Lecture #12
Scale-Up Biomanufacturing

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http://courses. engr.illinois.edu/bioe498gu/
Scale-Down & Scale-Up of Stem Cell Culture

Microfluidic
- Scale down
- 2D adherent culture
  - Precise spatial/temporal dynamics
  - High-throughput screening

Static
- 10 mL

Bioreactor
- Scale up
- 3D suspension culture
  - Continuous monitoring & feedback
  - Scalable production

Fridley et. al, Stem Cell Res Ther, 2012
Scaling Up Production

Needs:

1) Scalable self-renewal
2) Scalable differentiation
Scaling Up Production

Commercial Scale Cell Therapy Production

<table>
<thead>
<tr>
<th>IND</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Commercialization</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 patients</td>
<td>10 patients</td>
<td>20 patients</td>
<td>50 patients</td>
<td>100 patients</td>
</tr>
<tr>
<td>1000 patients</td>
<td>10000 patients</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Scaling Up Production

Challenges:

1) Fundamental limitations due to scale required.
2) Need for patient-specific products.
Scaling Up Production

Estimates of cell numbers required for clinical applications:

- Parkinson’s disease: $10^5$ dopaminergic neurons
- Myocardial infarction: $10^9$ cardiomyocytes
- Type I diabetes: $10^9$ beta cells
- Liver or Heart tissue engineering: $10^{10}$ cells
- Screening of large chemical library one time: $10^{10}$ cells
Scaling Up Production

500 million twinkies made each year

https://www.youtube.com/watch?v=UvK5u0mRSms#aid=P8THLdp59XA
Scaling Up Production

Manufacturing Flexibility – Understanding scale up for your Cell Therapy

The need for analysis and foresight – automated, closed and scalable processing is critical for commercial success but how do you decide what to invest in and when?

World Stem Cell and Regenerative Medicine Congress - May 2013
Richard Grant

Invetech
Upstream & Downstream Bioprocessing

Example: Recombinant Virus

- Baculovirus production
- Cell expansion
- Media components

Bioreactor

- Upstream Processing
- Downstream Processing

- Cell Disruption
  - Thermal shock
  - Chemical
  - Mechanical

- Clarification
  - Microfiltration/UF
  - Centrifugation

- Purification
  - Ultracentrifugation
  - Affinity chromatography
  - Ionic exchange chromatography
  - Depth end filtration
  - Tangential flow filtration
  - Hollow fiber
Scale-Up of Stem Cell Culture
Bioreactor Stem Cell Culture

Common platforms for suspension culture:
1. Microcarrier beads (adherent cells on the outside of solid beads or throughout porous materials).
2. Aggregates +/- biomaterial
3. Microencapsulation (singles cells or aggregates)
Bioreactor Stem Cell Culture-Microencapsulation

Wilson & McDevitt, Biotech Bioeng, 2013
Microencapsulation Materials

Alginate

G block

M block

High guluronic (G) acid alginate

High mannuronic (M) acid alginate

G residue

M residue

Calcium cross-link

Single cells

Aggregates

Uncoated

Solid matrix

Liquid core

PLL-coated

Wilson & McDevitt, Biotech Bioeng, 2013
# Microencapsulation Materials

## Table 1. Maintenance of viability and/or potency within microcapsules.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Capsule material</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSCs</td>
<td>Alginate</td>
<td>Goren et al. (2010),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Penolazzi et al. (2010),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trouche et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>Alginate w/RGD sites</td>
<td>Markusen et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Agarose-collagen</td>
<td>Batorsky et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Agarose-chitosan-PEG</td>
<td>Paul et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>Agarose w/fibronectin</td>
<td>Karoubi et al. (2009)</td>
</tr>
<tr>
<td>ESCs</td>
<td>Alginate</td>
<td>Maguire et al. (2005),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Siti-Ismail et al. (2008),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wang et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>Agarose</td>
<td>Dang et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Agarose w/alginate core</td>
<td>Sakai et al. (2008)</td>
</tr>
<tr>
<td>NSCs</td>
<td>Alginate</td>
<td>Li et al. (2006),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Purcell et al. (2009)</td>
</tr>
</tbody>
</table>
## Microencapsulation Materials

<table>
<thead>
<tr>
<th>Differentiation lineage</th>
<th>Starting cell type</th>
<th>Capsule material</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteogenic</td>
<td>MSCs</td>
<td>Alginate</td>
<td>Ding et al. (2007), Penolazzi et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Collagen</td>
<td>Chan et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>ESCs</td>
<td>Alginate</td>
<td>Hwang et al. (2009), Tang et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>ADSCs</td>
<td>Alginate</td>
<td>Abbah et al. (2006)</td>
</tr>
<tr>
<td>Chondrogenic</td>
<td>MSCs</td>
<td>Alginate</td>
<td>Babister et al. (2008), Endres et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Collagen</td>
<td>Hui et al. (2008), Li et al. (2011)</td>
</tr>
<tr>
<td>Cardiac</td>
<td>ESCs</td>
<td>Alginate</td>
<td>Jing et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agarose</td>
<td>Bauwens et al. (2005)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>ESCs</td>
<td>Alginate</td>
<td>Chayosumrit et al. (2010), Wang et al. (2009)</td>
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<tr>
<td>Hepatocytic</td>
<td>ESCs</td>
<td>Alginate</td>
<td>Fang et al. (2007), Maguire et al. (2005)</td>
</tr>
<tr>
<td>Adipogenic</td>
<td>ADSCs</td>
<td>Alginate-gelatin</td>
<td>Yao et al. (2012)</td>
</tr>
<tr>
<td>Neural</td>
<td>ESCs</td>
<td>Alginate</td>
<td>Li et al. (2011b)</td>
</tr>
<tr>
<td>Hematopoietic</td>
<td>ESCs</td>
<td>Agarose</td>
<td>Dang et al. (2004), Rahman et al. (2010)</td>
</tr>
</tbody>
</table>
PNIPAAm-PEG hydrogel
(poly N-isopropylacrylamide co-polyethylene glycol)

Liquid at room temperature, gel at 37 degrees

20-fold expansion for 5 day passage, $10^{72}$-fold expansion over 280 days

Previously: 10-20 fold expansion after 20 days (alginate)
PNIPAAm-PEG: human pluripotent stem cells

Lei & Schaffer, PNAS, 2013
Downstream Bioprocessing-
Stem Cell Cultures

Challenges as scale increases:

1) Exchanging or reducing media volume
2) Separating cells
3) Storing (cryopreservation)
Current Good Manufacturing Practices (cGMP)

Defined by FDA and European Medicines Agency- Requirement for Clinical-Grade Cells

A) Optimization and standardization of hESC culture protocols for clinical use

B) GMP-grade hESCs

C) Optimization and standardization of hESC differentiation protocols towards clinical use

D) GMP-grade hESC-derived cell line approved for specific clinical application (as in B)

Example: UC Davis Facility

Unger et al, Human Mol Gen, 2008
### Example: Expansion of human hematopoietic stem cells (UCB)

Table 1. Summary of the main results focusing on umbilical cord blood ex vivo expansion under static conditions, either in liquid suspension cultures or in the presence of mesenchymal stem/stromal-derived feeder layers

<table>
<thead>
<tr>
<th>Expansion system</th>
<th>Cytokines/other molecules</th>
<th>Serum</th>
<th>Days in culture</th>
<th>Fold expansion TNC</th>
<th>CD34⁺</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liquid suspension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCF, TPO, G-CSF</td>
<td></td>
<td>No</td>
<td>10</td>
<td>56</td>
<td>4</td>
<td>(Shpall et al., 2002)</td>
</tr>
<tr>
<td>SCF; Flt-3L; IL-6; TPO; TEPA</td>
<td></td>
<td>10% FBS</td>
<td>21</td>
<td>219</td>
<td>6</td>
<td>(de Lima et al., 2008)</td>
</tr>
<tr>
<td>Notch ligand 6-1; SCF; Flt-3L, IL-6; TPO; IL-3</td>
<td></td>
<td>No</td>
<td>16</td>
<td>660</td>
<td>160</td>
<td>(Delaney et al., 2010)</td>
</tr>
<tr>
<td>IL-6, TPO; SCF, SR-1, Flt-3L</td>
<td></td>
<td>No</td>
<td>7</td>
<td>51</td>
<td>24</td>
<td>(Boitano et al., 2010)</td>
</tr>
<tr>
<td>SCF, Flt-3L, TPO, Angpt5</td>
<td></td>
<td>No</td>
<td>10</td>
<td>220</td>
<td>n.a.</td>
<td>(Zhang et al., 2008)</td>
</tr>
<tr>
<td>SCF, Flt-3L, TPO, IL-3</td>
<td></td>
<td>No</td>
<td>10</td>
<td>300</td>
<td>12</td>
<td>(Zhang et al., 2006)</td>
</tr>
<tr>
<td>SCF, TPO, Flt-3L, IL-3, G-CSF, GM-CSF and IL-6</td>
<td></td>
<td>No</td>
<td>7</td>
<td>n.a.</td>
<td>66</td>
<td>(Yao et al., 2006)</td>
</tr>
<tr>
<td>SCF, Flt-3L, MGDF, IL-3</td>
<td></td>
<td>30% FBS</td>
<td>9</td>
<td>n.a.</td>
<td>2.5</td>
<td>(Araki et al., 2006)</td>
</tr>
<tr>
<td>SCF, Flt-3L, MGDF, IL-3, 5-AzaD, TSA</td>
<td></td>
<td>30% FBS</td>
<td>9</td>
<td>n.a.</td>
<td>5</td>
<td>(Araki et al., 2006)</td>
</tr>
<tr>
<td>IL-6, TPO, SCF, Flt-3L</td>
<td></td>
<td>10% FBS</td>
<td>21</td>
<td>n.a.</td>
<td>9.5 (CD34⁺)</td>
<td>(Gunetti et al., 2008)</td>
</tr>
<tr>
<td>SCF, TPO, Garcinol</td>
<td></td>
<td>No</td>
<td>7</td>
<td>n.a.</td>
<td>7.4 (CD34⁺ CD38⁻ cells)</td>
<td>(Nishino et al., 2011)</td>
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<tr>
<td>SCF, TPO, Flt-3L</td>
<td></td>
<td>No</td>
<td>14</td>
<td>34</td>
<td>8.9 (CD34⁺)</td>
<td>(Du et al., 2012)</td>
</tr>
<tr>
<td>SCF, Flt-3L, TPO, G-CSF</td>
<td></td>
<td>No</td>
<td>14</td>
<td>400</td>
<td>80</td>
<td>(Duchez et al., 2012)</td>
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<tr>
<td>SCF, Flt-3L, TPO, IL-6 (10% O₂)</td>
<td></td>
<td>No</td>
<td>8</td>
<td>n.a.</td>
<td>20</td>
<td>(Tursky et al., 2012)</td>
</tr>
<tr>
<td><strong>Stromal co-culture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM MSC</td>
<td>SCF, TPO, Flt-3L</td>
<td>No</td>
<td>7</td>
<td>25</td>
<td>15</td>
<td>(Andrade et al., 2010)</td>
</tr>
<tr>
<td>BM MSC</td>
<td>SCF, TPO, G-CSF</td>
<td>No</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>(Robinson et al., 2006)</td>
</tr>
<tr>
<td>BM MSC</td>
<td>SCF, Flt-3L, bFGF, LIF</td>
<td>No</td>
<td>26</td>
<td>241</td>
<td>35</td>
<td>(da Silva et al., 2005)</td>
</tr>
<tr>
<td>BM MSC</td>
<td>SCF, Flt-3L, bFGF, LIF</td>
<td>No</td>
<td>18</td>
<td>280</td>
<td>35</td>
<td>(da Silva et al., 2010)</td>
</tr>
<tr>
<td>BM MSC</td>
<td>SCF, IL-3, G-CSF</td>
<td>No</td>
<td>12</td>
<td>21</td>
<td>21.4</td>
<td>(Madkaikar et al., 2007)</td>
</tr>
<tr>
<td>BM MSC</td>
<td>SCF, Flt-3L, TPO, IL-3</td>
<td>No</td>
<td>10</td>
<td>500</td>
<td>50</td>
<td>(Zhang et al., 2006)</td>
</tr>
<tr>
<td>PMSC</td>
<td>SCF, TPO, Flt-3L</td>
<td>No</td>
<td>14</td>
<td>99</td>
<td>13</td>
<td>(Luan et al.)</td>
</tr>
<tr>
<td>BM MSC</td>
<td>SCF, TPO, bFGF</td>
<td>No</td>
<td>7</td>
<td>n.a.</td>
<td>35</td>
<td>(Walenda et al., 2011)</td>
</tr>
</tbody>
</table>

SCF, stem cell factor; TPO, thrombopoietin; G-CSF, granulocyte colony-stimulating factor; Flt-3L, Fms-like tyrosine kinase-3 ligand; TEPA, tetraethylpenepentamine; IL, interleukin; Angpt5, angiopoietin-5; MGDF, megakaryocyte growth and development factor; 5-azaD, 5-aza-2'-deoxycytidine; TSA, trichostatin A; bFGF, basic fibroblast growth factor; SR-1, stem reginin 1; FBS, fetal bovine serum; FCS, fetal calf serum; TNC, total nucleated cells; PMSC, placental-derived MSCs.

Andrade et. al, J Tissue Eng Regen Med, 2013
Example: Expansion of human hematopoietic stem cells (UCB)

- a) Stirred Tank
- b) Roller bottles (spinner flasks)
- c) Rotating wall vessel
- d) Packed-bed bioreactor
- e) Wave bioreactor
- f) Perfusion chamber

Example: Expansion of human hematopoietic stem cells (UCB)

Csaszar et. al, Biotech Bioeng, 2013
Example: Expansion of human hematopoietic stem cells (UCB)

LAP=Latency Associated Peptide; complexed with TGFb1, use as surrogate measurement for TGFb1 concentration

Csaszar et. al, Biotech Bioeng, 2013
Example: Expansion of human hematopoietic stem cells (UCB)

Csaszar et. al, Biotech Bioeng, 2013
Example: Expansion of human hematopoietic stem cells (UCB)

Csaszar et. al, Biotech Bioeng, 2013
Example: Expansion of human hematopoietic stem cells (UCB)

Csaszar et. al, Biotech Bioeng, 2013
Example: Expansion of human mesenchymal stem cells

GMP conditions: Xeno-free, serum free
Cell isolation & thawing: CellStart CTS flasks
Serum free medium: StemPro, human albumin

Microcarriers
Non-porous polystyrene beads, CellStart coated
Example: Expansion of human mesenchymal stem cells

**Spinner flask**
- 100mL volume
- 4 days

**Stirred-Tank**
- 800mL volume
- Additional 7 days

Human bone marrow mesenchymal stem cells (black squares), final = 1.1 x10^8
Human adipose-derived stem cells (white diamonds), final = 4.5 x10^7

Dos Santos et al, Biotech Bioeng, 2014
Example: Expansion of human mesenchymal stem cells

Marker Expression & Differentiation

Dos Santos et. al, Biotech Bioeng, 2014
Example: Expansion of human mesenchymal stem cells

Human MSC clinical application
(e.g. graft vs. host disease, immune-regulation)

1x10^6/kg

150 lb (68 kg) person, would need 68x10^6
Scale-Down & Scale-Up of Stem Cell Culture

**Microfluidic**
- Scale down
  - 2D adherent culture
  - Precise spatial/temporal dynamics
  - High-throughput screening
  - $10^3$-$10^4$ mL
  - 500 μm

**Static**
- 10 mL
- 20 mm

**Bioreactor**
- Scale up
  - 10 mL
  - 20 mm
  - 3D suspension culture
  - Continuous monitoring & feedback
  - Scalable production

Fridley et. al, Stem Cell Res Ther, 2012
Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation


Dana-Farber Cancer Institute, Boston, MA; Harvard Stem Cell Institute, Cambridge, MA; Fate Therapeutics, San Diego, CA; Massachusetts General Hospital, Boston, MA; Indiana University, Indianapolis, IN; Children’s Hospital, Boston, MA; Brigham and Women’s Hospital, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA; and Howard Hughes Medical Institute, Chevy Chase, MD

Key Points

- Molecular profiling was used to optimize an ex vivo modulation protocol with dmPGE2 for UCB transplantation.
- Pulse treatment of UCB with dmPGE2 is safe and may lead to accelerated UCB engraftment and preferential cord chimerism.

Umbilical cord blood (UCB) is a valuable source of hematopoietic stem cells (HSCs) for use in allogeneic transplantation. Key advantages of UCB are rapid availability and less stringent requirements for HLA matching. However, UCB contains an inherently limited HSC count, which is associated with delayed time to engraftment, high graft failure rates, and early mortality. 16,16-Dimethyl prostaglandin E2 (dmPGE2) was previously identified to be a critical regulator of HSC homeostasis, and we hypothesized that brief ex vivo modulation with dmPGE2 could improve patient outcomes by increasing the “effective dose” of HSCs. Molecular profiling approaches were used to determine the optimal ex vivo modulation conditions (temperature, time, concentration, and media) for use in the clinical setting. A phase 1 trial was performed to evaluate the safety and therapeutic potential of ex vivo modulation of a single UCB unit using dmPGE2 before reduced-intensity, double UCB transplantation. Results from this study demonstrated clear safety with durable, multilineage engraftment of dmPGE2-treated UCB units. We observed encouraging trends in efficacy, with accelerated neutrophil recovery (17.5 vs 21 days, P = .045), coupled with preferential, long-term engraftment of the dmPGE2-treated UCB unit in 10 of 12 treated participants. This study was registered at www.clinicaltrials.gov as #NCT00890500. (Blood. 2013;122(17):3074-3081)