Perfusion and Beyond
The XCell™ ATF System

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Field Application Scientist

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Inspiring advances in bioprocessing since 1985

Repligen Corporation: one of the fastest-growing companies in North America

Most of the world’s monoclonal antibodies are purified on Repligen’s Protein A

Upstream and Downstream Applications
- Chromatography: 19%
- Proteins: 38%
- Filtration: 43%

AN INDUSTRY LEADER IN
- Pre-packed Chromatography Columns
- Cell Culture process intensification
- Single-use Tangential Flow Filtration
- Protein A Affinity ligands manufacturing

20 of the Top 25 Pharma companies use Repligen products

Multiple global GMP manufacturing sites
Rapid growth through innovative launches and strategic acquisitions

Protein A ligands commercial manufacturing
Launched Immobilized Protein A resins
Acquired BioFlash technology: Pre-packed columns
Acquired Novozymes Biopharma: Native Protein A, growth factors
Launched OPUS® pre-packed columns
Acquired Refine XCell™ ATF technology: XCell™ ATF
Launched OPUS® 45/60
Launched XCell™ Single-use Systems
Launched XCell™ Single-use Systems
Launched XCell™ Single-use Systems
Launched OPUS® R
Launched XCell™ 10 Single-use
Acquired Atoll: OPUS™ PD
Acquired Spectrum® HF TFF Systems
Acquired TangenX™ TFF Cassettes

Repligen is one of the fastest growing companies in North America
Repligen products used in PD workflow
Repligen products used in Commercial bioprocessing
Agenda

Perfusion Basics

XCell™ ATF Introduction

XCell™ ATF Applications
• Perfusion
• CFB
• HD Cell Banking
• N-1
Perfusion Basics

Traditional bioreactor processes

Perfusion Process

Benefits

Drivers
The traditional forms of cell culture processes are Batch, Fed-batch, Chemostat and Perfusion.

Fed-batch and Perfusion are typically used as a manufacturing platform.
Continuous Process - Perfusion

What is Perfusion?

- A cell culture process involving the continuous replenishment of cell culture media, removing of waste products, and harvesting of the product of interest, all while retaining the cells within the bioreactor system.
- Result = higher cell densities, longer runs, and higher productivity.
Perfusion Benefits:

- Economic benefits
  - Higher cell densities -> Higher productivity
  - Longer runs -> Less turnaround
  - Smaller bioreactors -> Lower capital investment costs -> Single-use friendly
  - Smaller facilities -> Lower operating costs
  - R&D scale is commercial scale

- Benefits to cell culture and product quality
  - Removal of waste products
  - Replenishment of nutrients, fresh environment
  - Stabilized cell environment “Steady State”
  - Product is available almost immediately

<table>
<thead>
<tr>
<th></th>
<th>Fedbatch</th>
<th>Perfusion</th>
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<tbody>
<tr>
<td>Typical Bioreactor size</td>
<td>5,000 – 20,000</td>
<td>500 – 2000L</td>
</tr>
<tr>
<td>Single-use friendly</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Typical Run length</td>
<td>~2 weeks</td>
<td>1- 2 months</td>
</tr>
<tr>
<td>Estimated runs per year</td>
<td>20 - 30</td>
<td>5 - 10</td>
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<tr>
<td>Steady state environment</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Typical Cell Density</td>
<td>5 - 25 e⁶</td>
<td>50 - 100e⁶</td>
</tr>
<tr>
<td>Productivity per run</td>
<td>1x</td>
<td>5-20x</td>
</tr>
<tr>
<td>Product Quality Consistency</td>
<td>+</td>
<td>++</td>
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</table>
Drivers

Product Quality Drivers

<table>
<thead>
<tr>
<th>Protein</th>
<th>Trade name/Company</th>
<th>Driver</th>
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<tbody>
<tr>
<td>rFVII</td>
<td>NovoSeven (NovoNordisk)</td>
<td>product quality (γ-carboxylated, labile)</td>
</tr>
<tr>
<td>rFVIII</td>
<td>Kogenate (Bayer)</td>
<td>Product quality (labile)</td>
</tr>
<tr>
<td></td>
<td>Refacto (Pfizer)</td>
<td>Product quality (labile)</td>
</tr>
<tr>
<td>rProtein C</td>
<td>Xigris (Eli Lilly)</td>
<td>Product quality (γ-carboxylated, labile)</td>
</tr>
<tr>
<td>EPO</td>
<td>Epogen (Amgen), other EPO</td>
<td>Product quality (highly glycosylated)</td>
</tr>
<tr>
<td>mAbs</td>
<td>Reopro, Remicade (Janzen)</td>
<td>Space-time yield</td>
</tr>
<tr>
<td></td>
<td>Simulect (Novartis)</td>
<td>Scale of production</td>
</tr>
<tr>
<td></td>
<td>Rebif (Merck-Serono)</td>
<td></td>
</tr>
<tr>
<td>Enzymes</td>
<td>Sanofi - Genzyme</td>
<td>Product quality (labile)</td>
</tr>
<tr>
<td></td>
<td>Shire</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biogenin</td>
<td></td>
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</table>

Trends Driving Change

- **Process intensification / Capex reduction**
  - High density cell cultures from cell bank to final bioreactor
  - Reduction in size and number of unit operations
  - Capacity increases within existing infrastructure

- **CoG now a process consideration**
  - Drive to improve facility productivity
  - Small, modular, facilities can now provide equivalent drug quantities using ATF Technology

- **Drive to towards mindset of Continuous Processing**
Xcell™ ATF System

What is ATF

Backflush action

System sizing

Bioreactor connection
XCell™ ATF system Overview

- A cell retention device based on Alternating Tangential Flow (ATF) Filtration
- Cross flow is derived from a diaphragm pump.
- Filter is based on hollow fiber technology.
- Dynamic back flushing effect amounts to a practically self-cleaning filter!
XCell™ ATF System – How it works

Unique self cleaning action - Animation
XCell™ ATF Flow

Pressure cycle (P-cycle)
XCell™ ATF Flow

Exhaust cycle (E-cycle)

1. EXHAUST

2. DIAPHRAGM moves down
   BACKFLUSH ACTION
   Cleans filter

3. RAPID FLOW THROUGH FIBERS

4. FLOW OUT OF BIOREACTOR

Filtered Outflow
XCell™ ATF System
Backflush

- Transmembrane pressure was measured at both ends of the filter.

- Higher XCell™ ATF rate results in greater TMP changes.

- Higher XCell™ ATF rate = stronger backflush.

- Backflush occurs on both P and E cycles.
  - In **exhaust** cycle, back flush was observed in bottom part of the filter as TMP (P2-P3B) was negative.
  - In **pressure** cycle, back flush was observed in top part of the filter as TMP (P1-P3A) was negative.
Overview

XCell™ ATF Perfusion System

Media-In Level Control

Cell Bleed

Harvest

ATF System

Fresh Media

Bleed Waste

Pump

Harvest/Spent Media

Air

Vac
XCell™ ATF System Connection

Bioreactor connection

- Only a one connection required.
- Easily swappable if configured properly.
- Small scale connection is typically via bioreactor headplate. Large scale is via bioreactor sideport.
### Guideline bioreactor volume for each XCell™ System for Perfusion

<table>
<thead>
<tr>
<th>System</th>
<th>Filter Effective Surface Area (m²)</th>
<th>Pump Displacement Volume (L)</th>
<th>ATF Flow Range (LPM)</th>
<th>Typical Bioreactor Sizes (L)</th>
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</thead>
<tbody>
<tr>
<td>ATF2</td>
<td>0.13</td>
<td>0.1</td>
<td>0.3 - 1.5</td>
<td>2 - 10 L</td>
</tr>
<tr>
<td>ATF4</td>
<td>0.77</td>
<td>0.4</td>
<td>2 - 10</td>
<td>10 - 50 L</td>
</tr>
<tr>
<td>ATF6</td>
<td>2.5</td>
<td>1.3</td>
<td>5 - 20</td>
<td>50 - 200 L</td>
</tr>
<tr>
<td>ATF10</td>
<td>11</td>
<td>6</td>
<td>20 - 90</td>
<td>200 - 1000 L +</td>
</tr>
</tbody>
</table>

- Single-Use available

- **NOTE:** These numbers are just guidelines, actual capacity and vessel size depend upon process conditions.

- Systems are already used in both clinical manufacture and commercial manufacturing and come with validation packages.
XCell™ ATF
System Family

DESIGNED FOR SCALABILITY

Similar fiber lengths, just increasing fiber count to increase SA

The XCell™ ATF Single-use uses the same filters

NOTE | The XCell™ ATF 4 is 30cm and a little more attention is required to scale-up accurately
The XCell™ ATF System

Bioreactor Connection – SS bioreactor
The XCell™ ATF System

Bioreactor Connection – Single-use bioreactor
The XCell™ ATF System

Bioreactor Connection – SU-ATF to SUB

SU ATF6

SU ATF10
XCell™ ATF Applications

Perfusion – Continuous Processing

High Density Cell Banking

N-1

Extended Harvest
What is a batch in perfusion?

- Continuous harvest, batches determined by DSP

Microfiltration (0.2-0.5µ) filter retains cells inside bioreactor while protein passes through the filter.
ATF Perfusion vs. Fedbatch

“Medium” cell density

Fedbatch was a typical 14 day culture, while ATF Perfusion was run for 25 days.

Maximum VCD in Fed-Batch culture was 14e6 cells/mL whereas in ATF Perfusion the VCD was maintained at 40-60e6 cells/mL at higher viability.

Drop in viability during ATF Perfusion was due to bioreactor issue, and not typical.
ATF Perfusion vs. Fedbatch

“Medium” cell density

- The cumulative amount of IgG produced in ATF Perfusion culture is 3 times higher than in Fed-Batch culture after 14 days of cultivation
  - Fed-Batch: 1.42g (14 days)
  - ATF Perfusion: 4.41g (14 days)
  - 10.8g (25 days)

- The cell specific productivity in ATF Perfusion culture is slightly higher than in Fed-Batch culture
  - Fed-Batch: 6.3 pg/cell/day
  - ATF Perfusion: 7.5 pg/cell/day
High density perfusion run was maintained at “steady state” at high VCD of approx. 100e6 cells/ml and consistent viability >90%.
High Cell Density Perfusion

“High” cell density

- No change in cell specific productivity throughout the run, even at high 100e6 cells/m cell density.

\[ y = 4.9809x + 202597 \]

\[ R^2 = 0.9992 \]
Continuous Processing

Linking Upstream and Downstream unit operations.

- Perfusion can result in larger harvest volumes, depending on perfusion rates.
- A continuous concentration step can be implemented to minimize volume and control desired concentration for DSP.
Concentrating a process to produce higher cell densities and higher product yields in shorter time.

Reducing the number of upstream steps through increased cell concentration at each step

- Cell banking
- High density seed transfers
- N-1 perfusion
- Concentrated perfusion
- Concentrated fed-batch

HD seed transfers remove whole unit operations, increasing efficiency
Using the ATF to create HD Cryo-Seed Intermediate (HDCSI) bags

**Procedure Overview**

- Expand existing bank into a reactor
- Run perfusion process desired cell concentration, e.g. 50e6/ml, in 4-7 days
- Cool down bioreactor contents to room temperature
- Concentrate cells through the ATF System in 5-15 minutes
- Add freezing medium into the reactor, while mixing
- Fill bag manifolds with cell suspension directly from reactor
- Freeze bags in blood bank type freezer

**Advantages**

- All steps are performed within the sterile reactor environment
- No centrifuge required
- Same cell concentration & volume in each bag
- First & last bag from the same environment
- Reduced manual handling of cells
- Faster bank creation: complete <2 hours from harvest
Industry case study
Genentech
“FASTEC” – Frozen
Accelerated Seed Train for
Execution of a Campaign

Source: Gargi Seth, Development of a New Bioprocess Scheme Using Frozen Seed Train Intermediates to Initiate CHO Cell Culture Manufacturing Campaigns, Biotechnology and Bioengineering, 2012
N-1 Perfusion

Seed or N-1 Perfusion
Reducing the number of upstream steps by eliminating one or more steps through high cell density seed transfers

N-1 or N-2 Benefits
- Reduced seed expansion bioreactors
- Reduced operational risk
- Minimize time in production bioreactor
- Reduced bioreactor footprint & cost

Using the ATF to reduce number and size of expansion bioreactors
N-1 Perfusion with high seed in FB

Intensified N-1 used to inoculate Fedbatch bioreactor at higher seeding density

- Using cells from the N-1, cells were individually inoculated into low seeding density (0.5E6 cells/mL) and high seeding density (10E6 cells/mL) fed-batch production cultures.

- The low seed culture reached a max VCD of 19E6 cells/mL in a two week run, whereas the high seed culture reached 25E6 cells/mL in a one week run.

- Note: A 3L bioreactor (2L wv) was used to mimic a 2000L production fed batch culture.
N-1 Perfusion with high seed in FB

Intensified N-1 used to inoculate Fedbatch bioreactor at higher seeding density

The high seed culture was able to produce same amount of protein in 7 days as the low seed in 14 days.

No difference was observed in cell specific productivity between low and high seed cultures.
N-1 Perfusion with high seed in FB

Intensified N-1 used to inoculate Fedbatch bioreactor at higher seeding density

### Protein Quality

<table>
<thead>
<tr>
<th>Conditions (Seeding Density)</th>
<th>Glycan Analysis</th>
<th>Size Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%G0F</td>
<td>%G1F</td>
</tr>
<tr>
<td>Low Seed (0.5E⁶ cells/mL)</td>
<td>79.7%</td>
<td>15.8%</td>
</tr>
<tr>
<td>High Seed (10E⁶ cells/mL)</td>
<td>82.2%</td>
<td>14.4%</td>
</tr>
</tbody>
</table>

- Equivalent protein quality, no significant changes in protein aggregation and glycosylation
- Note: Small percentages of G0 and G2F were also observed in the protein samples. Total percentage of aggregates were measured by comparing to the monomer
Process Intensification

HD seed transfers and N-1 remove whole unit operations, increasing efficiency.

Conventional Fed-Batch Process

Efficient Fed-Batch Process using ATF
eXtended Harvest

Extended Harvest using the XCell ATF to increase final yields

Conventional Clarification

- Fed-batch BR (Typically 14 days)
- Centrifugation
- Depth Filtration
- DSP

eXtended Harvest using XCell ATF

- Fed-batch BR
  - Day 1 - 10
  - Fresh Media
  - Harvest
- Fed-batch BR
  - Day 11 - 14
  - XCell ATF
- DSP
Extended Harvest using the XCell ATF to increase final yields

- Viable cell density increased by more than 3-fold in fed-batch cultures using eXtended Harvest compared to traditional FB.

- Viability in fed-batch cultures using eXtended Harvest stayed at >90% until the end of the run, a healthier environment for the cells and the product of interest.
Extended Harvest using the XCell ATF to increase final yields

- Cell specific productivity is higher in eXtended Harvest process compared to traditional FB process
- Higher perfusion rate (1vvd vs 0.5vvd) shows higher cell specific productivity.
Extended Harvest using the XCell ATF to increase final yields

- FB using eXtended Harvest process produced up to 2X more IgG compared to traditional FB within the same culture time.

- Harvested product is 0.2µm filtered and ready for downstream processing.
eXtended Harvest using XCell ATF

Benefits
- 2X increase in protein production w/o additional time
- Sterile, closed system and single step process
- Keeps cells healthy until the end of the run
- Healthy cells = Better control over product quality
- Reduction in residence time of product in reactor
- Harvested material is ready for DSP

Limitations
- Expanded harvest volume
- Higher media consumption
Applications
summary

Where ATF is used today
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