

Master Research project

Novel strategies for production, purification and maturation of cardiomyocytes derived from human pluripotent stem cells

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Background

Heart failure is a leading cause of mortality in developed countries. It is estimated that up to a billion of cardiomyocytes (CMs) can be damaged after acute myocardial infarction, which corresponds to one quarter of the whole myocardium. An experimental approach for the treatment of heart failure may be to utilize the emerging technologies of stem cells, cell therapy and tissue engineering to repopulate the injured heart with new CMs.

Human pluripotent stem cells (hPSCs), including embryonic and induced pluripotent stem cells (hESCs and hiPSCs, respectively), are the most powerful cells for cardiac cell-based therapies. The inherent capacity to grow indefinitely (self-renewal) and to differentiate into all mature cells of the human body (pluripotency), makes hPSCs the only cell source that can provide, *ex-vivo*, an unlimited number of human functional CMs for transplantation. However, for the successful implementation of these technologies it is essential to establish efficient and scalable bioprocesses to produce CMs in high quantity, relevant purity and consistent quality. Another major hurdle in cardiac cell therapy is the poor survival of the differentiated CMs within the infarcted region after transplantation. Early cell loss due to the harsh ischemic environment of the infarcted area, lack of supporting extracellular matrix and immature phenotype of the differentiated CMs, are frequent problems in transplantation studies. A promising approach to overcome these issues includes the use of bioactive and biocompatible materials as supporting matrices to more efficiently deliver CMs. These biomaterials not only retain cells in the infarcted area but also provide a highly controlled 3D environment that is structurally and biochemically similar to native ECM topology, potentiating further CM survival, maturation and function.

Objectives

This project will focus on the development of integrated bioprocesses and clinical-compatible tools for:

- i) Scalable differentiation and purification of human PSC towards CM lineage. For this purpose, different 3D culture strategies and cocktails of inductive factors will be evaluated using environmentally controlled bioreactor systems and perfusion approaches.
- ii) Efficient maturation of CMs. After differentiation and cell lineage selection, CMs will be further cultured on the surface and/or entrapped in different biomaterials aiming at developing 3D constructs suitable for CM maturation and tissue engraftment.

TASK 1. Production and purification of human PSC-derived CMs in environmentally controlled bioreactors

Protocols for cardiac differentiation of hPSC, based on chemically defined media, will be evaluated in environmentally controlled stirred tank bioreactors. Different hPSC lines (including hESCs and hiPSCs) will be cultured in a 3D configuration (as 3D aggregates and/or immobilized on microcarriers) and a screening of a cocktail of growth factors and/or small molecules will be carried out in bioreactors operating in perfusion. The impact of specific environmental parameters (including dissolved oxygen, and agitation type and profile) known

to affect cardiac differentiation of hPSC (Serra et al 2012, Correia et al submitted) will be evaluated using different types of bioreactor systems.

During cell expansion and cardiomyocyte differentiation, cultures will be monitored in terms of cell concentration, viability and cell composition, evaluated by phenotypic assessment and gene expression analysis. Process analytical technologies (e.g. NMR spectrometry) will be also implemented for monitoring of cell metabolism. Metabolome and fluxome analysis will be pursued to disclose which metabolic pathways are differentially activated/repressed by different cell populations at different stages of the cardiomyocyte differentiation process. Within this context, cell-lineage purification strategies will be designed based on the specific metabolic signatures of cardiomyocytes.

TASK 2. Maturation of human iPSC-derived CMs

In this task, a strategy for CM maturation will be developed. The main aim is to use natural hydrogels (e.g. collagen, gelatin, hyaluronic acid, chitosan and alginate) suitable for cardiac tissue engineering due to their soft and viscoelastic nature. Cells will be entrapped within these biomaterials, which can be functionalized with specific growth factors and/or small molecules to potentiate further CM viability and maturity. The development of a mature phenotype will be addressed by evaluating the molecular, biochemical, ultrastructural and functional nature of CMs. In particular, electrophysiological studies will be carried out, in order to confirm that the hPSC-derived CMs exhibit typical cardiac-like depolarization patterns with action potentials representing the atrial-, ventricular- and pacemaker-like morphologies.

Task 3. Master thesis preparation

In this final task the student will be focused on writing and preparing the master thesis.