The Use of Disposable and Alternative Purification Technologies for Biopharmaceuticals

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Presentation outline

- Driving forces for use of disposable and alternative purification technologies
- Examples of current technologies
- Obstacles to implementation
- Future possibilities
Driving Forces
Driving forces for disposable and alternative purification technologies

- Business drivers for alternative approaches to biopharmaceutical manufacturing:
  - Need to reduce capital investment in high-risk projects
  - Speed to clinical proof-of-concept and commercial launch

- Operational trends favoring disposables:
  - Increasing focus on cleaning and cleaning validation
  - Increasing availability of suitable disposable processing equipment throughout the biopharmaceutical flowpath:
    - Flexible bioreactors
    - Media and buffer bags
    - Disposable aseptic filling equipment

- Trend towards large volume monoclonal antibody processes:
  - Increasing productivity and scale of upstream processes
  - Purification increasingly becoming a bottleneck
The majority of manufacturing costs for most biopharmaceuticals are fixed costs.

Data from a process economic model of a typical mammalian cell culture-derived monoclonal antibody.
Project risk and capital cost decrease significantly as development progresses.

* - Cost of Capital estimate based on discount factors from a risk-adjusted NPV analysis.
Technologies that
  • reduce capital investment and/or
  • increase speed to risk-reduction milestones
are inherently more valuable in high-risk projects

⇒ There is a compelling financial and risk-management argument for disposable manufacturing technologies in early-stage biopharmaceutical development
Examples of Process Use of Disposable Bioreactors

➢ Use of disposable bioreactors for production at 100L+ scale:

➢ Use of disposable bioreactors for production at smaller scales:

➢ Use of disposable bioreactors to replace conventional bioreactors in the inoculum train of large scale processes:
  • Knevelman, C et al, “Characterisation and Operation of a Disposable Bioreactor as a Replacement for Conventional Steam in Place Inoculum Bioreactors for Mammalian Cell Culture Processes,” ACS poster presentation (2002).
Disposable products* exist across most of the biopharmaceutical manufacturing flowpath

Wave Bioreactors

- Fermentation
- Media Prep/ Storage

Limited

- Purification
- Buffer Prep/ Storage

Formulation/ Fill

Stedim Bags, Hynetics Mixers

* - Suppliers listed are examples, not an endorsement of any specific company or technology
Downstream processing unit operations

- **Normal Flow Filtration**
  - Depth filtration for clarification
  - Nanofiltration for virus removal
  - Sterile filtration

- **Tangential Flow Filtration**
  - Ultrafiltration for concentration and buffer exchange
  - Microfiltration for clarification

- **Centrifugation**
  - Clarification
  - Inclusion body isolation

- **Cell Breakage/Homogenization**
  - For recovery of products expressed intracellularly

- **Refolding**
  - For some *E. coli* products

- **Crystallization/Precipitation**

- **Chromatography and adsorptive separations**
  - Typical downstream process includes 3 – 4 chromatography and/or membrane adsorber steps
    - Ion exchange
    - Hydrophobic interaction
    - Affinity
    - Size exclusion
    - Reverse phase
• Fastest growing commercial biopharmaceutical segment is monoclonal antibodies at 40%/year
• Trend for commercial products is from agonists to antagonists -> larger volume, less expensive ($/g) products

Source: BPTC 2004 Annual Manufacturing Capacity Analysis
Clinical biopharmaceutical pipeline is weighted towards antibody-based products

- Biopharmaceutical pipeline is 70% mammalian cell culture
- Significant trend towards antibody-based products is ~ 85% of mammalian cell culture pipeline
- This analysis covers recombinant protein and monoclonal antibody product candidates only

*Source: BPTC 2004 Annual Manufacturing Capacity Analysis*
Typical material requirements – clinical development

- Pre-clinical development: 0.1 - 2 kg
- Phase I: 0.1 – 1 kg
- Phase II: 0.5 – 5 kg
- Phase III: 1 – 20 kg
Material requirements – commercial biopharmaceuticals

- Highly product dependent
- For microbial fermentation-derived products, commercial requirements range from:
  - ~10 g/yr (e.g., Infergen) to…
  - ~5,000 kg/yr (Novolin)
  - Most products require 0.5-50 kg/yr
- For mammalian cell culture-derived products, commercial requirements range from:
  - ~10 g/yr (e.g., Bexxar/Zevalin) to…
  - ~750 kg/yr (Rituxan)
  - Most non-antibody products require <10 kg/yr
  - Most antibody products require 10-500 kg/yr
Total estimated bulk product requirements for mammalian cell culture commercial products (2004):
- All mammalian products – 2,875 kg/yr
- Monoclonal antibodies – 2,810 kg/yr
- rProteins – 65 kg/yr

Significant growth in bulk requirements over recent years due to successful MAbs:

Source: BPTC 2004 Annual Manufacturing Capacity Analysis
Current Approaches
Examples of current “disposable format” purification products and technologies

- Membrane adsorbers
- Pre-packed chromatography columns or cartridges
- Disposable flow-path system concepts
Sartobind® Membrane Adsorbers

- Matrix: stabilized and cross-linked cellulose >3\(\mu\)m pore size
- Q,S,C,D and affinity ligands (ProtA, IDA, pABA, etc)
- Very low unspecific adsorption
- High chemical resistance against solvents, acids and caustic solutions and autoclaving
DNA removal

...clears the DNA below detection limit


- Average of eight 2,000 liter batches using 70 ml Sartobind
- Average of three 12,500 liter batches using 500 ml Sartobind
Other examples of process use of membrane adsorbers

BIOFLASH 12™ and BIOFLASH 80™ Prepacked Columns

Column Specifications
Pressure Rating 10-20 bar
   w/ module 33 bar
Diameters 1.2, 8, and 20 cm
Bed Length 5 – 30 cm
Bed Volume 5 mL – 5+ L

Courtesy of BioSepTec, Inc.
High performance packing in a disposable format

CM Toyopearl 650
BioFlash 80
(10 cm H)

Efficiency measured using 5% acetone in 0.1M NaCl as mobile phase.

Ret. Time 4.77
Efficiency 823
Assym. 1.03

Courtesy of BioSepTec, Inc.
Scale-up of recombinant protein separation on BioFlash pre-packed column

Purification of EPI-HNE-4 on Macroprep 25S at 100 cm/hr.

Introducing...

The Millipore Pod Filter Platform

- Improves process flexibility
- Decreases processing time
- More robust and scalable
- Easier to setup and use
- Minimizes product loss
- Enhances operator safety
- Reduces cleaning requirements
- Improves process economics
Improved Handling and Ease of Use

- 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 to set up and use
  - No hoist or high ceiling required
Impact of increasing cell culture titer and scale on downstream processes

- Cell culture titers of 4 g/L are now achievable for monoclonal antibodies:

- New facilities are increasing bioreactor scale to 20+ m³ to meet requirements of large volume products:
  - Lonza Portsmouth: 3 x 20,000 L (on-line) + 1 x 20,000 L (2006)
  - Genentech Vacaville CCP-2: 8 x 25,000 L (2009)

- At 4 g/L, a 25,000 L bioreactor will yield 100 kg per batch

- Increasing production scale and titers present challenges and opportunities for downstream process operation
Current approaches to managing high volume processes

- At 4 g/L, a 25,000 L bioreactor yields 100 kg per batch
  - At a loading capacity of 20 g/L during capture chromatography,
    - 8 cycles on a 2 m (ID) x 20 cm (H) column (CV ~ 630 L) are required
    - At 15 CV buffer per cycle, 75,000 L of buffer are needed per batch per chromatography step

- Current approaches based on maximizing value of existing conventional technology approaches (cf Smith*):
  - On-line dilution to reduce buffer storage requirements
  - Move to more rigid capture media to achieve higher velocities on chromatography steps
  - Multiple chromatography cycles (5-20) per batch
  - Load chromatography columns at/near break-through
  - Optimize UF steps to improve utilization of membranes

- These approaches work, but may have a limited lifespan -> parallel track investment in novel technologies

Obstacles and Future Possibilities
Potential obstacles to implementation of disposable or alternative technologies

- Cost
- Extractables and Leachates
- Inertia
The use of disposable-format purification technology will generally:

• Reduce capital costs
• Reduce labor requirements for setup/cleaning/cleaning validation
• Increase direct materials costs

The magnitude of the direct material cost impact for disposable chromatography is assessed for two situations:

• Clinical manufacturing for an outsourced early-stage product
• Commercial large-scale manufacturing
**Clinical Process and Analytical Development**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Time</th>
<th>Approximate Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Line Development (includes Vector Construction, Transfection, Selection, Identification of Final Clone)*</td>
<td>4 – 6 months</td>
<td>$150,000 – 500,000</td>
</tr>
<tr>
<td>Master and Working Cell Bank Generation*</td>
<td>4 – 6 months</td>
<td>$75,000 – 200,000</td>
</tr>
<tr>
<td>Process Development</td>
<td>8 months</td>
<td>$500,000 – 1,500,000</td>
</tr>
<tr>
<td>Process Scale-up</td>
<td>3 months</td>
<td>$300,000</td>
</tr>
<tr>
<td>Analytical Development and Qualification</td>
<td>9 months</td>
<td>$150,000</td>
</tr>
<tr>
<td>Viral Clearance Validation* – (1-3 model virus)</td>
<td>6 – 12 months</td>
<td>$150,000 - $250,000</td>
</tr>
</tbody>
</table>

**TOTAL**                                                        | -             | ~ $1.5-3 million       |

**cGMP Manufacturing**

- Clinical engineering and cGMP mfg lots ~$400k – 600k per batch (bulk)
- cGMP aseptic filling* of clinical lots ~$75k – 125k per lot

* - Activities that are frequently sub-contracted to other service providers
## Cost impact for disposable chromatography in clinical manufacturing

<table>
<thead>
<tr>
<th>Total Project Costs - Clinical Manufacturing</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process/Analytical Development Costs</td>
<td>$1,500</td>
<td>$3,000</td>
</tr>
<tr>
<td>Total Manufacturing Costs</td>
<td>$475</td>
<td>$2,525</td>
</tr>
<tr>
<td>Cost per lot</td>
<td>$400</td>
<td>$600</td>
</tr>
<tr>
<td># of bulk lots</td>
<td>$1</td>
<td>$4</td>
</tr>
<tr>
<td>Cost of DP mfg</td>
<td>$75</td>
<td>$125</td>
</tr>
<tr>
<td>TOTAL COSTS</td>
<td>$1,975</td>
<td>$5,525</td>
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</tbody>
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<tr>
<th>Media cost of using disposable capture affinity chromatography</th>
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<tr>
<td>Assumptions</td>
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<tr>
<td>Cost of media</td>
</tr>
<tr>
<td># of cycles per lot</td>
</tr>
<tr>
<td>Binding capacity</td>
</tr>
<tr>
<td>Volume of media</td>
</tr>
<tr>
<td>Cost of use as a disposable</td>
</tr>
<tr>
<td>As a % of Total Costs</td>
</tr>
<tr>
<td>As a % of Manufacturing Costs</td>
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<tr>
<th>Media cost of using disposable ion exchange chromatography</th>
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- With non-disposable approach, initial investment in media will be at least equivalent to single lot cost.
- Benefits of using disposable chromatography approach may be significant enough in this application to justify modest additional operating costs:
  - Improved manufacturing efficiency
  - Improved process portability
  - Reduced risk of cross-contamination
Summary of COG estimates for a “typical” commercial-scale monoclonal antibody process
Cost impact for disposable chromatography in large-scale manufacturing

<table>
<thead>
<tr>
<th>Commercial Manufacturing Costs</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Manufacturing Costs ($M/yr)</td>
<td>$40</td>
<td>$150</td>
</tr>
<tr>
<td>Cost per gram</td>
<td>$400</td>
<td>$150</td>
</tr>
<tr>
<td>Annual requirements (kg/yr)</td>
<td>100</td>
<td>1,000</td>
</tr>
<tr>
<td>Total Materials Costs ($M/yr)</td>
<td>$5</td>
<td>$44</td>
</tr>
<tr>
<td>Materials Costs as % of Total Costs</td>
<td>13%</td>
<td>29%</td>
</tr>
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<tr>
<th>Process Assumptions</th>
</tr>
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<tbody>
<tr>
<td>Scale</td>
</tr>
<tr>
<td>Titer</td>
</tr>
<tr>
<td>Yield</td>
</tr>
<tr>
<td>Output</td>
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<td># of batches/yr</td>
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<td>Cost of use as a disposable ($M/yr)</td>
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<td>Cost per g product produced ($/g)</td>
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- Operating cost impact of disposable chromatography approach far greater in this application.
- Alternative approaches required to significantly impact costs in large-scale commercial manufacturing.
Extractables and leachates are a potential concern with any disposable technology. Level of concern increases as product purity increases. Weidner presented work at Biogen Idec to address regulatory requests related to extractables:

- Risk-based assessment of extractables based on
  - Process step
  - Contact time
  - Temperature
  - Solvent
  - Stage of development
  - Vendor provided information on extractables and toxicological testing
- Conduct extractable tests where warranted based on potential risk
  - Mass transfer principles guide test design
  - Analytical methods for quantification and identification appropriate to situation
  - Results assessed against acceptance criteria or by evaluation of toxicological risk

Inertia: driving forces against use of novel technology and approaches

- There are significant costs and risks associated with process innovation in any highly regulated industry
  - Conservative approach to implementation of new technologies
  - Security of supply is a concern that must be addressed
- The complexity of biopharmaceutical processes provide additional challenges
- There is no good time to innovate: significant obstacles to implementation of new technologies exist at every stage of development

Result: New technologies often take longer than anticipated to implement, even when a compelling need exists.
Future trends

- Increased use of disposable purification technologies, particularly in smaller volume processes, driven by:
  - Continued advances in and increased use of disposable systems across the biopharmaceutical manufacturing flowpath
  - Implementation of new materials that significantly reduce per liter costs and allow increased throughput, potentially including:
    - Cost-effective affinity media
    - Novel membrane materials and adsorptive separation supports

- Increasing interest in alternative technologies and operating approaches for large-volume processes as optimization of conventional technologies matures, including:
  - Operating approaches that move away from “big batch” and towards continuous purification processes (e.g., SMB-like processes)
  - Integration of unit operations (i.e., semi-continuous capture and clarification)
  - Implementation of novel (to biotech) technologies and unit operations
Summary

- Current existing technology meets *some* of the requirements for cost-effective disposable purification solutions
  - Membrane adsorbers
  - Pre-packed columns
  - Disposable-format systems

- Trends in biopharmaceutical manufacturing are increasing the need for disposable or alternative purification technologies:
  - Increasing use of disposable technologies to reduce capital investment and product development times
  - Increasing titer and scale of monoclonal antibody cell culture production

- The use of disposable and alternative purification technologies will increase as:
  - The trends towards disposable equipment and large volume processes continue
  - New technology and viable solutions are introduced that provide solutions more broadly for purification unit operations
Thank you!

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Carousel-type SMB evaluation of Protein A separation

- Columns are mounted on a slowly rotating carousel
- The columns on the carousel are connected to the pumps and vessels via a rotary valve
- Switching mode simulates continuous adsorbent flow