miR-OptofectArray

Delivering non-viral silencing RNA (microRNA) using automated femtosecond lasers into stem cells for cardiac reprogramming and characterization using non-destructive optical techniques (01/08/2013 - 31/07/2015)

Final Publishable Summary

miR-OptoFectArray Objectives

The primary goal of miR-OptoFectArray is to develop a high throughput, automated non-invasive system of transfection and characterization to elucidate the role of various exogenous microRNAs and combinations thereof in the adjustment of cellular phenotype. This system will involve the use of a femtosecond (Fs) laser to enable highly efficient transfection of miRNAs (miRs) via the formation of transient nanopores under robotic control and the use of Raman microspectroscopy to characterize the effect of the miRs on cell proliferation, apoptosis, necrosis and differentiation towards therapeutic phenotypes. The secondary objective of this project is to determine the cohort of miRs involved in cardiomyogenesis of mouse embryonic stem cells.

To test the hypothesis of this project, a series of research objectives were formulated:
(i) A robotically controlled microarray Fs Laser Optotransfection system will be developed for the high throughput transfection the cell type described;
(ii) Proof of concept studies will be performed and optimized to validate the use of Fs Laser Optotransfection as an appropriate tool to deliver non-viral miRs;
(iii) Cardiomyogenesis will be assessed and screened using Raman spectroscopy building a library for future analysis;
(iv) The automated high throughput system will be validated and optimized to enable a high throughput, non-invasive screening tool to identify candidate miRs as inducers of cardiomyogenesis in mouse embryonic stem cells.

Work performed since the beginning of the project

The activities of the project are organized in four work packages. The activities are here summarized according to the WP organization of the project:

WP1: Fs Laser Optotransfection of cells with miR, differentiation studies, α-MHC fluorescence

α-MHC GFP mouse ES cells cultures; optimization of culture and propagation conditions with considerations of the logistics of the host laboratory and shared research facilities at Fraunhofer IGB and UKT has been achieved.
The fellow has collaborated with the group of Dr. Ali Nsair in the Department of Cardiology (UCLA) and has obtained mouse embryonic fibroblasts (MEFs) which have been modified to express the Green Fluorescent Protein at the same time that they begin to express alpha Myosin Heavy Chain (α-MHC) which indicates that they are becoming cardiomyocytes.
An extensive search of the literature has been performed with regard to the direct programming of cells towards a cardiomyocytes phenotype. The fellow has established (in co-ordination with the Department of Cardiology, UCLA) protocols for the pre-conditioning of MEFs to make them more susceptible towards epigenetic programming using miRs. This includes the use of hypoxic incubation, inhibition of the Wnt pathway and histone deacetylases using small molecules and culturing on a natural extracellular matrix. Additionally, the fellow has established the delivery of plasmid DNA encoding transcription factors to drive cardiomyogenesis as a parallel control to that of delivering miRs. Transition to a cardiomyocyte phenotype has been monitored via the expression of GFP (driven by the expression of α-MHC) and immunofluorescent staining for cardiac markers such as cardiac troponin t (Ctnt) and connexin 43 (Cnx43).

WP2: Using Raman spectroscopy to define representative spectra of cardiomyogenesis of mouse embryonic stem cells treated with miRs

The fellow has performed research on the use of Raman Spectroscopy to elucidate the biochemical footprint of cardiomyogenesis. Raman Spectroscopy is a powerful tool to obtain such information. However, it can be time-consuming and requires substantial input into data analysis. The results of this work are currently in revision for publication based on reviewer's corrections.

The fellow has replaced Raman Spectroscopy with fluorescent lifetime imaging (FLIM) as the laser transfection system has been designed to have time correlated single photon counting (TCSPC) in the same optical pathways as the laser. This alternative method has several advantages over Raman Spectroscopy. It allow one to obtain qualitative images of cells in the field of view, allows evaluation of cellular metabolic activity through NADPH auto fluorescence, detection of exogenous fluorescence (e.g. GFP expression), and can enable the distinction of cells of varying phenotypes. This deviation was anticipated in Part B of the original project application and therefore is not so unexpected. Additionally, it does not significantly change the description of the project

as it was always the intention to characterize cells using a non-invasive optical technique. So far, cellular analysis of cells has been presented at the TERMIS 2014 EU Meeting, Genoa, Italy and the 10th Workshop on Advanced Multiphoton and Fluorescence Lifetime Techniques, 2015. The fellow has also attended workshops and conferences to become skilled in the performance and interpretation of FLIM spectra.

WP3: Research, Development and Design of automated seeding, miR addition and Fs Laser Optotransfection of miR-OptoFectArray system
The fellow has setup and directed the installation of the femtosecond laser transfection system in the host's laboratory. This has been coordinated with the company JenLab and collaborating with the group of Prof. Karsten König in the University of Saarbrucken, Germany. This laser has been used in the process of transfecting cells and optimizing power and laser settings to achieve cellular transfection.
Previously, the optotransfection procedure was laborious and only facilitated the optoporation of single cells. With the König laboratory in the University of Saarbrucken, a semi-automated transfection protocol has been established. This enables the identification of cells and subsequent patterning and execution of transfection.
Additionally, as the optics of the FLIM laser are coincident with that of the laser optoporation optics, simultaneous monitoring of the cells can be performed.

WP4: To validate, perform and elucidate the miR-OptoFectArray system to create representative heat maps of Raman Spectra, action potential measurements and GFP expression in a high throughput array system for commercialization purposes
Within this workpackage, the use of Raman Spectra has been replaced by FLIM as a method to define a representative measurement of cardiomyogenesis. In the course of this project period this work package has not been completely finished, however there are measures in place that this workpackage is achieved outside of this project duration.

Main results achieved so far:
WP1: Pre-conditioning of cells has been optimized to enable more efficient programming of cells into cardiomyocytes. These protocols have been applied successfully to α-MHC GFP MEFS and human foetal cardiac fibroblasts. Transfection of the transcription factors GATA4, Mef2c and Tbx5 was achieved using laser optoporation which increased the number of cells expressing cardiomyocyte specific markers.

WP2: Raman spectroscopy has been investigated with regard to its suitability to evaluation of cardiomyogenesis in this system. These results are currently being submitted for publication.
FLIM has been adopted (as anticipated in the original research proposal) to elucidate cardiomyogenesis due to its quicker acquisition time and more detailed interpretation. Preliminary results have been presented at the TERMIS EU 2014 Conference and the 10th Workshop on Advanced Multiphoton and Fluorescence Lifetime Techniques 2015.

WP3: A semi-automated transfection system has been developed by the König group which is adapted into the laser transfection system. This allows a more powerful adaption of laser optoporation.
Additionally, the laser optoporation pathways have been modified to be coincident with multiphoton imaging, SHG imaging, and FLIM imaging. This allows the simultaneous imaging of cells and also can facilitate the identification of GFP expressing cells.

The expected final results and their potential impact and use
It is expected that this project will progress to establish optoporation as an effective tool for induction of cardiomyogenesis. Furthermore, this system will be patented licensed and marketed as a tool for determining the simultaneous effect of miRs, combinations of genes (through plasmid DNA), building up worldwide collaborations, and establishing the fellow and the host institution as leaders in this field. The use of FLIM as a non-invasive method of cellular profiling in this project, although not originally planned, has become a strong aspect of workpackages 2 and 4. It is expected that the fellow will establish this method as a robust tool to investigate and characterise cardiomyogenesis.

For more information, please visit
the project website:
http://www.mir-optofectarray.eu/

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