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

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Key Words: PSC Culture Scale-up, Fed-batch Culture, Media Optimization

3D suspension culture enables scale-up of human pluripotent stem cell (hPSC) manufacturing. However, media and methods optimized for 2D adherent cultures can lead to low volumetric productivity and laborious workflow in suspension cultures. To overcome these limitations we developed fed-batch media based on either mTeSR™1 (BSA-containing) or TeSR™-E8™ (animal component-free) for hPSC expansion as aggregates in suspension cultures. Fed-batch feeding protocols are more efficient and cost-effective than batch media changes because only exhausted components are replenished. Optimization studies were performed using human embryonic (H7 and H9), and human induced pluripotent (WLS-1C and STiPS-M001) stem cell lines. Suspension cultures were fed daily using either 50% medium exchanges of standard 2D media, or fed-batch optimized media and protocols. hPSC aggregate diameter must be kept below 350 µm to maintain cell viability and phenotype. With observed growth rates, aggregates required passaging every 3 or 4 days into clumps of 5-10 cells with Gentle Cell Dissociation Reagent. Clumps were re-seeded into fresh test medium plus 10 µM Y-27632. Passaging and feeding cycles were repeated for at least 5 passages. Optimization was performed by iteratively modifying the feed solution to maintain consistent nutrient levels and maximal growth rate while maintaining cell quality. Control and optimized fed-batch formulations demonstrated between 1.4 and 1.8-fold expansion per day, >90% viability, Oct4 and TRA-1-60 expression >90%, *in vitro* trilineage differentiation, and normal karyotype (n=8 independent cultures). Suspension culture optimized mTeSR™-3D or TeSR™-E8™3D fed-batch media enables the cost-effective production of hPSCs as aggregates with efficient workflow and high cell quality.