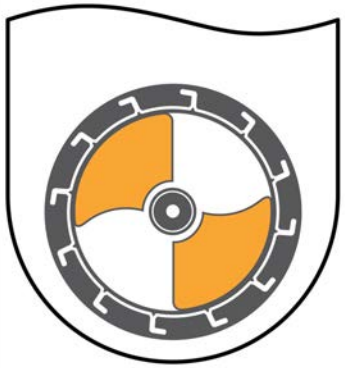
PBS BIOTECH®

Challenges and Solutions for Allogeneic Cell Therapy Manufacturing

Brian Lee, Ph.D.
CEO and Founder

VERTICAL-WHEEL®
BIOREACTORS



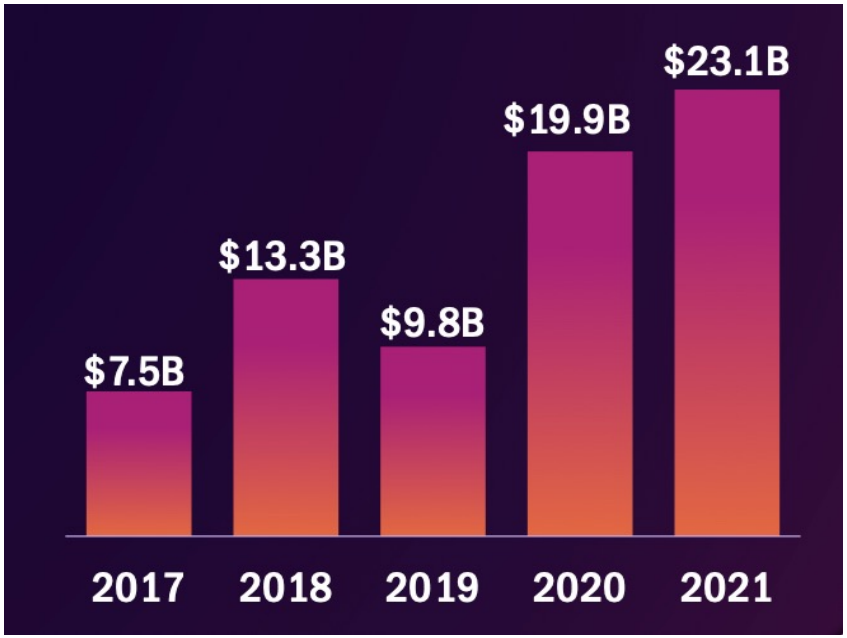
The next generation of single use bioreactors inspired by forward thinking

ECI Conference,
"Advancing Manufacture of Cell and Gene Therapies VII"

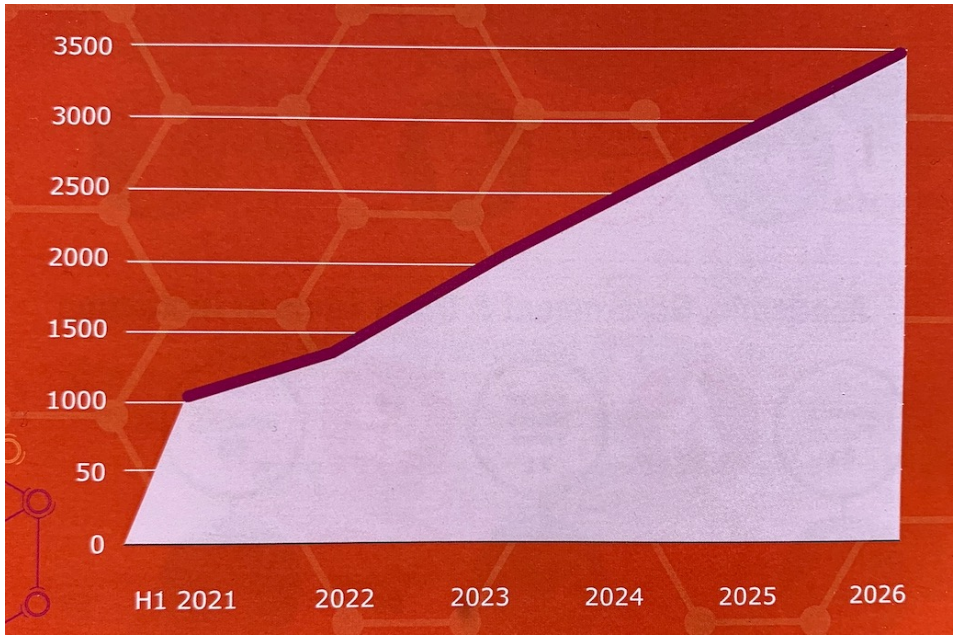
February 8th, 2022
Coronado, CA

Trend of Financing and Development for Regenerative Medicine

Global Financing for Regenerative Medicine



Products in Development: 5 Years Forecast



- Alliance of Regenerative Medicine Website and Stem Cell on the Mesa Conference2021

Clinical Milestones of hPSC-derived Therapy

A Cure for Type 1 Diabetes? For One Man, It Seems to Have Worked.

A new treatment using stem cells that produce insulin has surprised experts and given them hope for the 1.5 million Americans living with the disease.

VERTEX

Brian Shelton may be the first person cured of Type 1 diabetes. "It's a whole new life," Mr. Shelton said. "It's like a miracle."

The New York Times Nov. 27, 2021, 12:41 p.m. ET

Clinical Milestones of hPSC-derived Therapy - continued

CRISPR Therapeutics and ViaCyte, Inc. Announce First Patient Dosed in Phase 1 Clinical Trial of Novel Gene-Edited Cell Replacement Therapy for Treatment of Type 1 Diabetes (T1D)

CRISPR THERAPEUTICS®

VIACYTE

February 02, 2022 08:30 ET | Source: CRISPR Therapeutics AG

VCTX210 is an investigational, allogeneic, gene-edited, stem cell-derived product developed in collaboration by applying CRISPR Therapeutics' gene-editing technology to ViaCyte's proprietary stem cell capabilities for the generation of pancreatic cells designed to evade recognition by the immune system. This immune-evasive cell replacement therapy is designed to enable patients to produce their own insulin.

Large-Scale Autologous Cell Therapy Application

- 6 M heart failure patients in the US and a heart transplant is the best option
- Decellularize porcine heart scaffolds with patient's iPSC-derived heart cells
- Eliminate needs for immunosuppressants

Patient-derived Stem Cells

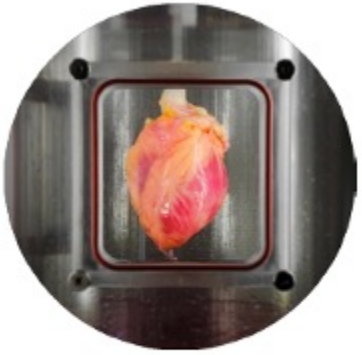
+

Large Scale Expansion and Differentiation into Cardiovascular and Parenchymal Cells

+

Healthy Mammalian ECM (Ghost Heart)

=



Bioengineered Autologous Heart

Bioreactor Scales Needed for Commercial Manufacturing

- **Diabetes Cure by PSC-derived β-cells:**
  - 50 Million insulin users worldwide (1.5M Type 1 in the US)
  - Each dose requires 1L cell culture
  - Treating 1 million patients/year = 200 x **500L** bioreactors x 10 batch/year
- **Heart Failure Treatment by PSC-derived Cardiomyocytes:**
  - 26 Million Globally Heart Failure
  - Each dose requires 0.5L cell culture
  - Treating 1 million patients/year = 100 x **500L** bioreactors x 10 batch/year
- **Bioengineered Hearts:**
  - 300,000 end-stage HF patients and only 3,600 heart transplants per year
  - ~200 B heart cells are needed to build a bioengineered heart
  - With 1-2 B cells/L productivity in bioreactor, a **100L** bioreactor per patient

Challenges of Cell Therapy Manufacturing in Bioreactors

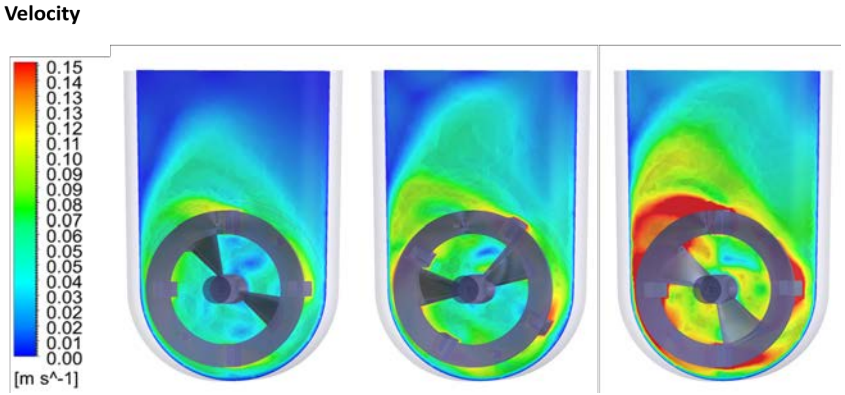
"Significantly different from biotech manufacturing experience"

- **Living Cell** itself is the **product**
- **Anchorage-dependent** cells grown in suspension on surface of **microcarriers** or as **aggregates in spheroid** (200 - 400 μm diameter)
- Suspension of these **large particles** in bioreactors requires **high power inputs** (agitation rates)
- Human cell growth and differentiation yields are **sensitive** to a bioreactor's **hydrodynamic conditions**
- **Sensitivity levels may be different** depending on cell types (PSC, MSC) and final target cells (β-Cells, Cardiomyocytes, Neural Organoids)
- Difficult to maintain **consistent hydrodynamic conditions** inside bioreactors as **vessel size increases**

CFD Analysis and Experimental Validation of Vertical-Wheel® Bioreactors

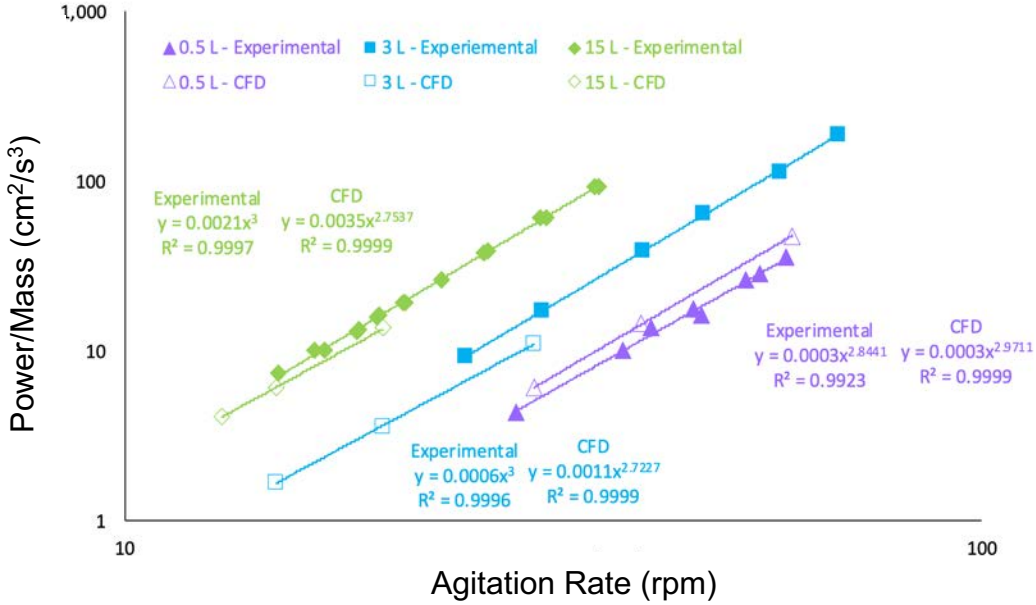
- **CFD Model Development**

T. Dang et al. / Can J Chem Eng. 24253 (2021) 1-18



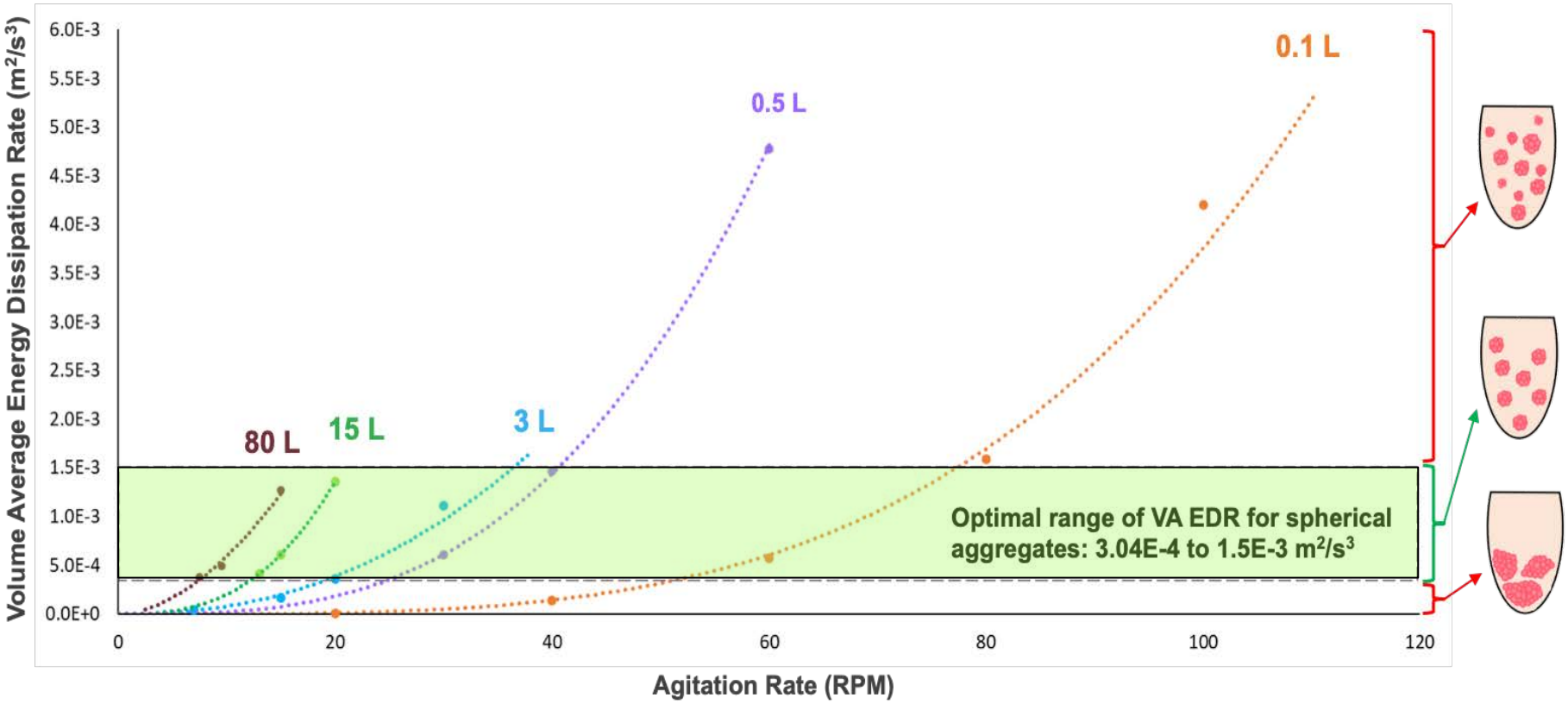
- **Experimental Validation**

M. Croughan et al. / Can J Chem Eng. (2022) In print



Comparison of measured data with CFD predicted values validates the CFD model

Scale-Up Correlations Using CFD Generated Hydrodynamic Variables

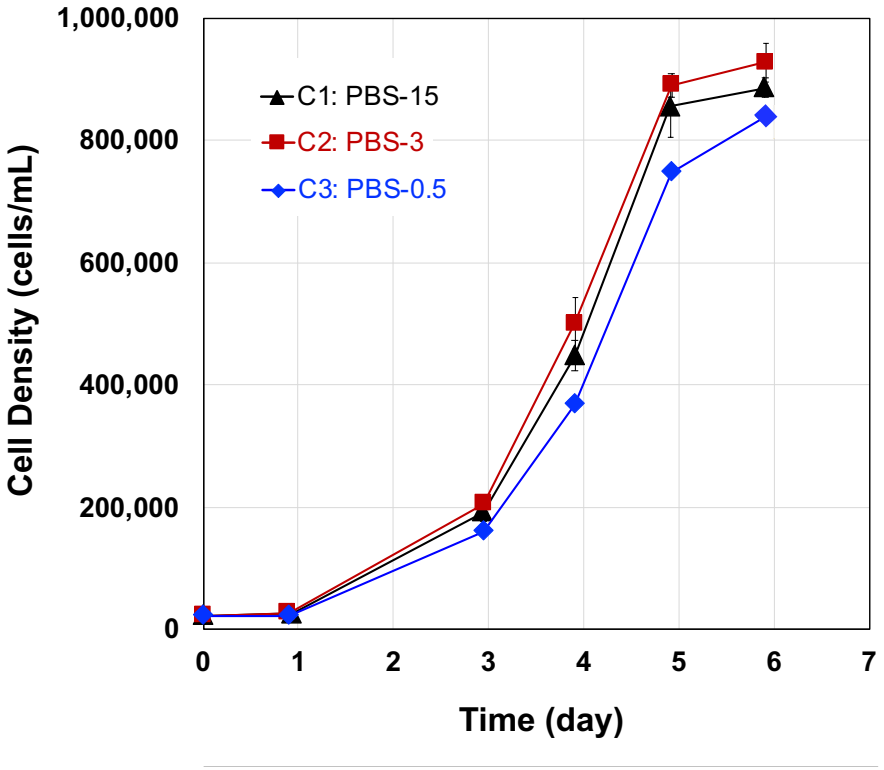


T. Dang et al. / Can J Chem Eng. 24253 (2021) 1-18

Patent Pending

Consistent Expansion of MSCs in Various Size VW Bioreactors

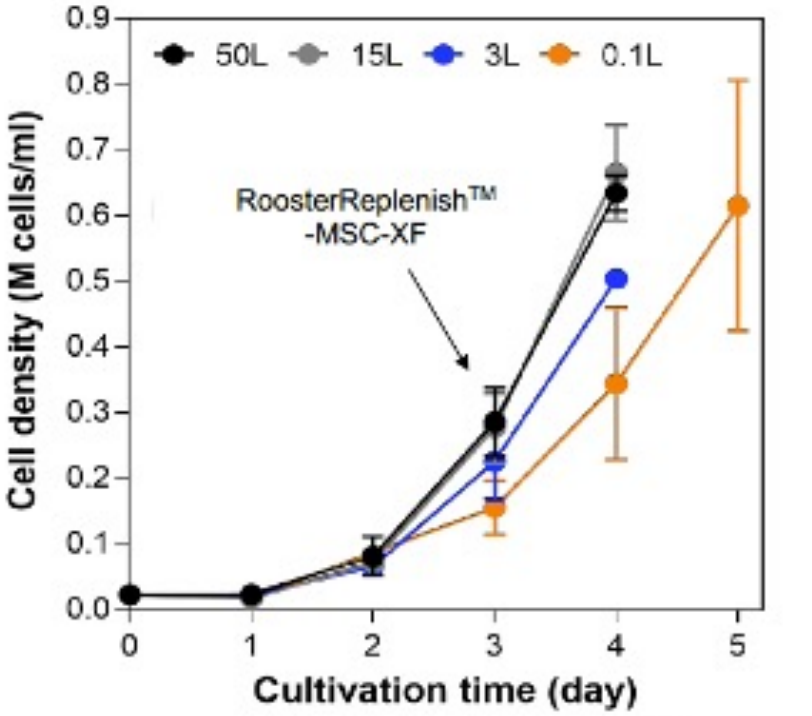
SCM Lifescience



- Over 800,000 cells/mL achieved at 0.5L, 3L, and 15L

RoosterBio® Radically Simplifying Use of MSCs

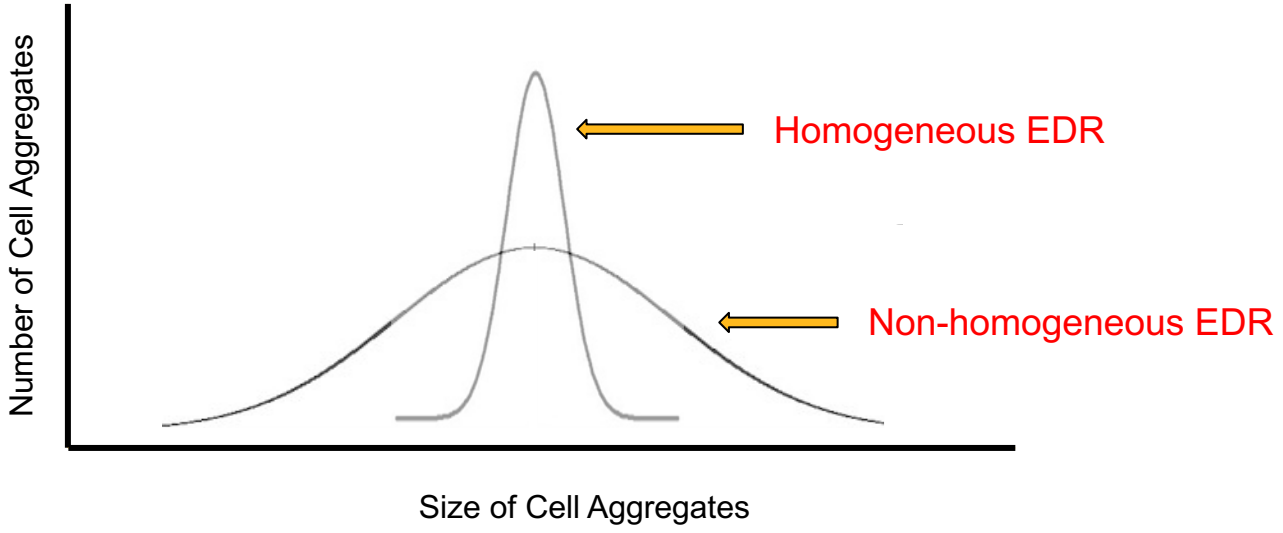
(n=3 for each size)



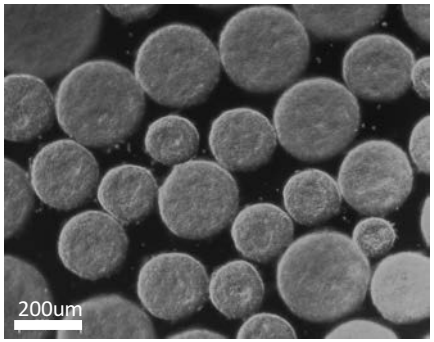
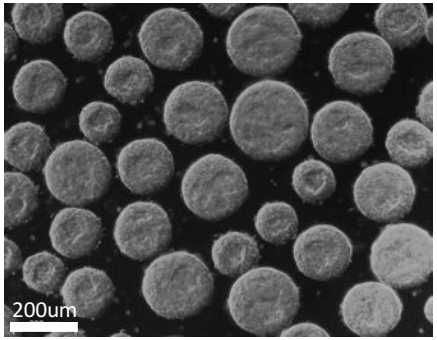
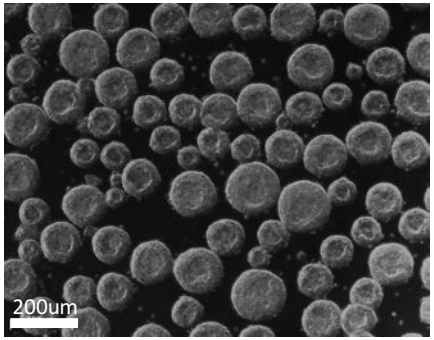
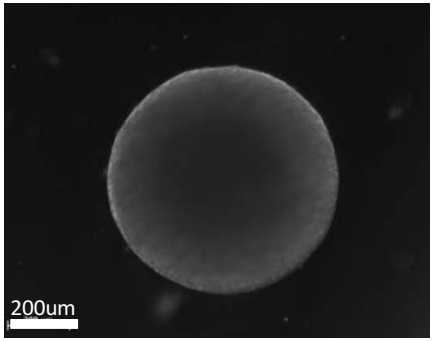
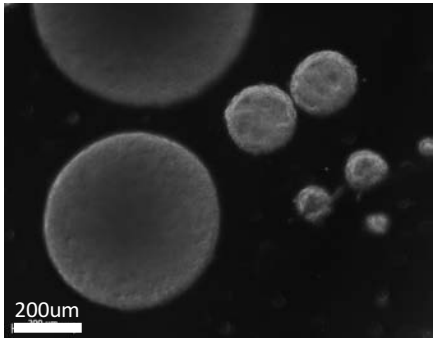
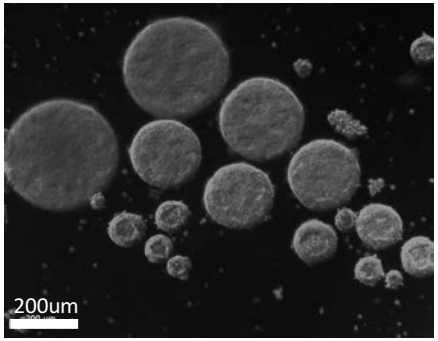
- Over 0.5 M cells/mL achieved at 0.1L – 50L

PSC Aggregate Sizes Are Critical for Differentiation Processes

- Size of cell aggregates varies inversely with the local hydrodynamic conditions (Energy Dissipation Rates)
  - ↑ EDR condition → ↓ size aggregates
  - ↓ EDR condition → ↑ size aggregates
- Typical Size Distribution of Cell Aggregates in a Bioreactor



Comparison of Cell Aggregate Size Formation in Different Bioreactors with Various Agitation Speeds

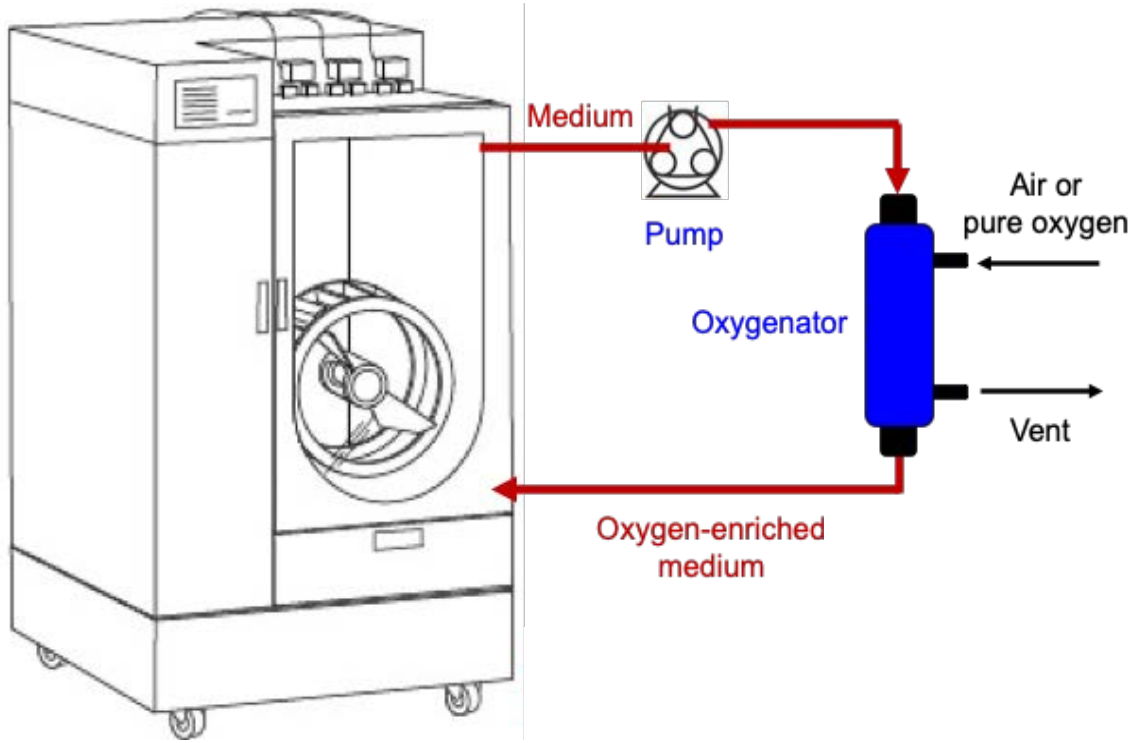
	40 rpm (D5)	60 rpm (D5)	80 rpm (D5)
PBS (Vertical-Wheel)			
Spinner (Horizontal-Blade)			

(100 mL Working Volume)

Borys et al. Stem Cell Research & Therapy (2021) 12:55

Enhanced Gas Exchange Without Sparging At Large Scale

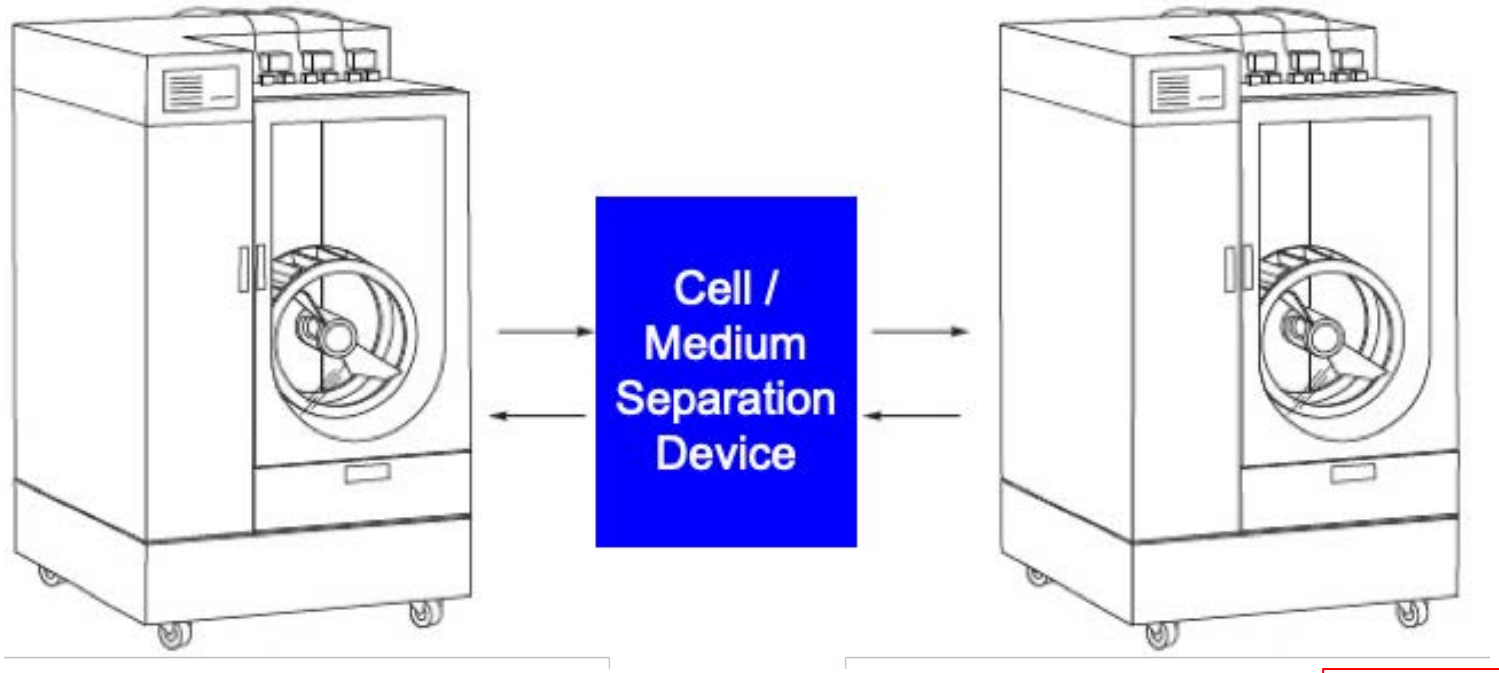
- Bubble bursts during gas sparging can damage the cells by shear stress and splashing the cell aggregates to the side wall
- Use of anti-foaming agent or shear protectant is NOT desirable



Patent Pending

Rapid and Complete Media Exchange Method At Large Scale

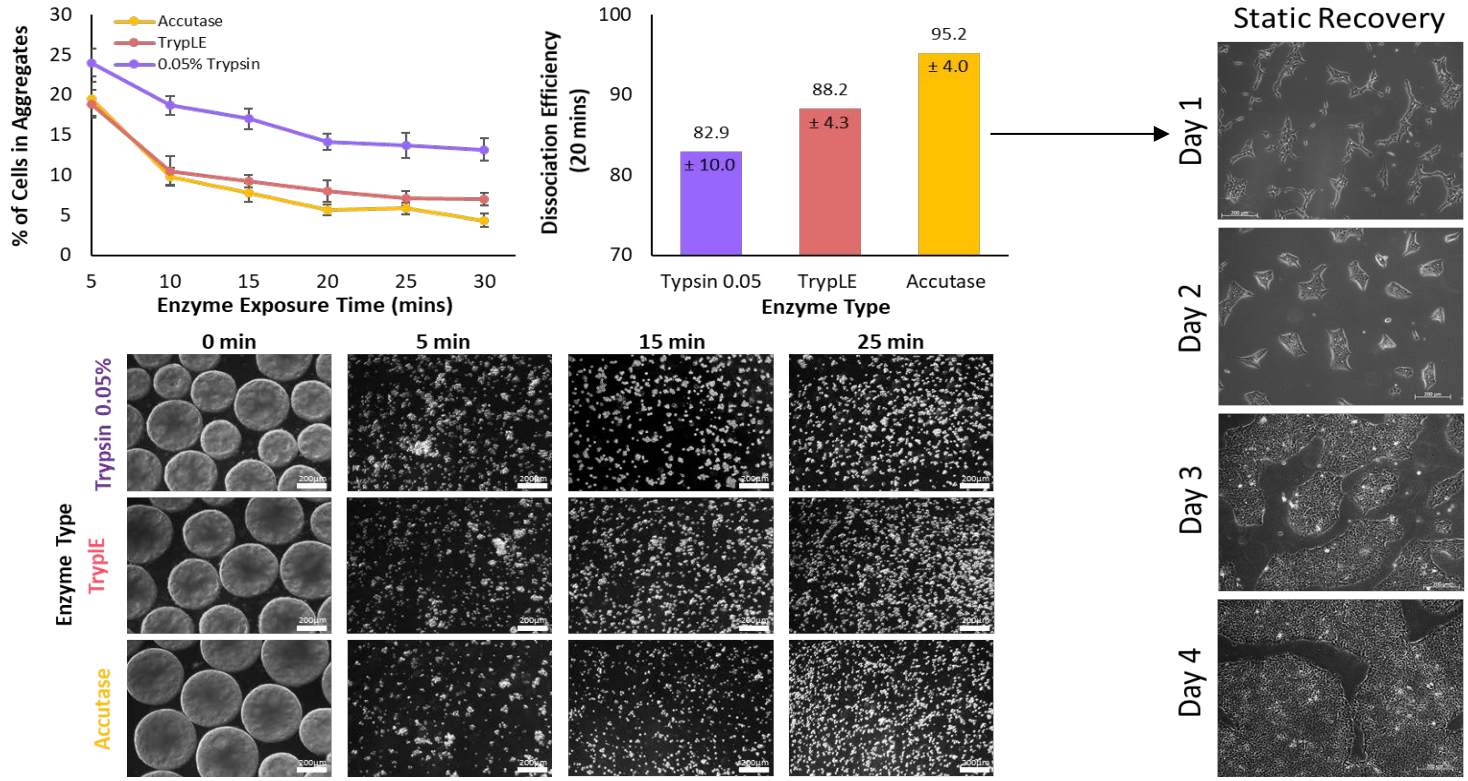
- Cell culture and differentiation process involves multiple media exchanges
- Prolonged duration of media exchange unit operation for large-scales
- Differentiation process requires step-wise and complete media exchanges



Patent Pending

Cell Dissociation and Harvest Conditions For Scale Up

- Test of Proteolytic Enzymes and Dissociation Time with Agitation in VW Bioreactor



- High speed agitation in Accutase + ROCi for 20 min led to 95% dissociation efficiency

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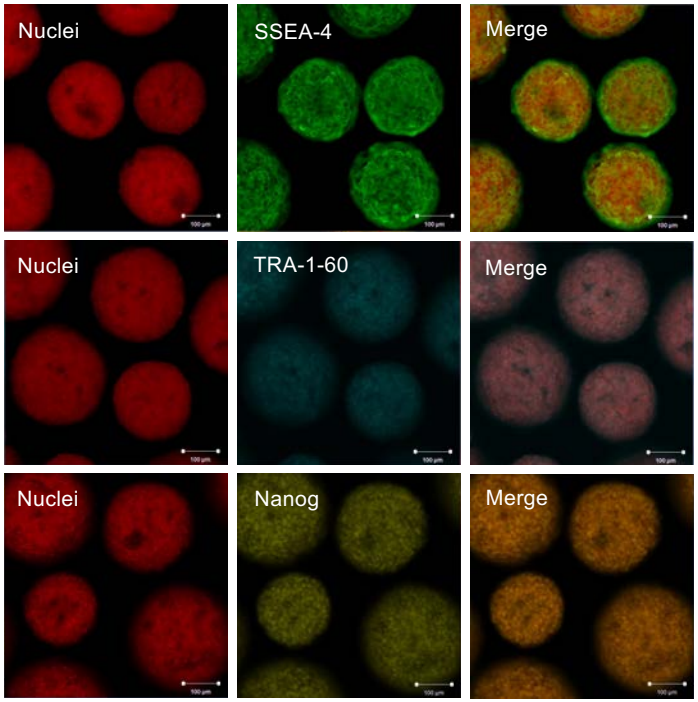
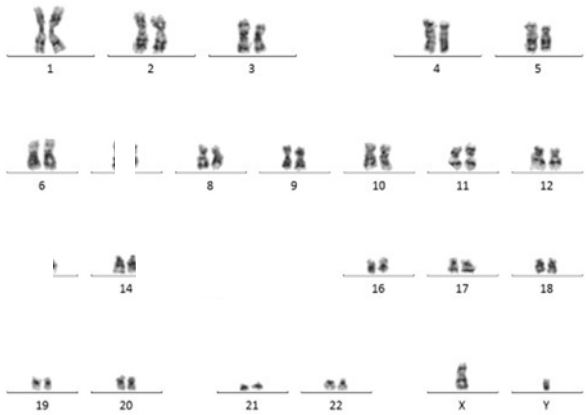
Maintenance of Quality Attributes After Cell Expansion and Harvest

hiPSC maintain high quality pluripotent characteristics following serial passages and harvest

Confocal Staining

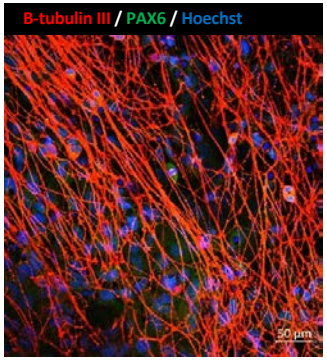


Karyotype

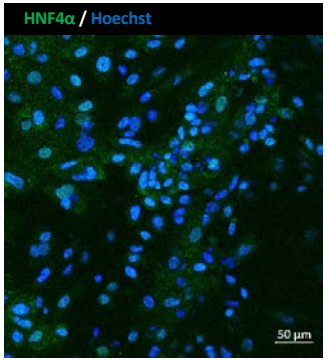


Directed Differentiation

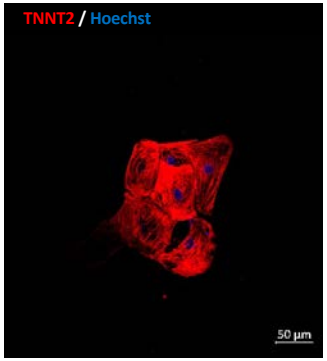
Ectoderm
Neural cells



Endoderm
Hepatocytes



Mesoderm
Cardiomyocytes



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Conclusions

- Culturing human cells in bioreactor requires unique hydrodynamic conditions.
- The process conditions of small-scale bioreactors need to be reproduced in large-scale bioreactors.
- Other areas to be optimized for large-scale bioreactor operations:
  - Reliable seed expansion process
  - Rapid and complete medium exchange for differentiation process
  - Efficient gas exchange method without using chemicals
  - Scalable cell harvest process

Thank you for your attention!

Q & A