SCALING UP A CHEMICALLY-DEFINED AGGREGATE-BASED SUSPENSION CULTURE SYSTEM FOR NEURAL COMMITMENT OF HUMAN PLURIPOTENT STEM CELLS

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The demand of high cell numbers for applications in cellular therapies and drug screening requires the development of scalable platforms capable to generating highly pure populations of tissue-specific cells from human pluripotent stem cells. This work describes the scaling-up of an aggregate-based culture system for neural induction of human induced pluripotent stem cells (hiPSCs) under chemically-defined conditions.

Since initial cell density and aggregate size have an important impact in the expansion and commitment of these cells into a particular lineage, a combination of non-enzymatic dissociation and rotary agitation was successfully used to produce homogeneous populations of hiPSC aggregates with an optimal (140 µm) and narrow distribution of diameters (coefficient of variation of 21.6%). Scalable neural commitment of hiPSCs as 3D aggregates was then performed in 50 mL spinner flasks, and process optimization using a factorial design approach was developed involving parameters such as agitation rate and seeding density. We were able to produce neural progenitor cell cultures, that at the end of a 6-day neural induction process contained less than 3% of Oct4-positive cells and that, after replating, retained more than 60% of Pax6-positive neural cells. Furthermore, after scalable differentiation, hiPSC-derived neural progenitors still retained their multipotent potential, being able to give rise to neuronal and glial cells.

The results presented in this work should set the stage for the future generation of a clinically relevant number of human neural progenitors for transplantation and other biomedical applications using totally controlled, automated and reproducible large-scale bioreactor culture systems.