Review of Single Use Technologies in BioManufacturing

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Outline

• Introduction – Strategic Outlook for BioPharma
• Advantages and Limitations of Single Use Systems
• Integration/Scale Up Challenges of Disposables
• Single Use Bioreactor Data
• Conclusions
BioPharma Strategy - New Paradigm

**Industry Growth**
- 15% annual avg.
- >15 approved mAbs
- >150 mAbs in clinic
- expanding pipelines

**New Technology**
- Better process yields
- Potent compounds
- Drug delivery

**Smaller Markets**
- Fewer blockbusters
- Personalized medicine
- Genetic screening

**Cost Pressures**
- Health care reform
- Pricing controls
- Biosimilar drugs

- Capacity demands
- Smaller batch sizes
- Smaller R&D budgets

**Need for more efficient PD & Mfg.**
fast, flexible and inexpensive manufacturing capacity
Xcellerex’s Technology will be Commercial Scale
Future Quantity Per Drug Demand for Biologics is decreasing

- Traditional Platform Scale – 2,000L to 20,000L
- Xcellerex XDR Platform Scale – 200L to 2,000L

Niche markets
- Improved potency
- Improving yields
Advantages of Single Use Systems

Reductions in:
– Cleaning
– Sterilization
– Engineering cost
– Equipment lead time
– Utility requirements
– Validation
– Quality / Regulatory burden
– Space
– Labor
– Waste generation

Improvements in:
– Manufacturing quality
– Capital investment
– Facility buildout time
– Cycle time
– Flexibility
– Environmental impact
– COGS
Limitation of Single Use Systems

- Mixing/Buffer/Media prep – Rate of liquid transfers
- Bioreaction – Lab to commercial scalability within the same reactor design/configuration
- Cell Harvest/TFF - Recirculating processes that require high pressure and high flow rates
- Disposable Chromatography is still TBD
- Non-standard, multiple connection options
- Disposable sensors are limited
Technology Survey

Products and vendors listed in the following slides are provided for reference and do not constitute a complete list or an endorsement of any specific vendor or product.
Enabling Single Use Technologies

- Bioprocess bags
- Cell culture bioreactors
- Separations (Cfg., TFF, rotary drum, filters)
  - Harvest
  - Virus removal / sterilization
  - Concentration / buffer exchange
- Purification – membranes
- Tubing welders / connectors / sealers
- Integrating stainless and disposables connectors
- Sensors
Mixing Systems

**Applications:** Media, buffer, product processing, formulation

**Capacity:** 10 L to 10,000 L

**Vendors/Types:** Hyclone MixTainer, LevTech/Sartorius levitated prop tank, Wave FlexMixer, Xcellerex XDM stirred tank

**Integration Challenges:** powder addition, connectors

**Scale Up Challenges:** powerful mixing, bags that flex to achieve mixing rely heavily on bag seam strength and durability
XDM-100 and 200 Disposable Tank Mixer
Invitrogen/Xcellerex XDM Commercial Disposable Mixers for powdered media mixing and delivery

XDM-100
Powdered media/buffer mixing

XDM-200
Powdered media/buffer mixing
BioProcess Bags/Tank Liners

**Applications:** culture media and buffers, product, samples or waste.

**Capacity:** 10 mL to 2500 L (up to 10,000 L)

**Vendors:** HyClone, Stedim, TC Tech, Charter, Newport: USP Class VI tested, gamma irradiated

**Types:** Monolayer, multilayer, LDPE, EVA, etc.

**Integration Challenge:** connectors not common

**Scale Up Challenge:** bag seam weld strength, rate of fluid transfers, robust, cheap tubing >1” diameter
Small Scale Cell Culture Bioreactors

**Application:** Culture of eukaryotic cells

**Capacity:** ~10 mL – ~10 L / 25K cm²

**Vendors:** Corning, Nunc, Wave, Bellco

**Types:** TC flasks, rollers, spinners, shake flasks, hollow fibers, expanded T-flasks (Cell Factory, Cell Cube), novel bioreactors (Wave, BelloCell)

**Integration challenges:** tubing/connectors sizes and compatibility

**Scale Up Challenge:** model system that scales to 10,000L
Mid Scale Cell Culture Bioreactors

**Applications:** Culture of mammalian, insect or plant cells in suspension. (Many vendors supply large hollow fiber systems for anchorage dependent cell culture).

**Capacity:** 1 L – 200 L

**Vendors:** HyClone SUB stirred tank, Wave Biotech rocking system, Xcellerex XDR stirred tank bioreactor

**Integration Challenge:** liners, connectors, sensors, filters, controllers

**Scale Up Challenge:** model system that scales to 10,000L
Xcellerex
XDR-200
working volume
disposable stirred tank reactor
Large Scale Cell Culture Bioreactors

**Application:** Culture of mammalian, bacterial, yeast, insect or plant cells in suspension.

**Capacity:** 200-1,000L working volume

**Vendors:** Wave Biotech 500L rocker, HyClone SUB stirred tank, Xcellerex 1,000L stirred tank bioreactor

**Integration Challenge:** liners, connectors, sensors, filters, controllers

**Scale Up Challenge:** avoid stressing bag seams, small scale modeling system that scales to 10,000L
Xcellerex 1,000L (wv) Disposable Stirred Tank Reactor - XDR™
Cell Harvest

**Application:** Separation of cells from growth medium during perfusion or for terminal cell harvest.

**Capacity:** Up to 100-200 L/hr

**Vendors/Types:** Kendro (centrifuge), Steadfast (rotary drum filter), Spectrum and GE (recirc. hollow fiber), Millipore POD system (dead end), Cuno depth filtration. All product contact surfaces disposable

**Integration Challenges:** connectors

**Scale Up Challenges:** recirculating systems: disposable tubing not amenable to high flow rates and pressures
Dead End/Depth Filtration

**Application:** Clarification / sterilization of media, buffers and process intermediates, cell harvest, and removal of particulates.

**Capacity:** Syringe filters, 30” capsules, flat membrane generally available, (larger by custom order)

**Vendors/Types:** Millipore POD, Pall, Sartorius, Meissner, Cuno – larger capsules coming available, many available pre-sterilized and integrity tested.

**Integration challenges:** connector compatibility

**Scale Up challenges:** >1000L capacity is lacking
Tangential Flow Filtration

**Application:** Perfusion, cell harvest, purification, concentration, and formulation / buffer exchange.

**Capacity:** Up to 5.6 m²

**Vendors/Types:** Spectrum HF, GE hollow fiber

**Integration:** disposable pump integration that is durable yet disposable

**Scale Up Challenge:** recirculating systems: disposable tubing not amenable to high flows/pressures
Virus Reduction

**Application:** Mechanical reduction of viral load by nanofiltration.

**Capacity:** 15 - 200 L/hr. (depending on pore size, filter medium & process stream)

**Vendors/Types:** Millipore dead end, Pall dead end, Asahi-Kasei

**Integration Challenges:** connectors

**Scale Up Challenges:** larger scale requires more area
Purification – Membranes

**Application:** Flow-through removal of contaminants, bind-and-elute purification of small or dilute process streams.

**Capacity:** 20 L/min., 5g DNA binding capacity

**Vendors/Types:** Pall, Millipore and Sartorius functionalized filter membranes.

**Integration Challenges:** connectors, area

**Scale Up Challenges:** less binding capacity compared to chromatography resins in general
Vial Filling

Applications: Aseptic filling into vials
Capacity: Clinical to commercial {?}
Vendors/Types: Millipore Acerta bag based filling system, MedInstill injection filling/laser sealing
Integration Challenges: connectors
Scale Up Challenges: not clear yet
Sensors

**Applications:** Process wide

**Capacity:** N/A

**Vendors/Types:** Wave Biotech, (pH, DO2), Fluorometrix and PreSens optical sensors, microprobes

**Integration Challenges:** Cytoxicity, irradaitability, fit up into bags, tubing, dead zone elimination, signal response time

**Scale Up Challenges:** stability, non-fouling, validatable
Sterile Tubing Connectors

**Applications**: Aseptic / sterile connections

**Capacity**: 1/4” to 3/4” OD tubing

**Vendors/Types**: CPT (C-Flex), Pall ACDs, BioQuate, Millipore. CPT connector is similar to a tubing welder. Pall & BioQuate connectors are similar to a quick-connect which can make sterile connections in a non-sterile environment.

**Integration Challenges**: no one system connects all and they are one time use (versus welders that can reweld)

**Scale Up Challenges**: connectors >1” diameter
Connectors - Tubing Welders

**Application:** Aseptic / sterile connections
**Capacity:** 1/4” to 3/4” OD tubing

**Vendors/Types:** Terumo, Wave, Sebra. Several devices have been validated by the vendor and/or biopharm manufacturers. Can be used on PVC and EVA (Sebra), or Tygon, C-flex and Pharmed (Terumo, Wave) tubing.

**Integration Challenges:** no one system welds all

**Scale Up Challenges:** welding tubing >1” diameter
SIP Tubing Connectors

**Application:** SIP-able connections between disposable tubing to stainless steel valves.

**Capacity:** 1/2” ID x 3/4” OD

**Vendors:** Millipore, Colder, TC Tech

**Comments:** Can be added as option to tubing on bioprocess bags for sterile transfer to or from stainless steel systems.

**Integration Challenges:** no one connects all

**Scale Up Challenges:** none
SIP Filter Capsules

**Application:** Disposable filter capsule capable of withstanding temperature and pressure required for steam-in-place.

**Capacity:** 5” housing

**Vendors/Types:** Pall - Can house vent or liquid sterilizing filters or virus removal filters.

**Integration Challenges:** connections

**Scale Up Challenges:** larger size not available
Sampling System

**Applications:** Used in place of sampling ports on stainless steel tanks.

**Capacity:** 20 mL - 1 L sample bags

**Vendors/Types:** NovAseptic - sheathed cannula, tubing set and bag as a pre-sterilized, closed system. Outside of sheath sterilized during vessel SIP. Can load multiple cannulae into single septum sample port.

**Integration Challenges:** none

**Scale Up Challenges:** none
Technology Trends

• Standard connectors
• Non-recirculating TFF
• Integration of existing stainless to disposable
• Development of new enabling technology
  – Sensors
  – Chromatography (scaleable and economical)
• Fully disposable biomanufacturing >1,000L
• Yeast, bacteria, fungal systems coming!
## Types of Cells Grown in XDRs

<table>
<thead>
<tr>
<th>Cell type/product</th>
<th>Mode</th>
<th>scale of runs done L (wv)</th>
<th>2007 planned runs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybridoma/mab</td>
<td>fedbatch</td>
<td>2x 200 - GMP</td>
<td>200 - GMP</td>
</tr>
<tr>
<td>CHO/mab-fusion</td>
<td>batch</td>
<td>2x200, 2 x 1,000L</td>
<td>1,000L</td>
</tr>
<tr>
<td>CHO/fus. protein</td>
<td>fed batch</td>
<td>2x 200, 3 x 500 GMP</td>
<td>200, 500 - GMP</td>
</tr>
<tr>
<td>Insect S2/vaccine</td>
<td>fed batch</td>
<td>200</td>
<td>200 - GMP</td>
</tr>
<tr>
<td>Insect SF9/vaccine</td>
<td>fed batch</td>
<td></td>
<td>200, 2,000</td>
</tr>
<tr>
<td>Human/fus. protein</td>
<td>perfusion</td>
<td>200</td>
<td>1,000</td>
</tr>
<tr>
<td>Yeast (Sacc.)</td>
<td>fed-batch</td>
<td>150</td>
<td>1,000</td>
</tr>
<tr>
<td>E. Coli</td>
<td>fed-batch</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>Vero/vaccine</td>
<td>batch</td>
<td></td>
<td>200, 1,000</td>
</tr>
</tbody>
</table>
GMP XDR-200 and 1000 Systems
BioNet DeltaV GMP Process Controller
XDR-1000 DO Control, fusion protein

DO

Time

5/14/06 7:21
5/14/06 20:55
5/15/06 10:29
5/16/06 0:03
5/16/06 13:36
5/17/06 3:12
5/18/06 16:44
5/18/06 19:52
5/19/06 9:26

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XDR-1000 pH control, fusion protein
XDR-1000 Temp Control

878-03 1000L Bioreactor Run, Temperatures, 3/11/2006

Temperature in Degrees C

Bioreactor Temp
Ambient Temp
XDR-1000 Mass Transfer
KLα measurements, single sparger

<table>
<thead>
<tr>
<th>XDR-1000 - F impellers, Air Only, 37C, 6 g/L Salt, F-68 1 g/L, polyol antifoam</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpm</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>132</td>
</tr>
<tr>
<td>167</td>
</tr>
</tbody>
</table>
XDR-1000L KLa hr-1 vs RPM and Sparge Rate (Multiple spargers)
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XDR-200 Comparison, Viability
XDR-200 Comparison Titer

Production Titer

Age (hrs) vs. Titer (mg/mL)

- 3000L eng
- GMP1
- GMP2
- GMP3
- 300L
- XDR200
- XDR200-2

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XDR-200 Comparison, pCO2

Production pCO2 (mmHg)

Age (hours)

pCO2 (mmHg)

3000L eng
GMP1
GMP2
GMP3
300L
XDR200
XDR2002-2

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XDR-200 CHO Perfusion Culture - Viable Cell Density

XDR200 vs 10L BR (Viable Cell Density)

Perfusion rate = 1 VVD

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XDR-200 CHO Perfusion Culture - % Viability

Days in Production
60.0
70.0
80.0
90.0
100.0

Viability (%)
-5.0
0.0
5.0
10.0
15.0
20.0
25.0

XDR200 vs 10L BR (Viability)

XDR200
10L BR
XDR-200 Insect Cells S2
- fed batch

XDR-200 Growth Curves Vials #21

Highest cell density achieved in stainless steel bioreactors was 32 x E6 cell/ml

Induce with 0.2 M CuSO₄
Added Glucose
Added Antifoam
Added Antifoam and Glucose

#21-1A Viable cells/ml
#21-1A Viability (%)

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XDR-200 Insect Cells S2 - fed batch - pCO2

pO2 and pCO2

Time (h)

pO2 (mm Hg)

pCO2 (mm Hg)
XDR-200 Yeast - S. cerevisiae

Run 130-999 - XDR-200: S. cerevisiae fermentation

- OD600
- EtOH
- Glucose
- 2 per. Mov. Avg. (Glucose)
- Poly. (Density)
XDR-1000, CHO, fusion mAb viable cell density

XDR-1000 Bioreactor Runs
CHO Cell Line
Viable Cell Density

Run#2
Run#1

Viable Cell/mL

0.00E+00 1.00E+06 2.00E+06 3.00E+06 4.00E+06 5.00E+06 6.00E+06

0 25 52 74 97 122 145 168 195 217 239

Hours
XDR-1000, CHO, engineered mAb cell viability

![Graph showing cell viability over time for two runs of XDR-1000 Bioreactor. The graph displays the percentage of viable cells over hours, with two runs labeled Run#1 and Run#2. The graph shows a decrease in cell viability over time for both runs, with Run#2 showing a more significant drop beyond 200 hours.]
Progress for Single Use Systems

- Wide acceptance of bioprocess bags
- Single use bioreactors are scalable and performance is comparable to SS bioreactors
- 1,000L (wv) stirred tank bioreactor breakthrough opens large scale/commercial applications
- Single pass cell clarification/removal (POD) simplifies 1° recovery
- Membranes for purification improving
Summary - Challenges for Disposables

- Mixing/Buffer/Media prep – Rate of liquid transfers
- Bioreaction – Lab to commercial scalability within the same reactor design/configuration
- Cell Harvest/TFF - Recirculating processes that require high pressure and high flow rates
- Membrane Purification – capacity and DNA/virus clearance
- Disposable Chromatography is still TBD
- Non-standard, multiple connection options
- Disposable sensors are limited
- Plastic durability and weld strength needed for scale up
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