a demand of 10,000 cells per patient.

required to meet the batch size decreases such that a 500 L bioreactor becomes optimal. Fig. 7 confirms the conclusion drawn from Fig. 2b, where the cost parameter with the greatest impact on the total COG in large scale scenarios is the media costs.

Fig. 7 also highlights the considerable impact that the cell doubling time has on the productivity of a cell therapy processes. In this figure, the capacity of the single use bioreactor (surface area per litre) was not varied, as this parameter has no bearing on the number of cells produced. The number of cells initially loaded into the process, the DSP yield and the cell detachment efficiency were varied by ± 25% of the base case instead. As expected, increasing any of these parameters by 25% increases the number of maximum doses produced by 25%.

3.3.3. *Optimization case study*

Having identified the key parameters affecting the profitability and capacity of technologies for adherent cell culture, this section will determine the development effort required to overcome current process and economic challenges.

Fig. 8 shows how varying the different parameters highlighted by the sensitivity analysis affects the COG as % sales for a selling price of $40,000. The base case scenario presented in this figure shows that in order to achieve COG as 15% sales, the surface area per litre inside the bioreactor must be at least 20,000 cm^2. If the cell doubling time is decreased (Scenario 2), the COG as 15% sales target can be reached by almost doubling the current bioreactor capacity (5,540 cm^2 per litre) to 10,000 cm^2 per litre in combination with increasing the detachment yield from 75% to 100%. Solutions for increasing the detachment yield have previously been discussed in Section 3.1. The same COG target can be achieved without changing the proliferation rate of the cells if the DSP yield can be enhanced from 61 to 81% (Scenario 4). The DSP yield is a combination of microcarrier removal, cell wash, concentration and cryopreservation yields, with the values of 90%, 85% and 80% [63,91] respectively. In this analysis, an assumption was made that the FBC would be used to remove the microcarriers from the cell containing solution. An alternative way to increase the yield of this step is the employment of the harvestainer™ (Thermoscientific, Waltham, MA, USA) technology instead of the FBC system for microcarrier removal. Another alternative to increase the overall DSP yield is to use a different cryoprotectant to DMSO in order to decrease cell loss during cryopreservation [92,93]. Moreover, additional strategies to increase the DSP yield include decreasing the concentration of the cryoprotectant used and optimizing freezing process.

Fig. 9 shows how varying the key parameters which influence the productivity of a manufacturing process for MSCs affects its ability to achieve 100,000 doses per year of 1billion cells. Fig. 9 shows that the process throughput is dependent on the DSP yield, and, that the maximum number of doses achievable across all scenarios is 75,600. This target is only achievable if the DSP yield is increased to 81% (Scenario 3 & 5). Fig. 9 also shows that excessively increasing the number of cells initially added to the process under the base case detachment yield would not increase productivity; although this would result in a higher number of cells achieved during the expansion process, the maximum capacity of a single FBC system under 4 h is ~1 trillion cells, which translates into 1.8 billion cells initially added to the process. Furthermore, Fig. 9 shows that the capacity of current DSP technologies is the key obstacle to commercial scale manufacture of MSC-based cell therapy products. Moreover, improving the performance of these technologies will result in lower COG as COG decreases with increasing scale, hence the development effort should be shifted towards the downstream process.

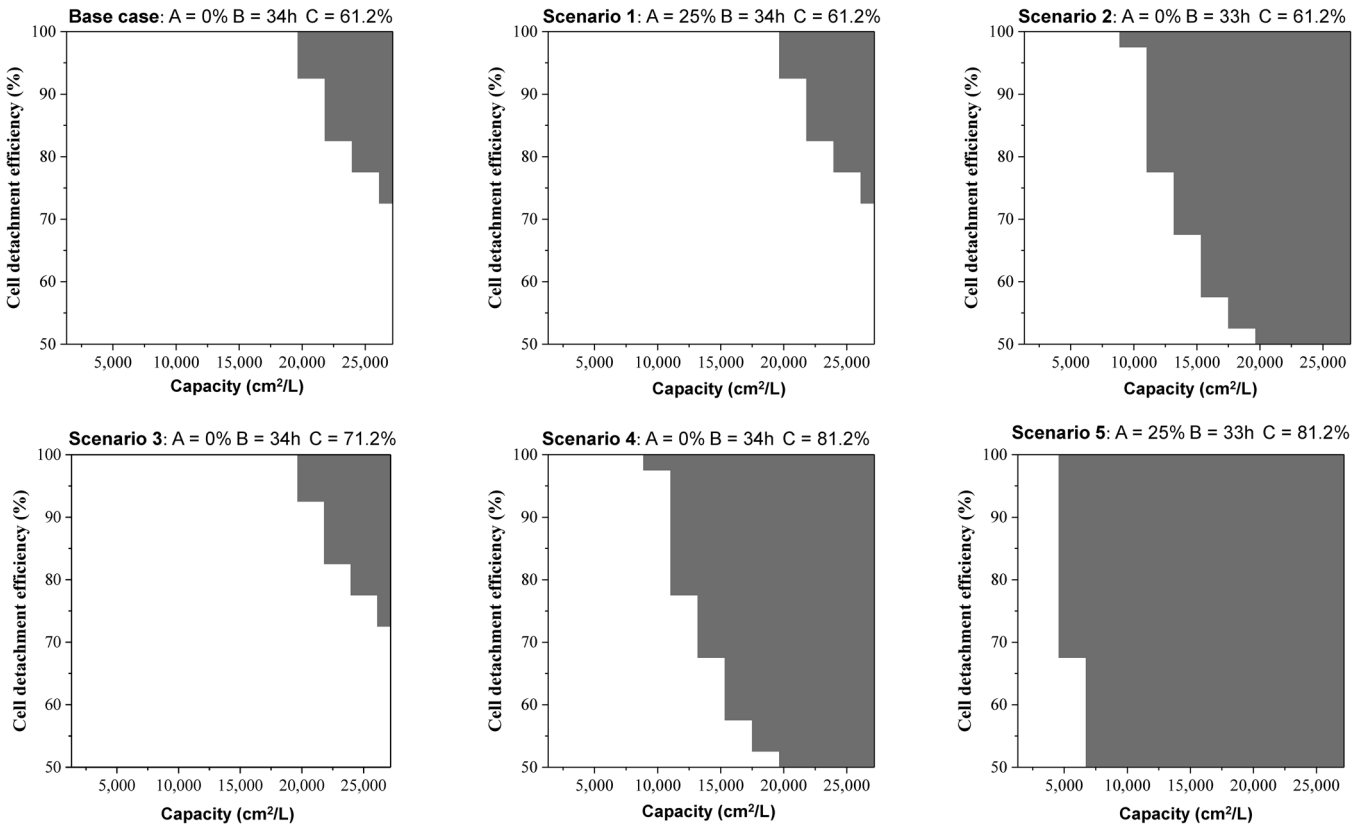

Fig. 8. Measurement of the impact of different parameters on the ability of reaching COG as 15% sales for a selling price of $40,000 per dose, an annual demand of 10,000 doses and a dose size of 1 billion cells. The shaded are represents scenarios where COG as 15% sales is reached. A = discount on media costs, B = cell doubling time and C = DSP yield.

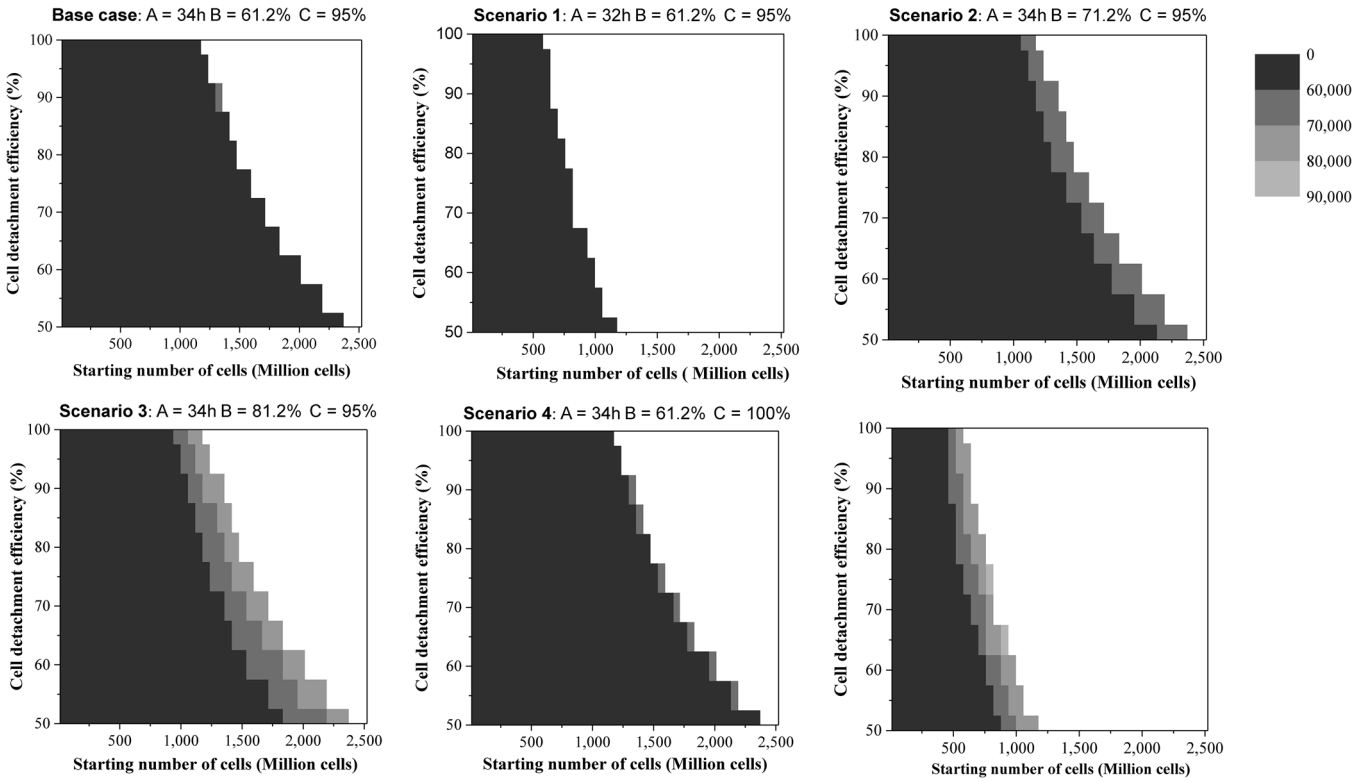

Fig. 9. Measurement of the impact of different parameters on the ability of reaching 100,000 doses per year of 1 billion cells. A = Cell doubling time. B = Batch success rate; C = DSP yield.

4. Conclusion

This study has explored the economic and operational performance of four candidate technologies for the commercial scale expansion of MSCs in order to evaluate the probability of each of these technologies leading to a feasible business model. The results show that from an economic perspective, planar manufacturing platforms are most cost-effective at smaller scales ($\leq$ 1B cells/batch) whilst microcarrier systems are more cost-effective at medium to large scales (10-100B cells/batch). The results have revealed that for applications with low dose sizes (10 million cells), the COG/dose vary between $485 and $1750 and for applications with high dose sizes (1 billion cells), the COG/dose vary between $13,134 and $111,488 depending on the technology and manufacturing scale selected. The results also show that the superior operational characteristics of multi-plate bioreactors allows them to closely compete with microcarrier systems even at larger scales. However, ultimately, microcarriers are the optimal technology for large-scale expansion of allogeneic MSC-based cell therapy products. Furthermore, this study highlights that in order to achieve commercial success under current reimbursement strategies significant improvement is required in the sector for treatments with large dose sizes and that the market penetration of certain indications is limited by the capacity of the current technologies. This study has also shown that future resources for the development of technologies for commercial scale manufacture of cell therapy products should be focused on DSP technologies. This tool can be used to understand and quantify the current limitations and characteristics of the different technologies for mesenchymal cell expansion and can be extended to explore further options such as the use of cultures based on aggregates or spheroids. Such analyses help predict the minimum reimbursement levels for different MSC-based cell therapy products that allow for feasible business models.

Acknowledgements

Financial support from the UK Engineering and Physical Sciences Research Council (EPSRC) and Pall Life Sciences is gratefully acknowledged (Grant Code: EP/G034656/1). The Advanced Centre for Biochemical Engineering at UCL hosts the Future Targeted Healthcare Manufacturing Hub in collaboration with UK universities and with funding from the EPSRC and a consortium of industry and government users. Feedback from a number of industry experts including Jon Rowley (RoosterBio Inc.), Reinout Hesselink (eXmoor pharma concepts Ltd) and Philippe Willemsen (Belgian Volition) is gratefully acknowledged.

Appendix 1.

N^{n}_{unit} Number of cell culture vessels required for the expansion stage n0
$N_{cells/batch}$ Number of cells produced per batch
y_{DSP} DSP yield
$y_{cell\ detachment}$ Cell detachment yield
$y_{batch\ sucess}$ Batch success rate
a_t Surface area for cell growth of a cell culture vessel
$Density_{microcarrier}$ Microcarrier's seeding density
$Utilisation_{cell\ culture\ vessel}$ % utilization a cell culture vessel
$a_{per\ gram}$ Surface area per gram of microcarrier
C_{media} Media costs
$C_{media/ml}$ Media costs per ml
$V_{media/a}$ Volume of media required per unit surface are of cell culture vessel
$C_{trypsin}$ Trypsin costs
$C_{trypsin/ml}$ Trypsin costs/ml
$V_{trypsin/a}$ Volume of trypsin required per unit surface are of cell culture vessel
$C_{consumables}$ Consumables costs
$C_{consumable/unit}$ Consumables costs per cell culture vessel
C_{labour} Labour costs
$C_{operator}$ Operator salary
$R_{documentation/executing}$ Ratio between the documentation operator and the executing operator
$R_{Overhead/operator}$ Ratio between labour overheads and operator salary
t_{step} Time required per cell culture vessel to perform a particular activity
$t_{step\ max}$ Maximum time allowed for a particular activity
FCI Fixed capital investment
$C_{equipment}$ Equipment costs
L_f Lang factor
$C_{equipmnet/unit}$ **U**nit costs for a particular type of equipment
$Capacity_{equipment}$ Max number of cell culture vessels that the equipment can handle
C_{BSC} Biosafety cabinet costs
$C_{BSC/unit}$ Biosafety cabinet costs per unit

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