Visiting Student Project Description

Sydney Pharmacy School

Faculty of Medicine and Health

The University of Sydney, Australia

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Prof. Wojciech Chrzanowski

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- **Research Group**: High-degree research students, visiting scholars/students and honors students

PROJECT TITLE: The cellular factory: extracellular vesicles as the next-generation drug delivery vehicles

PROJECT AIMS AND BACKGROUND

Conventional delivery vehicles such as liposomes and polymeric nanoparticles suffer from exceptionally high off-target delivery [1]. They possess broad in vivo biodistribution, lack the ability to cross cellular barriers, and undergo unspecific cellular uptake/decomposition [2]. Despite encouraging in vitro studies, it has been reported that only 1-2 % of nano-based vehicles reach their target [3]. This means that the use of these vehicles may lead to severe side effects and is associated with a substantial misuse of expensive active compounds. To reduce both the waste of active compounds, and potential side effects we need a new class of vehicles that have superior abilities to (1) cross physical cellular barriers, (2) target specific cells and tissues, and (3) exhibit low toxicity & low immunogenicity.

Extracellular vesicles (EVs), which are membrane-surrounded nanoscale structures secreted ubiquitously by all cells, are an ideal candidate for drug delivery because they possess the intrinsic ability to target cells, low toxicity & low immunogenicity, as well as the capability to translocate through biological/cellular barriers effectively [4]. The latter is considered the 'Holy Grail' in drug delivery, and it is unachievable using traditional delivery vehicles (such as liposomes). This feature makes EVs superior to other drug carriers. EVs can also be bioengineered by inserting exogenous compounds [5]. This means that active compounds (e.g., miRNA) can be added to EVs and delivered to a specific site using the inherent ability of EVs to target different cells [6]. Importantly, EVs are 'unpacked' effectively inside the cells, and release the cargo where it is most needed to promote desired biological effects.

The aim of the project is to modify EV composition with exogenous compounds and demonstrated their stability and delivery effectiveness.

Here we will employ nano-bioreactors to either mechano- or electroporate the membrane of EVs, enabling the effective incorporation of exogenous compounds into EVs in a finely controlled manner.

This project will develop technology to modify EV cargo with exogenous compounds. The knowledge about how to enrich EVs' cargo can be utilised to engineer precision delivery vehicles for different sectors including the food and plant science, agriculture, veterinary and pharmaceutical industries.

Dr Philip Chi Lip Kwok

Dr Philip Chi Lip Kwok is a Senior Lecturer in Pharmaceutical Sciences in the School of Pharmacy, The University of Sydney. He obtained his Bachelor of Pharmacy degree with First Class Honours in 2002 from this Faculty. He became a registered pharmacist after one year of training in a community pharmacy. Dr Kwok then undertook his PhD studies on pharmaceutical aerosol electrostatics in the Faculty of Pharmacy at The University of Sydney and graduated in 2007. He was a research associate in the same group until August 2011 and became an assistant professor in the Department of Pharmacology and Pharmacy at The University of Hong Kong in September 2011. Dr Kwok returned to The University of Sydney as a Lecturer in Pharmaceutical Sciences at the end of July 2017. His research is in pulmonary drug delivery. In particular, he specialises in the engineering, physicochemical characterisation, and electrostatics of pharmaceutical aerosol formulations. He has collaborated with academic and industrial researchers, both locally and internationally, on formulation-focused as well as cross-disciplinary projects.

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- **Research Group**: 4 PhD students

Project:

Inhalable probiotic aerosols for respiratory infections

Co-Supervisor:

Prof Hak-Kim Chan

Project Summary:

Conventional management of respiratory infections include symptomatic or antimicrobial therapies, with prevention regarded better than treatment. Current preventive measures include vaccination and physical barriers, e.g., face masks, social distancing, and isolation. However, vaccines are only available for a few respiratory pathogens, while physical barriers create social obstacles. Furthermore, vaccines only protect against specific pathogen strains and need constant reformulation according to mutation trends. On the other hand, frequent use of antimicrobials exacerbates resistance development. Therefore, the ideal strategy is a platform method that can strengthen the immunity against a broad range of pathogens to reduce pathogen growth, symptom severity, and mortality if infected.

A platform for the delivery of inhaled probiotics is potentially a plausible preventive strategy against respiratory infections. The immunomodulatory effects and health benefits that probiotics emanate have attracted significant interest in their potential clinical applications. Orally delivered probiotics (e.g.

Lactobacillus spp.) have demonstrated protective and anti-inflammatory effects in respiratory infections and non-infective airway inflammation. Although the underlying mechanism remains elusive, the immunomodulation effect is attributed to the intricate physiological and immunological network that exists between the gastrointestinal and respiratory tracts, known as the gut-lung axis. However, delivering probiotics orally for lung conditions is indirect, as it relies on the proper functioning of the highly complex gut-lung axis, which may render the efficacy slow and/or variable. We hypothesise that probiotic bacteria delivered as aerosols into the respiratory tract can elicit a local, favourable immunomodulatory effect that reduces the burden of infection and pathogen load, as well as increasing survival, when subsequently exposed to the pathogen. To test this hypothesis, we will:

- **Aim 1.** Formulate inhalable probiotic aerosols and characterise their *in vitro* aerosol performance; and
- **Aim 2.** Test the *in vitro* inhibitory effects of these formulations against a respiratory pathogen, e.g. *P. aeruginosa*.

Techniques/Methods:

Spray drying, nebulisation, high performance liquid chromatography, laser diffraction, cascade impaction, *in vitro* bacterial culturing

Selected Publications:

1. X. Li, Q. Wang, X. Hu, W. Liu, Current status of probiotics as supplements in the prevention and treatment of infectious diseases, Frontiers in Cellular and Infection Microbiology, 12 (2022) 789063.
2. K. Martens, B. Pugin, I. De Boeck, I. Spacova, B. Steelant, S.F. Seys, S. Lebeer, P.W. Hellings, Probiotics for the airways: Potential to improve epithelial and immune homeostasis, Allergy, 73 (2018) 1954-1963.
3. S. Alvarez, C. Herrero, E. Bru, G. Perdigon, Effect of *Lactobacillus casei* and yogurt administration on prevention of *Pseudomonas aeruginosa* infection in young mice, Journal of Food Protection, 64 (2001) 1768-1774.
4. L. Khailova, C.H. Baird, A.A. Rush, E.N. McNamee, P.E. Wischmeyer, *Lactobacillus rhamnosus GG* improves outcome in experimental *Pseudomonas aeruginosa* pneumonia: Potential role of regulatory T cells, Shock, 40 (2013) 496-503.
5. M.S. Fangous, Y. Alexandre, N. Hymery, S. Gouriou, D. Arzur, G. Le Blay, R. Le Berre, Lactobacilli intra-tracheal administration protects from *Pseudomonas aeruginosa* pulmonary infection in mice – a proof of concept, Beneficial Microbes, 10 (2019) 893-900.
6. M.S. Fangous, P. Gosset, N. Galakhoff, S. Gouriou, C.-A. Guilloux, C. Payan, S. Vallet, G. Héry-Arnaud, R. Le Berre, Priming with intranasal lactobacilli prevents *Pseudomonas aeruginosa* acute pneumonia in mice, BMC Microbiology, 21 (2021) 195.
7. G. Harata, F. He, N. Hiruta, M. Kawase, A. Kubota, M. Hiramatsu, H. Yausi, Intranasal administration of *Lactobacillus rhamnosus GG* protects mice from H1N1 influenza virus infection by regulating respiratory immune responses, Letters in Applied Microbiology, 2010 (2010) 597-602.
8. H. Zelaya, A. Tada, M.G. Vizoso-Pinto, S. Salva, P. Kanmani, G. Agüero, S. Alvarez, H. Kitazawa, J. Villena, Nasal priming with immunobiotic *Lactobacillus rhamnosus* modulates inflammation–coagulation interactions and reduces influenza virus-associated pulmonary damage, Inflammation Research, 64 (2015) 589-602.

9. M.-K. Park, V. Ngo, Y.-M. Kwon, Y.-T. Lee, S. Yoo, Y.-H. Cho, S.-M. Hong, H.S. Hwang, E.-J. Ko, Y.-J. Jung, D.-W. Moon, E.-J. Jeong, C. Kim, Y.-N. Lee, J.-H. Jang, J.-S. Oh, C.-H. Kim, S.-M. Kang, Lactobacillus plantarum DK119 as a probiotic confers protection against influenza virus by modulating innate immunity, PLoS One, 8 (2013) e75368.
10. Y. Tomosada, E. Chiba, H. Zelaya, T. Takahashi, K. Tsukida, H. Kitazawa, S. Alvarez, J. Villena, Nasally administered *Lactobacillus rhamnosus* strains differentially modulate respiratory antiviral immune responses and induce protection against respiratory syncytial virus infection, BMC Immunology, 14 (2013) 40.

Senior Lecturer, Dr Slade Matthews

Research Passion – I am passionate about finding ways computer technology can improve human understanding of both wanted and unwanted chemical effects in the body. My lab works in a Python environment using scientific computing libraries including scikitlearn, pandas, rdkit, Pytorch to build QS(P/A)R models.

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- https://scholar.google.com/citations?user=P1JKDSgAAAAJ&hl=en
- **Research Group**: Computational Pharmacology & Toxicology Laboratory: https://github.com/cptlab

Project 1: Investigation of molecular representations for toxicity prediction

Co-Supervisor:

Project Summary: In this project we will use the large NIEHS CATMoS (Collaborative Acute Toxicity Modeling Suite) dataset of over 50, 000 molecules with acute toxicity endpoints to investigate molecular representations for association with toxicity apical endpoints.

Techniques/Methods: Python environment using scientific computing libraries including scikitlearn, pandas, rdkit, Pytorch to build QS(P/A)R models.

Selected Publication: Lui, R., D. Guan, and S. Matthews, *A comparison of molecular representations for lipophilicity quantitative structure-property relationships with results from the SAMPL6 logP Prediction Challenge*. J Comput Aided Mol Des, 2020.

Project 2: Modelling the toxic effects of antimicrobial peptides on kidney using pfeature and literature based endpoint data.

Co-Supervisor:

Project Summary: Antimicrobial peptides (AMPs) have emerged as promising therapeutic agents against a wide range of bacterial infections. However, their potential toxicity towards mammalian cells, particularly the kidney, has raised concerns. Computational approaches that can predict the toxic effects of AMPs on kidney could aid in the development of safe and effective AMP-based therapies. In this project, we propose to use pfeature, a Python-based tool for molecular feature calculation, and literature data sources to model the toxic effects of AMPs on kidney.

Techniques/Methods: Python environment using scientific computing libraries including scikitlearn, pandas, rdkit, Pytorch to build QS(P/A)R models.

Selected Publication: Tse, E.G., et al., *An Open Drug Discovery Competition: Experimental Validation of Predictive Models in a Series of Novel Antimalarials*. Journal of Medicinal Chemistry, 2021.

A/Prof. Fanfan Zhou

My research group is a young and dynamic team with PhD students, visiting scholars/students and honors students. Our research is primarily focused on two themes:1) drug discovery and development for human eye diseases and cancers; 2) drug design and optimisation targeting human Solute Carrier Transporters that control drug uptake into tissues. I have established a research consortium that includes national and international experts in these two fields and welcome you joining our group.

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- **Research Group**: High-degree research students, visiting scholars/students and honors students

Project 1: Explore the differential profiles of mitochondrial health and function in human Uveal melanoma cells.
Co-Supervisor: A/Prof. Thomas Grewal
Project Summary: Uveal melanoma (UM) accounts for ~85% of all ocular melanomas in humans. Up to 50% of patients develop metastatic disease that has a poor survival of <18 months. Mitochondria are the powerhouse of a cell, involved in cell survival, proliferation, death etc. It is well known that mitochondrial health and function are distinguished in tumour *vs.* normal cells. Moreover, in anti-cancer drug development, mitochondria have been considered as a key therapeutic target. Our laboratory has a collection of various UM immortalised cell lines as well as patient tumour-derived primary cultures, all of which cell models with distinguished genetic profiles. To better understand the role of mitochondria in the tumour progression of UM, it is essential to evaluate mitochondrial health and function in these cell models. Such information will contribute to elucidating the differential response of these cell models to therapeutic molecules targeting mitochondria. In this project we will use a range of classic molecular and biochemical approaches to define the mitochondrial health and function in a variety of UM cell models. The findings from this project will provide critical information to understand the pathogenesis of UM and may identify potential target(s) for therapeutic development against UM.
Techniques/Methods: tissue culture, cell viability and cell death assays, western blotting, flow cytometry, fluorescence microscopy, metabolism analysis
Selected Publication: Cell Oncol (Dordr). 2022 Aug;45(4):601-619 & FEBS 2021 Apr 10. doi: 10.1111/febs.15869.

Project 2:
Co-Supervisor: Dr. Ling Zhu
Project Summary: Human retina is the layer of cells situated at the back of eyes. It is the main site for human vision. Retinal degeneration is a main cause of blindness worldwide. It is triggered by many factors such as aging, UV exposure, oxidative stress etc. The major hurdle in the treatment of this condition is how we can deliver therapies to the targeted type of retinal cells. Our research focuses on developing new treatments for retinal degeneration as well as selecting the best drug delivery carrier(s) for these new treatments. In this project, we will utilise the nano technology to customise nanoparticles and explore their capacity in drug conjugation and more importantly, their targetability to the specific retinal cell types. The outcome of the project is to better deliver drugs to the eyes and improve the treatment outcome for retinal degeneration, which will eventually shift the way we fight with human blinding diseases.
Techniques/Methods: tissue culture, organ-on-a-chip technology, nanoparticle synthesis, western blotting, fluorescence microscopy
Selected Publication: Adv Drug Deliv Rev. 2023 Jun 13;199:114965 & Theranostics. 2022 Sep 11;12(15):6705-6722.

Dr Hien Duong

Our research is multidisciplinary which focuses on the new concepts and ideas to engineer novel materials/biomaterials and devices at nanoscale. The ultimate goal is to utilise the nanotechnology in the form of nanoparticles to extend our life in two ways: i) early detection of life-threatening diseases and ii) improvement of their current therapy. Our research area includes polymer synthesis, fabrication, nanomaterial characterisation, and biological testing.

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• **Research Group**: One PhD student, one MPhil student, two postdocs.

Project summary: Bacteriophage nanobots

Project Summary: We are facing an increasing problem of antimicrobial resistance (AMR) not just in Australia but worldwide. Hospital pressure and increased antibiotic usage in the COVID pandemic has increased the risk from AMR. Bacteriophages (phages) are natural predators of bacteria and have proven to be an alternative solution to AMR. In this project, we will match the most common bacterial targets to high-value phages and most important antibiotics and make these available for further R&D or clinical trials.

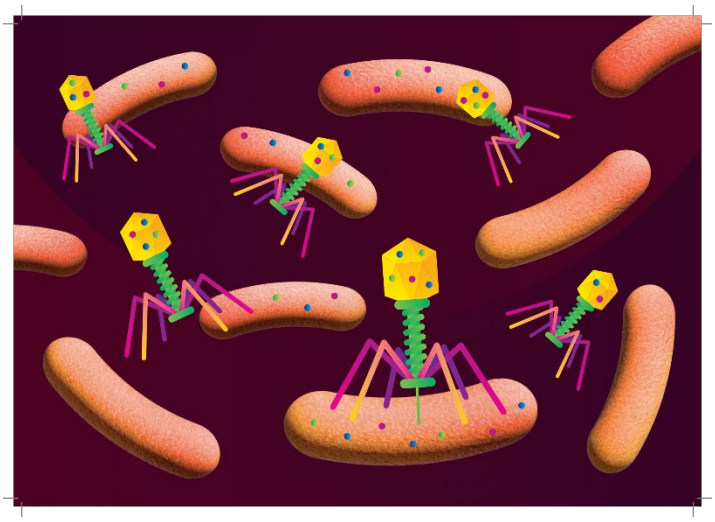

Figure. Phages and therapeutic agents to enhance the efficacy of each sole agent.

Techniques/Methods: Polymer synthesis and characterisation using advanced polymerization technique, nanoparticles preparation and characterisation, microscopy techniques, biological testing.