PROTOCOL DEVELOPMENT TO OVERCOME BIOPROCESS BOTTLENECKS IN THE LARGE-SCALE EXPANSION OF HIGH QUALITY HiPSC AGGREGATES IN VERTICAL-WHEEL BIOREACTORS

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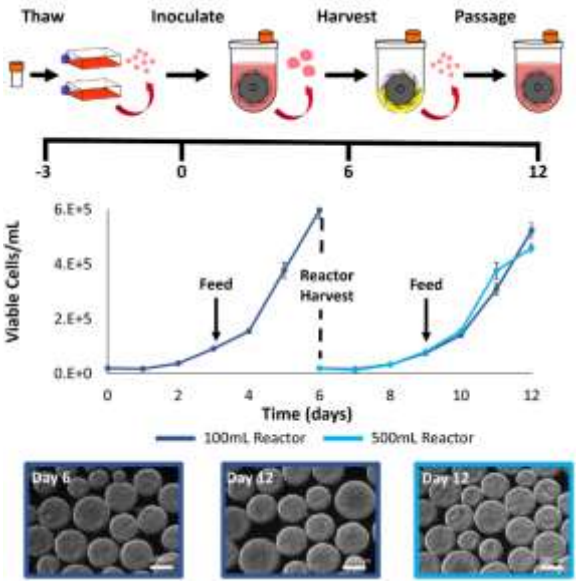
Human-induced pluripotent stem cells (hiPSCs) have generated a great deal of attention owing to their capacity for self-renewal and trilineage differentiation. hiPSCs are cultured as adherent colonies at small scale, which is sufficient to generate cells for experimental purposes but impractical to achieve large quantities for clinical applications. Bioreactor-based processes are the method of choice for efficient expansion and differentiation of cells. Current protocols for the expansion of hiPSCs, however, utilize horizontal impeller, paddle, or rocking wave mixing method bioreactors which require large static cell-culture starting populations and achieve only moderate cell fold increases within the bioreactor. We have recently demonstrated that the vertical-wheel bioreactor produces a unique fluid flow pattern that results in a homogeneous distribution of hydrodynamic forces, making it the opportune environment for systematic bioprocess optimization of hiPSC expansion.

In this study, bioprocess optimization was first carried out at the 0.1L vertical-wheel bioreactor scale. The bioreactor was modeled with CFD simulation software Fluent at agitation rates between 20rpm and 100rpm. These models produced fluid flow patterns that mapped out the hydrodynamic environment to guide in the development of hiPSC inoculation and in-vessel aggregate dissociation protocols. While clump seeding has been widely reported as an inoculation strategy for bioreactor culture, it produces a bottleneck in scalability. It is also difficult to control the clump size, resulting in heterogeneity in bioreactor aggregates leading to increases apoptosis and spontaneous differentiation. The effect of single-cell inoculation on aggregate formation and growth was tested at select CFD modeled agitation rates and feeding regimes in the vertical-wheel bioreactor.



Single cell inoculation followed by in vessel aggregate dissociation and harvest for scale-up serial expansion of hiPSCs

Downstream hiPSC bioreactor operations also lack scalable protocols. Harvesting is a critical step in serial passaging and recovery of the final cell product, but excessive shear during the harvesting process can alter cell phenotype. Few studies have investigated potential methods for full bioreactor harvesting of hiPSC aggregate culture. Publications only collect small (1-5 mL) aggregate samples from the bioreactor to dissociate for cell counts using enzymatic and mechanical dissociation techniques which cannot be translated to harvesting. An in-vessel dissociation protocol was developed through the testing of various proteolytic enzymes and reduced working volume agitation exposure times.

We were successful in developing a scalable, single-cell inoculation protocol for the culture of hiPSCs as aggregates in the vertical-wheel bioreactor, achieving over 30-fold expansion in 6 days without sacrificing cell quality. We have produced the first published protocol for in-vessel hiPSC aggregate dissociation, permitting the entire bioreactor volume to be harvested into single-cells for serial passaging into larger scale reactors.

Importantly, the cells harvested and re-inoculated into *scaled-up vertical-wheel bioreactors not only maintained* consistent growth kinetics, they maintained a normal karyotype and pluripotent characterization and function. Taken together, these protocols provide a feasible solution for the culture of high quality hiPSCs at a clinical and manufacturing scale by overcoming some of the major documented bioprocess bottlenecks.