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Single use disposable BioSettler removes the dead cells and cell debris selectively to increase the viability percentage of mammalian cells (e.g., CAR-T) during expansion

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Single Use Disposable BioSettler:
A closed bench-top automated flow system for autologous cell therapy purification, washing and concentration of CAR-T, MSC, islet cells, etc.

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Unique Solution for an Urgent Need

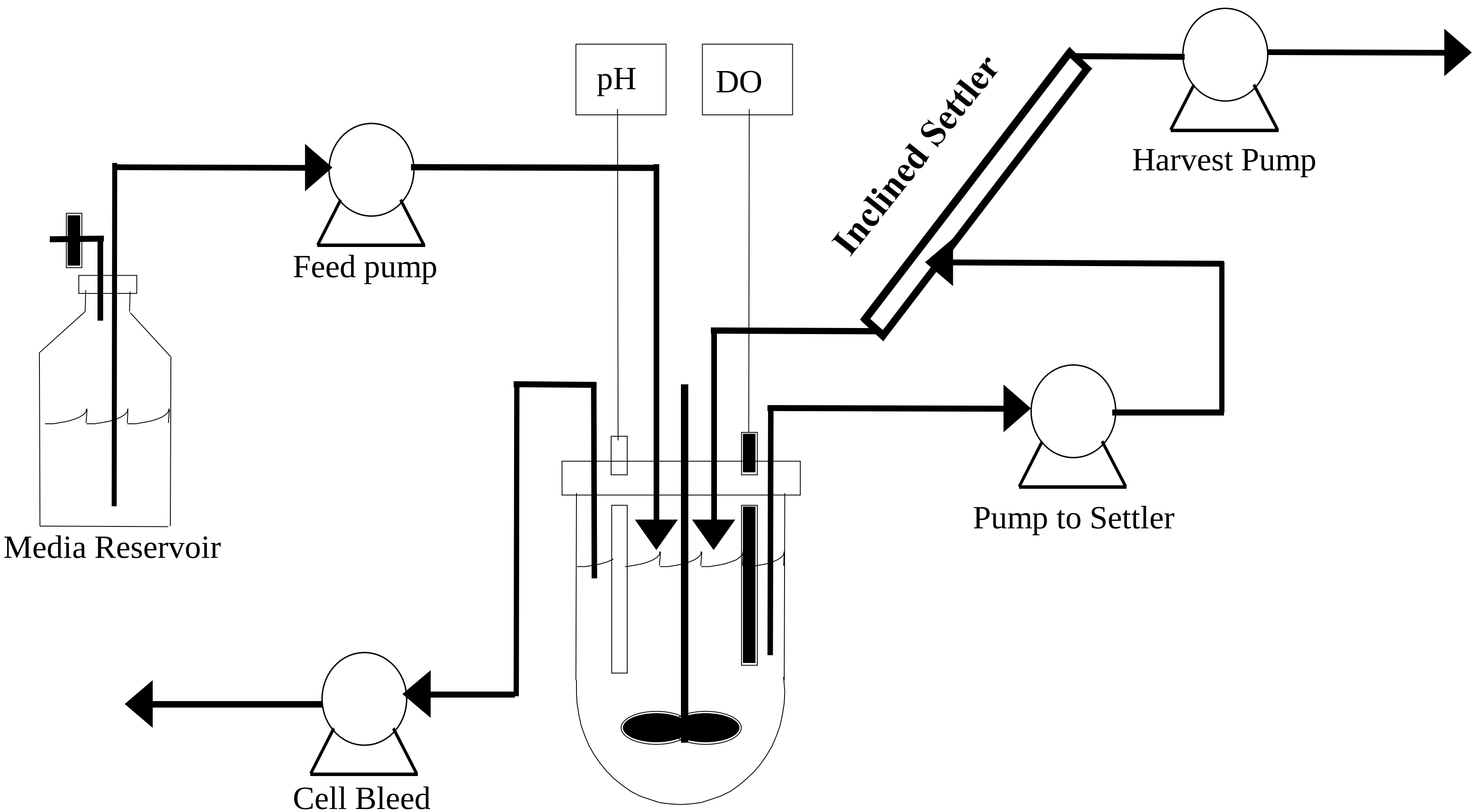
- Current challenge in Kymriah manufacturing for adult cell therapy seems to be the ***lower viability*** of CAR-T cells during cell expansion in bioreactor.
- This problem can be easily solved by a BioSettler, which can selectively remove the dead cells and cell debris from the expanding cell suspension.
- BioSettler technology, selective removal of dead cells and recycle of live cells, has been proven with mammalian cells in large perfusion bioreactors.
- Now this technology is available as single use disposable plastic BioSettlers for autologous cell therapy manufacturing applications.

Inclined Settler – a selective cell retention device

- Simple, passive, robust & powerful technology
- Uses no membrane barriers that can clog!
- Uses no moving parts that can break down!
- Proven at large scale for mammalian cell cultures
- Selective removal of dead cells and cell debris
- Complete recycle of live cells to bioreactor
- High *viable* cell density and productivity
- Perfusion bioreactors can be operated indefinitely
- Not demonstrated successfully for microbial cells

Inclined Settler as a selective cell retention device

Batt, Davis & Kompala, Biotech Prog, **6**:458-464,*1990*;
Searles, Todd & Kompala, Biotech Prog, **10**:198-206,*1994*



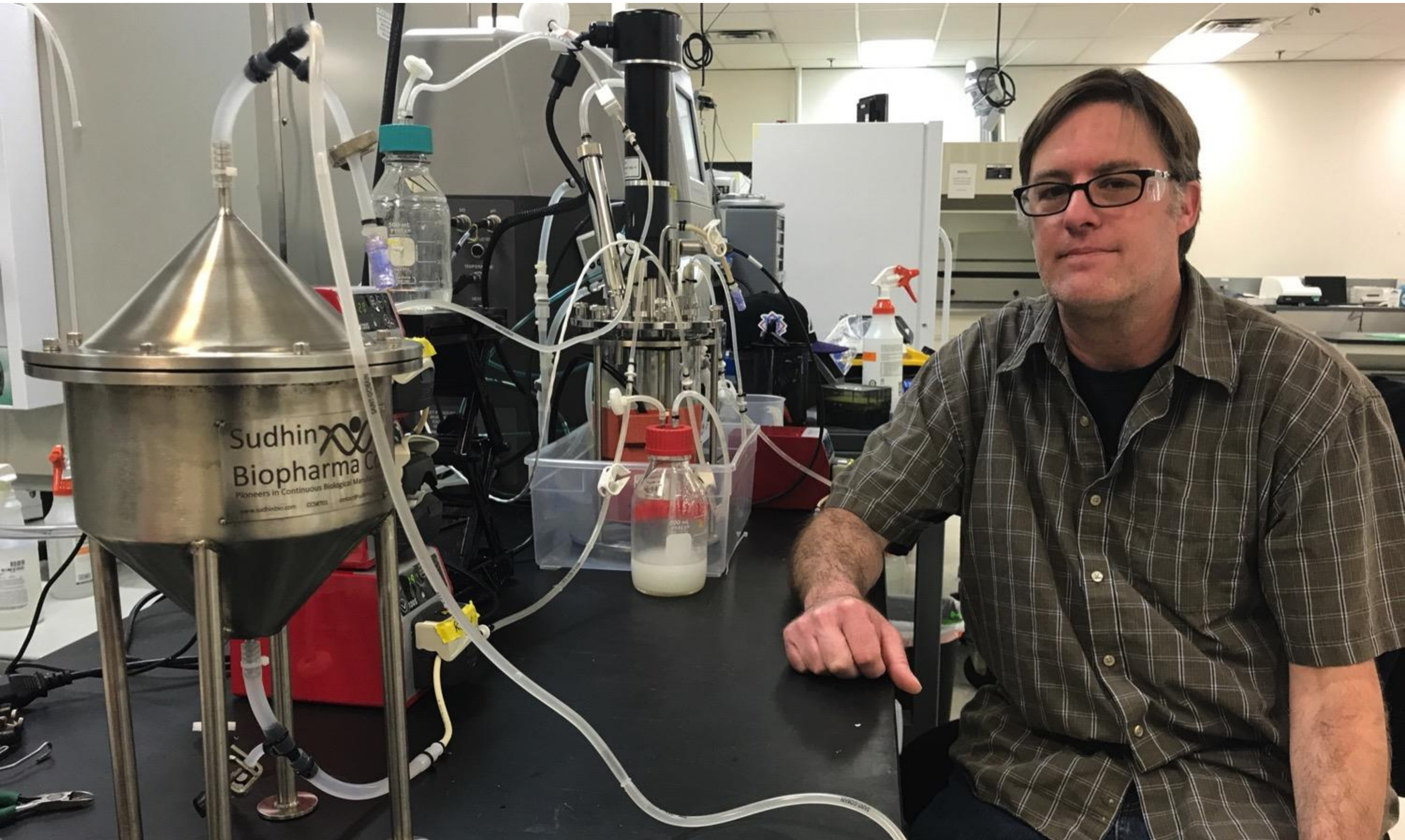
Inclined settler scaled up for a 3000 L perfusion bioreactor (replacing n-1 seed train)
Pohlscheidt, et al. Biotech Prog. (2013)29:222-229, Roche/Genentech, Germany

Novel Compact Cell Settlers

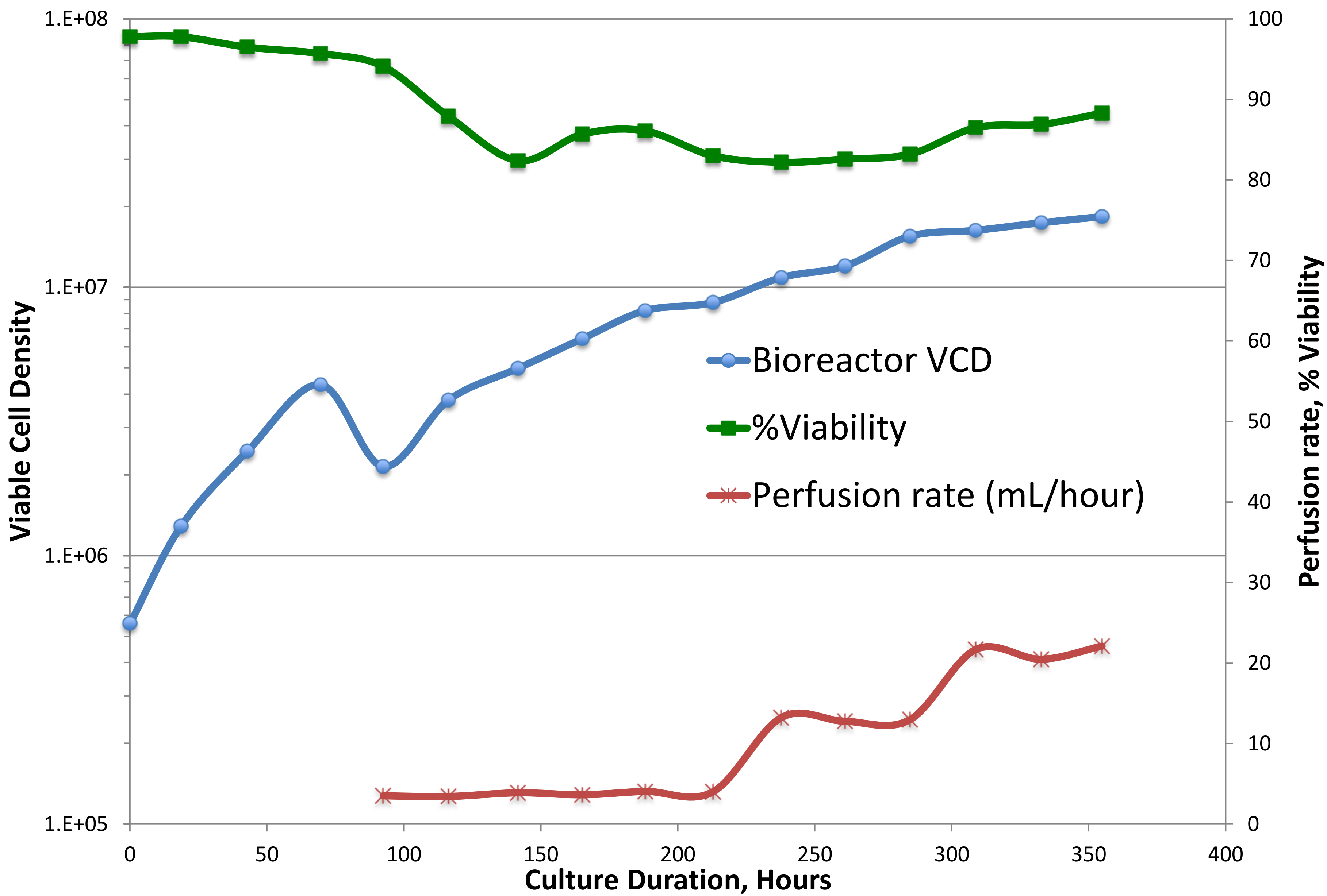
Easily scalable cylindrical & conical design

6 – 10 x more settling area for the same footprint

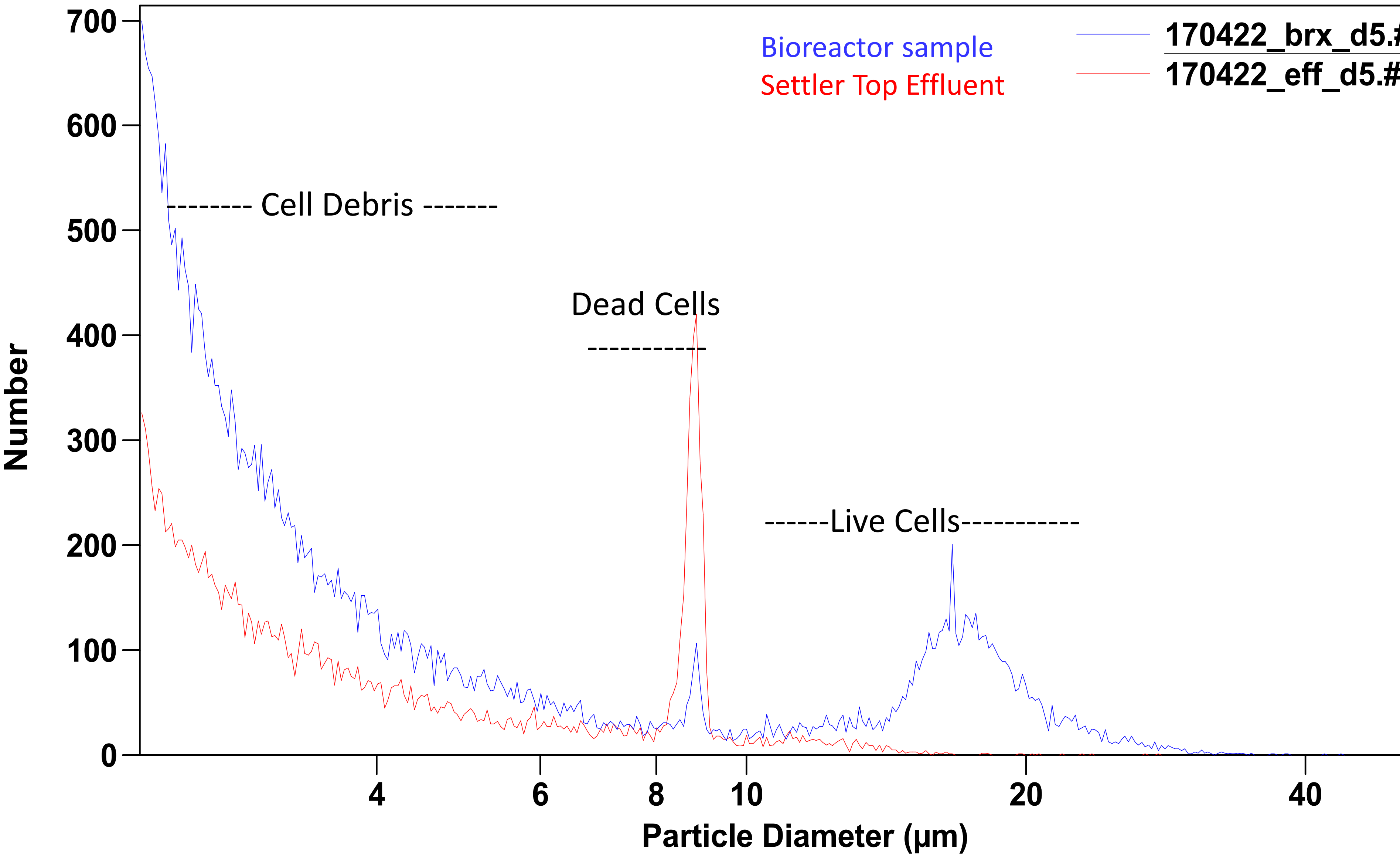
6” diameter Compact Cell Settler attached to a 1.2-liter Celligen CHO Cell Bioreactor



Perfusion Bioreactor of CHO cells with our Novel Compact Cell Settler as the Selective Cell Retention Device



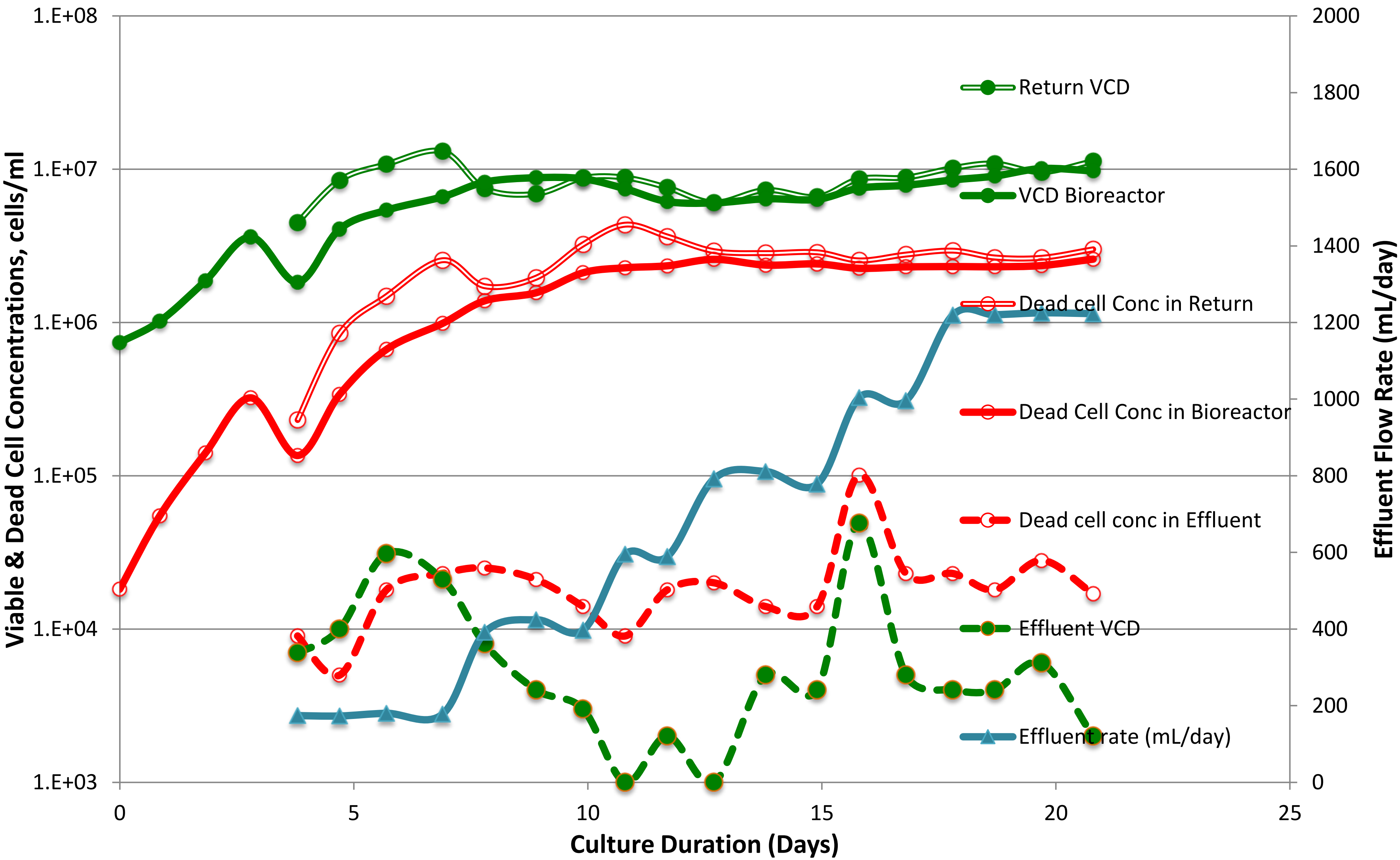
Selective Removal of Smaller Dead Cells & Cell Debris in BioSettler Top Effluent



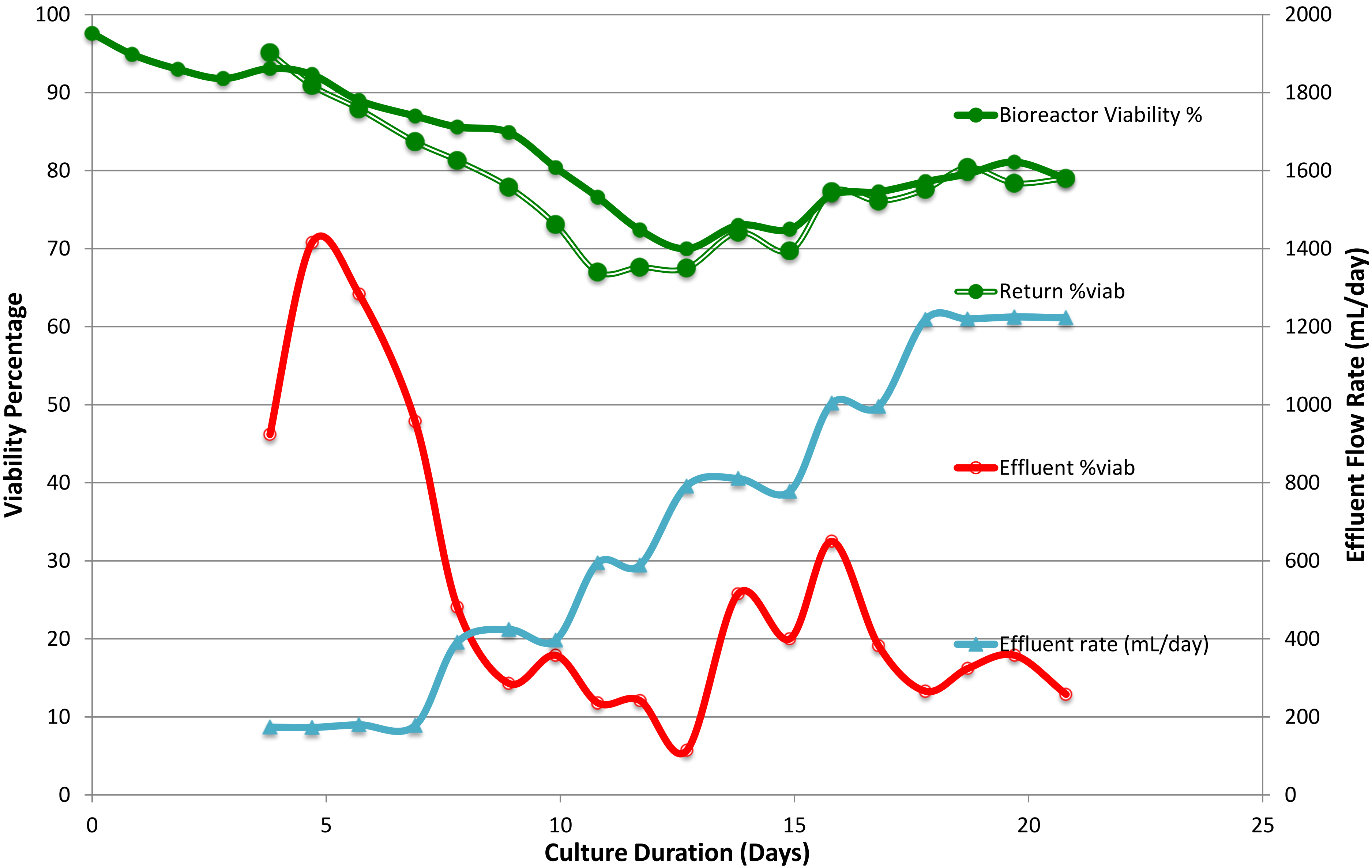
Single Use Disposable BioSettler 150 developed with NIIMBL funding

BioSettler for 1. CHO Perfusion Bioreactor

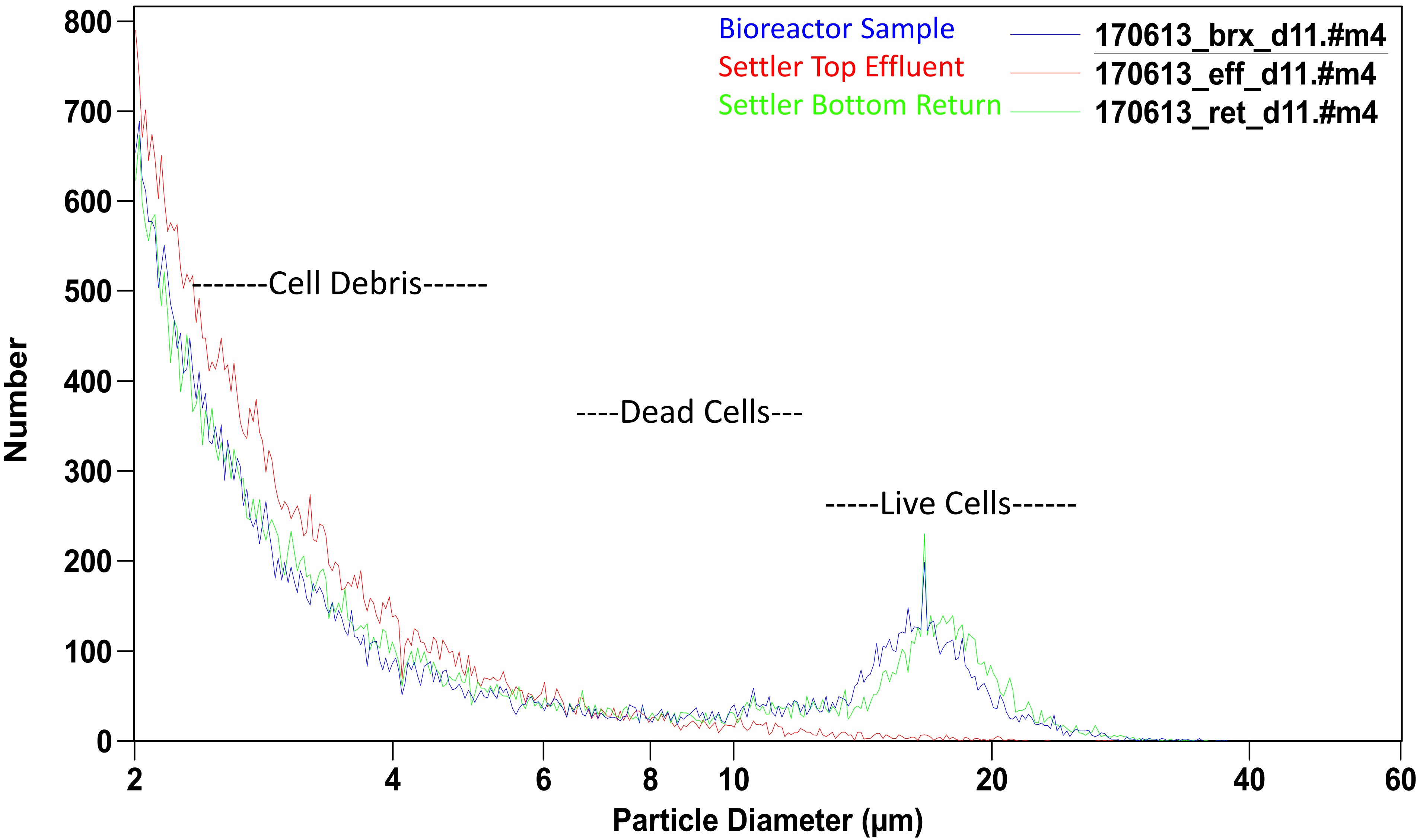
CHO Cell Density profiles from our first perfusion bioreactor expt w BioSettler150



Cell Viability profiles during our first perfusion bioreactor expt w plastic settler



Size distribution of cells in samples from Bioreactor, Settler Effluent & Return



2. Clarification of Cell Culture Broth in the BioSettler150 at Sudhin Biopharma

Reducing culture broth's pH from 7 to 5 or lower by adding a small amount of acetic or citric acid causes cell clumping and rapid settling of clumps.

Fast settling cell clumps may be removed from the bottom port with a peristaltic pump quickly before they get packed or compacted. After draining out the cell clumps, the clarified supernatant can be collected via bottom port.

Alternately, the secreted product in the settling cell clumps may be removed by pumping in a buffer via bottom port to wash out the product from these cell clumps and collecting the diluted and clarified supernatant from the top port.

This photograph shows the collected cell clumps (~70 ml) at the bottom of settler from 3.5 liters of cell culture broth at 2 – 4 million cells/ml plus about 300 ml of acetic acid at pH 4.

2a. Washing of Settled Cell Clumps

2b. Draining of Cell Clumps from bottom

2c. Fed-Batch Cell Clump & Clarification

3.4 liters of cell culture broth (pH 7) were pumped with about 400 ml of citric acid (pH 4) in to fill the settler (3.8 liters) over 30 minutes and reduce the pH to below 6.

More cell culture broth (4.6 liters) and citric acid (600 ml) were pumped in at a lower rate over the next 3 hours, as the clarified supernatant is collected at 1.8 liters/hr from the top port.

Total of 8 liters of cell culture broth was pumped in the settler along with 1 liter of citric acid and was clarified in < 4 hours while the cell clumps were retained in the single use disposable biosettler.

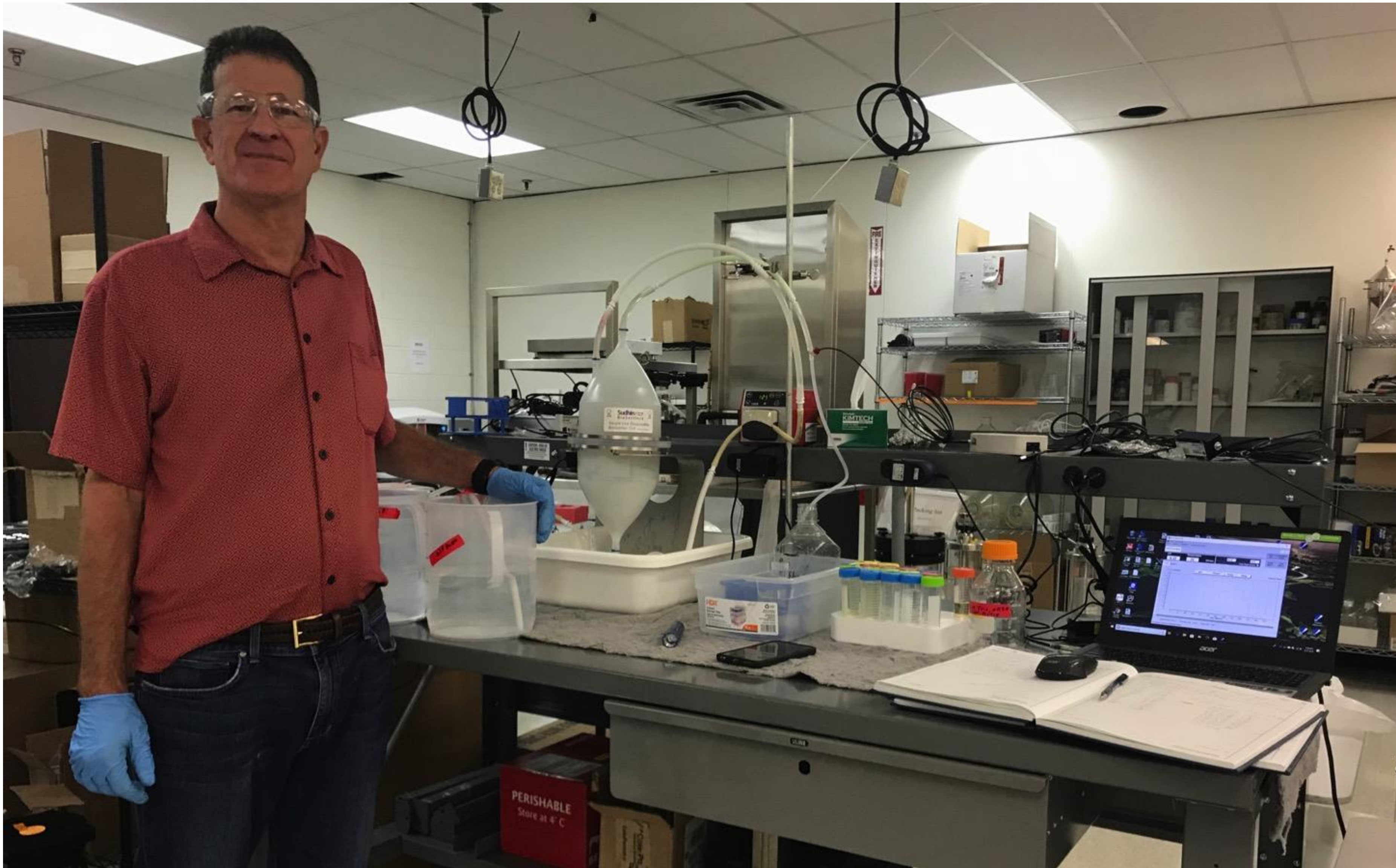
91.4% of secreted antibody product was recovered in the settler clarified supernatant, plus an additional 7.3% of IgG was recovered after centrifuging the cell clumps.

Centrifugation of the clarified supernatant resulted in a packed cell volume (PCV) of 0.5%, whereas the PCV in the starting cell culture broth was 3.0% (reduction of 83.3 %).

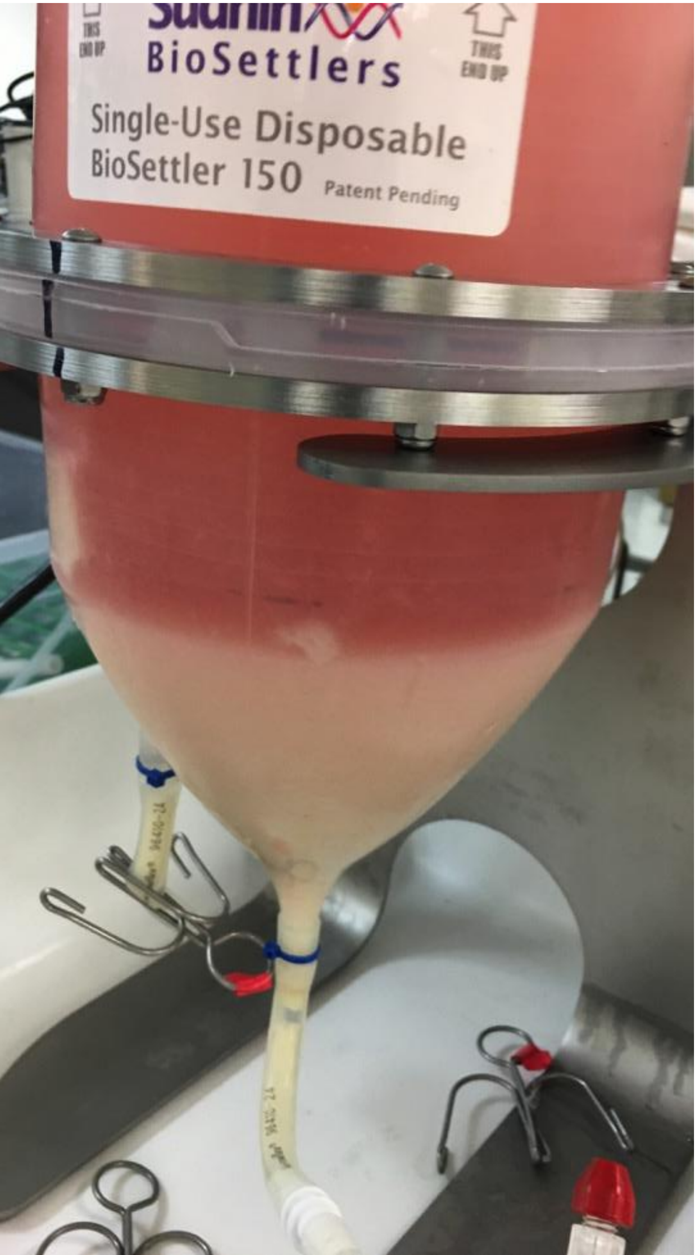
3. Affinity Capture of Secreted Antibodies on Protein A beads suspended in settler

- Protein A beads (GE MabSelect SuRe LX) have a size range of 28 – 82 microns(μ), with a median of 43 μ.
- Size range of live CHO cells is 10-20 μ, dead cells: 8 – 10μ, and cell debris: < 8μ.
- Cell culture broth from end of Fed-batch bioreactor can be directly loaded into our settler, along with Protein A beads.
- After secreted antibodies are bound to the larger beads suspended in the settler, all smaller cells, debris and unbound host cell proteins can be washed out the top.
- Elution and regeneration steps can also be carried out sequentially with the beads suspended in the settler.

3. Affinity Capture Experimental Setup



Sudhin BioSettler for Cell Therapy Applications



Selective Removal of Dead Cells and Cell Debris to increase viability percentage in expanded CAR-T cells (e.g. Kymriah)

Harvesting, Washing and Concentration of many *in vitro* expanded cells in a closed, bench-top automated device.

Harvesting and Purification of ex vivo expanded MSCs after expansion on microcarriers and enzymatic detachment.

Harvesting of Organoids, such as the larger b cell islets, from smaller iPSCs and other differentiating cells.

Harvesting of Gene Therapy vectors (AAV) from HEK cells (being tested at NCState Univ through a NIIMBL SP grant).

Clarification of Cell Culture Broth from fed-batch culture (being tested at Genentech through NIIMBL QSP grant).

Selective Cell Retention Device for achieving high cell densities and viabilities in perfusion bioreactors (Sudhin).

Key References

- Batt, B.C., R.H. Davis, and D.S. Kompala, Biotechnology Progress 6: 458-464, 1990
- Searles, J.A., P.W. Todd, and D.S. Kompala, Biotechnology Progress 10: 198-206, 1994.
- Freeman, C.A., P.S.D. Samuel and D.S. Kompala, Biotechnology Progress 33: 913- 922, 2017

Acknowledgements

- National Science Foundation, Presidential Young Investigator award to DSK, $500,000; 1988-1993
- National Science Foundation, Small Business Innovation Research Phase I grant $180,000; 2015
- National Science Foundation, Small Business Innovation Research Phase I grant $225,000; 2016
- National Institute for Innovation in Manufacturing (NIIMBL) Biopharmaceuticals, QSP 1.18, $1.5 M, 2017-2019