ADAPTATION OF SINGLE-USE TECHNOLOGY IN PILOT PLANT OPERATIONS

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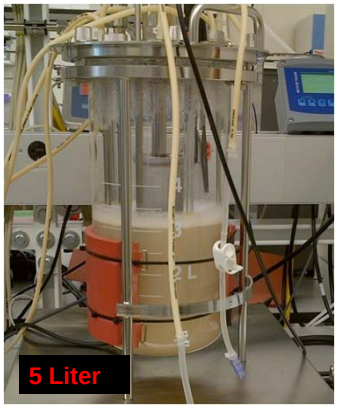
Outline

- Scope of case study
- New technology evaluation and adoption
- Carr Centritech Cell II introduction and background
- Centrifuge redesign work
- ATF6 introduction and background
- ATF6 redesign work
- Points to consider before adopting new science

Scope of Case Study

Challenge: 5 liter perfusion process using spin filters needed to be scaled up to PPO. Spin filters do not exist for disposable reactors



Strategy: A new science needed to be found and tested.

New Technology Evaluation

Who else is using them?

- Process length?
- What application?
- For what purpose?
- Scalable?

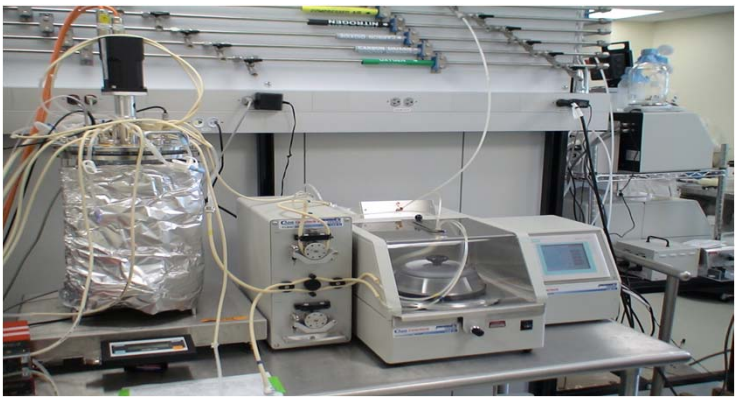
Characterization:

Do suppliers have dependable characterization data?

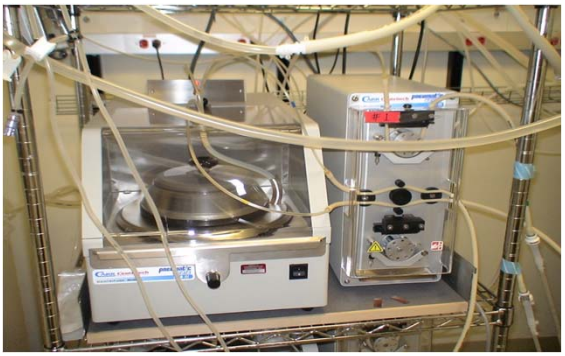
- Can they supply the data?
- Can we replicate their data?
- Can they support our development?

Is this new science plug and play?

- **Companies claim out of the box integration**
- **Trial and error characterization for small scale development**
- **10L characterization was successful!**



Points to Consider for Scale-Up of Disposables



- Throughput
- Cell shear
- Residence time
- Connectivity
- Fluid additions
  - Top
  - Sub-surface
- Line dimensions
- Temperature loss
- Material of construction and their dependability

Scaling Up Disposables Does Not Mean Connecting Multiple Lab Bench Models Per Bioreactor

3 R's for Connections:

- Reduce
- Re-size
- Re-form

Reducers and hose barbs should be removed wherever possible!

Molded assemblies provide the least amount of shear stress and concern.

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Introduction of the Carr Centritech Cell II into the PPO

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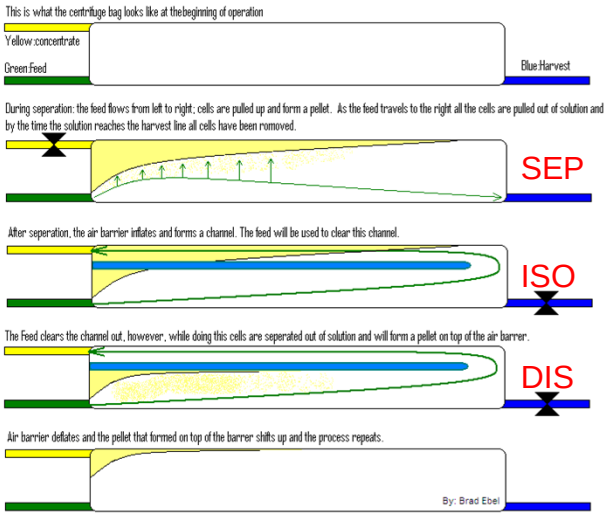
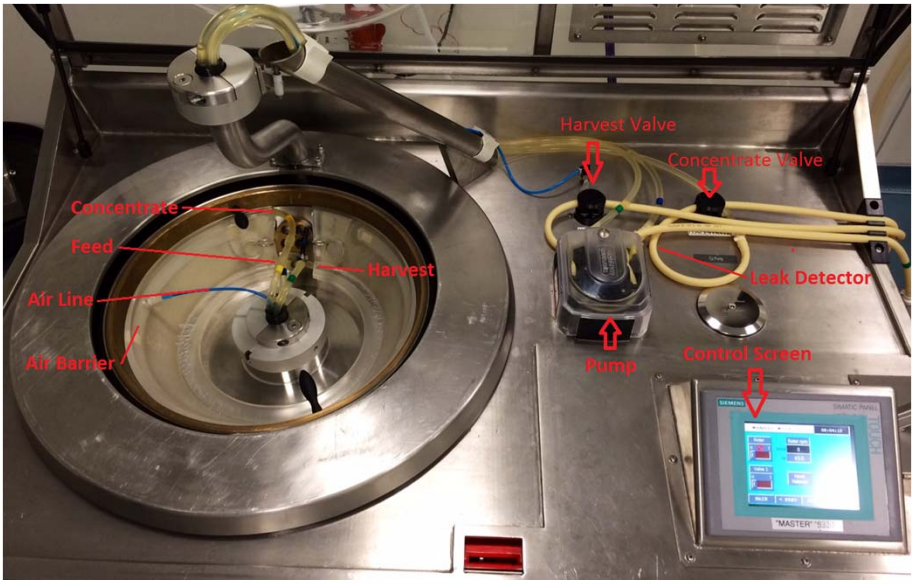
So how does a disposable centrifuge work?

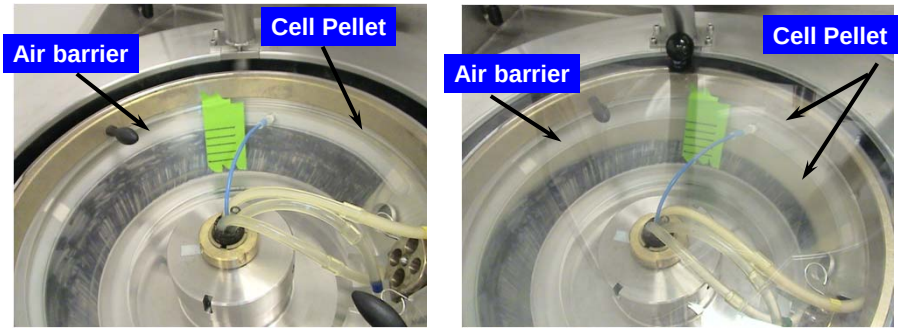


Top Deck of Centrifuge.



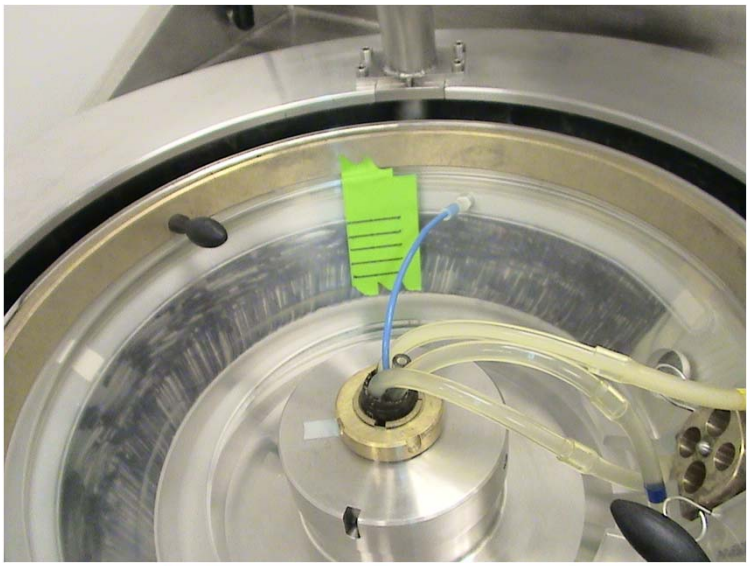
Process Characterization

Separation Time (s)	% chamber Filled	Throughput (L/day)	Cell Loss (%)	Pellet Clearing
5	33	1229	1.2	Clear
7	44	1408	1.5	Clear
8	49	1474	1.7	Clear
9	55	1531	NA	Build up

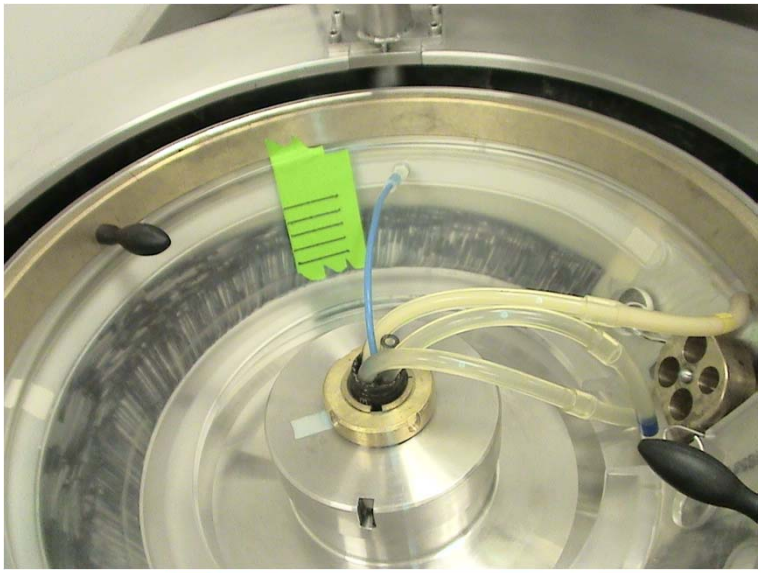


Limiting Factor: Pellet Buildup

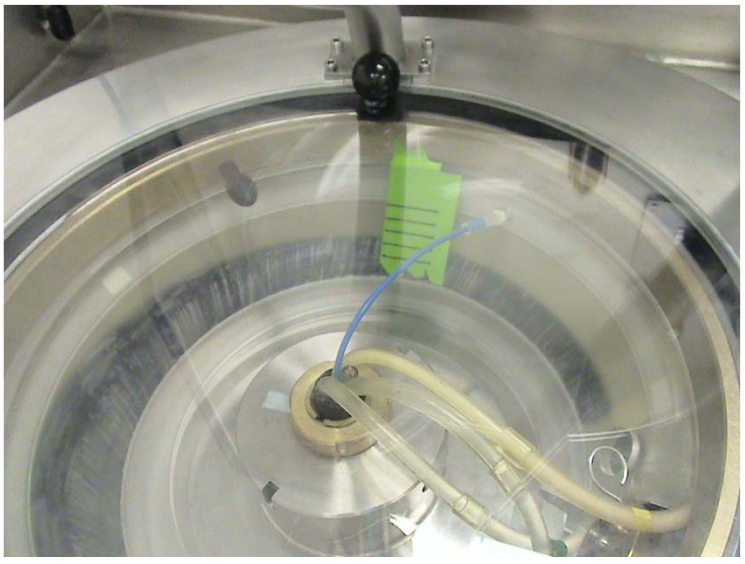
700 RPM



750 RPM



800 RPM



Key parameters for optimizing centrifuge operation:

- Discharge pump speed and time
- Isolation time of air barrier inflation
- **Separation pump speed and time**
- RPM of Bowl
  - Repeat separation pump speed and time to develop range
- Percent solids (CPV)
  - Repeat separation pump speed and time for optimal operation range
- Determine safety factor

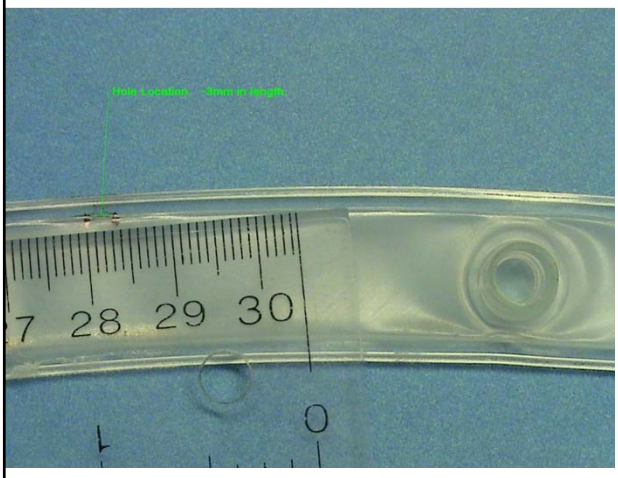
➢**Now that the characterization of the centrifuge is finalized is your development completed?**

Centrifuge Redesign Work

- **Air Barrier**
- **Insert**
- **Belts and Pulleys**
- **Pump Head and Gaskets**
- **Seals**

Air Barrier Redesign

Several air barrier failures using the 4 ply air barrier were observed

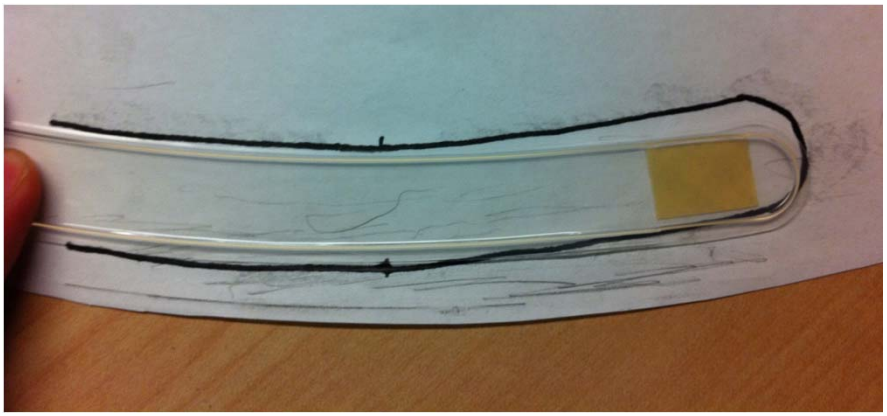


- Standard 4 ply design was experiencing issues in manufacturing process due to difficulty with the RF weld process resulting in failures on the welded edge.

Air barrier failures

2 Ply Air barrier

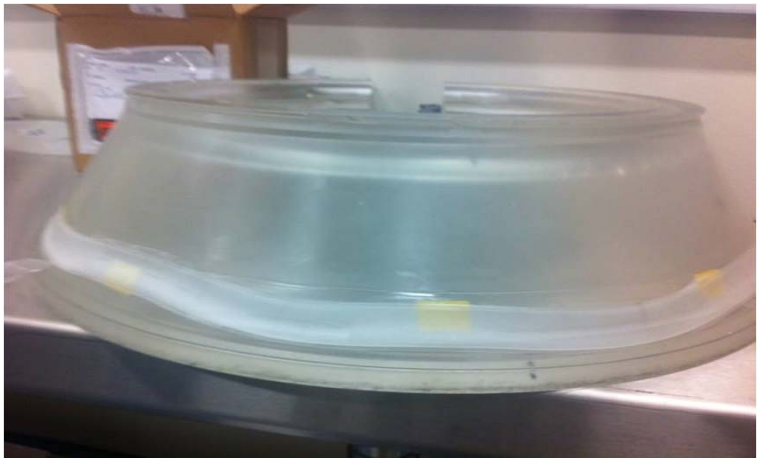
- It was believed that the 2 ply air barrier would work better because it was more rigid.
- Ended up being not as effective as the 4ply.



Air Barrier: 2 Ply Air Barrier with “4 Ply Material”

- Material was too flexible and deformed too easy

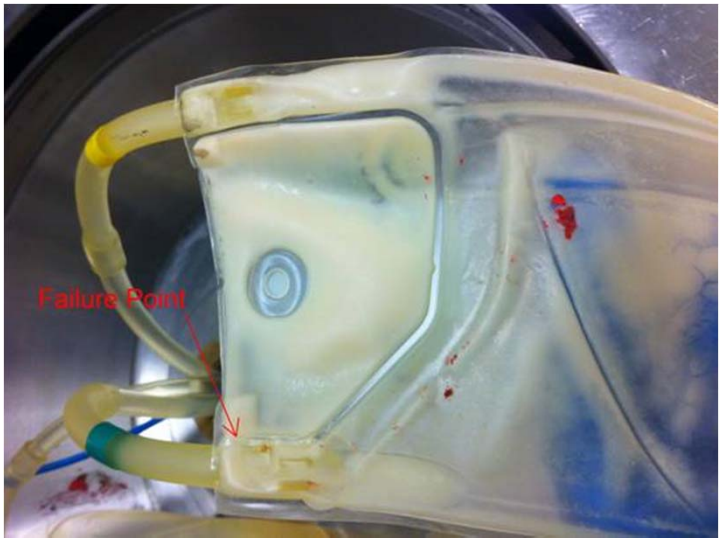
Solution: changed material to a thicker polyester polyurethane



Deformation of Insert

•Air pressure of air barrier had to be closely monitored to insure the air barrier was not negatively affecting the insert.

Insert Redesign



Centrifuge Insert QC

- Actions were put into place to insure the insert went trough a complete QC inspection.
- The insert can not be treated in the same manor as a “harvest bag” that is sitting on the shelf.

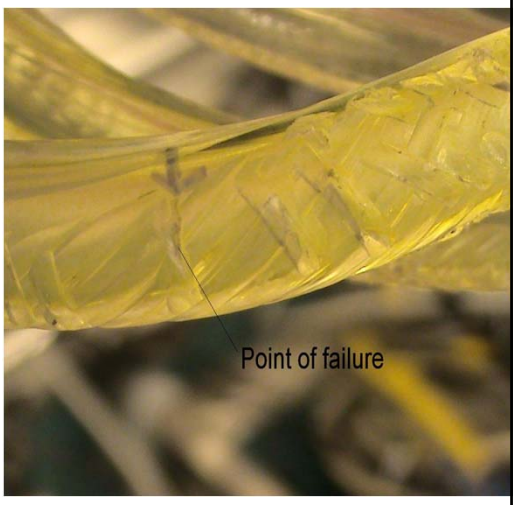
Tubing Braid Fit
Goldilocks Paradigm- Too Tight or Too Loose?

Issue: Excessive tubing wear brought on by a combination of:

- The twist of the braid.
- Tightness of the wrap
- Tubing length specifications

Results:

- Failure due to split in tubing caused by cuts from braid
- Failure due to excessive rubbing



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Tubing Braid Fit:
Goldilocks Paradigm- Too Tight or Too Loose?

Recommendations for remedial actions:

Throw away visually defective braid.
Execute "Braid Wear Characterization" test program
Measure Braids per centimeter and set specification.
Remove oven heating operation from braid installation.
Add ¼ turn marks on the wall to insure a smooth twist
Only allow 5 turns at 40.5"

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How many revolutions can your insert go?

Centrifuge Belt Redesign

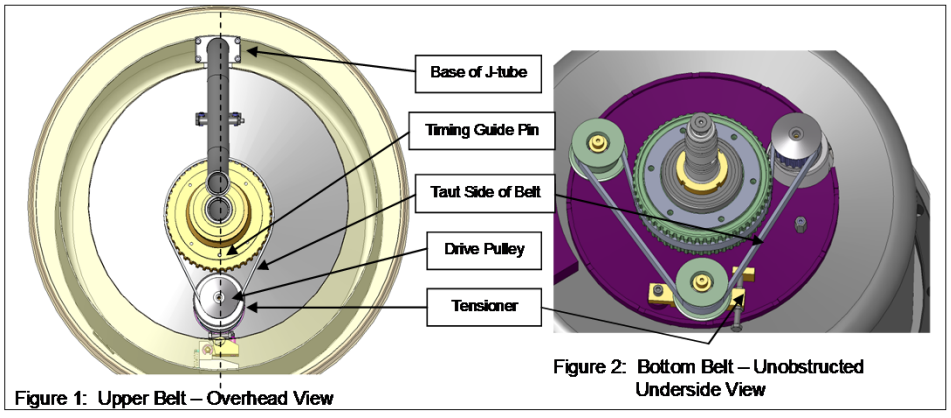


Figure 1: Upper Belt – Overhead View

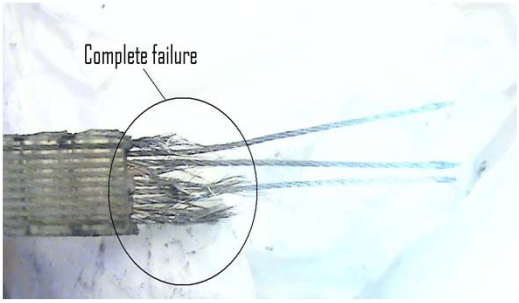
Figure 2: Bottom Belt – Unobstructed Underside View

Drive Belt Failures

- Centrifuge was experiencing belt failures on a regular basis.
- Initial thought was the insert retention ring was being overtighten…
- However, during the investigation more was found!

Belt MFG Consideration:

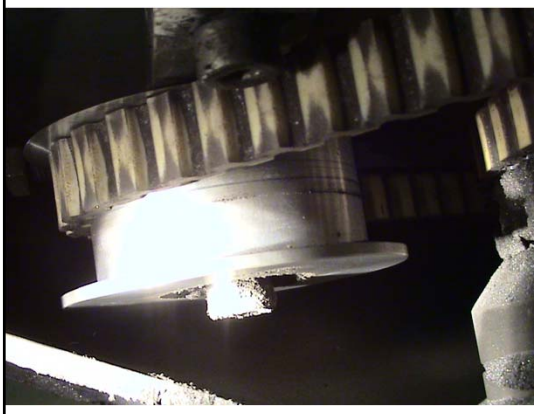
- Appropriate belt tensioning and use of grease
- Installation issues.
  - Over or under tensioning.
  - Stress due to other failures.
- Hardware issues
  - Pulley alignment
  - Correct parts
  - Tensioning bolts



Lower Drive Belt

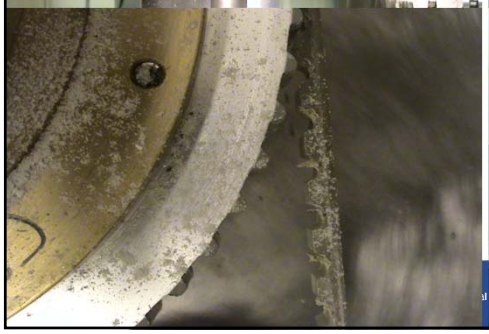
Flange Wear

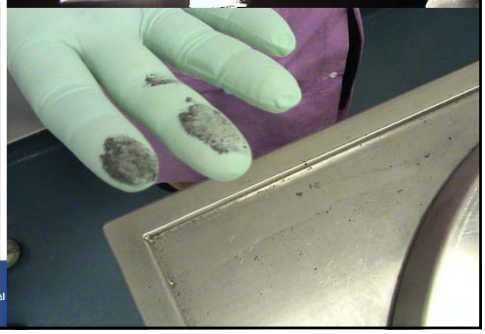
Excessive wear and belt positioning.

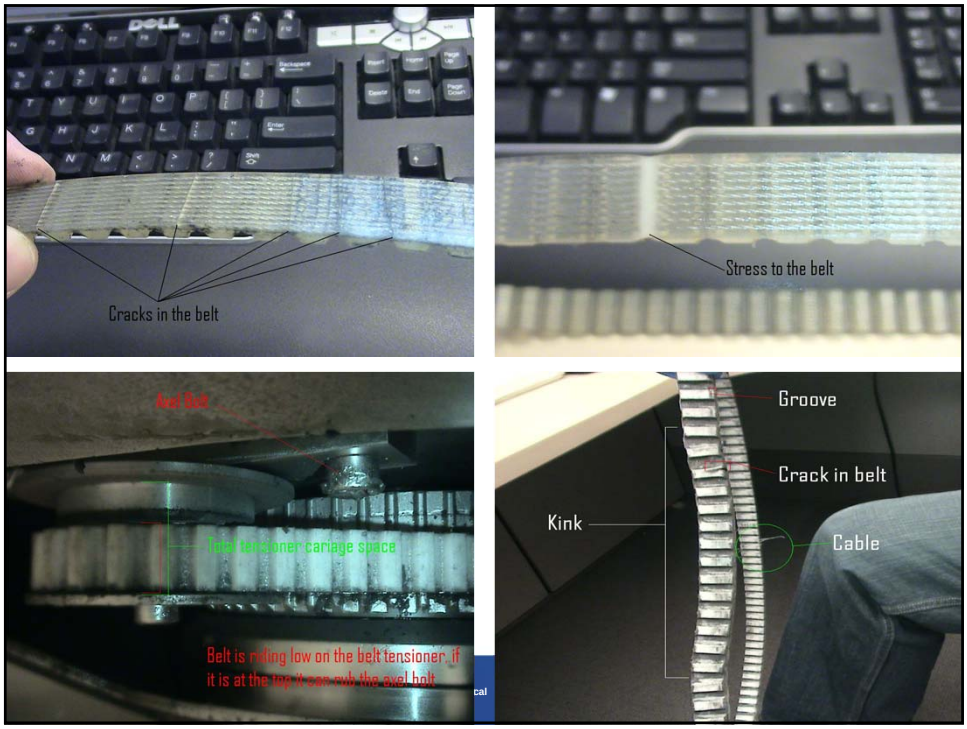


Centrifuge Belts Degradation

•Particulate generation of the deteriorating belts

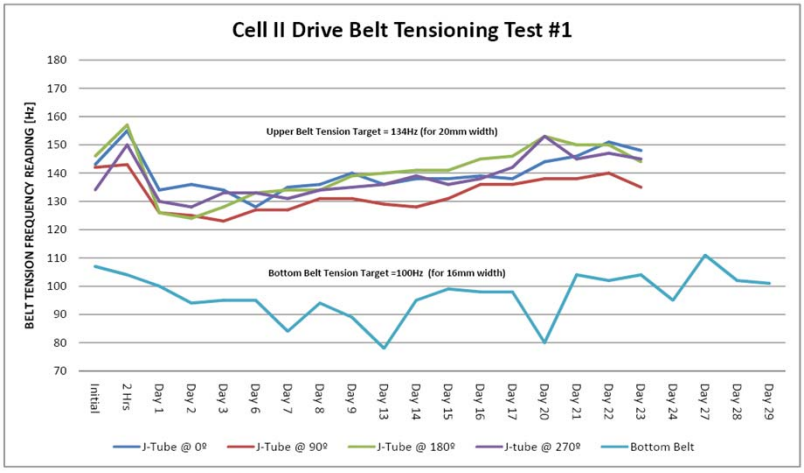




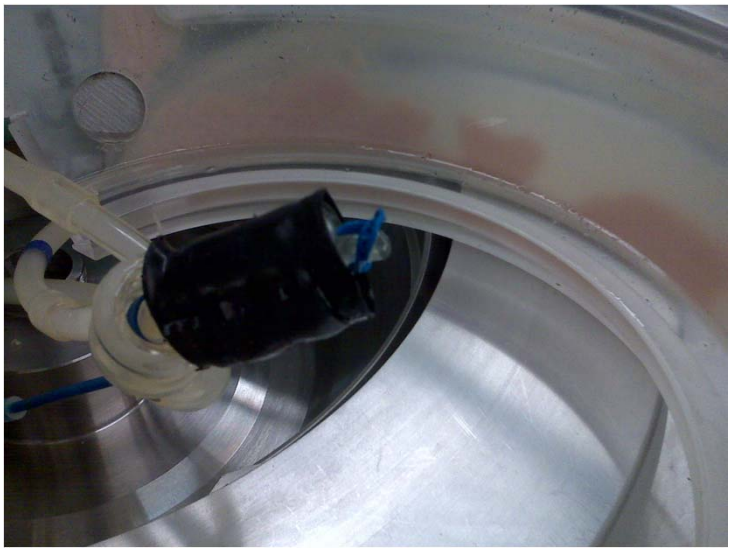


Solution: Belt Tensioning Frequency Reading and New MFG

- Identified new belts and designed a study to test the optimal tension of the belts by measuring their frequency



What Happens if the belt breaks during processing?

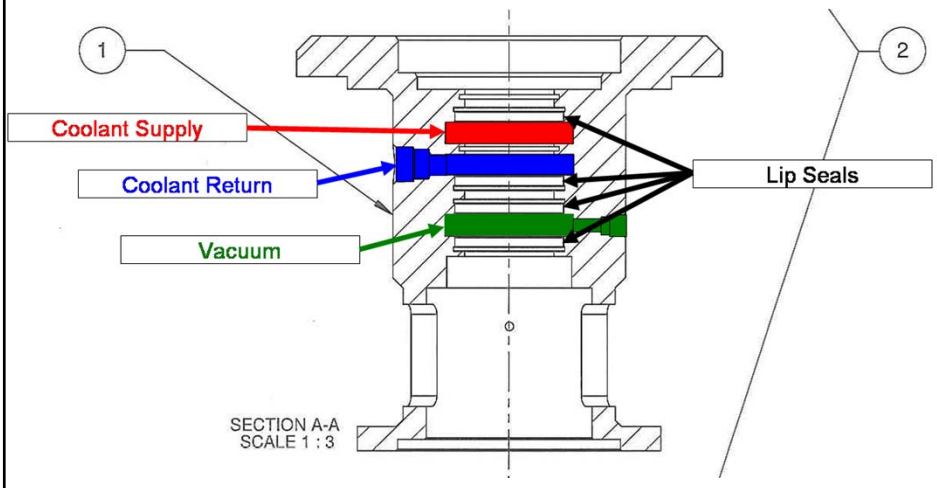


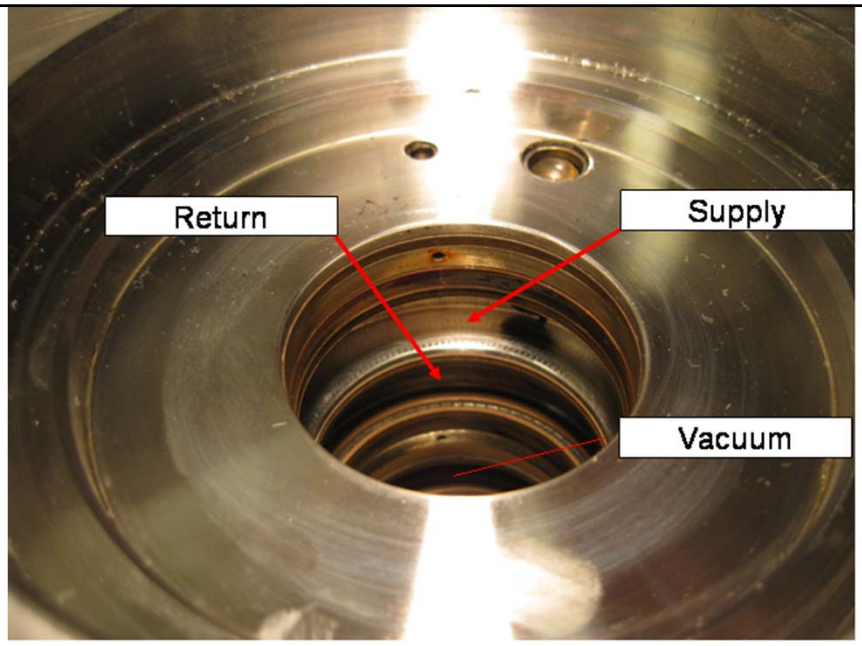
Pump Head Gasket Redesign

- **During engineering testing, water leaked onto the centrifuge deck and got into the pump head housing.**
- **Why was the water not detected by the leak detector and how did the pump head fill with water?**

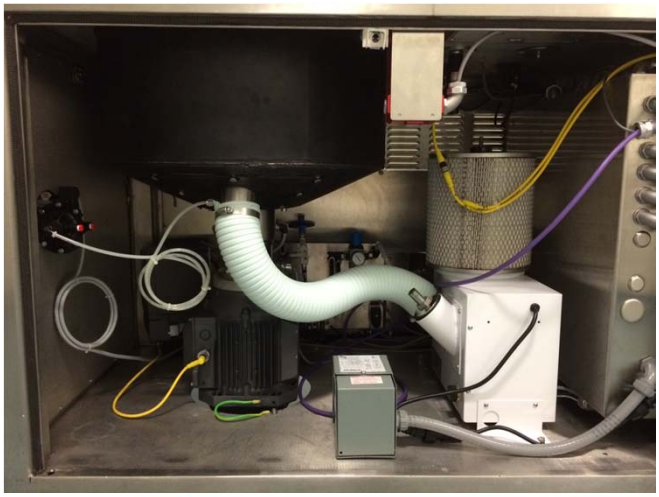
<u>Solution:</u> Redesign of pump head and gasket material

Leaking Seals and False Leak Detections Redesign





Leaky Seal Solution: Removed water cooled seals and implemented a HEPA filtered air cooling system.



Success!

- Centrifuge is now one of the most dependable pieces of equipment in the lab
- PPO has been contamination free for >4years
- Centrifuge is now a streamlined process - setup time has drastically reduced
- Great working relationship with vendor (PSA)

Key Components:

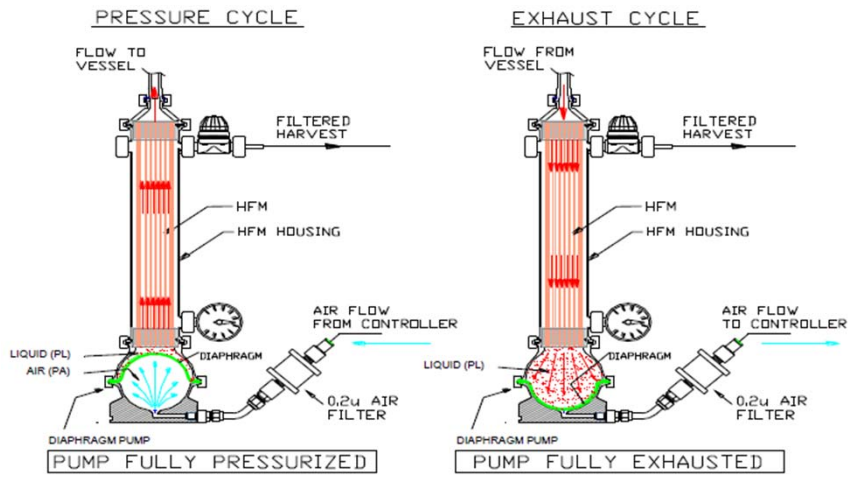
- New MOC for the Air Barrier
- Water Cooled to Air Cooled
- Re-Orientation for Tubing Length and Braid Tightness Consistency
- New Geared Idler Pulleys
- New Gates Belts
- New Pump Head
- Bleed Pump Removal
- New Non-Acrylic Dome
- New Non-Acrylic Dome
- New VFD Tuning
- New Cover Position Clips
- New VVD Separation Pump Timer Offset
- New ABB Motor
- New Top and Bottom Locking Collars
- Spanning Cam Retention Pin
- Etc….

Introduction of the ATF (Alternating Tangential Flow) into PPO

Advantages of Using ATF

- **Continuous: provides clarified harvest ready for capture chromatography**
- **Efficient: delivers 100% cell retention, increases productivity**
- **Intensified: generates high cell concentration and viability**
- **Flexible: connects to any bioreactor.**
- **Gentle: reduces cell stress, increases cell viability and productivity**
- **Scalable: adapts from development-scale to production-scale**
- **Self-cleaning: reduces biofilm build up on the hollow fiber filter**
- **Easy to use: reduces setup time and user training**

So how does a ATF work?



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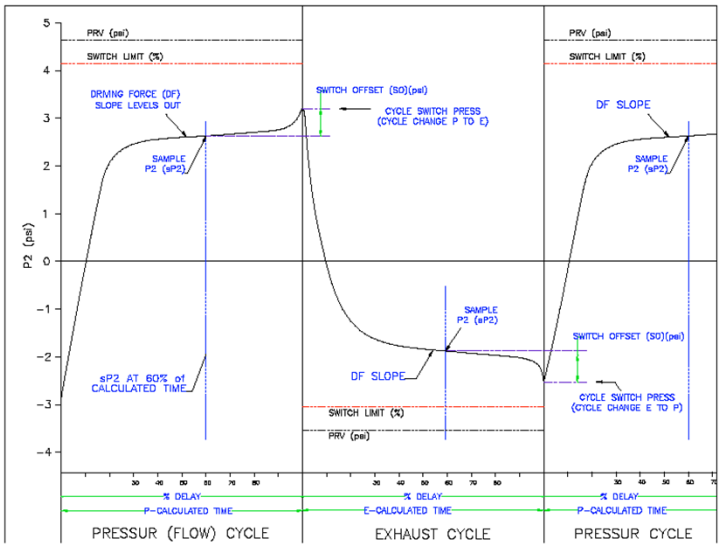


Process Characterization

- **ATF System Scale-up Calculator supplied by Repligen**

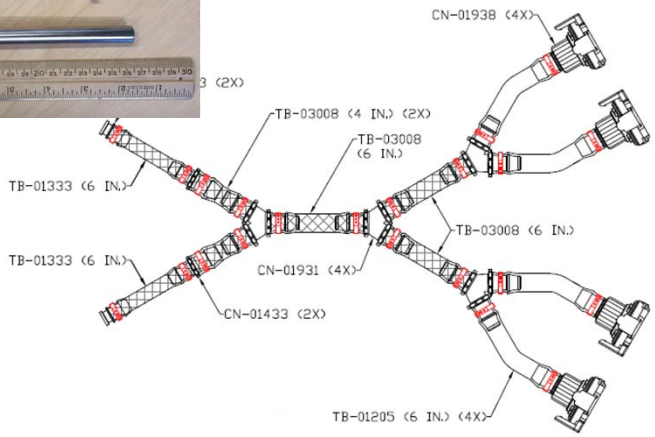
	Effective SA	Fiber count	Fiber length	ATF rate	Cycle Time	Residence Time	Process	Bioreactor Volume	Filtration rate				Scale-up Parameters	Flow per fiber	Shear	Flux	ATF to Filtration
	m2	nominal	cm	LPM	sec	sec		L	L/hr	LPM	L/day	wv/day		(ml/min)	sec^{-1}	LMH	RATIO
ATF2H	0.13	75	61	0.70	8.6	41.8		5.0	0.2	0.003	5.0	1.00		9.3	1584	1.60	202
ATF6	2.5	1440	63.5	14.3	5.5	34.4		100	4.0	0.067	100	1.00		9.9	1686	1.60	215
ATF10H	11	6720	61	63	5.7	35.4		422	17.6	0.293	422	1.00		9.3	1584	1.60	214

- **Residence Time: Cells are exposed to the same level of DO stress**
  - **Residence time is defined as 4 complete ATF Cycles, assumes 97% of the original volume is cleared.**
- **wv/Day: should be equal so the specific perfusion rates are equal across scales**
- **Flow per Fiber: should be equal so that cell shear is maintained across all scales.**
- **Flux: should be equal so that filtration capacity is consistent**
- **ATF to Filtration Ratio: approximately equal so that back flush efficiency is maintained across all scales**

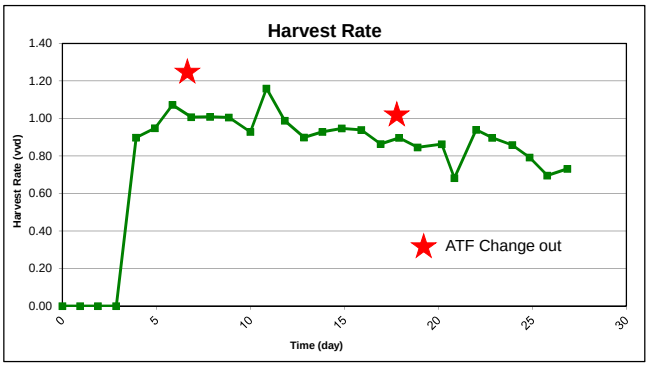
Introduction of the ATF into PPO

- **ATF 2 located in BPD**
- **Fits on bench top**
- **Autoclaved out of place and attached to sterile connections to a subsurface dib tube**
- **Small scale data shows that 1 ATF can remain in place for entire 26 harvest day process**
- **Transferred process to PPO that allows for single change out.**

Connecting ATF to reactor



ATF Performance



- **ATF system was not on our Delta V control system and data was not able to transfer to the OSI Pi data capture system**
- **Monitored the performance of the ATF system off single data points on the ATF controller (Harvest Line Pressure and Harvest weight)**
- **1 ATF was used in BRX at 120L Working Volume.**
- **We considered our first ATF production run a success.**

Over 4 Years Without Perfusion Issues

New project introduced to the PPO that utilizes ATF

- **New project will run reactor at 200L**
  - **This requires (2) ATF 6 systems to be connected to BRX**
  - **200L bioreactor bag revised to handle new system**
  - **Connection rig has to be reengineered.**

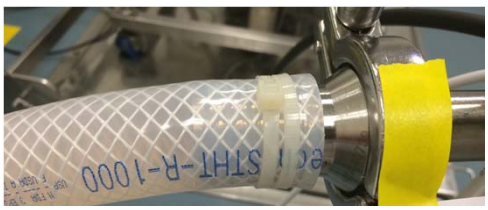
Bioreactor System	Seed Date	Termination Date	Duration prior to contamination
Xcellerex 200 L	26-Aug-15	21-Sep-15	Day 26 or H18 (5 days after ATF filter change-out)
	28-Oct-15	31-Oct-15	Day 3 (1 day after perfusion started)
Hyclone 50 L	14-Oct-15	22-Oct-15	Day 7 or H2 (5 days after perfusion started)

Warped Plastic Flange Connectors Were Replaced with Stainless Steel Prior to Next Run

Failure Identification

Mono-barbed, plastic connector (Run 1)

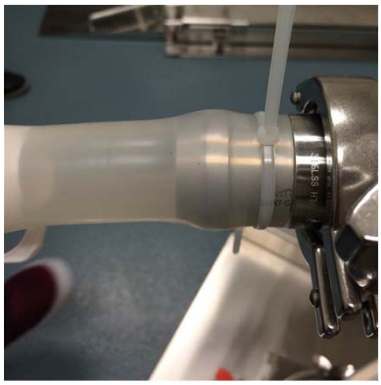
Multi-barbed, SS connector (Run 2 and 3)



- **Run 1 Failed because plastic mono barb did not work well with SS.**
- **Run 2 and 3: moved to a SS mulitbarbed connector**
  - **Multibarbed has a 1” OD**
  - **Webbed tubing has a 1” ID**
- **Run 2 and 3 failed because the webbed tubing does not bound to the multibarbed connector well.**

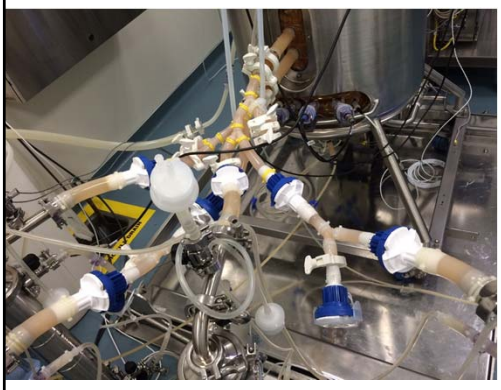
SS to Disposable Fix:

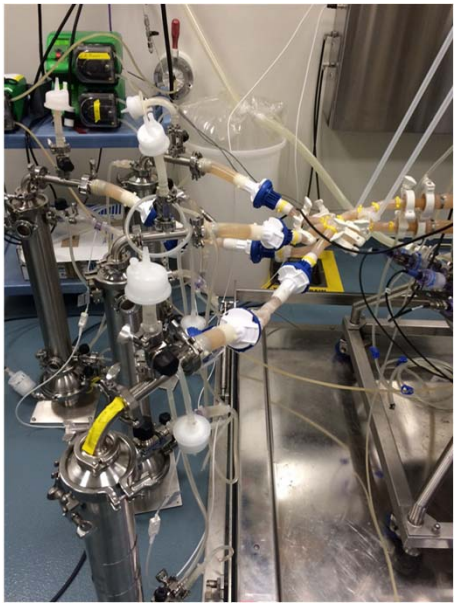
- **SS to C-Flex needs to be as close to "molded" as possible.**
- **C-Flex ID: 1", custom made Monobarb OD: 1.25"**
- **Stop testing failures with production runs.**
  - **Static and Dynamic media holds were tested on the ATF systems with the new monobarb for 14 days with no contamination.**



Remember the 3 R's?:

- **Reduce**
- **Re-size**
- **Re-form**





Tips for Evaluating New Technologies

- **Make sure you are getting involved with a company that is willing to make improvements.**
- **The adoption of new single-use systems significantly alters the development model**
  - equipment characterization needs to be included in the scope of "process development"
- **Single-use systems are not as "plug-n-play" as advertised**
  - extensive equipment characterization and process modeling studies
  - Scalability studies and process integration need to be mapped
- **Single-use technology platforms require careful consideration of the human factor**
  - skilled art along with the exact science
- **Integrate Supply Chain, Facilities, Engineering, Safety and Quality early in the process and truly partner with your supplier**
  - Detailed planning and supplier qualification MUST be part of your development process
  - Avoid "long term" side effects

Acknowledgements

Thank You to the following:
- **Pneumatic Scale Angelus (PSA)**
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- **Shire Bioprocess Development (BPD)**
- **Shire Engineering and Technical Services (ETS)**
- **Shire Pilot Plant Operations (PPO)**

Adaptation of Single-Use Technology in Pilot Plant Operations

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