Protocol Development to Overcome Bioprocess Bottlenecks in the Large-Scale Expansion of High Quality hiPSC Aggregates in Vertical-Wheel Bioreactors

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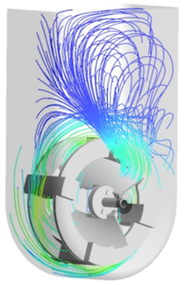
BACKGROUND

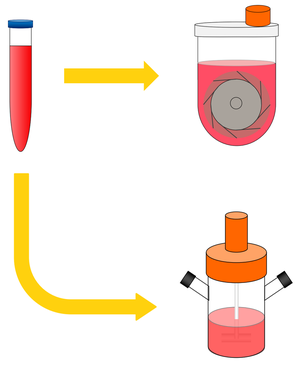
Human induced pluripotent stem cells (hiPSCs) have generated significant interest in various medical and biomedical fields due to their capacity for self-renewal and trilineage differentiation [1-3]. To achieve clinically relevant hiPSC numbers, scalable bioreactor-based processes are required. While there exist studies in scaling up the expansion of hiPSCs, the use of conventional bioreactors with horizontal-impeller, paddle, or rocking-wave mixing mechanisms have demonstrated several challenges including unfavourable hydrodynamic environments and poor scalability. Additionally, many of these processes require large static seed trains and achieve only moderate cell fold increases [4-6]. An alternative to these traditional geometries is the Vertical-Wheel® (VW) bioreactor. We have previously demonstrated that the VW bioreactor combines radial and axial mixing to uniquely produce uniform distributions of hydrodynamic forces which can be favourable for the cultivation of shear-sensitive cell types including hiPSCs grown as cell aggregates [7].



PROJECT OVERVIEW

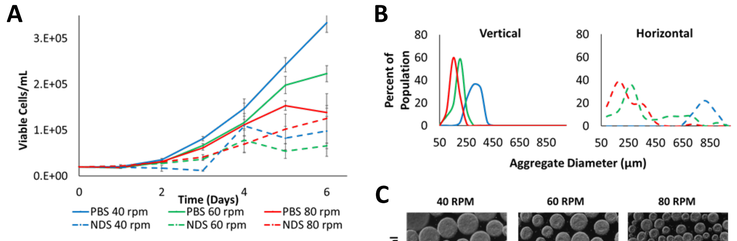
- The objective of this project was to demonstrate that the VW bioreactor could be used to overcome current challenges associated with scaling-up production of hiPSCs.
- Bottlenecks associated with **inoculation**, **expansion**, and **aggregate dissociation** phases were investigated at the 0.1L scale.
- Protocol robustness was validated by **serial passaging** hiPSCs from a 0.1 L VW bioreactor into a secondary 0.1 L and 0.5 L VW bioreactor. Successful culture was defined by growth kinetics, aggregate morphology, and biological testing.

COMPARING SINGLE CELL INOCULATION IN DIFFERENT GEOMETRIES



Vertical-Wheel (PBS) Single Cell (20,000 cells/mL)

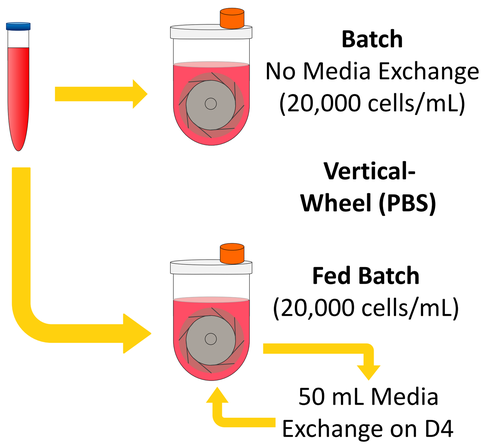
Horizontal Blade (NDS) Single Cell (20,000 cells/mL)



Day 6 Fold Expansion	PBS	NDS
40 rpm	16.7 ± 1.1	4.9 ± 2.8
60 rpm	11.2 ± 0.9	3.3 ± 0.3
80 rpm	7.7 ± 0.5	6.3 ± 2.7

Figure 1: Single cell expansion was successful in VW bioreactors, but not in traditional horizontal-blade bioreactors as demonstrated by the growth kinetics **(A)**. The VW bioreactor also resulted in more uniform aggregate size distributions as shown in the Day 6 distributions **(B)** and images **(C)**. Scale represents 200 µm.

IMPROVING CELL EXPANSION USING DIFFERENT FEEDING REGIMES

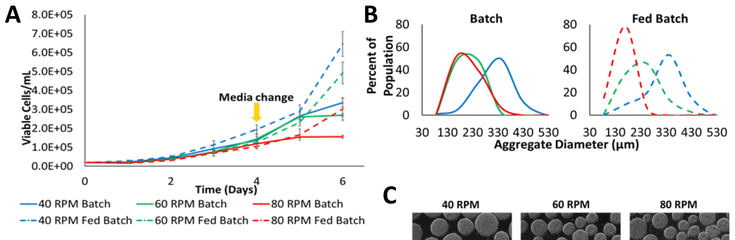


Batch No Media Exchange (20,000 cells/mL)

Vertical-Wheel (PBS)

Fed Batch (20,000 cells/mL)

50 mL Media Exchange on D4

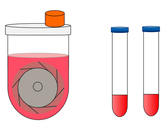


Day 6 Fold Expansion	Batch	Fed Batch
40 rpm	16.8 ± 1.1	32.3 ± 3.2
60 rpm	13.5 ± 0.3	24.6 ± 2.9
80 rpm	7.8 ± 0.4	15.1 ± 3.0

Figure 2: Fed-batch culture in VW bioreactors resulted in approximately 2 times higher cell expansion at all agitation rates with a maximum of 32 at 40 RPM **(A)**. The use of a fed batch strategy also resulted in more optimal aggregate diameters as shown in aggregate size distributions **(B)** and pictures from Day 6 **(C)**.

DEVELOPING AN IN-VESSEL AGGREGATE DISSOCIATION PROTOCOL

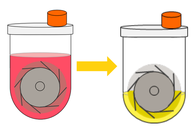
Pre Dissociation Count



1 mL samples taken and counted

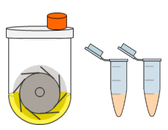
A = Average Cell Count * Working Volume

Aggregate Dissociation



Aggregates settled, media removed, 20 mL enzyme added, and agitated at 80 RPM

Post Dissociation Count



200 µL samples taken and counted

B = Average Cell Count * Enzyme Volume

Dissociation Efficiency (%) =

((B/A)*100%) - (% cells in aggregates)

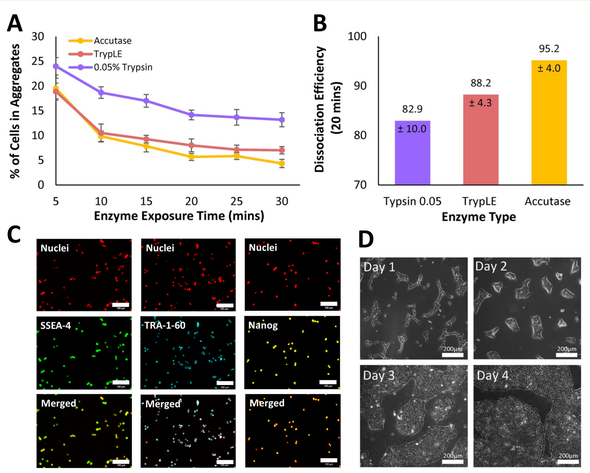
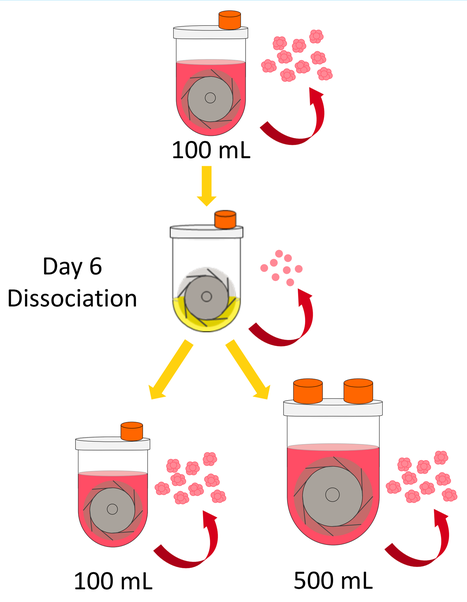


Figure 3: Accutase at 20 minutes was selected as it had the lowest percentage of cells remaining in aggregates **(A)** and a 95% dissociation efficiency **(B)**. Dissociated cells maintained positive expression of pluripotency markers **(C)** (scale represents 100 µm) and recovered in static **(D)** (scale represents 200 µm).

VALIDATING THE OPTIMIZED PROTOCOLS VIA SERIAL PASSAGING



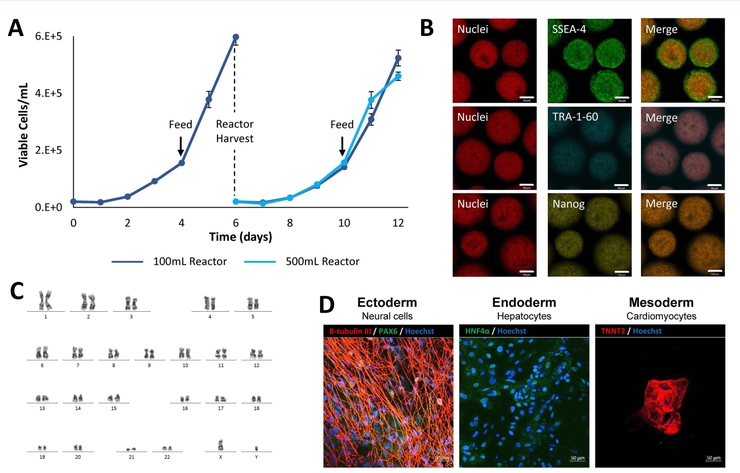


Figure 4: Using the optimized protocols, hiPSCs that were serial passaged into 100 mL and 500 mL VW bioreactors maintained similar growth kinetics **(A)**, positive expression of pluripotent markers **(B)** (scale bar represents 100 µm), normal karyotypes **(C)**, and differentiated into cell types from all three germ layers **(D)** (scale bar represents 50 µm) at the end of 12 days.

CONCLUSIONS

In this study, various bioprocess bottlenecks associated with the large scale expansion of hiPSCs were addressed. This included developing strategies to use single cell inoculation, methods to improve cell fold expansion using different feeding regimes, and a protocol for in-vessel aggregate dissociation. Under optimized single-cell inoculation conditions, we achieved a 32-fold expansion in six days with uniform aggregate size distribution. We also achieved recoveries of over 90% using optimized harvesting parameters while maintaining positive expression of pluripotency markers. Additionally, we demonstrated that cell growth and pluripotency were maintained while serial passaging using these protocols. Through these findings, we demonstrated that Vertical-Wheel bioreactors can be used to overcome the current challenges associated with scaling-up production of hiPSCs.

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ACKNOWLEDGMENTS

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