OPTIMIZED MEDIA AND WORKFLOW FOR THE EXPANSION OF HUMAN PLURIPOTENT STEM CELLS AS AGGREGATES IN SUSPENSION

Eric Jervis, STEMCELL Technologies, Inc., Vancouver, B.C., Canada
eric.jervis@stemcell.com
Angela McLaughlin, STEMCELL Technologies, Inc., Vancouver, B.C., Canada
Kyle Hukezalie, STEMCELL Technologies, Inc., Vancouver, B.C., Canada
Steven Woodside, STEMCELL Technologies, Inc., Vancouver, B.C., Canada
Terry E. Thomas, STEMCELL Technologies, Inc., Vancouver, B.C., Canada
Allen C. Eaves, STEMCELL Technologies, Inc.; Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, B.C., Canada
Sharon A. Louis, STEMCELL Technologies, Inc., Vancouver, B.C., Canada

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3D suspension culture enables the efficient and cost-effective scale-up of human pluripotent stem cell (hPSCs) manufacturing. However, media optimized for 2D adherent cultures can lead to low volumetric productivity and inefficient workflow. To overcome these limitations we developed mTeSR™3D, a defined medium based on mTeSR™1, and novel protocols for fed-batch culture of hPSC aggregates. Human embryonic stem cell (hESC) lines (H1 or H9) or human induced pluripotent stem cell (hiPSC) lines (WLS-1C or STiPS-M001) that were previously maintained in 2D mTeSR™1 culture were seeded into multiple suspension culture vessels containing mTeSR™3D Seed Medium plus 10 µM Y-27632 ROCK inhibitor. 3D cultures were maintained using either daily 50% mTeSR™1 medium exchanges (control) or using a fed-batch protocol whereby the culture medium was supplemented daily with mTeSR™3D Feed Medium. After 3 or 4 days in suspension culture, aggregates were harvested, dissociated into small clumps with Gentle Cell Dissociation Reagent (GCDR) or single cell suspensions enzymatically, and re-seeded in mTeSR™3D Seed Medium plus 10 µM Y-27632. Passaging and feeding cycles were repeated for at least 5 passages. 3D cultures were assessed for growth, viability, hPSC marker expression, *in vitro* differentiation potential, and karyotype. In addition, media was analyzed for molar glucose to lactate yield to characterize metabolism. By day 4, aggregates cultured in mTeSR™3D typically grew to a mean diameter of 350 µm, with a 5-fold increase in cell number. Using mTeSR™3D up to 10^9 cells can be produced from a single plate within 2-3 weeks representing a greater than 500-fold expansion. hPSC cultures maintained in mTeSR™3D differentiated into all 3 germ layers with high efficiency. The average volumetric productivities were 0.7, 3.1 and 6.9 ($x10^5$) viable cells / mL in 2D, daily 50% media exchange, and mTeSR™3D cultures, respectively. Using the GCDR clump passaging protocol, mTeSR™3D cultured hPSCs retained normal karyotypes. Culture performance was evaluated in shaker bottles, spinner flasks and bioreactors. Performance in each culture system was comparable confirming straightforward scale-up and wide applicability. Typical growth rates were on the order of 1.5-fold expansion per day. Metabolic activity as assessed by the moles lactate produced to glucose consumed was 1.7, consistent with a primarily glycolytic metabolism. Image analysis was performed to estimate aggregate size during growth. Adaptation times for cells moving from 2D to 3D aggregate culture varied with different cell lines; typically one passage in 3D was required before consistent expansion passage over passage was obtained. Additionally, protocols were developed for use on a Hamilton® robotic platform for reproducible, matrix-free, high-throughput hPSC suspension culture at a small scale. mTeSR™3D enables efficient scale-up and scale-down of hPSC cultures with greatly simplified workflow.