Process Evolution from the Iron Age to the New Age – A Case Study

A Transition from Stainless Steel and Glass to a Fully Disposable Upstream Process During Clinical Development

BIO International
Chicago, 2013
Avid Bioservices – Substantial Experience in both Stainless Steel (SS) and Single Use Stirred-Tank Reactors (SUB)

- Considerable experience in cGMP production
  - Over 200 cGMP lots produced to date
  - Stainless Steel bioreactor production since 1997
  - 1st CMO in the west coast implementing SUB production in 2008
- 1,000 liter scale in both SS and SUB
- Significant experience in regulatory inspections with over 17 successful US FDA and European inspections
- Commercial production in SS reactors since 2005

Customer Perspective:
1. Prefer Stainless Steel reactor based processes
2. Prefer SUB based processes
3. Flexible and have no preference
The Case for Single Use Technologies
Lower initial investment cost

– Less manufacturing infrastructure

– Ease of implementation
  • Easy to retrofit into existing facility without building modifications
  • Smaller footprint eases space restrictions
  • Expand capacity through multiple reactors
Disposables – Process Perspective

• **More efficient production processes**
  – No cleaning validation reduces turnaround time

• **Multi-Product facility risk reduction**
  – Eliminate the potential for product cross contamination
  – Eliminates potential reservoirs for virus contaminations

*Everything was going along fine until they discovered their HeLa cell line expressed Y chromosome markers.*
Disposables – Is Avid Just Drinking the Koolaid

• 57.1% of Biomanufacturing Firms to Focus on Scaling up Single Use Systems to Commercial Manufacturing in 2012
  – Survey conducted by

• The biomanufacturing community is focused on replacing traditional facilities with single-use systems to improve flexibility, efficiency, and savings.
Disposables – OK its Not All a Bed of Roses

• Leachables and Extractables
• Must show process and product comparability when switching from Stainless Steel to Single-Use Bioreactors
• Robustness of plastic bag construction
• Difficulty of growing lipid dependent cell lines
• Dependency on vendors for single-use bioprocess containers
• Limitation in single-use bioreactor size
  – 2000 L largest available
<table>
<thead>
<tr>
<th>Challenges</th>
<th>Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leachable and Extractable</td>
<td>Leachable and Extractable testing performed by manufacturers or contract testing labs</td>
</tr>
<tr>
<td>Show process and product comparability when switching from Stainless Steel to Single-Use Bioreactors</td>
<td>Successfully demonstrated comparability between Single Use and Stainless Steel processes with the FDA</td>
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<td>Robustness of plastic bag construction</td>
<td>Single-use bioreactor containers are pressure integrity tested by the manufacturer</td>
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<td>Difficulty of growing lipid dependent cell lines</td>
<td>Recent data shows feasibility to grow lipid dependent cell lines in Disposable Vessels</td>
</tr>
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<td>Dependency in vendor single-use bioprocess containers</td>
<td>Integrated Supply Chain Materials Management System working closely with vendors to maintain inventory for production campaigns</td>
</tr>
<tr>
<td>SUB = Stainless Steel Bioreactors</td>
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</tr>
<tr>
<td>Limitation in single-use bioreactor size</td>
<td>Process improvement to increase yield; SUB manufacturers are continuing implementing larger vessels</td>
</tr>
<tr>
<td>Ease of expanding capacity with same process and same SUB size</td>
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Case Study: Client That Required Multiple Process Changes During Clinical Development

- **Phase 1 Clinical Trials (20-100 patient trials)**
  - Need to move quickly resulting in limited process development
  - Result was sub-optimal yields yet adequate to support early development

- **Phase 2 Clinical Trials (70-250 patient trials)**
  - Implemented new cell line to improve yields and process potential
  - Must maintain product comparability to Phase 1 material

- **Phase 3 Clinical Trials (500+ patients trials)**
  - Larger trials require up to multiple kg yields
  - Process with improved performance that can be well characterized and validated during phase 3
  - Must maintain product comparability to Phase 1/Phase 2 material
Process Evolution

• Iron Age
  – Original process – sub-optimal yield (<200 mg/L)
  – Early Iron Age at 300 L
  – Late Iron Age at 1000 L Stainless Steel

• Middle Age
  – Cell line change – Mid Yield (<600 mg/L)
  – Non-disposable inoculum
  – 1000 L Stainless Steel or Single Use Bioreactors

• New Age
  – Optimized medium & process (>2g/liter)
  – Upstream process with new media and feeds
  – Completely disposable inoculum train
  – 1000 L Single Use Bioreactors
Upstream Process Successfully Transitioned to Completely Disposable Process

Iron Age
- Vessel 1
- Vessel 2
- Vessel 3
- Vessel 4

Middle Age
- Vessel 1
- Vessel 2
- Vessel 3
- Vessel 4

New Age
- Vessel 1
- Vessel 2
- Vessel 3
- Vessel 4
- Vessel 5

300 L Stainless Steel
100 L Stainless Steel
1000 L Stainless Steel
1000 L Single Use
Process Evolution – Regulatory Approach

Iron Age
- Sub-optimal yield
- Early Iron Age (300 L SS)
- Late Iron Age (1000 L SS)

Middle Age
- Change of cell line resulted in mid Yield (<600mg/l)
- Non-disposable inoculum
- 1000 L Stainless Steel or Single Use Bioreactors

Regulatory Filings

Demonstrate Process Comparability

Demonstrate Product Comparability through Analytical Characterization
Comparable Upstream Process between 1,000 L SS vs. 1,000 L SUB

- No significant difference in cell growth or titer

Cell Growth
- Stainless Steel (n=9)
- Single Use (n=4)

Titer
- Stainless Steel (n=9)
- Single Use (n=4)
## Product Comparability Demonstrated

<table>
<thead>
<tr>
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<th>Additional Characterization</th>
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<td>✓ Carbohydrate Analysis</td>
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<td>✓ Residual Protein A</td>
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<tr>
<td></td>
<td>✓ SDS-PAGE</td>
</tr>
<tr>
<td></td>
<td>✓ Visual Inspection</td>
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Iron Age to Middle Age: Comparable Product Peptide Map

- Middle Age (1000L Single Use)
- Middle Age (1000L SS)
- Late Iron Age (1000L SS)
- Early Iron Age (300L SS)
Iron Age to Middle Age Comparison

• No significant difference in Product Quality Attributes

• Received FDA approval for:
  – Manufactured product interchangeably in Stainless Steel and Single Use Bioreactor
  – Post process changes (ie. cell line and downstream process)

• Provided adequate drug product for several Phase II clinical studies
Iron Age to Middle Age: Labor comparison

• Turnaround time:
  – Stainless Steel is ~10 days
    • Break down and CIP: 3 days
    • Quality Control testing: 3 days
    • Release for use of next product: 2 days
    • SIP: 1 day
  – Single Use is 1 day
    • None of the above required
• Stainless Steel has considerably higher associated labor costs
Process Evolution – Regulatory Approach

Middle Age
- Change of cell line resulted in mid Yield (<600mg/l)
- Non-disposable inoculum
- 1000 L Stainless Steel or Single Use Bioreactors

New Age
- Optimized medium & process
- Upstream process with new media and feeds
- Completely disposable inoculum train
- 1000 L Single Use Bioreactors

Regulatory Filing
Demonstrate Product Comparability
Inoculum Expansion Comparison

Middle Age with Non-Disposable Vessels

New Age with Disposable Vessels

Time in Vessels
Iron Age to New Age

- Better cell growth with New Age Process
- Significantly increased titer with New Age Process
- No impact on Product Quality Attributes
## Product Comparability Demonstrated

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<tr>
<td>✓ Peptide Mapping</td>
<td></td>
</tr>
<tr>
<td>✓ pH</td>
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Middle Age to New Age
Product Comparability Peptide Map

Received FDA Approval for Late Stage Development
Iron Age to Middle Age
• Labor & Overhead Costs for an entire production run at same scale and same process costs ~ **25-30 % less** with disposable process

Middle Age to New Age
• **Requires ~35 hours** for Non-Disposable Inoculum Process
  – Process (clean and autoclave) all spinner flasks for one production run
  – Clean, perform cleaning verification (including testing), assembly, process, and post-use clean
  – Documentation and review of all paperwork

• 3-5 hours of prep time for completely disposable process
Scale-up from Process Development to Full Scale Production: No Problem!

- Aspect ratios and bioreactor parameters are kept constant for all size reactors making scale down verification prior to process transfer makes it easy and representative.

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<tr>
<th>Parameter</th>
<th>50L</th>
<th>100L</th>
<th>250L</th>
<th>500L</th>
<th>1000L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid Geometry @ Working Volume (height/diameter) Ratio</td>
<td>1.5</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>Overall Reactor Geometry (height/diameter) Ratio</td>
<td>1.9</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>Impeller (quantity X blade count)</td>
<td>1 x 3</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>Impeller Scaling (impeller diameter/tank diameter)</td>
<td>1/3</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>Impeller Blade Pitch (angle)</td>
<td>45°</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
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<tr>
<td>Impeller - Calculated Power Number (N)</td>
<td>2.1</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>Nominal Agitation Rating - Power/Volume Ratio</td>
<td>0.1 hp/1000 gal (19.7 W/1000 liter)</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>Agitation Shaft Resolved Angle</td>
<td>19.6°</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
<td>Same</td>
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**Cell Growth**
- 100 L Scale (n=1)
- 1000 L Scale (n=2)

**Titer**
- 100 L Scale (n=1)
- 1000 L Scale (n=2)
Summary and Conclusions

✓ Single-use processes are transforming Bio-manufacturing and Avid has embraced this new technology early on

✓ Avid Bioservices Inc. is leading the way
  - Through extensive experience and expertise
  - Active role in making single-use product improvements
  - In constant communication with manufacturers for up-to-date progress in single-use container characterization and robustness

✓ We’re setting the trend
  - Flexible manufacturing scale solutions for all project types
  - Single-Use fleet consists of 1000 L, 200 L, 100 L and 50 L
  - Avid has successfully demonstrated comparability between Single Use and Stainless Steel Bioreactors with the FDA