Digital Profiling of Circulating Extracellular Vesicles at Single-Upconversion Nanoparticle Sensitivity and Resolution

by Guan HUANG

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Doctor of Philosophy

under the supervision of Prof. Dayong Jin,
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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Guan Huang declare that this thesis is submitted to 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.

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Signature of Student:

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List of publications

1. **G. Huang**, Y. Zhu, S. Wen, H. Mei, Y. Liu, D. Wang, M. Maddahfar, Q. P. Su, G. Lin, Y. Chen and D. Jin. Single Small Extracellular Vesicle (sEV) Quantification by Upconversion Nanoparticles. *Nano Letters*. 22, 3761–3769 (2022).
2. **Guan Huang**, Yongtao Liu, Dejiang Wang, Ying Zhu, Shihui Wen, Dayong Jin*. Upconversion Nanoparticles for Super-resolution Imaging of Single Small Extracellular Vesicles (in preparation)
3. **Guan Huang**, Laura Laura Rodriguez de la Fuente, David Gallego-Ortega, Ying Zhu, Yongtao Liu, Dayong Jin*. Preclinical detection of circulating EVs (in preparation)
4. **Huang, G.**; Lin, G.; Zhu, Y.; Duan, W.; Jin, D. Emerging Technologies for Profiling Extracellular Vesicle Heterogeneity. *Lab on a Chip* **20**, 2423–2437 (2020) (highlighted on front cover)
5. Chen, Y, Shimoni, O, **Huang, G**, Wen, S, Liao, J, Duong, HTT, et al. Upconversion nanoparticle-assisted single-molecule assay for detecting circulating antigens of aggressive prostate cancer. *Cytometry Part A*. 2021; 1– 11.
6. Liu, Y., Lin, G., Bao, G., Guan, M., Yang, L., Liu, Y., Wang, D., Zhang, X., Liao, J., Fang, G., Di, X., **Huang, G.**, Zhou, J., Cheng, Y., and Jin, D. Stratified Disk Microrobots with Dynamic Maneuverability and Proton-Activatable Luminescence for in Vivo Imaging. *ACS Nano* 2021 15 (12), 19924-19937
7. Lin, G., Liu, Y., **Huang, G.**, Chen, Y., Makarov, D., Lin, J., Quan, Z. and Jin, D.3D Rotation-Trackable and Differentiable Micromachines with Dimer-Type Structures for Dynamic Bioanalysis. 2021. *Adv. Intell. Syst.*, 3: 2000205.

([1,2,3,4,5] are closely related to my PhD program)

Conferences

1. Background-free single luminescent nanoparticle assay for detecting tumor-derived extracellular vesicles, Oral Poster, Thomas Ashworth CTC & Liquid Biopsy Symposium 2021, Sydney, Australia
2. Profiling Single Extracellular Vesicles from Single Cells, Oral Presentation, Australasian Extracellular Vesicles Conference 2021, Auckland, New Zealand

Award

1. Vice-Chancellor's Postgraduate Research Conference Fund 2021, UTS, Australia.

2. TCRN PhD Scholarship Top-up Awards 2020, Translational Cancer Research Network, Australia
3. Australasian Extracellular Vesicles Conference Outstanding student presentation award 2020, ANZSEV, Australia and New Zealand

Structure of Thesis

This thesis has five chapters. Chapter 1 is on the basic knowledge and literature review. Chapter 2-4 are the three core result chapters reporting the experiments, the research results and discussions of developing nanoparticles and imaging tools to detect and qualify the surface biomarkers of single extracellular vesicles (EVs). In chapter 2, the types and numbers of EV surface markers have been quantified and profiled at single nanoparticle sensitivity. In chapter 3, super-resolution imaging techniques have been used to digitize the number of nanoparticles on single EVs. In chapter 4, the developed technology platform has been applied to prognose the cancer metastasis on two mouse models, both relevant breast cancer cohort. Chapter 5 is the conclusion of this thesis and discussions for future works. I organize the five chapters following the flowchart below:

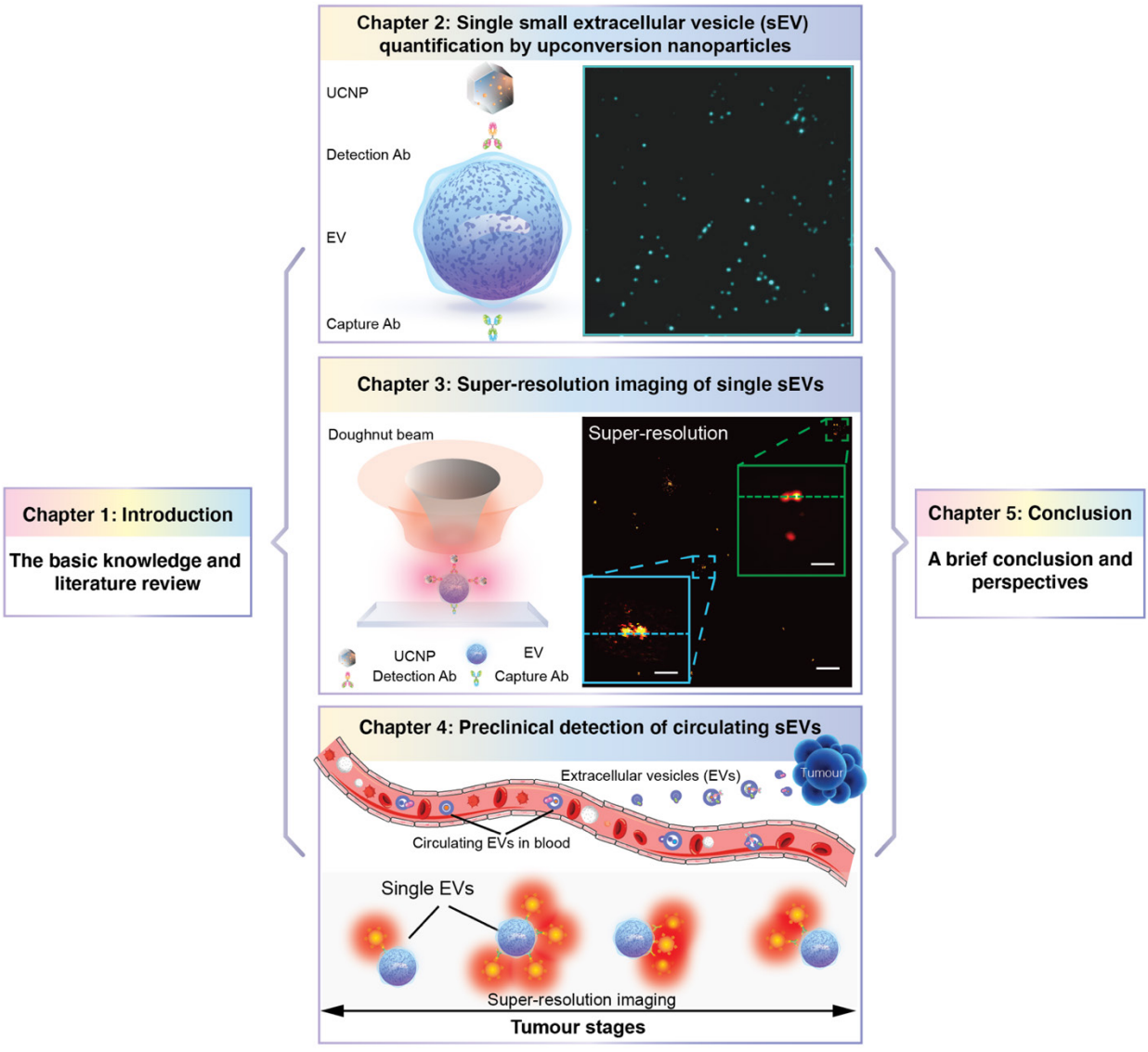

Table of Contents

Abstract

Circulating extracellular vesicles (EVs) carry significant information about the progression stages of tumour sites. Quantification of low-abundant EVs and statistical profiling of the heterogeneity of single EVs, particularly from liquid biopsy sampling, will guide clinical decisions on the stages of tumour progression. However, the nanoscopic sizes (typically 40-200 nm) and the extremely small quantity of cargo materials demand the high detection sensitivity, stability, resolution and throughput to be simultaneously achieved.

Nanotechnology has been broadly used in the field of liquid biopsy. This thesis explores a new strategy for ultra-sensitive, photo-stable, and super-resolution immunoassay of single EVs, which is based on the development, bio-conjugation and application of upconversion nanoparticles (UCNPs). In chapter 2, we apply UCNPs for direct enumeration of single CD9 and EpCAM positive EVs ($^{CD9+EpCAM+}$EVs). The achieved single-molecule sensitivity results in a femtomolar detection limit (1.8×10^6 EVs mL^{-1}), which was nearly 3 orders of magnitude lower than the standard enzyme-linked immunosorbent assay (ELISA). Compared with previous luminescence resonance energy transfer (LRET) method using UCNPs for detection of EVs, our technique achieves single tumour-derived sEV quantification. In chapter 3, we report super-resolution imaging technique for single $^{CD9+EpCAM+}$EV analysis. The upconversion luminescence of single UCNPs can nonlinearly response to a donut-shaped scanning beam, so that a resolution better than 40 nm can be achieved beyond the diffraction limit. In chapter 4, with the ultra-sensitivity and photo stability achieved by UCNPs as well as super resolution offered by a donut-shaped scanning, the preclinical translation capability of the integrated technology platform has been examined by two types of breast cancer mouse models. Our results suggest that the population of cancer-derived circulating EVs, detected and classified by the number of UCNPs, can be used to monitor the metastatic tumour progression, including non-metastasis/high-metastasis and low-metastasis/high-metastasis mouse models. Furthermore, we find that the number of UCNPs on single EVs can be used to index the stage of metastatic tumour progression. In chapter 5, we discuss the challenges and opportunities of this thesis towards clinical translation, which suggests a new scope of research by integrating nanotechnology, microscopy imaging and lab-on-a-chip devices for EV research and applications. This thesis presents a viable approach of using the EVs-based liquid biopsy for tumour diagnosis and prognosis.

Key words: extracellular vesicles, upconversion nanoparticles, super-resolution, cancer metastases, liquid biopsy