ADVANCING MANUFACTURE OF HIPSC-DERIVED HEPATOCYTES WITH IMPROVED FUNCTIONALITY: A NATURE-INSPIRED PROTOCOL

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Key Words: hiPSC-derived hepatocytes, maturation, bioreactors, PAT, omics technologies.

Hepatocyte-like cells differentiated from human induced pluripotent stem cells (hiPSC-Hep) provide unprecedented opportunities for hepatic cell-based therapies. Regrettably, more than 15 years of research in the field were not enough to convert hiPSC-Hep into an advanced therapy medicinal product. The often hybrid fetal-adult phenotype exhibited by these cells, the lack of reliable and reproducible protocols for their scalable production and the uncertainties regarding their engraftment ability are some of the reasons that hamper their clinical application. In this study, we aim to obtain relevant numbers of mature HLC for regenerative medicine applications. To address that, we designed a nature-inspired strategy and combined advanced manufacturing platforms with omics technologies and process analytical tools to better recapitulate and monitor the microenvironment of physiological liver development during hiPSC-Hep bioprocessing.

hiPSC-Hep were generated as 3D cell aggregates using stirred-tank bioreactors (STBR) operated in perfusion with an *in situ* capacitance probe. When dissolved oxygen was controlled at low levels during hepatic specification stage, higher hiPSC-Hep production (2×10^6 cell/mL) and differentiation efficiencies (> 80% $Albumin^+$ cells) were obtained when compared to uncontrolled condition (0.6×10^6 cell/mL and <45% $Albumin^+$ cells). The generated hiPSC-Hep showed synthesis of key hepatic proteins (albumin, alpha 1 antitrypsin), urea and bile acids secretion as well as drug metabolization capacity, CYP450 activity and glycogen storage. Our results also show a good correlation between the cell permittivity measured online and the aggregate biovolume measured by standard offline methods, demonstrating the potential of dielectric spectroscopy to monitor hiPSC expansion and differentiation in STBR.

The hepatic maturation step was induced by culturing hiPSC-Hep with the secretome of human intestinal microbiota, recapitulating what happens in liver development [1]. hiPSC-Hep treated with microbiome secretome showed improved expression of critical hallmarks of human hepatocytes and preserved functionality. Importantly, we used transcriptomic analysis (RNA-Seq) to confirm, for the first time, that hiPSC-Hep maturation levels modulate the “machinery” that mediates cell engraftment and identifiy the cell maturation stage that ensures efficient cell’ engraftment *in vitro* and *in vivo*, with preservation of hepatic functionality.

References: [1] Almeida et al 2022. Hallmarks of the human intestinal microbiome on liver maturation and function, J Hepatol, doi: 10.1016/j.jhep.2021.10.015.

Funding: This work was supported by a grant from ERA-NET-Rare 3research program, JTC ERAdicatPH (E-Rare3/0002/2015); iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associate Laboratory LS4FUTURE (LA/P/0087/2020); FCT fellowships SFRH/BD/116780/2016 and SFRH/BD/145767/2019; European Union’s Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No. 813453. Please proofread your abstract carefully prior to submission.