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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

A Micro Array Chip based Single Cell Manipulation System for Characterization of Electrical Stimulation Induced Stem Cell Differentiation

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. SUN Dong	Prof. ZHU Rong
Post	Chair Professor	Professor
Unit / Department / Institution	City University of Hong Kong	Tsinghua University
Contact Information	bmehead@cityu.edu.hk	ZhuRong@noemail.com
Co-investigator(s) *(with title and institution)*	Prof. LI Gang, The Chinese University of Hong Kong	Prof. LIU Peng, Tsinghua University

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	01/01/2016		
Project Completion date	31/12/2019		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	31/12/2020		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Develop a single cell manipulation system that enables generation of electrical stimulus to direct stem cell differentiation by using technologies of micro array chip and robotically controlled optical tweezers, allowing groups of cells to be positioned accurately in the electrode for simultaneous processing.
2. Investigate and develop optimal protocols for the stem cell osteogenic differentiation in the micro array chip developed, and quantitatively analyze the electrical stimulation induced differentiation of mesenchymal stem cells (MSCs).

3. Characterize mechanobiological and physiological electrical properties of MSCs such as cytoskeleton vicissitude during cell stimulation and differentiation and specific markers for the mature Osteoblast, and perform *in vivo* testing of the osteogenic potentials of 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)

We have performed this project study in the following three aspects to achieve the project objectives.

1. Development a single cell manipulation system

We reported the development of a novel microfluidic platform that can individually array and culture hundreds of cells under chemical and electrical stimuli in *Biomicrofluidics* (2017). The size of the microchamber can be adjusted to fit different cell culture times, and this characteristic enables remarkable scalability. Transparent indium tin oxide microelectrodes were integrated with the single-cell array platform for on-chip electrical stimuli. The platform exhibited nearly 90% single-cell efficiency and facilitated week-scale clonal expansion of different types of single cells. By tracking clonal expansion of cells under chemical/electrical stimuli for relatively long periods, the proposed platform can facilitate the screening of the cell subpopulation with a favorable growth phenotype for drug testing and cell therapy. We also reported a new type of DEP-enabled 3D scaffold for active cell seeding and patterning in *Biomedical Microdevices* (2017). The proposed scaffold design can enable formation of multiple ring patterns via DEP and the properties of the scaffold are suitable for bone tissue culture. We have further developed a simplified sheathless cell separation technology by using combined gravitational sedimentation based prefocusing and dielectrophoretic separation technologies in *Lab on a Chip* (2019).

2. Investigation and development of optimal protocols for stem cell osteogenic differentiation

Osteogenic differentiation MSCs, especially through the electrical stimulation (ES), plays an important role in bone healing. Direct (DC) and alternating (AC) currents are used clinically to stimulate osteogenic differentiation; however, information on conducting effective differentiation for clinical treatment remains lacking. We developed a method to optimize ES parameters based on the calcium spike patterns of MSCs. Calcium spike frequency decreases as the MSC osteogenic differentiation progresses. Using the optimal parameters of AC, including voltage, wave, frequency and duty time, we efficiently initiated the process of osteogenic differentiation in MSCs. This method provides a new aspect for

optimizing osteogenic differentiation; moreover, it has potential uses in clinical treatments, such as in bone fractures. This work was reported in the *IEEE Transactions on Nanobioscience* (2019). In addition, we have investigated a novel ES based microelectrode array chip to enhance osteogenic differentiation of MSCs, and reported this work in *Lab on a Chip* (2020).

3. Characterization of mechanobiological and physiological electrical properties of MSCs

As reported in our paper in *Lab on a Chip* (2020), when using the microelectrode unit array on the microchip to generate an inhomogeneous electric field to stimulate MSCs, osteogenic differentiation with nodular structures and tissue formation could be more effectively enhanced. The osteogenic differentiation was assayed by using alizarin red staining and morphology observation as well as immunocytochemistry, which provided in situ differentiation assessment on a chip with ES and without ES. According to the results, the osteogenic differentiation of MSCs was enhanced by in-plant stimulation with an array-distributed inhomogeneous electric field, and especially for the combination of induced conditions with osteogenic medium (ES + OM), the differentiation efficiency was remarkably increased. The microelectrode-array-chip-based electrical stimulation method can also be applied onto differentiation of MSCs as a fundamental platform which can be further integrated with in situ cell assay approaches for the applications in MSCs assay and bone tissue therapies. In our another paper published in *Biomicrofluidics* (2018), given the stimulation of the periodic mechanical confinement on-chip, the migration ability of cells was promoted, and moreover, the migration speed increased as the stimulation was enhanced. Both AFM nanoindentation and optical stretching tests on cells were performed to measure their mechanical property.

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

The future development includes the optimization of protocols for stem cell osteogenic differentiation, and characterization of mechanobiological and physiological electrical properties of MSCs with possible in vivo testing results of the osteogenic potentials.

7. The Layman's Summary

(describe <u>in layman's language</u> the nature, significance and value of the research project, in no more than 200 words)

Electrical stimulation is a physical induction method that can induce the proliferation and differentiation of stem cells with less damage. Currently, due to the lack of effective methods for measuring accurate information during differentiation, the actual differentiation efficiency under electrical stimulation is quite low. The project aims to solve this problem by developing a new micro array chip based single cell manipulation system to induce the proliferation and differentiation of MSCs. This research was conducted from three aspects: the development of a single cell manipulation system, the study of optimal protocols for stem cell osteogenic differentiation, and the characterization of the mechanical biology and physiological electrical characteristics of MSCs. This research broadens our understanding of how to manipulate stem cells at the single-cell level and induce ideal electrical stimulation conditions for stem cell differentiation, thereby helping us develop new treatment options for tissue repair and regeneration.

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)*						
2017				T. Luo, J. Hou, S. Chen, Y. T. Chow, R. Wang, D. Ma, **R. Zhu**, and **D. Sun***	"Microfluidic single-cell array platform enabling week-scale clonal expansion under chemical/electrical stimuli," Biomicrofluidics, vol. 11, no. 5, 054103, 2017	Yes	Yes	Yes	Yes
2017				W. Ma, J. Li, F. Niu, H. Ji, and **D. Sun***	"Robust control to manipulate a microparticle with electromagnetic coil system," IEEE Trans. Industrial Electronics, vol. 64, no. 11, pp. 8566-8577, 2017.	Yes	Yes	Yes	Yes
2017				Z. Huang, H. Chu, H. Liu, J. Yang, and **D. Sun***	"Engineered bone scaffolds with dielectrophoresis-based patterning using 3D printing," Biomedical Microdevices, vol. 19, no. 4, November 2017.	Yes	Yes	Yes	Yes
2017				Lin SE, Lee WYW, Xu LL, Wang YJ, Chen YF, Ho KW, Qin L, Jiang XH, Cui L, **Li G***	Stepwise preconditioning enhances mesenchymal stem cell-based cartilage regeneration through epigenetic modification. Osteoarthritis and Cartilage; 2017 Sep;25(9):1541-1550.	Yes	Yes	Yes	Yes
2017				Sun YX, Xu J, Xu LL, Zhang JF, Chan KM, Pan XH, **Li G***	MiR-503 promotes bone formation in distraction osteogenesis through suppressing Smurf1 expression. Scientific Reports, 2017; Mar 24; 7(1):409.	Yes	Yes	Yes	Yes

2017				Sun YX, Zhang JF, Li DJ, Xu LL, Pan XH, **Li G**.*	Comparing the osteoconductive potential between tubular and cylindrical beta-tricalcium phosphate scaffolds: an experimental study in rats. Journal of Biomedical Materials Research: Part B - Applied Biomaterials, 2017 Sep 29. doi: 10.1002/jbm.b.34011.	Yes	Yes	Yes	Yes
2018				T Luo, L. Fan, Y. Zeng, Y. Liu, S. Chen, J. Hou, Z. Guan, D. Ma, T. Wei, Q. Tan, R. H. W. Lam, **D. Sun***	A simplified sheathless cell separation approach using combined gravitational sedimentation based prefocusing and dielectrophoretic separation. Lab on a Chip, vol. 18, no. 11, pp. 1521-1532.	No	Yes	Yes	Yes
2018				Xiangpeng Li, H. Yang, H. Huang, **D. Sun***	A switching controller for high speed cell transportation by using a robot-aided optical tweezers system. Automatica, vol. 89, pp. 308-315.	No	Yes	Yes	Yes
2018				Dongce Ma, R. Wang, S. Chen, T. Luo, Y. T. Chow, **D. Sun***	Microfluidic platform for probing cancer cells migration property under periodic mechanical confinement. Biomicrofluidics, vol. 12, issue 2, 024118.	No	Yes	Yes	Yes
2019				J. Hou, T. Luo, S. Chen, S. Lin, M. M. Yang, **G. Li, D. Sun***	Calcium spike patterns reveal linkage of electrical stimulus and MSC osteogenic differentiation. IEEE Transactions on Nanobioscience, vol. 18, no. 1, pp. 3-9.	No	Yes	Yes	Yes

2019				M. Xie, A. Shakoor, Y. Shen, J. K. Mills, **D Sun***	Out-of-plane rotation control of biological cells with a robot-tweezers manipulation system for orientation-based cell surgery. IEEE Transactions on Biomedical Engineering, vol. 66, no. 1, pp. 199-207.	No	Yes	Yes	Yes
2019				K. Meng, H. Yang, Y. Wang, **D. Sun***	Modeling and control of single-cell migration induced by a chemoattractant-loaded microbead. IEEE Transactions on Cybernetics, vol. 49, no. 2, pp. 427-439.	No	Yes	Yes	Yes
2019				T. Luo, L. Fan, **R. Zhu, D. Sun***	Microfluidic single-cell manipulation and analysis: methods and applications. Micromachines, vol. 10, no. 2, article 104.	No	Yes	Yes	Yes
2020				T. Zheng, Z. Zhang, **R. Zhu*, D. Sun**	"A microelectrode array chip for osteogenic differentiation of mesenchymal stem cells under electrical stimulation," Lab on a Chip, vol. 20, no. 2, pp. 373-383,	No	Yes	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)*
2016	A microarray platform for high-throughput single-cell capture and culture	IEEE International Conference on Nano/Molecular Medicine and Engineering, Macau, Oct. 30-Nov. 2, 2016.	Yes	Yes	Yes	Yes

2017	Design of an automated controller with collision-avoidance capability for in-vivo transportation of biological cells	IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS), Vancouver, Canada, Sep. 24-28, 2017	Yes	Yes	Yes	Yes
2018	High-throughput single cell trapping and patterning using a sandwiched microfluidic chip	IEEE International Conference on Robotics and Biomimetics, Kuala Lumpur, Malaysia, December 12-15, 2018	No	Yes	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
Jundi Hou	PhD	September 2014	January 2018
Yuxin Sun	PhD	August 2013	August 2016
Tao Luo	PhD	September 2015	June 2018
Dongce Ma	PhD	September 2015	July 2018

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

Book Chapter:
T. Luo, L. Fan, **R. Zhu**, and **D. Sun***, "Microfluidic single-cell manipulation and analysis: methods and applications," *Microfluidics for Cells and Other Organisms (volume 1)*, pp. 104-134, edited by Danny van Noort, MDPI, March 2020.

Grant Patent:
D. Sun, Y. T. Chow, and Ran Wang, "System and method for delivery of substance into mammalian cells", USA Patent, US 10,011,848, July 2018.