HIGH DENSITY CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN STIRRED TANK BIOREAKTORS FOR REGENERATIVE THERAPIES

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Background & Aim: Human induced pluripotent stem cells (iPSCs) are a unique source for the production of functional human cell types, fueling advanced regenerative therapies. This, however, will require the constant supply of billions of cells generated by robust, economically viable, and regulatory compliant bioprocesses. We here show, how we instrumented stirred tank bioreactor (STBR) technology to take iPSC cultivation to the next level.

Methods, Results & Conclusion: Perfused suspension culture (3D) of matrix-free iPSC aggregates in STBRs was applied to identify and control process-limiting parameters including pH, dissolved oxygen, Glucose and Lactate levels, and the obviation of osmolality peaks. Media supplements promoted single cell-based process inoculation and hydrodynamic aggregate size control. Wet lab-derived process characteristics enabled predictive *in silico* modeling as a new rational for iPSC bioprocess development. As a result, long-term maintenance of exponential cell proliferation was enabled achieving 70-fold cell expansion, an unmatched density of 35 x 10^6 cells/mL, and, in parallel, reducing media requirements by 75%. The strategy was successfully applied to numerous independent iPSC lines, despite cell-line specific differences in culture properties such as aggregate size patterns.

Furthermore, we have developed a systematic approach enabling maintenance of aggregate size distribution patterns across different STBR platforms and process scales. Consequently, robust upscaling from 150 mL, to 500 mL, and ultimately 2,000 mL process scale in 3 independent STBR platforms was achieved.

The mass-produced hPS cells showed a high degree of pluripotency, homogeneity, and unrestricted differentiation potential.

The study provides a deeper understanding of iPSC's physiology, better definition of culture requirements, and a straightforward strategy for the controlled production of iPSCs and their progenies for regenerative therapy research and development.