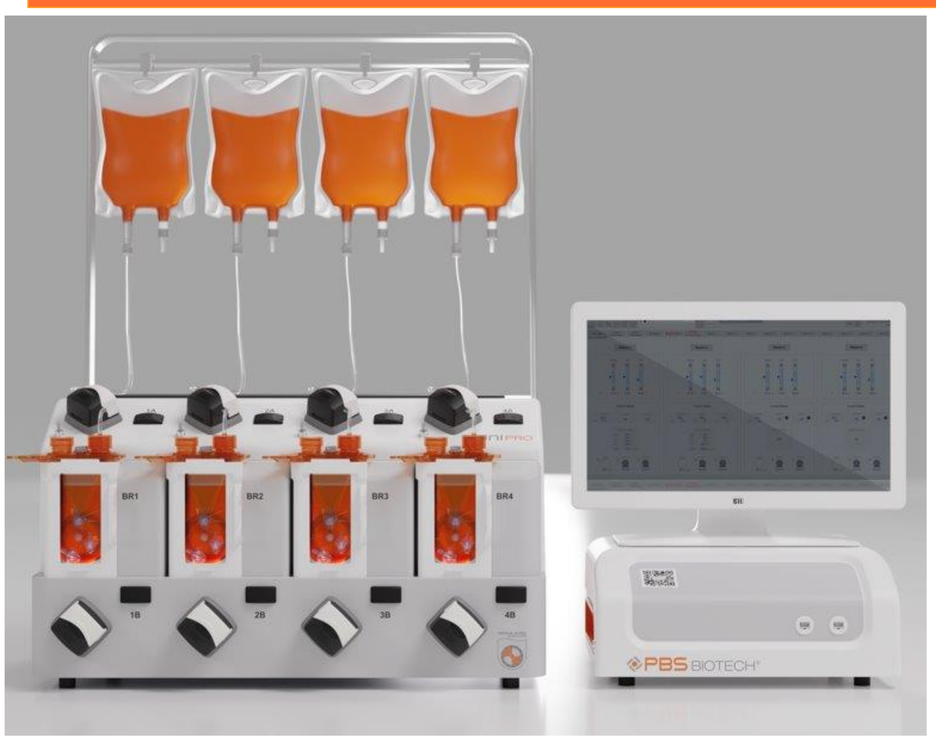
Optimizing Closed-System iPSC Processes Using a Scaled-Down, Computer-Controlled Parallel Bioreactor Platform

Breanna S. Borys[1], Omokhowa M. Agbojo[1], Govanni Estrada[1], Walongorn Kritalukkul[1], Kenny Cruz[1], Brett D. Abraham[1], Sharon Harvey[1], Sunghoon Jung[1]

[1]PBS Biotech Inc., Camarillo, California, USA

Introducing MiniPRO



Independent operation of 1-24 controlled bioreactors

pH and dissolved oxygen feedback control via sensor spots (no additional probes impacting hydrodynamics)

Increased cell yield and cell quality control

Controls reduce fluctuations in the physiochemical environment and overcome O_2 supply limitations that prevent continued expansion

Integrated large-particle perfusion capabilities

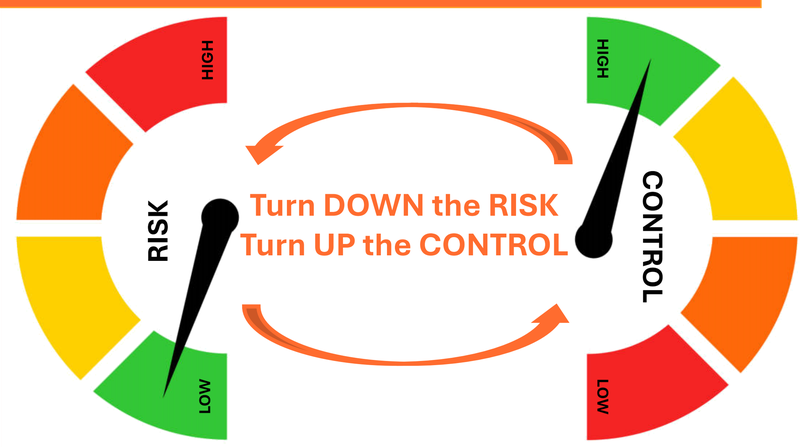
Each single-use vessel is equipped with a pre-installed 60 um retention filter and tubing to enable perfusion with the on-board MiniPRO pumps

Closed-system sterility

Easily batch, inoculate, MX, and sample using the medium lines on the perfusion cap and the front of the vessel ports (at 60 mL or 30 mL levels)

Linear scalability to GMP-ready systems

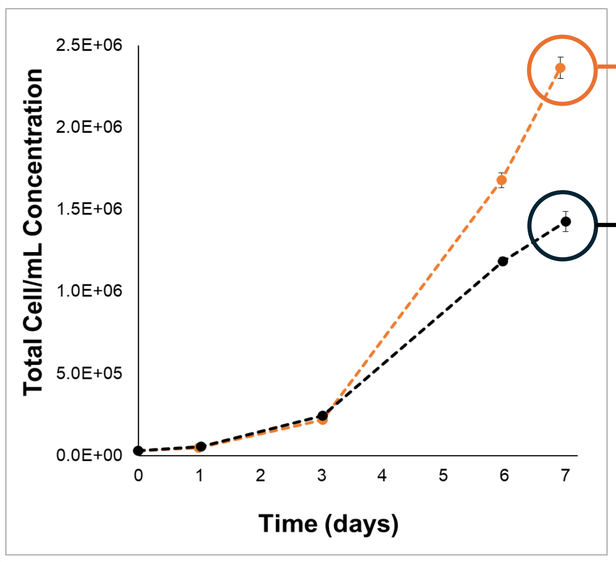
Utilize recipe building features to design gassing control strategies at small-scale to obtain results that match those from clinical scale reactors

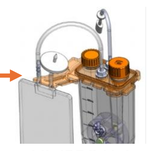


While efforts have been made to develop scalable processes for producing human induced pluripotent stem cell (iPSC)-derived products, cell therapy manufacturing processes are complex, involving various unit operations such as expansion, differentiation, harvest, fill/finish, and cryopreservation. Developing each of the processes at scale for sensitive iPSCs and derivatives is extremely challenging and resource intensive. However, without an effective scale-down tool, unknown process risks are often taken at clinical scales with multiple reports of costly manufacturing failures. This study is the first to utilize a novel, scaled-down parallel bioreactor system from the family of Vertical-Wheel® (VW) bioreactors. The MiniPRO system demonstrates the advantages of control systems to overcome oxygen limitations and provides a better scaled-down model to work from.

Increased Cell Yield and Cell Quality Control

1. Experiment Workflow = Thaw into 2D T-Flasks (P+1 and P+2) and Passaged into 3D Bioreactors (P+3)



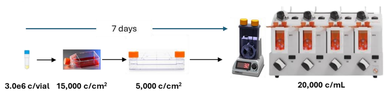


MiniPRO Unit
O_2 Controlled

PBS-0.5L (Incubator)
O_2 Not-Controlled

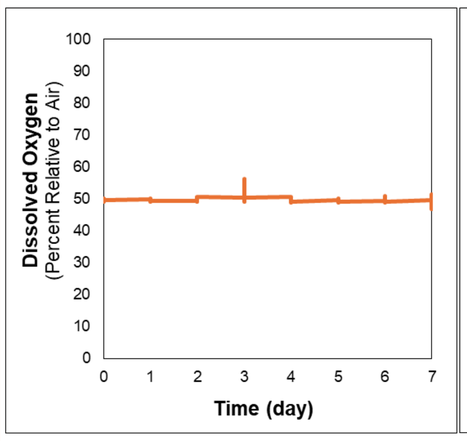
Methods /PIVs

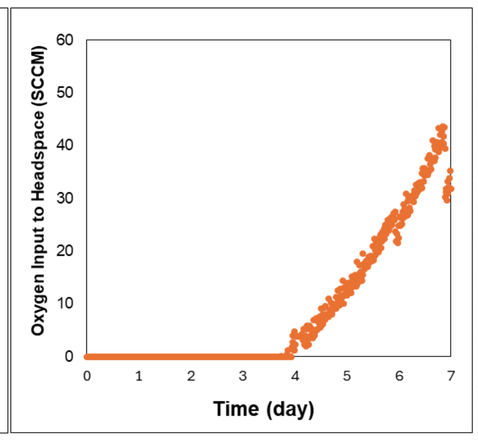
- **Cells/workflow** = iPSCs (TC1133) cryopreserved at 3.0e6 cell/vial from 2D culture were thawed and seeded into 2D culture for two passages prior to bioreactor inoculation



- **3D inoculation density** = 2.0e4 cells/mL (single-cells)
- **Expansion media** = mTeSR1 (supplemented with 10uM Y-27632 D0)
- **Feeding protocol** = Built-in retention filter utilized for perfusion
- **Feeding regime** = 0.5VVD D3-D5 and 0.8VVD D5-D7

Oxygen Control and Gassing Input on the MiniPro Unit



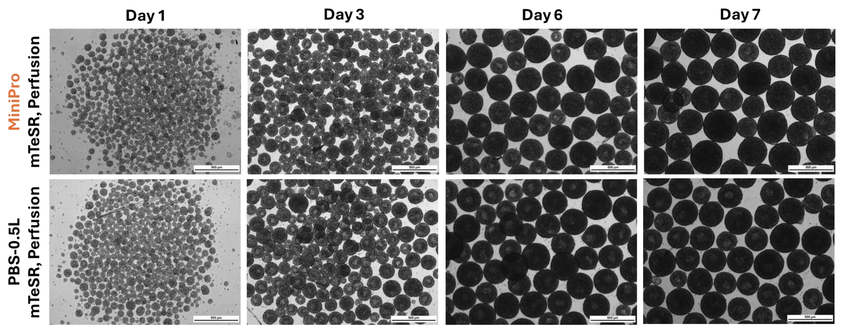


Gassing into the MiniPro headspace is set at a flowrate of 60sccm. In this case, the unit is set to control the dissolved oxygen in the culture at 50% (relative to air). This is achieved through constant monitoring and feedback control to the system.

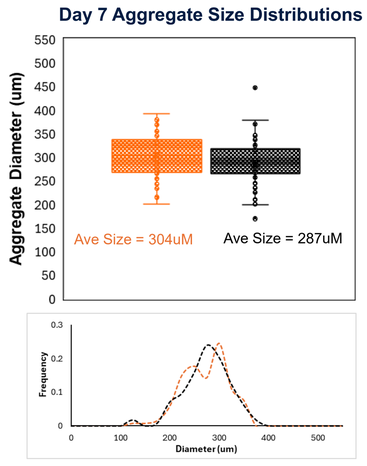
To maintain the oxygen levels, the unit starts to input oxygen on D4. The amount of oxygen going into the system increases as cell number increases to maintain control

With gassing control, oxygen is added to the system, overcoming limitations experienced in non-controlled vessels (incubator). This increases the physiochemical stability throughout the culture and increases the cell yield potential.

MiniPro Aggregate Size Homogeneity (Compared to PBS-0.5L Control)

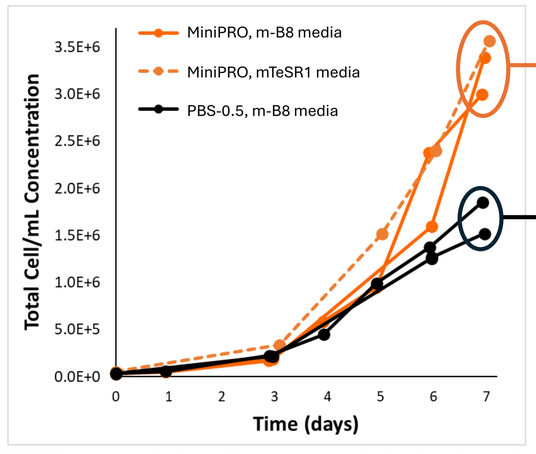


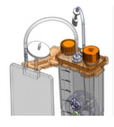
Representative microscope images taken throughout the culture period demonstrate morphologically healthy, uniform and relatively homogenous sized iPSC aggregates. Aggregate size distribution profiles between the MiniPro and PBS-0.5L are similar, which is a key indication that the vessel hydrodynamics between the two reactors are maintained.



Reproducible and Robust Results when Tested with Different Input Variables

2. Experiment Workflow = Direct Thaw of iPSCs into Bioreactors





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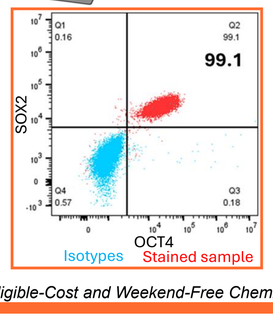
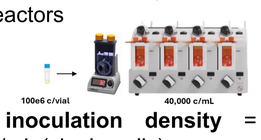


iPSCs expanded for 7 days from high-density cell banks (HD-CBs) directly thawed into 3D maintain quality markers (SOX2 and OCT4).

Methods /PIVs

- **Cells/workflow** = iPSCs (TC1133) cryopreserved at high density (100e6c/mL) from 3D culture were thawed and directly inoculated into bioreactors



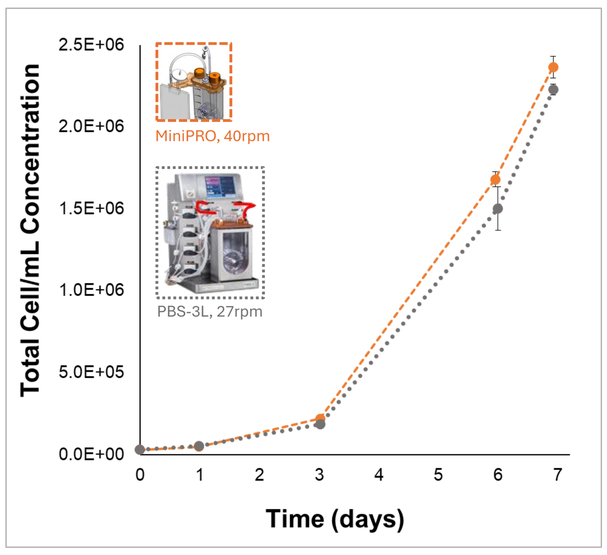
- **3D inoculation density** = 4.0e4 cells/mL (single-cells)
- **Expansion media** = mTeSR1 or m-B8* (supplemented with 10uM Y-27632 D0)
- **Feeding protocol** = Built-in retention filter utilized for perfusion MX
- **Feeding regime** = 0.5VVD D3-D5 and 0.8VVD D5-D7

m-B8 is a derivative of E8 medium modified from Kuo et al. (2020) "Negligible-Cost and Weekend-Free Chemically Defined Human iPSC Culture"

Linear Scalability to GMP-Ready Systems

Defining Linear Scalability:

Maintenance of growth rates (cell/mL concentration) between optimized scaled-down bioreactors and larger scaled-up bioreactors

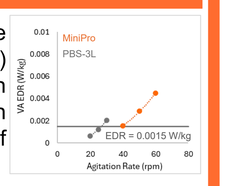


Methods /PIVs

- **Cells/workflow** = iPSCs (TC1133) cryopreserved at 3.0e6 cell/mL from 2D culture were thawed and seeded into 2D culture for two passages prior to bioreactor inoculation
- **Inoculation density** = 2.0e4 cells/mL (single-cells)
- **Expansion media** = mTeSR1 (supplemented with 10uM Y-27632 D0)
- **Feeding protocol** = Retention filter utilized for perfusion
- **Feeding regime** = 0.5VVD D3-D5 and 0.8VVD D5-D7
- **Gassing Controls** = Dissolved oxygen set to control at 50% (relative to air), carbon dioxide set to 5% addition

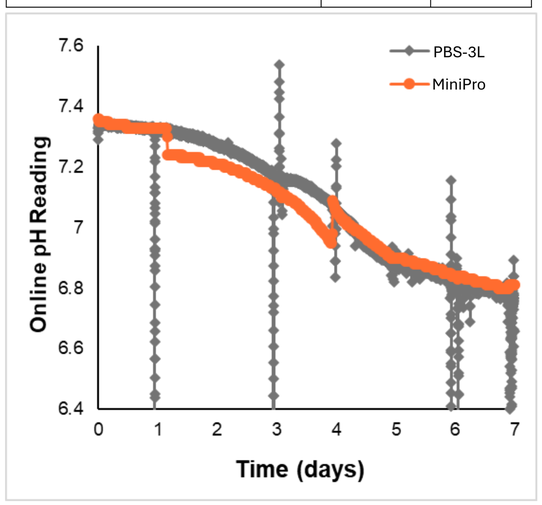
Scale-up Correlation

Scale-up agitation rates maintain volume average energy dissipation rate (VA EDR) constant. These scale-up correlation equations were developed through computational fluid dynamic simulations of MiniPro and PBS-3L.

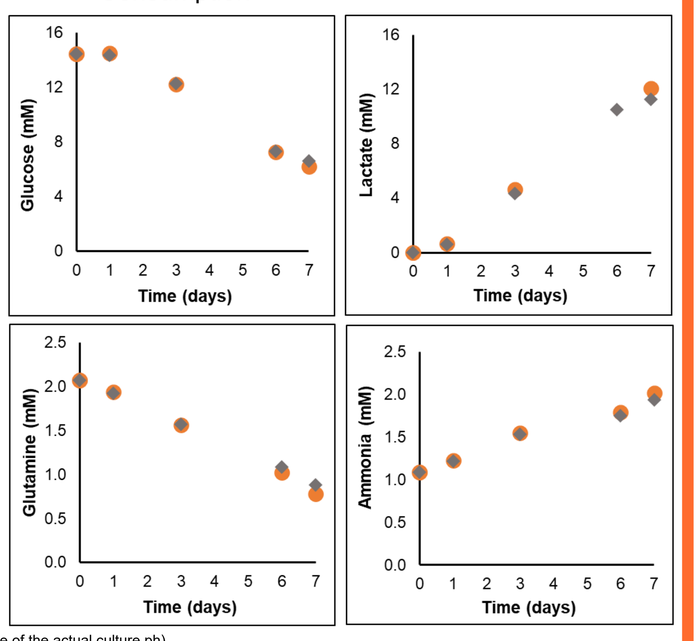


Growth Kinetics and Metabolites

	MiniPro	PBS-3L
Cumulative Growth Rate (h^{-1})	0.026	0.025
Population Doublings (D7)	6.3	6.0



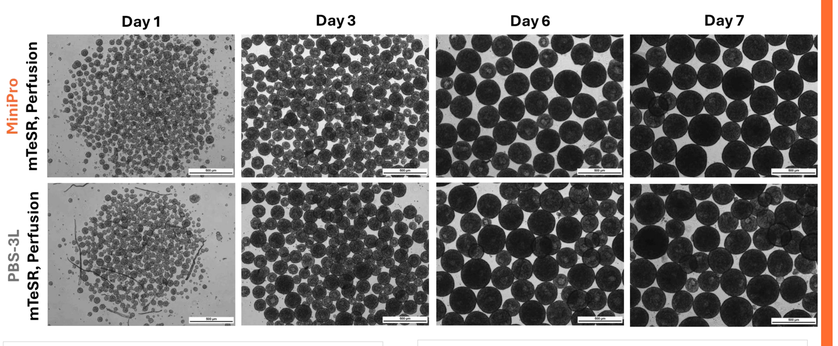
Spikes in online readings can occurring as an artifact of sampling (not representative of the actual culture ph)

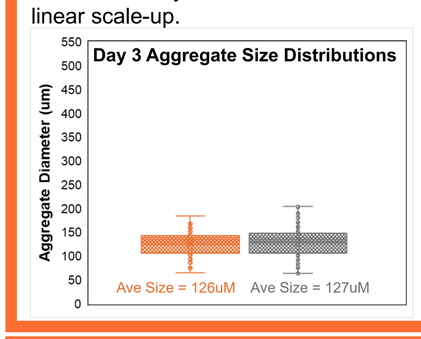


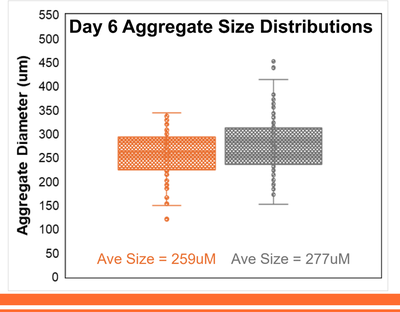
Monitoring the pH (online) and metabolic profiles (NovaFlex2) over the culture is a good way to validate cell counts. The similarity in profiles validate the linear scalability between the MiniPro and PBS-3L.

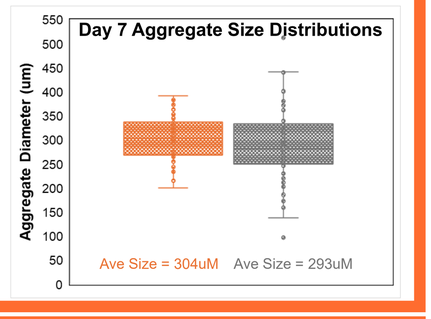
Maintenance of Aggregate Size and Size Distributions (MiniPro vs PBS-3L)

One of the most critical process attributes of 3D iPSC culture is control of aggregate size and aggregate size distributions. Utilizing the hydrodynamic relationships (scale-up maintaining VA EDR) we have demonstrated the maintenance of morphologically healthy, uniform and relatively homogenous sized aggregates. Aggregate size distribution profiles between the MiniPro and PBS-3L are similar throughout the culture which is a key indication of successful linear scale-up.









Summary

This work establishes the MiniPRO system as a powerful tool for identifying important scale-up PIVs in PSC bioprocessing. By accurately modeling manufacturing-scale conditions, the MiniPRO enables more efficient and cost-effective process development, ultimately increasing the speed and success at which PSC-based therapies can be translated from the laboratory to the clinic.