RGC Ref.: X-HKU701/14
(please insert ref. above)

The Research Grants Council of Hong Kong
SFC/RGC Joint Research Scheme
Completion Report

(Please attach a copy of the completion report submitted to the Scottish Funding Council by the Scottish researcher)

Part A: The Project and Investigator(s)

1. Project Title

Advancing cell-based therapies for Parkinson's disease through Surfaceome Analyses

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Scottish Team
Name of Principal Investigator *(with title)*	Professor Kenneth R. Boheler	Dr. Tilo Kunath
Post	Professor Deputy Director	Parkinson's UK Senior Research Fellow and Chancellor's Fellow
Unit / Department / Institution	Dept of Physiology (***Now*** *School of Biomedical Sciences*), LKS Faculty of Medicine, HKU	University of Edinburgh MRC Centre for Regenerative Medicine
Contact Information	bohelerk@hku.hk	Tilo.kunath@ed.ac.uk
Co-investigator(s) *(with title and Institution)*	Dr. Ellen Poon (Post-doctoral fellow, HKU)	

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1 Nov 2014		
Project Completion date	31 Oct 2015		

Duration *(in month)*	12 months		
Deadline for Submission of Completion Report	31 Oct 2016		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Generate functional hPSC-mDA neuronal precursors and assess the surfaceome using Cell Surface Capturing Technology
2. Compare surfaceome analysis with proprietary Cell Surface Protein Atlas to putatively identify positive and negative markers of mDA cells
3. Time permitting, test commercially available antibodies with reactivity to identified surface markers for improved sorting of mDA cells

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

Based on the tandem mass spectrometry output, we have successfully generated a unique and informative list of cell-surface proteins enriched on human ESC-derived midbrain dopaminergic cells. The data are being utilized for analyses aimed at immunophenotyping and ultimately isolation of therapeutically viable dopamine producing cells. This list represents a novel information set that will be critical to the characterization and purification of dopamine producing cells generated from human pluripotent stem cells. We have compared this list with our Cell Surface Protein Atlas and proprietary information not yet added to this Atlas. Moreover, we have begun to identify possible drug targets of the non-dopamine producing cells to identify possible mechanisms for eliminating contaminating cells.

Potential for further development of the research and the proposed course of action
(maximum half a page)

The cell protocols and data generated from this pilot project have been used to apply for one grant in Hong Kong. This grant was submitted to the HMRF and after review it was funded (HK$1,199,000) for 2 years. The title "Eliminating barriers to the use of GMP-qualified human pluripotent stem cell-derived midbrain dopaminergic neurons for cell based therapies, received funding from 15 Sept 2016 and the anticipated project end date is 14 Sept 2018. Dr. Kunath also applied for two grants, one of which was funded. In parallel and not part of these grants, the group of Tilo Kunath has generated Affymetrix HTA2.0 data on similarly treated cells, which will be used to help classify these surface proteins and identify those markers that can only be identified through protein and not transcriptomic analyses. Moreover, as we test the informative value of these markers, we will be able to immunophenotype these cells and ultimately produce cells that are clinically relevant for transplantation in patients with Parkinson's. These experiments will be conducted collaboratively with Dr. Tilo Kunath, and it represents a critical step towards our long-term goal of translating these cells as a viable therapy to patients with Parkinsonism. The seed funding from SFC/RGC was critical in helping us realize the first step in this long process of translating human pluripotent stem cell derivatives into effective treatments for human disease.

7. The Layman's Summary
(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Parkinson's disease (PD) is a progressive neurodegenerative condition that causes severe movement disorders and other non-motor symptoms. Transplantation of nerve cells may be a viable therapeutic option for Parkinson's patients. One potential renewable and inexhaustible source of neuronal cells is from human pluripotent stem cell (hPSC) lines. The procedure to transform hPSCs into dopaminergic neurons is now well established. However, the efficiency of converting hPSCs into this neuron type is about 70%-80%. The best solution to this problem of mixed cell types is to physically separate the desired dopaminergic neurons for the other cell

types using a technique called fluorescence-activated cell sorting (FACS). The procedure takes advantage of the fact that different cell types have different sets of proteins on their cell surface; however, we currently know very little about the unique cell surface proteins, or surfaceome, of midbrain dopaminergic neurons. In this study we used the highly specialized Cell Surface Capture (CSC) technology to precisely characterize the surfaceome of midbrain dopaminergic neurons derived from hPSCs. This knowledge will directly inform strategies for FACS purification of these neurons, and remove one of a few remaining barriers to advancing a cell-based therapy for Parkinson's.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.))

The Latest Status of Publications				Author(s) *(**bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/ Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered

(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)

Month/ Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

I have written one review (Boheler and Gundry, Stem Cells Transl Med, 2016) where we acknowledged support from this grant. I attach it with the final report, but it should be noted that this review publication did not come directly from this grant. The invited review that I wrote was based on output from multiple studies on the cell surface technology and use for drug screening that we pioneered. We did, however, refer to the utility of this approach when applied to Parkinsonism and how we will use this technology to address challenges associated with stem cell research on this disease.