Heart Research Institute

Research Activities and Teaching Capabilities

TODAY'S RESEARCH, TOMORROW'S CURE FOR HEART DISEASE

Cardiovascular disease is Australia's – and the world's – number one killer.

The Heart Research Institute (HRI) is an internationally recognised medical research institute that performs groundbreaking cardiovascular research.

The HRI's mission is to prevent death and suffering from cardiovascular diseases, a complex array of diseases affecting the heart and blood vessels.

We will address areas of unmet need in cardiovascular diseases including coronary artery disease, stroke, peripheral artery disease, hypertension, heart failure, preeclampsia, congenital heart disease and pulmonary vascular disease, as well as metabolic complications such as diabetes.

Specifically, our research programs will:

- Provide greater understanding of the pathogenesis, development, and early detection of cardiovascular diseases
- Develop new drug therapies and devices to prevent and treat cardiovascular diseases and translate these through to clinical trials
- Train the next generation of cardiovascular research leaders
- Connect cardiovascular research communities to maximise collaboration and research translation.

We collaborate with institutes in Australia and globally – with over 110+ collaborations across 44 countries – and are partnered with the Royal Prince Alfred Hospital, the Sydney Local Health District and Sydney Health Partners in Australia.

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JOIN THE HRI TEAM

HRI is committed to providing an exciting and encouraging environment of research excellence and mentoring for honours, graduate and postgraduate students to carry out their research and advance their scientific careers.

OPPORTUNITIES

Masters and PhD

HRI students are provided with a wide range of practical, intellectual and emotional support from their supervisors, membership to and the associated benefits of an early-mid career research (EMCR) group, as well as the support and collegiality of the research family at HRI.

Our PhD students benefit from world-class research training, interaction with a diverse array of research groups across our own institute and that of our collaborative partners, and a variety of opportunities to learn more widely from some of Australia's best medical researchers.

HRI offers advanced clinical trainees the opportunity to undertake a PhD with one of our research groups. HRI holds a longstanding relationship with the Royal Prince Alfred Hospital (RPA), with several of our group leaders sharing clinical appointments with RPA within the Departments of Cardiology and Haematology.

PhD candidates who are successful in securing an Australian Government Research Training Program (RTP) scholarship are also awarded an HRI top-up scholarship of $A6000.

Honours

HRI welcomes honours students from a variety of affiliated universities and disciplines, including The University of Sydney; the University of New South Wales (UNSW); and the University of Technology, Sydney (UTS).

The program is heavily devoted to the student's research project, and thereby emphasises the importance of experimental design, data collection and

"Spending a summer with HRI was an awesome change of gear. It was incredible to go from lectures and examinations in undergrad to critical thinking and hands-on experiments in the lab. The access to cutting-edge equipment and the support from successful researchers inspired me to return to HRI for my honours year."

– Emily McCarthy, NZ Summer Scholarship recipient and former honours student with Thrombosis Group

analysis, literature reviews and troubleshooting. Undertaking your honours degree with HRI is not only a great way to further investigate cardiovascular disease, it can also open up a plethora of career opportunities not limited to science.

All selected HRI honours students will receive a scholarship valued at $A2000.

Candidates will receive support and advice from their supervisor and the HRI research grants management team in applying for a number of available national postgraduate scholarships (government, NHMRC, NHF, other).

Undergraduate

HRI offers summer research placements to Australian and New Zealand undergraduate students through several scholarship programs. These placements provide high-achieving, research-focused students the opportunity to work on a medical research project directly related to cardiovascular disease, expand on their skills and knowledge, and be mentored in a world-class research institute.

These scholarships vary in duration from six to ten weeks, and can offer opportunities for further undergraduate and postgraduate study, beyond summer placement.

HOW TO APPLY

If you are interested in pursuing research experience with HRI, send a formal cover letter stating your reasons for this, and a copy of your CV and transcript to research@hri.org.au.

ARTERIAL INFLAMMATION & REDOX BIOLOGY

Prof Roland Stocker

Research overview

Atherosclerosis is the biggest cause of heart attack, stroke and death in Australia.

It occurs when there is a build-up to fatty deposits and inflammatory cells in the wall of your arteries, which carry oxygen to your heart and other tissues. There are two types of atherosclerotic lesions, stable and unstable. Stable plaque builds up over years and causes arteries to become hardened, eventually restricting blood flow to the heart and other organs, which can be readily detected and treated with appropriate intervention. Unstable plaque is vulnerable to 'rupture' before it substantially restricts blood flow. These plaques are difficult to detect/identify yet can be fatal when they rupture and cause acute thrombosis and occlusion of the artery.

Professor Roland Stocker and his team are trying to understand what changes in the arteries, how they become diseased and how atherosclerosis can be prevented. More specifically, they examine the contribution of arterial inflammation and oxidative processes to the formation of unstable plaque, and how we can use this information to detect high-risk plaque and interfere with the formation and rupture of these plaques.

Key research areas:

- High-risk atherosclerotic plaque, identification and treatment
- Arterial inflammation
- Arterial redox biology.

Research projects

Project 1. Non-invasive imaging of high-risk vulnerable atherosclerotic plaque

Acute myocardial infarction ('heart attack') remains the most common cause of death in Australia, and atherosclerotic plaque rupture with blood clotting and artery blockage accounts for the majority of myocardial infarctions. Despite this, assessment of specific characteristics that predispose plaques to rupture is not currently incorporated into standard diagnostic or treatment regimes. Non-invasive, molecular imaging of biological processes known to contribute to plaque rupture is an exciting new way to potentially identify patients at risk of heart attack. We recently discovered that the pro-inflammatory enzyme myeloperoxidase contributes to the formation of plaque

with features of plaque vulnerable to rupture (Eur Heart J 2018;39:3301). Therefore, it is a potential target for molecular imaging of high-risk plaque. Our laboratory is currently assessing the utility of molecular imaging of myeloperoxidase activity using state-of-the art imaging techniques including magnetic resonance imaging (MRI) to specifically identify vulnerable plaque in pre-clinical models of plaque instability and plaque rupture, as well as in humans. This work is a collaboration with Dr Alkystis Phinikaridou and Prof René Botnar (King's College, London) and Dr Imran Rashid (University Hospitals Cleveland) and A/Prof Andrew Jabbour. The validation of such diagnostic tools could have significant impact for the assessment management of heart disease.

Relevant publications from our group

- Rashid I, et al, Stocker R. Myeloperoxidase is a potential molecular imaging and therapeutic target for the identification and stabilization of high-risk atherosclerotic plaque. Eur Heart J 2018;39:3301-3310.

Project 2. Molecular mechanism leading to the destabilisation and rupture of atherosclerotic plaque

This project addresses the possibility that inhibiting the pro-inflammatory and oxidant-producing enzyme myeloperoxidase (MPO) may stabilise 'vulnerable' lesions and hence may prevent a heart attack or stroke. MPO is expressed in certain white blood cells where it plays an important role in the innate immune system by producing hypochlorite (bleach) to kill bacteria and other pathogens. However, there is also evidence for MPO contributing to vascular disease by inducing endothelial dysfunction (Arterioscl Thromb Vasc Biol 2019;39:1448) and destabilising atherosclerotic plaque (Eur Heart J 2018;39:3301). The project is a collaboration with Professor Tony Kettle (University of Otago, Christchurch), Dr Alkystis Phinikaridou and Professor René Botnar (King's College, London) and AstraZeneca. It makes use of a new class of molecules, i.e., 2-thioxanthines, that effectively inhibit MPO. We are currently investigating whether MPO inhibition can stabilise existing unstable plaque, and whether it can also prevent plaque rupture in pre-clinical models.

Relevant publications from our group

- Rashid I, et al, Stocker R. Myeloperoxidase is a potential molecular imaging and therapeutic target for the identification and stabilization of high-risk atherosclerotic plaque. Eur Heart J 2018;39:3301-3310.
- Rashid I, et al, Stocker R. Inhibition of MPO (myeloperoxidase) attenuates endothelial dysfunction in mouse models of vascular inflammation and atherosclerosis. Arterioscler Thromb Vasc Biol 2019;39:1448-1457.

Project 3. Biomarkers of unstable, high-risk atherosclerotic plaque.

Using state-of-the-art proteomics, metabolomics and lipidomics, this project characterises the molecular changes that distinguish stable from unstable atherosclerotic plaque in pre-clinical models and humans. The project also investigates whether some of these characteristic molecular changes can be detected in blood as potential novel biomarkers of the presence of high-risk plaque. If successful, these studies may open the way for the development of novel biomarkers for and treatments of patients at elevated risk of a heart attack or stroke.

Project 4. How does bilirubin prevent formation of unstable atherosclerotic plaque?

Bilirubin is a product of heme catabolism and formed from biliverdin by biliverdin reductase-a. Bilirubin has various protective properties, including antioxidant and anti-inflammatory activities, and the ability to inhibit and promote the growth of vascular smooth muscle and endothelial cells, respectively. Plasma bilirubin concentrations inversely associate with the risk of cardiovascular disease. The current project examines how a deficiency in bilirubin caused by deletion of the gene biliverdin reductase-a prevents both the formation of unstable atherosclerotic plaque and insulin resistance. By identifying the nature of the protective activity of bilirubin, this project has the potential to identify novel pathways to prevent heart attacks and diabetes.

Project 5. Novel regulatory pathway for the synthesis of the mitochondrial electron transfer agent coenzyme Q

Coenzyme Q (CoQ) is a lipid essential for mitochondrial energy production and normal cell function. CoQ concentrations are decreased in atherosclerotic lesions, and in the hearts of patients with heart failure. It is thought that CoQ deficiency contributes to atherosclerosis and heart failure progression. Unfortunately, supplemental CoQ is poorly absorbed into tissues like the heart, and relatively little is known about how cells regulate CoQ concentrations. Thus, improving our understanding of how cells regulate their CoQ content may lead to novel strategies to increase cellular CoQ in conditions of deficiency. We recently identified 27 novel genes from a yeast genome-wide screen that change cellular CoQ concentrations. We have characterised one of these genes and how it regulates cellular and mitochondrial CoQ concentrations in yeast, mammalian cells and mice, and how this protects the animals against insulin resistance. We now wish to extend these studies to elucidate whether and if so, how these genes protect against atherosclerosis, especially unstable plaque. These studies are carried out in collaboration with Professor Ian Dawes (University of New South Wales, Sydney), Professor Catherine Clarke (University of California, Los Angeles),

and Professors René Jacobs and Dennis Vance (University of Alberta, Edmonton).

Project 6. Arterial redox signalling

Our laboratory previously reported that, under conditions of inflammation, degradation of the amino acid tryptophan by the activity of the enzyme indoleamine 2,3-dioxygenase relaxes arteries and decreases blood pressure in inflammation (Nat Med 2010;16:279). In an international collaboration led by our laboratory and involving Professor Richard Payne (The University of Sydney), Professor Philip Eaton (King's College, London), Professor Paolo di Mascio (University of Sao Paolo) and Professor Yorihiro Yamamoto (Tokyo University of Technology), we have identified a tryptophan-derived hydroperoxide as the vasorelaxant agent formed in vivo (Nature 2019;566:548). In collaboration with Dr Christopher Stanley (Heart Research Institute), we are investigating how this amino acid-derived hydroperoxide redox signals in inflamed arteries, and how this relates to other pathways of redox signalling.

Relevant publications from our group

- Wang Y, et al, Stocker R. Kynurenine is an endothelium-derived relaxing factor produced during inflammation, Nat Med 2010, 16:279–285.
- Stanley CP, et al, Stocker R. Singlet molecular oxygen regulates vascular tone and blood pressure in inflammation. Nature 2019;566:548-522.

ATHEROSCLEROSIS & VASCULAR REMODELING

Dr Ashish Misra

Research focus

The main objective of our research program is to broaden our understanding of the cellular and molecular mechanisms involved in blood vessel wall patterning and define the role of these pathways in vascular abnormalities and complications, and then link these insights to translational research to improve the prevention and treatment of human cardiovascular disease. To this end, we employ a unique combination of mouse models, cultured cells as well as human samples, that together are aimed at unveiling the pathogenesis of cardiovascular diseases. Our goal is to reverse/prevent vascular disease and prevent heart attack and stroke.

Research projects

Project 1. Modulating coronary atherosclerosis through perivascular fat (PVAT)

Background: Organ-to-organ communications are vital for living systems and play critical roles in cellular homeostasis. Various studies have demonstrated the existence and significance of evolutionally conserved factors involved in inter-organ communication. Hindrance in this intricate network of inter-organ communication initiates development of disease (i.e., cancer, obesity, aging and vascular disorders). Perivascular adipose tissue (PVAT) anatomically proximal to vasculature has a distinctive cellular composition that modulates a range of cardiovascular disease (CVD) processes. Recently, it was shown that PVAT and the vessel wall communicate bidirectionally through release of inflammatory molecules, adipokines and oxidative products; as such, PVAT may be a potential therapeutic target in cardiovascular disease.

Rationale: Antonopolous et al (Science Translational Medicine, 2017) recently showed that inflammatory signals from the human arterial wall diffuse into the perivascular fat to influence adipocyte lipid content. We have previously shown that low dose colchicine therapy in patients with coronary disease significantly reduced inflammatory trans-coronary cytokine levels (Martinez et al, Journal of the American Heart Association, 2015). As such, we hypothesise that colchicine pre-treatment prior to cardiac surgery will reduce diffusion of inflammatory cytokines from the vessel wall, thereby inhibiting differentiation of pre-adipocytes into mature adipocytes. Using novel transgenic mouse model and lineage tracing methods and human patient samples, our team will identify if blocking inflammatory signals have any implication on progression of atherosclerosis.

Experiment plan: We will study atheroprone mice carrying a fluorescent reporter (GFP) in macrophages to determine effects of colchicine on macrophage proliferation, macrophage fate change (from M1 to M2), and their migration from the adventitial layer to the perivascular fat. We will further investigate whether colchicine can modulate established plaque through inhibiting differentiation of pre-adipocytes into mature adipocytes and reducing adipocyte inflammation. We will confirm these findings on human patient samples. Forty consecutive patients enrolled in the COLPOC study will undergo adipose tissue harvesting from the following sites: chest incision (EpAT) (control), central thoracic area attached to the pericardium (ThAT), and right atrioventricular groove (ScAT), away from visible vessels. Samples will be snap frozen immediately after harvesting and stored at -80°C for histology and gene expression studies. In detail, adipocyte size and adipocytes per field will be quantified from tissue sections of EpAT, ThAT, and ScAT from the same patients. Gene expression for FABP4, PPAR-g, CEBPA and FABP4 (key adipocyte differentiation markers) will also be assessed. Fat sampling will incur no additional risk to the patient or increase in operation time.

Significance: Ultimately, this novel project will uncover biological processes observed in one tissue (e.g., PVAT) may influence processes observed in a different tissue (e.g., blood vessel). In turn, understanding these inter-organ communications will reveal novel pathways with important implications for cardiovascular therapeutics.

Project 2. Colchicine – A novel role in stabilising vulnerable atherosclerotic plaque

Atherosclerosis is the leading cause of cardiovascular deaths, accounting for ~30% of all deaths in Australia in 2018. Atherosclerotic plaque rupture leads to heart failure and stroke. Decades of clinical research indicates that the key feature of plaque rupture is thinning of the fibrous cap due to loss of cap smooth muscle cells (SMCs). Although SMCs are the major component of plaques and a major cause of plaque complications, treatments for atherosclerosis are limited to lipid lowering and reducing inflammation in plaques and have failed to target plaque regression. In this proposal we are challenging existing dogmas and introducing a novel concept of utilising SMC plasticity to combat plaque rupture.

Our studies provide compelling evidence that SMCs play a much greater role in plaque stability than has been generally appreciated. We found that in atherosclerotic plaque or in pulmonary artery hypertension, (Nature Commn, 2018, and Sci Transl Med, 2015) SMCs clonally expand from a ‘rare’ smooth muscle (SM) progenitor and differentiate into diverse phenotypes; ~30% differentiate into atheroprone inflamed smooth muscle-derived macrophages

(VMϕs) in the plaque interior, whereas ~12% convert into atheroprotective smooth muscle (SM)-like cells which increase fibrous cap thickness. With the help of clinical samples, advanced transgenic mouse models and systems biology approaches, we are currently investigating if anti-inflammatory therapy can promote atherosclerotic plaque cap thickness by reversal of smooth muscle derived macrophages into atheroprotective smooth muscle like cells. Results from this work are spearheading a nested trial within the NHMRC-funded clinical trial for use of colchicine in persistently-inflamed survivors of acute coronary syndrome. To characterise the pathways involved, and strengthen its use clinically, we will interrogate colchicine's plaque stabilising effects using in vitro and in vivo models of atherosclerosis. This work is in collaboration with A/Prof Sanjay Patel (Royal Prince Alfred Hospital & Heart Research Institute) and Prof Edward Fisher (New York University, New York).

Significance: Ultimately, this novel project will uncover novel pathways and genes involved in plaque cap thickness. This will have important implications for cardiovascular therapeutics. The studies described are also likely to uncover novel anti-inflammatory mechanisms that could be relevant more broadly, and potentially developed for use in other chronic inflammatory diseases such as chronic kidney disease, diabetes and rheumatoid arthritis, themselves risk factors for CVD.

Project 3. Notch signaling in atherosclerosis: Friend or foe

Aim: The overall aim of this project is to understand the functional role of notch signalling in recruitment of smooth muscle cells/smooth muscle derived cells and macrophages into atherosclerotic plaques.

Background: Atherosclerosis is the underlying cause of most cardiovascular diseases, including coronary artery disease (CAD), aortic aneurysms, and many instances of heart failure and stroke. Atherosclerosis involves multiple processes including endothelial dysfunction, inflammation, vascular proliferation and matrix alteration. Recent studies have emphasised the involvement of inflammation and proliferation of vascular smooth muscle cells (VSMCs) in mediating different stages of atherosclerosis. Although much progress has been made in identifying the mechanisms that initiate the inflammatory cell recruitment and SMCs proliferation during atherosclerosis, less is known about the intrinsic pathways that counteract these events. Notch proteins are transmembrane receptors that drive signalling pathways required for vascular development and remodelling.

Project overview: Recent studies implicated Notch pathway genes in coronary artery disease; however, notch signalling in atherosclerosis is unexplored. Our initial studies with high fat fed ApoE(-/-) mice indicate that expression of Notch3 is upregulated in atherosclerosis and our preliminary

results indicate that deleting notch3 gene in ApoE (-/-) background reduces the plaque size. Furthermore, timeline analysis shows that the recruitment of SMCs in plaques is reduced significantly compared with ApoE(-/-), Notch3(+/+). Additionally, marker analysis of inflammatory cells showed marked reduction in number of macrophages in the lesion.

In this study using in vitro and in vivo mouse models, we will explore the role of notch signalling in recruitment of SMCs in the plaque and macrophage-SMCs interplay in atherogenesis. We will also test the hypothesis that reduction of Notch3 in SMCs reduces transdifferentiation of SMCs into macrophage-like cells and its effect in athero progression.

This work involves techniques such as in vitro cell culture, gene expression (PCR, Western blotting), molecular biology (e.g., luciferase assays, chromatin immunoprecipitation), creating transgenic animals, histology, bone marrow transplant, in vivo fate mapping and clonal analysis.

Relevant publications from our group

- Misra, A., et al., Integrin beta3 regulates clonality of smooth muscle-derived atherosclerotic plaque cells. Nat. Commun, 2018 May 25; 9(1):2073.
- Misra A., et al., Integrin beta3 inhibition is a therapeutic strategy for supravalvular aortic stenosis. J. Exp. Med. (2016), 213 (3):451.
- Misra A, Fisher EA, Translational research in culture: AADCA, diabetes and cardiovascular disease. Cell Stem Cell. 2020 Jul 2;27(1):6-7.
- Bradley T, et al, Patel S. Colchicine as a novel therapy for suppressing chemokine production in patients with an acute coronary syndrome – A pilot study. Clinical therapeutics. 2019 Oct;41(10):2172-2181.

CARDIOMETABOLIC DISEASE

A/Prof John O'Sullivan

Research focus

Heart failure describes the condition where your heart cannot pump blood to the rest of your body as well as it should, and as a consequence becomes enlarged, weak or stiff. "Stiff" heart failure, or Heart Failure with Preserved Ejection Fraction (HFpEF), represents the most common type of heart failure globally, and dysregulated myocardial metabolism is a key feature.

The heart is the organ with the highest energy expenditure and oxidative demand, hydrolysing 6-35kg of ATP daily. It derives this energy from three major classes of substrates – fatty acids (~70%), glucose (~30%), and ketone bodies (~1%) – under normal conditions. However, in different circumstances it can utilise these substrates in different ratios, along with other substrates such as branched chain amino acids. For example, in diabetics the heart can no longer use glucose due to cardiac insulin resistance. Instead it upregulates use of ketone bodies, considered to be a "thrifty" energy source, as they provide more ATP per molecule of oxygen invested. Infusion of ketone bodies has been shown to improve cardiac function in heart failure patients acutely, while their increased levels secondary to current strategies to lower blood glucose (SGLT2 inhibitors) are postulated to underlie the improved heart failure outcomes seen with these agents.

Understanding the role of energetic substrate alteration is critical to improving our understanding and treatment of heart failure. To this end, we have put together a HFpEF program with comprehensive resources and unique capabilities, including: a HFpEF clinic, cardiac MRI, advanced cardiac echocardiography, a myocardial biopsy program, a myocardial tissue slice model, access to the world's largest heart bank (Sydney Heart Bank), an ex vivo Working Heart model, several relevant murine and rat HFpEF models, and stable isotope tracing of myocardial energetic substrates. Using this unique combination of technologies, our research concentrates on examining the interaction of diet, metabolism, and cardiac disease.

Research projects

Project 1. Transforming diagnosis and treatment of stiff heart failure

Our unique collection of technologies/capabilities places us at the forefront of HFpEF research. Using these strategies, our aims are as follows:

1. Determine key mechanistic underpinnings in each subclass of HFpEF.
2. Develop novel management strategies and treatment guidelines to treat

each HFpEF subclass (with Dr Sean Lal, co-Director of HFpEF clinic and Director of Sydney Heart Bank).

3. Develop novel therapeutics to improve HFpEF outcome (with Dr Xuyu Liu, HRI).

Outcomes: New management guidelines and therapeutic agents for HFpEF, the most common form of heart failure globally that currently has no approved pharmacotherapies.

Project 2. Developmental and dietary origins of myocardial disease

We examine the effects of maternal diet on cardiac development in utero, and thereupon its effects on adult cardiac development and disease. The insights gained from our studies thus far have reframed understanding of dietary regulation of cardiac development in utero; for example, we have found that the type of monosaccharide ingested is a major determinant of foetal cardiac size. We will now explore the long-term effects of in utero cardiac thickening secondary to ingestion of fructose or sucrose-enriched diets.

With A/Prof Kim Bell-Anderson (Charles Perkins Centre), we have uncovered striking cardiac enlargement in the offspring of pregnant mice ingesting diets enriched with sucrose or fructose (compared to glucose and normal chow). To further explore this, we are performing RNA seq in all these foetal hearts.

Going forward, our aims are to:

1. Identify key mechanistic pathways underlying this phenotype.
2. Determine the relationship to subsequent cardiac development and disease.
3. Mechanistically determine the relationship of novel pathways to cardiac thickening and enlargement and target them therapeutically.

Outcomes: Completely reframe understanding of the relationship of maternal diet to cardiac thickening and subsequent disease.

Project 3. Uncovering the interaction of obstructive sleep apnoea with cardiac metabolism, function, and disease

Working with Dr Melissa Farnham and Dr Kristina Cook, we have recently uncovered that intermittent hypoxia (as seen in obstructive sleep apnoea) causes hallmarks of cardiac insulin resistance and changes in cardiac substrate utilisation such as upregulation of cardiac ketone bodies. We have developed a program that includes homes sleep studies in all our heart failure patients (with Prof Peter Cistulli, ResMed Chair in Sleep Medicine), a rat model of intermittent hypoxia (Dr Melissa Farnham, HRI), with expertise in the master regulator of hypoxia transcriptional change, HIF-1α (Dr Kristina Cook,

Charles Perkins Centre).

We aim to do the following:

1. Determine the mechanisms of intermittent hypoxia-induced metabolic dysregulation via HIF-1α and other regulators.
2. Develop novel therapeutics to mitigate these effects.
3. Explore the cardiovascular perturbations consequent upon these changes.
4. Examine the metabolic and cardiovascular consequences of obstructive sleep apnoea clinically using our advanced echocardiography, vascular measurements, cardiac MRI, and sophisticated metabolic profiling.

Outcomes: 1. The first rigorous exploration of the protean cardiometabolic changes induced by obstructive sleep apnoea. 2. Novel management strategies and therapeutic agents to target these cardiometabolic complications.

CARDIOVASCULAR MEDICAL DEVICES

Dr Anna Waterhouse

Research focus

Research in the Cardiovascular Medical Devices Group focuses on how medical devices, such as artificial hearts, stents and bypass machines, interact with the body. Our team applies cutting-edge bioengineering tools to develop new methodologies to assess and understand the interplay of events at the biointerface, where the devices interact with the patient, and manipulate this interplay to improve medical device function, create novel medical devices and diagnostics and both drug and non-drug based avenues for therapies.

Our research is focused on development of: (i) biointerfaces; (ii) biomimetic model systems; and (iii) bioengineering smart materials and nanorobots.

Research projects

Project 1. Developing models of biomaterial-device thrombosis

Aim: To develop novel bioengineering solutions to study how material properties and blood flow dynamics govern the initiation of biomaterial-induced thrombosis, with the ultimate aim of improving medical device function.

Background: Advances in micro and nanotechnology have revolutionised bioengineering, allowing high precision manipulation of materials for modelling medical devices in the lab. Using bioengineering strategies, increasingly sophisticated devices are being constructed. However, protein and cellular interactions with materials are still poorly understood. One such example where this lack of understanding causes detrimental outcomes is blood-material interactions causing medical device failure. Blood is one of the most complex biological fluids containing multiple proteins and cell types. When blood contacts foreign materials in medical devices, it can cause fatal thrombosis (blood clots).

Project overview: The majority of experimental systems to test biomaterial-induced thrombosis in vitro rely only on traditional in vitro clotting assays which are done in test tubes using solutions of individual enzymes, fibrinogen alone or platelet-free plasma. These systems do not account for the reaction dynamics of cellular components or physiological blood flow, both of which are integral to thrombosis. Microfluidic systems provide sophisticated, real-time analysis of proteins and blood components that drive thrombosis, combined with the ability to manipulate blood flow at physiologically

relevant rates (Fig 1). Utilising the new facilities at the University of Sydney Nano Institute, we aim to develop bioengineering solutions using microfluidics to investigate the protein and cellular interactions at the biointerface. Different medical device materials will be assessed for their mechanism of thrombosis initiation. Furthermore, this platform system could be used to evaluate novel bioengineered surfaces, such as repellent, immobilised liquid surfaces or tissue engineered materials.

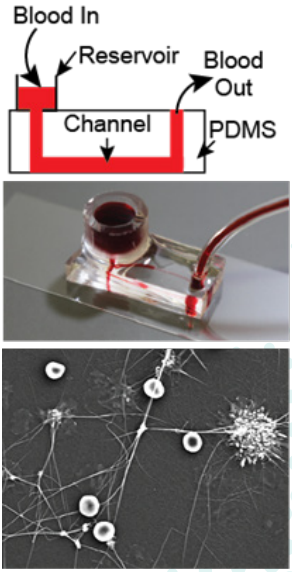


Fig 1. Schematic (top) and photo (middle) of a microfluidic channel for blood flow-material interaction analysis. Blood clot on polysulfone (bottom).

Project 2. Investigating the mechanism by which super repellent surface coatings reduce thrombosis of medical devices

Aim: To determine the mechanism by which the newly developed super repellent surface coating, Tethered-Liquid Perfluorocarbon (TLP) coating, reduces the thrombogenicity of medical devices.

Background: Thrombosis caused by medical devices can be costly and fatal for a number of reasons, including; 1) thrombosis can cause failure of device function requiring device replacement which can be expensive, or cessation of blood flow, which can be fatal, and 2) embolism of the thrombus can cause pulmonary embolism or stroke. Recently, slippery, liquid immobilised surfaces, TLP coatings, have been utilised to prevent thrombosis and biofouling by preventing surface adhesion of blood and pathogens.

Project overview: Tethered-liquid perfluorocarbon (TLP) coatings reduce fibrin polymerisation and platelet adhesion and activation in vitro under static and blood flow conditions. In vivo, an extracorporeal circuit consisting of TLP coated medically approved tubing and cannula, remained patent for at least 8 hours at 15L/hr of blood flow in a swine arteriovenous shunt model without the use of any antithrombotic medication (Leslie et al., 2014). However, the mechanism by which proteins and cells are repelled by TLP remains poorly understood. Here we aim to explore how plasma proteins, such as fibrinogen, and blood cells interact with TLP surfaces. This will have implications for how thrombus propagation is reduced on TLP surfaces. Utilising this system, the contribution of adhesion and local accumulation of blood components vs. protein and cellular activation to thrombosis and prevention of thrombosis could be elucidated. Understanding the mechanism of the low-thrombogenic, repellent properties of TLP coatings will enable improved application to

medical devices and provides insights for design improvements.

Project 3. Molecular nanorobotics for health: Hemocompatibility by design

Aim: To build molecular nanorobots, self-assembled from biomolecules, to navigate the body to detect and treat early, atherosclerotic disease.

Background: Atherosclerosis is one of the world's biggest killers, and current diagnostic methods are inadequate for early disease detection. Molecular-level changes in early atherosclerosis occur on the nanoscale. We are building molecular nanorobots, autonomous and programmable nanomachines self-assembled from molecules, for early detection and intervention of disease (Fig 1).

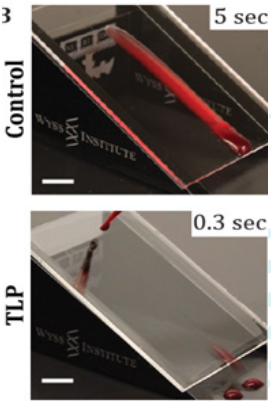


Fig 1. A drop of human blood adheres to uncoated acrylic (top) but is repelled by TLP coated acrylic (bottom) (Scale bar 1cm). Leslie et al. 2014.

Project overview: This project aims to make nanorobots hemocompatible by design. There is no 'one size fits all approach' to biocompatibility. Compatibility is a systems property, and is only meaningful in reference to specific scale, function, material and location in the body. It is essential that our nanorobots are intrinsically compatible with blood, vasculature and the immune system. This project will investigate the hematological and immunological compatibility of nanomaterials and establish biomimetic solutions for nanorobot development and in vitro and in vivo models for nanorobot evaluation. This is highly interdisciplinary and open to students from a broad range of backgrounds, including: chemistry, physics, biochemistry, pharmacology, biological and medical sciences, biomedical, materials, chemical and biomolecular engineering. This project is co-supervised by Dr Shelley Wickham (School of Chemistry and Physics) at The University of Sydney. On this project, you would be part of a multidisciplinary team of researchers across all Faculties and be part of the Sydney Nano Institute.

Relevant publications from our group

- Leslie, D. C. and Waterhouse, A. et al. Nature Biotechnology, 32 (11) 1134–1140 (2014).
- Waterhouse, A et al. Tissue Engineering Part B Reviews, 17 (2) 93-99 (2011).
- Waterhouse, A. et al. Biomaterials, 31 (32) 8332–8340 (2010).

CARDIOVASCULAR NEUROSCIENCE

Dr Melissa Farnham

Research focus

Our research focuses on derangements in the autonomic nervous system resulting in development of cardiometabolic diseases such as hypertension and diabetes. Obstructive sleep apnoea (OSA) is a condition characterised by repetitive airway collapse during sleep which causes persistent, excessive autonomic activity and is frequently associated with hypertension and diabetes, but mechanisms remain elusive. We employ a range of physiological, pharmacological, and genetic tools to interrogate and manipulate the neurocircuitry involved in this response to prove causation.

Research projects

Project 1. Sleep apnoea and cardiometabolic disease: What goes wrong in the brain?

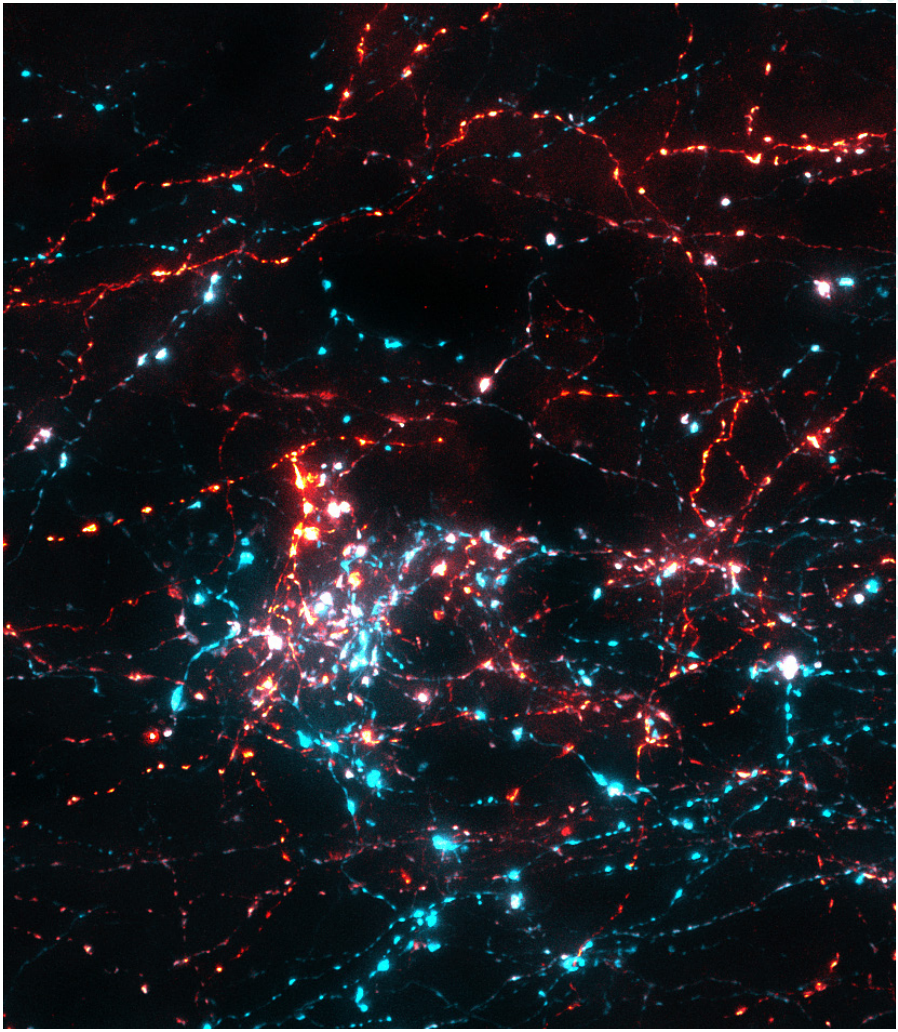
Aim: To define the mechanisms driving the cardiometabolic effects of OSA to spearhead the development of novel, and more effective, treatment strategies for OSA sufferers.

Background: Repetitive, or intermittent, hypoxia caused by repetitive airway collapse, is a key feature of OSA. Patients with OSA present with persistent increases in sympathetic activity, which is also a key feature of hypertension and evident prior to the onset of hypertension. In fact, OSA is now recognised as a leading cause of secondary hypertension, which is precursor to the more malignant cardiovascular disease. OSA is also present in >80% of diabetes patients. Although commonly thought of as a disease of obesity, more the 20% of people with OSA have normal BMI, are young, and otherwise "healthy". These "normal" OSA patients have far worse outcomes than their obese counterparts and are more difficult to treat. It is imperative that we understand the mechanisms driving these pathological cardiometabolic changes so that we can detect OSA earlier and develop more effective treatment strategies for the future.

Work from our lab has shown that the persistent increases in nerve activity and the increase in blood glucose, following a protocol of acute intermittent hypoxia, are dependent upon a neuropeptide acting at its receptors in sympathetic areas of the brain and spinal cord. We hypothesise that neuropeptides are driving the chronic, maladaptive increases in nerve activity, leading to hypertension and development of type 2 diabetes in human OSA conditions.

Project overview: We use acute and chronic rodent models of OSA, to measure blood pressure, heart rate and various sympathetic outputs following pharmacological and genetic manipulations of the central nervous system. The physiology experiments are combined with immunohistochemical, molecular and metabolomic experiments to assess both the signalling changes within the brain and spinal cord and the downstream metabolic consequences in critical target organs.

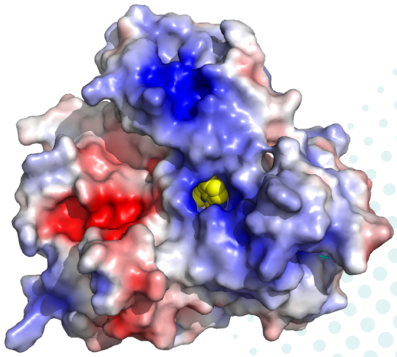
Neuronal projections in a rat spinal cord.
Image courtesy of Dr Polina Nedoboy, Cardiovascular Neuroscience Group.



CARDIOVASCULAR-PROTECTIVE SIGNALLING AND DRUG DISCOVERY

Dr Xuyu Liu

Research focus



Electrostatic feature of Akt1 oncogenic kinase with an allosteric inhibitor. Image courtesy of Dr Xuyu Liu, Cardiovascular-protective Signalling and Drug Discovery Group.

Despite the global burden of cardiovascular disease, the development of new cardiovascular drugs has stalled for over two decades. The primary reason is intolerance to drug-related side effects. Recently, there has been considerable interest in the development of natural supplements for cardiovascular-protective therapeutics owing to their inherent safety profiles and the clinical evidence for ameliorating chemotherapy-induced cardiovascular complications. However, it remains a huge challenge to understand the cardiovascular-protective mechanisms at the molecular level, which impedes pharmacological optimisation of these bioactive agents for therapeutic use. Therefore, we aim to apply cutting-edge chemoproteomics platforms to understand the intricate signalling interplay in cardiomyocytes in response to different natural products and to construct a comprehensive chemotype database for cardiovascular-protective drug discovery.

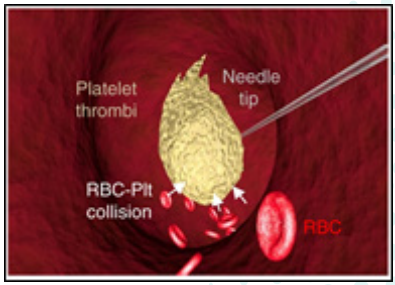
Research projects

Project 1: Developing safer anti-clotting agents derived from "Mother Nature" for the treatment of stroke

Thrombin is by far the most robust activator of blood clotting in both physiological haemostasis and pathological thrombotic response. Fibrin clots contain a large amount of thrombin, which is released into the circulation following administration of clot busting drugs to treat stroke (rtPA, thrombolysis). This pool of thrombin remains highly active and is responsible for the rethrombosis. While a strong rationale exists for the use of thrombin inhibitors as effective antithrombotic agents in enhancing clot lysis, all current thrombin inhibitors lead to severe bleeding complications, precluding their use in stroke, due to risk of intracerebral haemorrhage. We have identified novel anticlotting agents derived from naturally occurring proteins found in saliva of

the bush tick. Our studies have demonstrated that these bug-derived proteins are able to dissolve blood clots in a disease model of thrombosis with fewer bleeding complications. This project will involve the synthesis of novel anti-clotting proteins and characterisation of the mechanisms underpinning the safe antithrombotic mechanism, and testing whether administration of this novel drug in combination with rtPA provides for a safe approach to treatment of thromboembolic diseases such as stroke in the future. This project will be co-supervised by Dr Xuyu Liu, A/Prof Simone Schoenwaelder and Dr Jessica Maclean.

Project 2: PROTACs for cardiovascular disease



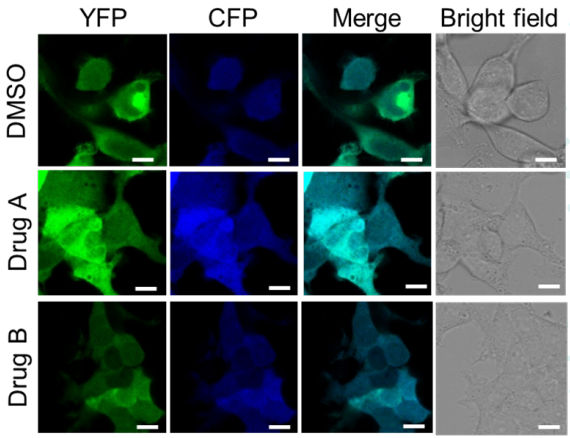
PROteolysis-TArgeting Chimeras (PROTACs) represent exciting new drug modalities. PROTACs are bifunctional molecules capable of binding simultaneously to a protein in the ubiquitinase complex as well as a protein target; this promotes selective proteolytic degradation of the target. This project aims to develop the first cardiovascular PROTACs for the treatment of a range of thrombosis/atherosclerosis-related cardiovascular diseases. The project will involve examining PROTAC libraries targeting three key kinase targets (Pyk2, PI3K and Akt) and novel redox regulators found in vasculature system harnessing advanced platelet and vascular in vitro and in vivo platforms established at HRI. This project will be co-supervised by Dr Xuyu Liu (Chemical biology), A/Prof Simone Schoenwaelder (Platelet biology), A/Prof John O'Sullivan (Cardiomyocyte biology) and Dr Christopher Stanley (Vascular biology).

Project 3: Development of high-affinity ACE2 mutants to combat COVID-19

The human surface-expressed angiotension-converting enzyme 2 (ACE2) receptor has been identified as a functional receptor to mediate cell-entry of SARS-CoV-2 – the etiological virus causing the current COVID-19 pandemic. SARS-CoV-2 infection has also been shown to lead to a substantial decrease in surface expression of ACE2 receptor, resulting in acute respiratory distress syndrome (ARDS) – one of the most devastating forms of acute lung failure. To address these ongoing issues, we aim to establish an expressed protein ligation platform (Nature Chemistry 2017, ACS Central Science 2018) to develop high-affinity ACE2 mutants which are capable of scavenging SARS-CoV-2 virus effectively in vivo whilst offering lung- and cardiovascular-protective effects.

Project 4: Application of natural product-based probes to discover novel cardiovascular-protective signalling

Despite the global burden of cardiovascular disease, the development of new cardiovascular drugs has stalled for over two decades. Recently, there is a considerable interest in the development of natural products extracted from healthy diets for cardiovascular-protective therapeutics. However, it remains a huge challenge to understand the molecular biology behind, which impedes pharmacological optimisation of these bioactive agents. In this honours project, we aim to apply cutting-edge molecular biology and chemical proteomics technologies (ACS Central Science 2020, Trends in Biochemical Sciences 2019, Current Opinion in Chemical Biology, 2019) to understand the intricate bio-activity of sulforaphane (SFN), a cardioprotective ingredient found in heart-healthy diets.



CORONARY DISEASE

A/Prof Sanjay Patel

Research focus

Acute Coronary Syndromes (ACS) are the leading cause of mortality in NSW and dominate heart disease presentations and health care costs. ACS account for 53% of all heart admissions and 87% of total heart health care expenditure; numbers are rapidly increasing and in NSW there were 10,172 hospitalisations in 2014–2015, costing $637 million. Moreover, 34% of all ACS are repeat events, which are twice as likely to be fatal, particularly in women and in rural, Indigenous and low socioeconomic populations. Failure to specifically target residual inflammation at vulnerable culprit and non-culprit sites drives recurrent events post-ACS. Therefore, development of treatment protocols using anti-inflammatory agents are urgently needed.

Benefits of specifically targeting inflammation in ACS: The CANTOS study using canakinumab, a monoclonal antibody directed against IL-1β, showed reduced recurrent cardiovascular events in patients with a previous MI and evidence of persistent inflammation (residual hs-CRP levels ≥2 mg/L), already on guideline-directed therapy Canakinumab, however, is unsuitable for mainstream use as it was associated with fatal sepsis, is not FDA approved and is prohibitively expensive. Supporting this notion, the neutral CIRT trial showed that methotrexate treatment, in patients not selected on the basis of elevated circulating hs-CRP, did NOT reduce CRP or future events, possibly because methotrexate does not target relevant vascular inflammatory pathways.

Role for colchicine in ACS patients: Colchicine is a cheap, well-tolerated, oral anti-inflammatory agent. The recent COLCOT study demonstrated that colchicine reduced clinical events (stroke and angina requiring revascularisation, not myocardial infarction) in a post-ACS population NOT chosen on the basis of a high hsCRP. We recently secured $4.2M from the NHMRC to conduct a colchicine RCT in 3000 post-ACS patients with persistently high hsCRP (>2mg/L), a much higher risk group likely to derive maximal benefit from an anti-inflammatory agent.

Research projects

Project 1. To embed imaging/biomarker studies within the larger study

To embed imaging/biomarker studies within the larger study, which is funded for clinical outcomes only. It will aim to:

1. Understand mechanisms of action of chronic colchicine therapy in post-

ACS patients.

2. Design a biomarker repertoire to assess early treatment response, which will be correlated with long term clinical outcomes.
3. Provide a more robust assessment of treatment efficacy by combining clinical and surrogate endpoints.

To perform these studies, we will assess the following disease surrogates:

1. Vulnerable carotid plaque with MRI: This can predict recurrent events; its superficial location lends itself to imaging and histologic assessment.
2. CT imaging of coronary vascular inflammation (NaF uptake for coronary plaque inflammation and perivascular adipose tissue (PVAT) imaging for coronary wall inflammation): Both modalities predict future plaque rupture in CAD patients.

These imaging endpoints will be used to track responses to colchicine and will be correlated with changes in inflammatory mediators in blood.

HAEMATOLOGY RESEARCH

Dr Freda Passam

Research focus

The Haematology Research Lab aims to discover novel pathways in blood clotting which can lead to the development of effective and safe drugs to treat thrombosis.

Research projects

Project 1. Defining the diabetic platelet proteome

Platelets are integral in forming and sustaining thrombus formation in healthy and diseased states. The differences in platelet function are predominantly due to changes in protein expression and post-translational modifications, given their anucleate nature. A major benefit of proteomic analysis of platelets is that there is little variation between healthy individuals for most of the expressed proteins. This allows easier comparison to patients with a disease state such as diabetes mellitus. There have been several studies in recent years applying platelet proteomics in the study of cardiovascular disease, however the information on the "diabetic" platelet proteome is limited. In this project, we will elucidate the proteomic phenotype of platelets in patients with diabetes to identify biomarkers of thrombotic risk and new therapeutic targets. This project will provide the opportunity to learn platelet function assays and platelet proteomics.

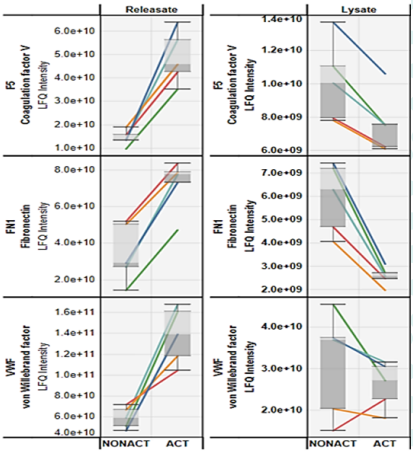


Figure 1: Line plots showing the quantification of selected proteins (F5, fibronectin, and vWF) for platelet releasates and lysates in response to thrombin activation (credit Dr Mark Larance).

Relevant publications from our group

- Velez P, Garcia A. Platelet proteomics in cardiovascular diseases. Transl Proteom, 2015 7:15-29.
- Springer DL, Miller JH, Spinelli SL, et al. Platelet proteome changes associated with diabetes and during platelet storage for transfusion. J Proteome Res, 2009. 8(5):2261-2272.
- Harney DJ, Hutchison AT, Hatchwell L, et al. Proteomic Analysis

of Human Plasma during Intermittent Fasting. J Proteome Res. 2019;18(5):2228-2240.

Project 2. Thiol isomerases as novel antithrombotic targets

Thiol isomerases constitute a new clotting pathway. Thiol isomerases are a group of enzymes that regulate the function of blood cell receptors and clotting proteins by reacting with their disulphide bonds. We have identified a thiol isomerase, named ERp5, which is released into the circulation from activated platelets and promotes clot formation in vivo. In this project we will dissect the role of ERp5 in platelet function and clot formation by using mice with genetic deletion of ERp5 in their platelets. We will investigate how this thiol isomerase regulates the interaction of platelets with clotting proteins (fibrinogen, von Willebrand factor) and vascular cells (endothelial cells and neutrophils). We will explore the potential of ERp5 inhibitors to prevent thrombus formation and become candidate antithrombotic drugs. This project will provide the opportunity to learn the method of intravital microscopy for the study of clot formation in mice, flow cytometry and platelet function assays.

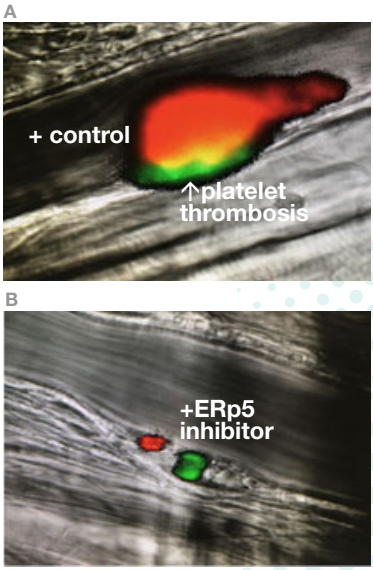


Figure 2. In vivo thrombus formation in the cremaster artery of (A) a mouse injected with inactive control compound and (B) a mouse injected with an ERp5 inhibitor. Platelets are labelled in red and fibrin in green.

Relevant publications from our group

- Passam F, et al, and Hogg PJ. Mechano-redox control of integrin de-adhesion. Elife. 2018 Jun 22;7:e34843.
- Passam FH, et al, and Furie BC, Furie B. Both platelet- and endothelial cell-derived ERp5 support thrombus formation in a laser-induced mouse model of thrombosis. Blood. 2015;125(14):2276-2285.
- Jasuja R, Passam FH, et al, Furie B, Furie BC, Flaumenhaft R. Protein disulfide isomerase inhibitors constitute a new class of antithrombotic agents. J Clin Invest. 2012;122(6):2104-2113.

Project 3. Developing biochips for the study of haemostasis and thrombosis

Many patients with bleeding and clotting disorders go undetected by routine laboratory tests in part because the available assays do not reflect the conditions in the circulation. The Haematology Research Group uses biochips in a microfluidic system that allows blood to flow through passages under controlled conditions. The passages are designed to mimic blood vessels and include features, e.g., stenosis, that simulate the circulation in stenosed vessels. The flow of blood through these biochips generates thrombi that can be visualised by real-time microscopy and quantified. This project will study blood cell adhesion and thrombus formation in the microfluidic devices to assess for persisting thrombotic tendency in patients with a history of thrombosis, who have completed treatment. Samples from patients with bleeding disorders on treatment will be assessed for haemostatic potential. A range of parameters, which participate in clot formation, will be measured in the microfluidics system including platelets, fibrin, neutrophil extracellular traps and von Willebrand factor. This project involves the preparation of the microfluidics chips, microscopy and image analysis.

Relevant publications from our group

- Dupuy A, Ju LA, Passam FH. Straight channel microfluidic chips for the study of platelet adhesion under flow. Bio-protocol; 2019.
- Lee KH, Cavanaugh L, Leung H, Yan F, Ahmadi Z, Chong BH, Passam F. Quantification of NETs-associated markers by flow cytometry and serum assays in patients with thrombosis and sepsis. Int J Lab Hematol; 2018;40(4):392-399.
- Zhang C, Neelamegham S. Application of microfluidic devices in studies of thrombosis and hemostasis. Platelets. 2017;28(5):434-440.

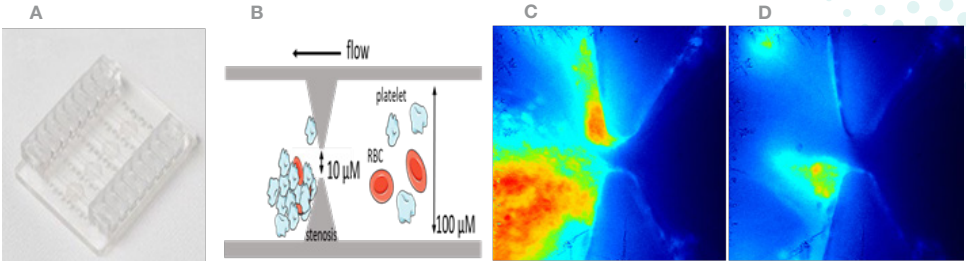


Figure 3. Microfluidic devices for measuring thrombotic and bleeding tendency. (A) Biochip containing channels for perfusion of blood. (B) Schematic of a 90% stenosed channel for the study of thrombus formation. (C) Blood sample with increased thrombus formation. (D) Decreased thrombus formation at the stenosis site.

MICROVASCULAR RESEARCH

Dr Christopher Stanley

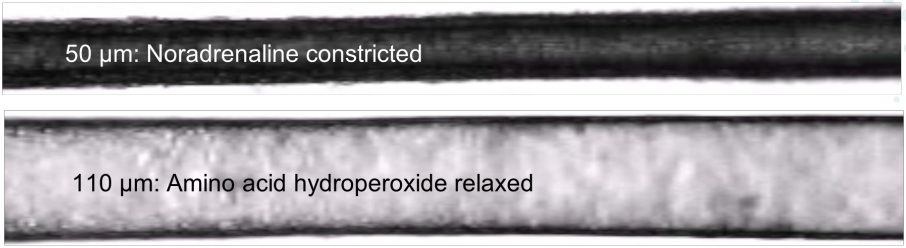
Research focus

We are interested in arteries that have a key role in the regulation of blood pressure. To understand how these arteries work we use staple scientific disciplines including: physiology, electrophysiology, pharmacology, microscopy and redox biology.

Hypotension (low blood pressure) in sepsis is an unmet clinical need and leads to inadequate tissue perfusion and death. Hypertension (high blood pressure) is a major risk factor for cardiovascular disease. Patients with hypertension have damaged arteries and over the long term they are at increased risk of cardiovascular diseases such as heart attack, stroke, diabetes and heart failure. The aim of the Microvascular Research Unit is to investigate how dysfunctional resistance arteries contribute to these pathologies. Resistance arteries are a subset of arteries that typically have diameters of ~100 mm and have crucial roles in regulating blood pressure and tissue perfusion. Thus, dysregulation of resistance artery signaling is likely a large contributor to, and therefore promising therapeutic target for the treatment of, hypotension/hypertension and other vascular complications.

Dr Chris Stanley uses pressure myography (Figure 1) to understand the signalling changes that contribute to resistance artery pathophysiology, and how these impact on blood pressure. More specifically, Dr Stanley combines pressure myography with microscopy, electrophysiology and calcium imaging to examine the contribution of arterial inflammation and oxidative processes in the regulation of resistance artery tone, membrane potential, calcium signalling, and structure.

Figure 1. Mouse mesenteric artery bio-assay (pressure myography)



Research projects

Project 1. Downstream signalling of oxidised protein kinase 1a

Non-canonical activation of protein kinase G1a (PKG1a), which occurs via oxidation of the kinase, has been shown to be involved in the regulation of blood pressure and contraction of the heart. We, and our collaborator Prof Philip Eaton (William Harvey Research Institute, London), have shown that oxidation of PKG1a leads to pathologically low blood pressure in models of sepsis (PNAS 2013;110:9909, Nature 2019;566:548). However, a mechanistic understanding of oxidised PKG1a signalling is yet to be detailed. We now wish to understand more about the downstream signalling of PKG1a to fully highlight the therapeutic potential of this pathway in combating hypotension associated with sepsis. This work will involve resistance arterial physiology, pharmacology, redox biology, electrophysiology, and calcium imaging. Techniques utilised will include myography, sharp electrode/patch clamping, and live tissue calcium imaging.

Relevant publications from our group

- Stanley CP*, Maghzal GJ*, et al, Stocker R. Singlet Molecular Oxygen Regulates Vascular Tone and Blood Pressure in Inflammation. Nature 2019; 566 p548.*Co-first authorship.
- Queiroz RF…Stanley CP, et al, Stocker R. Stereospecific impediment of reductive inactivation of tryptophan-derived hydroperoxide: a new model for mammalian redox signaling by hydrogen peroxide. Nat Commun 2020; manuscript under review.

Project 2. Indoleamine 2,3-dioxygenase-1 and nitric oxide in treatment of hypotension associated with sepsis

We have recently shown that tryptophan catabolising enzyme indoleamine 2,3-dioxygenase-1 (IDO1) plays a key role in the regulation of blood pressure in experimental models of sepsis (Nature 2019;566:548). However, previous work by others in experimental models of sepsis has implicated that inducible nitric oxide synthase may be the leading cause of hypotension. Studies by Prof Roland Stocker (a collaborator on this project) have demonstrated a link between these two enzymes, showing that nitric oxide can affect IDO1 activity (JBC 2007;282:23778). Furthermore, we now have unpublished data demonstrating that inhibition of both pathways leads to a better blood pressure outcome in models of sepsis. The aim of this project would be to investigate the interplay between IDO1 and nitric oxide in experimental sepsis. This work will involve resistance arterial physiology, pharmacology, imaging, and molecular and redox biology along with in vivo blood pressure measurements. Techniques utilised will include myography, confocal

microscopy, genetic manipulation of IDO1 and/or nitric oxide, and live animal blood pressure recordings.

Relevant publications from our group

- Stanley CP*, Maghzal GJ*, et al, Stocker R. Singlet Molecular Oxygen Regulates Vascular Tone and Blood Pressure in Inflammation. Nature 2019; 566 p548.*Co-first authorship.
- Queiroz RF…Stanley CP, et al, Stocker R. Stereospecific impediment of reductive inactivation of tryptophan-derived hydroperoxide: a new model for mammalian redox signaling by hydrogen peroxide. Nat Commun 2020; manuscript under review.

Project 3. Identification of novel metabolites in human plasma samples from patients with sepsis

Tryptophan catabolism via the enzyme indoleamine 2,3-dioxygenase-1 (IDO1) plays a key role in the regulation of blood pressure in animal models of sepsis (Nature 2019;566:548). This involves the generation of a high energy form of oxygen (singlet molecular oxygen) and subsequent conversion of tryptophan to several different chemical compounds including tryptophan-derived hydroperoxides and alcohols. Whilst we have established this pathway in mice and pigs, we have yet to confirm the activity of this pathway in humans. The aim of this project would be to establish for the first time the presence of this pathway in human plasma, thus validating the IDO1 pathway as a novel therapeutic target for the treatment of low blood pressure in sepsis. This project, done in collaboration with Prof Roland Stocker, will involve molecular biology, redox biology and biochemistry. Techniques utilised will include confocal microscopy, western blotting, RT-PCR and mass spectrometry.

Relevant publications from our group

- Stanley CP*, Maghzal GJ*, et al, Stocker R. Singlet Molecular Oxygen Regulates Vascular Tone and Blood Pressure in Inflammation. Nature 2019; 566 p548.*Co-first authorship.
- Queiroz RF…Stanley CP, et al, Stocker R. Stereospecific impediment of reductive inactivation of tryptophan-derived hydroperoxide: a new model for mammalian redox signaling by hydrogen peroxide. Nat Commun 2020; manuscript under review.

THROMBOSIS

Prof Shaun Jackson

Research focus

The Thrombosis Research Group aims to understand the events leading to blood vessel occlusion of the macro- and micro- vasculature, precipitating thrombosis and ischaemia reperfusion injury (IR) in cardiovascular disease. Research carried out in the Thrombosis Group focuses on the following themes:

1. Cell death pathways regulating vascular dysfunction
2. Mechanisms leading to microvascular dysfunction and poor cerebral perfusion in stroke and ischaemia/reperfusion injury
3. Discovery/preclinical development of novel antiplatelet and/or anticoagulant treatments for stroke
4. Investigating mechanosensitive pathways regulating thrombus formation.

Our approach to these research questions is to examine interactions between blood cells (platelets, leukocytes, erythrocytes) and injured blood vessels (primarily endothelial cells), in vitro and ex vivo, as well as in vivo using mouse models of thrombosis, ischaemic stroke and IR injury. We combine these approaches with cutting-edge technologies including: advanced microscopy techniques (intravital imaging, confocal, TIRF, super resolution, 2-photon, tissue clearing); molecular mouse models and genome editing; omic studies; biomechanics/microfluidics, biomembrane force probe (BFP) studies.

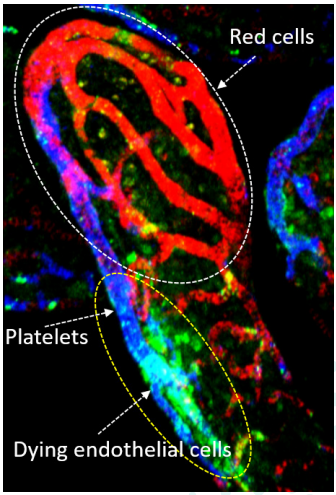
Whilst our studies are primarily aimed at defining new mechanisms promoting thrombosis and inflammation (termed thromboinflammation), we also actively translate our research discoveries into new therapeutic approaches.

Research projects

Project 1. Cell death pathways regulating vascular dysfunction

Acute myocardial infarction (AMI) and stroke are the major cause of disability and mortality globally. The primary focus of AMI and stroke therapy is to promptly re-open the blocked arteries to salvage the dying ischemic tissue. However, despite the re-opening of the culprit artery, blood perfusion in surrounding microvasculature supplying the tissue can remain poor, a common complication of ischemia reperfusion (IR) injury, known as microvascular obstruction (MVO). MVO occurs in 60% AMI patients and persistent MVO can lead to progressive worsening of heart function and infarction. Several pathogenic processes have been implicated in MVO,

however targeted therapies have not been effective in improving microvascular perfusion. This is due in part to the lack of suitable animal models and technical difficulties associated with performing real-time imaging on the microvasculature. In order to gain a better understanding of the temporal and spatial events leading to MVO, thus affording better insights into potential therapy options, we have established a mouse model of gut IR injury which allows access to the microvasculature in living animals during IR injury.



Using this model, combined with cutting edge confocal microscopy, we have observed previously unappreciated in vivo changes within the microvasculature during IR injury. There is increasing evidence that genetically regulated cell death pathways (necroptosis, apoptosis, pyroptosis, autophagy) play an important role in regulating the cardiovascular system in health and disease. We have recently uncovered new roles for apoptosis and necroptosis in regulating microvascular dysfunction during IR injury. Our ongoing studies aim to identify/characterise the cell death pathways promoting IR injury, and identify/test novel therapeutic targets which may reduce the impact of IR injury on end-organ function (heart, brain and gut). This is particularly important given that dysregulation of these pathways may also help explain the vascular problems experienced by COVID-19 patients.

Relevant publications from our group

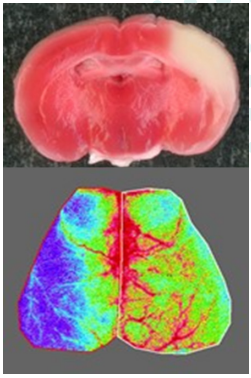
- Yuping Yuan et al, Sci. Trans. Med, 2017 Sep 27;9(409). pii: eaam5861. doi: 10.1126/scitranslmed.aam5861.
- Jackson SP. Nature Med. 17(11):1423-1436, 2011.
- Jackson et al, Blood, 133(9):906-918. doi: 10.1182/blood-2018-11-882993.

Project 2. Discovery and development of novel antithrombotics for the treatment of stroke

The development of a thrombosis or embolus in the cerebral circulation (ischaemic stroke [IS]) is the third most common cause of death and the most common cause of adult disability globally. Whilst considerable progress has been made in developing more effective treatments for coronary disease, progress in the management of stroke continues to be unsatisfactory. The

central goal of acute stroke therapy is the prompt re-opening of occluded blood vessels to minimise tissue death. The delivery of fibrinolytic agents modelled on tissue-type plasminogen activator (t-PA) is the only clinically approved thrombolytic agent for IS therapy. However, thrombolytic therapy is not without its limitations, with lysis resistant blood clots, as well as haemorrhage presenting as major complications. One of the main factors delaying reperfusion and increasing the risk of re-occlusion of cerebral vessels is the presence of platelets in arterial thrombi, with numerous preclinical and clinical studies demonstrating the benefits of adjunctive anti-platelet therapy to enhance cerebral reperfusion and reduce re-occlusion following thrombolysis. Unfortunately, in IS patients, the benefits of combined antiplatelet/thrombolytic therapy are partially offset by the increased risk of life-threatening intracerebral bleeding, limiting the widespread use of this approach.

Our laboratory has a longstanding interest in identifying pathways in platelets that are important for arterial thrombus formation, but less critical for haemostasis. One of these pathways involves shear activation of platelets through activation of the p110β isoform of PI3-kinase (PI3Kβ). This project will examine the mechanisms by which PI3Kβ inhibitors enhance reopening of the blood vessel, examine the impact of thrombus channel formation on blood flow, thrombus porosity and thrombus dissolution. Moreover, the impact of PI3Kβ inhibitors on end-organ damage, particularly in the stroke context, will also be examined. These studies will not only provide important insight into our understanding of blood clot formation but may also lead to new approaches to regulate the size and stability of blood clots forming in the body, providing major clinical benefit in the delivery of thrombolytic therapy (blood clot removal).



Studies involve the use of:

- in vivo models of thrombosis and thrombolysis
- genetic mouse models
- state-of-the-art imaging systems (tissue clearing techniques, confocal microscopy, intravital microscopy, laser doppler flowmetry and laser speckle contrast imaging)
- behavioural assessment to determine cerebral damage following recovery from stroke.

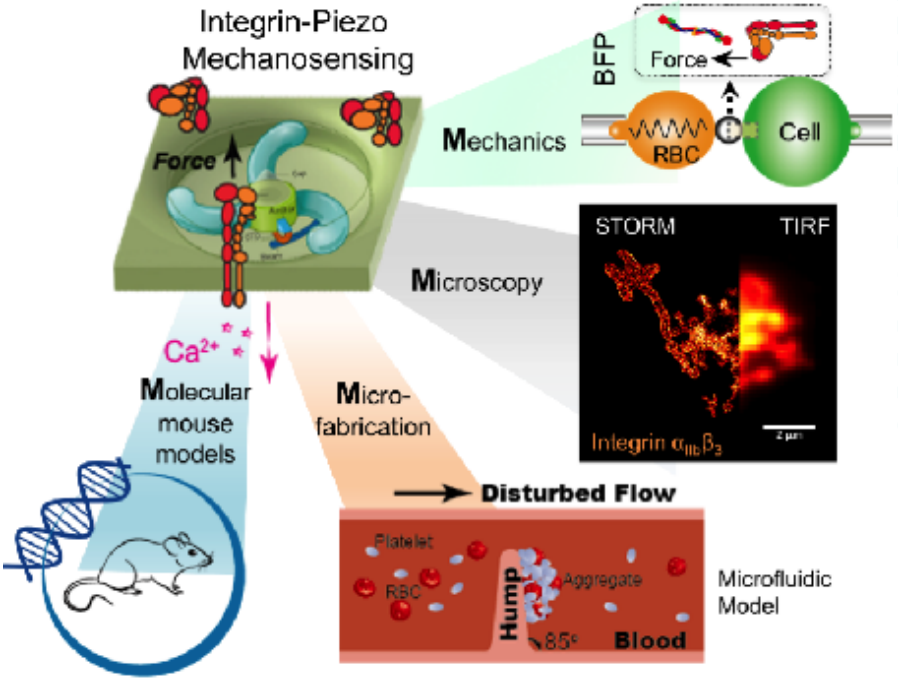
Relevant publications from our group

- Andre L Samson, et al, Endogenous fibrinolysis facilitates clot retraction in vivo. Blood 2017, Dec 7;130(23):2453-2462.

- Jackson SP and Schoenwaelder SM. Nature Reviews Drug Discovery, 2:775-789, 2003; Cell Mol Life Sci, 63(10):1085-90, 2006; Curr Top Microbiol Immunol, 346:203-24, 2010.
- Jackson SP, et al, Nature Medicine, 11(5):507-514, 2005.
- Schoenwaelder SM, et al, J Biol Chem, 282(39):28648-58, 2007; J Biol Chem, 285(4):2886-2896, 2010.
- Jackson SP. Nature Medicine. 17(11):1423-1436, 2011.

Project 3. Mechanosensitive pathways regulating thrombus formation – Multidisciplinary 4Ms approaches

To investigate platelet mechanobiology at cell-molecular scales, we have established the 4M's approaches in Australia: **M**echanics, **M**icroscopy, **M**icrofabrication & **M**olecular Mouse Models by combining the live-cell dynamic force spectroscopy BFP system with other complementary technologies including the TIRF/STORM super-resolution imaging, microfluidics, in vivo mouse models of thrombosis as summarised in the figure.



Relevant publications from our group:

- Lining Ju, et al. Compression Force Sensing Regulates Integrin αIIbβ3 Adhesive Function on Diabetic Platelets. Nat. Comm, 2018, mar14; 9(1):1087. Doi: 1038/s41467-018-03430-6.
- Jackson SP. Nature Med. 17(11):1423-1436, 2011.
- Jackson and Schoenwaelder. Nature Reviews Drug Discovery, 2:775-789, 2003.
- Nesbitt WS, et al. Nature Med. (Article) 15(6):665-673, 2009.

Project 4: Developing safer anti-clotting agents derived from "Mother Nature" for the treatment of stroke

See page 21, in collaboration with Dr Xuyu Liu, Cardiovascular-protective Signalling and Drug Discovery Group.

VASCULAR COMPLICATIONS

Dr Mary Kavurma

Research interest

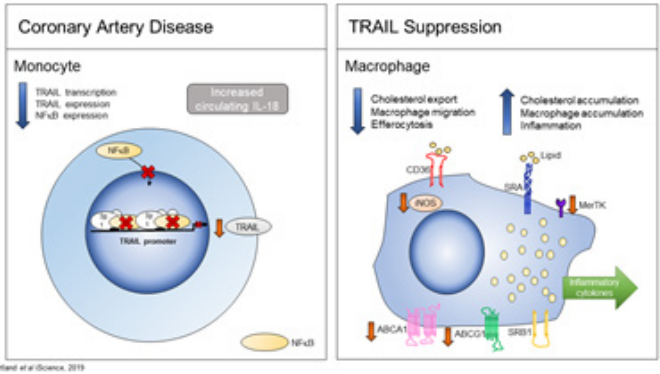
Our research aims to understand the molecular, biochemical, and cellular mechanisms underlying blood vessel diseases, focusing on atherosclerosis and its complications, including peripheral artery disease and diabetes. By providing new knowledge as to how blood vessels become dysregulated in cardiovascular disease (CVD) and related pathologies, our work will help uncover new strategies and therapeutics to combat disease, ultimately improving quality of life and life expectancy.

Research projects

Project 1. Can we improve atherosclerosis by targeting monocyte/macrophages for therapy?

Summary: This project will investigate whether increasing the level of TNF-related apoptosis-inducing ligand (TRAIL) specifically in monocyte/macrophages will improve their function and reduce atherosclerosis in mice.

Background: Atherosclerosis is the primary cause of CVD. In atherosclerosis, the blood vessels narrow, restricting blood flow, due to harmful fatty build-ups in the vessel wall. It is initiated by white blood cells (monocyte/macrophages), containing cholesterol, which accumulate around the site of an injury in the blood vessel wall. Our research shows that monocyte/macrophages expressing TRAIL is protective of atherosclerosis. We have discovered that in mouse models lacking TRAIL, monocyte/macrophages are more inflammatory, less able to regulate cholesterol, and less able to migrate out of the area of injury; accelerating atherosclerosis. Significantly, monocytes from people with coronary artery disease have reduced TRAIL expression.



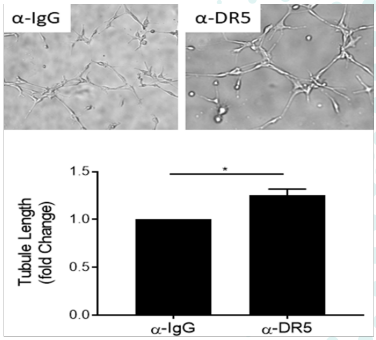
Overview: In this project, we will use liposome technology as a potential treatment. Atherosclerotic mice will be treated with liposomes containing TRAIL to specifically increase TRAIL levels in monocyte/macrophages. Atherosclerosis will be measured, as will plasma markers of CVD. We will also test the functional effects (e.g., cholesterol efflux, migration and inflammatory phenotype) of increasing TRAIL levels in macrophages ex vivo.

Relevant publications from our group:

- Kavurma MM, Rayner KJ, Karunakaran D. The walking dead: macrophage inflammation and death in atherosclerosis. Curr Opin Lipidol. 2017; 45(2):843-848.
- Cartland SP, et al., Kavurma MM. TRAIL-expressing monocyte/macrophages are critical for reducing inflammation and atherosclerosis. iScience. 2019; 12:41-52.

Project 2: Targeting death-inducing receptors in angiogenesis

Summary: This project seeks to investigate whether the TNF superfamily receptor, death receptor 5 (DR5) plays important role(s) in ischaemia-induced angiogenesis.



*α-DR5 agonistic mAb increases tubule formation in endothelial cells in vitro (n=3); t-test, *p<0.05.*

Background: Proliferation and apoptosis of cells is an intimately coupled process. We are interested in how molecules regulating aberrant proliferation and apoptosis of cells can lead to complications of CVDs including peripheral artery disease (PAD); a condition where arteries are narrowed due to atherosclerosis most commonly to the lower limbs. PAD is a major risk factor for amputation, heart attack and stroke. Restoring blood supply is critical but current interventions are often insufficient because extensive disease precludes revascularisation in many patients. An alternative approach is to stimulate the growth of new microvascular capillary networks (angiogenesis) to bypass the blockage and restore the nutrient diffusion necessary for tissue survival.

Overview: DR5 is a molecule known to kill cancer cells by inducing the caspase cascade. Our preliminary findings suggest that DR5 can also stimulate differentiation of cells to promote tube-like capillary structures in

vitro. In this project we will examine whether DR5 can promote blood vessel development in vitro, ex vivo and in vivo. A range of techniques will be used including proliferation, migration, and tubulogenesis assays, 3D angiogenic sprouting and in vivo models of angiogenesis involving hindlimb ischaemia. Additional techniques include Laser doppler perfusion, histology, gene expression (PCR, Western blotting) and ELISA.

Relevant publications from our group:

- Di Bartolo BA, Cartland SP, et al., Kavurma MM. Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) Promotes Angiogenesis and Ischemia-Induced Neovascularization Via NADPH Oxidase 4 (NOX4) and Nitric Oxide-Dependent Mechanisms. J Am Heart Assoc. 16;4(11). pii: e002527. doi: 10.1161/JAHA.115.002527.
- Cartland SP, Genner SW, Zahoor A, Kavurma MM. Comparative Evaluation of TRAIL, FGF-2 and VEGF-A-Induced Angiogenesis In Vitro and In Vivo. Int J Mol Sci. 2016 Dec 2;17(12). pii: E2025.

Project 3: Single cell transcriptome profiling of diabetic peripheral artery disease

Summary: This project seeks to investigate whether the TNF superfamily receptor, death receptor 5 (DR5) plays important role(s) in ischaemia-induced angiogenesis.

Background: Peripheral artery disease (PAD), where narrowed or blocked arteries reduce blood flow to limbs, is a disease with high human and social impact, significantly reducing quality of life. It is the third most prevalent form of atherosclerotic disease after heart disease and stroke. Restoring blood supply is critical but current interventions are insufficient as extensive disease precludes revascularisation in many patients. Significantly, patients can experience acute events, e.g., gangrene, necessitating surgical amputation of limbs. An alternative approach is to stimulate the growth of new micro blood vessels – a process called angiogenesis – to bypass the blockage and restore the nutrient diffusion necessary for tissue survival. To date, all large clinical trials delivering angiogenic factors to PAD patients have shown little benefit. It is now clear that these treatments do not adequately maintain stability of the newly formed vessels. Finding a pharmacological therapy for PAD that increases stable blood vessel formation and enables blood flow that could prevent amputation, would be life changing for these patients. To develop new therapies, we need greater understanding of the disease itself, particularly how genes regulate cell-cell interactions to promote stable micro blood vessel networks.

Overview: In this project single cell RNA sequencing (scRNAseq) will

be performed on limb tissues from control and diabetic mice with PAD. scRNAseq is a powerful, state-of-the-art technique that informs us of transcriptional activity of every gene in a cell for thousands of cells at the same time, with the potential to map in a 3D-manner, cell-cell, cell-gene and gene-gene interactions altered in PAD. Identifying genes that are differentially expressed in disease will provide a molecular signature that underpins PAD.

A range of techniques will be used in vivo models of angiogenesis involving microsurgery (hindlimb ischaemia), animal handling, Laser doppler perfusion, crypreservation, flow cytometry, histology, gene expression and simple bioinformatics platforms.

Relevant publications from our group:

- Di Bartolo BA, Cartland SP, et al., Kavurma MM. Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) Promotes Angiogenesis and Ischemia-Induced Neovascularization Via NADPH Oxidase 4 (NOX4) and Nitric Oxide-Dependent Mechanisms. J Am Heart Assoc. 16;4(11). pii: e002527. doi: 10.1161/JAHA.115.002527.

If you are interested in pursuing research experience with the Heart Research Institute, email **research@hri.org.au** for more information