Effective Scale-up of Human Induced Pluripotent Stem Cell Aggregate Culture in Vertical-Wheel Bioreactors through Computational Fluid Dynamics Hydrodynamic Characterization

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Figure 1: CFD Modelling of EDR in VW Bioreactors

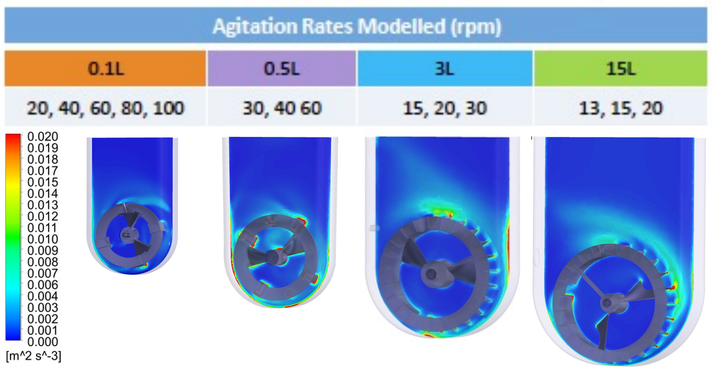


Figure 2: Scale-Up Curves & Equations: VA EDR vs. Agitation

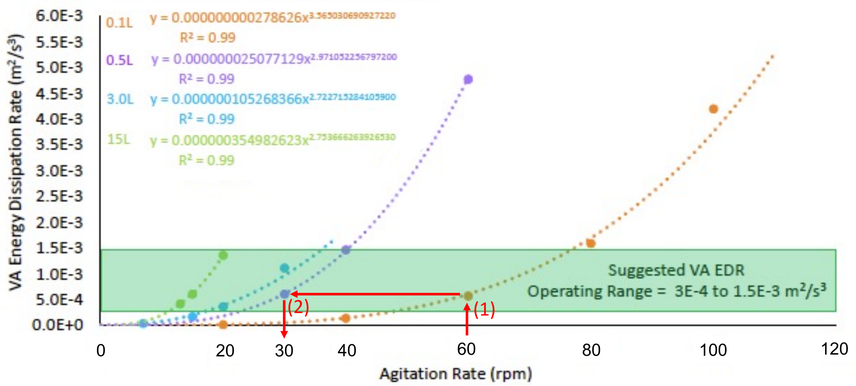
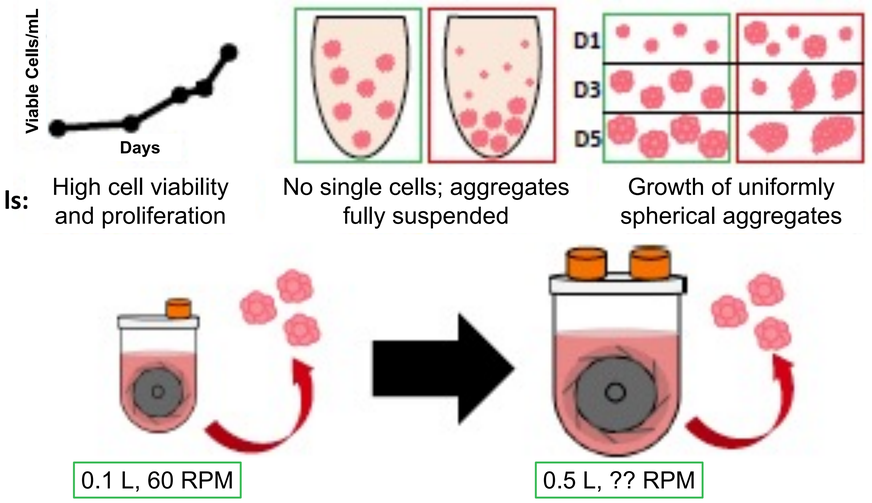


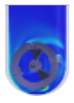
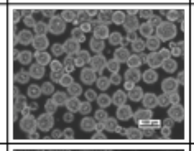
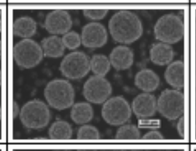
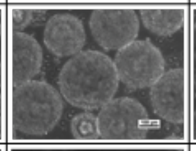
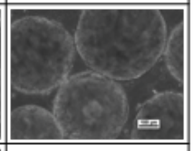
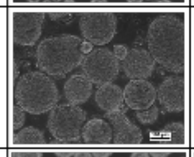
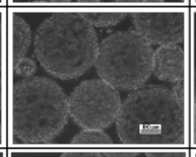
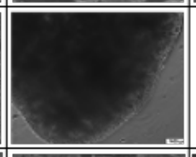
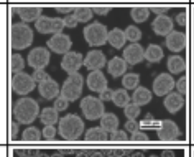
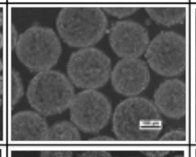
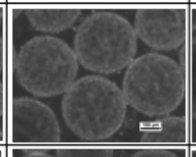
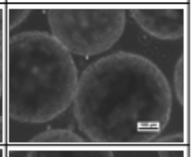
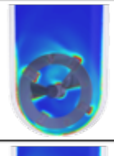
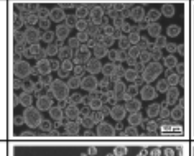
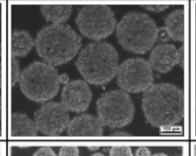
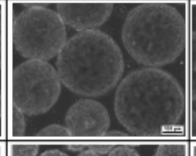
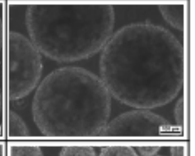
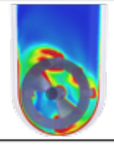
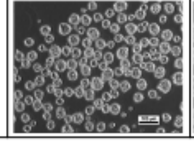
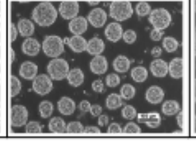
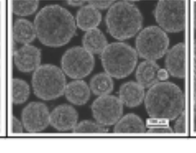
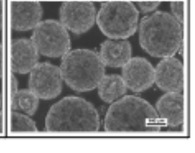
Figure 3: Biological Testing of hiPSC Aggregate Expansion



- Vertical-Wheel bioreactors uniquely generate a mixing environment with homogeneous distribution of turbulent energy dissipation rates (EDR)
- CFD models (Fig.1) were used to calculate the volume average EDR (VA EDR) for various combinations of working volume and agitation rate
- Plotting VA EDR values with best-fit curves generated the scale-up equations for each working volume (Fig. 2)
- A combination of volume and agitation that has a VA EDR within the suggested operating range yields uniformly spherical hiPSC aggregates of similar average diameter; the suggested range of **3.0E-4 – 1.5E-3 m^2/s^3** is based on results from multiple experiments with various collaborators
- VA EDR can be used as a predictive tool to minimize guesswork during process scale up:
  1) At small scale, determine the RPM that yields optimal aggregates → the corresponding VA EDR (within optimal range) is the "target VA EDR"
  2) Find the target VA EDR on larger scale curves → corresponding agitation rate can be expected to produce similarly optimal aggregates

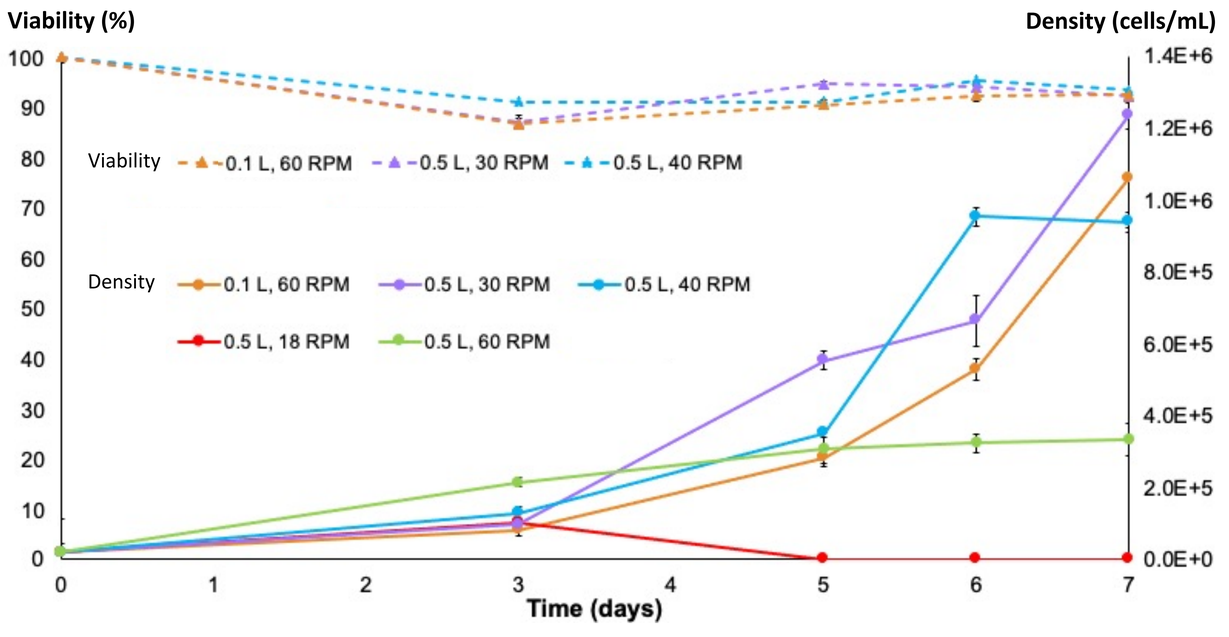
- Cell culture process was developed to compare expansion of hiPSC aggregates at 0.1 and 0.5 L working volumes
- At 0.1 L, 60 RPM resulted in optimal aggregate morphology and yield
- Various agitations were tested at 0.5 L to confirm that utilizing target VA EDR (in suggested range) results in similarly optimal aggregates

Figure 4: Correlation Between VA EDR and hiPSC Aggregate Morphology

Volume (L)	Agitation (RPM)	VA EDR (m^2/s^3)	CFD Model (EDR)	Day 1 (10x)	Day 3 (10x)	Day 5 (10x)	Day 7 (10x)
0.1	60	6.1E-4					
0.5	18	1.4E-4					Clumped cell mass too large to suspend
0.5	30	6.1E-4					
0.5	40	1.4E-3					
0.5	60	4.8E-3					

- 0.1 L, 60 RPM → uniformly spherical aggregates, with target VA EDR of 6.1E-4 m^2/s^3 (within operating range)
- Target VA EDR matched at 0.5 L, 30 RPM → confirm nearly identical aggregates at 0.1 and 0.5 L scale
- 0.5 L, 40 RPM: VA EDR near upper limit of optimal range → spherical but slightly smaller average diameter
- 0.5 L, 18 RPM & 0.5 L, 60 RPM: VA EDRs outside of suggested range → aggregates of varying morphologies

Figure 5: Correlation Between VA EDR and hiPSC Density and Yield



- 0.1 L, 60 RPM, 0.5 L, 30 RPM, & 0.5 L, 40 RPM have VA EDRs that fall within operating range → each has cell density of approximately 1.0 M cells/mL after day 7 and viability >90% throughout expansion process
- 0.5 L, 18 RPM & 0.5 L, 60 RPM have VA EDRs outside of suggested range → poor cell density and viability

Conclusion

- Similar aggregate morphology, cell density, and viability of hiPSCs were achieved for an expansion process at 0.1 and 0.5 L scale in Vertical-Wheel bioreactors, by maintaining target VA EDR within the operating range
- An optimal VA EDR to target will be beneficial for large scale expansion and differentiation process development
- Consistency of hydrodynamic conditions in Vertical-Wheel bioreactors can enable the scalable manufacturing of high-quality hiPSCs for clinical or commercial purposes