

BIOPROCESS OPTIMIZATION FOR GENERATION OF HEPATOCYTES DERIVED FROM hiPSC AND ITS APPLICATION IN PRIMARY HYPEROXALURIA TYPE 1 DISEASE MODELLING

Pedro Vicente, iBET, ITQB, Portugal
pvicente@ibet.pt

Joana I. Almeida, Inês E. Crespo, Inês A Isidro , iBET/ITQB-NOVA, Portugal
Maren Calleja, Felipe Prosper, Universidad de Navarra, Pamplona, Spain
Juan Rodriguez-Madoz, Felipe Prosper, Universidad de Navarra, Pamplona, Spain
Felipe Prosper, Universidad de Navarra, Pamplona, Spain
Anders Aspegren, Takara Bio Europe AB, Gothenburg, Sweden
Paula M Alves, Margarida Serra , iBET/ITQB-NOVA, Portugal

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Primary hyperoxaluria type 1 (PH1) is a rare metabolic disorder caused by mutations in the hepatic alanine-glyoxylate aminotransferase (AGT). Defective AGT in PH1 patients is characterized by excessive oxalate synthesis, which leads to a broad range of kidney complications including the end-stage renal disease [1]. Combined liver-kidney transplantation remains the only effective treatment; however significant morbidity, mortality and costs encouraged the development of advanced cell- and gene-based therapies for PH1. Thus, our aim was to implement a novel strategy to generate high numbers of functional hepatocyte-like cells (HLC) from PH1 patient derived human induced pluripotent stem cells (PH1.hiPSC), for PH1 disease modelling and further application in drug and therapeutics development.

PH1.HLC were differentiated as 3D aggregates in stirred-tank bioreactors (STB) operated in perfusion, according to the integrated bioprocess previously developed by our group [2,3]. Briefly, PH1.hiPSC were aggregated and expanded in STB for 4 days preceding the hepatic differentiation. hiPSC to HLC commitment begin by culturing the 3D aggregates in different medium formulations (from Takara BioEurope AB). Two different dissolved oxygen (pO₂) conditions were explored: a normoxia (pO₂: uncontrolled, 95% air, 5% CO₂) throughout the differentiation process (21 days) and a hypoxia with a low oxygen (pO₂: 4% O₂) environment between day 4 and day 14 of the differentiation.

Our results showed that PH1-hiPSC successfully proliferated as 3D aggregates with an expansion factor of 6-fold after 4 days in culture while maintaining their pluripotent phenotype. Low dissolved oxygen concentration during hepatic specification, generate higher yields of HLC and improve gene expression levels of ALB, A1AT and CYP3A4 hepatic markers when compared with HLC differentiated under uncontrolled pO₂ conditions. Moreover, Flow cytometry analysis, revealed a higher hepatocyte content of 80% (low pO₂) vs 43% (uncontrolled pO₂) for albumin, showing a higher process efficiency. Transcriptomic analysis using RNAseq confirmed that hepatocyte differentiation was enhanced in the low dissolved oxygen condition. In addition, these PH1.HLC showed functional characteristics typical of hepatocytes including production of important hepatic proteins (albumin, alpha 1 antitrypsin), urea and bile acids. PH1.HLC also display drug metabolization capacity, CYP450 activity and, by histological assessment, glycogen storage and positive staining for albumin and AFP markers. To further characterize the PH1 disease features, we performed a detailed metabolomic analysis and demonstrated that PH1.HLC show defective AGT activity with significantly higher production and secretion of oxalate for PH1.HLC when compared with HLC generated from healthy counterparts.

Overall, controlling the dissolved oxygen concentration at key stages of the hepatic differentiation process improved cell yield and the maturation status of HLC. The bioprocess developed and optimized in this work offers high relevance not only for generation of more accurate in vitro models to study PH1 rare disease, but also towards the development of novel therapies.

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