Optimizing Human Pluripotent Stem Cell (hiPSC) Production Process Using Vertical-Wheel Bioreactors

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Vertical-Wheel Stirred Suspension Bioreactors

Growth Platforms

Static
- Variability between flasks
- More difficult to regulate culture environment
- Labour intensive
- Batch or fed batch mode only

Bioreactor
- Well-mixed vessel
- Less labour intensive
- Easier to monitor and control process parameters
- Can operate in batch, perfusion mode, etc.
- Scalable

Vertical-Wheel Reactors

Horizontal Blade Suspension Bioreactor

PBS Vertical Wheel Bioreactor

Geometry of Vertical Wheel Bioreactor

Velocity though the Reactor Height (60rpm)
Inoculation of hiPSC Aggregates Pre-Formed in Static Culture

Early Studies by our Team using Spinners and Stirred-Tank Bioreactors: Unsuccessful cell growth and poor aggregate distributions when cells were inoculated as a single cell suspension. For this reason, the team developed a passaging method involving a pre-formation of hiPSCs in static culture prior to inoculating cells into a bioreactor.

1. Hydrodynamic Testing

2. Oxygen and Nutrient Testing

<table>
<thead>
<tr>
<th>Reference</th>
<th>Bioreactor Type</th>
<th>Fold/Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zweiggerdt et al. (2011)</td>
<td>Horizontal Blade</td>
<td>3-6 fold / 4-7 days</td>
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<tr>
<td>Abbasalizadeh et al. (2012)</td>
<td>Horizontal Blade</td>
<td>8 fold / 7-10 days</td>
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<tr>
<td>Haraguchi et al. (2015)</td>
<td>Horizontal Blade</td>
<td>10 fold / 12 days</td>
</tr>
<tr>
<td>Badenes et al. (2016)</td>
<td>Horizontal Blade</td>
<td>3.5 fold / 10 days</td>
</tr>
<tr>
<td>This Study</td>
<td>Vertical Wheel</td>
<td>32 fold / 6 days</td>
</tr>
</tbody>
</table>
Successful Serial Passage of hiPSCs in PBS 0.1L

Achieved a Total of >1E6-Fold Expansion (n=4)

Process

Results

![Graph showing cell expansion over time](image)

**Average Fold Expansion = 32**

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Serial Passaged hiPSC are of High Quality

After 28 days of VW bioreactor culture, hiPSCs maintain normal karyotype and characteristic pluripotent stem cell phenotype and function.

- **SSEA-4 (FITC)**: 98.1 ± 0.93
- **OCT-4 (Alexa Flour 700)**: 98.1 ± 1.07

**In vivo teratoma formation**

**Images:**
- A: A section of an in vivo teratoma with indicated differentiation into endoderm, ectoderm, and mesoderm.
- B: Endoderm differentiation.
- C: Ectoderm differentiation.
- D: Mesoderm differentiation.
Single cell inoculation led to successful cell growth in vertical-wheel bioreactors but not in traditional horizontal-blade bioreactors.
1. Single Cell vs Pre-formed Aggregate Inoculation in PBS 0.1

1. Single cell and pre-formed aggregate inoculation resulted in similar growth in the PBS-0.1 at all agitation rates

2. Single Cell Batch vs Fed-Batch in PBS 0.1

2. Fed-batch culture resulted in approximately 2X higher fold expansion at all agitation rates
Single Cell Harvest of hiPSCs from Cell Aggregates in PBS-0.1

Test of Proteolytic Enzymes and Dissociation Time with Agitation

- **Accutase**
- **TrypLE**
- **0.05% Trypsin**

Percentage of Cells in Aggregates vs. Enzyme Exposure Time (mins):

- 0 min
- 5 min
- 15 min
- 25 min
- 30 min

Dissociation Efficiency (20 mins):

- Typsin 0.05
- TrypLE
- Accutase

- Static Recovery

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20 min in Accutase + ROCi under agitation led to 95% dissociation efficiency
hiPSC cell growth remains high after bioreactor harvest and re-seeding of single cells into PBS 0.1 and 0.5.

Aggregate size and morphology remain healthy in both the PBS 0.1 and 0.5.
hiPSC maintain high quality pluripotent characteristics following optimized serial passage protocol with single cell inoculation and full bioreactor harvesting

Pluripotency Staining
Day 12 Bioreactor Aggregates

Tri-lineage Differentiation

Ectoderm
Neural cells

Endoderm
Hepatocytes

Mesoderm
Cardiomyocytes

Combined Process (Single Cell & Bioreactor Harvest)
Serial Passage in PBS-0.1 and PBS-0.5
1. Optimizing bioprocess variables (agitation, oxygen, and nutrients) in the PBS-0.1L reactor resulted in hiPSC expansion far greater than previously reported.

2. The expansion process is highly reproducible with the potential to generate over $1E12$ high quality PSC (starting from $2E6$) over 4 serial passages.

3. Bioprocess variables within the PBS reactor can be altered to overcome bioprocess bottlenecks in manufacturing hiPSCs (namely single cell seeding and full bioreactor harvests).