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INTEGRATION OF BIOPROCESS DESIGN WITH TRANSCRIPTOMIC AND METABOLOMIC CHARACTERIZATION FOR THE EXPANSION OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSC), including human embryonic stem cells (hESC), have an enormous potential as source for cell replacement therapies. However, the clinical application of hPSC has been hindered by the lack of robust protocols able to sustain production of high cell numbers maintaining their phenotype. To facilitate the bioprocess optimization aiming at higher cell yields, the analysis of transcriptomic and metabolomic profiling would provide important insights about stem cell phenotype in different culture conditions. Within this context, we developed a robust and well-characterized bioprocess for hESC expansion under fully defined conditions using stirred-tank bioreactors. Moreover, we explored the potential of transcriptomic and metabolomic tools for a more comprehensive assessment of culture system impact on cell proliferation, metabolism, and phenotype.

In this study two hESC lines displaying different growth characteristics: a feeder-dependent hESC line (growing as a colony) and a feeder-free hESC line (growing as a monolayer) (both from Cellectis Bioresearch) were cultured on different synthetic microcarriers (kindly provided by Corning Inc.) in stirred-tank bioreactors. Cell growth profile of all cultures was monitored daily and the undifferentiated phenotype was characterized by immunofluorescence microscopy, flow cytometry, RT-qPCR and *in vitro* pluripotency assays. Finally, both transcriptional and metabolic profiles of hESC cultured in stirred-tank bioreactors were analyzed and compared to the culture in 2-dimensional (2D) monolayer system.

Our results showed the efficiency of Synthemax-II polystyrene microcarriers to expand both hESC lines in stirred-tank bioreactors, with high viable cell recovery yields. Both hESC lines maintained the expression of stemness markers and presented high pluripotency scores (according to the PluriTest bioinformatic platform), showing similar values compared with 2D culture. Transcriptome signatures of both hESC lines were compared during the expansion process, showing clear convergence of hESCs when cultured on synthetic microcarriers using stirred culture systems. The most significant changes steering this convergence, were found at the level of central carbon metabolism and cytoskeleton and extracellular matrix rearrangements. Under low-oxygen tension, hESCs displayed a metabolic rearrangement with upregulation of the glycolytic machinery favoring an anaerobic glycolysis Warburg-effect-like phenotype, with no evidence of hypoxic stress response, in contrast to 2D culture [1]. This study provided relevant findings on the hESC physiological and metabolic changes during expansion, which can contribute to the design of improved scale-up production strategies as well as for media optimization to generate cells in higher quantity with improved quality, critical for their translation into clinic.

References: [1] Silva MM, et al. *Stem Cells Transl Med.* 2015;4(7):731-42.

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