Development of spheroids and organoids on the microfluidic chip

by Guocheng Fang

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Doctor of Philosophy

under the supervision of Prof. Dayong Jin, Dr. Hongxu Lu, Dr. Gungun Lin, A/Prof. David Gallego-Ortega

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Certificate of Original Authorship

I, Guocheng FANG, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Mathematical and Physical Sciences, Faculty of Science, at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution.

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Signature removed prior to publication.

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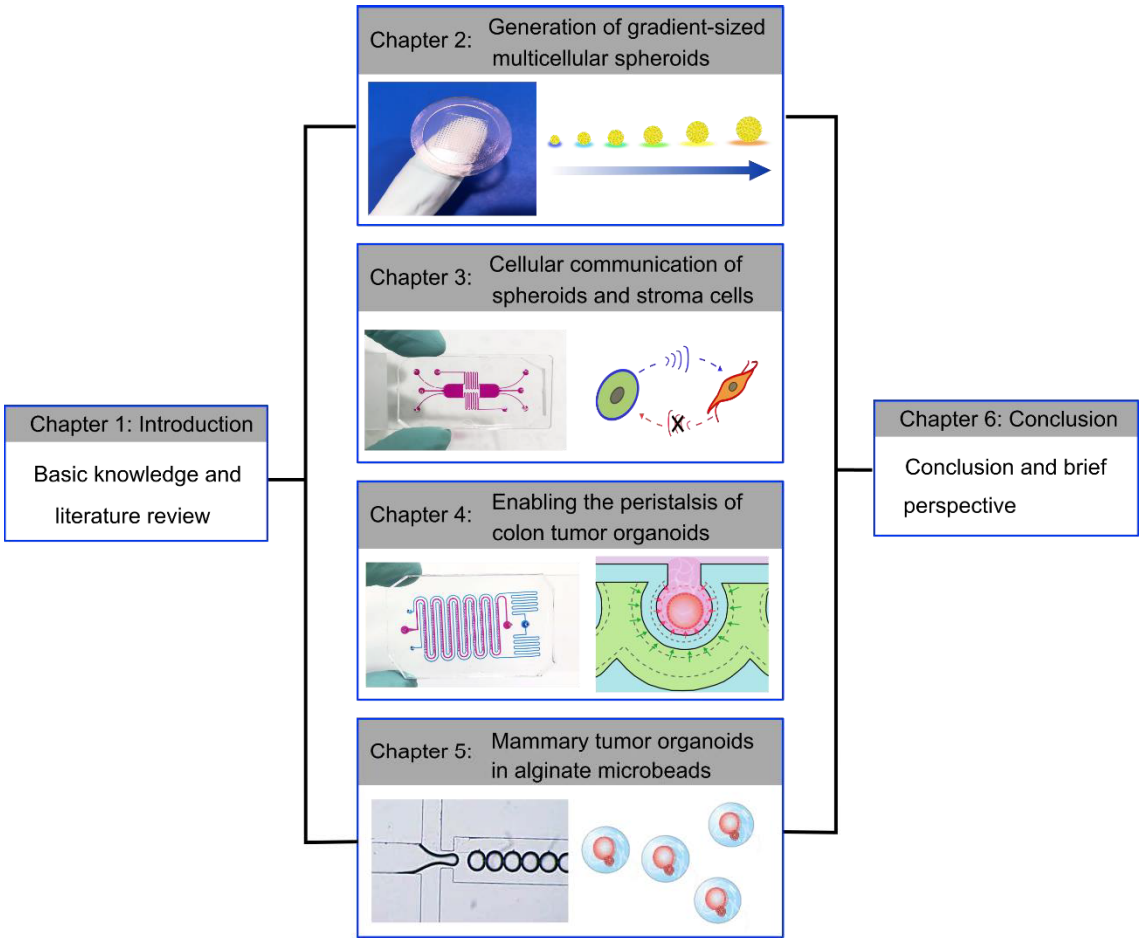
Format of Thesis

The thesis consists of six chapters. Chapter 1 introduces the biological fundamentals of multicellular spheroids and organoids and reviews the state-of-art microfluidic technology of spheroid and organoid culture. Chapters 2~5 cover the research in my PhD course that utilize microfluidic chips to culture spheroids and organoids and the sequential application of these models. Chapter 6 summarizes all the work and gives a brief perspective for potential future work. The thesis flow chat is illustrated below.



My PhD work aims to develop novel multicellular spheroids and organoids culture methods utilizing the microfluidic and microfabrication technology, also named on-chip technology. Chapter 2~3 focus on the spheroids and chapter 4~5 focus on the organoids. Chapter 2 describes a method to generate multicellular spheroids with gradient sizes on a single chip. Chapter 3 designed cell co-culture chip, which allows unidirectional signal communication, to investigate interactions between tumour spheroids and stromal cells.

In chapter 4, peristalsis is mimicked on microfluidic chips for the mechano-stimulated culture of human colon tumour organoids. In chapter 5, alginate is found to be a good candidate material for establishing the culture of mouse mammary tumour organoids. Taking the advantages of the droplet technique, we achieved high-throughput generation of mammary tumour organoids. Moreover, this model can be used to measure the luminal pressure during culture. In the last chapter, I conclude my PhD research work and briefly describe the potential future work.

List of Publications

➢ Articles

1. **G. Fang**, H. Lu*, R. Al-Nakashli, R. Chapman, G. Lin, D. Jin*. Enabling peristalsis of human colon tumour organoids on microfluidic chips. (Under revision in *Biofabrication*)
2. **G. Fang**, H. Lu*, L. Fuente, A. Law, G. Lin, D. Jin, D. Gallego-Ortega*. Mammary tumour organoids culture in non-adhesive alginate for luminal mechanics and drug screening. *Advanced Science (2021)* (DOI: 10.1002/advs.202102418).
3. **G. Fang**, H. Lu*, D. Jin*. Advances in spheroids and organoids on a chip. (In preparation).
4. **G. Fang**, H. Lu*, HA. Es, D. Wang, Y. Liu, ME. Warkiani, G. Lin, D. Jin*, Unidirectional intercellular communication on a microfluidic chip. *Biosensors and Bioelectronics (2020)* 175, 112833.
5. **G. Fang**, H. Lu*, A. Law, D. Gallego-Ortega, D. Jin, G. Lin, Gradient-sized control of tumour spheroids on a single chip. *Lab on a chip (2019)* 19 (24), 4093-4103.
6. A. Law, J. Chen, Y. Colino-Sanguino, S. Grimes, H. Lu, **G. Fang**, …, H. Ji and D. Gallego-Ortega*, ALTEN: a high-fidelity primary tissue-engineering platform to assess cellular responses in situ, submitted to *Advanced Science*, 2021
7. Y. Liu, F. Wang, H. Lu, **G. Fang**, S. Wen, …, L. Zhang, M. Stenzel, D. Jin*. Super-Resolution Mapping of Single Nanoparticles inside Tumour Spheroids. *Small (2020)* 16 (6), 2070030.
8. S. Jiang, M. Guan, J. Wu, **G. Fang**, X. Xu, …, S.Wang, P. Xi*. Frequency-domain diagonal extension imaging. *Advanced Photonics (2020)* 2 (3), 036005.
9. P. Jia†, **G. Fang**†, Z. Li, H. Liang, Y. Hong, T. Liang, J. Xiong*. Bellows spring-shaped ultrasensitive fiber-optic Fabry-Perot interferometric strain sensor. *Sensors and Actuators A: Physical* (2018) 277, 85-91.

([1-5] are closely related to my PhD program)

Abbreviations

2D	two-dimensional
3D	three-dimensional
aSCs	adult stem cells
BME	based membrane extract
BMP4	bone morphogenetic protein 4
BSA	bovine serum albumin
CAF	cancer-associated fibroblast
CAGR	compound annual growth rate
CE	counter electrode
CPA	chlorpromazine
DMD	digital micromirror device
DOX	doxorubicin
ECM	extracellular matrix
EGF	epithelial growth factor
ESCs	embryonic stem cells
FACS	fluorescence-activated cell sorting
FBS	fetal bovine serum
FoV	field of view
HR	hormone receptor
HUVECs	human umbilical vein endothelial cells
ICCP	interactive co-culture plates
IDTs	interdigital transducers
iPSCs	induced pluripotent stem cells
MSCs	mesenchyme stem cells

NA	numerical aperture
NK	natural killer
PAAm	prepolymer polyamide
PBS	phosphate buffer saline
PDMS	polydimethylsiloxane
PEG	polyethylene glycol
PHPMA	poly (N-(2-hydroxypropyl)methacrylamid)
PSCs	pluripotent stem cells
RE	reference electrode
RGD	arginylglycylaspartic acid
SIM	structured illumination microscopy
SPR	surface plasma resonance
SSAW	Surface acoustic wave
STORM	stochastic optical reconstruction microscopy
TGF-β1	transforming growth factor beta 1
TWPV	thick-wall pressure vessel
UV	ultraviolet
WE	working electrode
WNT	proto-oncogene protein
α-SMA	α-smooth muscle actin

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Abstract

Multicellular spheroids and organoids are typical *in vitro* models widely used in developmental biology, drug screening, precision medicine etc. Regulation and optimisation of these models and their residential microenvironments are crucial to maintaining their functions and behaviours. With the advances in microfabrication technology, microfluidic devices gradually become a useful tool for biomedical engineering of cultured spheroids and organoids. Building the complex multicellular systems on the microfluidic chips offers apparent advantages on these models (showcases in Chapter 1). The aim of this thesis is to implement a series of new designs of microfluidics devices and materials to improve the state-of-art cell co-culture and engineering of spheroids and organoids.

Multicellular spheroids are commonly used *in vitro* tumour models as they replicate the *in vivo* tumour. The size of spheroids plays a crucial role in cell responses during drug screening. Chapter 2 reports a method that can generate gradient-sized spheroids on a single chip with microwell arrays. As a liquid dome of cell suspension was formed on the chip, the size of spheroids can be regulated by the position of the microwells under the liquid dome.

Though tumour cells in the native microenvironment can be influenced by neighbouring stromal cells, the conventional co-culture cannot reveal the directional communications due to the random signal diffusion. In Chapter 3, a novel type of microfluidic chip was developed for the unidirectional communication between breast tumour spheroids and stromal cells.

The conventional culture of *in vitro* models lacks the mechanical cues, especially for the gastrointestinal organoids. In Chapter 4, a microfluidic chip that can mimic intestinal peristalsis was developed for human colon tumour organoids. The chip allows organoids' high-throughput dynamic culture individually and parallelly in a microwell array.

The matrix that supports 3D cell growth poses another challenge that currently hinders the developments of organoid culture, due to the high cost and batch-to-batch variations. Chapter 5 found that the naturally-derived polymer, alginate, can be used for the mouse mammary tumour organoids, especially for the luminal organoids.

In summary, this thesis has developed a series of microfluidic designs and techniques for spheroids/organoids culture towards their applications in drug screening, cell biology and nanomedicine. This thesis advances the potential of on-chip technology, materials and devices for biomedical engineering.