TOWARDS LARGE-SCALE PRODUCTION OF HUMAN-INDUCED PLURIPOTENT STEM CELL-DERIVED EXTRACELLULAR VESICLES IN STIRRED-TANK BIOREACTORS

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A significant bottleneck in the advancement of Extracellular Vesicle (EV)-based therapies is the efficient large-scale manufacture of clinical-grade EV. Upstream challenges include the selection of an appropriate EV parent cell and its large-scale expansion. We previously identified human induced Pluripotent Stem Cells (hiPSC) as a source of native EV for cardiac regeneration and pinpointed the bioactive signatures of hiPSC-EV. Currently, we are developing scalable bioprocesses for hiPSC expansion in Stirred-tank Bioreactors (STB) and modulating critical process parameters to intensify EV production.

Briefly, hiPSC were expanded as 3D aggregates in STB (DasGip Eppendorf AG), operated in perfusion (D=1.3 day^{-1}). Throughout hiPSC expansion, the dissolved O_2 concentration was controlled at low levels (4% O_2). EV were isolated by density gradient ultracentrifugation, probed for specific markers, and characterized in terms of particle size distribution and morphology. EV bioactivity was assessed in human umbilical vein endothelial cells. A 2.9-fold increase in cell concentration was observed in STB compared to the static 2D monolayer culture, which resulted in a 3.1 increase in total particles isolated per mL of conditioned medium. hiPSC-EV produced in STB presented a cup-shape morphology and were positive for EV markers. Tube formation assays showed increased pro-angiogenic activity for hiPSC-EV produced in STB versus static 2D monolayer culture.

Overall, our study validates hiPSC as cell biofactories for large-scale EV production in STB and provides insights into manufacturing EV-based products. Ongoing work aims at optimizing EV production yields and potency by further manipulating process parameters such as dissolved O_2, stirring rate and operation mode.

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