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INTEGRATED STRATEGIES FOR THE PRODUCTION, MATURATION AND STORAGE OF FUNCTIONAL CARDIOMYOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Key Words: human pluripotent stem cells derived cardiomyocytes (hPSC-CMs), bioprocess, differentiation, maturation, hypothermic storage.

The production of cardiomyocytes (CM) from human pluripotent stem cells (hPSC) holds great promise for cardiotoxicity drug testing, disease modeling and cardiac regeneration [1]. However, the complex nets of signalling pathways involved in cardiomyogenesis compromises the effectiveness of the existing differentiation protocols to reproducibly produce high-quality CM from hPSC (hPSC-CM). Produced hPSC-CM are immature compared with adult CMs, express typical fetal cardiac genes, have immature electrophysiological properties and use glucose as major energy source [2]. The applicability of hPSC-CM in the clinic/industry is also dependent on the development of efficient methods for worldwide shipment of these cells. In this study we aim to overcome these hurdles by devising an integrated strategy for scalable production, maturation and storage of functional hPSC-CM. hPSC (hiPSC and hESC lines) were cultured as aggregates in environmentally controlled bioreactors, where the necessary conditions to control stem cell fate are tightly tuned [3]. Dissolved oxygen and hydrodynamic forces were manipulated in order to improve CM differentiation yields [4]. CM differentiation was monitored using flow cytometry and qRT-PCR. Novel feeding strategies were tested aiming at improving hPSC-CM enrichment and maturation. We also evaluated the feasibility to cold store monolayers and aggregates of hPSC-CM using a clinical compatible preservation formulation. After storage the ultrastructure and functionality of the hPSC-CM were assessed by TEM and electrophysiology analyses.

Our results showed that hypoxia and hydrodynamic forces affect cell differentiation towards functional CM. The bioreactor protocol herein described (i.e. the controlled hypoxic and specific hydrodynamic environment) improved PSC differentiation by enhancing culture homogeneity, process reproducibility, and CM productivities. Moreover, enhanced CM maturation was attained when hPSC-CM were cultured in glucose depleted media supplemented with fatty acids; hPSC-CM showed a more elongated structure with organized sarcomeric pattern and displayed higher expression of genes responsible for contraction, calcium handling and electrophysiology. Noteworthy, using gas chromatography-mass spectrometry (GC-MS) analysis and 13C labeled subtracts we showed that hPSC-CM use fatty acid β-oxidation as energy source, a typical feature of adult CMs. At the end, we showed that monolayers of hPSC-CM and cardiospheres can be stored up to 7 days at hypothermic conditions without compromising cell viability, morphology and electrophysiological properties.

This work describes significant advances towards mass production of mature hPSC-CM and their short-term storage, meeting some of the needs of the cardiac regenerative medicine market and industrial field.

References

[1] Oh Y et al, 2012, Heart, 98:443. [2] Ribeiro M et al, 2015, Biomaterials 51:138. [3] Serra M et al, 2012, Trends Biotechnol, 30:350. [4] Correia C et al, Stem Cell Rev. Reports, 10:786.