Continuase™: Empowering continuous cell manufacturing on multiple technology platforms for different scaling strategies

Abstract

Increased biomanufacturing capacity is required to meet the future demands for cell products, regardless of the field being biopharmaceuticals or emerging technologies such as allogeneic cell products or cellular agriculture. Continuous biomanufacturing is a technological approach which creates a steady state in cell biomass, identified as a key enabling technology to meet the rising demand for cells and cell products. Conventional biomanufacturing processes are often time-consuming and labour-intensive with a high batch-to-batch variability, often facing production bottlenecks with associated escalating costs at research and development stage. These limitations can hinder biomanufacturing scaling in a consistent, timely and cost-effective manner. Continuous biomanufacturing technologies are addressing the status quo by offering streamlined (semi)automated approaches to produce biological products of consistent quality with shorter turnaround and more favourable scaling approaches. In this case study CellRev demonstrates how Continuase™, a proprietary media additive, is capable of controlling cell-to-surface attachment. Continuase™ was used to develop a first of its kind continuous biomanufacturing process on different bioreactors to showcase clear techno-economic advantages for the emerging industries which require to expand cells in a more cost-effective manner.

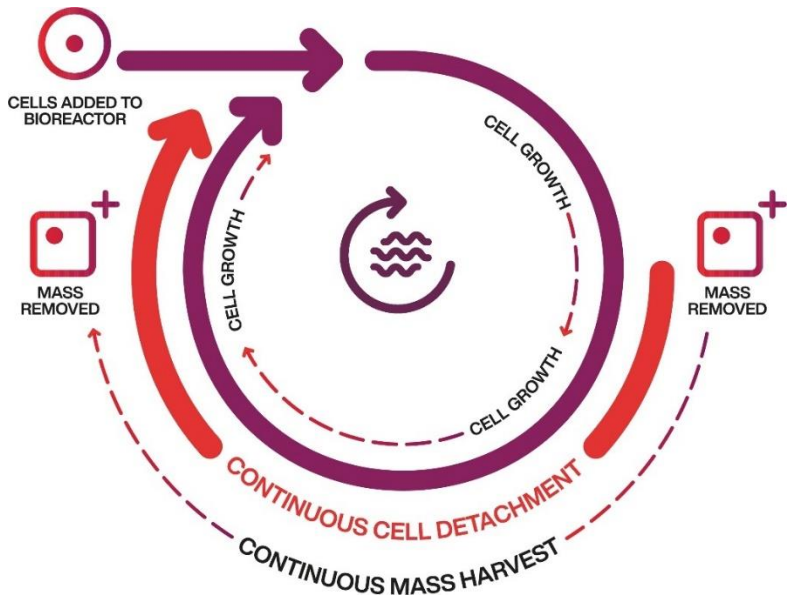
Introduction

The current state of biomanufacturing is inefficient with cell losses >30% deemed the norm and a loss of 5% - 20% deemed good, where batch-to-batch variation results in billions of USD lost annually[1]. Inefficiencies in biomanufacturing can be attributed to human error and lack of automation within archaic bioprocess design and infrastructure; factors which translate to high labour costs with associated integrative CAPEX investment for compensation. The intrinsic variability of mammalian cell behaviour within *in vitro* cell culture compounds existing bioprocess development challenges in batch-to-batch variability, not considering batch losses due to microbial contamination or biological debris causing blockages in fluidic systems (i.e., filters, tubing, valves). The above limitations, once a concern for cell-derived biopharmaceutical manufacturing, prove to be a technological bottleneck for industries where the cell itself is the product of interest, i.e., (i) regenerative medicine (RMED); (ii) cell and gene therapies (C&GT) as well as (iii) cellular agriculture (CA). These emerging fields are at the forefront of advancing biomanufacturing science and technology, developing innovative solutions to scale biomanufacturing more cost-effectively and pushing the technological envelope to address the current and future biomass demands of mammalian cells.

Continuous biomanufacturing (CBM) has been identified as a key technological approach to meet the cell biomass demands. Such approach is already being implemented for the manufacture of cell-derived products[2-4], showing that persistent biomanufacturing scaling[5-9] is not required to meet production demands. CBM keeps the cell biomass in a steady-state for longer process duration, ensuring a reliable supply of cell-derived products with reduced production time. The CBM offer presents with more productive process scalability, improved

batch-to-batch consistency and improved product quality when compared to conventional biomanufacturing approaches.

In this case study, CellRev demonstrates CBM of adherent mammalian cells with the use of Continuase™, a proprietary media additive which controls the cell-to-surface (C2S) attachment of cells in a dose-dependent manner. Adherent cells are allowed to reach ~75% confluency in the bioreactor at which time Continuase™ is added in a dose specific manner to trigger continuous cell detachment. Detached cells are continuously collected and periodically removed from the bioreactor (Schematic 1). Detached cells were phenotypically evaluated to determine doubling times, attachment capability as well as terminal differentiation[10]. The feasibility of CBM using Continuase™ was evaluated using three different bioreactor technologies, demonstrating process versatility. The aim of developing a first of a kind CBM was to establish a proof-of-concept application of maintaining a steady-state between cell confluency and Continuase™ dependent cell detachment for downstream collection, with cells maintaining initial phenotypic characteristics. A techno-economic assessment (TEA) demonstrates advantages of a CBM over conventional biomanufacturing approaches.

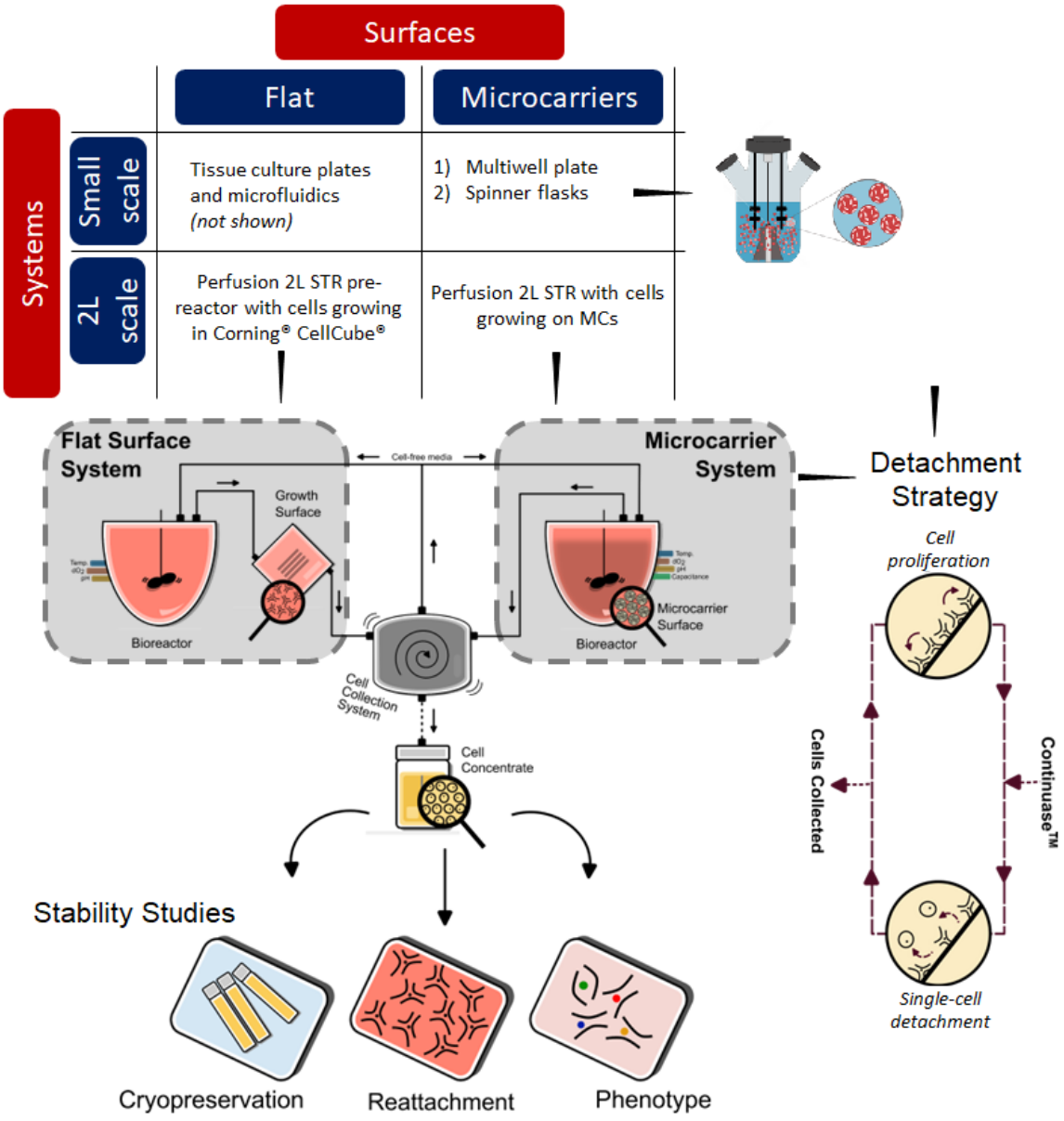


Schematic 1: A general depiction of a CBM using Continuase™. Adherent cells are dose-dependently detached from the cell culture surface and removed from the bioreactor. Media and Continuase™ replenishment occur at regular intervals, dependent on cell doubling time and media composition.

Materials and Methods

General methods and materials (**Table 1**) are provided in the paragraphs below, with bioreactor specific information in later sections. A general schematic (**Schematic 2**) shows the bioreactor platforms used to develop Continuase™-CBM applications, specifically:

(i) planar culture using a Corning® CellCube®
(ii) microcarrier (MC) culture in disposable Corning® spinner flasks
(iii) microcarrier (MC) culture in a 2L stirred tank reactor (STR)



Schematic 2: An overview of how Continuase™ was implemented to develop multiple biomanufacturing approaches. The Corning® CellCube® is an ideal planar scale out technology, whereas MCs from spinner flasks to 2L STR demonstrate scaling up. The planar scaling out and MCs 2L STR scale-up approaches were both connected to a semi-automated centrifuge for continuous cell collection. Downstream analysis post cell harvest was done to determine if Continuase™ influenced myoblast cells characteristics.

Platform specific methods and materials will be in their respective sections, with commonly used method and materials reported below in Table 1.

Table 1: Materials used for common methodologies used across all bioreactor platforms, i.e., seed train, cell biology and cell quality assessment.

Components	Description
Cell line	C2C12 (ATCC: CRL-1772)
Composition of Medium	DMEM/F12 (Gibco™ Cat. No. 32500035) enriched with sodium bicarbonate (Thermo Scientific Cat No: 12675057), 1% or 5% FBS (BioSera Cat No: 1001 018135116) and Penicillin-Streptomycin (Gibco™ Ca. No. 51540122).
Composition of Differentiation Medium	DMEM/F12 (Gibco™ Cat. No. 32500035) enriched with sodium bicarbonate (Thermo Scientific Cat No: 12675057), 1mM ascorbic acid (Merck Cat No: A8960), Insulin-Transferrin-Selenium (Gibco™ Cat No: 12097549), and Penicillin-Streptomycin (Gibco™ Ca. No. 51540122).
Cell culture incubator	Humidified incubator set to 37°C and 5% CO_2
Wash Reagent	Phosphate-buffered saline (PBS) (Gibco™ Cat. No. 18912014).
Dissociation Agent	Gibco™ TrypLE™ Express Enzyme
Cell Culture Flask, Seed train	CELLdisc 8 Layer (Greiner Bio-One Cat. No. 678108).
Cell Counter	Countess II (Thermo Fisher Scientific Cat No: 15397802)
Nuclear stain	Hoechst (Invitrogen™ Cat No: H3570)
Live cell stain	Calcein AM (Invitrogen™, Cat No: C3100MP)
Dead cell stain	Propidium Iodide (Molecular Probes™, Cat No: P3566)
Undifferentiated cell stain	Anti-PAX7 (Abcam Cat No: ab187339)
Differentiated cell stain	Anti-MYH (Santa Cruz Biotechnology Cat No: sc-376157)

Cell Culture: C2C12 mouse myoblast cells were grown in DMEM/F12 supplemented with 5% FBS and 1% Penicillin-Streptomycin, maintained in CELLdisc 8-Layer culture flasks prior to seeding the respective bioreactors.

Reattachment of Collected Cells: Collected cells, from Continuase™ detachment, were seeded in cell culture plates and allowed to attach and recover (~18 hours), before being fixed and nuclei stained (Hoechst 33342). Nuclei were counted with automated fluorescence microscopy (BioTek Cytation 5, Agilent) and expressed as fold change compared to seeding density.

Proliferation Rate of Collected Cells: Like for reattachment of cells above, collected cells were seeded in cell culture plates, where cells were fixed every 72-96 hours and nuclei stained (Hoechst 33342) followed by automated fluorescence microscopy counting. Cell doubling time was determined by analysing the fold increase in cells between periodic fixed samples.

Retention of Myoblast Progenitor State: Collected cells were seeded into cell culture plates and fixed (~18 hours). Immunofluorescence staining was done with anti-PAX7 and Hoechst. The percentage of PAX7 expressing cells was calculated relative to stained nuclei using automated fluorescence microscopy.

Myotube Differentiation: Differentiation of C2C12 myoblasts was induced when collected cells were cultured to confluency with standard medium, followed by culturing in differentiation

medium for 7 days. Cells were washed, fixed and stained (Table 1: Hoechst, PAX7, MHC). Cells were imaged with automated fluorescence microscopy.

(i) Corning® CellCube® experiments (planar, adherent)

The Corning® CellCube® culture system can be used in static and dynamic culture conditions. Developing a CBM approach using Continuase™ required a semi-automated bioprocess design, requiring a dynamic culture approach to ensure medium formulation and bioreactor conditions were similar for cells in the bioreactor between batch and CBM experiments. A summary of equipment is given in Table 2 with a brief discussion on methods.

Table 2: Materials and instruments used for Corning® CellCube® experiments (planar).

Components	Description	
Bioprocess Setup	Process control	Applikon ez-2 control tower (Getinge/Applikon)
	Software	Lucullus® PIMS
	Pre-reactor	2L Stirred tank reactor (STR) (Getinge/Applikon)
	Bioreactor	Corning® CellCube®
	Cell collection device	UFmini Centrifuge (CARR Biosystems) + 100mL centrifuge collection bowls
	Tubing / Valves	Pharmaceutical grade
Continuase™		Developed by CellRev.

CellCube® Batch Culture: Cells were seeded in a 10-layer CellCube® (10 000cells/cm^2 on 8500cm^2) and introduced into the process setup. Medium flow was from the pre-reactor to the CellCube® and recirculated back to the pre-reactor, bypassing the centrifuge. The pre-reactor serves as a culture medium conditioning (pH, aeration, temperature) vessel. When cells reached high confluency (80-90%), the CellCube® was detached from the process and cells were harvested using trypLE.

CellCube® Continuous Culture: Cell seeding was performed as for batch culture. Continuase™ was added in a predetermined dosage once cells reached ~75% confluency. The process flow had culture medium flowing from the pre-reactor to the CellCube®, subsequently entering in the centrifuge as a cell collection device. Medium would return from the centrifuge to the pre-reactor, forming a cyclical process (**Schematic 2**). Cells were manually collected from the centrifuge periodically throughout the day (~10 hours), with cells collected overnight being discarded at 8:00am daily. Partial medium replenishment (25 – 40%), and Continuase™ maintenance, was done to compensate for culture medium used during cell collection from the centrifuge. Cells were harvested from the CellCube® at the end of the experiment, as described above for batch culture.

(ii) MC adherent cell culture (Impeller-based bioreactors)

Adherent MC cell culture offers more cell culture surface area per volume than planar culture approaches. The feasibility of a CBM was evaluated using a myoblast cell line, grown on MC. The process design for a MC bioprocess was simplified, requiring only a 2L STR and a centrifuge for continuous collection of cells (**Schematic 2**). The summarised materials used for MC studies can be found in Table 3.

Table 3: Materials and instruments used for MC experiments

Components	Description	
MC type		Corning® untreated (Corning® Cat No: 3772)
Culture Vessel		Spinner flask 125 mL (Corning® Cat. No. CLS3152)
Agitation		bioMIX stirrer (2mag)
Cell Counter		NucleoCounter ® NC-202™ (ChemoMetec Cat No: 991-2020)
Bioprocess Setup	Process control Software	Livit ACE control tower (Getinge/Applikon) Lucullus® PIMS
	Pre-reactor	2L Stirred tank reactor (STR) (Getinge/Applikon)
	Bioreactor	Corning® CellCube®
	Cell collection device	UFmini Centrifuge (CARR Biosystems) + 100mL centrifuge collection bowls
	Tubing / Valves	Pharmaceutical grade
Continuase™		Developed by CellRev.

MC Preparation: Corning® untreated MCs were prepared following manufacturer's protocol.
Sample analysis: Samples were taken regularly for counting adherent cells on MCs as well as cells in suspension, with immunofluorescence microscopy for live-dead analysis. Phenotypic evaluation was performed as described earlier. Metabolites (glucose, lactate) were tested daily.
100 mL Spinner flask MC Batch cultures: MCs were transferred to a 100mL spinner flask with 50mL medium and left to acclimate for 30 min. Cells were seeded onto the MCs (10 000cells/cm^2 for 720cm^2) followed by an on-off agitation (5 min – 20 min) for 5 hours to promote cell attachment. Total culture volume was 100 mL for the rest of the experiment after addition of 50mL medium.
100 mL Spinner flask MC CBM cultures: All experiments were performed following the protocol mentioned above for batch culture, with the difference in having Continuase™ being added when cells reached a ~70% confluency (typically on day 3). The Continuase™-detached cells were manually collected once a day, for a total of 14 days. Partial media exchanges were performed to refresh Continuase™ and replenish nutrients every 48 hours.
2L STR MC Batch cultures: MCs were transferred to a 2L STR with 300mL medium and left to acclimate for 30 min. Cells were seeded onto the MCs (10 000cells/cm^2 for 4320cm^2), followed by an on-off agitation (5 min-20 min) for 5 hours to promote cell attachment. The total culture volume was 1 500mL for the remainder of the experiment, after 1 200mL medium was added post MCs seeding.
2L STR MC CBM cultures: All experiments were performed following the protocol mentioned above for batch culture, with the difference in having Continuase™ being added when cells reached a ~70% confluency. The process required a 2L STR and a centrifuge (**Schematic 2**), with culture medium circulating back into the bioreactor after returning from the centrifuge. Cells were manually collected from the centrifuge periodically throughout the day (~10 hours), with cells collected overnight being discarded at 8:00am daily. Partial culture medium replenishment (25 – 40%), and Continuase™ maintenance, was performed to compensate for medium used during cell collection from the centrifuge.

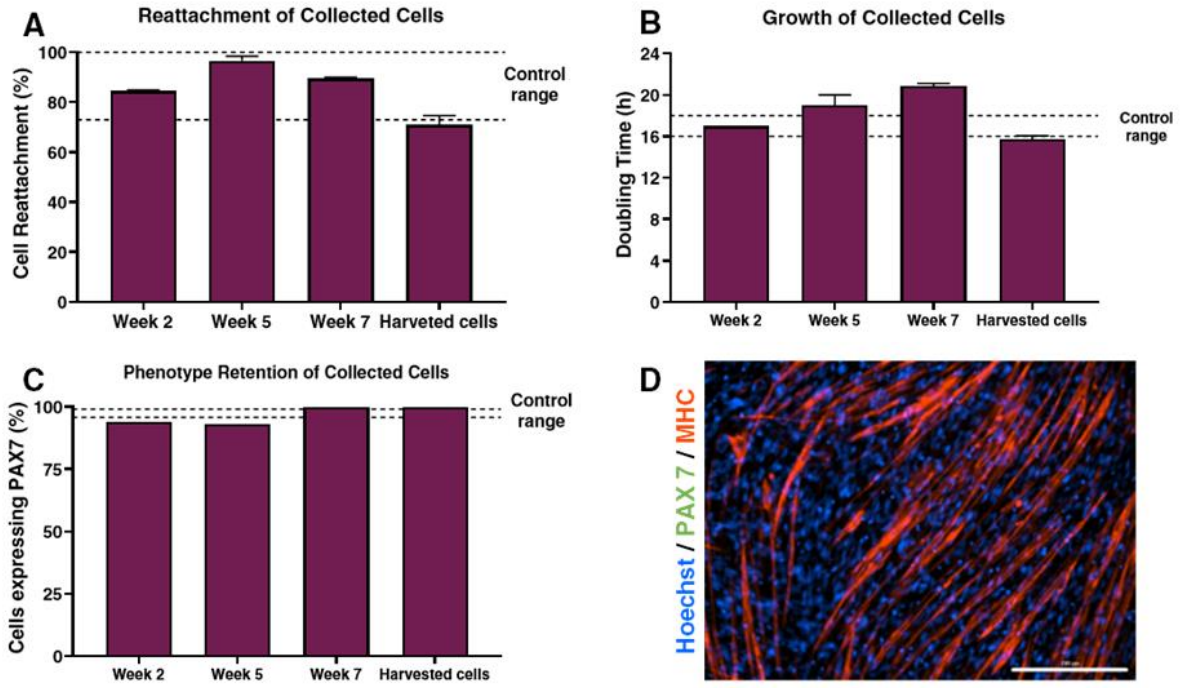
Results and Discussion

Continuous growth and collection of myoblasts in the Corning® CellCube®

The Continuase™ CBM experiment had a duration of 61 days with cells constantly being maintained at ~75% confluency on the surface of the CellCube®. Comparatively, batch experiments had an average duration of 6 – 7 days, of which, 4-5 days were necessary for the cells to reach confluency, plus2 days were included for process turnover. The doubling time of the myoblasts from the CBM experiment was found within the expected range (**Fig. 1B, ~20 hours**).

The effect of prolonged (57 days) and continuous exposure of myoblasts to Continuase™ was evaluated via a panel of stability studies (Figure 1). The first study assessed whether the cells could adhere to standard cell culture surfaces when harvested from the centrifuge (**Schematic 2**). Cells collected during the experiment showed a >80% attachment rate (**Fig. 1A**). Similarly, when assessing preservation of cell progenitor markers, such as PAX7, >90% of cells retained the marker (**Fig. 1C**), with full capability to differentiate into myotubes (**Fig. 1D**). The cumulative cells collected from a CBM using Continuase™ over 57 days process times (**Fig. 1E**) was compared to a representative batch process, showing that 10 batch processes were required to deliver the same total cell yield as a single CBM process.

Interestingly, variations in the CellCube® CBM process flow designs (**Schematic 2**), led to binary extremes on cell viability and bioreactor productivity. Whenever the process yielded a high productivity in cell number (*data not shown*), the cell quality was compromised with low viability as the norm. Parameters which favoured cell quality, as presented in the data here, had significant lower bioreactor productivity for cell expansion.



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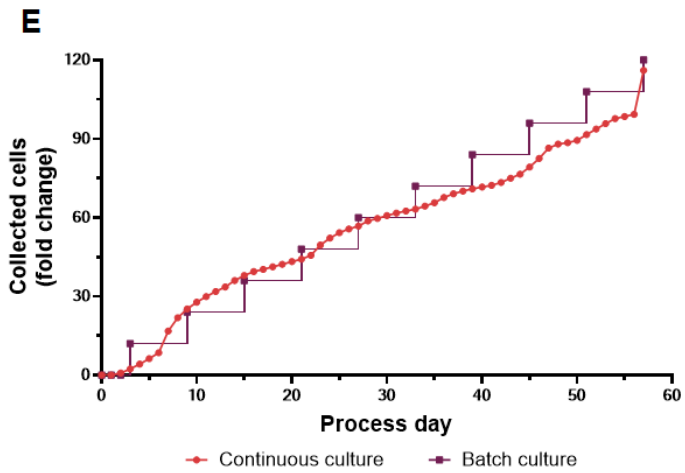

Figure1: Stability studies and productivity of the CellCube® CBM process. Myoblasts that were detached and collected from a continuous culture were routinely assessed for reattachment (A); proliferation rate (B); progenitor phenotype retention (C) and ability to differentiate into myotubes (D). For bar charts (A-C), dotted lines represent range measured from cells grown in T-flasks (control). Error bars represent SEM. For cell phenotype evaluation after induction of differentiation (D), expression of undifferentiation cell marker PAX7 (green stain), and muscle differentiation cell marker myosin heavy chain (MHC, red stain) were analysed from collected cells. Nuclei were stained with Hoechst (blue). CBM collected cells (E) were counted daily, with cells harvested from the bioreactor on the final day added to the collected cell count. Batch culture data points represent average yield from cells harvested for an average experiment duration of 6 – 7 days (inclusive of 2 days for process turnover). Cell numbers are represented as fold change compared to the initial seeding density. Scale bar: 200µm.

Continuous growth and collection of myoblasts on MCs

MC adherent cultures offer several advantages over flat surface cultures, particularly for process scaling, providing larger surface area to volume utilisation. The dynamic cell culture environments for MC-adherent cells aim to create homogenous cell culture environments, however biomechanical stress on the cells can come from mixing as well as aeration methodologies. An additional technical challenge in MC-adherent cell culture includes cell-to-cell (C2C) induced clustering and subsequent aggregate formation, resulting in reduced cell culture homogeneity and increase in batch-to-batch variation. Planar cell culture environments, static (i.e. T-flask) or perfused multilayered vessels (i.e. Corning® CellCube®), have limited challenges in cell clumping and cell-associated aggregates when compared to MC-adherent cell culture, but are limited in scalability.

Myoblasts were seeded onto MCs and grown in 100mL working volume spinner flasks, with and without Continuase™ (**Fig. 2**). Myoblasts grown without Continuase™ (batch mode, control) showed severe C2C aggregation by day 7 (**Fig. 2A**), with initial aggregates forming by day 5. Subsequent spinner flask batch experiments were ended on day 4 when cells reached ~80% confluency. Continuase™ supplementation to the media started between day 3 and 4 with a total cell culture time of 14 days (**Fig. 2B, Day 1 - 14**). No C2C-associated aggregates were observed for the full 14-day experimental duration, with a confluency steady-state reached, and cells being collected daily. The total amount of cells collected from the spinner flask experiments was compared between a representative batch and continuous experiment (**Fig. 2C**), with initial number of cells seeded ~7 million. Cells were not harvested, with total cells collected being extrapolated via a sample reading out of the NucleoCounter. Total cells collected for the continuous spinner flask experiment over 14 days was the equivalent of 4 representative batch experiments. The batch experimental duration did not include experimental turnover days as for the CellCube® experiments due to the consumables being sterile packed and physical experimental setup requiring minimal labour.

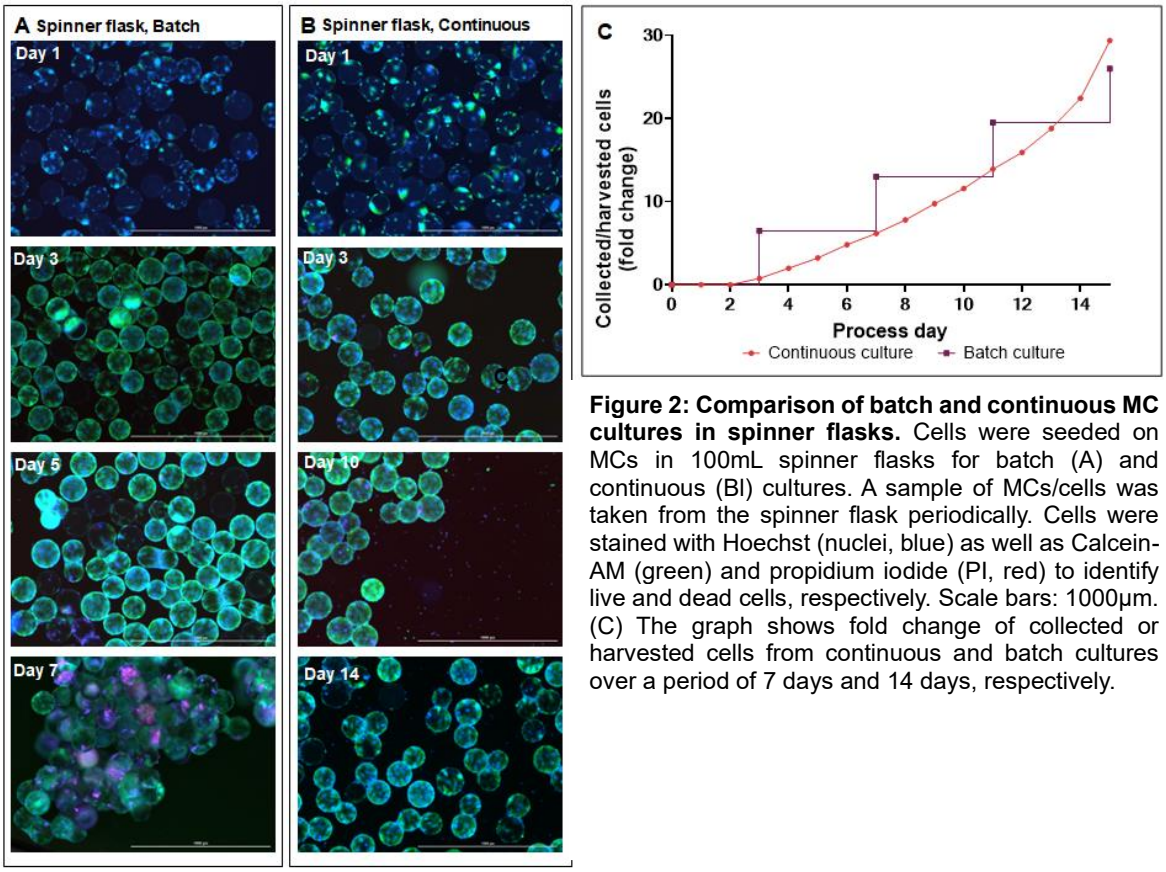

Figure 2: Comparison of batch and continuous MC cultures in spinner flasks. Cells were seeded on MCs in 100mL spinner flasks for batch (A) and continuous (BI) cultures. A sample of MCs/cells was taken from the spinner flask periodically. Cells were stained with Hoechst (nuclei, blue) as well as Calcein-AM (green) and propidium iodide (PI, red) to identify live and dead cells, respectively. Scale bars: 1000µm. (C) The graph shows fold change of collected or harvested cells from continuous and batch cultures over a period of 7 days and 14 days, respectively.

A CBM using Continuase™ was deemed feasible based on the spinner flask experiments as cell confluency was maintained using Continuase™ with viable cells being collected daily. The low working volume and labour requirement of spinner flask experiments limited the experimental duration, parameters investigated (i.e. phenotype analysis, etc.) and assessment of scalability. Scaling the MC-based CBM process to a 2L STR (**Schematic 2**) was done using the Livit ACE platform (Getinge/Applikon) to facilitate a semi-automated bioprocess. A batch mode experiment was used to determine basic process parameters (i.e. agitation, aeration), myoblasts behaviour in the dynamic 2L STR cell culture environment and optimise Continuase™ supplementation protocol. The batch mode experiment did not include the centrifuge or Continuase™ in the process setup.

Myoblast cells grown on the MCs in the batch mode (**Fig. 3A**, **3D**) showed strong C2C-associated aggregation on day 3 with systematic increase in aggregation and cell loss from day 3 to day 7, with an increase in dead cells in culture suspension. The cell number on day 3 was close to theoretical ~75% confluency and was used as a reference point for the continuous experiment to begin the supplementation protocol of Continuase™. Batch mode experiments persistently showed a 14 – 18-fold increase in cell number over 3 – 4 days of cell culture, with initial seeding cell number 43 million cells. The continuous experiment showed a that the cell distribution on the MCs covered most of the surface area (**Fig. 3E**) for 21 days of experimental duration, with a growth curve showing ~25-fold increase in cell number over 3 days, with the addition of Continuase™ on day 3. A steady state of cells on the MC surface (**Fig. 3B**) was maintained for the full process duration, facilitated by detaching cells enzymatically with Continuase™. The only difference between batch- and continuous modes in the first 3 days of cell culture was the inclusion of the centrifuge (**Schematic 2**), all other

parameters were identical. The dosage of Continuase™ was adjusted until a steady state between ~75% MC surface confluency and detaching cells was found.

The batch process was considered to last for an average of 4 days for peak cell number, which includes two days turnover, then compared to the continuous bioprocess (**Fig. 3C**). The same cell yield from batch mode would require at least 4 repetitions to match the cell yield of the continuous process – a similar trend as observed for spinner flask experiments. The viability of the cells was dependent on the frequency of collection, showing ~85% viability for frequent collections and ~20% viability if collections were done only once a day.

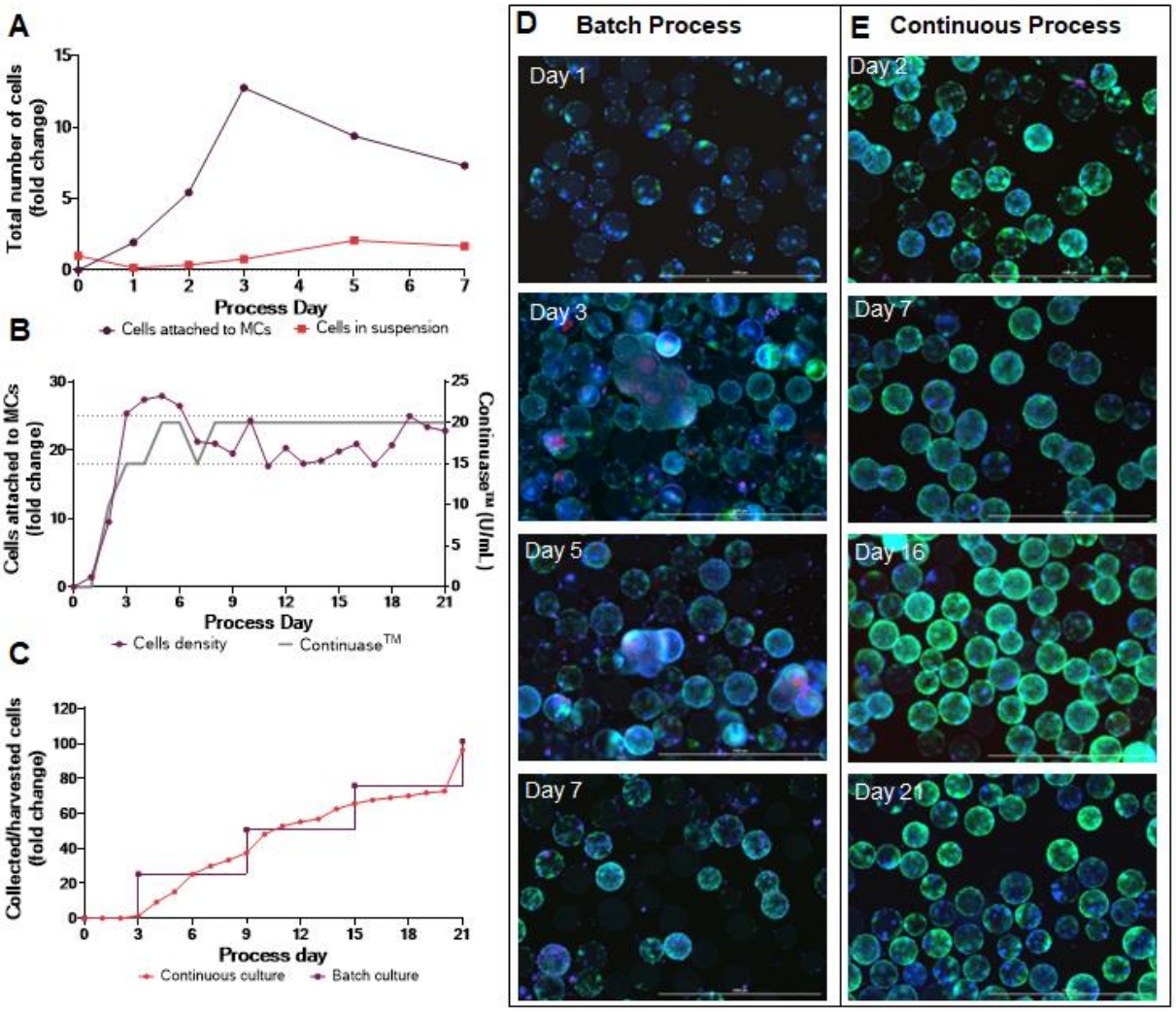

Figure 3: Comparison of batch and continuous MC cultures in the Livit ACE platform. Cells were seeded on Corning® untreated MCs in a 2L STR and cultured in 1L of medium. (A) Samples of MCs/cells from a batch culture were taken periodically to count the number of cells attached to MCs and the number of cells in suspension. (B) Continuase™ was added to continuously grow, detach, and collect cells for 21 days. (C) Fold change of collected or harvested cells from continuous and batch cultures over a period of 21 days. Detached cells were collected and counted daily. The average cell yield of four batch experiments were used to extrapolate how many cells could be collected over 21 days. Cell numbers are represented as fold change relative to input cell numbers. The final continuous data point also includes cells harvested from the MCs at the end of the process. MC samples were also stained with Hoechst (nuclei, blue) as well as Calcein-AM (green) and propidium iodide (PI, red) to identify live and dead cells, respectively, in batch (D) and continuous (E) processes. Scale bars: 1000µm.

Throughout the continuous process cells were collected periodically for quality assessment to determine whether there was a change in the cell doubling time or phenotypic characteristics (i.e., progenitor markers, differentiation capability). Cell which enzymatically detached from the MCs during the continuous process showed a ~60% attachment rate on average, with a peak of (**Fig. 4A**), attributed to the challenging dynamic cell culture environment of a 2L STR. Collected and harvested cells retained their ability to differentiate into myoblasts at similar efficiencies as control cells grown in standard tissue culture flasks (**Fig. 4B, 4C**).

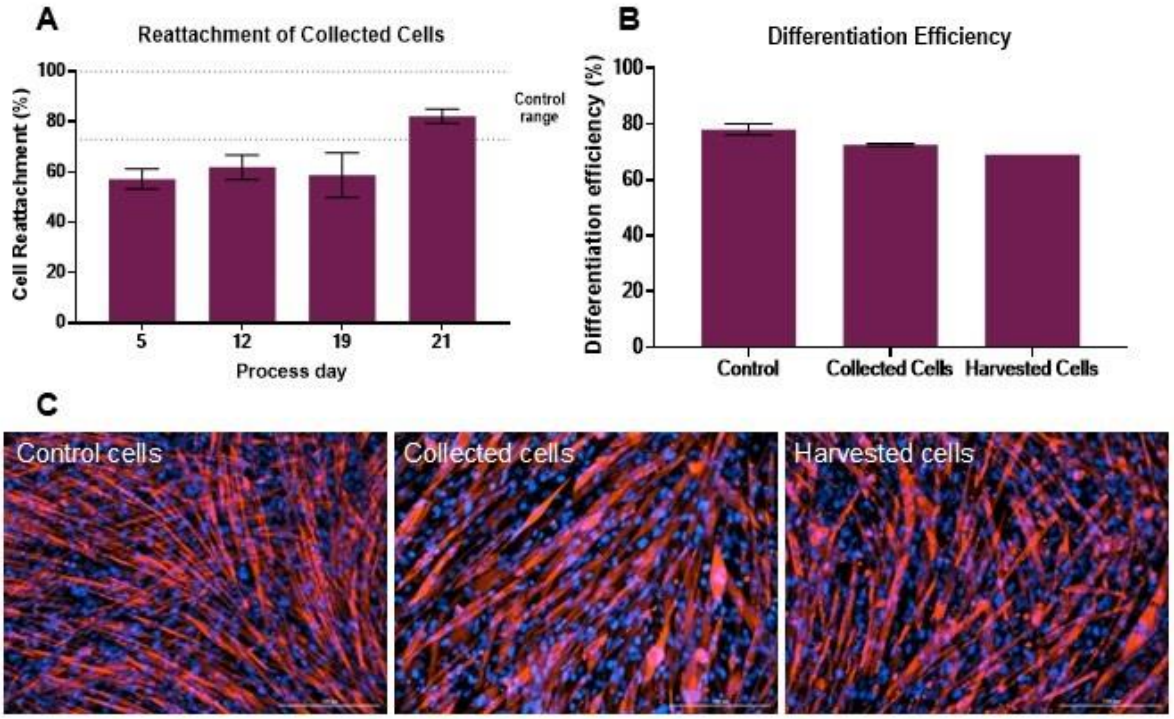

Figure 4: Stability studies of cells collected from the continuous process using the Livit ACE platform (MCs). Cells that were detached and collected from a continuous culture were routinely assessed for reattachment to tissue culture flasks (A) and ability to differentiate into myotubes (B-C). (A) Collected cells exhibited reattachment efficiencies close to the control cell range represented by the dotted lines. (B-C) Myotube differentiation was induced on collected cells and harvested cells at the end of the process and compared to control cells grown in tissue culture flasks. Cells were stained with Hoechst (nuclei, blue), PAX 7 (undifferentiated cells, green), and muscle differentiation cell marker myosin heavy chain (MHC, red stain). The total area of the culture vessel covered by MHC positive cells was measured to extrapolate the differentiation efficiency. Error bars in bar graphs represent SEM. Scale bars in immunofluorescence images: 200µm.

An optimisation point for the developed MC-based CBM process is to improve cell viability. Biocompatibility assays (*data not shown*) and stability studies indicate that cell viability is decoupled from the biochemistry/cell biology, thus most probably requires more process optimisation. The journey of the cells through the process, once detached from the MCs, requires more experimentation to be optimised (**Schematic 2**):

(i) The frequency of Continuase™-detached cells being removed from the 2L STR to prevent excessive time spent in suspension.
(ii) The duration of the centrifugation steps and general cell collection frequency.
(iii) Minimise the fluid shear stress the cells experience throughout the whole process (i.e. 2L STR agitation; spin filter flow rates; centrifugation parameters).

Regardless of the collected cells viability challenge, it is important to highlight that a steady state of cells on MCs surface was achieved in a dosage dependent manner using Continuase™. This is a first-in-class approach allowing for a CBM process for continuous cell expansion.

A techno-economic analysis (TEA) comparing a CellRev CBM process with classical batch process approaches.

The total cells collected during the CBM process (Corning® CellCube®, 2L STR MCs) showed being equivalent to multiple batch process experiments. The potential of continuous cell expansion, despite the cell viability challenge, suggests that it would have several advantages over batch, such as reduced costs (i.e., labour; reagents; consumables, etc.) and batch-to-batch variation.

A TEA was generated using the data acquired in the experiments discussed in this case study, comparing the results obtained when running a batch process and Continuase™ associated CBM. For the TEA preparation, the following assumptions were made:

(i) The experimental turnaround time to setup a new batch bioprocess (**Schematic 2**) was set at 48 hours.
(ii) 330 days a year were set for constant biomanufacturing work.
(iii) The values for media consumption, consumables and reagents usage, and hours of labour, are empirically derived from the experiments performed and discussed in this case study.
(iv) CAPEX and depreciation were accounted for.

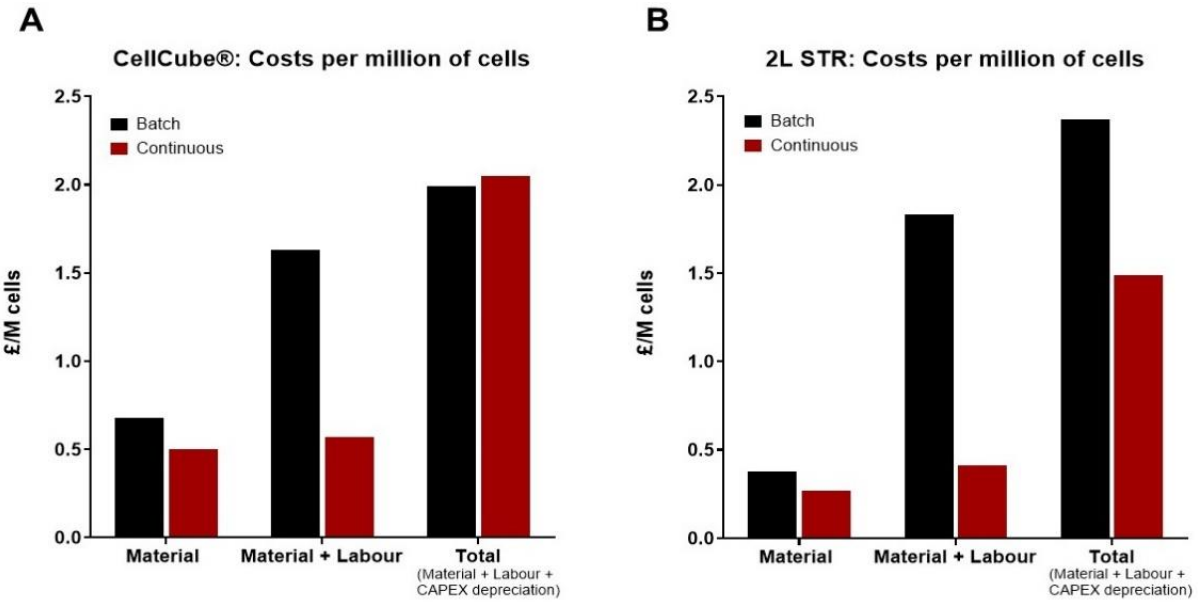

Figure 5: Techno-economic analysis comparison of batch and CBM processes for myoblast cells on the Corning® CellCube® and MC-based 2L STR platforms.

The bioreactor productivity factor was expressed as projected annual output for the respective bioprocesses, with an increase of 1.2-fold for the Corning® CellCube® and 2.5-fold for the MC processes whenever running in a continuous mode. The CBM process on the Corning® CellCube® (**Fig. 5**) showed a 27% cost reduction on materials and 65% on materials with labour included. On the other hand, when CAPEX with linear depreciation was included, CBM showed a 3% increase in costs compared to batch. The MC-based CBM showed significant benefits when compared to batch processes. Indeed, the CBM showed a 30% cost reduction on materials, 78% saving when labour was included and an overall 37% cost reduction when CAPEX with linear depreciation was accounted for.

Conclusion

An optimised CBM approach has clear advantages over conventional batch methodologies when materials, labour and logistical considerations are accounted for. Emerging fields requiring large number of cells (i.e. allogeneic cell therapies, regenerative medicine, and cellular agriculture) need to implement more efficient processes to meet cell demand.

CellRev have demonstrated a first-in-class approach capable of producing cells as final product, in a continuous manner, via Continuase™ supplementation. Despite optimisation requirements in improving cell viability, the continuously collected live cells were functional. Indeed, they were able to re-adhere to culture surfaces, proliferate at the expected growth rate, retain their phenotype and differentiation potential.

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