

Plano de trabalhos para tese de Mestrado

Tema: Produção de cardiomiócitos para terapia celular

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Duração da componente experimental: 1 ano lectivo

State of the art

Embryonic stem cells (ESCs) constitute an exciting emerging field. The inherent capacity of these cells to grow indefinitely (self-renewal) and their ability to differentiate into all mature cells of the human body (pluripotency), have made them an extremely attractive tool for cell therapy, to treat devastating maladies such as cardiovascular diseases.

Another important and promising achievement in the stem cell field was the reversion of somatic cells (e.g. fibroblasts, keratinocytes) to a state of pluripotency using defined reprogramming strategies. The creation of these induced pluripotent stem cells (iPSCs) elicited an explosion of scientific curiosity and industrial interest. This is mainly because iPSCs are similar to ESCs (namely cell morphology, self-renewal ability, potential to differentiate into cells derived from all three germ layers) and thereby could potentially replace ESCs for clinical applications, circumventing the ethical concerns regarding the use of embryos. Additionally, iPSCs present the benefit of being patient-derived cells, avoiding immune rejection in cell therapy applications.

The successful translation of ESCs and iPSCs to this field requires the development of robust bioprocesses for the production of stem cells and/or their progeny in (i) high purity, (ii) consistent quality and (iii) relevant quantities that satisfy clinical demands. Currently, two dimensional (2D) culture systems are well established for routine stem cell cultivation. However, the inherent variability, lack of environment control and the low productions yields associated with these 2-D culturing approaches are the main drawbacks limiting their use in clinical or industrial scale.

Objectives and Methodologies

The main aim of this project is to develop a robust and scalable bioprocess for the efficient production of cardiomyocytes, derived from pluripotent stem cells, to use in cell therapy applications. Transgenic murine ES and iPS cell lines, provided by the University of Cologne, will be used. These cell systems allow easy monitoring of cell differentiation and lineage selection (since cell lines were transfected with α PIG vector in which the promoter of the cardiomyocyte lineage marker, α -myosin heavy chain, enhance both GFP and puromycin resistance gene expression).

In this project, the differentiation of ES and iPS cells into cardiomyocytes will be performed by cultivating these cells as 3D aggregates in fully controlled stirred tank bioreactors (500 ml vessels with temperature, pH and pO_2 control). In order to establish the optimal conditions for cardiac differentiation, different culture parameters/variables will be evaluated (e.g. inoculum concentration,

pO₂, reefered strategies, pH, medium composition) taking into account promising results already reported in the literature (Bauwens et al., 2005; Schroeder et al., 2005; Zandstra et al., 2003; Niebruegge et al., 2008).

In this project the hypothesis of developing methods for non-invasive and on-line monitoring of the cardiomyogenic differentiation process will be also investigated by incorporating a fluorescence probe in the bioreactor apparatus. The data generated will be compared and validated taking into account the results obtained by standard characterization protocols (e.g. flow cytometry analysis).

References

Bauwens C et al., 2004. *Biotechnology and Bioengineering*, 90(4), 452-461.

Schroeder M et al., 2005. *Biotechnology and Bioengineering*, 92(7), 920-933.

Zandstra PW et al., 2003. *Tissue Engineering*, 9(4), 767-778.

Niebruegge S et al., 2008. *Biotechnology and Bioengineering*, 102(2), 493-507.