Scalable Manufacturing of Allogeneic Cell Therapy Products Using Vertical-Wheel Bioreactors

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VERTICAL-WHEEL™ BIOREACTORS

The next generation of single-use bioreactors, inspired by forward thinking

Presentation Topics

- Challenges of Manufacturing Allogeneic Cells
- Benefits of Vertical-Wheel Bioreactors
- Examples of Microcarrier-Based Processes
- Examples of Aggregate-Based Processes
- Key Takeaways

Choosing the Right Allogeneic Manufacturing Platform

- Scalable technology needed for large patient populations
- Static planar platforms adequate for R&D or clinical scale but not commercial
  - Limited process monitoring & control
  - Labor and material expenses
  - Variability among planar units

- Simaria A. et al., *Biotech & Bioeng, 2014*
- Campbell A. et al., *Stem Cells Trans. Med., 2015*

Single-Use Bioreactor As Cost-Effective Solution For Scale Up

- Achieve greater lot sizes while reducing manufacturing cost per dose

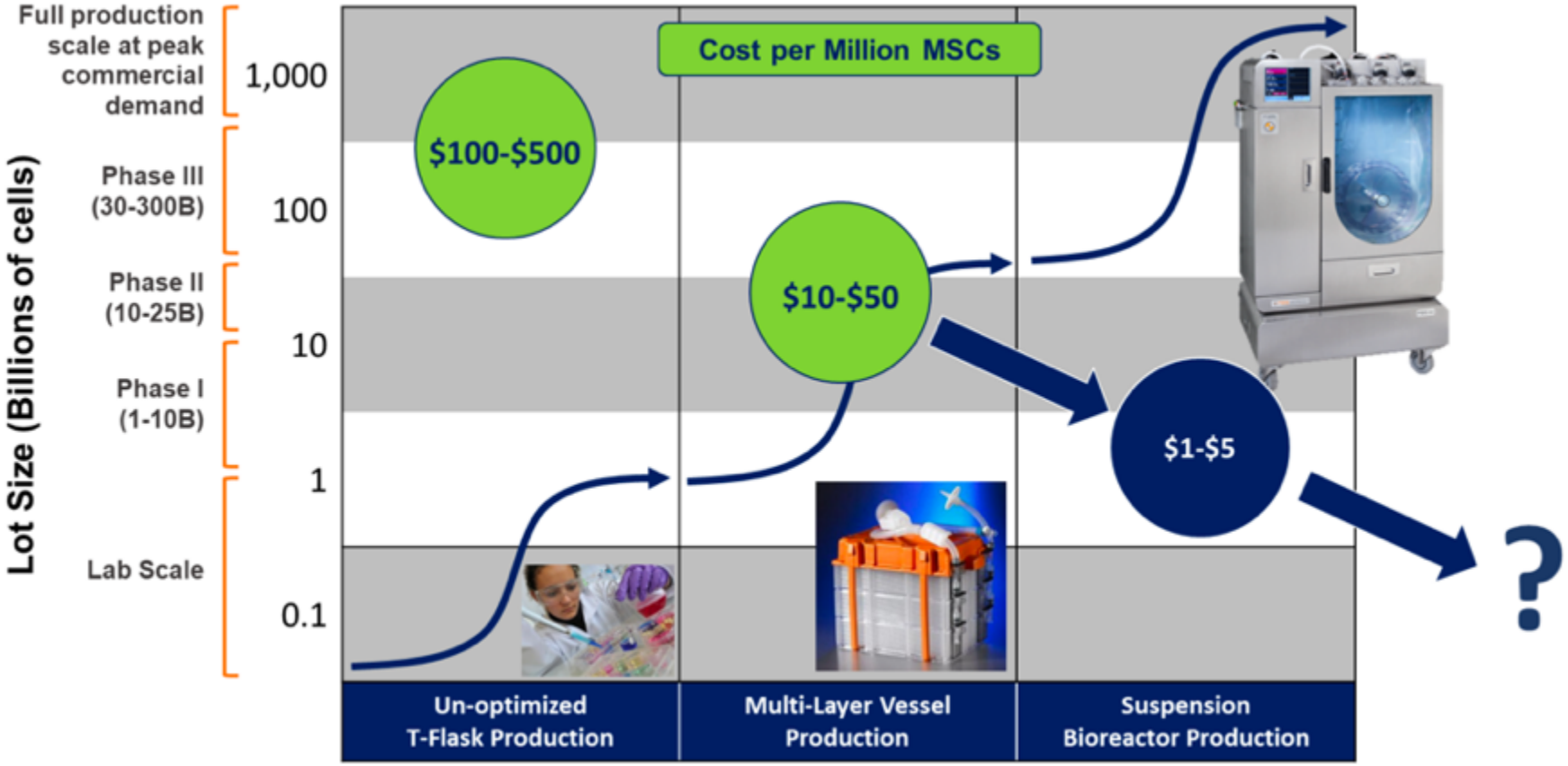
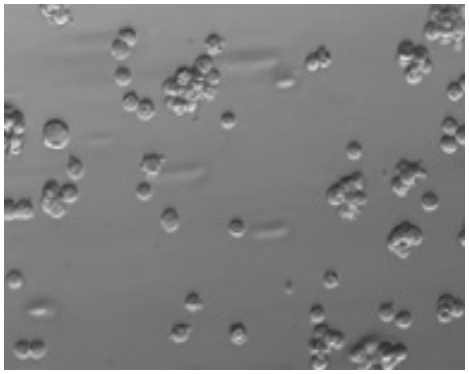


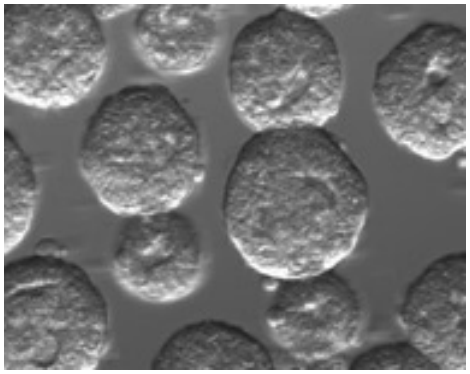
Figure adapted from Rowley & Montgomery, *BioProcess International* 2018

Manufacturing Different Cell Types in Suspension Culture

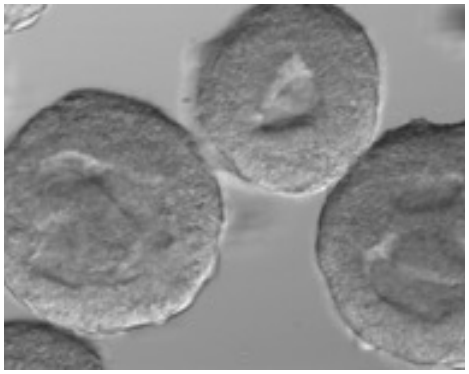
- **PSCs & ESCs**: self-forming cell aggregates



0 hrs

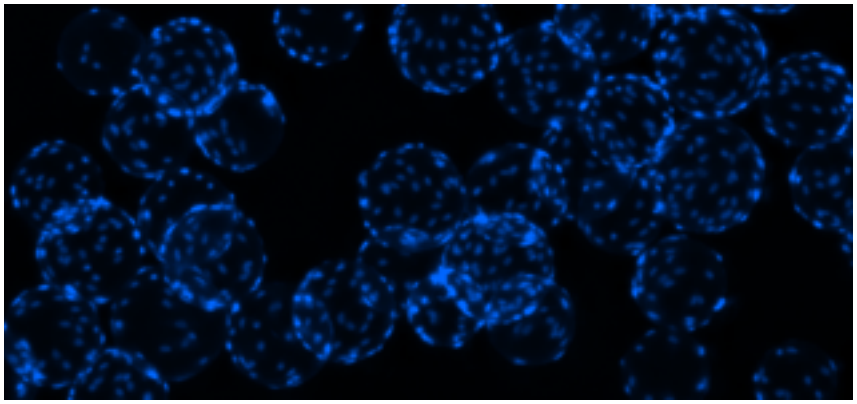


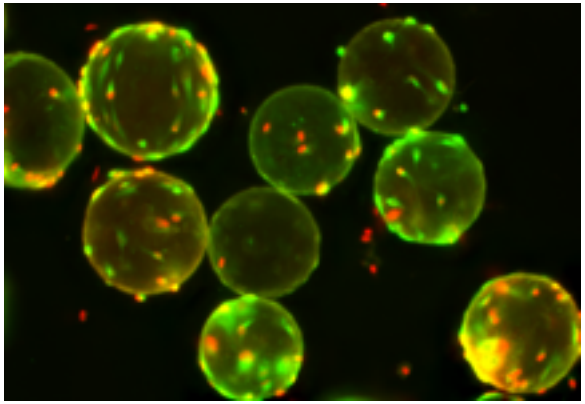
24 hrs



72 hrs

- **MSCs & Primary Cells**: grown on surface of microcarriers



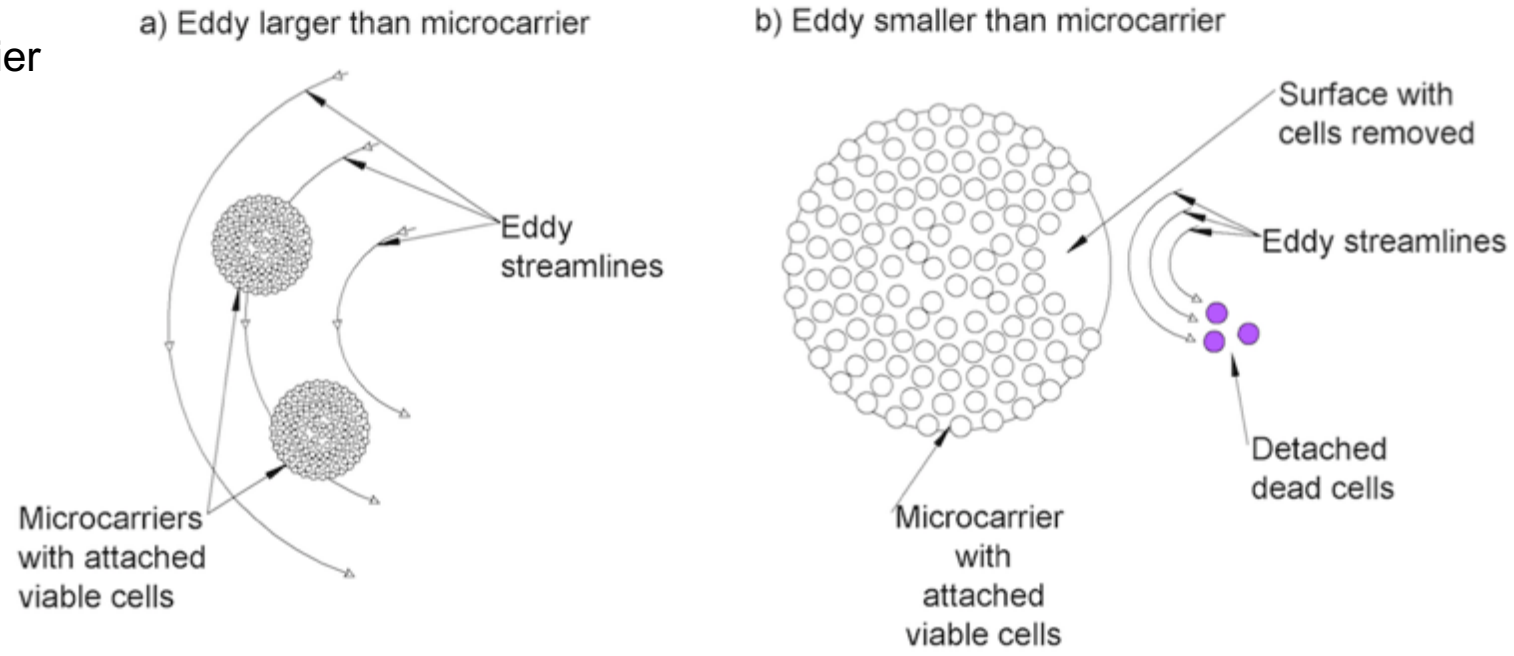


- **NK & T cells:** suspended single cells or loose clumps

Hydrodynamic Considerations for Suspension Cultures

Fluid shear forces (microcarrier example):

Originally proposed by Croughan et al. (1985) and confirmed for FS-4 cells (1987)



- Increased agitation rate → smaller Kolmogorov eddies → potential shear stress

Fluid environment for cell aggregates:

- Variable fluid flow → variable shapes and sizes
- Homogeneous environment → uniformly spherical
- Shear forces → limit on maximum size

Challenges for Allogeneic Cell Therapy Manufacturing

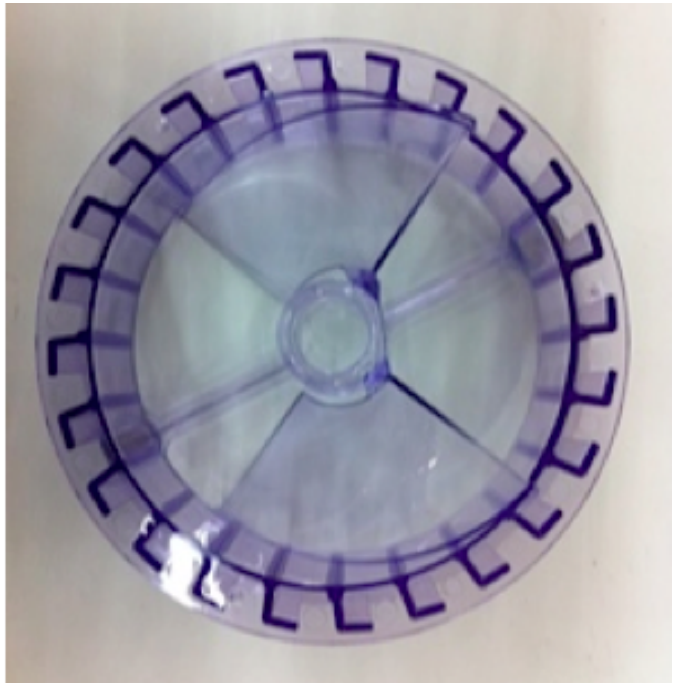
- Significantly different from traditional biotech
  - Biological considerations
    - Anchorage-dependent & mortal cells are final product
  - Bioreactor manufacturing considerations
    - Effects of hydrodynamic conditions on cells
    - Scalability of hydrodynamic environment

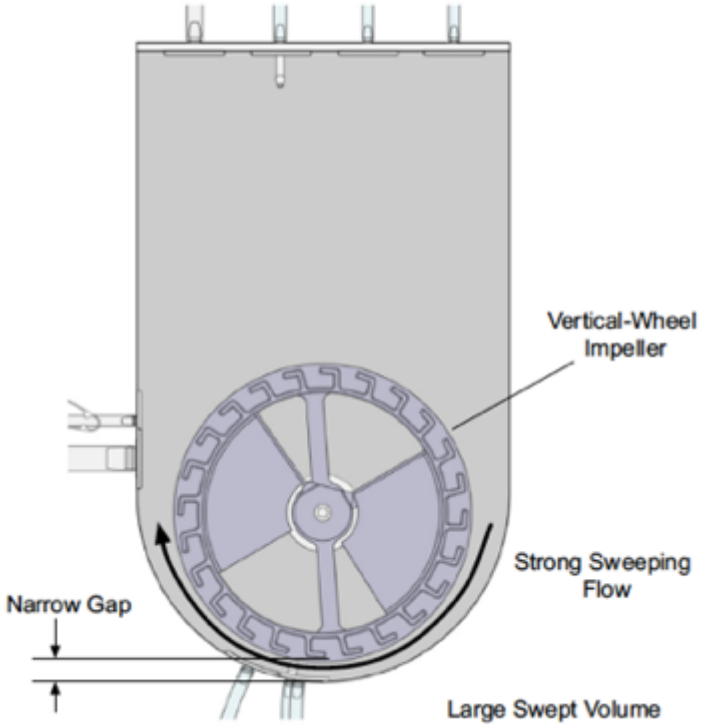
Vertical-Wheel (VW) Bioreactors as Enabling Technology

PBS 15

- Single-use system that is plug-and-play
- Wide range of volumetric scales
- True Scalability: scale down models with representative performance

Combination of Unique VW Impeller and U-Shaped Vessel



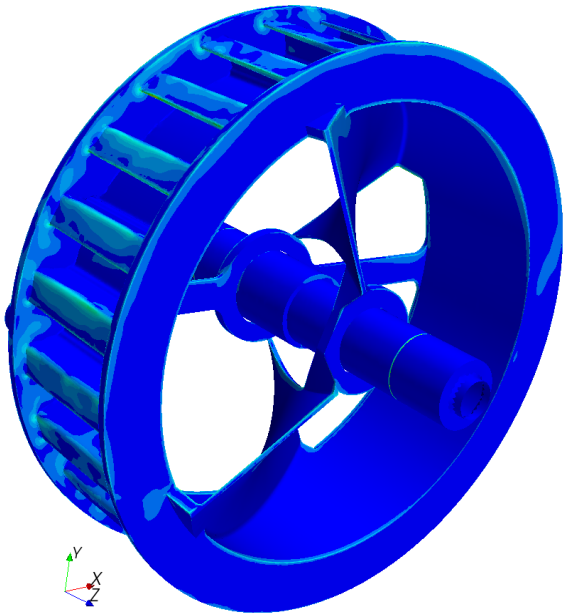


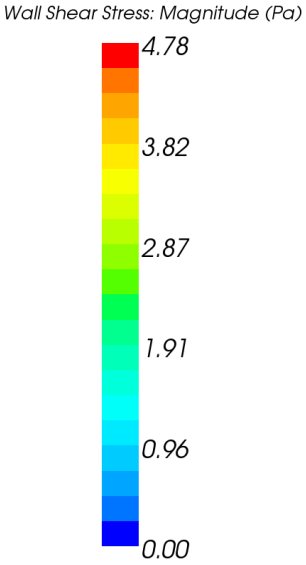
- Large diameter & vane surface areas
- Internal vanes to promote “cutting & folding” action of fluid
- Combination of tangential and axial fluid flow

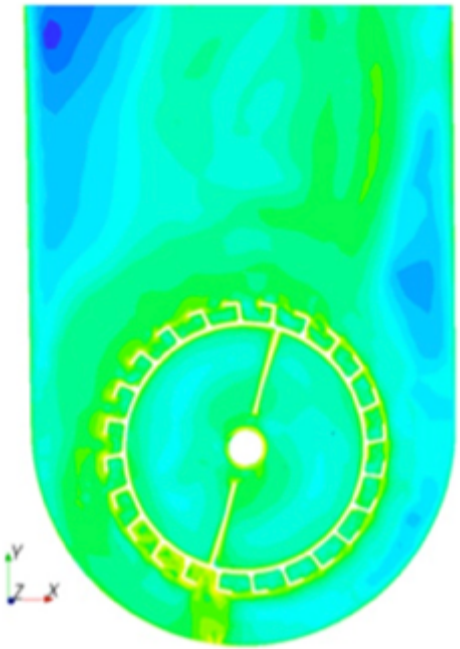
- Radius and placement of impeller creates strong sweeping flow along U-shaped bottom
- Homogeneous fluid mixing environment throughout vessel with no dead zones

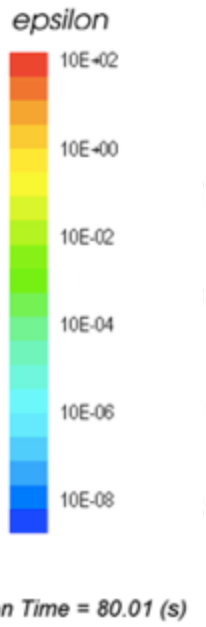
CFD Analysis of VW Bioreactor's Hydrodynamic Environment

3L scale







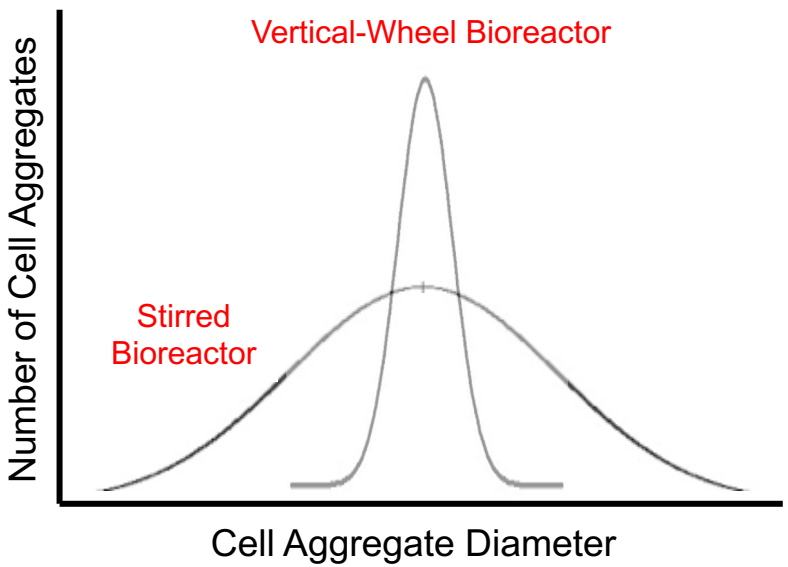


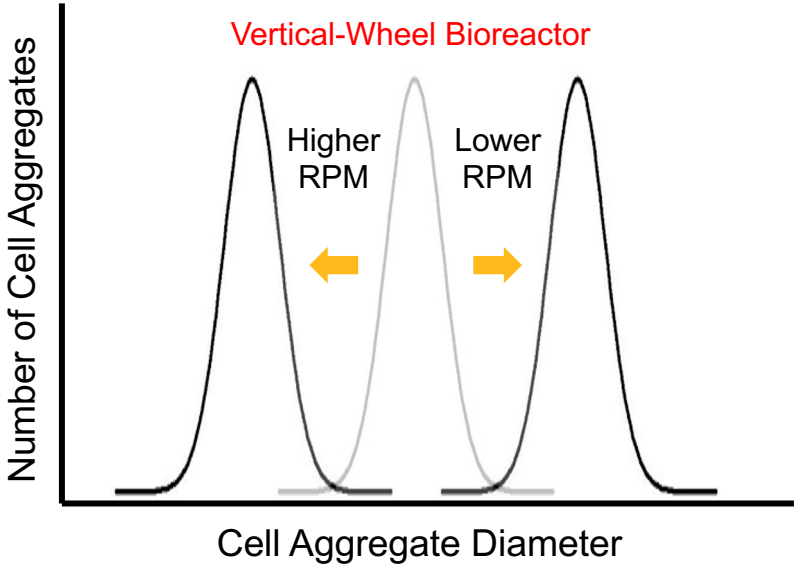
- Consistent shear stress levels across entire surface of impeller with no hot spots
- Homogeneous distribution of turbulent energy dissipation rates throughout vessel

Croughan M. et al., Stem Cell Manufacturing, Elsevier, 2016

Benefits of VW Bioreactors For Cell Aggregate Morphology

- Spherical aggregates ideal for expansion and differentiation
  - Optimal diameter for even diffusion of nutrients and growth factors
  - Avoid necrosis or heterogeneous differentiation in central cells

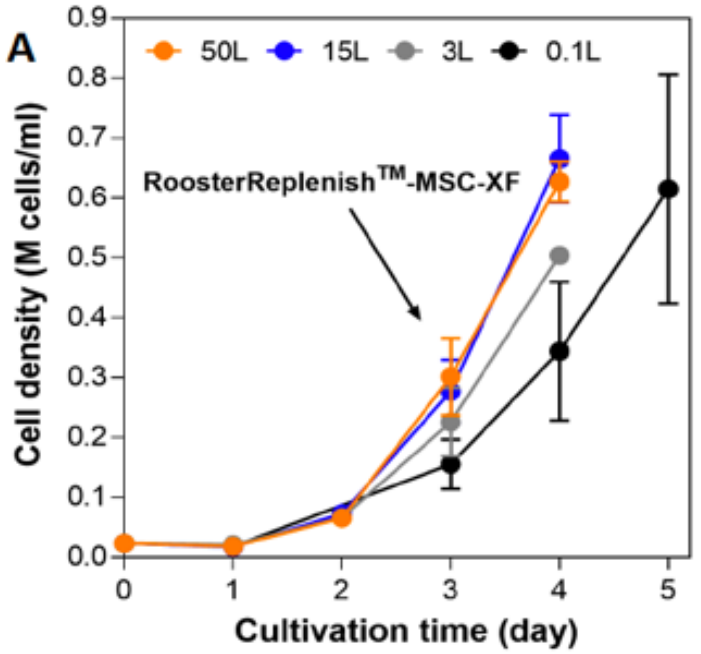


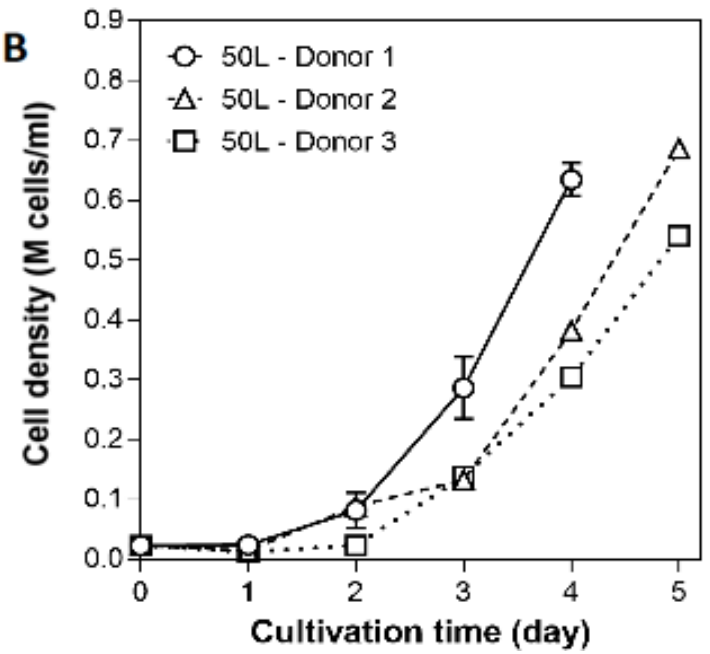


- Narrower diameter distribution in VW bioreactor compared to stirred bioreactor
- Inverse correlation between agitation rate and average diameter

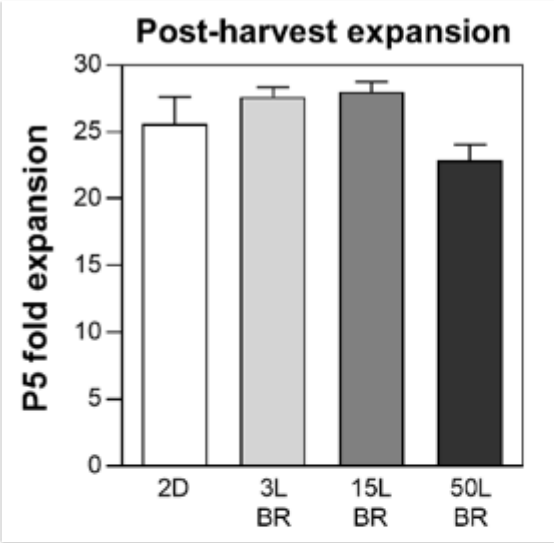
Scalable Expansion of hMSCs in VW Bioreactors

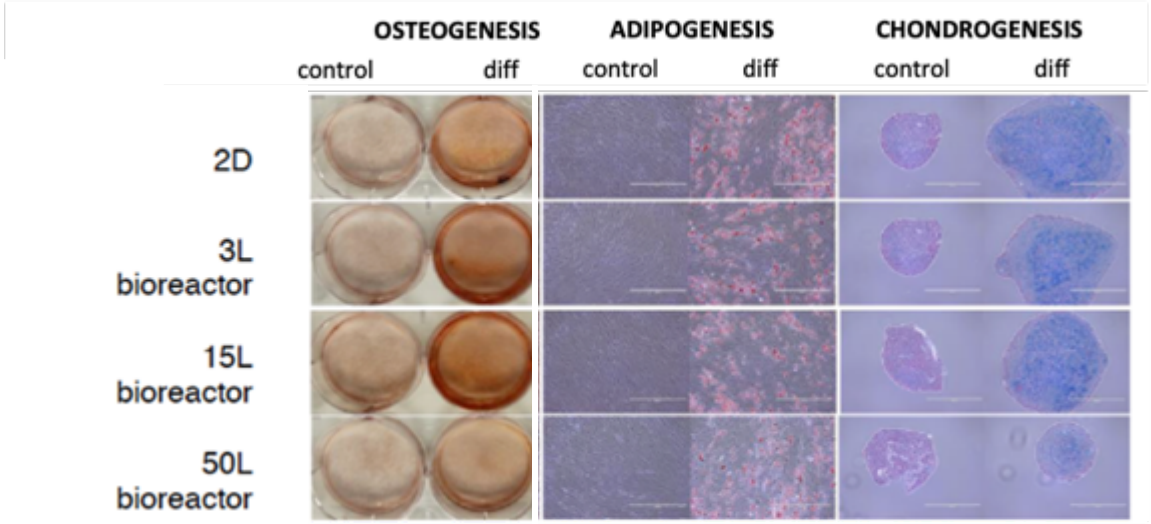
Scalable expansion process for hMSCs:

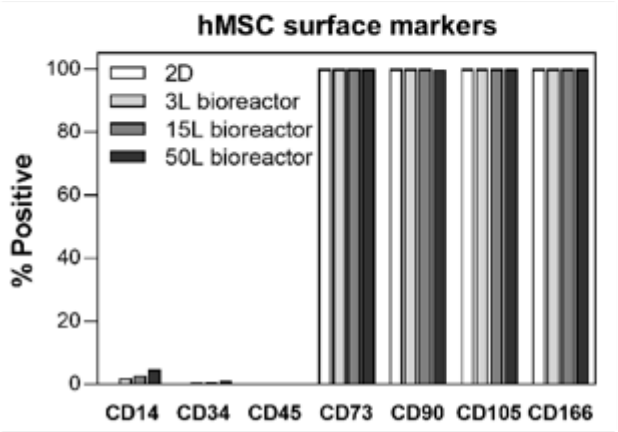


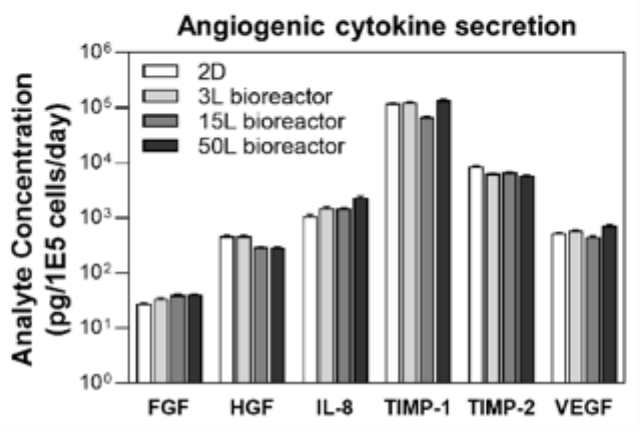


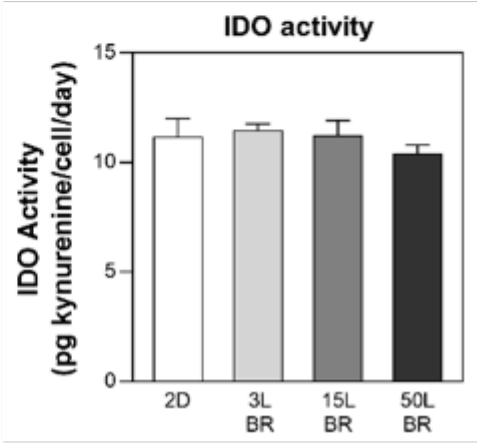
CQAs and Functionality of hMSCs in VW Bioreactors





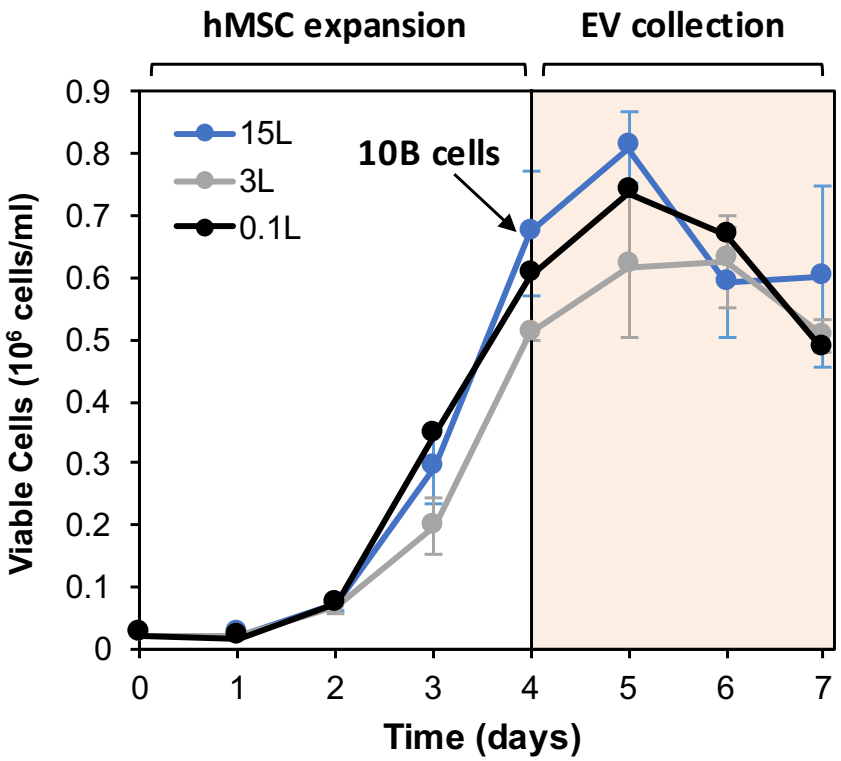






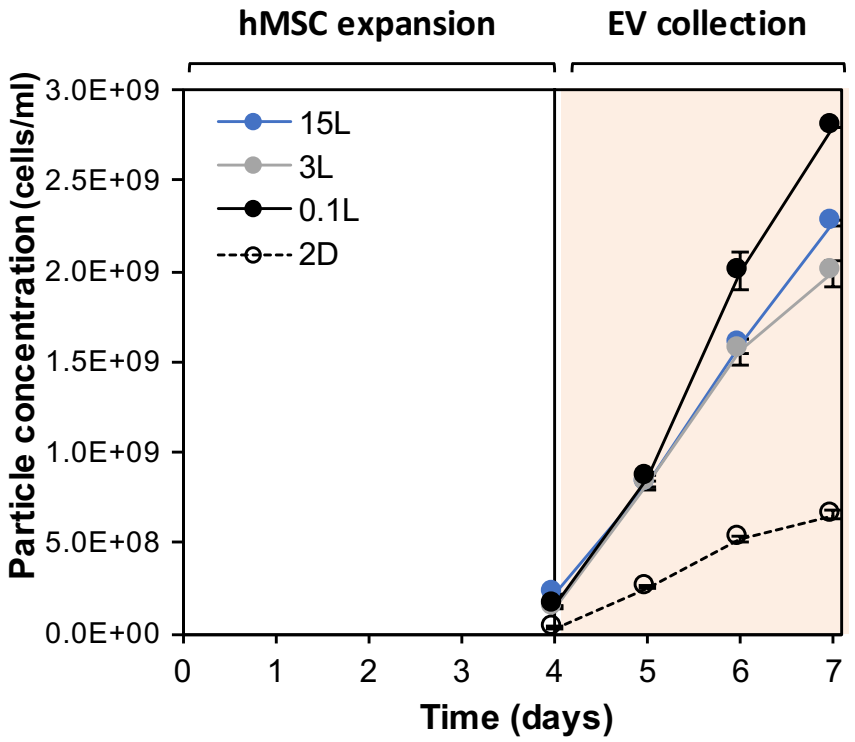
Production of hMSC-Derived EVs in VW Bioreactors

Cell counts across scales



- Peak concentration of of hMSCs reached on day 5 for all scales of VW bioreactors

EV production across scales

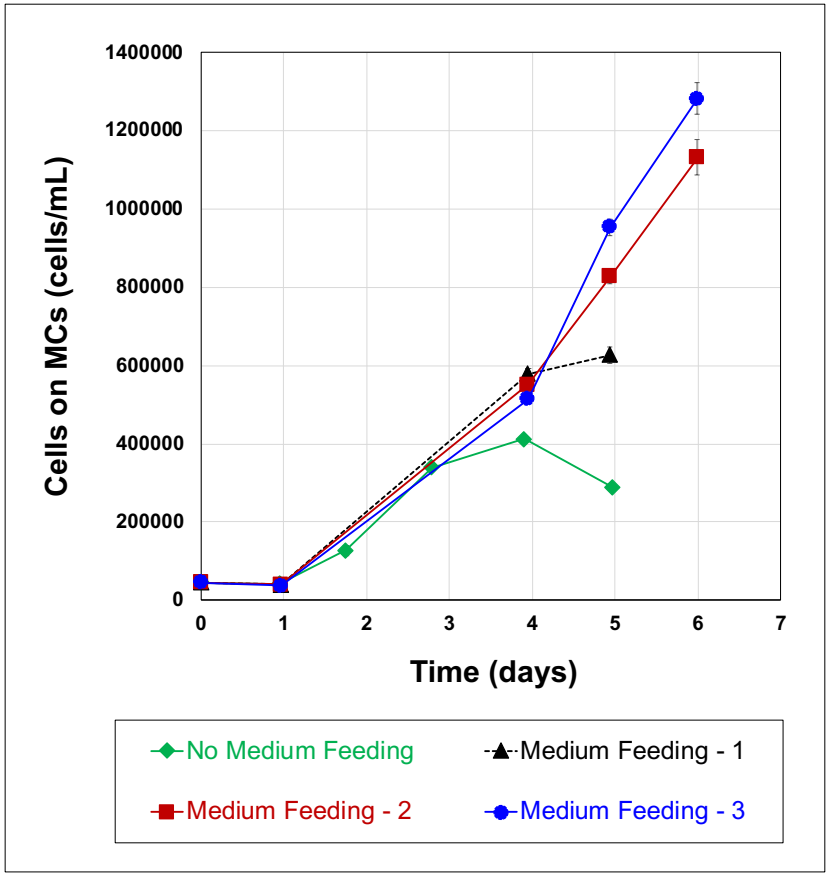


- Superior production of EVs in all scales of VW bioreactors compared to 2D platform

Scalable Expansion of eMSCs in VW Bioreactors

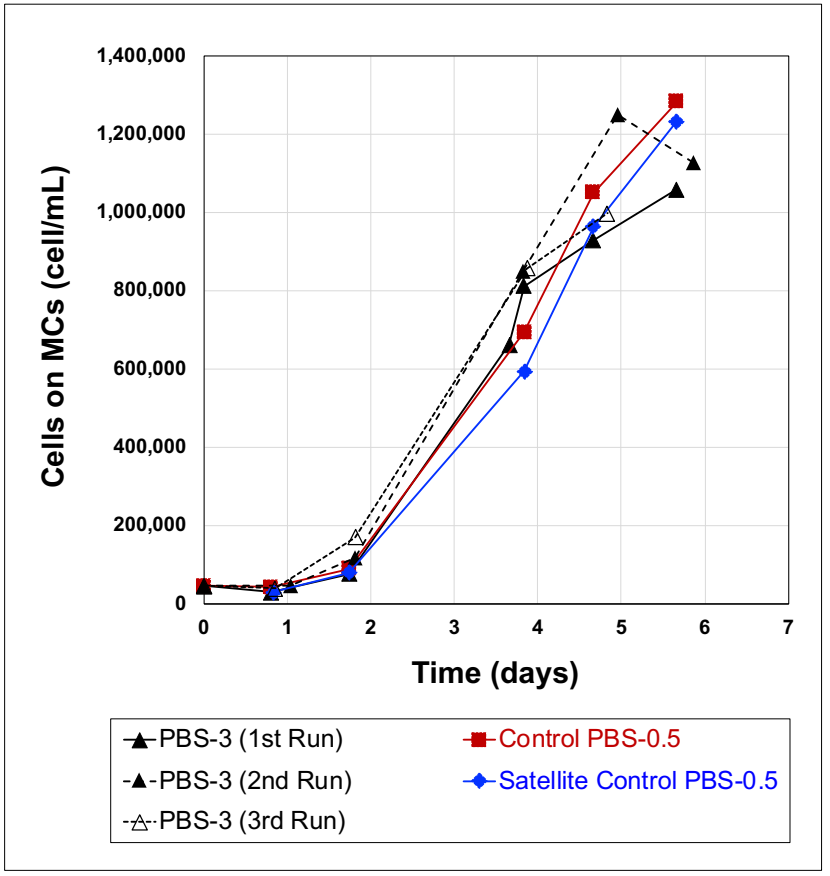
Process Optimization

Effects of Medium Feeding Regimes on eMSC Growth at 0.5 L Scale



Scale-Up from 0.5L to 3L

Comparison of eMSC Growth at 0.5L and 3L Scales



Example of PSC-Derived Therapy For Serious Disease Indication

Diabetes is a Large Burden on Patients

Disease management and risk of secondary complications

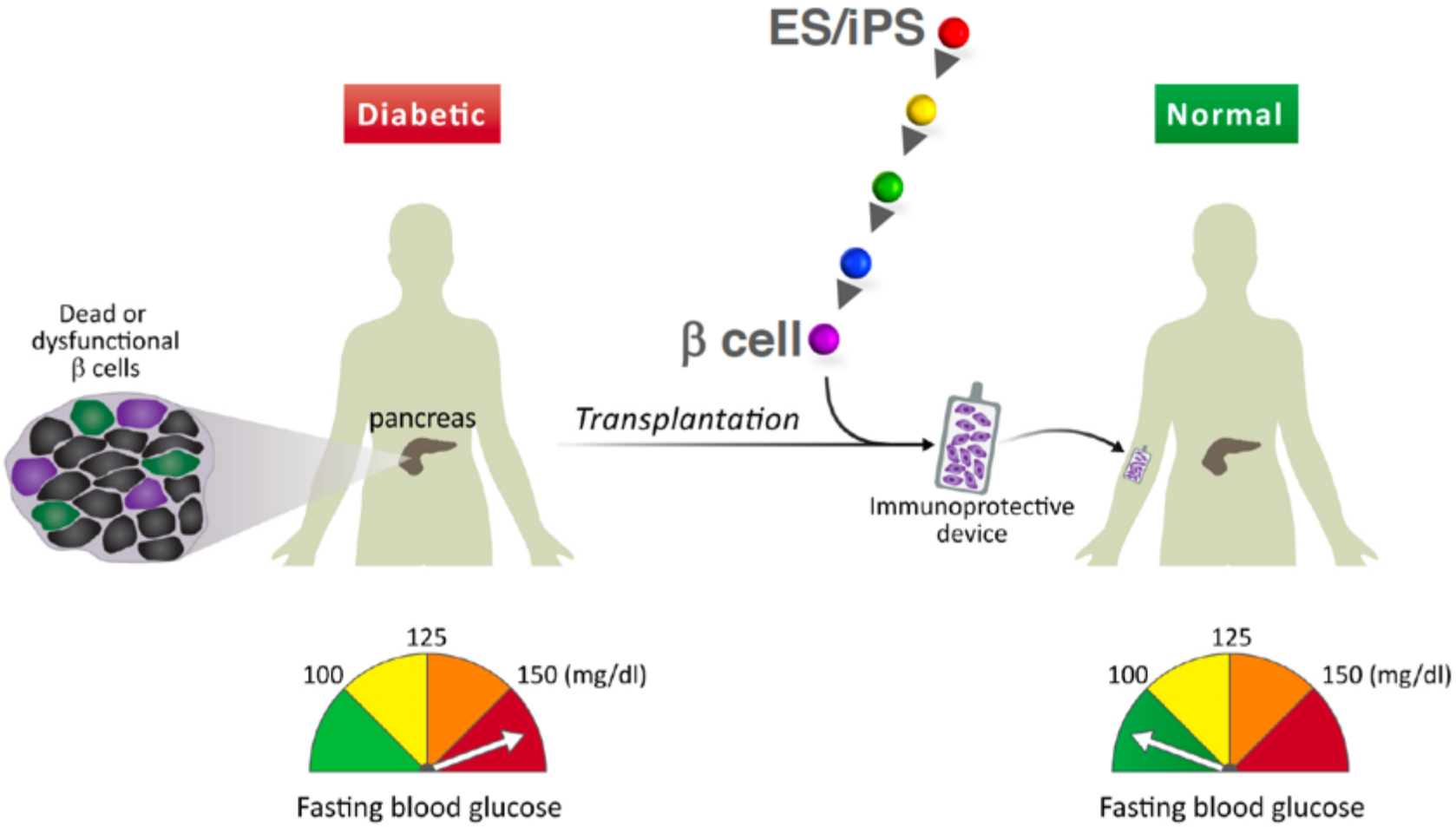
A Year in the Life of a Type 1 Diabetes Patient

5 glucose tests/day	4 insulin injections/day
x	x
365 days/year	365 days/year
=	=
1825 finger pricks	**1460 injections**

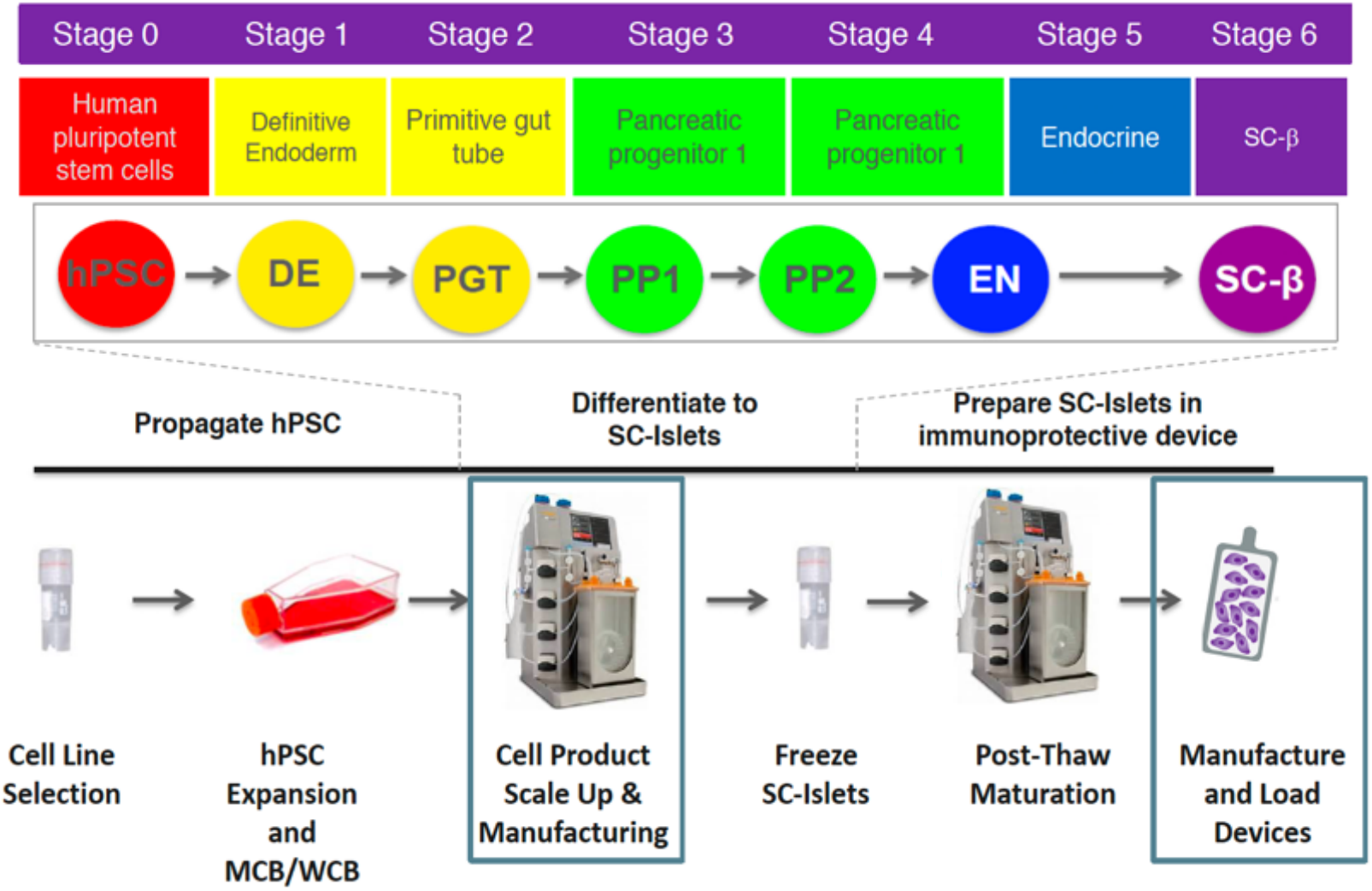
- **Type 1 Diabetes Patients in US: 1 M**
- **Type 2 Diabetes Patients in US: 28M**

Despite insulin therapy, diabetics face increased risk of amputation, renal and retinal failure, and lasting nerve damage

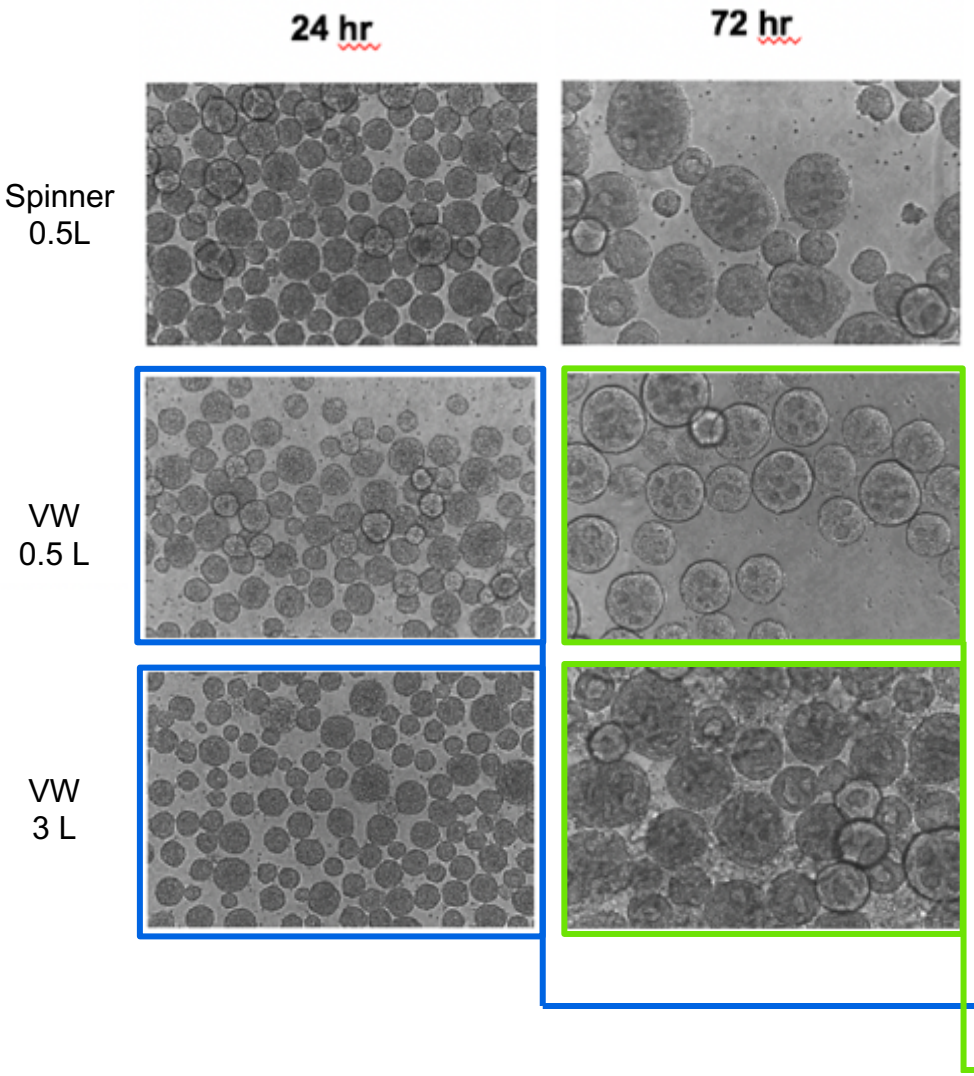
Transplantation of β Cells Derived from PSCs



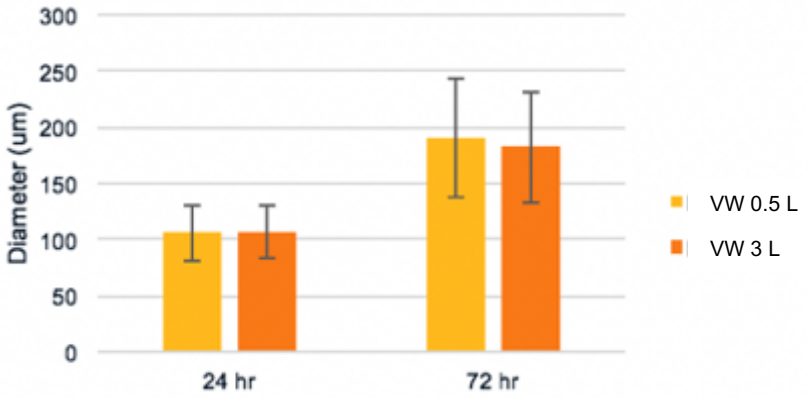
Multi-Step Expansion and Differentiation Process for β Cells



Comparison of Cell Aggregate Expansion



- Greater variation in morphology after 72 hours for aggregates grown in spinner
- Consistent aggregate morphology maintained during scale up from 0.5 to 3L VW bioreactors



MANUFACTURING FOR REGENERATIVE MEDICINE

STEM CELLS TRANSLATIONAL MEDICINE

Optimized serial expansion of human induced pluripotent stem cells using low-density inoculation to generate clinically relevant quantities in vertical-wheel bioreactors

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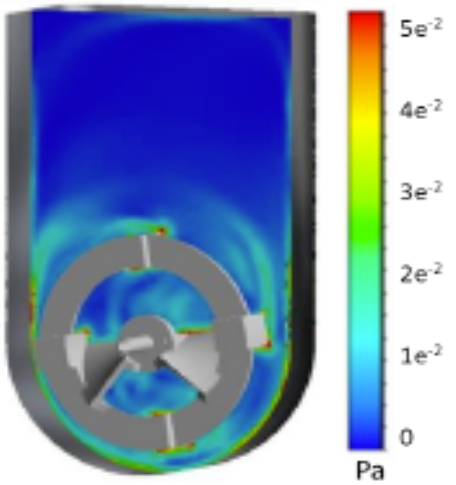
CFD Analysis of 0.1 L VW Bioreactor

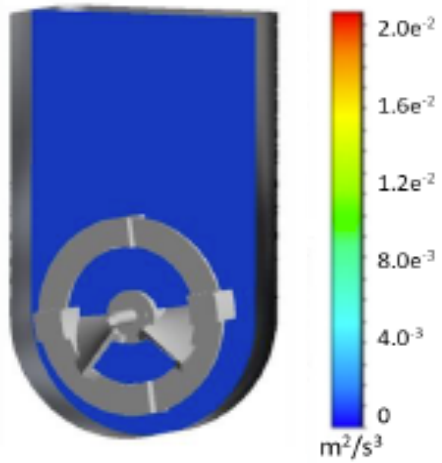
Shear Stress

40rpm

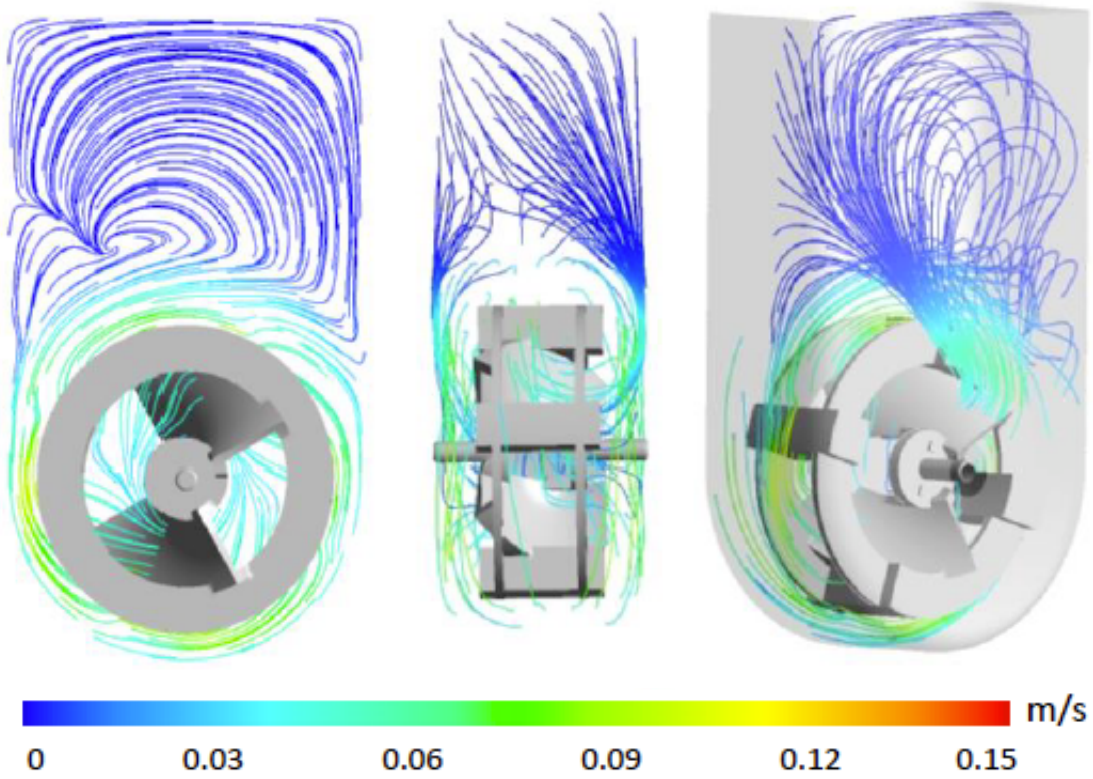


Energy Dissipation

40rpm



Velocity Streamline through VW Bioreactor Height (60rpm)



Comparison of iPSC Aggregate Yield

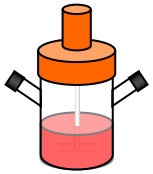
Single cell inoculation, 20,000 cells/ml seeding, batch culture

Vertical-Wheel (VW)

40 rpm
60 rpm
80 rpm

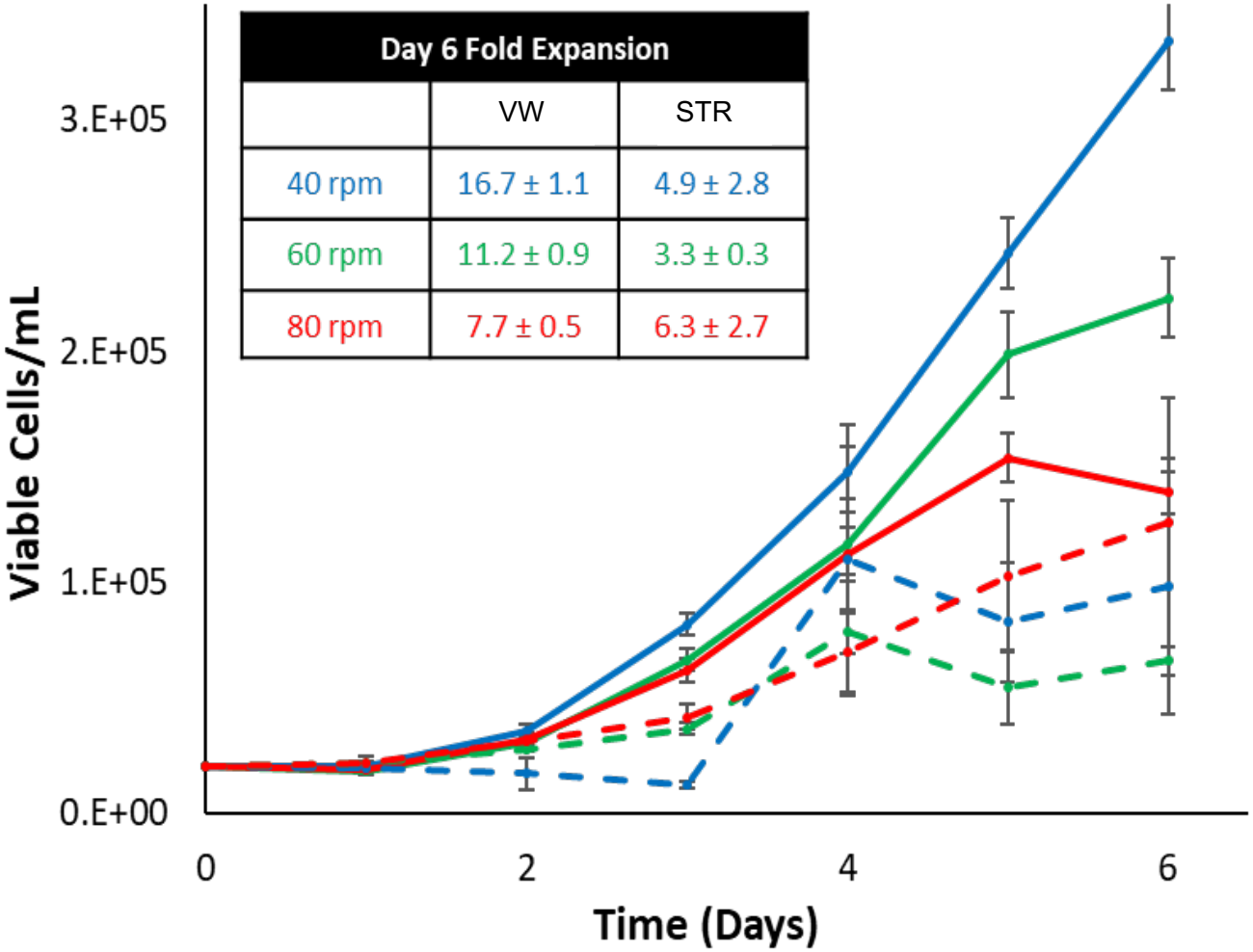
0.1 L vessels

Spinner (STR)



40 rpm
60 rpm
80 rpm

Day 6 Fold Expansion		
	VW	STR
40 rpm	16.7 ± 1.1	4.9 ± 2.8
60 rpm	11.2 ± 0.9	3.3 ± 0.3
80 rpm	7.7 ± 0.5	6.3 ± 2.7



Comparison of iPSC Aggregate Morphology

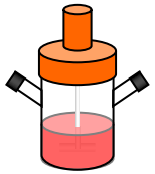
Single cell inoculation, 20,000 cells/ml seeding, batch culture

Vertical-Wheel (VW)

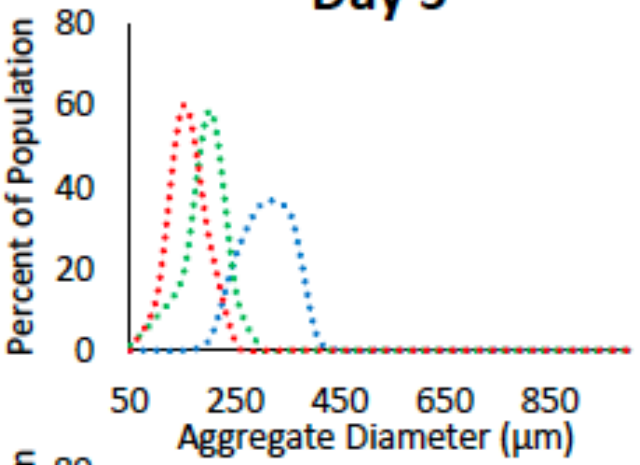
40 rpm
60 rpm
80 rpm

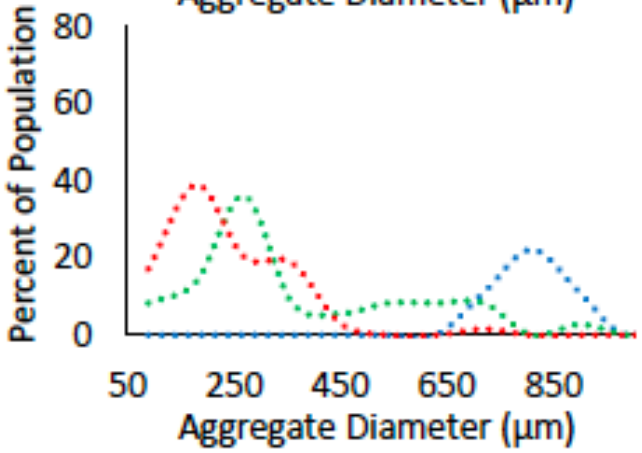
0.1L scale

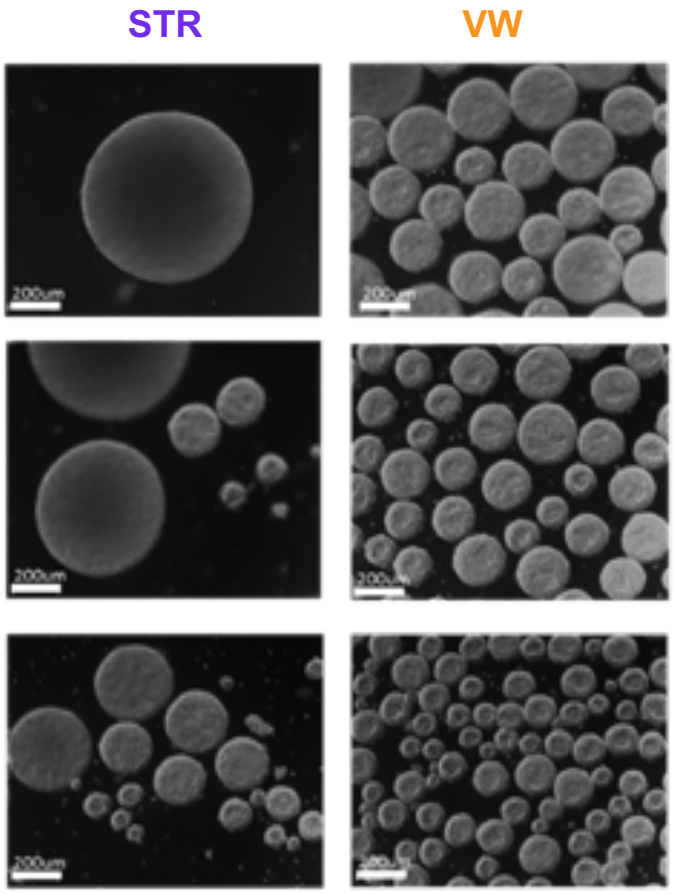
Spinner (STR)



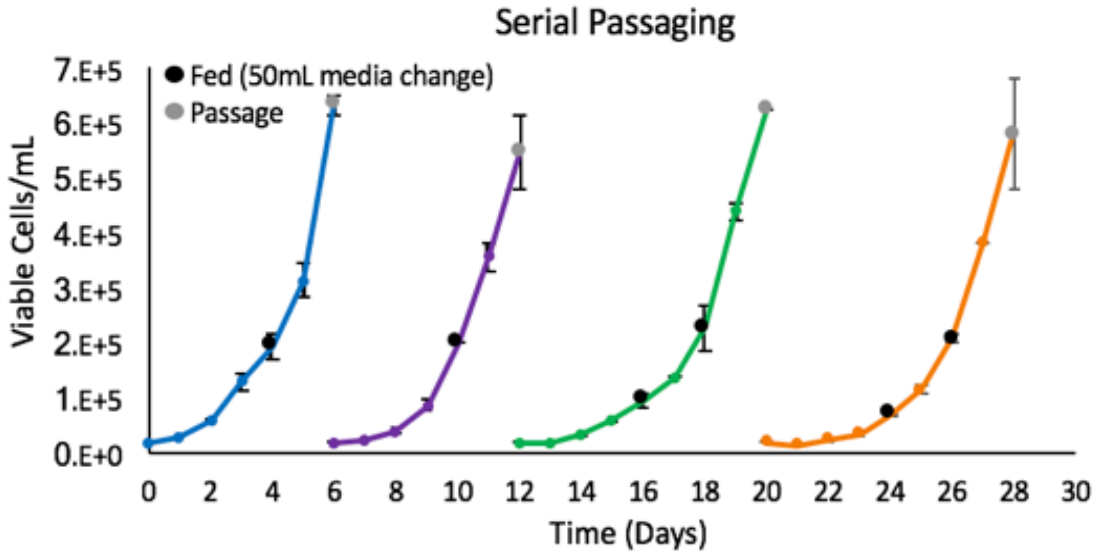
40 rpm
60 rpm
80 rpm





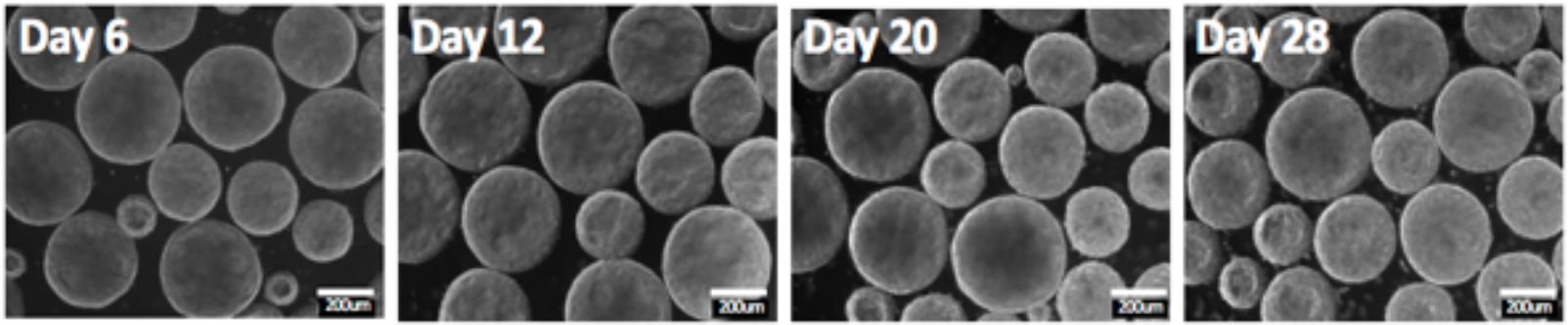


Effective Serial Passaging of iPSCs in VW Bioreactors



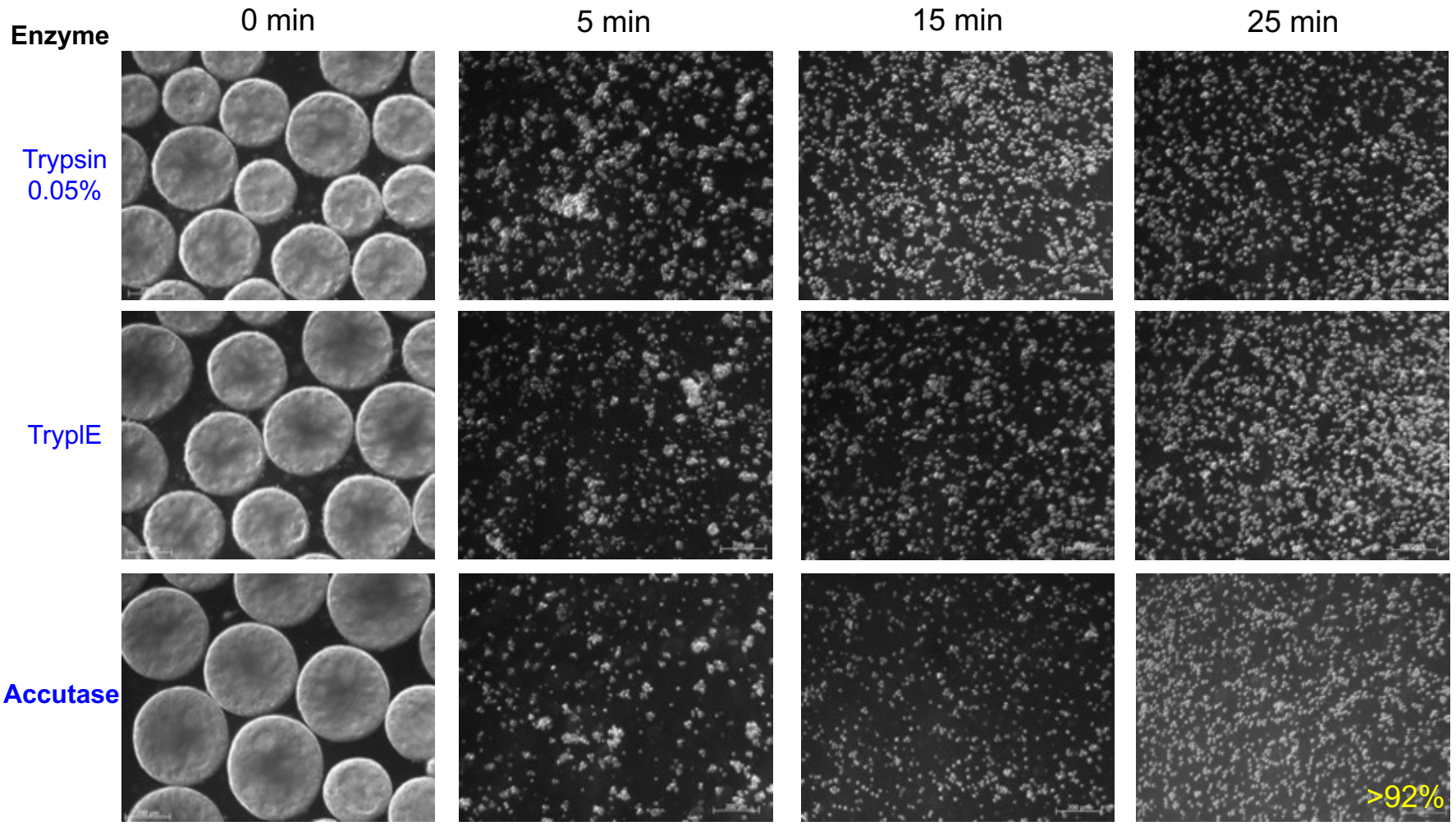
n=1 bioreactor/passage

0.1L working volume and 40 rpm



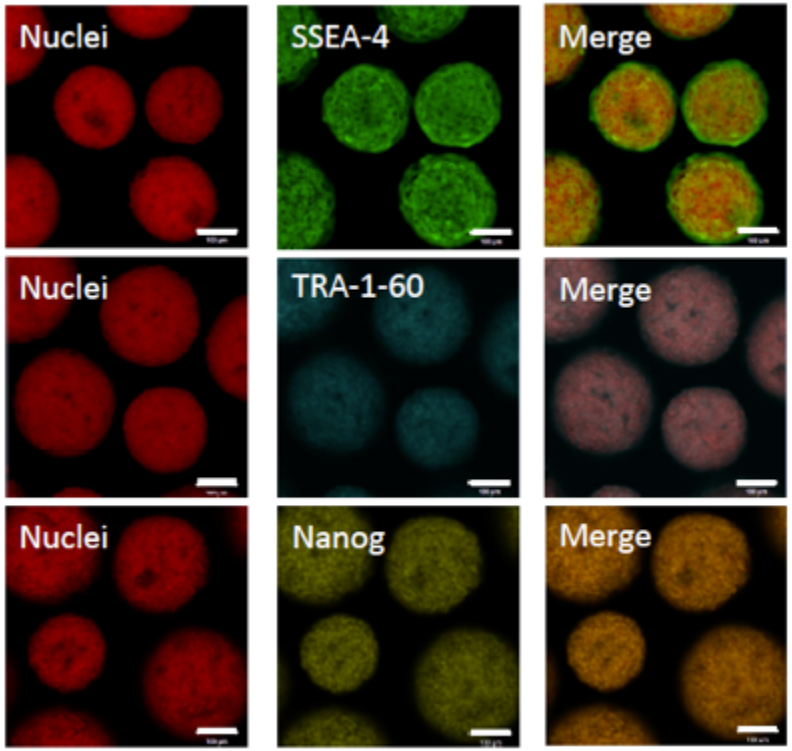
- Consistent formation of uniform aggregates even after multiple passages

Dissociation of hiPSC Aggregates in VW Bioreactor

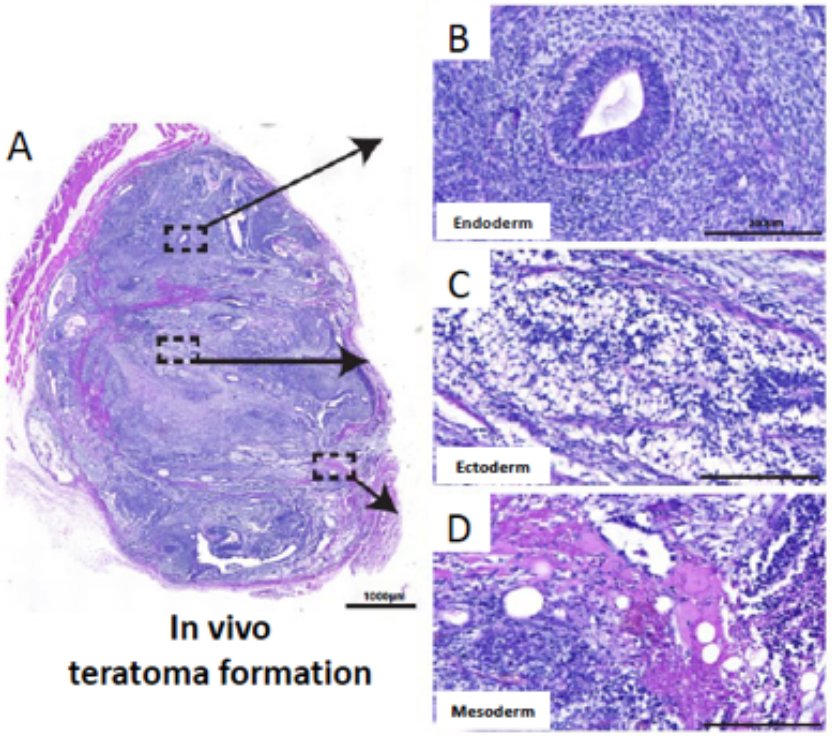


Pluripotency and Functionality of Serially-Passaged iPSCs

Traditional human pluripotency markers



Function

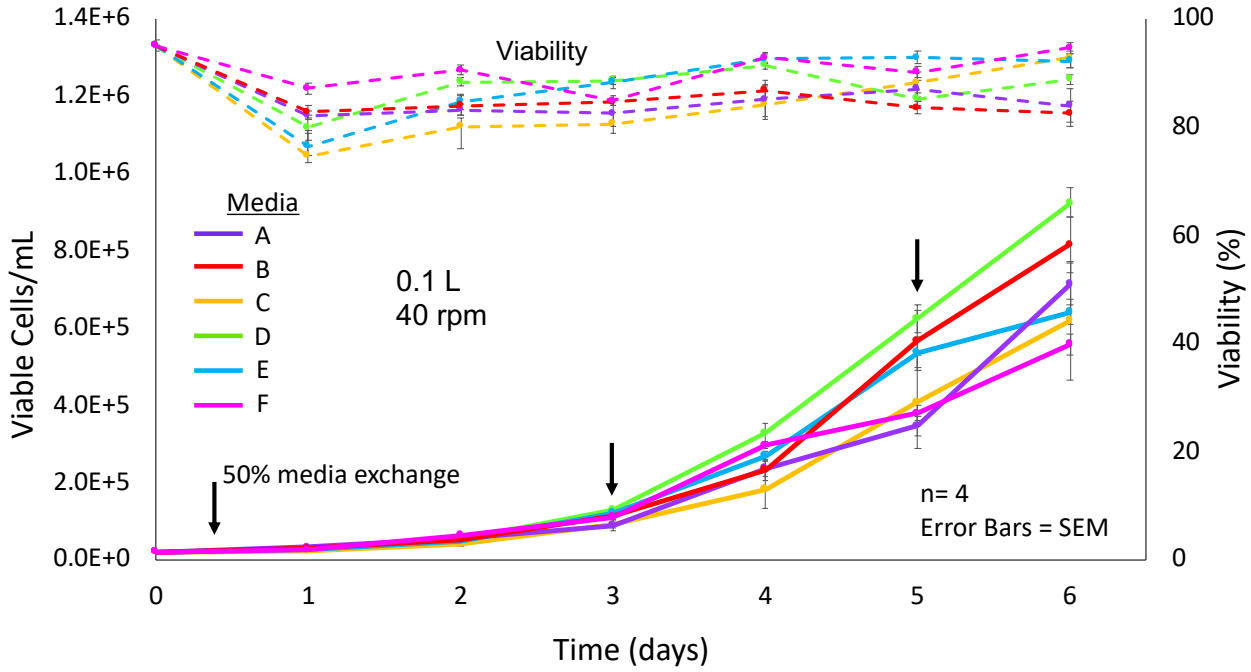


- iPSC pluripotency and ability to form teratoma germ layers maintained after serial passaging

Testing Various PSC Expansion Media in VW Bioreactor

Media (Blinded Name)	cGMP Grade?	FDA Master File?	Comments
A	Yes ✓	Yes ✓	Most widely published
B	No ✗	No ✗	Stabilized FGF2
C	Yes ✓	Yes ✓	Human origin free
D	Yes ✓	No ✗	Supports single-cells
E	No ✗	No ✗	Supports single-cells
F	Yes ✓	Yes ✓	Low levels of bFGF

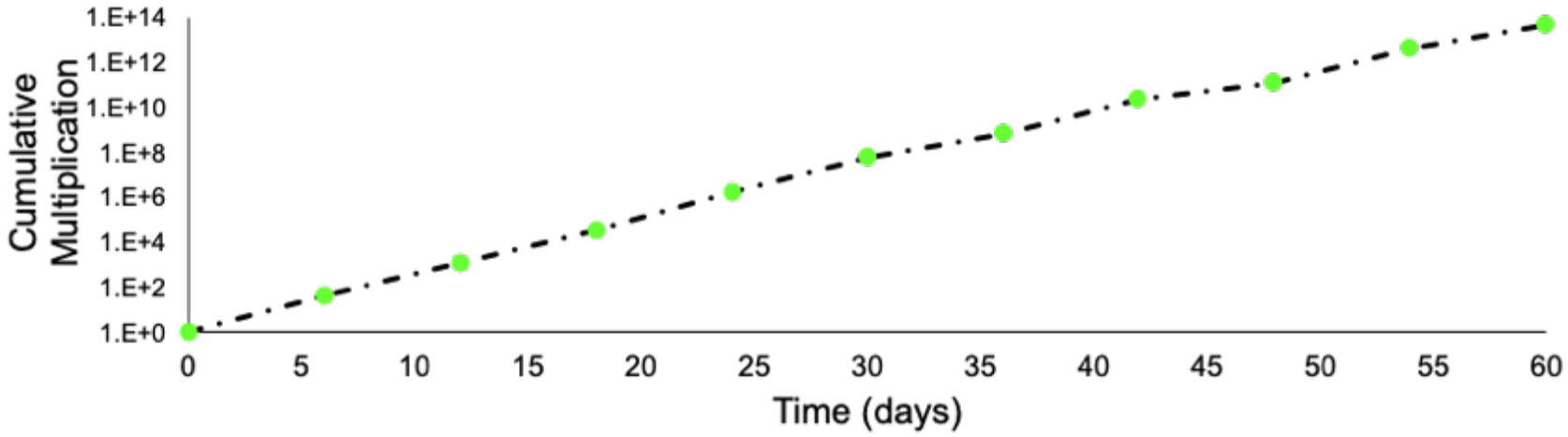
- All 6 media designed for static cultures
- Optimized media will improve scalable PSC expansion in VW bioreactors

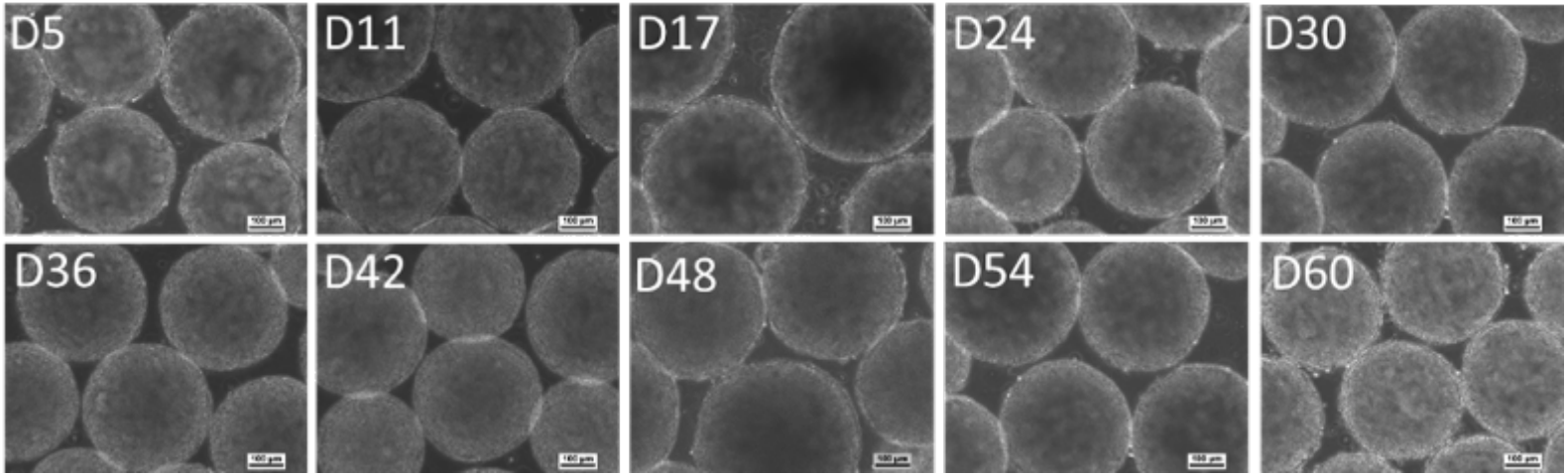


Media	Fold Expansion (Day 6)
A	36
B	41
C	31
D	46
E	31
F	28

Long-Term Expansion of iPSC Aggregates in VW Bioreactors

- Process: 10 serial passages over 60 days, 0.1L scale, 40 rpm, media “C”
- Consistent aggregate morphology with cumulative cell multiplication of 4.6E13





PBS BIOTECH®

Proposed Commercial Manufacturing Using VW Bioreactors

Example Protocol for PSC Manufacturing	
Cell inoculation density	20,000 cells/mL
Medium feeding	50% exchange on day 3 or 4
Cell density, post-expansion	>600,000 cells/mL (>30-fold)
Cell harvest efficiency	>80%
Culture period	6 days per passage

Working Cell Bank

Seed 2 M cells

P+1 Stage

1 x PBS-0.1

0.1 L working volume
Cell yield: 48 M

P+2

1 x PBS-3

2.4 L working volume
Cell yield: 1.15 B

P+3

1 x PBS-80

55 L working volume
Cell yield: 26.4 B

Differentiation or Downstream Processes

>600 B cells

3x

P+4

3 x PBS-500

440 L working volume each
Combined cell yield: 634 B

24 Days

jove

Scalable Generation of Mature Cerebellar Organoids from Human Pluripotent Stem Cells and Characterization by Immunostaining

Teresa P. Silva[1,2], **Tiago G. Fernandes**[1], **Diogo E. S. Nogueira**[1], **Carlos A. V. Rodrigues**[1], **Evguenia P. Bekman**[1,2,3], **Yas Hashimura**[4], **Sunghoon Jung**[4], **Brian Lee**[4], **Maria Carmo-Fonseca**[2], **Joaquim M. S. Cabral**[1]

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Silva, T.P., Fernandes, T.G., Nogueira, D.E.S., Rodrigues, C.A.V., Bekman, E.P., Hashimura, Y., Jung, S., Lee, B., Carmo-Fonseca, M., Cabral, J.M.S. Scalable Generation of Mature Cerebellar Organoids from Human Pluripotent Stem Cells and Characterization by Immunostaining. *J. Vis. Exp.* (160), e61143, doi:10.3791/61143 (2020).

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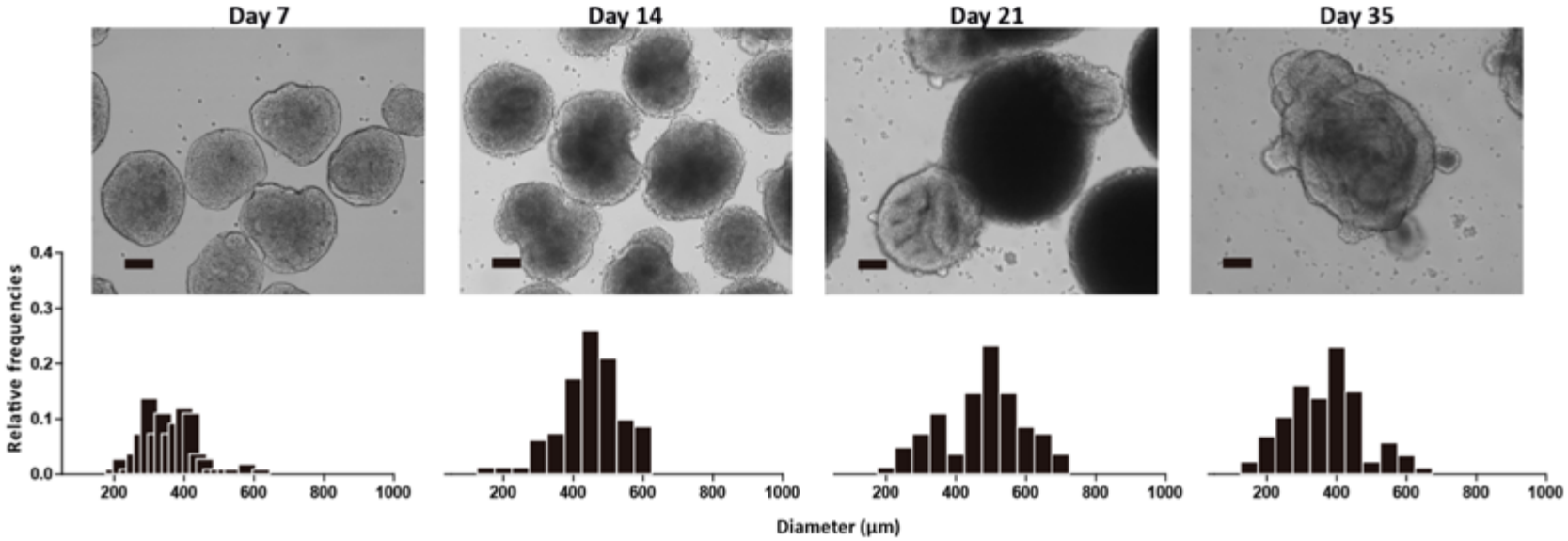
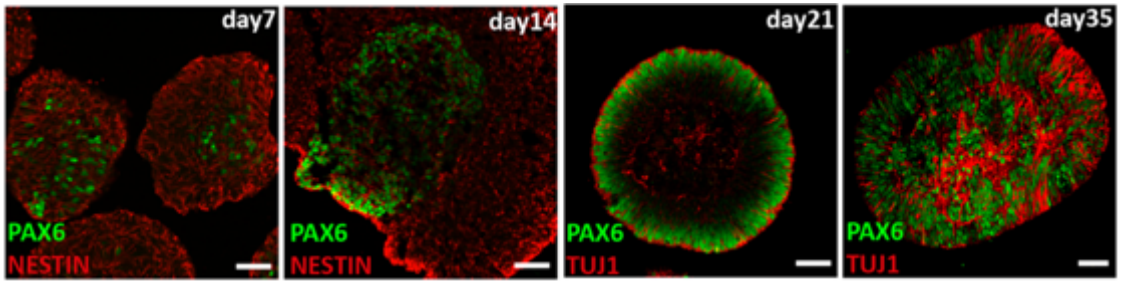
jove.com/video/61143

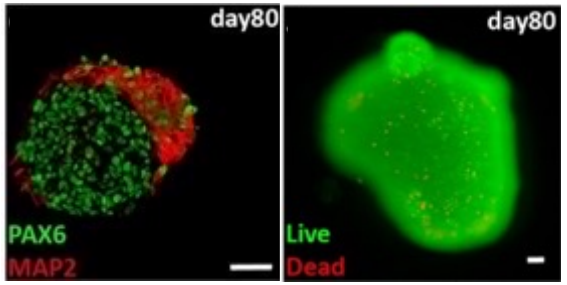
Differentiation of iPSCs Into Cerebellar Organoids in VW Bioreactor



- Homogeneous distribution of organoid morphology throughout differentiation (0.1 L vessel)



- Immunofluorescence shows efficient neural induction during differentiation process



- Mature granule cells and high cell viability after 80 days

Key Takeaways from this Webinar

- Bioreactor should provide optimal hydrodynamic conditions
- Unique benefits of VW bioreactors, backed up by data:
  - Consistently low shear particularly for cells on microcarriers
  - Homogenous energy dissipation rates for spherical aggregates
  - Size control through inverse correlation with agitation rate
  - True scalability for potential commercial manufacturing

Additional Publications: MSCs

Production of Oncolytic Adenovirus and Human Mesenchymal Stem Cells in a Single-Use, Vertical-Wheel Bioreactor System: Impact of Bioreactor Design on Performance of Microcarrier-Based Cell Culture Processes

Marcos F. Q. Sousa, Marta M. Silva, António Roldão, Paula M. Alves, and Margarida Serra
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bioengineering

MDPI

Bioreactor Parameters for Microcarrier-Based Human MSC Expansion under Xeno-Free Conditions in a Vertical-Wheel System

Josephine Lembong, Robert Kirian, Joseph D. Takacs, Timothy R. Olsen, Lye Theng Lock, Jon A. Rowley and Tabassum Ahsan *
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Additional Publications: PSCs

Research Article

SCI

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(wileyonlinelibrary.com) DOI 10.1002/jctb.5738

Scalable culture of human induced pluripotent cells on microcarriers under xeno-free conditions using single-use vertical-wheel™ bioreactors

Carlos AV Rodrigues,[a,b*] Teresa P Silva,[a,b,c] Diogo ES Nogueira,[a,b] Tiago G Fernandes,[a,b] Yas Hashimura,[d] Robin Wesselschmidt,[d] Maria Margarida Diogo,[a,b] Brian Lee[d] and Joaquim MS Cabral[a,b]

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Nogueira *et al. Journal of Biological Engineering* (2019) 13:74
https://doi.org/10.1186/s13036-019-0204-1

Strategies for the expansion of human induced pluripotent stem cells as aggregates in single-use Vertical-Wheel™ bioreactors

Diogo E. S. Nogueira[1,2], Carlos A. V. Rodrigues[1,2*], Marta S. Carvalho[1,2], Cláudia C. Miranda[1,2], Yas Hashimura[3], Sunghoon Jung[3], Brian Lee[3] and Joaquim M. S. Cabral[1,2]