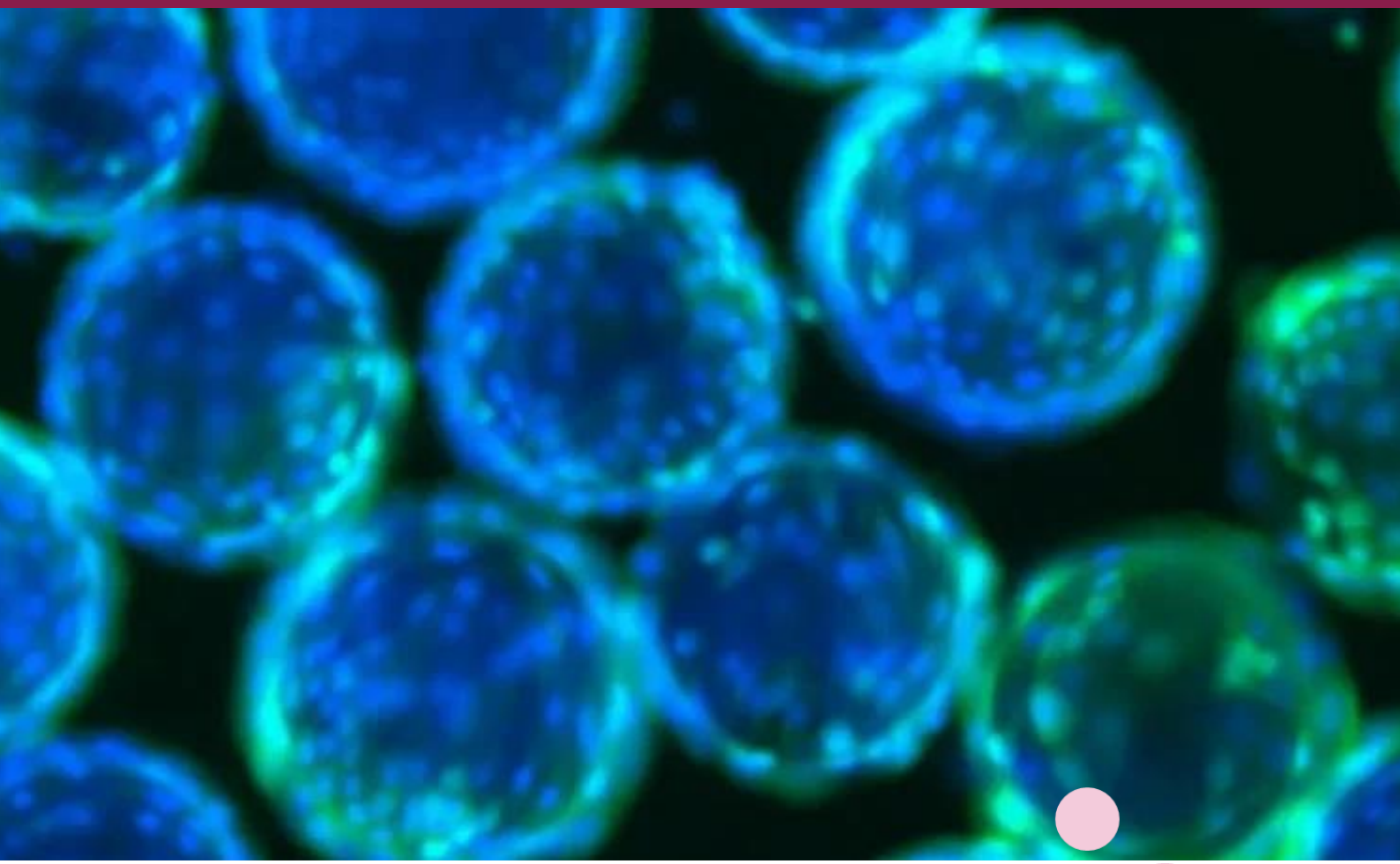
IN-DEPTH PROCESS UNDERSTANDING TO UNDERPIN

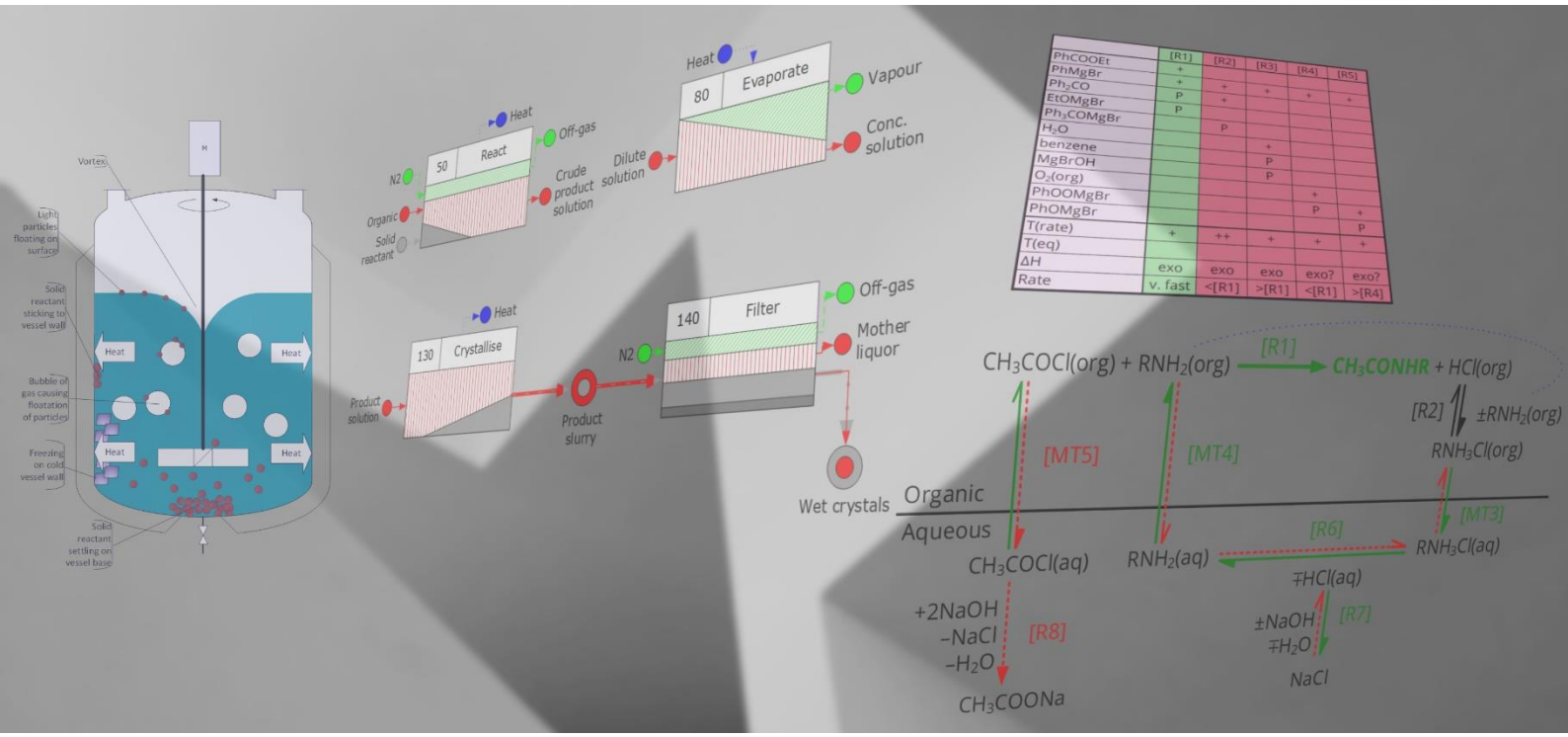
A CONTINUOUS BIOPROCESS MANUFACTURING PLATFORM



BRITEST LIMITED & CELLREV | AUGUST 2024

Britest

Generating value from process understanding



Britest is a not-for-profit membership-based organisation and consultancy which champions effective whole process design and open innovation throughout the chemical, biochemical and related process industries.

Britest's specialist technical facilitators help multidisciplinary development and manufacturing teams within companies, across supply chains, and in collaborative projects turn their working knowledge into impactful process understanding capable of driving innovation. Visually rich tools for information capture and structuring enable our clients to assimilate and communicate insight critical to product and process development, successful problem solving, and process improvement. The Britest approach has successfully delivered innovative solutions to key process and manufacturing challenges since 2001.

Visit our website at www.britest.co.uk

CELLREV

Breakthrough solutions to enable cost-effective and scalable cell culture

CellRev is a solutions provider in controlling cell-to-cell and cell-to-surface attachment, enhancing biomanufacturing for cell-based vaccines and biologics as well as cell and gene therapies.

CellRev span out of Newcastle University in 2019 and has since gone on to create a cutting-edge bioprocessing solution to solve many of the challenges associated with scaling up an adherent cell culture process. CellRev's breakthrough solution is founded on the ability to continuously grow, detach, and collect adherent cells. This industry-leading manufacturing solution enables more efficient and affordable cell processing.

For more information see www.cellrev.co.uk

SUMMARY

CellRev's core technology in enzymatic cell detachment offers a breakthrough in the ability to control and balance the rates of cell growth and detachment, opening the way to a scalable, continuous process for adherent cell expansion. The challenges of *productivity* – (achieving very large cell counts) and *product flexibility* (maximizing the compatibility of the technology platform with clients' pre-existing systems) are, however, inherent to all CellRev's target markets.

A bespoke Britest facilitated study was designed in consultation with senior leaders at CellRev to capture critical process understanding to enable subsequent inter-company technology transfer with Cellrev's chosen development partner for a new continuous manufacturing platform.

Conducted over three sessions, the study translated the knowledge of CellRev's study team into a series of working descriptive models and a prioritized risk assessment. The study also generated thirty follow-up actions for further development of concepts, validation of hypotheses and assumptions, plugging knowledge gaps, and pursuing new ideas. This output formed an important element in shaping the focus of CellRev's R&D programme leading up to the successful launch of the Livit ACE continuous manufacturing platform in March 2024.

INTRODUCTION

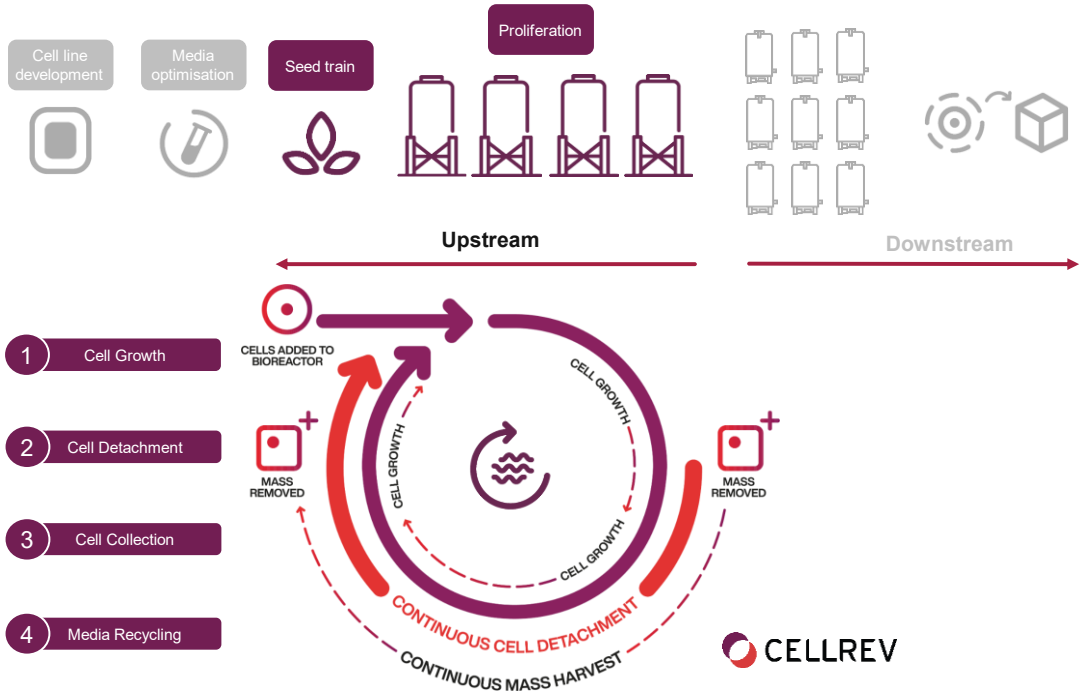
CellRev is an innovative supplier of processing solutions to improve the efficiency of cell-based manufacturing. The company works with cell-based vaccine and therapy developers to reduce cost, increase productivity, and unlock process bottlenecks to ensure more patients have access to life-saving treatments. Continuous bioprocessing offerings a compelling solution to the limitations and inefficiencies commonly associated with existing cell culture processes within the cell therapy and cellular agriculture market.[1] For cellular product developers it presents the opportunity to improve cost, process stability, and consistency of products variously through

- Elimination of manual handling and human error
- Increased quality assurance through online monitoring and control
- Reduce manufacturing time and increased efficiency
- Reduced capital costs and manufacturing space demands due to smaller equipment per unit output
- Operational flexibility and efficient response, e.g. to supply shortage.

CONTINUOUS BIOPROCESSING OFFERS A COMPELLING SOLUTION TO THE LIMITATIONS AND INEFFICIENCIES COMMONLY ASSOCIATED WITH EXISTING CELL CULTURE PROCESSES.

CellRev's game changing technology is founded on the ability to continuously grow, detach, and collect adherent cells (cells which need to be attached to a surface for growth to occur). Adherent cells are conventionally grown in batches on flat surfaces, a manually intensive, painstaking and repetitive process to manage and operate. Furthermore, the technology is inherently non-scalable as it depends on the provision of increasing surface area for cell growth. Overcoming this constraint to enable continuous production offers access to a more readily scalable technology.

Whilst cell growth in suspension can be more readily scaled, it only provides a niche solution as many types of commercially interesting adherent cells are, almost by definition, disinclined to grow in suspension. The only other route to an intensified process of suitable commercial readiness is to grow the cells on suitable suspended particles, referred to as microcarriers. This approach is already used in batch processes, but prior to CellRev's breakthrough technology there has been no continuous bioreactor technology for adherent cell culture where the cells are the product. CellRev's protected and proprietary core technology in enzymatic cell detachment enables controlled, targeted cell detachment at a rate which, crucially, can be matched to that of cell growth, opening the way to a scalable, continuous process.

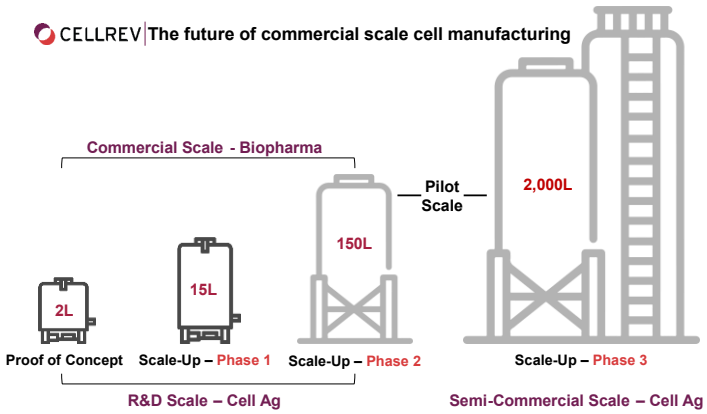


Continuous cell growth and harvesting (lower section of diagram) overcomes many of the inherent challenges of scaling up conventional batchwise upstream biomanufacturing

THE CHALLENGE

Productivity is a major factor in all CellRev's target markets. Whether it is cell-based therapeutics or cellular agriculture, cost-effective manufacturing of very large cell counts is required. Current approaches simply cannot meet the demand, and existing infrastructures cannot deliver to scale.

Product flexibility is also central to CellRev's business model. When the company engaged with Britest (early in the summer of 2023) they had identified the need to establish a technology platform for adherent cell manufacture that was not just scalable, but that would also be both flexibly compatible with their clients' pre-existing systems and the foundation of a service-based offering, supporting clients on their journey from batch to continuous operation.



The challenge of productivity is central to CellRev's target markets

To do so, they needed to:

- Capture process understanding to enable subsequent inter-company technology transfer with their chosen development partner
- Understand the key requirements to control cell detachment and enable process control at scale
- Identify opportunities for process intensification
- Understand what would be needed to achieve the necessary flexibility to process a range of cell types
- Identify the risks associated with scale-up and strategies to mitigate them.

AN AGREED APPROACH

Working together, Britest's technical facilitator and senior technical leaders from CellRev defined an agreed scope of work to address their challenge. The Britest process understanding tools would be used to help the project team **understand the required processing experience** of the materials (e.g. molecules, particles, organisms, enzymes), relating these to underlying scientific principles and, in so doing, identify any gaps in the technical team's understanding. By thoroughly reviewing the process defined in this way, the **main scale-up risks would be identified**, translated into a risk register, and **prioritised mitigation strategies developed**. To support equipment selection and further detailed process design engineering work, a list of key practical, design, and operational requirements to deliver the required process experience(s) would be generated.

The study was conducted over three facilitated team sessions during July and August 2023. In the first session Initial Screening Analysis was used to help ensure that the subsequent more technically orientated sessions were related back to CellRev's business goals and aims. In total the study involved 14 people and approximately 100 hours of staff time.

Initial Screening Analysis

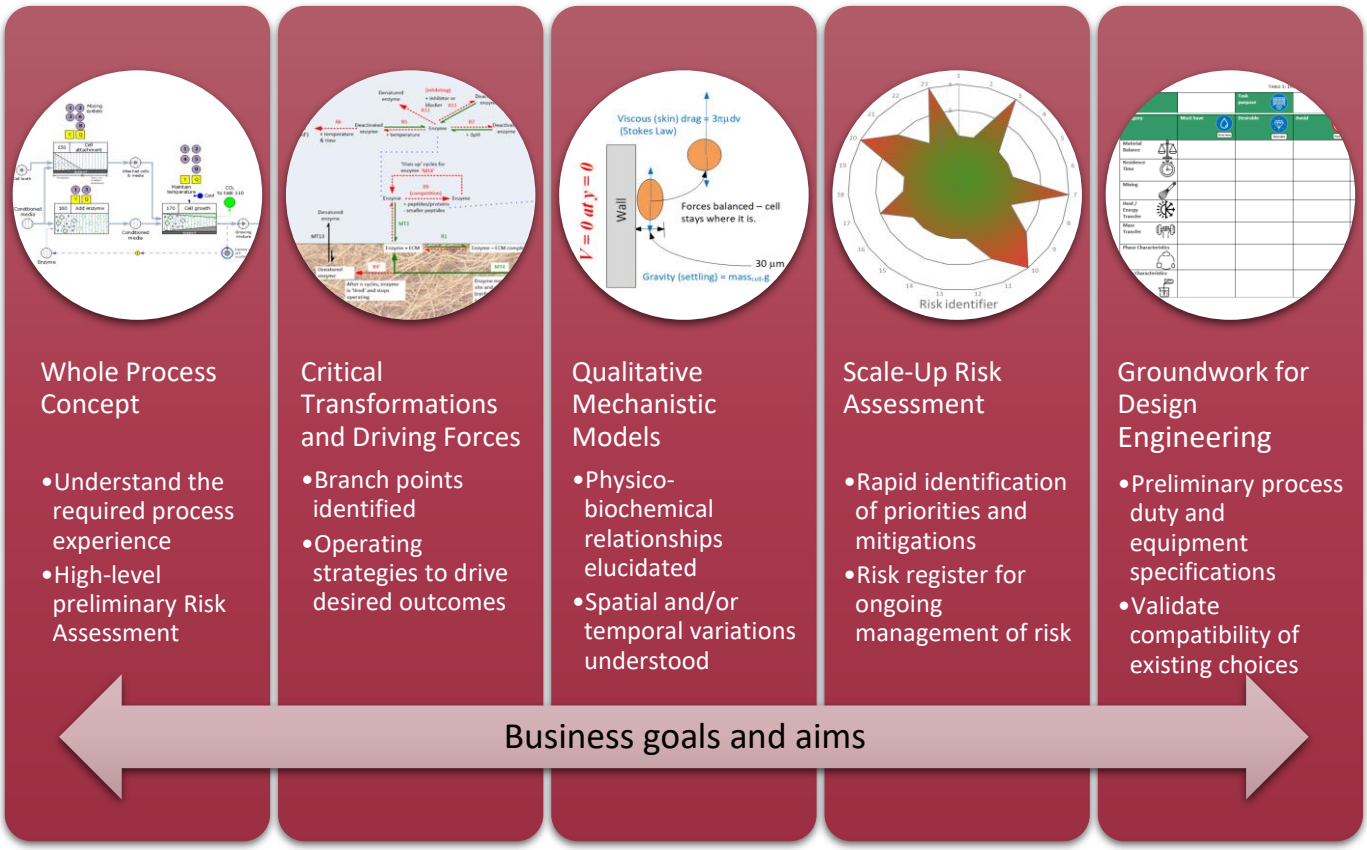
- Set scope
- Establish shared understanding of the business context and aims

1st Technical Session

- Capture process understanding and identify gaps
- Identify scale-up risks
- Explore driving forces

2nd Technical Session

- Drill into driving forces
- Manage scale-up risks
- Support Process Design



Outline of preliminary study plan

A FACILITATED SOLUTION

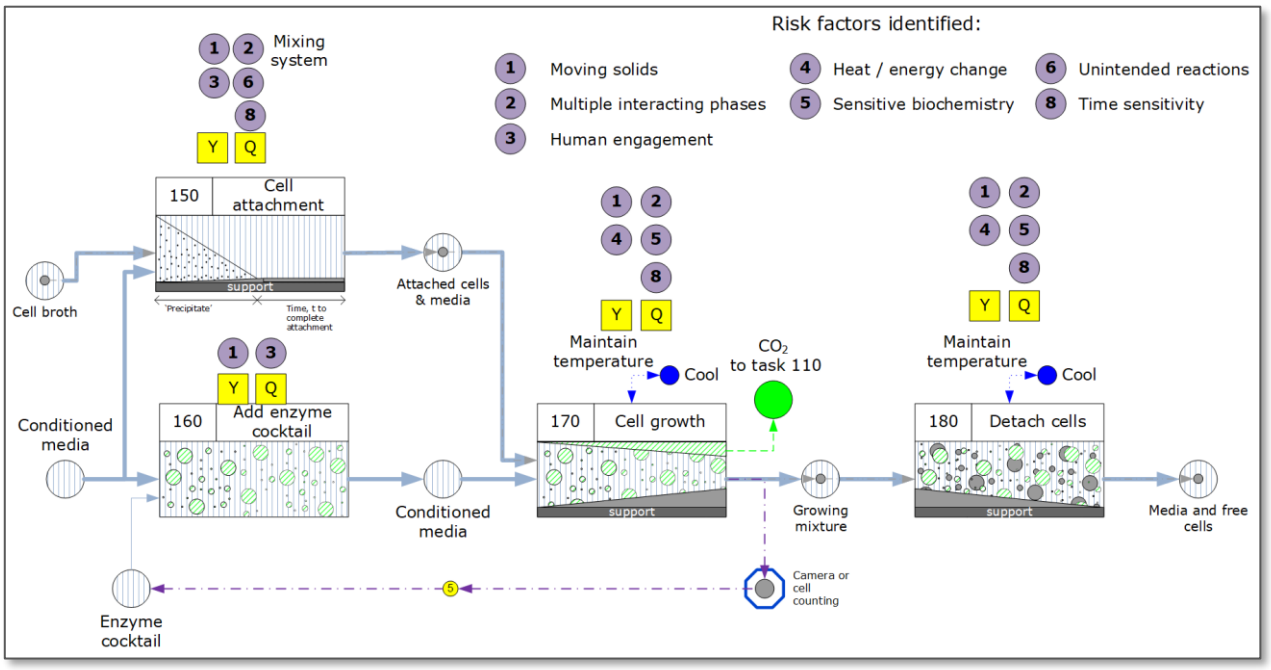
CAPTURING A RICH WHOLE PROCESS DESCRIPTION

Guided by Britest's specialist technical facilitator, the project team first developed a visually rich representation of the whole process concept in the form of a Process Definition Diagram (PDD).[2] This representation, in the form of an enhanced state-task network, enabled the process technologists to describe the process independently of scale and equipment, and enumerate process material introductions and removals from the system, the phases present at different stages of the process, and phase changes. Whilst the PDD is a well-proven and flexible tool for the representation of conventional chemical reaction-based processes, the successful application to CellRev's adherent cell process represented a notable extension into the field of biotechnology, part of an emerging trend in Britest's coverage and demonstrated capabilities. Adaptations to the standard tool's approach to phase representations and phase changes over time (for batch concepts) or space (for continuous) were successfully used to capture bioprocess relevant tasks such as media preparation, sterilization, cell attachment, growth, detachment, and separation.

ADAPTATIONS TO THE PDD's REPRESENTATIONS OF PHASES AND PHASE CHANGES IN SPACE / TIME WERE SUCCESSFULLY USED TO CAPTURE BIOPROCESS RELEVANT TASKS.

The PDD was subsequently used as the basis of a scale-up risk assessment. Here the PDD was annotated using parallel sets of prompts for *known scale-up risk factors* (such as operations requiring movement of solids, multiple interacting phases, human involvement, sensitive biochemistry etc.) and *the nature of the impacts* arising from them (impacts upon the product quality or yield and/or the process operability / safety). The section of the PDD dealing with cell attachment, growth and detachment is shown below with the associated risk annotation. The importance of mitigating

the numerous risks associated with process yield and quality inherent in these core tasks through effective process design and control strategies is evident.

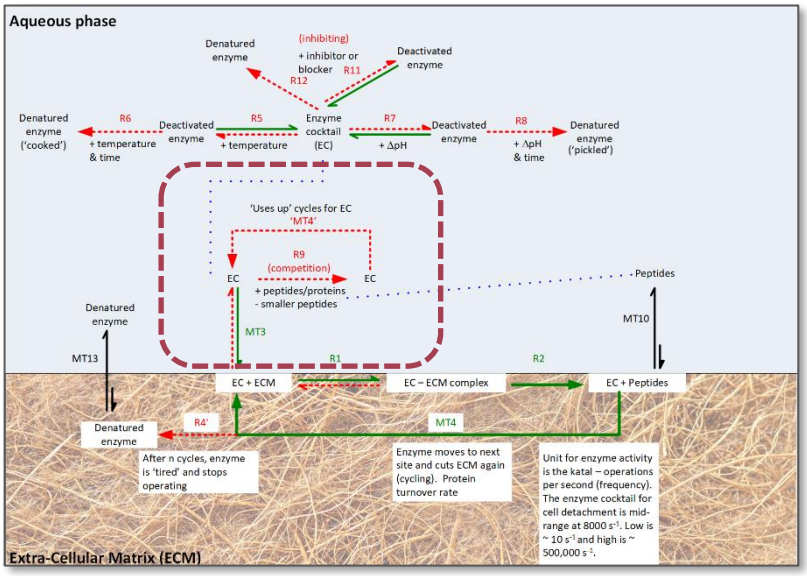


Extract from PDD showing cell attachment, growth and detachment tasks with scale-up risk and impact annotation added

EXPLORING THE TRANSFORMATIONS BEHIND THE PROCESS

To better explore and understand the nature of the underlying physical and biochemical transformations occurring within these key process tasks so that risk mitigation strategies and process control concepts might be developed, the project team were supported in constructing a Transformation Map representing the action of the detachment enzyme cocktail (EC) at the heart of the biochemistry, and a Rich Cartoon of the various transformations occurring during cell attachment, growth and detachment.

A Transformation Map is used to graphically portray the network of (bio)chemical and/or physical transformations that convert raw materials into products within a process task. They usually include both desired and undesired transformations, to aid the identification of "branch points" (points of divergence from the desired outcome) within the process.



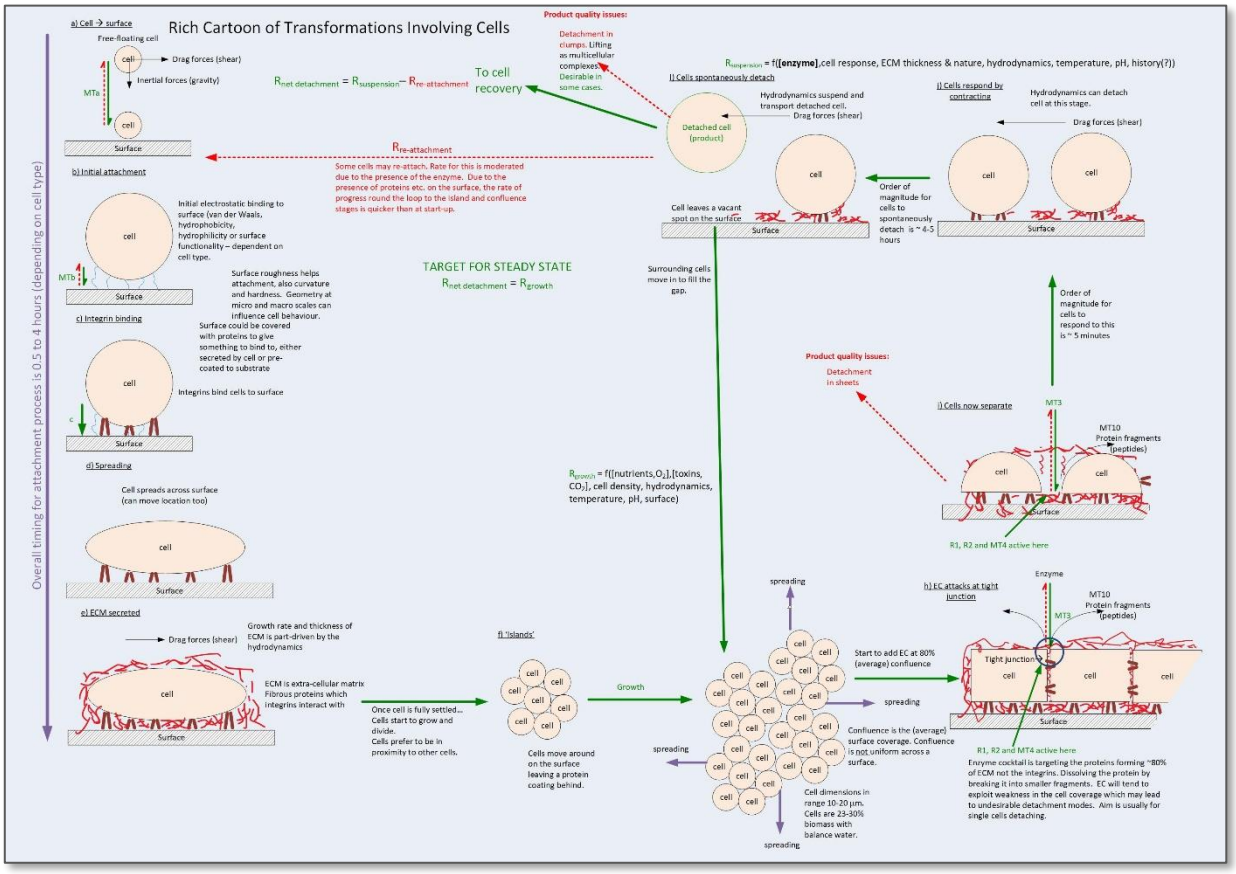
Transformation Map for detachment enzyme cocktail action

All these features are evident in the Transformation Map for the EC developed for CellRev (see opposite). There is one significant branch point (within the dashed envelope) where a series of side reactions with various sorts of cell debris present in the aqueous reaction medium compete with the desired mass transfer of the EC into the extra-cellular medium (ECM) where its bond-breaking activity is targeted as intended towards cell detachment.

Several risk-mitigating operating strategies were developed from this working model:

- Ensuring precise temperature and pH uniformity not just at the macro-scale but avoiding localised temperature and pH gradients through attention to mixing design, injection points and fluid dynamics
- Removing, scavenging or otherwise limiting the concentration of unwanted peptides and other debris
- Closer characterization of potential blocking/inhibiting species (towards their elimination from the medium)
- Enhancing the mass transfer rate of the enzyme cocktail toward the ECM.

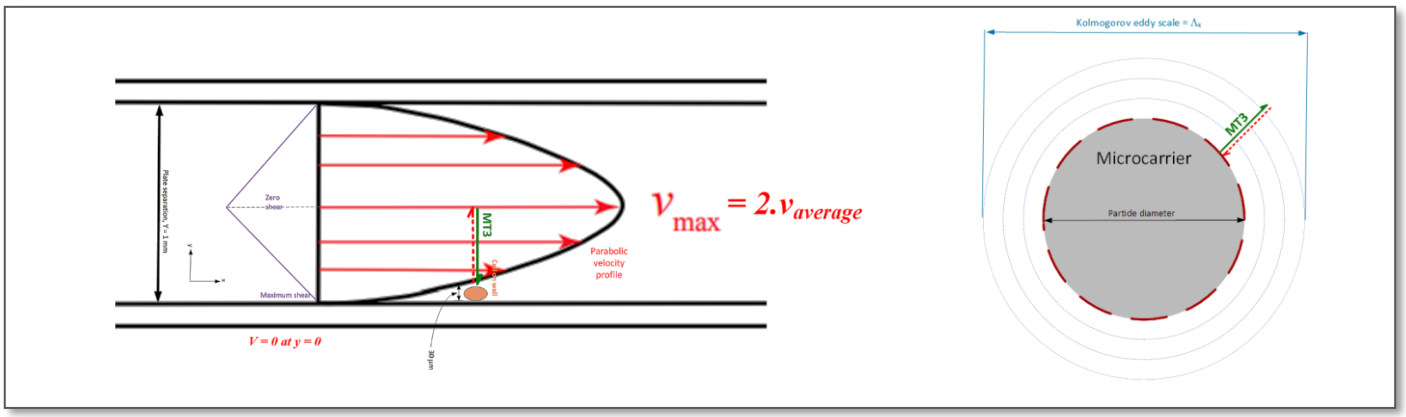
The Rich Cartoon developed by the team (below) helped visualise and analyze more deeply the relationships between the concurrent rate processes and the influencing factors that form the dynamically interacting cycle of attachment, growth and detachment. In combination with a Driving Force Analysis taking account of the influence of gaseous mass transfer (O_2 and CO_2 are both important in cell growth) it was possible to develop a meaningful qualitative model of the balance between the growth of the cell, the rate of suspension (i.e. detachment) and the rate of re-attachment. Since system hydrodynamics are a factor in all the transformations described, the key control challenge is essentially a hydrodynamical one: how to move the detached cells to become remote from the surfaces as quickly as possible, without damaging either the cells in suspension or those remaining on the surface.



Rich Cartoon of cell transformations

UNDERSTANDING THE FORCES AT PLAY

Responding to the process understanding already developed, the project team decided to take an extra step on the way to their desired end. Before trying to capture the key requirements for the equipment needed for continuous cell production, they further considered the differences between cell production when based upon planar and spherical support surfaces respectively, with the focus primarily upon understanding how the critical mass transfer which brings the EC to the ECM is controlled, and the nature of the cell detachment forces, under the two scenarios.

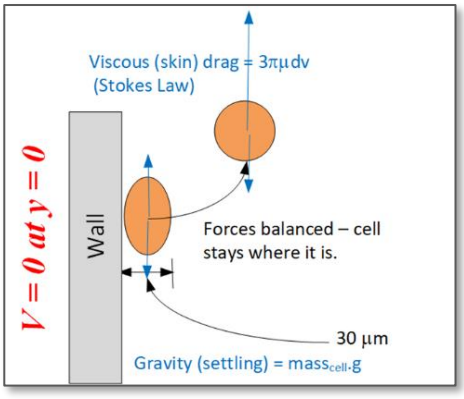


Rich Pictures illustrating the hydrodynamic environment for enzyme mass transfer (MT3) under plane (left) and spherically supported (right) conditions.

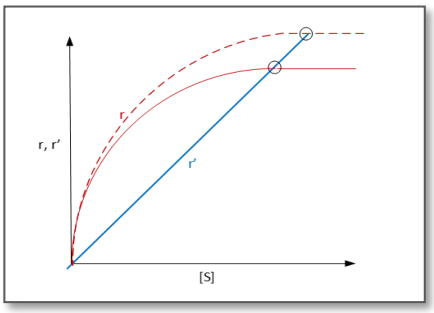
In a planar, plate-based system flow is laminar. In practice, changes of flow direction dictated by equipment layout considerations allow so-called Dean vortices to form enabling (predominantly diffusion limited) mixing. Cell detachment (left) is governed approximately by Stokes Law, where an imbalance between the drag and inertial forces (gravity) allows the detached cell to be transported by the flow (above right).

The beads employed as cell micro-carriers are sized to be on the scale of the Kolomogorov length scale, the size of the smallest turbulent eddies present in the agitated medium (the system operates as a quasi-continuous stirred tank reactor with periodic addition of feedstocks and removal of product). By considering diffusion in spheres of this size the mixing time can be estimated and, in principle, matched to the order of the net rate of mass transfer of enzyme into the ECM.

The performance of flat surface-based systems is known to be sensitive to the enzyme concentration under a range of bulk agitation conditions. Microcarriers, operating with a very different bulk agitation, are much less sensitive. Complications can arise in either case as non-enzyme-controlled detachment processes come into play, limiting the ability to measure cell growth rate accurately, but in essence a control/operating point may be defined where the rates of ECM digestion (r) and production (r') are in balance (below right).



Forces acting on cell during detachment



Control points for continuous operation

SCALE-UP RISK REGISTER

Building on the priority concerns identified through the scale-up risk assessment described earlier, a scale-up risk register was developed, initially for two risks around cell detachment associated with incorrect control of enzyme concentration and excessive bead to bead collisions associated with process intensification respectively, as a template for further work by Cellrev's technical team.

Risk Number	PDD Task Number	Task Description	Risk Factors	Risk / opportunity and triggers	Impact	Failure Probability F	Consequences C	Understanding U	Risk Priority P	Reasoning	Mitigation Strategy
14	180	Detach cells: Separate cells from surfaces to allow harvesting.	1, 2, 4, 5, 8	Unstable/variable cell detachment rates caused by incorrect [enzyme]. Process run terminated. Increased process operating costs.	Y, Q	3	3	3	2	Process is reliant currently on biomass feedback control. Significant production loss on failure. Detachment is broadly understood but gaps in picture.	**Action 29:** Confirm detachment works as expected under more intense conditions. Prove this translates scale to scale. Develop understanding of alternative detachment processes which may occur.
15	180	Detach cells: Separate cells from surfaces to allow harvesting.	1	Variable cell detachment rates from increased bead to bead collisions as process intensified	Y, Q	3	2	3	2	More beads leading to more collisions.	**Action 30:** Controlled experiments to investigate effect of increasing process intensity, consider bead shape.

Example entries in scale-up risk register

OUTCOMES AND BENEFITS

In addition to the process understanding, working descriptive models, and risk assessments summarized in the previous section, the Britest study activity generated a set of thirty associated follow-up actions variously aimed at developing concepts more fully, validating hypotheses and assumptions, plugging knowledge gaps (through experimentation or literature-based research), and pursuing new ideas arising.

The study helped in consolidating various aspects of biology and bioprocessing knowledge and created a shared foundation within the team. The output formed an important element to shape the focus of the ongoing R&D programme for CellRev.

CellRev's patented bioprocess technology, delivered through the proprietary media additive Continuase™, keeps cell detachment equal to growth rate to allow steady state operation at high cell densities. The Livit ACE continuous manufacturing platform[3] combines the best of CellRev's technologies with Getinge's expertise and equipment in a complete bioprocessing solution offering superior productivity, scalability, automation, and stability versus existing technologies.

BRITEST PLAYED AN IMPORTANT ROLE IN IMPROVING PROCESS UNDERSTANDING FOR A BREAKTHROUGH IN CONTINUOUS BIOPROCESSING TECHNOLOGY.

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[1] M. Miotto, R. Gouveia, F. Z. Abidin, F. Figueiredo, and C. J. Connon, ACS Appl. Mater. Interfaces 2017, 9, 47, 41131–41142. https://doi.org/10.1021/acsami.7b09809

[2] K. Wall, P.N. Sharratt, N. Sadr-Kazemi, and J.N. Borland, Organic Process Research & Development5, 434-437, 2001. https://pubs.acs.org/doi/10.1021/op010002j

[3] See https://cellrev.co.uk/livit-ace/ and https://www.getinge.com/uk/products/livit-ace/ for further details.

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