Decisional tool for cost of goods analysis of bioartificial liver devices for routine clinical use

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Abstract

Background aims: Bioartificial liver devices (BALs) are categorised as advanced therapy medicinal products (ATMPs) with the potential to provide temporary liver support for liver failure patients. However, to meet commercial demands, next generation BAL manufacturing processes need to be designed that are scalable and financially feasible. We describe the development and application of a process economics decisional tool to determine the cost of goods (COG) of alternative BAL process flowsheets across a range of industrial scales.

Methods: The decisional tool comprised an information database linked to a process economics engine with equipment sizing, resource consumption, capital investment and COG calculations for the whole bioprocess, from cell expansion and encapsulation to fluidised bed bioreactor (FBB) culture, to cryopreservation and cryorecovery. Four different flowsheet configurations were evaluated across demands, with cell factories or microcarriers in suspension culture for the cell expansion step and single-use or stainless steel technology for the fluidised bed bioreactor culture step.

Results: The tool outputs demonstrated that the lowest COG was achieved with microcarriers and stainless steel technology, independently of the annual demand (1,500 to 30,000 BALs/year). The analysis identified the key cost drivers were parameters impacting the media volume and cost. To achieve the target cost of goods of £25k/BAL, optimisation of process and economic parameters that impact the culture media cost is required. The tool was used to identify the critical combination of reductions in plasma price, FBB culture time and nutrient supplement price required to achieve the target cost.

Conclusions: The tool outputs can be used to identify cost-effective and scalable bioprocesses early in the development process and minimise the risk of failing to meet commercial demands due to technology choices. The tool predictions serve as a useful benchmark for manufacturing ATMPs.

Keywords: Bioartificial liver, Cost of goods, Cell factories, Microcarriers, Stainless steel, Single use

1. Introduction

Bioartificial liver devices (BALs) have increasingly gained clinical relevance for treatment of several liver failure conditions and/or as a bridge until a donor organ can be sourced for liver transplant. The BAL provides improved liver function to the patient, "buying time" either for the native liver to recover after an insult such as acute liver failure, or until a suitable donor organ is found. However, its commercialisation as a therapy depends on the technology scalability and economic feasibility of the manufacturing bioprocess. Cost of goods (COG) analysis enables assessment of the financial feasibility of a process by identifying alternative technologies and key cost drivers and exploring how best to meet predefined cost targets and thus achieve commercial success. We present data to assess the cost of BAL manufacturing at industrial scale evaluating the benefits of different technological options across a range of demands, using HepatiCan, a bioartificial liver developed at UCL.

The BAL is an extracorporeal organ support system comprised of a cell-housing bioreactor, where patient's blood or plasma is perfused, capable of mimicking the metabolic, synthetic and detoxifying functions of the liver. These devices differ from each other essentially in the bioreactor design, cell type and source, with current designs including hollow-fibre cartridge-based systems, encapsulated cells in perfused beds [1–3].

HepatiCan is a pre-clinically tested BAL device composed of a fluidised bed bioreactor (FBB) with alginate encapsulated HepG2 cell spheroids, which are initially encapsulated as single cells and cultured in the same bioreactor design for up to 12 days [1,4,5]. After culture to 'performance competence', the biomass is cryopreserved and recovered upon demand for patient use. For recovery, encapsulated cells are transferred to an equivalent BAL vessel, which is then shipped to the patient bedside for treatment. FBB design promotes a high mass transfer between the biomass and the perfusate (e.g. patient's plasma). Encapsulated cell spheroids are easily manipulatable and cryopreservable leading to constituting a product that can be stored at cryogenic temperatures and be available 'on demand' [6–9]. Moreover, HepG2 cells benefit from unlimited proliferation, stable phenotype, low culture cost and survival in human liver-failure plasma, and their 3D tissue-like structure improves function and performance compared to their phenotype in monolayer culture [10–13].

In pre-clinical trials, HepatiCan has demonstrated, in a porcine ischaemic acute liver failure model, improvement in coagulation, blood pH, intracranial pressure, brain oxygenation and reduction in vasopressor requirements to maintain blood pressure, all important parameters in the clinical prognosis of the condition [1,5].

At the current scale, only one BAL is produced per batch but to meet commercial demands relevant to routine clinical adoption, the bioprocess needs to be scaled up. The scalability of the manufacturing process required depends on the clinical conditions and percentage of patients addressable by the BAL, and its market

penetration (Table 1 and Table 2). With increased demand there is an increase in technological constraints and consequent decrease of feasible candidate technologies to meet production requirements.

For example, the standard for expansion technology for adherent cell types in the laboratory is the conventional tissue culture flask. However, industrial scenarios demand a higher number of cells than those normally produced in the laboratory, highlighting the limitations of tissue culture flasks: labour-intensive manipulations, small surface area to volume ratio and inability of inline monitoring. More automated systems, are less labour-intensive, have a high surface area to volume ratio and larger volume capacities and constitute a better solution for large scale processes [14]. Agitated suspension culture using microcarriers could be considered a successful alternative since it combines all these advantages [15,16]. However, it has been reported that, when using this technology, there are increased difficulties in harvesting cells, diffusion of nutrients may be restricted in large microcarriers of high cell densities, and it can be perceived as requiring more expensive capital investment [17].

Moreover, throughout the years industrial bioprocesses have relied on stainless steel equipment. These vessels accommodate large volumes, endure high temperature and pressure and have a high stain and corrosion resistance [18]. They require cleaning, sterilisation and validation which are costly and time-consuming activities as well as a high initial capital investment. Although stainless steel facilities still dominate, accounting for 85% of the market, there is a growing adoption by industry of disposable/ single-use systems [19]. This technology, composed mainly of plastic components supported by rigid containers (e.g. stainless steel), eliminates extensive cleaning and sterilisation with the associated validation, reduces the risk of contamination, has a smaller facility footprint and lower investment and construction costs because of the simpler facility infrastructure required [20]. However, due to its limited scalability (typically up to 2,000 L, with some vendors commercialising up to 6,000 L), high consumable cost, concerns about breakage and 'leachables and extractables', to date, disposable systems have been more commonly seen at pre-commercial scale [21].

The use of decisional tools has proven useful in identifying and assessing different bioprocess designs *in silico*, in order to make decisions early on about the manufacturing process that will lead to the most cost-effective design with suitable technology selections and equipment sizes. A specific field that has benefited historically from decisional tools, and that in turn has supported their development, is the production of monoclonal antibodies (mAbs). These tools have been applied to multiple decisions including batch versus continuous modes of operation [22–24]. They have also been extended to cell manufacturing processes of patient-derived induced pluripotent stem cells (iPSCs), allogeneic mesenchymal stromal cells (MSCs) and chimeric antigen receptor T (CAR-T) cells. More specifically, COG analysis has assessed: the impact of using manual vs automated technologies for iPSCs across different scales [25]; the use of planar or microcarrier-based technologies for cell expansion of MSCs and the most cost-effective single-use technologies for downstream

processing according to demand levels [26–28]; and to determine the optimal flowsheet configuration for allogeneic CAR-T cell manufacture [29].

As yet, few cost analyses or decisional tools have been published about combined advanced therapy medicinal products (ATMP) such as the BAL. A health economics cost-effectiveness analysis conducted by Hessel examined the treatment of acute-on-chronic liver failure with an artificial liver system MARS (i.e. a system without biomass content) [30]. However, the scope of this analysis was limited to the treatment phase and not the manufacturing of the artificial liver device or its consumables; the device was purely artificial, thus not containing a biological component.

The present study describes the development and application of a decisional tool built to capture the resources required and associated COG for manufacturing BALs, particularly the HepatiCan, for treatment of several liver conditions (Table 1). It assesses the use of different manufacturing technologies and determines the cost-effectiveness of the process for different flowsheet configurations. The tool also identified the economic drivers for all investigated configurations, and sensitivity and target analyses were used to probe the effects on COG of altering process and economic parameters. This is the first time this type of analysis has been applied to the production of a combined ATMP-medical device such as a bioartificial liver system.

2. Materials and methods

2.1. Tool description

The databases contained unit costs of materials (e.g., alginate, plasma, cryoprotectant), equipment (e.g., incubator, fluidised bed bioreactor, Jetcutter™ encapsulator) and labour (e.g., operators) (Table 3). It captured also the key input assumptions for the bioprocess protocols to enable mass balance and equipment sizing calculations (Table 4); these included generic inputs for each unit operation such as the step yields and specific inputs such as the initial and final cell densities and the media to encapsulated cells ratio for the FBB culture.

The tool includes an interface where the scale, the demand and the process flowsheet are defined. For each scenario, the model imported information from the database, performed the mass balances, equipment sizing and labour calculations, and determined the COG and FCI. A snapshot of the inputs and outputs page of the model is shown in Figure S1.

2.2. Bioprocess economics model

The economic parameters used to calculate the total COG, COG/batch and COG/BAL comprised direct, labour and indirect costs. Direct and indirect costs were calculated following previously described methods [25,26,28]. The direct cost comprised the materials cost that included process reagents such as media, process

consumables such as plasticware, and quality control (QC) testing materials. The materials cost was determined considering the amounts required determined from the mass balance calculations and their unit costs. The indirect cost captured the depreciation, maintenance, general utilities, insurance and taxes that were a function of the fixed capital investment (FCI). The FCI was estimated using the Lang factor method with a Lang factor of 8.13 [25].

Labour costs were determined based on the number of units to be manipulated in each flowsheet configuration and scaled up with demand. It was assumed that a minimum of eight operators were required for the peak of the manufacturing activities (the end of the cell expansion, cell encapsulation and start of FBB culture – Figure 2) and a 10% safety factor was included. The total labour cost per year was estimated as:

$$c_{lab} = \sum \left(\frac{u_{peak}}{\omega_u}\right) \times \alpha \times c_{salary} \times \beta \times \gamma \quad [1]$$

where u_{peak} is the unit to be manipulated during the peak activities, ω_u is the number of units an operator could handle, c_{salary} is the operator's annual salary, α is the safety factor, β and γ are factors accounting for salary overheads and management and supervision costs, respectively.

The total labour cost per year was estimated as:

$$c_{lab} = \sum \left(\frac{u_{peak}}{\omega_u}\right) \times \alpha \times c_{salary}(1 + \beta + \gamma) \quad [1]$$

where u_{peak} is the number of units to be manipulated during the peak activities, ω_u is the number of units an operator could handle, c_{salary} is the operator's annual salary, α is the safety factor, β and γ are factors accounting for salary overheads and management and supervision costs, respectively.

Moreover, the bioprocess economics model was configured to calculate mass balances and the size of key equipment units according to the demand modelled. The expressions used to determine these values have been applied in other studies [25–28]. However, specific equipment associated with the BAL manufacturing process included the stainless-steel fluidised bed bioreactor (SSFBB). Across scales, key parameters that should be maintained constant in the FBB design are the ratio of the height of the packed bed of alginate encapsulated cells to the internal diameter ($x = H_b/D$), and the ratio of the total bioreactor height to the internal diameter (H_T:D). The volume of the packed bed of alginate encapsulated cells can be described as the volume of a cylinder:

$$V = \pi r^2 h \quad [2]$$

where, $h = H_b$ is the height of the settled packed bed of alginate encapsulated cells and $V = V_{cell}$ is the total volume of the packed bed of alginate encapsulated cells to be cultured in the SSFBB (in cm^3).

Rearranging this equation for the bed height H_b and substituting the radius with the ratio x gives:

$$H_b = \left(\frac{V_{cell} 4x^2}{\pi}\right)^{\frac{1}{3}} \quad [3]$$

Subsequently and given $H_b/D = 1$, the internal diameter was established as:

$$D = \frac{H_b}{1} \quad [4]$$

And for H_T:D = 2.4, the total height of the SSFFB comes as:

$$H_T = 2.4 \times D \quad [5]$$

3. Case study

This decisional tool was applied to a case study evaluating the capacity of the manufacturing process to respond to the demand for a bioartificial liver cell therapy and use of the different technologies to deliver that bioprocess. The annual demand for the BAL is likely to follow the clinical indications described in Table 1 and, different market penetrations as in Table 2 make the BAL addressable to variable numbers of patients. Independent of the clinical condition, on average, each patient may require 1 – 3 BALs during treatment. Therefore, taking the Europe market as a case study (Table 2), the projected annual demand for the BAL is 1,500 to 30,000 BALs/year, depending on market penetration of 1 - 20%. The dose of each BAL is fixed at 2.5 L of alginate encapsulated cells containing a total of 60 – 70 billion cells, equivalent to one- to two-thirds of an adult liver mass. Using the economics model, the fixed capital investment and COG were computed for the different BAL annual demands and bioprocess flowsheet configurations, according to the technology selected.

The HepatiCan bioprocess is outlined in Figure 1 and draws on the authors' experience during preclinical trials in pigs with human sized livers [1,5]. The manufacturing schedule was modelled in staggered batches, avoiding an overlap of the FBB culture step (Figure 2), and resulting in a maximum of 21 batches per year. The batch size is variable with annual demand.

The process starts with a cell expansion step where HepG2 cells (HB-8065; American Type Culture Collection, Manassas, VA, USA) are cultured as monolayers during 4 stages lasting a total of 25 days (Figure 2) with regular media exchanges and cell passage between stages. The culture media is supplemented with 10% (v/v) fetal bovine serum. At this step, there is a choice of available technologies between multi-layer flasks (e.g. cell factories) or suspension culture using microcarriers in a stirred tank single-use bioreactor (SUB). Regardless of the choice of technology, this step is followed by cell dissociation (involving washing and proteolytic enzyme detachment steps) and then cell concentration using a spinning filter membrane system (e.g., LOVO Cell Processing System, IL, USA; membrane pore size: 4 µm). Thereafter, the cells are resuspended as a single cell suspension. Simultaneously, a 2% (w/v) alginate solution is prepared in a HEPES/saline buffer,

and both cell suspension and alginate solution are mixed in a 1:1 ratio in a pressure vessel which feeds the Jetcutter™ encapsulation system (geniaLab Biotechnologie, Germany). The mixed suspension is cut into droplets, which fall into a bath of 0.2 M $CaCl_2$ where they are crosslinked into semi-solid micro-spheres (i.e., beads). These alginate encapsulated cells constitute the biomass of the BAL and, after washing to remove any calcium excess, are transferred to the FBB culture step.

During FBB culture, alginate encapsulated cells are maintained for up to 12 days in a fluidised bed bioreactor (FBB), perfused continuously and expanded from single cells at a density of 2 million cells/mL beads to several cell spheroids per bead with a final density of ~30 million cells/mL bead. Several batch media exchanges are performed to replenish nutrient and remove toxin build-up. The culture media is supplemented with 10% (v/v) human plasma.

At this stage, there is another technology choice between disposable FBB (DFBB) and stainless-steel FBB setup (SSFBB) configurations. The former consists of multiple disposable fluidisation chambers, each harbouring 2.5L of alginate encapsulated cells, all connected to a SUB serving as a reservoir for constant media recirculation; whereas the SSFBB comprises one customised stainless-steel fluidisation chamber harbouring the total volume of alginate encapsulated cells and connected to a stainless-steel stirred tank bioreactor serving as a reservoir for media recirculation.

After FBB culture, the alginate encapsulated cells as “cell-spheroids” are harvested and cryopreserved immediately. The volume of harvested beads is equilibrated with cryopreservation solution and added to several cryopreservation cryocassettes (e.g., cryobags), each holding 1 L of biomass. These are then cooled in a controlled rate freezer until a final temperature of -100°C and stored in the vapour phase of liquid nitrogen. Upon demand, 3 cryobags per dose are thawed, the content washed to remove the cryoprotectant solution and added to disposable fluidisation chambers connected to a SUB. The biomass is cultured (in culture media supplemented with 10% (v/v) human plasma) for an additional 1 – 3 days to recover from cryoinjury sustained during the cryopreservation process that might compromise the final cell density and biological performance, before being shipped for patient treatment in a sterile disposable HepatiCan chamber. For simplification and to incorporate the impact of this step in the economic model, it was assumed that the whole production per batch was immediately cryorecovered. Of note, the cryopreservation step described in the Results and Discussion section and depicted in Figure 1 includes both the freezing and cryorecovery processes.

Overall, the BAL bioprocess has four different flowsheet configurations: using cell factories and DFBB, cell factories and SSFBB, microcarriers and DFBB, or microcarriers and SSFBB. Key process and cost assumptions of materials, equipment and labour are described in Table 3 and Table 4, respectively. Moreover, the overall yield from cell expansion through to cell concentration and cell encapsulation is 72% and the overall yield from FBB culture through to cryorecovery is 86%.

The cost of BAL manufacturing per flowsheet configuration and annual demand was determined. The target cost of goods value was set at £25,000 per BAL. In order to recommend improvements to the process to meet the target cost of goods value, a sensitivity analysis was carried out to identify important process and economic parameters that drive the COG/BAL. Input parameters varied individually between a minimum and maximum value that represent the best- and worst-case scenarios as indicated in Figure 6 A. The rationale for the values is provided in the Results and Discussion section. The factors with the highest impact on the COG/BAL were then selected and combined during optimisation analysis so as to identify the window of opportunity that would enable the COG target to be met and consequently, the economic feasibility of the bioprocess. The COG was compared to the estimated reimbursement levels of £100,000-£250,000 depending on the condition (Table 1); these reimbursement values were based on meeting the UK's National Institute of Health and Care Excellence (NICE) cost threshold for a therapy of £30,000 per quality adjusted life year (QALY) [31–33].

4. Results and Discussion

This section presents insights from the cost modelling of the production of the bioartificial liver device (BAL). More specifically, it discusses the cost-effectiveness across different demands and flowsheet configurations, provides a breakdown of the COG categories, identifies the key cost drivers of the process and how they each significantly impact the COG. To meet the target COG, key parameters which influenced cost-effectiveness were optimised.

4.1. Bioprocess economic model results

The cost-effectiveness of the BAL bioprocess was investigated at different flowsheet configurations and product demand. Figure 3 illustrates the COG per BAL (COG/BAL) and how it changes with the annual demand and technology used for the cell expansion and fluidised bed bioreactor (FBB) culture steps.

The COG/BAL varies between £34.3k to £44.6k for the considered demands and flowsheet configurations with the overall trend displaying a decrease of COG/BAL as the annual demand increases (Figure 3 A). In all cases, materials cost dominates the COG (81 – 92%), followed by indirect (6 – 13%) and labour (1 – 7%) costs. This dominance can be attributed to the high annual demand, even at the lowest scale of production (1,500 BALs/year), which results in labour and indirect costs, being spread over the high number of BALs produced per year. Other cell therapy economic models have highlighted similar trends where the indirect costs have a small influence on COG [26,27,29]. Examining COG/BAL by stage (Figure 3 B) highlights that the FBB culture is the cost dominant step of the bioprocess (40 – 48%), followed by cryopreservation (29 – 34%) and cell expansion (14 – 25%) (Figure 3 B). Of note, the cryopreservation step includes the freezing and recovery processes.

In terms of technology, independent of the scale of production, microcarriers (M) offer cost advantages over cell factories (CF). At lower demands (1,500 BALs/year) the reduction in COG/BAL when resorting to microcarriers is 9% (£42.7 - £44.6k for cell factories to £38.9 – £40.7k for microcarriers). This impact is reflected only in the cell expansion stage where this technology is employed (Figure 3 B). It results from a decrease in indirect costs, consequent on the smaller number of equipment units needed for fewer cell expansion vessels (e.g. 5 vessels for microcarriers *vs* 185 for cell factories - Figure 3 C). As the scale of production increases, cost savings increase and a reduction of 14% in COG by changing from cell factories (£39.8 – £42.8k) to microcarriers (£34.3 – £38.2k) is observed. The advantage of the microcarriers for the current scale of production ($1x10^{12}$ to $2x10^{13}$ cells/batch) is supported by others who identified microcarrier-based systems as the most cost-effective technology for allogeneic stem cell manufacturing of more than 10^{10} cells/batch, up to a maximum of $3x10^{12}$ cells/batch [26,28]. This bottleneck at high demands was based on the assumption that the largest SUB was 2,000 L, which in the present study is no longer a constraint due to industry efforts in scaling up SUB units to larger working volumes (e.g., 6,000 L).

Employing stainless steel FBB (SSFBB) technology also brings benefits compared to disposable FBB (DFBB): COG is reduced by 4% at 1,500 BALs/year and 10% at 30,000 BALs/year relative to DFBB configurations. This change is manifest at the FBB culture step with a decrease in indirect and materials costs of 8% (Figure 3 B). The difference is attributed to the high number of disposable vessels necessary to meet the demand, resulting in a total investment which is not substantially different from conventional stainless-steel technology, even if the unit price of the single-use hardware is lower. However, the greatest impact is reflected in the labour cost (Figure 3 A). For example, to manufacture 15,000 BALs/year using cell factories and DFBB, 106 operators are needed in contrast to 42 operators for cell factories and SSFBB (Figure 3 C). DFBB configurations are very labour-intensive because manipulating a high number of vessels requires more operators, rather than the SSFBB whose higher volumetric capacity harbours equivalent volumes in fewer vessels and consequently, fewer manipulations and operators are necessary.

Although there are incentives to adopt disposable technology in bioprocess manufacturing, benefits of lower costs are typically seen in scenarios that require capacities below the maximum scale of the single-use technology [26,34,35]. Once the maximum volume of the SUBs is exceeded, scale-out is required rather than scale-up and this no longer benefits from economies of scale. In fact, stainless steel equipment benefits much more from economies of scale as demonstrated by SUBs being economically disadvantageous for downstream processing scales of 10,000 L when producing recombinant proteins [36–38].

Overall, the cost benefit of using microcarriers instead of cell factories is higher than SSFBB vs DFBB. Yet, combining the two technological alternatives, i.e. microcarriers and SSFBB instead of cell factories and DFBB, significantly impacts the cost, and increasingly so with demand: the COG/BAL decreases 13% at 1,500 BALs/year (£44.6k to £38.9k) and 20% at 30,000 BALs/year (£42.8k to £34.3k). Although COG/BAL is reduced based on the technology selected and the increase in annual demand, for all flowsheet configurations the

projected cost exceeds the target cost of £25k. At the best configuration of M + SSFBB, the cost is 1.6-fold higher at 1,500 BALs/year and 1.4-fold higher at 15,000 and 30,000 BALs/year than the target value. This suggests that the manufacturing strategy needs to be refined to improve the economic competitiveness of the BAL bioprocess, although it is well-known that in today's climate ATMPs are more costly than simple device-based therapies.

4.2. Material cost breakdown

To enhance the economic feasibility of the BAL bioprocess it is important to understand the main contributors to the COG/BAL, and the decisional tool enabled a breakdown of the material cost, which is relevant due to its dominance in COG as previously determined in Figure 3 A. For this analysis, the annual demand of 15,000 BALs/year was selected across all flowsheet configurations.

The total material cost varies between £31.8k and £35.8k according to the flowsheet configuration, with the highest cost for CF+DFBB, followed by CF+SSFBB, M+DFBB and M+SSFBB (Figure 4). The similarity in cost between configurations is explained by the main cost driver of the bioprocess being the culture media (≥ 83%), regardless of the configuration. Culture media is used in all steps of the bioprocess and particularly, at a high media to encapsulated cells ratio during the FBB culture and cryopreservation (particularly recovery process) steps (Table 4), therefore cost is not dependent on the selected technology, but rather a function of the dose (i.e. volume of encapsulated cells) necessary per BAL.

A further breakdown of the culture media cost (Figure 5) identifies that the key drivers are the human plasma (30%), fetal bovine serum (22%), alpha MEM (17%) and DMEM (1%) (Figure 5 A). Although "Supplements" has a similar contribution to that of plasma, this category combines several different components which individually embody 10% or less of the media cost. As demonstrated by Figures 5 A and B, the impact of each component on the media cost does not exclusively depend on the used volumes in the bioprocess, it also depends on the respective unit cost. For example, although Alpha MEM is the main component of the culture media, accounting for 83% of the total volume (Figure 5 B), it only represents 17% of the cost (Figure 5 A) due to its low unit price (£8/L) (Table 3).

Conversely, the chamber and plasticware costs are a function of the flowsheet configuration. The chamber cost relates to the cost of the disposable fluidisation chamber and its accessories which in the DFBB configuration is employed in two steps of the process (FBB culture and cryopreservation, particularly in the cryorecovery process); while its use in the SSFBB configuration is limited to the cryopreservation step. Thus, the cost of the chamber decreases from £2.2k/BAL in DFBB (Figure 4 A and C) to £1.1k/BAL in SSFBB configuration (Figure 4 B and D), explaining its smaller representation in the material cost in the latter.

Plasticware usage also changes significantly when switching from cell factories to microcarriers because the several 40-layer cell factories (L-40) are replaced by a few SUB bags (Figure 3 C). Although the unit cost of an L-40 is considerably less than a SUB bag (Table 3), the substantial decrease in the number of vessels used when adopting microcarriers diminishes the contribution of plasticware to the material cost to £0.9k/BAL, from £1.8k/BAL in a cell factory configuration (Figure 4 B and D). Reducing the number of vessels also minimises the losses in the bioprocess associated with manual handling errors that can occur in complex large scale manufacturing processes.

4.3. Sensitivity analysis

As the culture media is the main cost driver of BAL bioprocess, a sensitivity analysis was carried out to identify the significant process and economic parameters in the manufacturing process that could influence the culture media cost and consequently, COG. The tornado diagrams in Figure 6 depict the effect of changing each parameter by the variations denoted in Figure 6 A on COG/BAL. This analysis was conducted for a demand of 15,000 BALs/year and selecting the SSFBB technology, where the labour and indirect costs are minimised compared to DFBB. The impact was assessed when using cell factories (Figure 6 B) and microcarriers (Figure 6 C).

The variation of the parameters was based either on information from vendors (e.g. ,the plasma price), the known flexibility of the bioprocess (e.g., FBB culture time) or varied by ± 25% of their original value (e.g., microcarrier concentration).

Independent of the technology used for the cell expansion step (Figure 6 B and C), the plasma price, FBB culture time and nutrient supplement price are the top three key cost drivers, with plasma price leading the ranking. The impact of these parameters predominantly results from a direct correlation with the culture media cost, as discussed below.

Different grades of plasma exist that meet different regulatory specifications and commercial prices [39]. Here, the base case assumes the use of clinical grade plasma (£135/L), although a less stringent grade (£16/L) could be supplemented while still meeting regulations [40]. Plasma price at £16/L results in a 19% - 22% decrease of the COG/BAL (Figure 6 A and B, red bars). The reduction in price has a direct impact on COG as the plasma is the main media cost (Figure 5 A) due to its unit price and usage. It represents 9% of the culture media volume (65 L of a total of 741 L/BAL - Figure 5 B) as it is employed, at 10% (v/v), in the two steps of the process that consume the largest media volumes: FBB culture and cryopreservation (particularly cryorecovery process) steps require a 46:1 ratio of media relative to the volume of encapsulated cells to be cultured.

A 20% decrease in COG/BAL is observed when reducing the FBB culture time from 12 to 8 days. The authors observed that 8 days is the minimum time necessary to achieve a cell density that will not be compromised

by cryopreservation and cryorecovery and still yield the necessary dose per patient (i.e. 60 – 70 million cells/BAL). For the same demand, decreasing the number of FBB culture days enables the production of more batches per year. For example: to produce 15,000 BALs/year, if the FBB culture time lasts 12 days, 21 batches of 714 BALs are produced, while if reduced to 8 days, it will be possible to fit 30 batches of 500 BALs per year. This results in the spread of the indirect cost over more batches per year, contributing to the overall reduction in COG.

The effect of the nutrient supplement on the cost is driven by its price rather than its volume. The base case uses fetal bovine serum and, although it is mainly restricted to the cell expansion stage only representing 1% of the total culture media volume used in the bioprocess (Figure 5 B), its high unit price (£996/L) outweighs its low usage (7 L/BAL), placing nutrient supplement as a key cost parameter. The modelled best-case scenario replaces the serum with a serum-free supplement with a unit price of £100/L (Figure 6 A). This decreases the COG/BAL by 14% and circumvents the use of xenogeneic components in cell therapy products to minimise contamination, immunogenic factors and batch-to-batch variability [41].

The ratio of "media to encapsulated cells" directly dictates the volume of media that will be used in the FBB culture and cryopreservation steps. This parameter has been extensively optimised and its substantial alteration would compromise the biological performance of the encapsulated cells and consequently, the efficacy of the BAL [5]. Hence, changes in this parameter result in only a ±5% change in the COG/BAL.

Interestingly, changes to the yield of the different steps (± 5%) have a smaller impact on COG, between -4% and 4%. The variation of these parameters will result in a higher or lower number of cells obtained per step, and consequently, vary the number of consumable units needed (e.g., number of cell factories or mass of microcarriers) and the volume of media required to achieve the intended demand. Since, the yields of the bioprocess steps are already highly optimised and less flexible to improvement, the change in cell number is small. Of note, from the steps analysed, the thawing yield impacts COG/BAL the most as it determines the final volume of alginate encapsulated cells. Because the dose per patient is fixed (60 – 70 billion cells), changes to this yield dictate that more or less biomass should be produced in the previous steps (e.g. a higher number of cells at the cell expansion stage) to account for losses or gains, in order to meet the required number of cells per dose and the subsequent annual demand.

4.4. Target COG analysis

Having identified the key cost drivers of the BAL manufacturing process, the sensitivity analysis demonstrated that changing only one of the parameters did not improve sufficiently the COG/BAL to achieve the target value of £25k. The most impactful change in cost observed in Figure 6 B and C is of 20%, but as highlighted in Figure 3 A, there is a difference of at least 37% between the base case COG/BAL and the target value.

Thus, a target analysis was carried out to identify the necessary combination of improvements in the model parameters by including the simultaneous variation of two or more parameters to achieve the intended COG. It was performed for the same flowsheet configurations and annual demand as the sensitivity analysis: 15,000 BALs/year using cell factories plus SSFBB and microcarriers plus SSFBB.

The parameters chosen were the top three ranking in the sensitivity analysis: plasma price, FBB culture time and nutrient supplement price. Whereas the plasma price and the FBB culture time varied across a range of values, the nutrient supplement price was maintained at two fixed scenarios: fetal bovine serum (Figure 7 A and B) or the serum-free alternative (Figure 7 C and D).

The tool highlighted that adopting cell factories for cell expansion always exceeds the target COG/BAL value when fetal bovine serum is used to supplement the culture media; reducing the FBB culture time to 8 days and the plasma price to £15/L was not sufficient to achieve a cost of £25k per BAL in this case (Figure 7 A). Cell factories cannot compete with microcarriers in the serum scenario. When using microcarriers the target cost (≤ £25 000/BAL) is met in the window indicated by the blue region (Figure 7 B). There are multiple combinations of FBB culture time and plasma price that meet the target with the two extremes consisting of a culture time of 8 days with a plasma price of £55/L, or 10 days of culture with a plasma price of £15/L.

Alternatively, when fetal bovine serum is replaced by a serum-free supplement, both cell factories and microcarriers become attractive technologies with respective adjustments to the FBB culture time and plasma price (Figure 7 C and D). In this case, for cell factories to meet the COG target, FBB culture should be shortened to 10 days with a corresponding plasma price of £35/L; if the culture is shortened to 8 days a smaller reduction in plasma price, to £95/L, is sufficient (Figure 7 C). These scenarios widen the window of operation where COG/BAL ≤ £25k. When utilising microcarriers, the size of the window increases significantly, and the target cost can be met just by reducing the FBB culture days to 8, while maintaining the base cost of the plasma (£135/L) (Figure 7 D). Conversely, if the plasma price is subjected to improvements and decreases to £55/L, the FBB culture step can be maintained at 12 days. If all parameters are improved to their minimum values (8 days culture and plasma at £15/L), the COG/BAL drops below £20k, creating savings up to 31% (£17.3k) from the target value.

Overall, this decisional tool demonstrates that for the projected annual commercial demands, the most beneficial technological configuration of the bioprocess is to use microcarrier technology for cell expansion and SSFBB setup for the FBB culture step. The generated outputs highlight that those adjustments alone are not sufficient to meet the target cost value without further process optimisation and intensification. The authors provide insights on how to improve the current process, supporting efforts at the R&D level to validate whether time in the FBB could be shortened, or by extensively testing the impact of serum-free supplements on cell growth and performance. The benefits of reducing the COG/BAL whilst still meeting the NICE QALY threshold, would positively impact the economic feasibility of the process. It will satisfy the

reimbursement strategy defined by the NICE guidelines of £100,000 - £250,000 per patient, with the COG/BAL representing on average 15-45% of sales depending on the number of BALs required per treatment [42].

The estimated cost of goods values are in the range of cell therapy products, which normally have a cost per dose varying between US$10,000 to US$100,000. Compared to allogeneic stem cell manufacturing[27,28], the cost per dose of the BAL can be an order of magnitude higher. The difference may be attributed to a lower number of cells per dose in allogeneic stem cell treatment (1 million to 1 billion cells compared to 50-70 billion cells per BAL) and process steps with fewer material requirements, since the BAL process involves two cell growth steps (cell expansion and FBB culture to 70 billion cells as organoids) which is not common in other bioprocesses. However, for autologous products which require genetic manipulation and that are produced at smaller scales, the COG/dose can be similar or higher to that of the BAL.

5. Conclusion

A case study has been presented where a decisional tool, integrating an information database and a bioprocess economic model, has been developed to provide information on the bioprocess design for manufacturing BAL devices. From the alternative flowsheet configurations, the tool identified using microcarriers and stainless steel FBB setup as the most cost-effective configuration. A sensitivity analysis indicated that the plasma price, the FBB culture days and the serum price were the top three key cost drivers of the process and their optimisation will be required to reduce the COG/BAL to the target value of £25k. In addition to a decisional tool, this model also provides an overview of the cost associated with scaling up a cell therapy to commercial manufacturing. These values could be defined as benchmarks for the materials costs of BAL devices and other ATMPs, serving to highlight competitiveness and points for improvements between products in the industry. Future work will consider the potential for optimising other stages of the BAL process such as cryopreservation strategies and the impact of shop-floor complexity in the high demand scenarios.

Acknowledgements

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List of Tables

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Table 1 - BAL projections for Europe market for different clinical conditions.

	Acute liver failure	Acute-on-chronic failure	Peri-transplantation	Resectable liver cancer
Total Patients/year	8,500	192,000	7,000	180,000
% Addressable by BAL	50%	20%	20%	1%
Addressed Patients	4,250	38,400	1,400	1,800
Revenue per clinical condition	£253 k	£114 k	£120 k	£100 k
Total Revenue/year*	£1,074 M	£4,369 M	£168 M	£180 M

*Total Revenue/year is estimated based on the reimbursement value that meets the NICE threshold of £30,000 per quality-adjusted life year (QALY) [31].

Table 2 - BAL demand at different market penetrations in Europe.

Market penetration	Patients/year	BALs/year
1%	459	1,376
5%	2,293	6,878
10%	4,585	13,755
15%	6,878	20,633
20%	9,170	27,510

Table 3 – Key cost input assumptions of the BAL bioprocess.

Cost parameter	*Unit cost*
Materials	
Alginate	£495/Kg
Alpha MEM culture media	£8/L
DMEM culture media	£9/L
Fetal bovine serum	£996/L
Plasma	£135/L
Microcarriers	£3/g
Cryopreservation solution	£135/L
Cryoprotectant (DMSO)	£28/L
Multi-layer planar technologies	£127 (L-1); £189 (L-4); £599 (L-10); £781 (L-40)
Cell dissociation kit	£2927
Single-use bioreactor bag	£3,800 (6000 L)
Disposable chamber	£1,116
Cryobag	£600 (2L)
Reference Equipment	
L-10/L-40 incubator	£90,760
L-40 manipulator	£256,120
Cell dissociation system	£65,000
Jetcutter™ encapsulator	£91,100 (P.V.*100 L)
Single-use bioreactor hardware	£418,100 (6,000 L)
Stainless steel FBB**	£303,960 (100 L)
Stainless steel stirred tank	£841,710 (10,000 L)***
Cryopreservation equipment	£129,165
Quality control (QC)	
Sterility tests	£700/BAL
External QC tests	£14,490/batch
Automated cell counter	£18,340
Fluorescence microscope	£42,600
Labour	
Operator salary	£78,000/year

*P.V. – pressure vessel of 100 L associated to the Jetcutter™ encapsulator where the solution to encapsulate is held and mixed.

**FBB = fluidised bed bioreactor

***Cost of the bioreactor is scaled up with a 0.38 size factor using the ”six-tenths” rule.

Table 4 – Key process input assumptions of the BAL bioprocess.

Input parameter	Value
Batches/year	21
Dose/BAL	1
Dose volume	2.5L
Cells/dose	70 billion cells
No. BALs/patient	3
Depreciation time	10 years
Cell expansion	
Number of expansion stages	4
Cell detachment yield	90%
Concentration factor	28
Cell concentration yield	89%
Cell factories seeding cell density	22,000 cells/cm^2
Cell factories harvest cell density	360,000 cells/cm^2
Microcarriers surface area	515 cm^2/g
Microcarriers seed concentration	5 g/L
Microcarriers seeding cell density	20,000 cells/cm^2
Microcarriers harvest cell density	388,000 cells/cm^2
Cell encapsulation	
Cell density of encapsulation mix	2 million cells/mL
Encapsulated cell density	1.75 million cells/mL beads
Encapsulation mix	1:1
Encapsulation yield	90%
Collection yield	90%
FBB culture	
Media : encapsulated cells ratio	46:1
Days of operation	12
Bead swelling	4%
Bead harvesting yield	95%
Media change	50% d4; 60% d7; 70% d9; 80% d11
Initial cell density	1.75 million cells/mL beads
Final cell density	30 million cells/mL beads
Cryopreservation	
Bead shrinkage after freezing	15%
Beads : washing media ratio	1:2
Recovery days	3
Media : encapsulated cells ratio	46:1
Thawing yield	90%
Bead swelling after washes	9%
Quality Control (QC) release testing*	
FBB culture step	23 mL beads/vessel**
	34 mL culture media/vessel
Cryorecovery step	15 mL beads/vessel
	23 mL culture media/vessel

*Note: QC release testing involves cell counting, viability, metabolic and synthetic functions, sterility (incl. mycoplasma and endotoxin).

**The number of vessels depends on the selection of DFBB or SSFBB

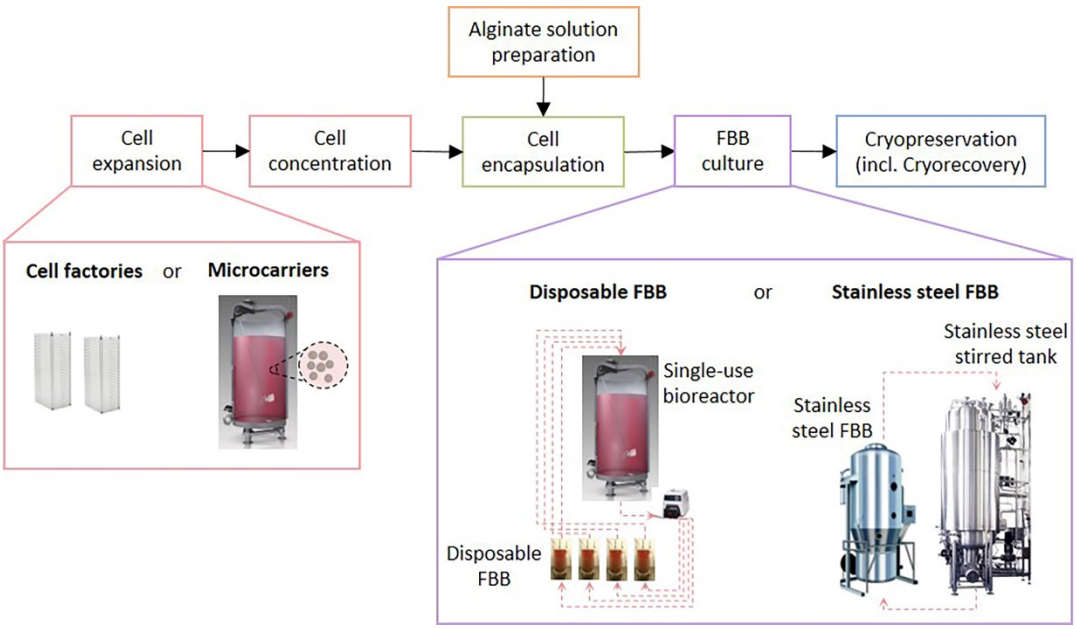


Figure 3 - Bioartificial liver process flowsheet. The flowsheet can assume different configurations according to the technology considered for the cell expansion (cell factories vs microcarriers) and FBB culture (disposable FBB vs Stainless steel FBB) steps. The configurations discussed in this case study are: Cell factories plus Disposable FBB, Cell factories plus Stainless steel FBB, Microcarriers plus Disposable FBB, Microcarriers plus Stainless steel FBB. Incl., including.

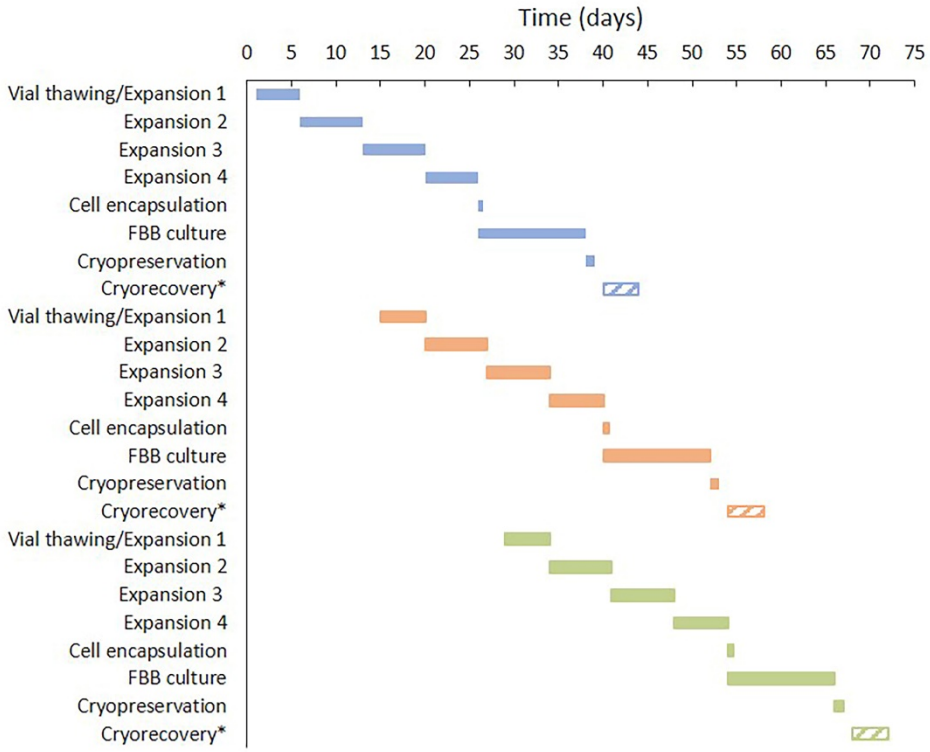
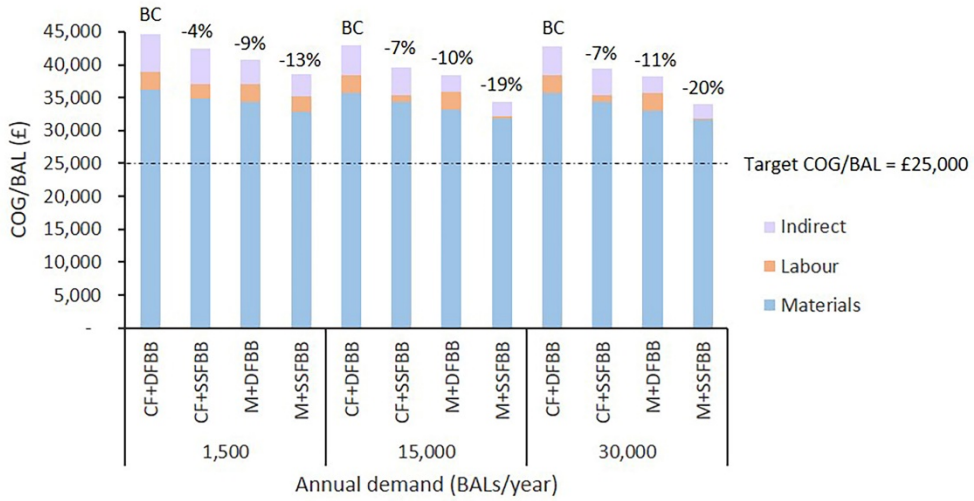


Figure 4 - Bioartificial liver device production schedule. Staggering of three production batches. *Cryorecovery depends on the frequency of patient demand and does not have to follow immediately the production phase.

A



B

C

Annual demand (BALs/year)	*1,500*				*15,000*				*30,000*			
Cell expansion technology	*CF*		*M*		*CF*		*M*		*CF*		*M*	
FBB culture technology	*DFBB*	*SSFBB*	*DFBB*	*SSFBB*	*DFBB*	*SSFBB*	*DFBB*	*SSFBB*	*DFBB*	*SSFBB*	*DFBB*	*SSFBB*
Cell Factories	185		4		1,829		20		3,656		55	
SUB_M	-		1		-		2		-		5	
SUB_M volume	2,000 L				6,000 L				6,000 L			
Encapsulators	5				34				95			
Disposable FBB	76	-	76	-	768	-	768	-	1,536	-	1,536	-
SUB_{DFBB}	2	-	2	-	15	-	15	-	30	-	30	-
SUB_{DFBB} volume	6,000 L				6,000 L				6,000 L			
Stainless steel FBB setup	-	1	-	1	-	5	-	5	-	9	-	9
Stainless steel FBB volume	460 L				1,031 L				1,031 L			
Stainless steel stirred tank volume	10,000 L				20,000 L				20,000 L			
Operators	11	9	9	9	106	42	106	11	211	83	211	13

Figure 5 - COG per bioartificial liver machine produced across varying annual demands and flowsheet configurations. (A) COG breakdown by category against annual BAL demand for each flowsheet configuration with different technologies for cell expansion (CF and M) and FBB culture (DFBB and SSFBB). Highlighted percentages mark difference in COG/BAL relative to the BC of CF+DFBB within each annual demand. (B) Breakdown of the material (mat) cost and facility indirect cost (IC) per process stage. (D) Details regarding the number and size of key consumables and equipment of the bioprocess according to the flowsheet configuration. BC, base case; CF, cell factories; DFBB, disposable FBB; IC, indirect cost; M, microcarriers; mat, materials costs.; SSFBB, stainless steel FBB; SUB, single-use bioreactor.

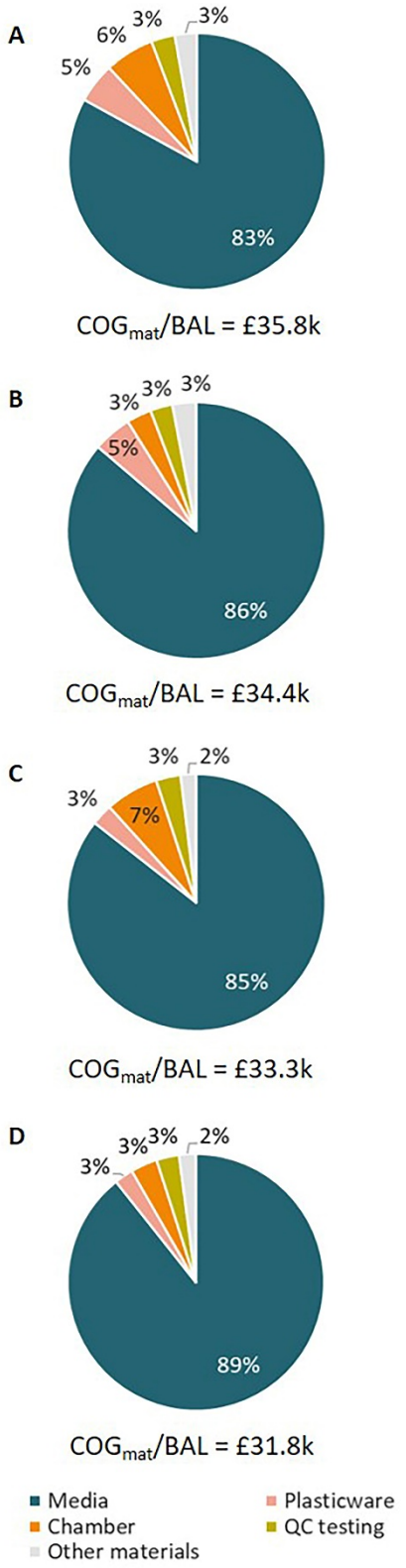


Figure 6 – Material cost breakdown for the BAL production bioprocess at an annual demand of 15,000 BALs for different flowsheet configurations. (A) cell factories plus disposable FBB, (B) cell factories plus stainless steel FBB, (C) microcarriers plus disposable FBB, (D) microcarriers plus stainless steel FBB.

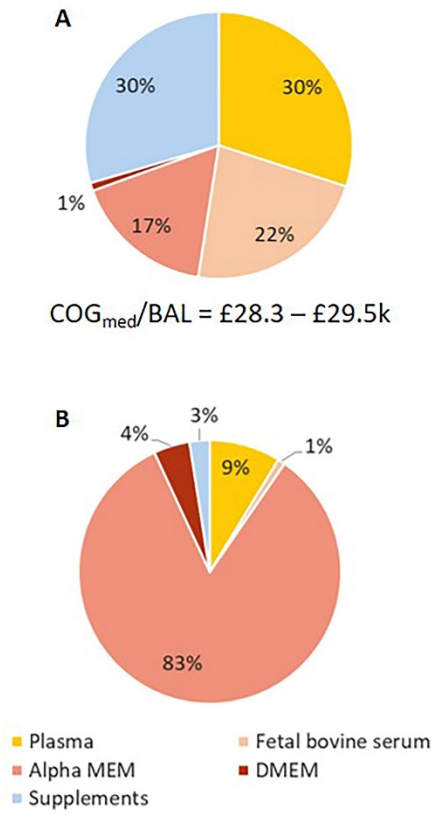
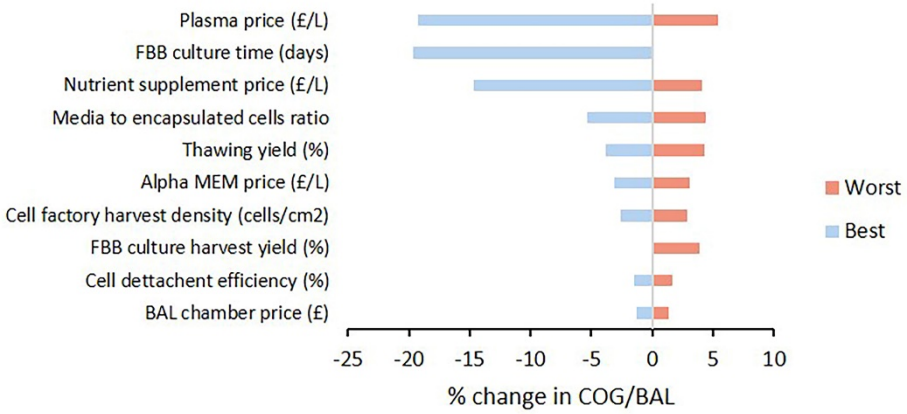


Figure 5 – Contribution of the culture medium components per batch in the BAL bioprocess in terms of (A) cost and (B) volume.

A

Parameter	Case		
	Best	Base	**Worst**
Nutrient supplement price (£/L)	100	996	1,245
Plasma price (£/L)	16	135	169
Alpha MEM price (£/L)	6	8	10
BAL chamber price (£)	500	1,000	1,500
Cell detachment efficiency (%)	95	90	85
Cell factory harvest density (cells/cm²)	3.80E+05	3.60E+05	3.40E+05
Microcarrier harvest density (cells/microcarrier)	455	364	273
Microcarrier concentration (g/L)	6.25	5	3.75
FBB culture time (days)	8	12	
Media to encapsulated cells ratio	42	46	50
FBB culture harvest yield (%)		95	90
Thawing yield (%)	95	90	85

B



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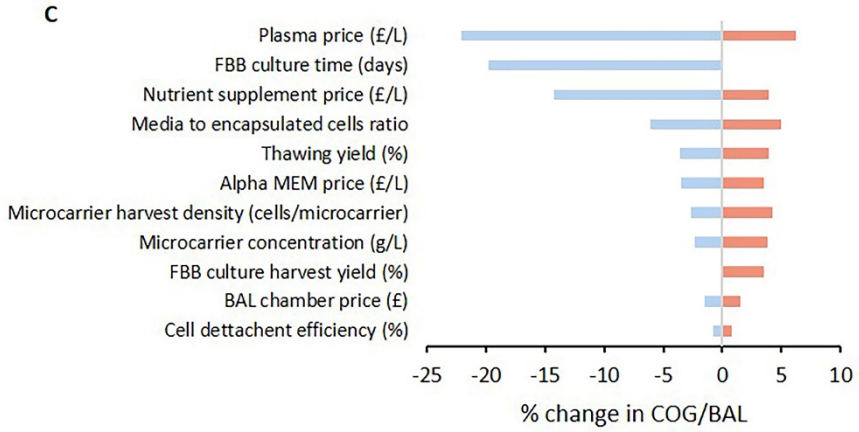

Figure 6 - Sensitivity analysis of COG/BAL to key bioprocess and cost parameters. (A) Best, worst, and base case values for parameters in sensitivity analysis. Impact of parameter variation on COG/BAL at an annual demand of 15,000 BALs for (B) cell factories plus SSFBB and (C) microcarriers plus SSFBB.

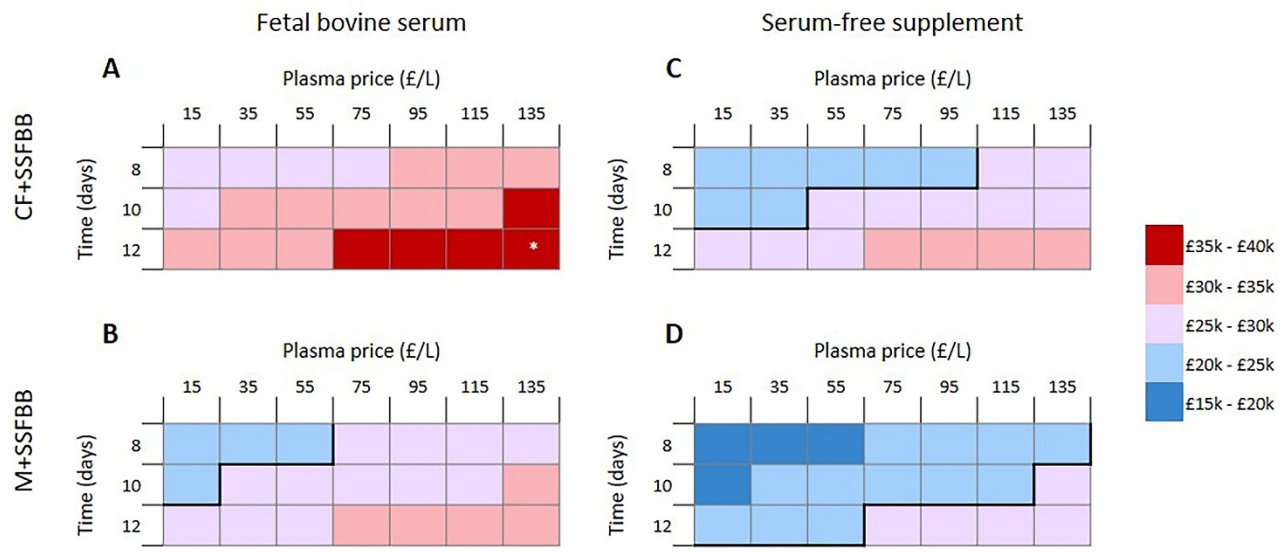

Figure 7 - Contour plots measuring the impact of different parameters on the ability to reach COG/BAL of £25k for an annual demand of 15,000 BALs. Target analysis combining variation in plasma price and FBB culture time when using fetal bovine serum (£996/L) (A,B) or serum-free supplement (£100/L) (C,D) for CF+SSFBB (A,C) and M+SSFBB (B,D). The area containing the target value is delimited by the thicker black line which includes light and dark blue boxes. *The base case is plasma price £135/L and 12 days FBB culture using fetal bovine serum. CF, cell factories; M, microcarriers; SSFBB, stainless steel FBB.

Supplementary material

Decisional tool for cost of goods analysis of bioartificial liver devices for routine clinical use

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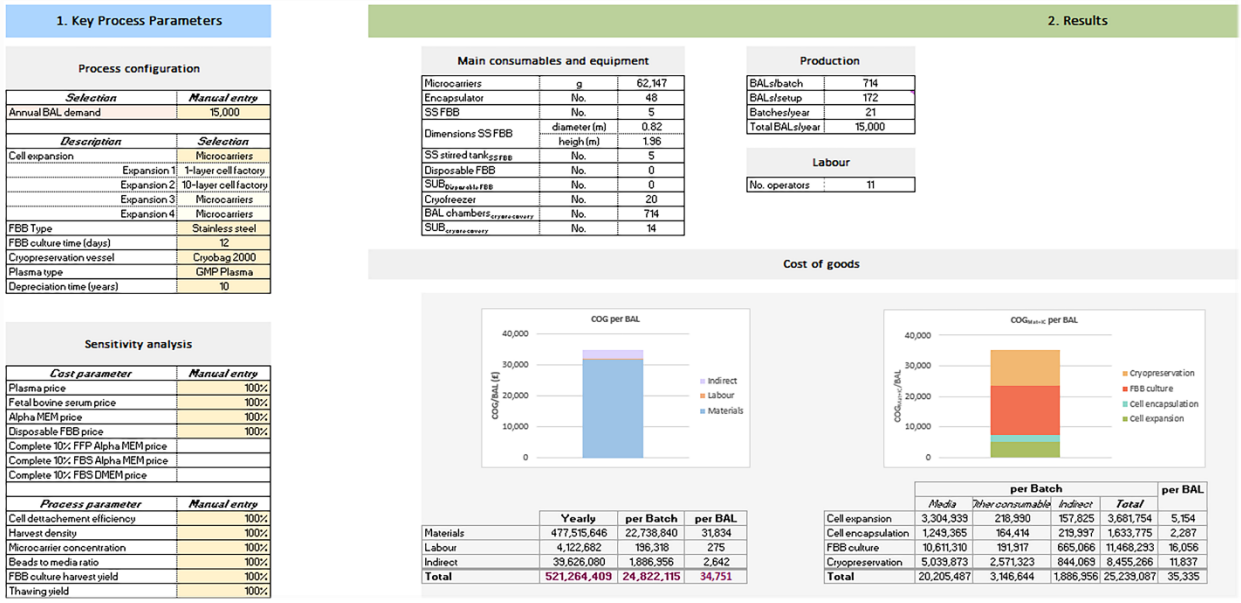

Figure S1 – Screenshot of decisional tool input and output page in Microsoft Excel.