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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

Rationalizing scaffold design with optimal cell niche for mesenchymal stem cell (MSC)-based therapy in disc degeneration

理性化支架設計用于優化口胞微環境以輔助間質幹細胞治療椎間盤退變

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Barbara Pui Chan 陳佩 (教授)	Prof. Yanan Du 杜亚楠 (教授)
Post	Professor (教授)	Professor (教授)
Unit / Department / Institution	Medical Engineering, Department of Mechanical Engineering, the University of Hong Kong 香港大学機械工程學系醫學工程	Department of Biomedical Engineering, Tsing Hua University, Beijing, China 清華大學生物醫學工程系
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Co-investigator(s) *(with title and institution)*	Nil	Nil

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1 Jan 2015		
Project Completion date	31 Dec 2018		
Duration *(in month)*	48		

Deadline for Submission of Completion Report	31 Dec 2019		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To **screen for the optimal cell niche** maintaining phenotype and function of NP cells;
2. To **study MSC fate and phenotypic changes** upon exposure to the optimal cell niche;
3. To **translate the optimal cell niche into scaffold design** for IVD tissue engineering

5.2 Revised Objectives

Nil

6. Research Outcome

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

Aim 1 – To screen for the optimal cell niche maintaining phenotype and function of NP cells
Native bovine NPCs are round cells with thin layer of cortical actin and are expressing phenotypic markers including type II collagen, keratin 8 and SNAP25 at both gene and protein level. These markers have been used to evaluate the effects of niche factors on NPC maintenance. When bovine NPCs were cultured as 3D monolayers, they lost their round shape and expressed intensive actin stress fibers rather than thin cortical actin ring. They still express phenotype markers including type II collagen but the matrix markers only localize intracellularly but not extracellularly, suggesting that the phenotype of NPC were not maintained. Laser parameters such as power, exposure time, zoom, etc have been found to influence mechanical properties and porosity with linear or non-linear association. The topological features including flat matrix, gratings with hierarchical organization, concave and convex structures, microwells, micropillars and random fiber-beads micropatterns can be fabricated. A number of commonly used ECM niche factors including fibronectin, vitronectin, fibrinogen and laminin have been fabricated and their local density can be controlled. A library of ROIs has been developed to define the topological features. How do different levels of these parameters affect the phenotype markers, particularly Col 2, keratin 8 and SNAP25, of NPCs have been defined, In brief, softer substrate or micropillars, presence of any level of laminin and vitronectin, lower level of fibronectin, and micropillar arrays and fiber-beads topology better maintained the NPC phenotype.

Aim 2 – To study mesenchymal cell fate and phenotypic changes upon exposure to the optimal cell niche
Combinations of individual cell niche factors were fabricated before culturing bovine NPCs. We found that the topological and matrix niche combinations of micropillar array+vitronectin+laminin, or fiber-beads+vitronectin+laminin resulted in the best maintenance of the phenotype markers including the round morphology, type 2 collage,

keratin 8 and SNAP25 expression. This knowledge provides the rationale for the subsequent experiment of incorporating laminin into collagen microspheres, as well as incorporating GAGs (beads) into collagen fiber, referring to the fiber-beads topological feature. Human fibroblasts sense the geometry or topology dependent stiffness rather than the intrinsic material property elastic modulus of the cell niche mechanical factors in terms of larger and more mature matrix adhesion. Human MSCs and fibroblasts all generate stronger traction force over the micropillars when the stiffness increases. When the lateral or in plane stiffness increases, human MSCs interact with the matrix strongly with more mature integrin alpha 5-based fibrillar adhesions, which are known to mediate cell fate changes including proliferation and migration. Human MSCs were also found to preferably interact with fibronectin as compared with the other matrix proteins in terms of adhesion and attachment. When decoupling the matrix niche with the mechanical niche, hMSCs were found to prefer attachment towards stiffer niche regardless of the type of matrix factors. On stiffer micropillars, mesenchymal cells expressed much higher level of Runx2 than that on the softer ones, suggesting the cell niche factors may affect their cell fate.

Aim 3 – To translate the optimal cell niche into scaffold design for IVD tissue engineering
Using a top down approach, rabbit NPCs were able to remodel the collagen microsphere with a complex matrix niche including GAGs, type II collagen, and TGFbeta as well as its membrane-bound receptor (TGFbeta R1) have been found in the acellular matrix. Human fibroblasts repopulating the acellular NPC-derived matrix niche were able to survive and alter their phenotype by expressing higher level of collagen type II and CA12, which are rabbit NPC markers. This suggests that NPC-derived matrix niche may affect the cell fate of fibroblasts exhibiting plasticity. Using a bottom-up approach, extracellular matrix components that are critical to NPC phenotype maintenance such as laminin (optimal matrix) and GAGs (optimal matrix and optimal topological) were chemically conjugated into the collagen fibrous meshwork of the microsphere platform using EDC chemical conjugation. Bovine NPCs were encapsulated in collagen-laminin and collagen-GAG scaffolds and their phenotype maintenance were compared using 2D cultures and 3D collagen microsphere as controls. It was found that NPCs can survive in these scaffolds and col-laminin gave a better morphology with cortical actin expression but 2D monolayer culture exhibited a largely spread morphology although the phenotype markers all express at a high level. Col-GAG showed better GAG staining because it was chemically incorporated. Collagen-GAG seems to give better collagen II and keratin 19 staining but the gene expression was not better than the collagen control group. Collagen-GAG recapitulates the fiber-beads topological feature in NP tissue and in the in vitro screening platform but its chemical properties need to be optimized and the mechanical properties need to be controlled.

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

A collection of cell niche micropatterns and microstructures has been developed in this study. This knowledge can be translated into some methods and products on protein cell niche biochips. One biochip can be universal for all cell types, for screening of optimal cell niche factors for phenotype maintenance. Another biochip can be made-to-order, based on the information collected from the first screening biochip, for reconstituting the optimal cell niche for cell culture with phenotype maintained. NPC has been used as an example cell system in this work. We shall further validate this platform by using other cell types with unknown culture conditions for phenotype maintenance. Future funding will be sought to develop these biochips and the associated applications.

The cell niche factors resulting in optimal phenotype maintenance of NPCs have been identified in this study. Some of these optimal cell niche factors such as laminin, GAGs and the fiber-beads topology have been translated into scaffold designs. However, the optimal cell niche is a multi-factorial event and translating a few of the individual factors is not sufficient to achieve optimal phenotype maintenance in developing scaffolds. Further optimization of scaffolds by including other favorable cell niche factors including mechanical properties, other matrix factors such as vitronectin, are to be investigated in the future. Moreover, the top down approach on cell-derived matrix niche in 3D has been developed in this study and this approach can be used to develop acellular matrix from other cell types and the acellular matrix developed can be used as a scaffold or 3D culture substrates.

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)

Using intervertebral disc as an example, this work demonstrates the process of rationalizing scaffold design by screening cell niche factors able to maintain the phenotype of intervertebral disc cells. Cell niche refers to the complex microenvironment that maintains cell phenotype and determines cellular fates. It consists of multiple interactive factors such as extracellular matrix, mechanical signals and topological features. The approach we used was to fabricate a wide range of protein microstructures and micropatterns with precisely controllable cell niche factors using a multiphoton biofabrication platform previously developed. Novel knowledge on the cell niche factors giving rise to the optimal intervertebral disc cell phenotype maintenance have been identified. The fabricated micropatterns and microstructures of the cell niches can be further developed into cell niche protein biochips for cell niche screening purpose for other cell types. Some of these factors have been translated into rationalized scaffold designs for future tissue engineering applications but incorporating other niche factors into the scaffold design warrants further investigations before an optimal scaffold can be developed. This study generates new knowledge, developed potential products and applications, and contribute to future development of optimal scaffolds and intervertebral disc tissue engineering.

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

2018				**Huang N**, Li CW, **Chan BP***. Multiphoton 3D micro-printing of protein micro-patterns with spatially controlled heterogeneity – A platform for single cell matrix niche studies.	Advanced Biosystems 2018 2(8):1800053.	2016	Yes	Yes	
2018				**Yuan MT**, Pai PJ, Liu XF, Lam H, **Chan BP***. Proteomic analysis of nucleus pulposus cell-derived extracellular matrix niche and its effect on phenotypic alteration of dermal fibroblasts – An in vitro model for cell niche interaction studies.	Scientific Reports 2018, 8:1512		Yes	Yes	
2018				Jiang SM, Li SC, Huang CY, **Chan BP, Du YN***. Physical properties of implanted porous bioscaffolds regulate skin repair: Focusing on mechanical and structural features.	Advanced Healthcare Materials Mar 21 2018, 7(6): 1700894. Doi: 10.1002/ADHM.201700894.		Yes	Yes	

2017				**Tong MH, Huang N,** Ngan AHW, **Du YN, Chan BP***. Preferential sensing and response to microenvironment stiffness of human dermal fibroblast cultured on protein micropatterns fabricated by 3D multiphoton biofabrication.	Scientific Reports, 2017 Sep 29; 7(1):12402. doi: 10.1038/s41598-017-12604-z.		Yes	Yes	
2017				**Ma JN**, Li CW, **Huang N, Wang XN, Tong MH**, Ngan AHW, **Chan BP***. Multiphoton fabrication of fibronectin-functionalized protein micropatterns – Stiffness-induced maturation of cell-matrix adhesions in human mesenchymal stem cells.	ACS Applied Materials & Interfaces Aug 24 2017 doi: 10.1021/acsami.7b07064.		Yes	Yes	

2017				Fan X, Zhu L, Wang K, Wang B, Wu Y, Xie W, Huang C, **Chan BP, Du Y***. Stiffness-Controlled Thermoresponsive Hydrogels for Cell Harvesting with Sustained Mechanical Memory.	Adv Healthc Mater. 2017 Mar;6(5). doi: 10.1002/adhm.201601152. Epub 2017 Jan 20.		Yes	Yes	
2016				**Tong MH, Huang N**, Zhang W, Zhou ZL, Ngan AHW, **Chan BP***. Multiphoton photochemical crosslinking-based fabrication of protein micropatterns with controllable mechanical properties for single cell traction force measurement.	Scientific Reports (2016); 6:20063. doi: 10.1038/srep20063.	2016	No	Yes	

2015				Zeng Y, Chen C, Liu W, Fu Q, Han Z, Li Y, Feng S, Li X, Qi C, Wu J, Wang D, Corbett C, **Chan BP**, Ruan D, **Du Y***. Injectable microcryogels reinforced alginate encapsulation of mesenchymal stromal cells for leak-proof delivery and alleviation of canine disc degeneration.	Biomaterials. 2015; 59:53-65.		Yes	Yes	
2019			✓	**Li HY,** Chooi WH, **Huang N,** Li YY, Chan D, Cheah K, **Chan BP*.** Collagen-based 3D culture systems for bovine nucleus pulposus cells (bNPCs)			Yes	Yes	
2019			✓	**Wang XN, Chan BP***. Multi-photon fabrication based micro-patterning of soluble niche factors: BMP2 as an example. (In preparation)			No	Yes	

			✓	**Yip CH, Chan BP*.** Cell niche factor screening by multiphoton biofabrication platform – Phenotype maintenance of bovine nucleus pulposus cells (bNPCs) (In preparation)			No	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)*
12-14 Sep 2018.	**Wang XN, Chan BP*.** Gradient immobilization of Wnt proteins by multiphoton biofabrication technology.	European Wnt Meeting. Heidelberg, Germany.		Yes	Yes	

14-17 Jun 2017. Boston, USA.	**Ma JN, Wang XN,** Chan CW, **Huang N, Chan BP*.** Multiphoton fabrication of functionalized protein micropatterns – an in vitro model for study on cell-matrix interactions in human mesenchymal stem cells.	International Society for Stem Cell Research Annual Meeting 2017.		Yes	Yes	
14-17 Jun 2017. Boston, USA.	**Yip CH, Chan BP*.** Developing a multiplex microscale screening platform by multi-photon biofabrication technology for maintenance of cell phenotype.	International Society for Stem Cell Research.		Yes	Yes	
30 Jul-2 Aug 2017. Hong Kong, China.	**Wang XN, Chan BP*.** Two-photon photochemical crosslinking-based immobilization of neutravidin on protein matrix surface.	8th WACBE World Congress on Bioengineering		Yes	Yes	
5-7 Jan 2017. Guangzhou, China.	**Huang N, Chan BP*.** Spatial control of ECM proteins in BSA microstructures with two photon crosslinking for cell matrix interaction study.	The 2nd International Symposium on Translational Nanomedicine.	2016	No	Yes	

3-6 September, 2016, Tamsui, Taipei.	**Wang X, Huang N, Chan BP.** Two-photon excited immobilization of BMP-2 on micrometer-scaled protein structure.	Tissue Engineering and Regenerative Medicine International Society-Asia Pacific Meeting.	2016	No	Yes	
3-6 September, 2016, Tamsui, Taipei.	**Yip CH, Huang N, Chan BP.** Coating of Monoclonal Antibodies Anti-integrin β1 and R-Phycoerythrin-preconjugated Anti-CD73 on Bovine Serum Albumin Matrixes by Two-photon Crosslinking.	Tissue Engineering and Regenerative Medicine International Society-Asia Pacific Meeting.	2016	No	Yes	
3-6 September, 2016, Tamsui, Taipei.	**Huang N, Chan BP.** Multiphoton-based Artificial Cell Niche Platform for Cell Matrix Interaction Study.	Tissue Engineering and Regenerative Medicine International Society-Asia Pacific Meeting.	2016	No	Yes	
July 6-8, 2015, Singapore.	**Tong MH, Chan BP.** Mechanical regulation of cell behaviors on multi-photon photochemical-crosslinking-based bovine serum albumin only micropillars with extremely low stiffness.	The 7th WACBE World Congress on Bioengineering.	2016	No	Yes	

July 6-8, 2015, Singapore.	**Tong MH, Huang N,** Zhou ZL, Ngan AHW, **Chan BP.** Multiphoton photochemical crosslinking-based fabrication of protein microstructures with controllable mechanical properties.	The 7th WACBE World Congress on Bioengineering.	2016	No	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
TONG Ming Hui	PhD	1 Sep 2012	31 Aug 2016
LI Hong Yi	PhD	1 Sep 2012	31 Aug 2016
HUANG Nan	PhD	1 Sep 2013	31 Aug 2017
WANG Xinna	PhD	1 Sep 2015	31 Aug 2019
YIP Chi Hung	PhD	1 Sep 2015	31 Aug 2019

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