



# Optimizing Human Pluripotent Stem Cell (hiPSC) Production Process Using Vertical-Wheel Bioreactors

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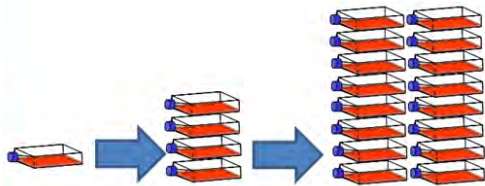
Phacilitate World Stem Cell Summit

Miami, FL

# Vertical-Wheel Stirred Suspension Bioreactors

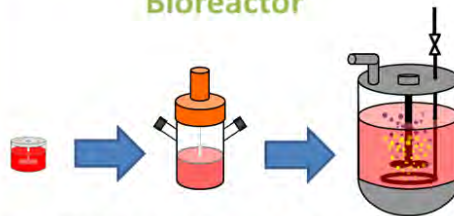
## Growth Platforms

### Static



- ✗ Variability between flasks
- ✗ More difficult to regulate culture environment
- ✗ Labour intensive
- ✗ Batch or fed batch mode only

### Bioreactor



- ✓ Well-mixed vessel
- ✓ Less labour intensive
- ✓ Easier to monitor and control process parameters
- ✓ Can operate in batch, perfusion mode, etc.
- ✓ Scalable

## Vertical-Wheel Reactors

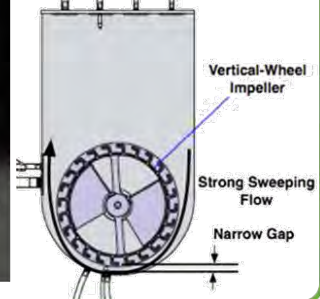
Horizontal Blade  
Suspension Bioreactor



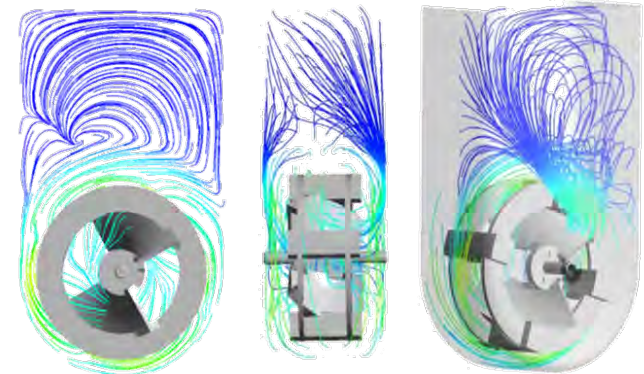
PBS Vertical  
Wheel Bioreactor



Geometry of Vertical  
Wheel Bioreactor



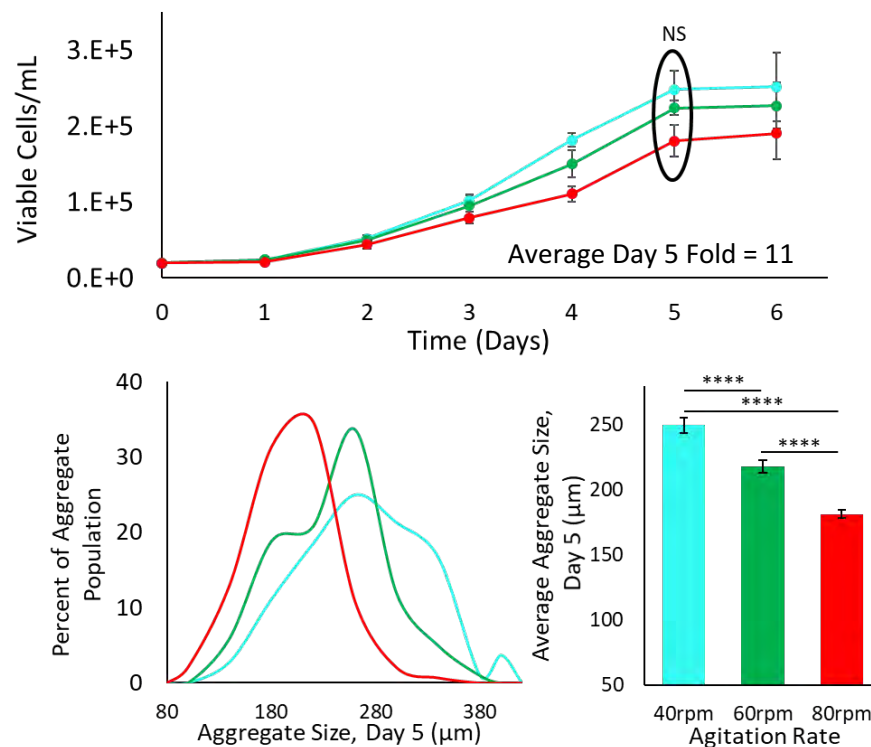
Velocity through the Reactor Height (60rpm)



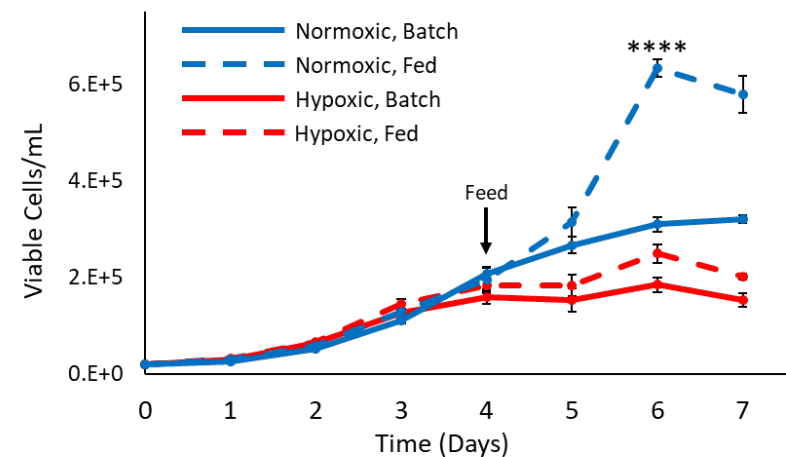
## Inoculation of hiPSC Aggregates Pre-Formed in Static Culture

**Early Studies by our Team using Spinners and Stirred-Tank Bioreactors:** Unsuccessful cell growth and poor aggregate distributions when cells were inoculated as a single cell suspension. For this reason, the team developed a passing method involving a pre-formation of hiPSCs in static culture prior to inoculating cells into a bioreactor.

### 1. Hydrodynamic Testing



### 2. Oxygen and Nutrient Testing

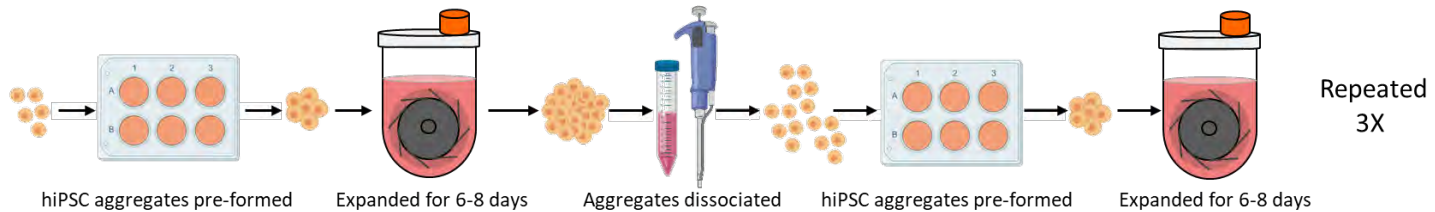


Reference	Bioreactor Type	Fold/Days
Zweiggerdt et al. (2011)	Horizontal Blade	3-6 fold / 4-7 days
Abbasalizadeh et al. (2012)	Horizontal Blade	8 fold / 7-10 days
Haraguchi et al. (2015)	Horizontal Blade	10 fold / 12 days
Badenes et al. (2016)	Horizontal Blade	3.5 fold / 10 days
<b>This Study</b>	<b>Vertical Wheel</b>	<b>32 fold / 6 days</b>

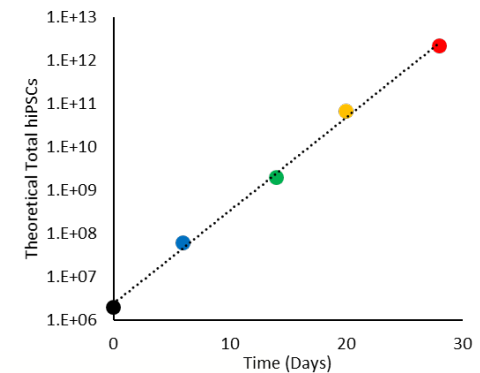
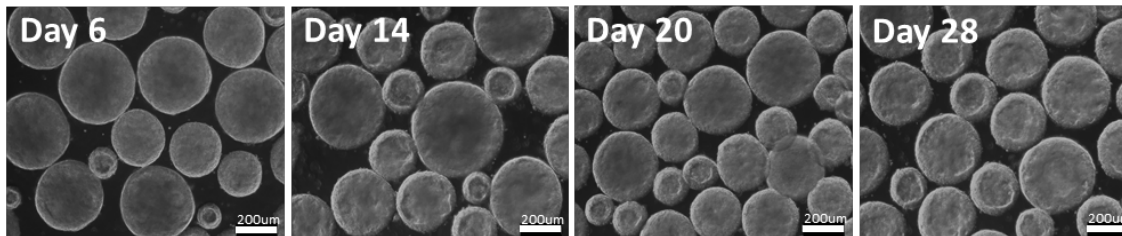
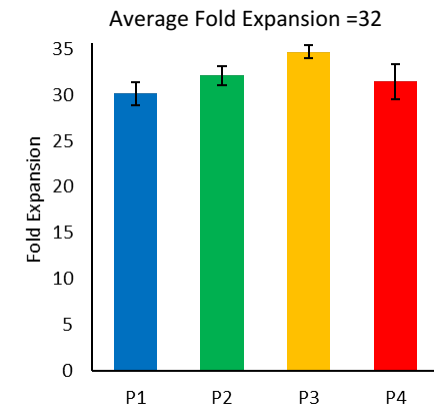
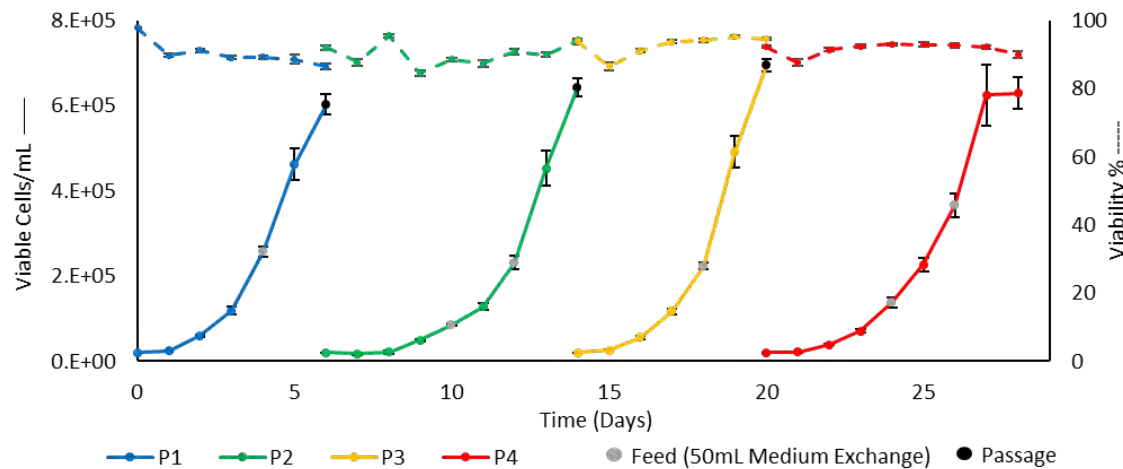
# Successful Serial Passage of hiPSCs in PBS 0.1L

## Achieved a Total of >1E6-Fold Expansion (n=4)

### Process



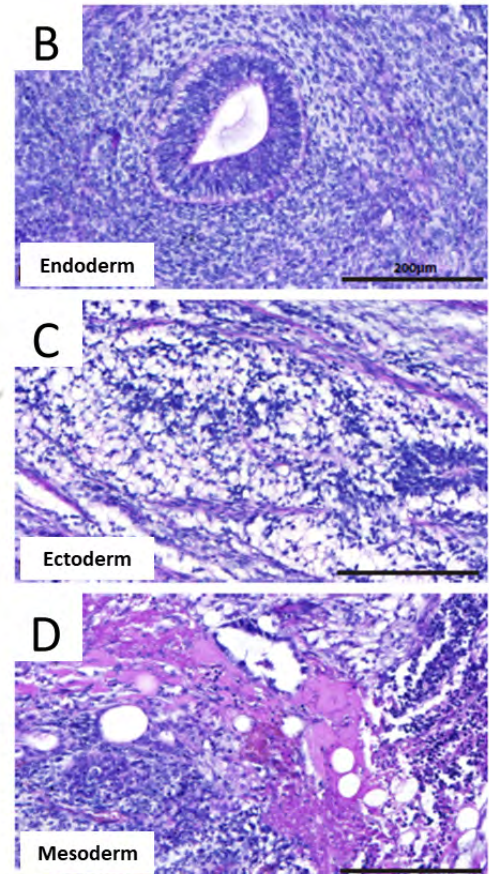
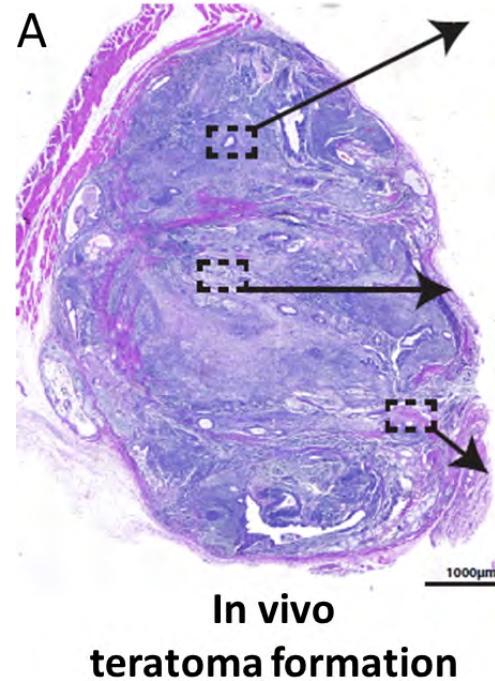
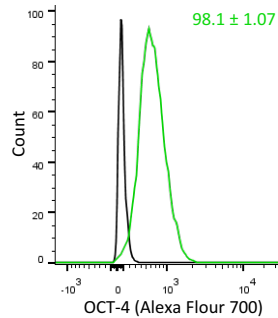
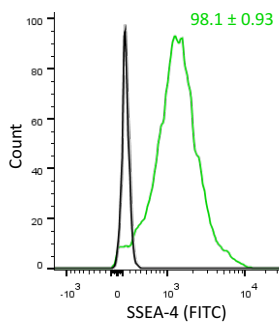
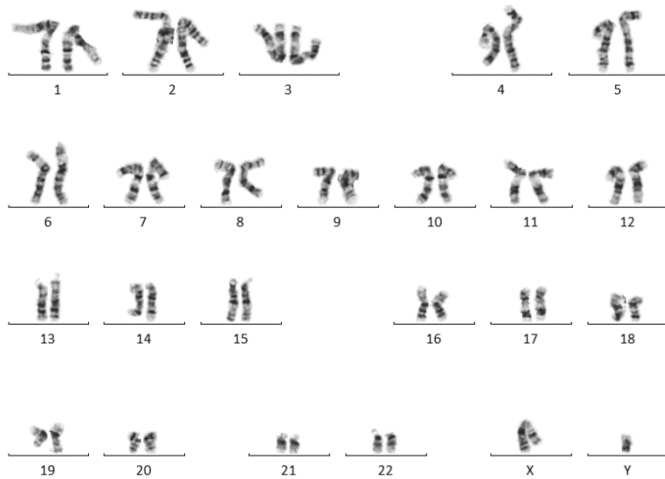
### Results





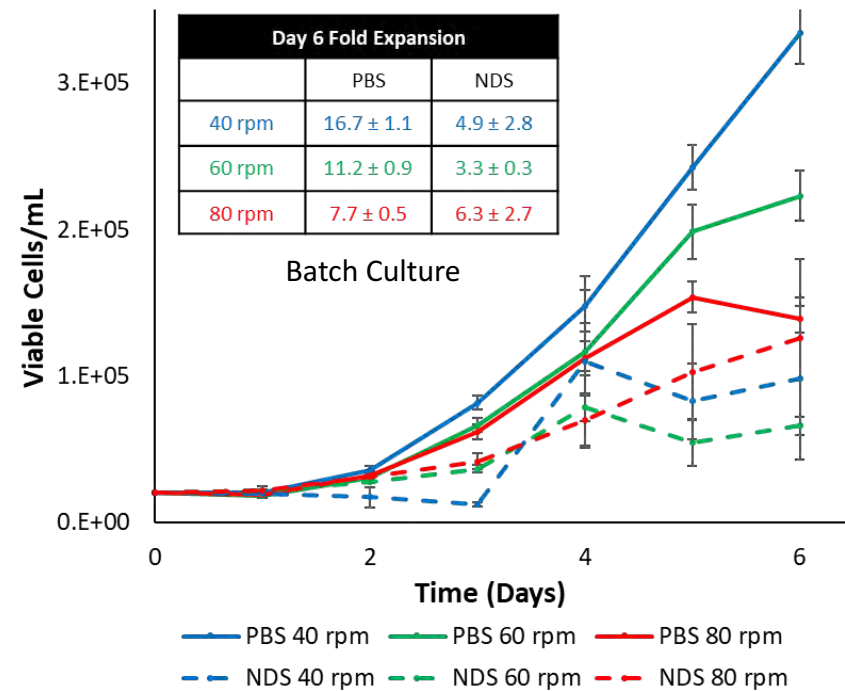
# Serial Passaged hiPSC are of High Quality

After 28 days of VW bioreactor culture, hiPSCs maintain normal karyotype and characteristic pluripotent stem cell phenotype and function



# Single Cell hiPSC Inoculation in PBS-0.1 Vessels vs Spinner (NDS)

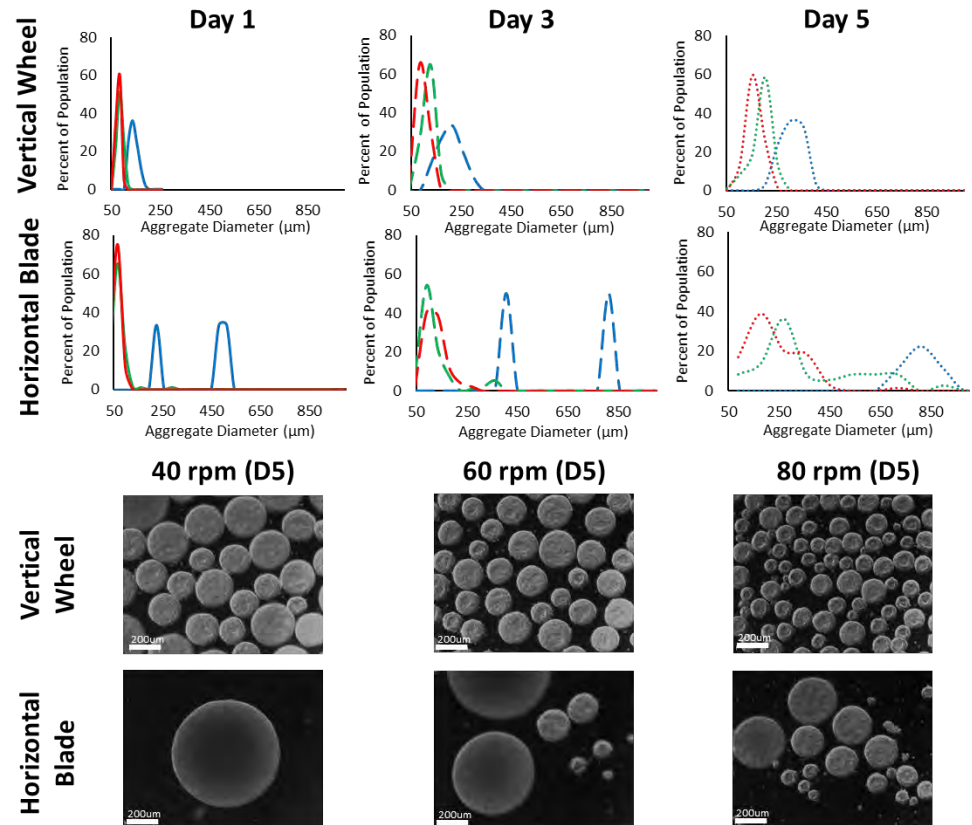
Single cell inoculation led to successful cell growth in vertical-wheel bioreactors but not in traditional horizontal-blade bioreactors



Horizontal Blade  
(NDS)



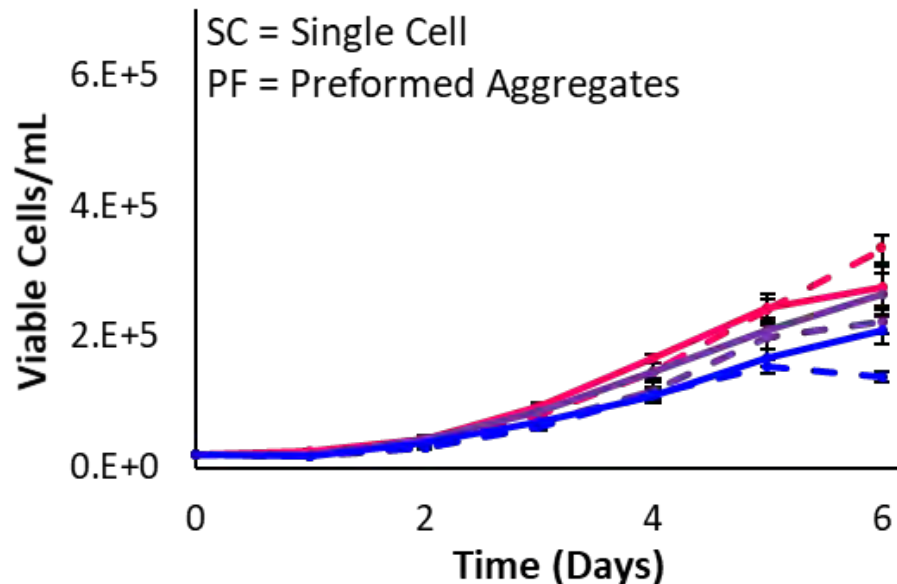
Vertical Wheel  
(PBS)



# 1. Single Cell vs Pre-formed Aggregate Inoculation in PBS 0.1

## 2. Single Cell Batch vs Fed-Batch in PBS 0.1

1

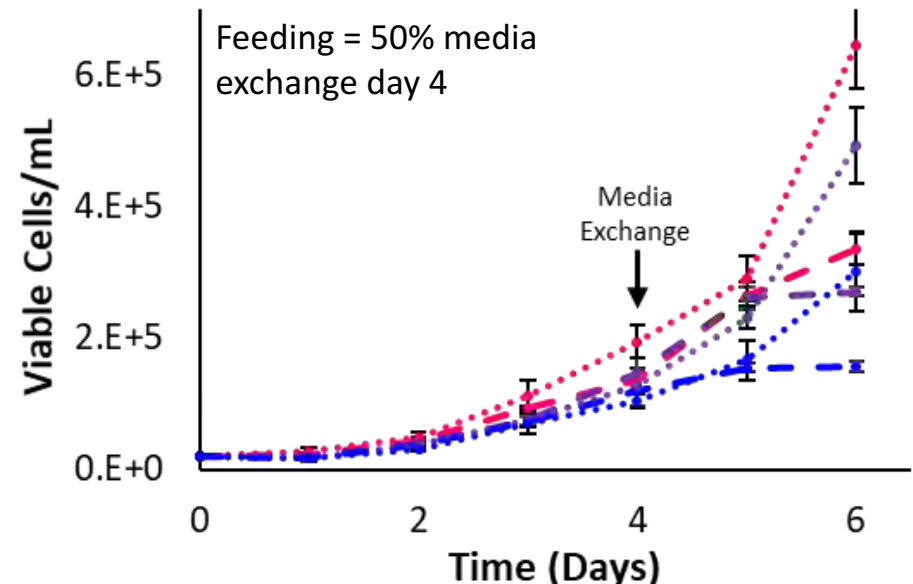


— PF Batch 40rpm  
- - SC Batch 40rpm  
... SC Fed 40rpm

— PF Batch 60rpm  
- - SC Batch 60rpm  
... SC Fed 60rpm

1. Single cell and pre-formed aggregate inoculation resulted in similar growth in the PBS-0.1 at all agitation rates

2

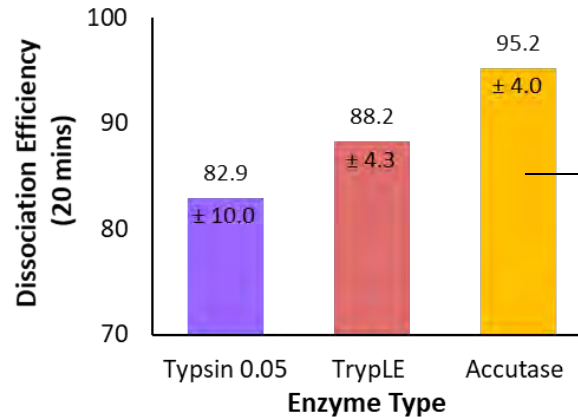
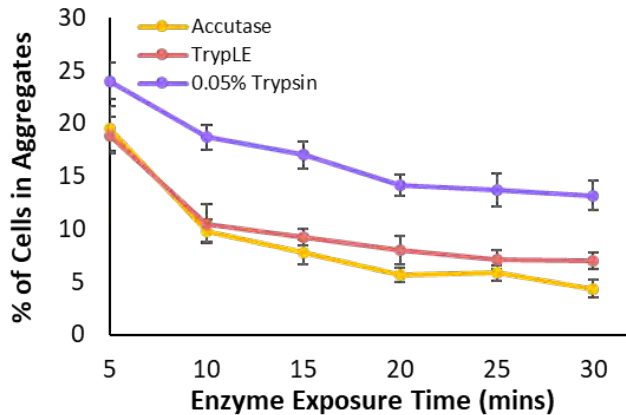


— PF Batch 80rpm  
- - SC Batch 80rpm  
... SC Fed 80rpm

2. Fed-batch culture resulted in approximately 2X higher fold expansion at all agitation rates

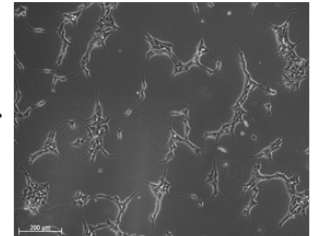


## Test of Proteolytic Enzymes and Dissociation Time with Agitation

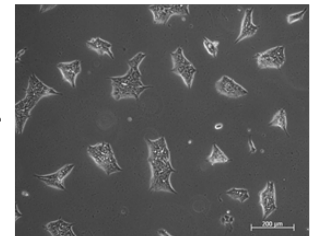


### Static Recovery

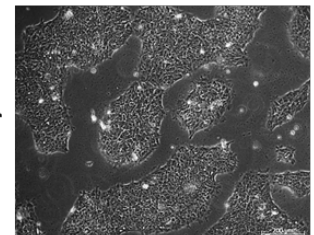
Day 1



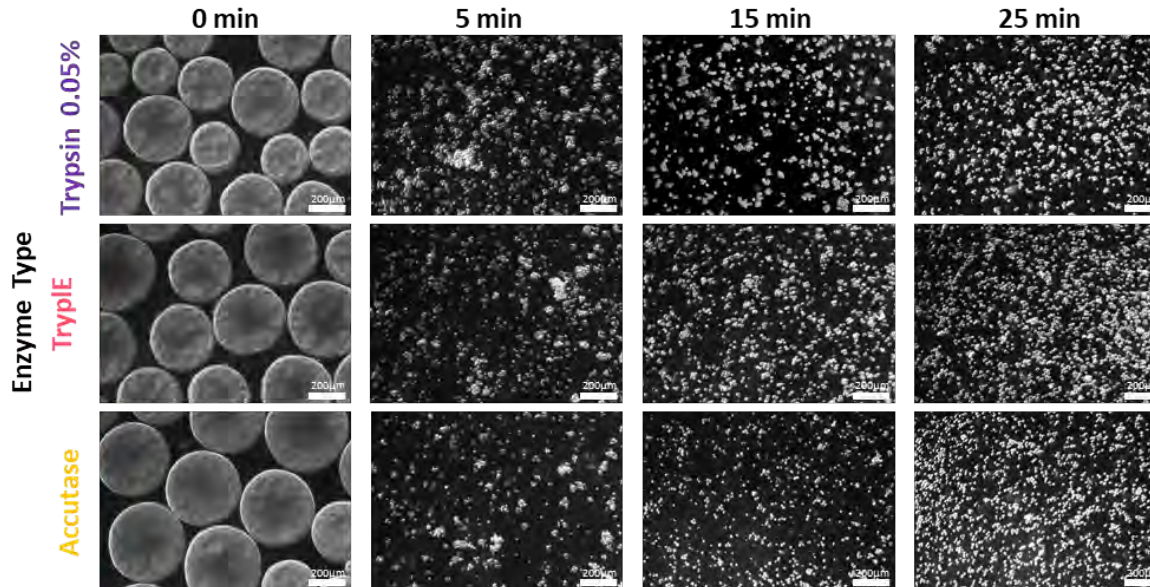
Day 2



Day 3



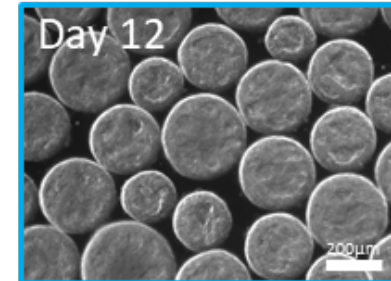
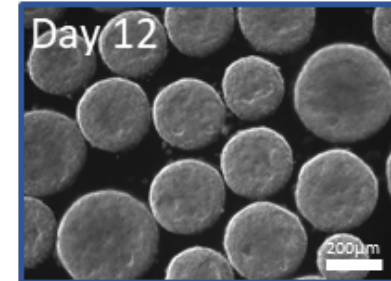
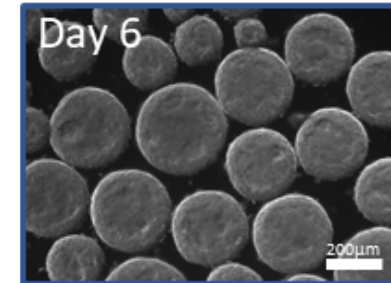
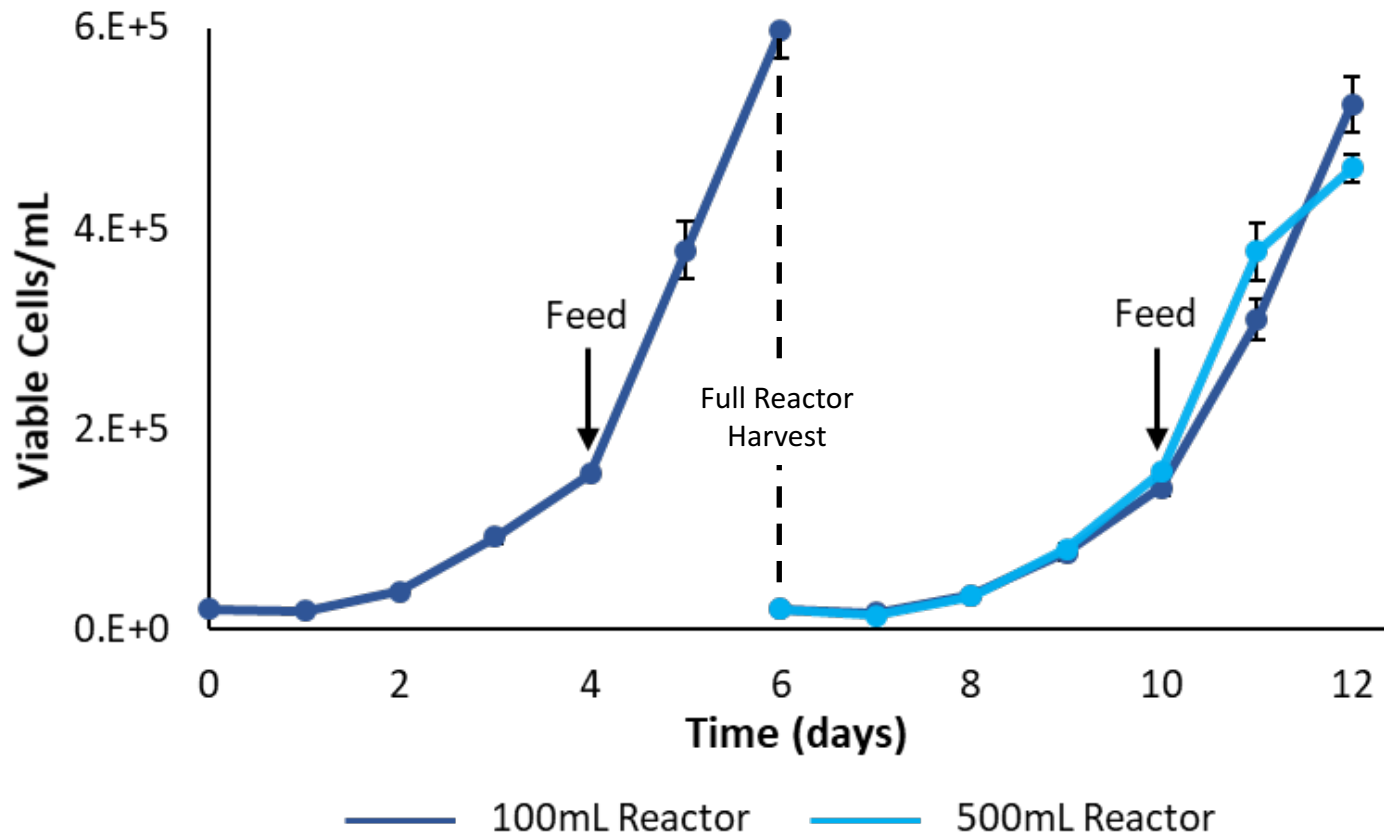
Day 4



20 min in Accutase + ROCi under agitation led to 95% dissociation efficiency



# Combined Process (Single Cell & Bioreactor Harvest) Serial Passage in PBS-0.1 and PBS-0.5



hiPSC cell growth remains high after bioreactor harvest and re-seeding of single cells into PBS 0.1 and 0.5

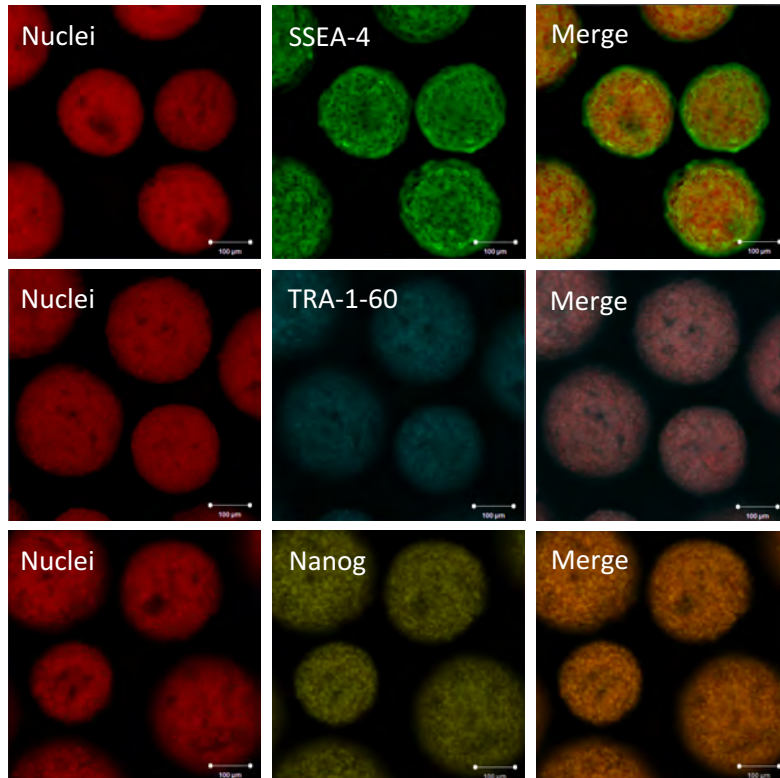
Aggregate size and morphology remain healthy in both the PBS 0.1 and 0.5

# Combined Process (Single Cell & Bioreactor Harvest) Serial Passage in PBS-0.1 and PBS-0.5

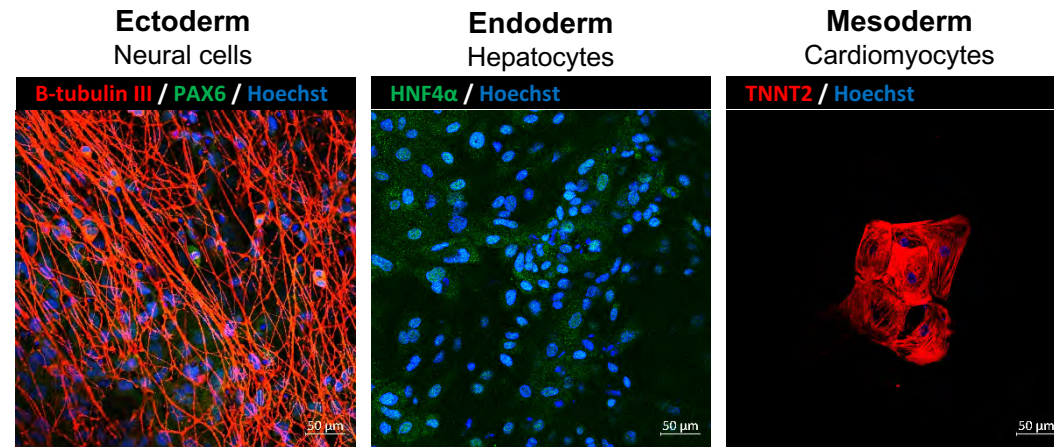
hiPSC maintain high quality pluripotent characteristics following optimized serial passage protocol with single cell inoculation and full bioreactor harvesting

## Pluripotency Staining

Day 12 Bioreactor Aggregates



## Tri-lineage Differentiation



- 1. Optimizing bioprocess variables (agitation, oxygen, and nutrients) in the PBS-0.1L reactor resulted in hiPSC expansion far greater than previously reported**
- 2. The expansion process is highly reproducible with the potential to generate over 1E12 high quality PSC (starting from 2E6) over 4 serial passages**
- 3. Bioprocess variables within the PBS reactor can be altered to overcome bioprocess bottlenecks in manufacturing hiPSCs  
(namely single cell seeding and full bioreactor harvests)**