The Use of Disposable Technology for Downstream Processing

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Current Biomanufacturing Facilities

- Large hard-piped, stainless steel based facilities with stainless steel bioreactors
- Very expensive to build and validate
  - Construction costs \( \geq \$300 \text{ Million} \)
  - Construction timelines 2-5 years or more

- Controlled environment, highly classified suites
  - Tightly controlled flow of people, materials, and equipment
- Huge utilities for WFI, HVAC, Clean steam, CIP
  - Extensive piping, transfer panels, complex operations

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Photos courtesy of Lonza Biologics
A Brief History of Disposable Systems in Biomanufacturing

- **1970s:** Use of flasks, pipettes, filters, blood bags
- **1990s:** Bags for media, harvest, buffer prep
- **1996:** Introduction of the Wave bioreactor
- **1998:** Introduction of first membrane adsorbers
- **2004:** First 250 L disposable stirred-tank bioreactor
- **2006:** First 1,000 L disposable stirred-tank bioreactor
- **2009:** First 2,000 L disposable stirred-tank bioreactor

Latest implementation of disposables include:

- Harvest clarification
- Cell concentration
- Downstream processing
- Fill/finish operations

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Disposable Options Across Entire Manufacturing Flowpath

Cell Culture → Recovery/Downstream Processing → Formulation/Fill

Disposable Sensors

Media Prep/Storage → Buffer Prep/Storage

All conventional unit operations now have disposable format solutions
Disposable Systems for Buffer and Media Preparation

Thermo Scientific HyClone Single-Use Mixer (S.U.M.)

ATMI Pad-Drive™ Single-Use Mixing System

Mobius® Single-Use Mixing System (Millipore)

Systems not yet in routine use but adoption is growing

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Disposable Systems for Media and Buffer Storage

Routinely used in biomanufacturing

Technology available since 1990s

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<table>
<thead>
<tr>
<th>Vendor</th>
<th>Scale</th>
<th>Product Name</th>
<th>Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xcellerex</td>
<td>Up to 2,000 L</td>
<td>XDR™ Bioreactor</td>
<td>Stirred-tank</td>
</tr>
<tr>
<td>Thermo Fisher</td>
<td>Up to 2,000 L</td>
<td>Single-use Bioreactor (S.U.B.)</td>
<td>Stirred-tank</td>
</tr>
<tr>
<td>(Hyclone)</td>
<td></td>
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</table>
## More Process Scale Disposable Bioreactors

<table>
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<th>Technology</th>
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<tbody>
<tr>
<td>Sartorius</td>
<td>Up to 1,000 L</td>
<td>Biostat® Culti-bag</td>
<td>Stirred-tank</td>
</tr>
<tr>
<td>GE Healthcare (Wave)</td>
<td>Up to 1,000 L</td>
<td>Wave Bioreactor</td>
<td>Rocking Platform</td>
</tr>
</tbody>
</table>

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Stainless Steel vs. Disposable Bioreactors

- Comparable cell growth and productivity
- No cleaning or sterilization required
- Fast turnaround

- Minimal validation requirements
- Increased flexibility and process portability
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**Downstream Processing Unit Operations**

- **Normal Flow Filtration**
  - Clarification
  - Virus removal
  - Aseptic processing

- **Tangential Flow Filtration**
  - Concentration
  - Buffer exchange
  - Clarification

- **Centrifugation**
  - Clarification

- **Cell Breakage/Homogenization**
  - Recovery of intracellular products

- **Refolding**
  - For inclusion body products

- **Crystallization/Precipitation**

- **Chromatography**
  - Typical downstream process includes 3 – 4 steps
    - Ion exchange
    - Hydrophobic interaction
    - Affinity
    - Size exclusion
    - Reverse phase
Disposable Technologies for Filtration

Millistak® Pod Disposable Depth Filter System

Zeta Plus™ Encapsulated System

Routinely used for media and buffer prep; increasing use in bioreactor harvesting and other downstream processes
Disposable Format for Depth Filtration

➢ Improved CIP of Hardware
  • Self-contained, disposable Pods
  • Disposable feed ports and fittings
  • No product contact with endplates or process skid

➢ Improved Handling
  • No messy spent filters
  • Lightweight, easy set up and use
  • No hoist or high ceiling required

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Photos courtesy of Millipore
Disposable Formats for Chromatography

- Membrane adsorbers
  - Sartorius Sartobind®
  - Pall Mustang®
  - Natrix Adsept™

- Monoliths
  - BIA Separation

- Disposable chromatography columns
  - GE Ready-to-Process™
  - Repligen BioFlash™

- Other
  - Simulated moving bed
  - Scouting columns and technologies

Suppliers profiled are not a complete list nor an endorsement of any specific company or technology.
Advantages of Disposable Membrane Adsorbers

- Much smaller size/volume compared to conventional columns
- Minimize manufacturing time
- Minimize buffer requirements and tank usage
- No batch to batch carry-over

- High capacity, high flow rate leading to high throughput
- No column packing
- Elimination of cleaning validation, protein carry-over validation, reuse validation and storage validation

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DNA Removal with Membrane Adsorbers

- Membrane run in flow-through mode
  - Product flows through
  - DNA remains bound to membrane

Product Capture on a Membrane Adsorber

Photos and data courtesy of Natrix Bioseparations
Monoliths for Downstream Processing

- Continuous stationary phase with tightly controlled channel sizes cast as a homogeneous unit
  - Large channels make the adsorptive surface directly accessible to solutes as they pass through the column
  - Laminar flow eliminates peak broadening
  - Capacity and resolution relatively unaffected by flow rate
- Less sensitive to variations in flow rate, column configuration, and residence time
Monoliths Idea for Purification of Viral Vaccines

0.34 ml disk  8 ml column  80 ml column  800 ml column  8000 ml column
3-8 ml/min  10-40 ml/min  40-250 ml/min  400-2000 ml/min  2000-10000 ml/min

Photos and data courtesy of BIA Separations
Purification of Live Replication-dependent Influenza H1N1

Purification on CIM® DEAE Monolith

- Flow rate: 43 mL/min
- Load: 140 mL partially purified H1N1 solution
- Wash: Equilibration buffer
- Elute: 500 mM NaCl
- Clean: 2 M NaCl

Photos and data courtesy of BIA Separations
Comparison of New and Old Purification Process

**MONOLITH BASED PURIFICATION PLATFORM**
- Expansion of Vero cells
- Infection
- Harvest and Clearance
- Benzonase
- TFF
- CIM QA Monolith: Anion exchange chromatography (AIEX)
- Size Exclusion (SEC)

**CENTRIFUGATION BASED PURIFICATION PLATFORM**
- Expansion of Vero cells
- Infection
- Harvest and Clearance
- Benzonase
- Tangential Flow Filtration (TFF)
- Ultracentrifugation (UCF)
- Adjustment to final formulation

<table>
<thead>
<tr>
<th>Process</th>
<th>Monolith</th>
<th>Centrifugation</th>
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<tbody>
<tr>
<td>Infectious virus yield</td>
<td>47.3%</td>
<td>11.4%</td>
</tr>
<tr>
<td>DNA removal</td>
<td>99.96%</td>
<td>99.50%</td>
</tr>
<tr>
<td>Protein removal</td>
<td>97.8%</td>
<td>97.4%</td>
</tr>
</tbody>
</table>

Photos and data courtesy of BIA Separations
Disposable Chromatography Examples

Ready-to-Process Chromatography Columns (GE Healthcare)
12.6, 25.1, and 35.9 cm ID x 20 cm H
Columns pre-packed with conventional media

BioFlash Disposable Format Chromatography (DFC™) Columns (Repligen)
1.2, 8, and 20 cm ID x variable H
Columns pre-packed with conventional media

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Continuous Purification with Simulated Moving Bed

- SMB is extremely scalable and leads to substantial reduction in manufacturing costs
- Fully disposable processing train can be paired with disposable bioreactors resulting in smaller process footprint
- Higher throughput per square foot of manufacturing space enables smaller manufacturing facilities

Photos courtesy of Tarpon Biosystems

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History of Simulated Moving Bed Technology

Simulated Moving Bed continuous chromatography is a well established unit operation.

Advantages include 40-60% reduction in buffers, solvents, and chromatography media.

History of SMB:

- 1950 – 1960 SMB developed for petrochemical industry
- 1960 – 1970 First applications in food industries
- 1980 – 1990 Fine chemical industries applications
- 1990 – 2000 Chiral separations for pharma industry
- 2000 – 2010 Biopharmaceutical applications
Continuous Downstream Processing with BioSMB™

- Process uses same fundamental phenomena as batch processes
  - No change in media and buffer composition
  - Same steps for binding, washing, and elution as in the corresponding batch process
- System volume depends on mass transfer kinetics, not binding capacity or titer

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<thead>
<tr>
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<th>Batch</th>
<th>BioSMB</th>
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<tbody>
<tr>
<td>Titer</td>
<td>3.5 gm/L</td>
<td></td>
</tr>
<tr>
<td>Batch size</td>
<td>2000 L</td>
<td></td>
</tr>
<tr>
<td>Productivity [g/L/day]</td>
<td>360</td>
<td>2630</td>
</tr>
<tr>
<td>Processing time</td>
<td>05:10 08:00 12:00 24:00</td>
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</tr>
<tr>
<td>Protein A media [L]</td>
<td>88 8.0 5.2 2.6</td>
<td></td>
</tr>
<tr>
<td>Buffer [L]</td>
<td>4600 3100 3350</td>
<td></td>
</tr>
<tr>
<td>Number of columns</td>
<td>1 8 8 12</td>
<td></td>
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<tr>
<td>Cycles per batch</td>
<td>2 19 29 58</td>
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<tr>
<td>Protein A Media Costs</td>
<td>$ 880k $ 80k $ 52k $ 26k</td>
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</tbody>
</table>

Data courtesy of Tarpon Biosystems
BioSMB™ Makes Size Exclusion Chromatography Feasible

Continuous gel filtration chromatography of a vaccine VLP using a 12 column process set-up

Photos and data courtesy of Tarpon Biosystems
Freeze/thaw of Process Intermediates and Final Bulk Systems provide more homogeneous and gentle freezing of biopharmaceuticals.
Disposable Container/Closure Systems for Aseptic Filling

Aseptic Technologies *Crystal® Closed Vial Technology*

- No need to wash, depyrogenate, or sterilize (hot air-tunnel)
  - No need for WFI
  - Vials come pre-sterilized with intact seal
  - No conventional capping required
Influenza Vaccine Manufacturing Using Disposables

Cell Substrate Preparation → Infect & Incubate → Remove Cells, Purify VLPs → Inactivate Virus

Insect Cell Culture-Based Flu Vaccine Production in Disposable Mfg Systems:

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Driving Forces for Single-Use Technologies

- Improved return on capital
  - Reduced and deferred capital investment
  - Increased speed of deployment
  - Cost structure shifted to variable costs
    - Significant reduction in capital equipment costs (>70%)
- Reduced process equipment complexity
  - Process and product flexibility
  - Improved process control and portability
- Reduced facility complexity and cost
  - Faster construction, commissioning, and launch
  - No change-over cleaning/validation between strains/products
  - Significant reduction in facility/equipment validation

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Current Status of Disposable Systems

- Disposables are accepted industry-wide for development, clinical, and manufacturing use
- Almost all the unit operations and process components used in biomanufacturing can be replaced by disposables
- The cost benefit, convenience, and flexibility of moving to disposables are well documented
- More and more vendors are developing single use and disposable products
- Companies are now moving to disposables for clinical and potentially commercial manufacturing
- A completely disposable manufacturing flowpath should be possible in the foreseeable future
Increased facility utilization by reducing change-over time
Reduced fixed piping
Reducing cleaning and validation costs in multi-product operations
Improved process portability
Easier to manage and implement process changes

Increased operational flexibility by minimizing or eliminating multi-use equipment

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Photo courtesy of Acceleron Pharma
Thank you!

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