Improving downstream processing unit operations for cellular therapies

Nik Willoughby
Cellular Bioprocessing
Institute of Biological Chemistry, Biophysics and Bioengineering
Heriot Watt University

24th Sept 2013
Overview

• Cellular therapy purification challenges
• Scale up or scale out?
• Strengths and weaknesses of current approaches
• Possible future directions of development
• Conclusions
Cellular Therapy Challenges

• Therapeutic model? Clinical or Biopharma?
  • Business Model
  • Production and development
  • Supply Chain
  • Regulatory Issues
• Or will we have a completely new model?
## Cellular Therapies

<table>
<thead>
<tr>
<th></th>
<th>Autologous</th>
<th>Allogeneic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor</strong></td>
<td><img src="image1.png" alt="Person" /></td>
<td><img src="image2.png" alt="Person" /></td>
</tr>
<tr>
<td><strong>Process</strong></td>
<td><img src="image3.png" alt="Image1" /></td>
<td><img src="image4.png" alt="Image2" /></td>
</tr>
<tr>
<td><strong>Cell numbers</strong></td>
<td>$10^4$-$10^8$</td>
<td>$10^8$-$10^{18}$</td>
</tr>
<tr>
<td><strong>Recipient</strong></td>
<td><img src="image8.png" alt="Person" /></td>
<td><img src="image9.png" alt="Group_of_People" /></td>
</tr>
</tbody>
</table>
Allogeneic Cellular Therapy Challenges

- Immunosuppression
- Donor sourcing
- Large scale culture (suspension)
- **Purification**
  - Regulation
  - Storage
  - Administration
Scale-up Challenges (purification)

- Purification of end target (cells)
  - Complexity of target is orders of magnitude higher than proteins
  - Target may be challenging to “define”
  - Target cells will be majority of final culture
  - Target cells have distinct morphology
  - “Contaminating” cells will be a mixture of different stages of differentiation
  - Scales involved could dwarf conventionally available cell separation technology
  - Solution may be to exploit distinct physical characteristics of cell types
## Current Techniques

<table>
<thead>
<tr>
<th></th>
<th>FACS</th>
<th>MACS</th>
<th>Ultracentrifugation</th>
<th>Chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scalable</td>
<td>✗</td>
<td>?</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Maximum cell throughput (cells/min)</td>
<td>$10^6$</td>
<td>$10^8$</td>
<td>$10^9$</td>
<td>?</td>
</tr>
<tr>
<td>Maximum cell throughput (cells/run)</td>
<td>$10^{10}$</td>
<td>$10^9$</td>
<td>$10^{11}$</td>
<td>?</td>
</tr>
<tr>
<td>Optimised for cell targets</td>
<td>✓</td>
<td>✓</td>
<td>?</td>
<td>✗</td>
</tr>
<tr>
<td>Label-free</td>
<td>✗</td>
<td>?</td>
<td>✓</td>
<td>?</td>
</tr>
</tbody>
</table>
Downstream Processing

Purity

Throughput

Yield
Downstream Processing

- Purification of a product requires more than one step
- Losses at each step become increasingly problematic
Importance of interdisciplinary links
Large-scale Purification of Cellular Therapies

- Weaknesses in current techniques
- How pure do we need to be?
- Possible target properties
  - Size
  - Charge
  - Elasticity
  - Cell surface markers
Exploiting target properties

(image from www.postnova.com)
How to identify these properties?

- One way is to use force microscopy - setup consists of piezo stage, cantilever, laser and detector
- Deflection of tip moves laser on detector to show topography
- Analysis of piezo movement, laser displacement and cantilever properties gives force response as well as topography
- Modification of tip properties allows study of different force responses
Force Microscopy
A Tag-Less Method of Sorting Stem Cells from Clinical Specimens and Separating Mesenchymal from Epithelial Progenitor Cells

Barbara Roda,1,2* Pierluigi Reschiglioni,1,2 Andrea Zattoni,1,2 Francesco Alviano,2,3 Giacomo Lanzoni,2,3 Roberta Costa,2,3 Arianna Di Carlo,1 Cosetta Marchionni,2,3 Michele Franchina,1 Laura Bonsi,2,3 and Gian Paolo Bagnara2,3

1Department of Chemistry “G. Ciamician”, via Selmi 2, I-40126 Bologna, Italy
2interuniversity Consortium I.N.B.B., Rome, Italy
3Department of Histology, Embryology, and Applied Biology, via Belmeloro 8, I-40126 Bologna, Italy
4Department of Obstetrics and Gynecology, S. Orsola-Malpighi Hospital, University of Bologna, via Massarenti 13, I-40138 Bologna, Italy

---

*Corresponding author:
Barbara Roda
E-mail: barbara.roda@unibo.it

Sorting Stem Cells: Scientists Propose a New Way to Isolate Early Stage Embryonic Stem Cells

Jan. 3, 2013 — When an embryonic stem cell is in the first stage of its development it has the potential to grow into any type of cell in the body, a state scientists call undifferentiated. A team of researchers from Scotland has now demonstrated a way to easily distinguish undifferentiated embryonic stem cells from later-stage stem cells whose fate is sealed. The results are published in the American Institute of Physics’ (AIP) journal Biomedical Optics.

Related Topics

- Health & Medicine
  - Stem Cells
  - Skin Cancer
- Plants & Animals
  - Biotechnology
  - Developmental Biology
- Matter & Energy
  - Electricity

Share This:

- American Institute of Physics’ (AIP)
- Journal Biomedical Optics
Elasticity-based separation
Separation by size and shape

Sort fully differentiated red blood cells

Differentiated RBCs potentially have different size, density, compressibility, refractive index and/or shape

Acoustic scaling with optical dexterity

RBCs from white blood cells

M.P. MacDonald et al., “Microfluidic Optical Cell Sorting”, Nature

Trapping in an acoustic standing wave

VALOR: Vertical Acoustic Levitation with Optical Routing

Mixed analyte enters sorting volume

Base of chamber

Species A aspirated

Species B remains at base

Impurities removed

Species A further refined optically

Ultrasound standing wave node
Separation by affinity/surface markers

- MACS – limits of scale?
  - Magnetic systems and heat dissipation
  - Limits of size for batch processing
- Affinity systems on surfaces
  - Monolithic resins
  - Cryogels
  - Affinity membranes
  - Specifically designed surfaces
- Capacity issues
Some thoughts

• Cellular targets are vastly more complex
  • Learning the bioprocessing lessons of the past (e.g. MAb processes) is key
  • New approaches and new unit operations are needed; focused on key target properties
• Engineering knowledge does apply widely
  • Process design, unit operations and know-how are transferable
  • Whilst this itself is well-known, there is plenty of scope for improvements in many industries
• But interdisciplinary collaboration is the key
  • Working with wider fields of expertise increases knowledge and progress
Acknowledgements

Heriot-Watt University
- Henry Bock
- Fiona Dempsey
- Robert Kiss
- Debaditya Choudhury
- Craig Williams
- Ajoy Kar
- Lynn Paterson

University of Edinburgh
- Paul De Sousa
- Steve Pells
- Paz Freile

University of Glasgow
- Gordon McPhee
- Huabing Yin

University of Dundee
- Mike MacDonald