Computational Fluid Dynamics (CFD) Modeling of Single-Use, Vertical-Wheel Bioreactors as a Predictive Scale-up Tool for Large Scale Stem Cell Culture

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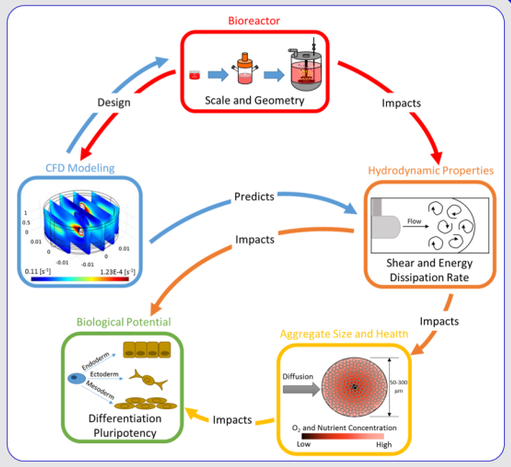
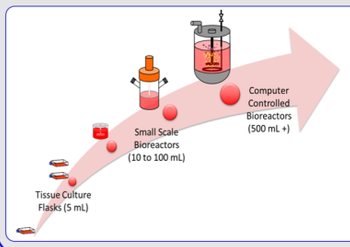
Using CFD Modeling as a Scale-up Tool

The therapeutic use of stem cells has become increasingly popular as a possible treatment option for a variety of diseases and injuries. Typically, 10^6 to 10^9 cells are required per patient, per treatment [1]. To achieve these numbers, large-scale production techniques must be implemented. Cellular aggregates can be expanded in stirred suspension bioreactors, achieving significantly greater cell quantities per volume compared to traditional static culture [2]. A change in bioreactor scale, however, results in altered hydrodynamic properties that affect cell products. Shear from the local fluid velocity gradient and cell interactions with turbulent eddies influence aggregate sizes, which impact cell pluripotency, differentiation, and proliferation potential [3]. Current bioreactor scale-up equations used for predicting agitation rates maintain single quantity variables that do not capture the variation and complexity of the bioreactor environment. As a result, predicted scale-up agitation rates are often ineffective and damage the cells. Computational Fluid Dynamic (CFD) modeling allows the user to customize geometry so that scale-up equations can be derived between reactors of any given shape and size. We have recently published data [4] that suggests maintaining the volume average energy dissipation rate, derived from CFD simulations, provides a robust method for scale-up of aggregate culture.

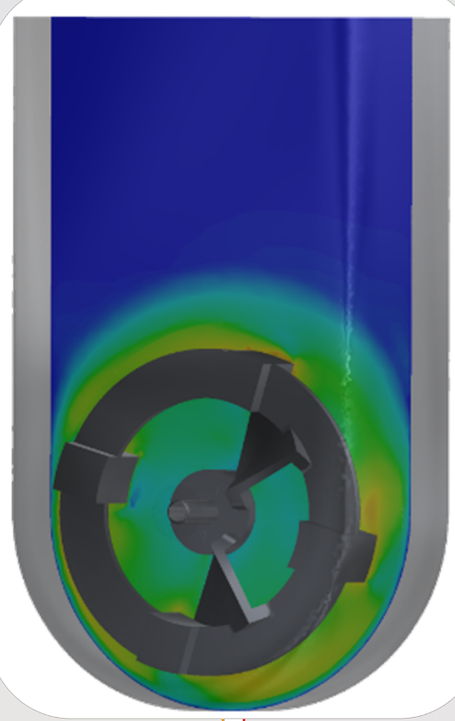


Project Overview



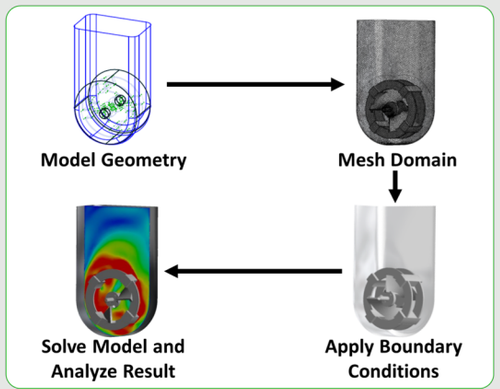
In this study:

- Ansys Fluent software was used to develop a complete (20rpm-100rpm) CFD model of the 0.1L PBS vertical-wheel reactor.
- Model variables (volume average and distributions) were compared to results from the 100mL NDS and the 500mL DasGip bioreactors.
- A hiPSC aggregate pre-formation protocol was developed (comparing pre-formation time and cell density) for bioreactor culture.
- hiPSCs were cultured in 0.1L PBS vertical-wheel reactors at various agitation rates to compare growth and aggregate size.

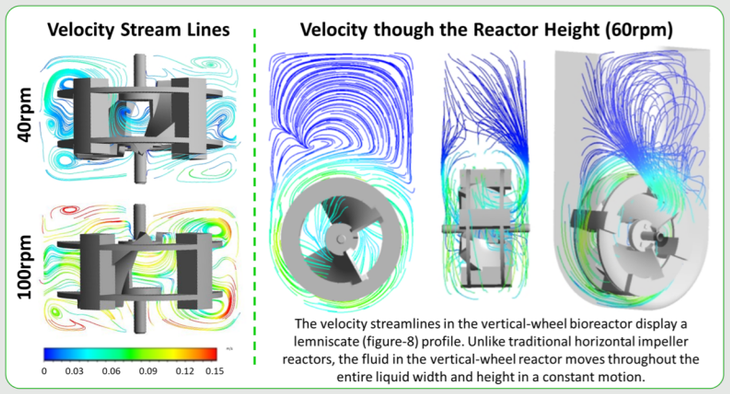


Single Use Vertical-Wheel Reactor Model (0.1L)

Methods

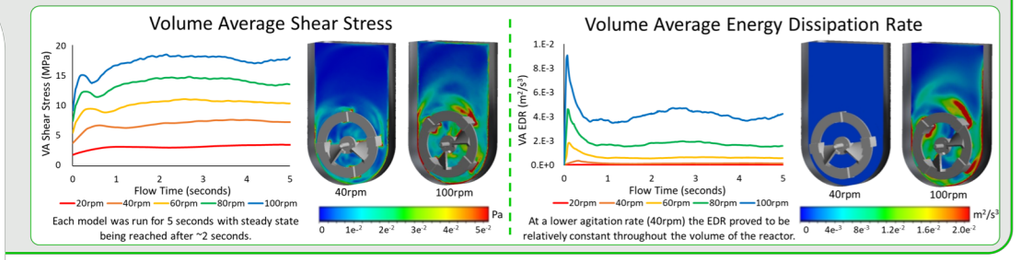


Velocity Profile



The velocity streamlines in the vertical-wheel bioreactor display a lemniscate (figure-8) profile. Unlike traditional horizontal impeller reactors, the fluid in the vertical-wheel reactor moves throughout the entire liquid width and height in a constant motion.

Shear Stress and Energy Dissipation Rate

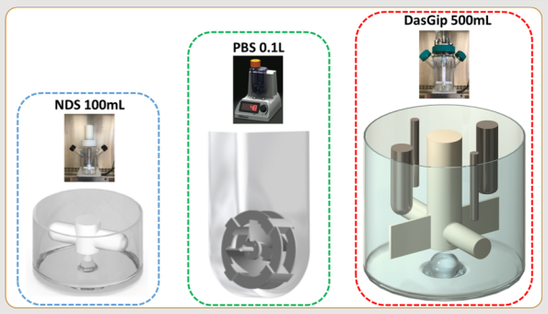


Each model was run for 5 seconds with steady state being reached after ~2 seconds.

At a lower agitation rate (40rpm) the EDR proved to be relatively constant throughout the volume of the reactor.

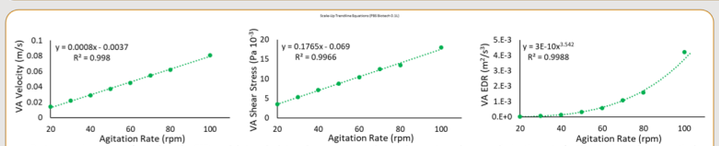
Bioreactor Model Comparison

Bioreactors Modeled

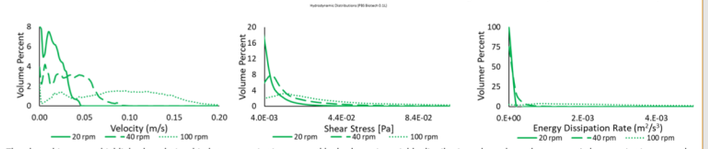


In this study, the 0.1L PBS vertical-wheel bioreactor was modeled at various agitation rates using CFD software Fluent. Results were compared to models generated for traditional horizontal-blade impeller 100mL NDS and 500mL DasGip bioreactors (modeled with the addition of control probes). It was discovered that while the volume average shear rates between the modeled reactors remained similar, the volume average energy dissipation rate in the 500mL DasGip was nearly 10 times higher than in the 100mL reactors. This increase can likely be attributed to the addition of control probes that occupy a large portion of the liquid headspace in the DasGip. These results suggest that a drastic decrease in agitation rate between the 100mL and 500mL reactors would be required for aggregate size to be maintained throughout scale-up.

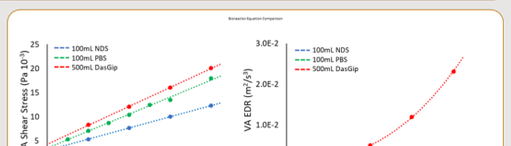
Equation Comparison and Hydrodynamic Variable Distributions



Post-processing was performed on the CFD models to calculate volume average velocity, shear rate and energy dissipation rate for the PBS Biotech 0.1L vertical-wheel reactor run at 20, 30, 40, 50, 60, 70, 80, and 100rpm. Variables were then plotted as a function of agitation rate and equations of lines were computed.



The above histograms highlight the relationship between agitation rate and hydrodynamic variable distributions throughout the reactor. At lower agitation rates, the hydrodynamics are relatively consistent throughout the reactor volume. As agitation rates increase, the volume fractions experiencing different forces also increase.



The trendline equations from the graphs can be used to predict agitation rates that maintain volume average hydrodynamic variables when scaling between the reactors.
For example, if 60rpm is determined optimum in the 0.1L PBS reactor

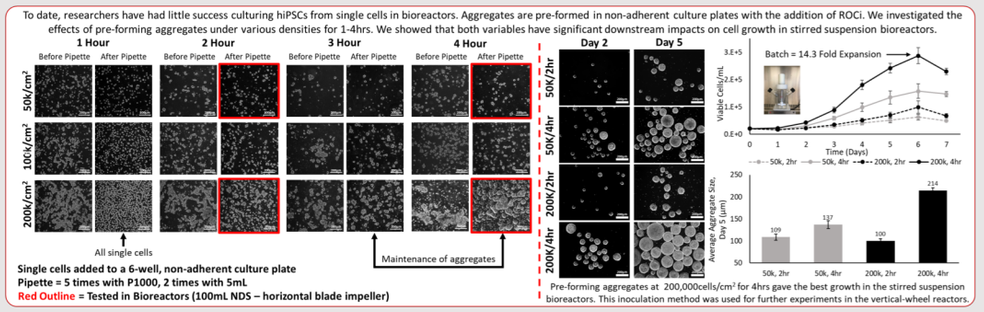
0.1L PBS Agitation	Variable Maintained	100mL NDS Agitation	500mL DasGip Agitation
60rpm	VA Shear Stress	84rpm	52rpm
	VA Energy Dissipation Rate	60rpm	30rpm

To maintain the 0.1L PBS reactor VA energy dissipation, the 100mL NDS reactor should be run at the same agitation rate (60rpm) but the 500mL DasGip reactor should be run at half the agitation rate (30rpm)

Growth of hiPSCs

Pre-forming Aggregates – Time and Density Results

To date, researchers have had little success culturing hiPSCs from single cells in bioreactors. Aggregates are pre-formed in non-adherent culture plates with the addition of ROCi. We investigated the effects of pre-forming aggregates under various densities for 1-4hrs. We showed that both variables have significant downstream impacts on cell growth in stirred suspension bioreactors.

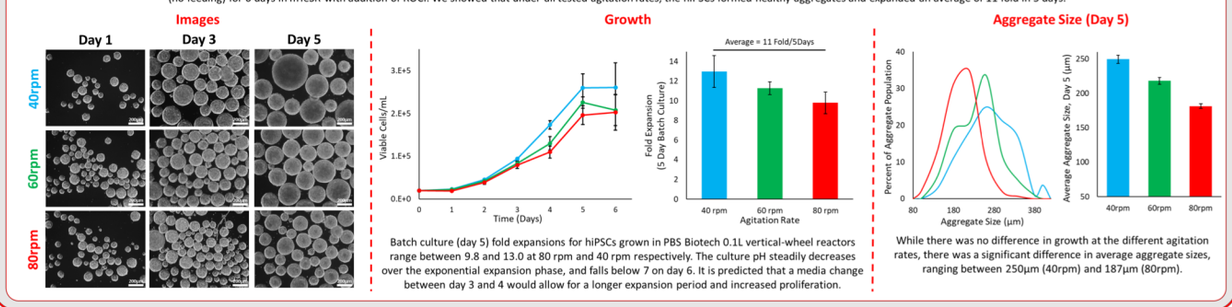


Single cells added to a 6-well, non-adherent culture plate
Pipette = 5 times with P1000, 2 times with 5mL
Red Outline = Tested in Bioreactors (100mL NDS – horizontal blade impeller)

Pre-forming aggregates at 200,000cells/cm² for 4hrs gave the best growth in the stirred suspension bioreactors. This inoculation method was used for further experiments in the vertical-wheel reactors.

Affects of Hydrodynamics on Growth and Aggregate Size

Using the optimized aggregate pre-formation method described above, hiPSCs were seeded into the 0.1L PBS vertical-wheel reactors at a density of 20,000cells/mL at agitation rates of 40, 60, and 80rpm. Cells were cultured under batch conditions (no feeding) for 6 days in mTeSR with addition of ROCi. We showed that under all tested agitation rates, the hiPSCs formed healthy aggregates and expanded an average of 11 fold in 5 days.



Batch culture (day 5) fold expansions for hiPSCs grown in PBS Biotech 0.1L vertical-wheel reactors range between 9.8 and 13.0 at 80 rpm and 40 rpm respectively. The culture pH steadily decreases over the exponential expansion phase, and falls below 7 on day 6. It is predicted that a media change between day 3 and 4 would allow for a longer expansion period and increased proliferation.

While there was no difference in growth at the different agitation rates, there was a significant difference in average aggregate sizes, ranging between 250µm (40rpm) and 187µm (80rpm).

Discussion

Data generated from the CFD model of the 0.1L PBS Biotech vertical-wheel reactor was used to gain a detailed understanding of the hydrodynamics within the reactor. Unlike traditional horizontal-blade impellers, streamlines in the vertical-wheel reactor form a figure-8 pattern, moving through the entire reactor height with each rotation. The volume average energy dissipation rate (a key variable controlling aggregate size) between the horizontal-blade and vertical-wheel 100mL reactors where found to be very similar. In contrast, the 500mL reactor modeled with control probes, had a much greater volume average energy dissipation rate and should be operated at an agitation rate of approximately half that of the 100mL reactors to maintain aggregate size throughout scale-up. Utilizing our understanding of the bioreactor hydrodynamic forces and control of environmental factors (nutrient and oxygen availability) we will continue to optimize growth and scale-up conditions for hiPSCs cultured as aggregates in the PBS Biotech vertical-wheel bioreactors at various scales.

References

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