SCALABLE GENERATION OF CEREBELLAR NEURONS FROM PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cells (iPSCs) have great potential for disease modeling and provide a valuable source for regenerative approaches. However, generating iPSC-derived models to study brain diseases remains a challenge. In particular, our ability to differentiate cerebellar neurons from pluripotent stem cells is still limited. Recently, we described the long-term culture of cerebellar neuroepithelium formed from human iPSCs, recapitulating the early developmental events of the cerebellum. Additionally, an efficient maturation of replated cerebellar progenitors into distinct types of functional cerebellar neurons was also achieved under defined and feeder-free conditions. However, developing a scalable protocol that allows to produce large numbers of organoids and high yields of mature neurons in a 3D bioreactor culture systems is still a difficult challenge. In this work, we present a new approach for the reproducible and scalable generation of mid-hindbrain organoids under chemically defined conditions by using the novel PBS 0.1 (100 mL) Vertical-Wheel single-use bioreactor. In this system, an efficient cell aggregation with shape and size-controlled aggregates can be obtained, which is important for homogeneous and efficient differentiation. Moreover, a larger amount of iPSC-derived aggregates can be generated without being excessively labour-intensive, achieving 431 ± 53.6 aggregates/mL at 24 hours after seeding. After differentiation, distinct types of cerebellar neurons were generated, including Purkinje cells (Calbindin$^+$), Granule cells (BARHL1$^+$ and Pax6$^+$), Golgi cells (Neurogranin$^+$ and GAD65$^+$), Deep cerebellar nuclei projection neurons (TBR1$^+$) and Non-Golgi-type interneurons (Parvalbumin$^+$ and Calbindin$^-$). These cells show signs of efficient maturation, staining positive for MAP2, and are able to change intracellular Ca^{2+} concentration following KCl stimulation. In this system, human iPSC-derived organoids are able to mature into different mature cerebellar neurons and to survive for up to 3 months, without replating and co-culture with feeder layers.