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

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ULTRA SCALE-DOWN MIMICS FOR PERFUSION CULTURE

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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 C2 may become limiting at HCD

Sedimentation

<table>
<thead>
<tr>
<th>Separation via</th>
<th>Gravitational</th>
<th>Centrifugation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Setting (Size, Density)</td>
<td>Gravitational Setting (Size, Density)</td>
<td></td>
</tr>
</tbody>
</table>

Advantages

- Low shear stress
- Separation time

Disadvantages

- Separation time, Variability
- Higher shear stress

OBJECTIVES

1. Design 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

Culture of GS-CHO in 24 round well plates at 37 C, 220rpm

Cells seeded at 2x10⁶ cells/mL in ~1mL in CD-CHO media

Fed-Batch

Sedimentation

Centrifugation

2% bolus feeding of efficient feed B commencing on day 3 for 5 consecutive days

Sediment from day 3

Centrifuge from day 3, exchange ~1mL media 1x daily

Media exchange rate ~1 VVD

RESULTS

Proof of Concept

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%, 10%, 20%, 30% and 45%.

Table 1. Feed B (%)

<table>
<thead>
<tr>
<th>Feed B (%)</th>
<th>Sedimentation</th>
<th>Centrifugation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>10%</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>20%</td>
<td>0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>30%</td>
<td>0.21</td>
<td>0.09</td>
</tr>
<tr>
<td>45%</td>
<td>0.31</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Fig. 4: Media exchange rate for Fed Batch and quasi-perfusion cultures.

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 stationary phase perfusion cultures exchanged with optimised media (media optimisation data not shown).

CONCLUSIONS

1. Microwell plates are able to mimic many characteristics of perfusion culture, including elevated cell densities and high productivities, C2 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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