RGC Ref.: N-HKU 741/11 NSFC Ref. : *(please insert ref. above)*

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

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

From bone marrow stromal cells to Schwann cells – *in vitro* route to specification and utility in re-myelination therapy

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Daisy Kwok-Yan SHUM	Dr. Qiang AO
Post	Professor	Associate Professor
Unit / Department / Institution	School of Biomedical Sciences, The University of Hong Kong	Department of Neurosurgery, Yuquan Hospital, Tsinghua University, Beijing 10049
Contact Information	shumdkhk@hku.hk	aoqiang@tsinghua.edu.cn
Co-investigator(s) *(with title and institution)*	Prof. Ying-Shing CHAN (School of Biomedical Sciences, The University of Hong Kong)	

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1 Jan 2012		
Project Completion date	31 Dec 2014		
Duration *(in month)*	36 months		
Deadline for Submission of Completion Report	31 Dec 2015		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To translate the rat protocol to a human protocol for derivation of fate-committed Schwann cells from bone marrow stromal cells (BMSCs).
2. To test for functions of derived Schwann cells in axonal re-growth and re-myelination with use of a new design of nerve conduit in a rat model of sciatic nerve injury.
3. To identify neuron-associated NRG-1 type III as a key effector that specifies commitment of SCLCs to the Schwann cell fate

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

6. Research Outcome

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

Mainland contribution: Dr. AO, Qiang and his group

Dr. Ao Qing and his group focused on the design and production of biocompatible nerve conduits. Chitosan was chosen as the conduit material due to its non-antigenic and biodegradable nature. To fulfill the needs of clinical translation, large mammals (goats) were used as animal model in the work. To demonstrate cell carrying and regeneration promoting capability, chitosan conduits pre-seeded with autologous goat bone marrow stromal cells were implanted to bridge a critical 30-mm gap in the severed sciatic nerve. Behavioral tests, high field functional MRI and electrophysiological examination were applied to evaluate locomotor function, *in vivo* structural regeneration and nerve conductivity. Data were collected for up to one year following the nerve-bridging implant before animals were sacrificed for histological analysis of structural regeneration down to cellular level. Results from chitosan conduit group were compared to i) autologous nerve graft group and ii) no conduit/graft control group.
One year after transplantation, animals from both conduit and autologous graft group were able to run and feed ad lib. MRI indicated successful regeneration of the bridged sciatic nerve, although the diameter of regenerated nerve was smaller than that receiving the autologous graft. Electrophysiological tests also revealed no significant difference in conductivity between the conduit group and autologous graft group. In contrast, the no conduit group failed to regenerate. Histological examination revealed significant amount of myelinated fibers in regenerated sciatic nerve of the conduit group. However, nerve fiber bundles of the conduit group were not as well organized as those of the autologous graft group. **See Part C: 8.1 - 8.4.** for research outcomes.
In conclusion, chitosan conduit represents a feasible alternative for autologous nerve graft. To further improve regeneration, conduits should be pre-seeded with Schwann cells derived by the HKU team.

Hong Kong contribution: Professor SHUM, Kwok Yan, Daisy and her group

Professor Shum and her group focused on three aspects:

(1) Translation to a **human protocol** for derivation of fate-committed Schwann cells from donor BMSCs was accomplished with (i) the derivation of human sensory neurons in an 8-day program from human iPSC, (ii) the conversion of human BMSCs to SCLCs and (iii) the coculture of the derived human neurons and SCLCs. Human Schwann cells can therefore be produced and purified for storage or for therapeutic applications. **See Part C: 8.5, 8.7; 9.1, 9.4, 9.11, 9.12, 9.14** for research outcomes.

(2) A"Schwann cell-unixially aligned chitosan nanofibre Swiss roll" was engineered as a new design of nerve conduit. This was viable in culture and amenable to guide axonal growth and to myelinate axons that sprouted from seeded neurons. **See Part C: 9.3 and 9.8.**

(3) Underlying mechanism that provides for the phenotypic stability of human BMSC-derived Schwann cells: Coculture of SCLCs with incipient neurons of rat embryonic DRGs allows contact-dependent signaling via (i) interaction of Notch ligands (expressed on neurons) with Notch-1 receptors (expressed on SCLCs) mediates upregulation of ErbB receptors on SCLCs; (ii) in turn, interaction of ErbB receptors (upregulated expression on SCLCs) with NRG-1 type III (expressed on neurons) effects commitment of SCLCs to the Schwann cell fate. **See Part C: 8.6; 9.2, 9.5, 9.6, 9.7, 9.13.**

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

Towards translational research:

- Following from **Objective 1**, an application for Research Grant (Ref: 03142536) under the Health and Medical Research Fund (HMRF) of the HK Food and Health Bureau "Towards clinical application of human bone marrow stromal cell-derived Schwann cells for repair and regeneration in nerve injury" was submitted in March 2015 but was not funded out of doubt that iPSC-derived sensory neurons cannot materialize. We expect to re-submit for the Research Grant with confidence backed by a manuscript submission to Stem Cell Translational Medicine on the subject.
- Following from **Objective 2**, we expect tests of the "Schwann cell-fibre Swiss roll" as a nerve conduit in a rat model of sciatic nerve injury will allow close collaboration with our Mainland partner, Dr. Ao, in testing the conduit design in a goat model of nerve injury.
- A spinoff from work under **Objective 3** is the emerging prospect of deriving oligodendrocyte precursor cells from bone marrow stromal cells for use in remyelination therapy. **[See Part C: 9.9, 9.10.]** This will provide preliminary results for another HMRF Grant application on the subject of remyelination therapy.

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)

The phenotypic instability of bone marrow-derived Schwann cell-like cells (SCLCs) is at issue with the intended use of these cells for nerve repair and regeneration following therapeutic transplantation. In this work, we demonstrate that commitment to the Schwann cell fate can be acquired by SCLCs in co-culture with sensory neurons via contact-dependent, Notch- and ErbB-mediated signaling, mimicking the niche within developing dorsal root ganglia. Though access to incipient neurons of developing dorsal root ganglia harvested from human embryos is limiting, here we report our use of a human iPSC line to bypass the translational barrier. By various combinations of small-molecule inhibitors of cellular signaling pathways, we arrived at an 8-day programme that effectively and efficiently fostered differentiation of a human iPSC line into sensory neurons. The human iPSC-derived sensory neurons therefore promise

utilization in the translation to a protocol whereby human bone marrow-derived Schwann cells achieve fate commitment and meet safety requirements for autologous transplantation and re-myelination therapy. In collaboration with our Mainland partner, we continue to engineer novel chitosan-based, Schwann cell-seeded nerve conduits for use in bridging critical gaps in severed nerves so as to improve functional recovery.

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)*						
1. 2012				Ma T., Niu Y., Zhao C., Su Z.Q., Ao Q., Zhang X.F., Zhao N.M., Gong Y.D.*	Amyloid precursor protein-mediated modulation of capacitive calcium entry. *Chin Sci Bull*, 57: 4552-4559.	n/a	Yes	Yes	
2. 2012				Wei Y.J., Gong K., Zuo H.C., Zhang X.F., Gong Y.D., Ao Q.*	ADSCs-Chitosan/silk fibroin hybrid enhances sciatic nerve regeneration in a rat model. *Chin J Cell Stem Cell*, 2: 160-168.	n/a	Yes	Yes	
3. 2013				Song B., Ao Q., Niu Y., Shen Q., Zuo H.C., Zhang X.F., Gong Y.D.*	Amyloid beta-peptide worsens cognitive impairment following cerebral ischemia-reperfusion injury. *Neural Regen Res* 8: 2449-2457.	n/a	Yes	Yes	
4. 2014				Liu H., Chen L, Wang Z., Liu W., Ji L. Ao Q.*	Control release of growth factors combined with small gap anastomosis promotes sciatic nerve regeneration after transaction in rats. *Chin J Neurosurg*, 24: 339-343.	n/a	Yes	Yes	

5. 2014				Cai S., Chan Y.S., Shum D.K.Y.*	Induced pluripotent stem cells and neurological disease models. *Acta Physiologica Sinica*, 66: 55-66.	n/a	Yes	Yes	
6.		2015		Tai E.W.Y., Shea G.K.H., Tsui A.Y.P., Mung A.K.L., Leung K.H.Y., Lam J.H.C., Chan Y.S.*, Shum D.K.Y.*	Juxtacrine signaling via Notch and ErbB receptors in the switch to tfate commitment of bone marrow-derived Schwann cells. *Glia* (submitted)	n/a	Yes	Yes	
7.		2015		Cai S., Han L., Ao Q., Chan Y.S.*, Shum D.K.Y.*	Induced pluripotent stem cell-derived sensory neurons for fate commitment of bone marrow-derived Schwann cells – Utilization in re-myelination therapy. *Stem Cells Translational Medicine* (submitted)	n/a	Yes	Yes	

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)*
1. 03/2012 Hong Kong	Characterization of human bone marrow stromal cells following differentiation into Schwann cells. Cai S., Tsui A.Y., Chan Y.S., Shum D.K.Y.	7th Int'l Symposium on Healthy Aging: Live Well, Age Well	2013	Yes	Yes	
2. 10/2012 New Orleans, USA	Notch signaling mediates the switch to fate commitment of bone marrow-derived Schwann cells. Shum D.K.Y., Tai E.W.Y., Shea G.K.H., Tsui A.Y.P., Leung K.H.Y., Chan Y.S.	Annual Meeting of the Society for Neuro-science (USA)	2013	Yes	Yes	

3. 03/2013 Hong Kong	Aligned chitosan nanofibers for culturing oriented Schwann cells to guide axonal growth. Tung W.Y., Tam K.W., Chan Y.S. , Shum D.K.Y.	8th Int'l Symposium on Healthy Aging: How to Age with Finesse	2013	Yes	Yes	
4. 03/2013 Hong Kong	Derivation of human neural crest stem cells from human induced pluripotent stem cells. Cai S., Tsui A.Y., Chan Y.S., Shum D.K.Y.	8th Int'l Symposium on Healthy Aging: How to Age with Finesse	2013	Yes	Yes	
5. 03/2013 Hong Kong	Neuregulin 1 type III in the switch of bone marrow stromal cells to fate-committed Schwann cells. Leung K.H., Tsui A.Y., Shea G.K., Tai E.W., Chan Y.S., Shum D.K.Y.	8th Int'l Symposium on Healthy Aging: How to Age with Finesse	2013	Yes	Yes	
6. 07/2013 Berlin, Germany.	Notch signaling in in in vitro derivation of Schwann cells from bone marrow stromal cells. Tai E.W.Y., Shea G.H.K., Tsui A.Y.P., Leung K.H.Y., Chan Y.S., Shum D.K.Y.	XI European Meeting on Glial Cells in Health and Disease.	2013	Yes	Yes	
7. 07/2013 Berlin, Germany	Roles of neuregulin 1 type III in the ex vivo generation of fate committed Schwann cells from bone marrow stromal cells. Leung K.H.Y., Tsui A.YP., Shea G.H.K., Tai E.W.Y., Chan Y.S., Shum D.K.Y	XI European Meeting on Glial Cells in Health and Disease.	2013	Yes	Yes	
8. 07/2013 Warwick, U.K.	Oriented Schwann cell culture on aligned chitosan nanofibres for axon growth guidance. Tung W.T., Tam K.W., Chan Y.S., Shum D.K.Y.	11th International Conference on Materials Chemistry	2013	Yes	Yes	

9. 07/2013 Birmingham, UK	Derivation of oligodendrocyte precursor cells from bone marrow stromal cells for remyelination therapy. Tsui Y.P., Li R.S., Lo A.C., Chan Y.S., Shum D.K.Y.	37th Int'l Congress of International Union of Physiological Sciences	n/a	Yes	Yes	
10. 11/2013 San Diego, USA	Towards remyelination therapy with oligodendrocyte precursor and Schwann cells derived from bone marrow stromal cells. Shum D.K.Y., Tsui Y.P., Tai W.Y., Chan Y.S.	Annual Meeting of the Society for Neuro-science (USA)	n/a	Yes	Yes	
11. 08/2014 Kaohsiung, Taiwan	Derivation of stable Schwann cells from human bone marrow stromal cells. Cai S., Chan Y.S., Shum D.K.Y.	11th Biennial Meeting of Asia-Pacific Society for Neurochemistry	n/a	Yes	Yes	
12. 11/2014 Washington DC, USA	Directed differentiation of human induced pluripotent stem cell to sensory neurons by combined small molecule inhibitors. Cai S., Chan Y.S., Shum D.K.Y.	Annual Meeting of the Society for Neuro-science (USA)	n/a	Yes	Yes	
13. 11/2014 Washington DC, USA	Juxtacrine signalling via Notch and ErbB receptors in the switch to fate commitment of bone marrow-derived Schwann cells. Shum D.K.Y., Tai E.W.Y., Shea G.H.K., Tsui A.Y.P., Leung K.H.Y., Chan Y.S.	Annual Meeting of the Society for Neuro-science (USA)	n/a	Yes	Yes	
14. 10/2015 Chicago, USA	Small molecule approach to direct differentiation of human induced pluripotent stem cells to sensory neurons. Shum D.K.Y., Cai S., Han L., Chan Y.S.	Annual Meeting of the Society for Neuro-science (USA)	n/a	Yes	Yes	

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
1. TSUI, Yat Ping	PhD	September 1, 2010	Thesis submitted in July 2013
2. LEUNG, Katherine Ho Yan	MPhil	September 1, 2011	Thesis submitted in August 2013
3. TUNG, Wing Tai	MPhil	September 1, 2011	Thesis submitted in February 2014
4. CHEN, Lin	PhD	September 1, 2010	Thesis submitted in August 2014
5. TAI, Evelyn Wing Yin	PhD	September 1, 2010	Thesis submitted in November 2014

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

Nil.