Computational Fluid Dynamic Characterization of Vertical-Wheel Bioreactors For Effective Scale-Up of Human Induced Pluripotent Stem Cell Aggregate Culture

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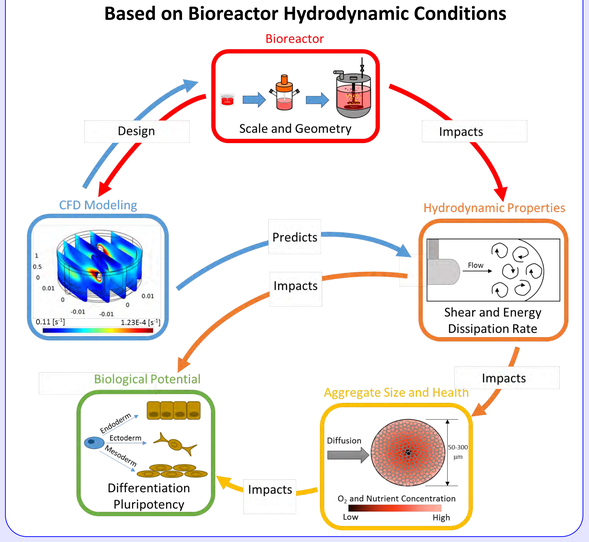
Using CFD Modelling as a Scale-Up Tool

Suspension-based cell culture processes in single-use bioreactors are the method of choice for efficient expansion of therapeutic cells in a controlled setting. However, choosing the optimal type of bioreactor for process development is critical as its hydrodynamic conditions will have a significant effect on biological performance, particularly for shear sensitive cells like induced pluripotent stem cells (iPSCs) grown as cell aggregates.

The size and shape of aggregates are critical for expansion and differentiation, as overly large or misshapen aggregates inhibit proper diffusion of nutrients, growth factors, and dissolved gases. Maintaining homogeneously spherical aggregates during scale-up will be critical for successful clinical or commercial manufacturing.

The PBS Vertical-Wheel® (VW) bioreactor combines radial and axial flow to produce uniform distributions of hydrodynamic forces, making it a promising platform to overcome scale-up challenges associated with aggregates. Effective scale-up equations allow small scale optimization to be translated to larger scales and choosing which variable to maintain constant throughout scale-up depends on culture conditions and critical quality attributes. We have demonstrated in an earlier publications [1] that volume average energy dissipation rate is a key variable that must be maintained for consistent aggregate size and size distributions during scale up.

Experimental Design Cycle to Predict Biological Performance Based on Bioreactor Hydrodynamic Conditions

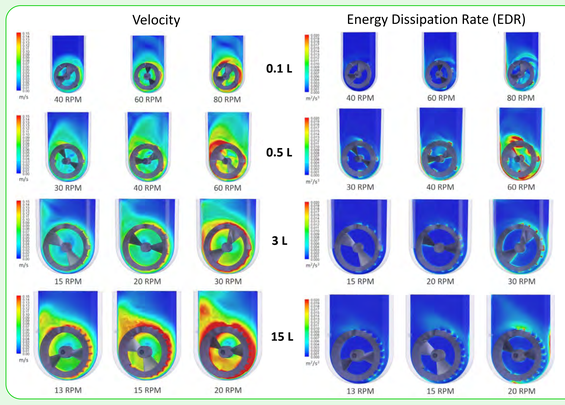


Project Overview

CFD models for each VW bioreactor volume (0.1, 0.5, 3, and 15 L) were run at a variety of agitation rates to generate scale-up correlation equations. An optimal operating range of hydrodynamic conditions was experimentally determined for the successful culture of iPSC aggregates across all tested volumes, with a successful culture defined by high cell proliferation rates, fully suspended aggregates (visually confirmed), and morphologically healthy cell expansion with homogeneous distributions of aggregate size (diameter) and shape (spherical). Various combinations of bioreactor volumes and agitation rates were tested to observe the effect on biological performance and aggregate formation.

Characterization of Bioreactor Hydrodynamics

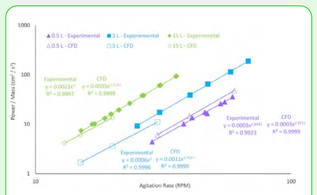
Hydrodynamic Profiles



For Velocity cut planes, the sweeping changes in colour from dark blue (0.0 m/s) to light blue (0.04 m/s) and green (0.08 m/s) in the upper corners of the VW vessels are indicative of simultaneous radial and axial mixing. These cut planes also indicate that certain combinations of volume and agitation rate may produce too low of a fluid velocity for effective mixing. For example, the model of 0.1 L vessel at 40rpm shows a substantial dark blue region of minimal fluid flow and agitation for cells.

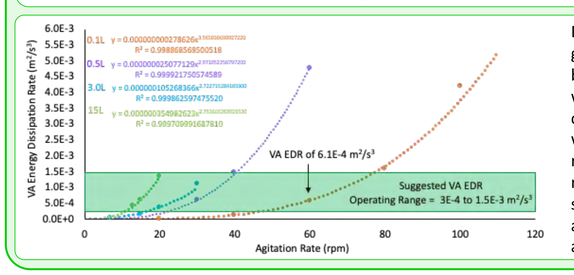
The EDR cut planes display less of a colour change through the bioreactor volume. The middle agitation rate modelled at each scale remains almost entirely dark blue (0.001 m^2/s^3), meaning the number of turbulent eddies should be limited throughout the volume. A narrow distribution of EDR values indicates hydrodynamic conditions that can be greatly beneficial when working with sensitive stem cell cultures.

Model Validation



Power/mass numbers for three agitation rates shown above compare experimentally derived data to CFD-predicted values. With some scatter, there is good correlation between the calculated and measured values, with an average difference of just 7.3%. For turbulent flow, the expected slope of VA EDR versus agitation rate is 3 [2]. All data sets show slopes near this expected value.

Energy Dissipation Rate Scale-Up Equations



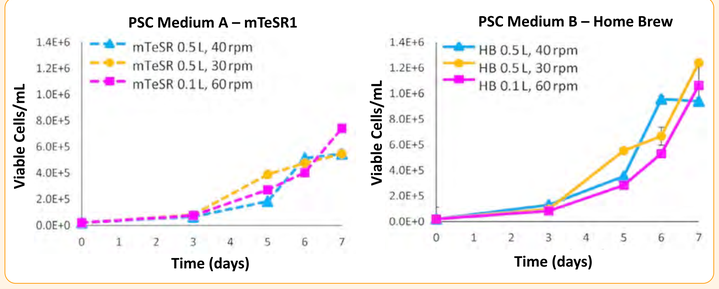
Each CFD model was run at a minimum of three different agitation rates, allowing for the generation of volume average (VA) EDR correlation scale-up equations for each scale of VW bioreactor. Once an optimal agitation rate is found at small scale (point on a curve that falls within the suggested VA EDR operating range), the equations can be used to predict the corresponding agitation rates needed at larger scales to achieve the same VA EDR and stay within the operating range, which will lead to optimal aggregate formation. For example, 60 rpm at 0.1 L scale (orange) produces a VA EDR of 6.1E-4 m^2/s^3 (within operating range): 30 rpm at 0.5 L (purple), 25 rpm at 3 L (blue), and 18 rpm at 15 L (green) will all produce the same VA EDR and beneficial hydrodynamic conditions for iPSC aggregates. The ability to achieve similar heterogeneous hydrodynamic conditions of narrow EDR distributions across a wide range of volumes demonstrates the true scalability of VW bioreactors.

Validation Within Operating Range

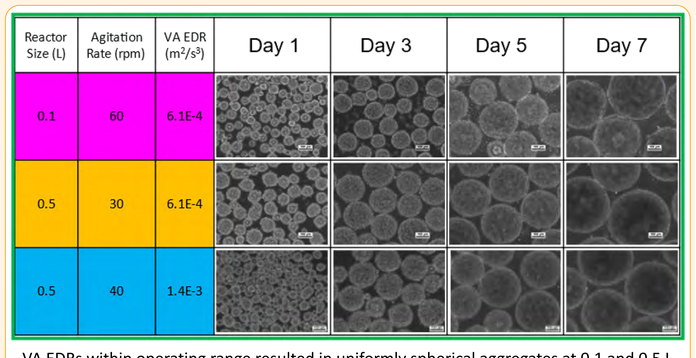
Growth in 0.1 L and 0.5 L VW Bioreactors Within Operating Range

VA EDRs within the operating range were tested with two different media types (commercially available mTeSR1 and home brew made in-house). 60 rpm at 0.1 L and 30 rpm at 0.5 L both produce a VA EDR of 6.1E-4 m^2/s^3 (within range) and uniformly spherical aggregates of nearly identical diameters. 40 rpm at 0.5 L has a VA EDR of 1.4E-3 m^2/s^3 near the upper limit of operating range and the resulting aggregates are slightly smaller but still uniformly spherical. There is an inverse correlation between agitation rate and aggregate diameter.

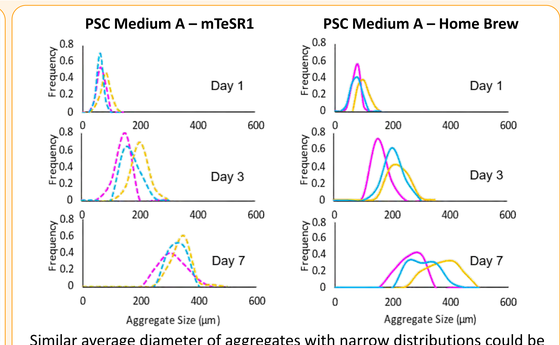
By day 7, hiPSCs cultured in mTeSR1 medium reached fold expansions between 27 ± 5.3 and 37 ± 1.9. This is in line with optimized published reports of hiPSCs previously cultured in VW bioreactors [3]. Cells cultured in home brew medium reached fold expansions between 47 ± 2.9 and 62 ± 3.5, although with slightly more heterogeneity.



Aggregate Morphologies and Size Distributions



VA EDRs within operating range resulted in uniformly spherical aggregates at 0.1 and 0.5 L.

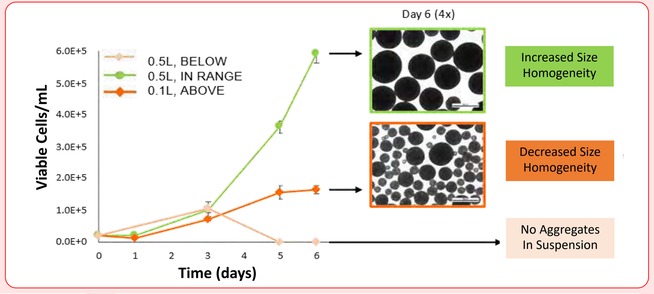


Similar average diameter of aggregates with narrow distributions could be achieved at 0.1 L (purple) and 0.5 L (orange and blue) using mTeSR1 (dashed).

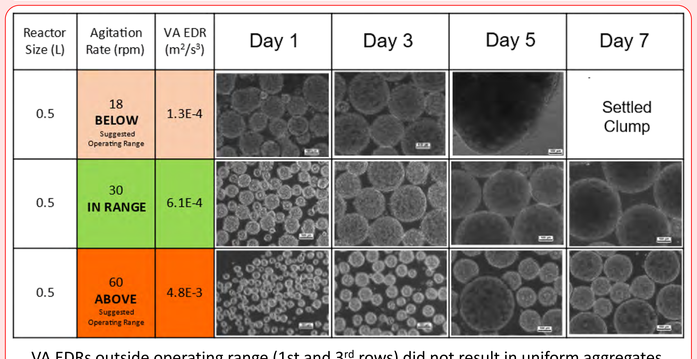
Validation Outside Operating Range

Growth in 0.5 L VW Bioreactors Outside of Operating Range

Conditions below (VA EDR of 1.3E-4 m^2/s^3 for 18 rpm at 0.5 L) and above (VA EDR of 4.38E-3 m^2/s^3 for 60 rpm at 0.5 L) suggested operating range were tested to compare growth and aggregate morphology to a control bioreactor operated within the suggested range (VA EDR of 6.1E-4 m^2/s^3 for 30 rpm at 0.5 L). As predicted, the agitation rates that produced VA EDRs outside the operating range did not support healthy aggregate growth during the entire culture period. For the VA EDR and hydrodynamic conditions below the operating range, the lack of agitation caused the aggregates to clump together into large flakes too heavy to be suspended, as well as allowing cells to stick to the bottom of the vessel. For the VA EDR above the operating range, there was much more heterogeneity in the size and shape of cell aggregates, which is undesirable for cell yield, quality, and efficiency of processes such as iPSC differentiation.

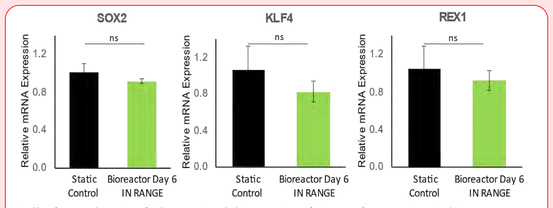


Aggregate Morphologies



VA EDRs outside operating range (1st and 3rd rows) did not result in uniform aggregates.

Pluripotency Maintenance



Cells from day 6 of the control bioreactor (30 rpm) were tested to compare relative expression of pluripotency genes SOX2, KLF4, and REX1 with static control hiPSCs cultured on Matrigel. There were no statistically significant differences in gene expression between the bioreactor culture and static controls, indicating maintenance of pluripotency. A limitation to this study is the functional testing of cell pluripotency following bioreactor expansion. Previous work using the same cell line, mTeSR1 medium, and 0.1 L VW bioreactors demonstrated that hiPSCs maintained pluripotency following several serial passages within the bioreactor based on karyotyping, teratoma formation, and directed differentiation [3].

Discussion and Conclusions

In this study, CFD modelling was used to characterize and analyze the hydrodynamic environment of various VW bioreactors, in order to assess their potential as a scalable platform for hiPSC aggregate production. Each bioreactor scale (0.1, 0.5, 3, and 15 L) was modelled at a minimum of three agitation rates to allow for the generation of scale-up correlation equations that can be used to predict operating conditions at larger scales, based on less costly experiments conducted at small scale. An optimal operating range of VA EDRs was defined for all VW bioreactor volumes. This suggested operating range indicates the combination of agitation rate and volume that produces optimal hydrodynamic conditions of narrow EDR distributions, which in turn is expected to result in the formation of spherical, high-quality aggregates that are desirable for scalable manufacturing. Optimal VA EDRs were biologically validated at the 0.1 and 0.5 L scales using two PSC culture media. Ultimately, it was shown that VW bioreactors can provide an optimal hydrodynamic environment for scalable production of hiPSC aggregates.

References

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