UTS UNIVERSITY OF TECHNOLOGY SYDNEY

3D Microfluidic System to Study of Tumour Microenvironment

by Hamidreza Aboulkhyer Estarabadi

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

under the supervision of Prof. Majid Ebrahimi Warkiani

University of Technology Sydney
Faculty of Engineering and Information Technology

April 2022

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, **Hamidreza Aboulkheyr Estarabadi** declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Biomedical Engineering 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. This research is supported by the Australian Government Research Training Program.

Signature: Date: 21-09-2022

Production Note:
Signature removed prior to publication.

Impact of COVID-19 on the completion of the thesis

This thesis's research aims, and objectives have been planned to complete within three years of candidature; however, the Covid-19 crisis and pandemics followed by long term lockdowns and restrictions in laboratory experiments significantly affected the few numbers of objectives of this research. Among all chapters, Chapter 8 of this thesis is affected extremely by Covid-19 restrictions ruled by the state government, campus lockdowns, and limitations in a daily laboratory working time. A detailed explanation of these impacts on methodology and results of Chapter 8 has been stated on the starting page of this chapter.

Acknowledgement

I would like to express my sincerest gratitude and appreciation to my supervisor, Prof. Majid Ebrahimi Warkiani, for his invaluable guidance, encouragement, patience, and, most importantly support through my PhD. He is an exceptional role model for both my academic and personal life. I am grateful for giving me the opportunity to be a member of his research team and for all that I have learned from him. My PhD research always benefited from his constructive and invaluable scientific guidance. I would like to thank my co-supervisor Prof. Dyong Jin, for his guidance and constant support through my PhD and for serving on my examination committee. Also, a sincere thank you to Prof. Thomas R Cox from the Garvan Institute of Medical Science for his insightful biological inputs and for sharing his invaluable expertise with me.

I extend my thanks to all past and present members of Warkiani Lab. It was an amazing experience and honour to work with a group of friendly, motivated, and knowledgeable scholars and students during my PhD. I would like to additionally thank to Sydney Vital Translational Cancer Research for awarding me a prestigious supporting grant.

Finally, my deepest gratitude and appreciation goes to my wonderful and lovely wife Monireh for her unconditional and endless encouragement and love. She dedicated her precious time and life to support me during my PhD journey. I am extremely lucky to have her in my life. Thank you, Monireh, for being my best friend and always believing in me. A special thank you to my father, Mohammad-Masoud and my mother, Malihe, for their unconditional love, dedication, and many years of moral support during my life. I am sincerely thankful to my brother and sister in Iran who have made it possible for me to focus on my work by supporting our parents.

Format of Thesis

This thesis is prepared in one introduction and six chapters, including published and under-publication research articles. The chapter 1; “Introduction” provides an overview of the tumour microenvironment (TME) and its cellular and molecular features, emphasizing the importance of modelling TME and current TME models. In Chapter 2, the current application of microfluidic technology in cancer research and therapy is discussed comprehensively and the remain challenges that required to be addressed are highlighted as well. Following these, in Chapter 3, a comprehensive in-silico data analysis has been performed to figure-out the association of cellular components of TME with tumour immunity and activation of invasion and migration. Moreover, in this chapter we highlighted potential of drug repurposing in cancer treatment and targeting TME. The findings and output of this chapter are modeled in our microfluidic system through the next chapters. Chapter 4 – Chapter 8 are the application-specific chapters showcasing the application of microfluidic-based 3D cell culture in particular modelling features of TME and drug discovery. Chapter 4 demonstrates modelling tumour stromal cells and cancer cell interaction and its effect on immune suppression on cancer cells which has not been before. Moreover, in this chapter, the application of an anti-fibrotic drug on reversion of immune sensitivity has been shown for the first time. Following this, in Chapter 5, the immune-modulation role of mesenchymal stem cells in the stimulation of immune escape and drug response is explored using a microfluidic device. To model the biological and molecular aspect of initiation and invasion of cancer cells, Chapter 6 illustrates the application of microfluidic for mimicking cancer and tumour-stromal cells invasion and cancer-stem cells formation using the culture of organotypic tumour spheroids containing stromal cells in the device. Moreover, this chapter highlights the therapeutic potential of an anti-fibrotic agent discovered in Chapter 3 on targeting key cytokines and suppression of invasion. As ECM is the main component of TME, in Chapter 7, we modeled the role of matrix stiffness on cancer cells immunity and invasion. In Chapter 8, the formation of microvasculature and pre-vascular tumour organoids in the microfluidic device has been shown. Finally, in last chapter, “Conclusion and Future Work”, the limitations and challenges of this thesis project and future research direction are discussed.

List of publications

- Azadi, Shohreh, Hamidreza Aboulkheyr Es, Arutha Kulasinghe, Pritam Bordhan, and Majid Ebrahimi Warkiani. "Application of microfluidic technology in cancer research and therapy." In Advances in clinical chemistry, vol. 99, pp. 193-235. Elsevier, 2020.

- Aboulkheyr Es, Hamidreza, Sareh Zhand, Jean Paul Thiery, and Majid Ebrahimi Warkiani. “Pirfenidone reduces immune-suppressive capacity of cancer-associated fibroblasts through targeting CCL17 and TNF-beta.” Integrative Biology 12, no. 7 (2020): 188-197.

- Aboulkheyr Es, Hamidreza, Bahareh Bigdeli, Sareh Zhand, Amir R. Aref, Jean P. Thiery, and Majid E. Warkiani. "Mesenchymal stem cells induce PD-L1 expression through the secretion of CCL5 in breast cancer cells." Journal of Cellular Physiology 236, no. 5 (2021): 3918-3928.

- Es, Hamidreza Aboulkheyr, Thomas R. Cox, Ehsan Sarafraz-Yazdi, Jean Paul Thiery, and Majid Ebrahimi Warkiani. "Pirfenidone Reduces Epithelial–Mesenchymal Transition and Spheroid Formation in Breast Carcinoma through Targeting Cancer-Associated Fibroblasts (CAFs)." Cancers 13, no. 20 (2021): 5118.

- Azadi, Shohreh, Hamidreza Aboulkheyr Es, Sajad Razavi Bazaz, Jean Paul Thiery, Mohsen Asadnia, and Majid Ebrahimi Warkiani. "Upregulation of PD-L1 expression in breast cancer cells through the formation of 3D multicellular cancer aggregates under different chemical and mechanical conditions." Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 1866, no. 12 (2019): 118526.

Table of Contents

List of Illustrations and Figures

List of Abbreviations

αSMA: Alpha Smooth Muscle Actin

CAF: Cancer-Associated Fibroblasts

ECM: Extra-Cellular Matrix

EGM: Endothelial Growth Medium

EMT: Epithelial-Mesenchymal Transition

FAP: Fibroblasts-Activated Protein

MCA: Multi-Cellular Aggregates

PD-L1: Programmed Death-Ligand 1

PFD: Pirfenidone

TAM: Tumour-Associated Mesenchymal Stem Cells

TCGA: The Cancer Genome Atlas

TIL: Tumour Infiltration Lymphocytes

TME: Tumour Microenvironment

TNBC: Triple-negative Breast Cancer

YAP: Yes-Activated Protein

3D microfluidic system to study of tumour microenvironment

Hamidreza Aboulkheyr Estarabadi
Doctor of Philosophy
School of Biomedical Engineering
University of Technology Sydney
2022

Abstract

The tumour microenvironment (TME) plays a crucial role in cancer initiation, progression, and development. To better understand the cellular and molecular feature of TME, various in-vitro, ex-vivo and in-vivo models of TME have been developed, ranging from 2D cancer cells lines, 3D organoids to genetically engineered animal models. However, these models faced a series of challenges and drawbacks limiting the study of the particular feature of TME in a specific type of cancer. Recently, microfluidic technology introduced a new platform to mimic the TME ecosystem at the micro-scale through an advanced engineered system. This platform enables modelling a wide range of TME properties from ECM to the vasculature and the complex cellular structure by co-culturing 3D tumour cells cost-efficient, real-time, and controllable fashion. However, the major focus of the current microfluidic models of TME is on cancer cells rather than tumor-stromal cells and their effects on tumour immunity and immunotherapy. Moreover, these models unbale to provide a space for down-stream molecular analysis including cytokines analysis or RNA-sequencing. Additionally, the design of majority of these models is complex which might limit broad application of these models.

This thesis focuses on addressing these challenges using a low-cost and easy to use microfluidic device which enable wide range of applications from in-vitro to ex-vivo and drug discovery and immune-oncology. Using developed model in this thesis, the most impactful contributions of this thesis was the discovery of the role of the tumor stromal cells including cancer-associated fibroblasts (CAFs) and tumour-associated mesenchymal stem cells (TAMs) in tumour immunity and that targeting these populations can significantly reduce immune-suppression capacity in TME. We discovered and introduced potential of an anti-fibrotic drug called Pirfenidone for targeting CAFs and secreted cytokines through a comprehensive in-silico data analysis at both bulk and single cell level in breast cancer for the first time. Using our developed microfluidic model of TME, we showed that how pirfenidone reduce CAFs and MSC activity, invasion, immune-suppression, and tumor initiation in breast carcinoma. Moreover, we modeled the effects of matrix stiffness which is regulated by CAFs on tumour immunity and expression of immune checkpoints. Finally, we tried to develop a novel method for generation of vascularized tumor organoids in device which extremely affected by Covid-19 pandemics and lockdowns.