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Ultra scale-down mimics for perfusion culture: Experimental study for rapid biopharmaceutical process development

Molly Tregidgo
Advanced Centre for Biochemical Engineering, University College London, molly.tregidgo.11@ucl.ac.uk

Martina Micheletti
Advanced Centre for Biochemical Engineering, University College London

David Pollard
Merck & Co., Inc., Rahway, New Jersey

ULTRA SCALE-DOWN MIMICS FOR PERFUSION CULTURE

MOLLY TREGIDGO[1] DAVID POLLARD[2] MARTINA MICHELETTI [1]

[1]Department of Biochemical Engineering, University College London, Torrington Place, London, WC1E 7JE
[2]Merck Sharp Dohme, NJ, USA

ABSTRACT

With the industry considering the implementation of end to end continuous bioprocesses, there is a need for the development of scale-down tools to provide the same consistency, reliability and throughput as the scale-down tools already available for traditional fed-batch processing. This work aims to develop a perfusion scale-down system capable of reproducing the specific characteristics of the perfusion culture process, namely cell retention capabilities, the ability to support high cell densities and to operate for extended periods compared to fed-batch cultures. Cell culture in microwell plates in fed-batch mode is well defined and is in widespread use; however to the best of our knowledge this represents the first attempt at the development of quasi-perfusion cell culture at this scale.

BACKGROUND

Quasi-Perfusion in microwell plates

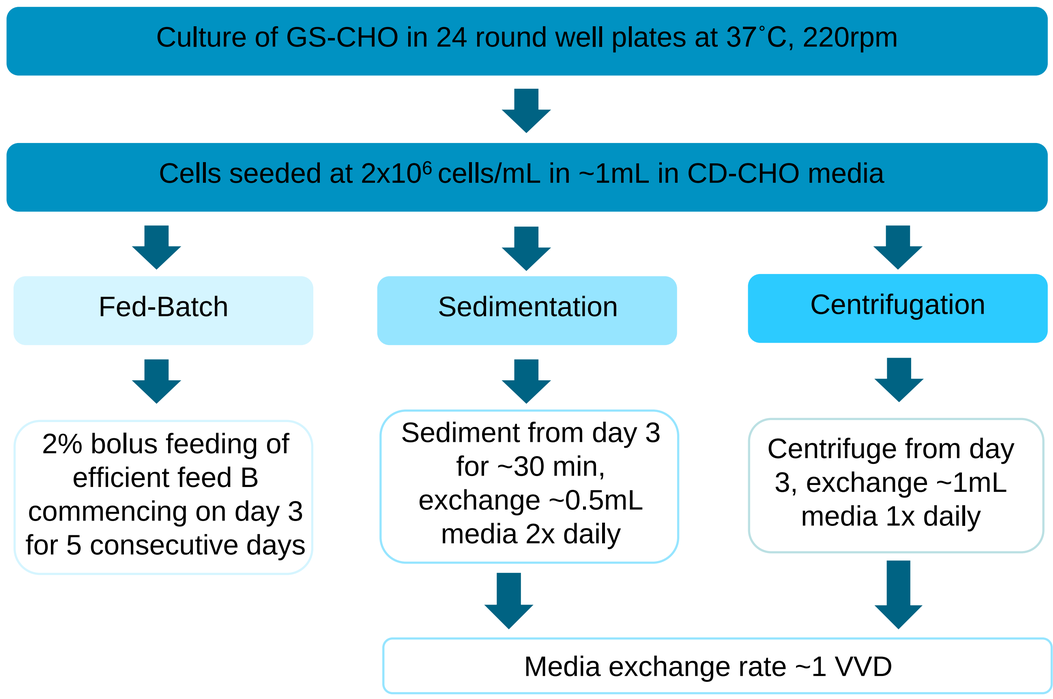
+ High throughput, low volume
+ Ability to automate if desired
− No on-line media or gas additions
  - Concerns O_2 may become limiting at HCD

	Sedimentation	Centrifugation
Separation via	Gravitational Settling (Size, Density)	Accelerated Gravitational Settling (Size, Density)
Advantages	Low shear stress	Separation time
Disadvantages	Separation time, Variability	Higher shear stress

OBJECTIVES

1. Design of a perfusion culture mimic capable of achieving the characteristics of large scale culture.
2. Implement developed quasi-perfusion techniques in microwell plate format.
3. Demonstrate the use of quasi-perfusion in microwells as a tool for the early phase development of perfusion culture.

EXPERIMENTAL METHODS



RESULTS

Proof of Concept

	Separation Efficiency (%)
Sedimentation	96.9
Centrifugation	94.5

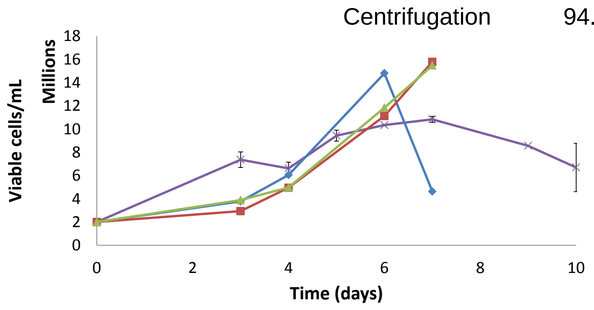


Fig. 1 *Viable cell density and for Fed-Batch and quasi-perfusion cultures exchanged with CD-CHO media and CD-CHO supplemented with glucose at 9g/L and 12g/L*

Media Optimisation

Improving cell densities by exchanging with CD-CHO supplemented with feeding media, increasing quantity of trace nutrients to alleviate limiting factor.

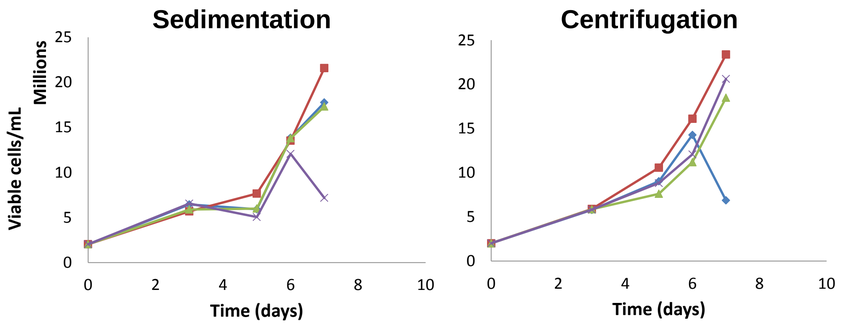


Fig. 2*. Viable cell density for Sed and Cent quasi-perfusion cultures exchanged with CD-CHO supplemented with feeding media at 5% 15% 30% and 45%*

Productivity

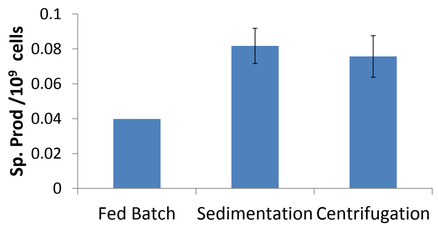


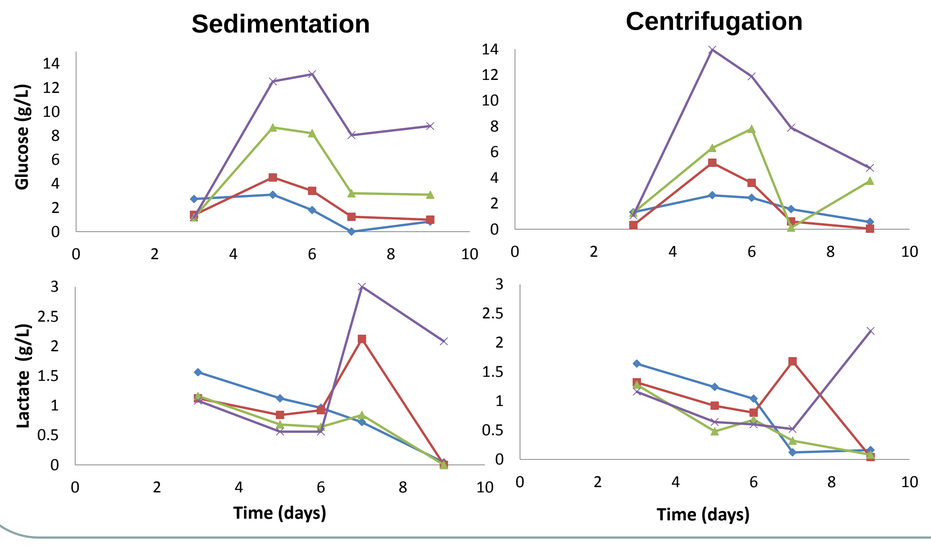
Fig. 3. *Average cell-specific productivity for Fed-Batch and quasi-perfusion cultures.*

Table 1. *Total mAb produced over the culture duration for quasi-perfusion cultures.*

	Total mAb Produced (g)			
Feed B (%)	**5**	**15**	**30**	**45**
Sedimentation	1.57	1.77	1.47	1.43
Centrifugation	1.64	1.76	1.70	1.28

Fed-Batch cultures produce a total of 1.06×10^{-3} g mAb, quasi-perfusion methods have 1000x greater volumetric productivity (with no impact on product quality – *data not shown*)

Metabolic profiles



Over-supplementation in 30and 45% cultures causes spikes high glucose concentrations.

Lactate largely in expected range.

Fig. 4*. Glucose and Lactate profiles for quasi-perfusion cultures exchanged with CD-CHO supplemented with feeding media at 5% 15% 30% and 45%*

Stationary Phase

Cells seeded at 40M cells/mL during the stationary phase. Determining the feasibility of using quasi-perfusion methodology to mimic the stationary phase of perfusion cultures.

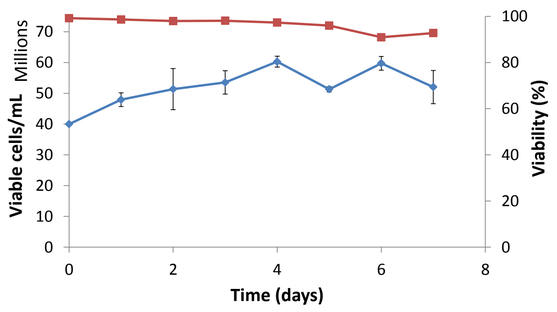


Fig. 5*. Viable cell density and viability for a stationary phase centrifugation quasi-perfusion culture exchanged using optimised media (media optimisation data not shown)*

Evaluating ability to mimic perfusion culture

1. Maintains elevated (high) cell density ✓
2. Cell retention ✓
3. Constant volume ✓
4. High productivity ✓
5. Continuous ×

Quasi-perfusion cultures in microwell plates are promising for use in early development. Data across the 24 wells is consistent (*data not shown*) allowing multiple cell lines and media compositions to be investigated.

CONCLUSIONS

1. Microwell plates are able to mimic many characteristics of perfusion culture, including elevated cell densities and high productivities, O2 not yet limiting
2. Sedimentation and Centrifugation quasi-perfusion cultures sensitive to media changes, promising for potential use in early phase development
3. High cell densities are able to be maintained in the microwell system, as demonstrated by the stationary phase culture

FUTURE WORK

1. Integration of microwell plate set up into automated liquid handling device in order to increase throughput and expand system application.
2. Utilisation of the developed protocol in the screening of cell lines and media for perfusion culture applications.
3. Set-up of a novel 250mL bioreactor to increase information output, maintain elevated cell densities for prolonged timescales and to operate continuously.

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Contact: molly.tregidgo.11@ucl.ac.uk