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8th March 2018

iCELLis® Bioreactors:
Virus Production from Bench to Industrial Scale

www.pall.com/bioreactors

Continuously Improving Bioprocesses
Challenges in Manufacturing Clinical Grade Virus for Gene Therapy

**Demand increase**
- Adherent cell culture on 2D growth surfaces limits capacity
- Cost of goods

**Robust processes**
- Process control: Batch to batch reproducibility and automation
- Reduce operator interventions

**Speed to product**
- Process: Reduced number of unit operations, simplicity
- Development: Process support and operator training
Scale Up Challenges: Vector Production

- Viral Titer = $5 \times 10^6$ vg/cm$^2$
- CS10 surface area = 6320 cm$^2$
- Total virus/CS10 = $3 \times 10^{10}$ vg

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dose</th>
<th>#CS10 Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ophthalmic</td>
<td>$2 \times 10^{11}$ vg/eye</td>
<td>7/patient</td>
</tr>
<tr>
<td>CAR-T</td>
<td>$2 \times 10^9$ vg/patient</td>
<td>15 patients from 1 CS10</td>
</tr>
<tr>
<td>Hemophilia</td>
<td>$2 \times 10^{15}$ vg/patient</td>
<td>66,667/patient</td>
</tr>
</tbody>
</table>

- System not scalable
- Limited automation
- No system control
iCELLis Fixed-Bed Bioreactor

- **Performance**
  - Proven technology for industrial-scale adherent cell culture
  - High and consistent virus titers

- **Usability**
  - Single-use bioreactor: no cleaning or cleaning validation
  - Reduced operator intervention
  - Reduced footprint: Up to 500 m² surface area ~ 790 10-layer vessels (CS10)

- **Process assurance**
  - Closed system
  - Automated and controlled
  - Validated control software

- **Scale-up**
  - Small scale system also available to facilitate process development
Macro-Carriers Compacted in Fixed-Bed

- Proprietary macro-carriers
- Microfibers of polyethylene terephthalate (PET), USP <87> Biological Reactivity Test, 
  *In Vitro*, Cytotoxicity and USP <88> Biological Reactivity Test, *In Vivo*, for Class VI - 50 °C Plastics, non woven
- Compressed into a doughnut shaped basket with surface from 4 m² up to 500 m²
- Different compaction levels and fixed-bed height available
- Growth surface areas of up to 20 m²/L in a controlled environment
Manufacturing Footprint Reduction

- **2.16 m² (23.25 ft²)** footprint
  - bioreactor controller and temperature control unit (TCU) on trolley
  - does not include surrounding single-use vessels and mixing equipment)

- ~ 535 kg (hardware) + 98 kg (filled SU bioreactor) = <300 kg/m²

Growth surface of 450 CS-10 in 2.16 m²
Process Control Capabilities

- High rates of gas exchange possible due to large surface area of thin falling film mass transfer (waterfall)
- **DO control**: gas exchange through a falling film or waterfall
  - Optimal gas transfer without sparging
  - Bubble free
  - High oxygenation capacity
  - Improved CO\(_2\) stripping
- **pH regulation**: base addition and CO\(_2\) exchange through headspace
- Gas flow control by 4 MFC
Cost of Goods

iCELLis bioreactors significantly increase LVV yields while reducing COGs

- Impact of scaling-out (20 - 200 MT10)
  - OPEX/L -29%  CAPEX/L ÷ 4; >18% of CoGs/L
- Impact of scaling-up (iCELLis 66 - 500 m²)
  - OPEX/L -48% CAPEX/L ÷ 7 ; < 10% of CoGs/L
  - 50% CoGs reduction with iCELLis bioreactor vs MT at similar scale
- Optimization to perfusion ➔ 1.6 - 1.9x CoGs reduction on pDNA mainly
- LVV throughput multiplied by 3 with Perfusion iCELLis bioreactor

Cost of Goods (OPEX+CAPEX) per liter of LVV bulk produced and LVV bulk annual throughput in MT10 and iCELLis bioreactors at small and large production scales

- CAPEX: Equipment, building, utilities
- OPEX: Labor, consumables, raw materials, waste
### iCELLis Bioreactor Formats and Comparable Flatware Equivalents

<table>
<thead>
<tr>
<th>iCELLis Bioreactor</th>
<th>Fixed-Bed Height</th>
<th>Fixed-Bed Volume (L)</th>
<th>Carrier Compaction (g/L)</th>
<th>Culture Surface Area (m²)</th>
<th>Equivalent CS-10</th>
<th>Equivalent HS-36</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>iCELLis Nano bioreactor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 cm</td>
<td>0.04</td>
<td>96</td>
<td>0.53</td>
<td>1</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>144</td>
<td>0.8</td>
<td>1</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>4 cm</td>
<td>0.08</td>
<td>96</td>
<td>1.06</td>
<td>2</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>144</td>
<td>1.6</td>
<td>2</td>
<td>0.8(\frac{9}{10})</td>
<td></td>
</tr>
<tr>
<td>10 cm</td>
<td>0.2</td>
<td>96</td>
<td>2.65</td>
<td>4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>144</td>
<td>4</td>
<td>6</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td><strong>iCELLis 500 bioreactor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 cm</td>
<td>5</td>
<td>96</td>
<td>66</td>
<td>105</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>144</td>
<td>100</td>
<td>159</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>4 cm</td>
<td>10</td>
<td>96</td>
<td>133</td>
<td>211</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>144</td>
<td>200</td>
<td>317</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>10 cm</td>
<td>25</td>
<td>96</td>
<td>333</td>
<td>528</td>
<td>185</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>144</td>
<td>500</td>
<td>794</td>
<td>278</td>
<td></td>
</tr>
</tbody>
</table>
## Viral Vector Production in iCELLlis Bioreactors – Results

<table>
<thead>
<tr>
<th>Vector</th>
<th>Cell</th>
<th>Size</th>
<th>Yield/cm²</th>
<th>Unit</th>
<th>Extrapolated to 500 m²</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV</td>
<td>HEK 293T</td>
<td>0.53 m²</td>
<td>4.7 x 10¹⁰ (transient)</td>
<td>VG</td>
<td>2.3 x 10¹⁷</td>
<td>University of Ulm (journal article, 2015)</td>
</tr>
<tr>
<td></td>
<td>HEK 293T/17</td>
<td>0.53 m²</td>
<td>3.6 x 10¹⁰ (transient)</td>
<td>VG</td>
<td>1.8 x 10¹⁷</td>
<td>St Jude (journal article, 2015)</td>
</tr>
<tr>
<td>Lenti</td>
<td>HEK 293T</td>
<td>0.53 m²</td>
<td>5.1 x 10⁶ (transient)</td>
<td>TU</td>
<td>2.5 x 10¹³</td>
<td>MolMed (conference presentation, 2016)</td>
</tr>
<tr>
<td>Retro</td>
<td>AM12</td>
<td>1.06 m²</td>
<td>7.3 x 10⁵ (transient)</td>
<td>TU</td>
<td>3.6 x 10¹²</td>
<td>MolMed (conference presentation, 2016)</td>
</tr>
<tr>
<td></td>
<td>HEK 293Vec</td>
<td>2.7 m²</td>
<td>9.26 x 10⁷ (stable)</td>
<td>VT</td>
<td>4.6 x 10¹⁴</td>
<td>Memorial Sloan Kettering (journal article, 2015)</td>
</tr>
<tr>
<td>Adeno</td>
<td>HEK 293</td>
<td>100 m²</td>
<td>6.1 x 10⁹ (infection)</td>
<td>VP</td>
<td>3.0 x 10¹⁶</td>
<td>FinVector (journal article, 2015)</td>
</tr>
</tbody>
</table>

VG = viral genomes, TU = transducing units, VT = viral titer, VP = viral particles
Pall Gene Therapy Hardware Platform

**Adherent Seed Train:**
Xpansion® multiplate bioreactor

**Adherent Bioreactor:**
iCELLis® 500

**Suspension Seed Train and Bioreactor:**
Allegro™ STR50, 200, 500, 1000

**Clarification:**
Stax®

**Purification:**
Allegro™ Single-Use Chromatography System

**Concentration:**
Allegro™ Single-Use Tangential Flow Filtration Systems

**Media/Buffer Mixing:**
Allegro™ Mixers

**Media/Buffer Storage/Handling:**
Allegro™ Plastic/Stainless Steel Totes

**Sterile Filtration:**
Allegro™ MVP Single-Use System
## Pall Gene Therapy Downstream Platform

<table>
<thead>
<tr>
<th>Crude harvest volume (L)</th>
<th>Clarification Depth filter</th>
<th>Chromatography</th>
<th>UFDF</th>
<th>Sterile Filtration (0.2µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.8</td>
<td>Supracap 50</td>
<td>Acrodisc XT</td>
<td>Cadence SUTFF module</td>
<td>Acrodisc Filters</td>
</tr>
<tr>
<td>1-10</td>
<td>Supracap 100</td>
<td>Mustang 60mL Capsule</td>
<td>Cadence SUTFF module</td>
<td>Mini Kleenpak 20 Capsule</td>
</tr>
<tr>
<td>10-1000</td>
<td>Stax disposable</td>
<td>Mustang XT5000</td>
<td>Cadence SUTFF module</td>
<td>Kleenpak Nova Capsule</td>
</tr>
<tr>
<td><strong>Hardware</strong></td>
<td>Allegro MVP</td>
<td>SU-Chrome</td>
<td>SU-TFF</td>
<td>Allegro filling system with capsule, bio containers and needles</td>
</tr>
</tbody>
</table>
Redefining Successful Development

Pall Biotech Process Development Services

**BETTER PROCESS**
Leveraging the unique features of our technologies and services results in higher performance unit operations, less process steps and reduced change over time

**FASTER DEVELOPMENT**
Our experts will efficiently transfer processes from any stage and provide targeted optimization and scale-up support up including consistency batches, SOP’s and more

**LOWEST COST**
Access to our experts and our well-equipped process laboratories gives you the opportunity to get most out of your process without impacting your schedule

Improvement without Compromise
Our complete single-use solutions include media preparation, cell harvest and separation, purification, formulation and filling.

Dedicated project engineers support the integration of all unit operations into a fully automated process, using Pall platform software solutions.

Automated single-use solutions for a total process.
Thank You
bioreactors@pall.com
www.pall.com/bioreactors

Continuously Improving Bioprocesses
Appendix 1: Global Installations for iCELLis Nano and iCELLis 500 Bioreactors
Global Installations for iCELLis Nano Bioreactors

Worldwide Install Base
220 iCELLis Nano bioreactors
- Human vaccines
- Animal vaccines
- Gene therapy
- Rec protein

Western Hemisphere
87 iCELLis Nano bioreactors

EU
107 iCELLis Nano bioreactors

Asia
26 Allegro iCELLis Nano bioreactors
Global Installations for iCELLis 500 Bioreactors

Worldwide Install Base
32 iCELLis 500 bioreactors
- Human vaccines
- Animal vaccines
- Gene therapy
- Rec protein

Western Hemisphere
6 users - 11 units
- 1 veterinary vaccine
- 4 viral vector production
- 1 CMO

EU
9 users - 12 units
- 3 viral vector production
- 1 CRO
- 4 vaccine
- 1 diagnostic

Asia
4 users - 9 units
- 2 rec protein
- 2 human vaccines
Appendix 2: References

Continuously Improving Bioprocesses
Journal Articles – Gene Therapy


Memorial Sloan Kettering Cancer Center. 2015. Large-scale clinical-grade retroviral vector production in a fixed-bed bioreactor. Journal of Immunotherapy.


University of Ulm. 2015. Rational plasmid design and bioprocess optimization to enhance recombinant adeno-associated virus (AAV) productivity in mammalian cells. Biotechnology Journal.
3rd Party Presentations, Posters and Case Studies (Partial List)

- SAFC, “Assessment of the iCELLis fixed-bed bioreactor for the production of viral vectors, the next step to scale-up adherent cultures.” Conference, ISBioTech, 2016*.
- University College London, “Adeno-associated virus production using a disposable fixed-bed bioreactor: from bench scale to industrial scale.” Conference, ESGCT, 2013*.

*Available upon request