INTENSIFYING THE MANUFACTURE OF HIPSC THERAPY PRODUCTS THROUGH METABOLIC AND PROCESS UNDERSTANDING

Margarida Serra, iBET/ITQB-NOVA, Portugal
mserra@ibet.pt
Pedro Vicente, iBET/ITQB-NOVA, Oeiras, Portugal
Catarina Gomes, iBET/ITQB-NOVA, Oeiras, Portugal
Joana Almeida, iBET/ITQB-NOVA, Oeiras, Portugal
Daniel AM Pais, iBET/ITQB-NOVA, Oeiras, Portugal
Bernardo Abecasis, iBET/ITQB-NOVA, Oeiras, Portugal
Marta Paiva, iBET/ITQB-NOVA, Oeiras, Portugal
Inês A Isidro, iBET/ITQB-NOVA, Oeiras, Portugal
Paula M Alves, iBET/ITQB-NOVA, Oeiras, Portugal
Rebeca Mertinez-Turrillas, CIMA/UNAV, Pamplona, Spain
Juan R. Rodriguez-Madoz, CIMA/UNAV, Pamplona, Spain
Anders Aspegren, Takara Bio Europe AB, Gothenburg, Sweden
Ibrahim Domian, Harvard Stem Cell Institute/Harvard Medical School, Cambridge, USA

Key Words: cell therapy, hiPSC-derived cardiomyocytes, hiPSC-derived hepatocytes, three-dimensional culture, perfusion, omics technologies

In vitro differentiation of human induced pluripotent stem cells into specific lineages such as cardiomyocytes (hPSC-CM) and hepatocytes (hPCS-Hep) is a crucial process to enable their application in cell therapy and drug discovery. Nevertheless, despite the remarkable efforts over the last decade towards the implementation of protocols for hPSC expansion and differentiation, there are some technological challenges remaining include the low scalability and differentiation yields. Additionally, generated cells are still immature, closely reminiscent of fetal/embryonic cells in what regards phenotype and function. In this study, we aim to overcome this hurdle by devising bioinspired and integrated strategies to improve the generation and functionality of these hiPSC-derivatives. We also applied robust multi-parametric techniques including proteomics, transcriptomics, metabolomics and fluxomics as complementary analytical tools to support bioprocess optimization and product characterization.

We cultured hiPSC as 3D aggregates in stirred-tank bioreactors (STB) operated in perfusion and used a capacitance probe for in situ monitoring of cell growth/differentiation. After cell expansion, the hepatic differentiation step was integrated by addition of key soluble factors and controlling the dissolved oxygen concentration at various stages of the process to generate populations enriched for definitive endoderm, hepatocyte progenitors and mature hepatocytes. The analyses of hepatic markers expression throughout the stages of the differentiation confirmed that hepatocyte differentiation was improved in 3D spheroids when compared to 2D culture. Noteworthy, these hiPSC-HLC exhibited functional characteristics typical of hepatocytes (albumin production, glycogen storage and CYP450 activity). We also demonstrate the potential of dielectric spectroscopy to monitor cell expansion and hepatic differentiation in STB.

For CM differentiation, we relied on the aggregation of hPSC-derived cardiac progenitors to establish a scalable differentiation protocol capable of generating highly pure CM aggregate cultures. We assessed if alteration of culture medium composition to mimic *in vivo* substrate usage during cardiac development improved further hPSC-CM maturation *in vitro*. Our results showed that shifting hPSC-CMs from glucose-containing to galactose- and fatty acid-containing medium promotes their fast maturation into adult-like CMs with higher oxidative metabolism, transcriptional signatures closer to those of adult ventricular tissue, higher myofibril density and alignment, improved calcium handling, enhanced contractility, and more physiological action potential kinetics. "-Omics" analyses showed that addition of galactose to culture medium and culturing the cells under perfusion improves total oxidative capacity of the cells and ameliorates fatty acid oxidation. This study demonstrated that metabolic shifts during differentiation/maturation of hPSC-CM are a cause, rather than a consequence, of the phenotypic and functional alterations observed. The metabolic-based strategy established herein holds technical and economic advantages over the existing protocols due to its scalability, simplicity and ease of application.
Funding: This work was supported by FCT-funded projects NETDIAMOND (SAICTPAC/0047/2015), MetaCardio (Ref.032566) and FCT/ERA-Net (ERAdicatPH; Ref. E-Rare3/0002/2015). iNOVA4Health Research Unit (LISBOA-01-0145-FEDER-007344) is also acknowledged.