Heart Research Institute

Research Opportunities

ABOUT THE HEART RESEARCH INSTITUTE

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

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

Our mission is to prevent death and suffering from cardiovascular disease through an understanding of the biological processes that cause atherosclerosis and thrombosis, the major underlying causes of most heart attacks and strokes.

We collaborate with institutes in Australia and globally – with over 60 collaborations across 25 countries – and are partnered with the Royal Prince Alfred Hospital, the Sydney Local Health District and Sydney Health Partners in Australia.

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 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.

Visit www.hri.org.au/join-us or email research@hri.org.au for more information

"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 honours student with Thrombosis Group

ATHEROSCLEROSIS AND VASCULAR REMODELLING GROUP
DR ASHISH MISRA

RESEARCH INTEREST

The main objective of our research program is to broaden 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 blending of mouse models and cultured cells, as well as human samples, with the aim of unveiling the pathogenesis of cardiovascular diseases. Our goal is to prevent and reverse vascular disease to prevent heart attack and stroke. Research carried out in this program focuses on the role of Notch signalling in atherosclerosis.

PROJECT 1 – NOTCH SIGNALLING IN ATHEROSCLEROSIS: FRIEND OR FOE

Aim: To understand the functional role of Notch signalling in the 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, 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 inflammatory cell recruitment and SMC 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 the Notch3 gene in the 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 the 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-SMC 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 (eg, PCR, Western blotting), molecular biology (eg, luciferase assays, chromatin immunoprecipitation), creating transgenic animals, histology, bone marrow transplant, *in vivo* fate mapping and clonal analysis.

Relevant publications

- 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. Using *in vivo* and tissue and cell explant approaches to study the morphogenesis and pathogenesis of the embryonic and perinatal aorta. Journal of Visualized Experiments, (2017), Sep 12;(127). doi: 10.3791/56039.
- Misra A et al. Integrin beta3 inhibition is a therapeutic strategy for supravalvular aortic stenosis. J. Exp. Med. (2016), 213 (3):451.

For more information contact: Dr Ashish Misra, Ashish.Misra@hri.org.au

ATHEROSCLEROSIS AND VASCULAR REMODELLING GROUP
DR ASHISH MISRA

PROJECT 2 – IMPACT OF DIABETES ON HAEMATOPOIETIC CELLS LINKED TO ATHEROSCLEROSIS

Background: Obesity and diabetes are major risk factors for a broad range of cardiovascular diseases. With three times as many people in the world estimated to die from over-nutrition than from starvation or malnutrition in today's society, the health implications of this "diabesity" epidemic are enormous. Based on current trends, this scenario will get worse, leading to a tsunami of cardiovascular diseases that could overwhelm a healthcare system already struggling to deal with an ageing population. Thus, there is an urgent need to uncover the fundamental mechanisms underlying the development of diabetes, including how cardiovascular risk factors affect atherosclerosis, in order to develop rational strategies for minimising the impact of these risk factors on our health and economy.

Project overview: Bone-marrow derived stem cells (BMDSCs) and progenitor cells are integral to tissue homeostasis and repair, and contribute to health through their ability to self-renew and commit to specialised effector cells. Importantly, defects in a variety of progenitor cell populations have been described in both preclinical and human diabetes. The general perception is that diabetes drives defects in BMDSCs, which accrue damage over time, disrupting tissue homeostasis and increasing risk of morbidity. However, the mechanisms by which defective BMDSCs can influence the pathology of individual plaque cells in atherosclerosis, and the subsequent impact this has on diabetes and obesity remains unknown.

In this study, we will be characterising effects of these BMDSCs on atherosclerotic plaque burden using state-of-the-art transgenic mouse models and cardiovascular genetics. We will be using the Cre-LoxP system, genetic knockouts, lineage tracing, clonal analysis, single-cell RNA sequencing, bone marrow transplant and culturing BMDSCs, histology of mouse and human patient samples.

For more information contact: Dr Ashish Misra, Ashish.Misra@hri.org.au

CARDIOMETABOLIC DISEASE GROUP
DR JOHN O'SULLIVAN

RESEARCH INTEREST

We aim to tackle the cardiovascular consequences of the obesity epidemic and to discover better diagnostic markers, predictors and therapies for cardiometabolic disease.

Obesity-driven metabolic disease such as insulin resistance, diabetes, fatty liver disease, hyperlipidaemia and hypertension are the major drivers of atherosclerotic cardiovascular disease in the modern era. This trend is continuing despite the best primary prevention efforts. These complex diseases are the consequence of gene-environment interactions, and to truly understand the various levels of dysregulation, both genomic data and environmental data must be captured. We probe carefully phenotyped patient cohorts using genome scanning and metabolomic profiling to discover novel disease markers that may have clinical utility, eg, by providing better diagnostic markers of disease, and allowing earlier intervention by predicting future disease. Furthermore, integration of genetic and metabolomic data allows delineation of disease pathways, which we then study in animal and cell models of disease. This allows us to determine disease-specific functional regulation, and potential for therapeutic intervention.

Our projects include: (i) a new therapy for fatty liver and diabetes; (ii) novel disease pathways in coronary artery disease; and (iii) discovering new disease pathways in young people at risk of cardiometabolic disease.

PROJECT 1 – GIVING THE FAILING HEART THE NUTRIENTS IT NEEDS

Project overview: The aim of this project is to determine the key cardiac substrates depleted in the failing heart, and to determine if replacing them can restore normal heart function. Heart failure (HF) associated with obesity and type 2 diabetes (leading to "stiff hearts", termed "Heart Failure preserved Ejection Fraction", or HFpEF), has exploded in prevalence, has no specific treatment, and is driven in large part by altered metabolism. Therefore, our specific aims are to: (i) measure cardiac substrate changes in human HFpEF and determine association with heart function and clinical outcome; (ii) determine stages of metabolic alteration, and substrate turnover, during the natural history of HFpEF using model systems; and (iii) investigate the effects, and underlying mechanism, of key substrate administration on cardiac function.

PROJECT 2 – PROBING MICROBIOME-METABOLOME-CARDIOVASCULAR DISEASE INTERACTIONS

Project overview: Recent research has shown significant health benefits deriving from high-dietary fibre/microbiome-accessible carbohydrate (MAC) consumption. Compared with native starch, dietary resistant starch is a high-MAC starch that significantly alters the gut microbiome. The aim of a recent study was to determine the systemic metabolic effects of MAC. Male C57BL/6 mice were divided into two groups and fed either native starch or resistant starch for 18 weeks (n = 20/group). Metabolomic analyses revealed plasma levels of numerous metabolites were significantly different between the resistant starch-fed and native starch-fed mice, many of which are microbiome-derived. Most strikingly, we observed a 22-fold increase in gut microbiome-derived tryptophan metabolite indole-3-propionate (IPA), which was positively correlated with several gut microbiota including Clostridiales, Allobaculum, Bifidobacterium and Prevotella; Allobaculum had the most consistently increased abundance of IPA-associated taxa across all resistant starch-fed mice.

In addition, major changes were observed for metabolites solely or primarily metabolised in the gut, eg, trimethylamine-N-oxide; metabolites that have a significant entero-hepatic circulation, ie, bile acids; lipid metabolites, eg, cholesterol sulfate; metabolites indicating increased energy turnover, eg, tricarboxylic acid cycle (TCA) intermediates and ketone bodies; and increased antioxidants such as reduced glutathione. Our findings reveal potentially novel mediators of high MAC-derived health benefits.

We will now extend this analysis and examine the role of IPA, and related indoles, as *the* major mediators of the cardiometabolic benefits of high-fibre diets.

For more information contact: Dr John O'Sullivan, John.O'Sullivan@hri.org.au

CARDIOVASCULAR-PROTECTIVE SIGNALLING AND DRUG DISCOVERY GROUP
DR XUYU LIU

RESEARCH INTEREST

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.

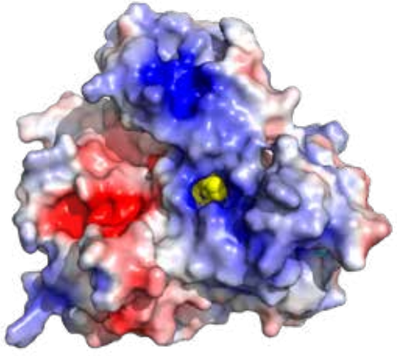
Recently, there has been considerable interest in the development of natural supplements for cardiovascular-protective therapeutics owing to their inherent safety profiles and the clinical evidence for ameliorating chemotherapy-induced cardiovascular complications. However, it remains a huge challenge 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.

PROJECT – UNDERSTANDING HEART-HEALTHY DIETS AT THE MOLECULAR LEVEL

Project overview: Sulforaphane and alliin are known to be the cardioprotective "ingredients" in broccoli and onion diets. They have been shown to promote cardiomyocyte survival against ischaemic injury and exhibit potent anticancer activity by potentiating apoptosis. However, the protein target spectra of these small molecules in cells remain unclear. There is no unified model to explain the cell-type-dependent phenotypes observed in the treatment. Therefore, the specific aims of the first arm of this project are to: (i) profile the cell-type-specific target spectra of sulforaphane and alliin and forge a molecular link between protein target engagement and phenotypic outcome; and (ii) engineer small-molecule transport proteins targeting specific organelles to enable protein target profiling of sulforaphane and alliin in a spatiotemporally controlled manner.

In collaboration with the Payne research group (School of Chemistry, USYD), the specific aims of the second arm of this project are to: (i) optimise the efficacy and mitigate the cardiotoxicity of current chemotherapy through conjugation with cardiovascular-protective natural products; and (ii) apply "click-and-release" chemistry to develop antibody-small-molecule conjugates enabling organ- and tissue-specific release of sulforaphane and alliin.



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

For more information contact: Dr Xuyu Liu, Xuyu.Liu@hri.org.au

CARDIOVASCULAR MEDICAL DEVICES GROUP
DR ANNA WATERHOUSE

RESEARCH INTEREST

Our research focuses on how medical devices – such as artificial hearts, stents and bypass machines – interact with the body. Cutting-edge bioengineering tools are applied 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.

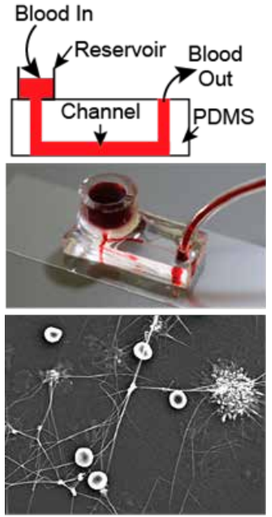
Our research is focused on development of: (i) biointerfaces; (ii) biomimetic model systems; and (iii) bioengineering smart materials and nanorobots.

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 that 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 (see figure). 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.



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

For more information contact: Dr Anna Waterhouse, Anna.Waterhouse@hri.org.au

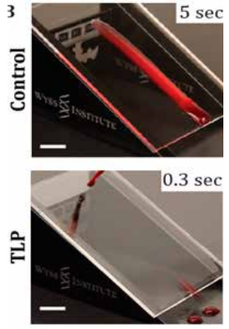
CARDIOVASCULAR MEDICAL DEVICES GROUP
DR ANNA WATERHOUSE

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), reduces the thrombogenicity of medical devices.

Background: Thrombosis caused by medical devices can be costly and fatal for a number of reasons, including: (i) failure of device function, requiring device replacement, which can be expensive, or cessation of blood flow, which can be fatal; and (ii) pulmonary embolism or stroke caused by embolism of the thrombus. 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: 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 provide insights for design improvements.



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.

For more information contact: Dr Anna Waterhouse, Anna.Waterhouse@hri.org.au

CARDIOVASCULAR MEDICAL DEVICES GROUP
DR ANNA WATERHOUSE

PROJECT 3 – MOLECULAR NANOROBOTICS FOR HEALTH: HAEMOCOMPATIBILITY BY DESIGN

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

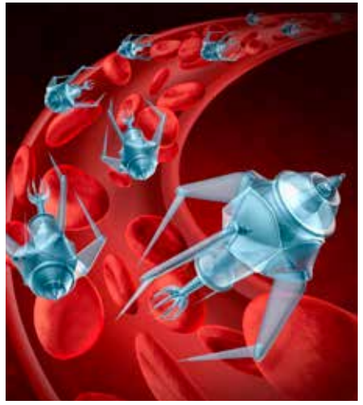
Background: Atherosclerosis is one of the world's biggest killers, and current diagnostic methods are inadequate for early disease detection. Molecular-level changes in early atherosclerosis occur on the nanoscale. We are building molecular nanorobots, autonomous and programmable nanomachines self-assembled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

Project overview: This project aims to make nanorobots haemocompatible 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 haematological and immunological compatibility of nanomaterials and establish biomimetic solutions for nanorobot development and *in vitro* and *in vivo* models for nanorobot evaluation. This is a highly interdisciplinary project 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

- 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).



Conceptual image of molecular nanorobots consisting of a core with the ability to move, sense, react and interact, inside a human artery.

For more information contact: Dr Anna Waterhouse, Anna.Waterhouse@hri.org.au

CARDIOVASCULAR NEUROSCIENCE GROUP
DR MELISSA FARNHAM

RESEARCH INTEREST

We are interested in the central control of blood pressure and breathing. For the last few years we have been focusing on a model of obstructive sleep apnoea (OSA) and the role of a neuropeptide called PACAP (pituitary adenylate cyclase activating polypeptide).

We also study how OSA causes diabetes and are trying to identify the central pathways that lead to glucose handling dysfunction in OSA.

PROJECT – SLEEP APNOEA AND HYPERTENSION: WHAT GOES WRONG IN THE BRAIN?

Aim: To uncover the mechanisms driving the cardiometabolic effects of OSA to spearhead the development of new strategies to treat OSA.

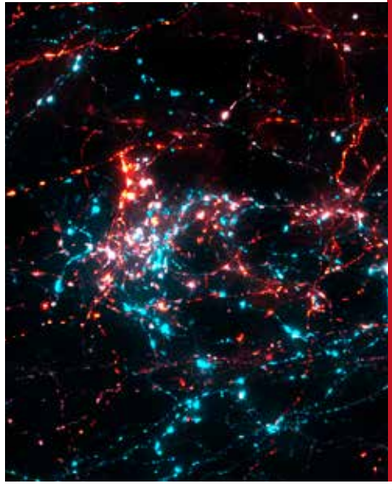
Background: Repetitive hypoxia is a feature of OSA, a condition characterised by intermittent airways obstruction. Patients with OSA present with persistent increases in sympathetic activity and commonly develop hypertension, which is precursor to the more malignant cardiovascular disease. Diabetes is also commonly associated with OSA. Work from our lab has shown that the persistent increases in nerve activity, following a protocol of acute intermittent hypoxia, are dependent upon activation of PACAP receptors in the spinal cord. PACAP is an excitatory neuropeptide found throughout the sympathetic nervous system. PACAP also acts to elevate blood glucose by stimulating adrenaline release. Other groups have shown, in both rats and humans, that intermittent hypoxia elevates blood glucose. We hypothesise that PACAP is driving the increase in nerve activity, leading to hypertension and increased adrenaline release and elevated glucose. These early changes could initiate triggers that promote insulin resistance and development of type 2 diabetes in human OSA conditions.

Project overview: We use rodent models of OSA, both anaesthetised and conscious to measure blood pressure, heart rate and various sympathetic nerve activities following pharmacological manipulations of the central nervous system. The physiology experiments are combined with immunohistochemistry and molecular experiments to assess the signalling changes within the brain and spinal cord.

Relevant publications

- Farnham MMJ, Li Q, Goodchild AK, Pilowsky PM. (2008) PACAP is expressed in sympathoexcitatory C1 neurons of the brainstem and increases sympathetic nerve activity *in vivo*. Am J Physiol 294:R1304-1311.
- Inglott MA, Lerner, E, Pilowsky PM, Farnham MMJ. (2012) Activation of PAC1 and VPAC receptor subtypes elicits differential physiological responses from sympathetic preganglionic neurons in the anaesthetised rat. Br J Pharmacol 167:1089-98.
- Kakall ZM, Pilowsky PM and Farnham MMJ. (2017) PACAP(6-38) or kynurenate microinjections into the RVLM prevent development of sympathetic long-term facilitation following acute intermittent hypoxia. Am J Physiol 314: H563-H572.

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



For more information contact: Dr Melissa Farnham, Melissa.Farnham@hri.org.au

CLINICAL RESEARCH GROUP
DR KEYVAN KARIMI

RESEARCH INTEREST

Pulmonary vasculature in health and disease – new therapies for pulmonary hypertension: Our research focus is in identifying the mechanisms that control perfusion in pulmonary vessels in physiology and changes that occur in pathophysiology. Specifically, we study the effects of hypoxia on pulmonary vasculature, which in animal models recapitulate the clinical phenotype in disease states such as pulmonary hypertension – a rare but deadly disease. Using the animal model, we study new pharmaco-therapies for pulmonary hypertension that target pulmonary vasoconstriction and proliferative phenotype in microvasculature that are hallmarks of this disease.

The eventual aim of our studies is to test these therapies in clinical trials for treatment of patients suffering from pulmonary hypertension.

PROJECT – MECHANISMS OF MICROVASCULAR INJURY IN THE HEART

Despite opening the blockage in coronary arteries in patients with acute heart attack, normal perfusion of the heart tissue is not fully restored in about 50 per cent of patients – a phenomenon that is clinically referred to as “no-reflow” and is mechanistically poorly understood. No-reflow is associated with significant increase in morbidity and mortality.

In collaboration with Professor Shaun Jackson (Thrombosis Group), we plan to examine the changes that occur during ischaemia and reperfusion in the heart vessels ex vivo using microscopy techniques. The ultimate aim of this project is to assess these changes in vivo by intravital microscopy of the beating heart.

For more information contact: Dr Keyvan Karimi, Keyvan.Karimi@hri.org.au

CORONARY DISEASES GROUP
ASSOCIATE PROFESSOR SANJAY PATEL

RESEARCH INTEREST

Our mission is to develop novel therapies to target atherosclerosis (arterial blockages) and its consequences – heart attack and stroke. Our treatment mission is to bypass arterial blockages by stimulating new blood vessel growth, to restore blood flow to affected regions.

Our research focus is to develop novel stem-cell based therapies for patients with blockages in the blood vessels of the leg and heart, including: (i) generation of induced pluripotent stem cells (iPSCs); (ii) testing the angiogenic potential of iPSCs *in vitro* and *in vivo*; (iii) testing the potential pro-atherosclerotic properties intra-vitreal VEGF inhibitors; and (iv) testing the anti-inflammatory effects of colchicine in patients with coronary artery disease.

PROJECT – DEVELOPING NOVEL STRATEGIES TO REDUCE ACUTE ATHEROSCLEROSIS-ASSOCIATED INFLAMMATION

Aim: The overall aim of this project is to comprehensively assess the acute and chronic anti-inflammatory effects of colchicine in atherosclerosis in patients and in animal models of disease. We will additionally assess the effects of colchicine on infarct-related inflammation.

Project overview: Inflammatory cells and mediators play a critical role in the progression of atherosclerotic plaques and can also deleteriously affect plaque stability. The period following a myocardial infarction phase is also characterised by a pro-inflammatory state that, if prolonged, can lead to ventricular remodelling, myocardial dysfunction and subsequent heart failure. Emerging data has identified that colchicine may be protective against future cardiovascular events in patients with established atherosclerosis. Colchicine is an established, potent and safe anti-inflammatory drug that is widely used in other inflammatory conditions, however the mechanisms by which it exerts its athero-protective effects are poorly understood. We expect that our work will contribute to a greater understanding into the cardioprotective effects of colchicine and may eventually lead to the inclusion of this safe and cost-effective drug as part of the standard drug armamentarium in the treatment of atherosclerosis and acute myocardial infarction. This is a truly translational project incorporating bench work, state-of-art molecular imaging in animal models of disease and patient studies in the cardiac catheterisation laboratory.

For more information contact: Associate Professor Sanjay Patel, Sanjay.Patel@hri.org.au

RESEARCH INTEREST

We aim to discover novel pathways in blood clotting that can lead to the development of effective and safe drugs to treat thrombosis.

PROJECT 1 – THIOL ISOMERASES AS NOVEL ANTITHROMBOTIC TARGETS

Aim: To discover alternative pathways in the clotting system that can be targeted to develop more efficient and safer antithrombotic drugs.

Background: It has recently been discovered that 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*.

Project overview: 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 and 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.

These studies will employ platelet function tests, cell perfusion assays, flow cytometry and confocal microscopy. This project will provide the opportunity to learn the method of intravital microscopy for the study of clot formation in mice.

Relevant publications

- Sharda A, Furie B. Regulatory role of thiol isomerases in thrombus formation. Expert Rev Hematol. 2018;11(5):437-448.
- Passam FH, Lin L, Gopal S, Stopa JD, Bellido-Martin L, Huang M, 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, Kennedy DR, Kim SH, van Hessem L, Lin L, Bowley SR, Joshi SS, Dilks JR, 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.

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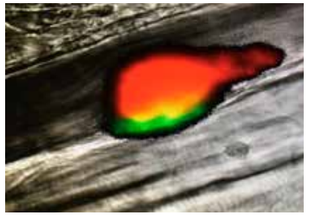

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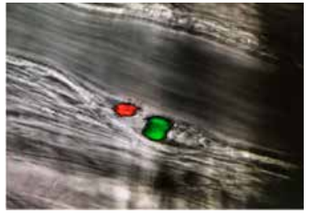


In vivo thrombus formation in the cremaster artery of (A) a mouse injected with inactive control compound and (B) a mouse injected with an ERp5 inhibitor. Platelets are labelled in red and fibrin in green.

For more information contact: Dr Freda Passam, Freda.Passam@hri.org.au

HAEMATOLOGY RESEARCH GROUP
DR FREDA PASSAM

PROJECT 2 – REDOX BIOMARKERS IN THROMBOTIC DISEASE

Aim: To identify new biological markers that can be used in the monitoring and treatment of patients with thrombotic disease.

Background: The redox balance (balance of reduction and oxidation reactions in our blood) is essential for a healthy circulation. Redox imbalance causes alterations of protein function contributing to the development of thrombosis. We are focused on redox modification of disulphide bonds in two proteins critical for thrombus formation: the platelet receptor integrin a2bb3 and the plasma protein von Willebrand factor. We have found that reduced forms of a2bb3 and vWF have decreased thrombotic activity and may therefore protect from thrombotic disease, such as venous clots.

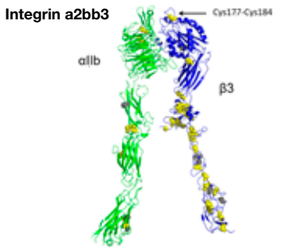
Project overview: We have developed assays which measure the redox balance in blood, including tests which measure the disulphide reducing activity of plasma and the production of reactive oxygen species by platelets. We will study the redox modifications of platelet a2bb3 and plasma vWF that occur in patients at high risk for thrombosis to identify those most likely to benefit from drugs that restore the normal redox balance.

This project employs mass spectrometry to study the posttranslational modifications of clotting proteins using disulphide labels specifically developed for this purpose. It also involves plasma and platelet functional assays.

Relevant publications

- Karimi Galougahi K, Antoniades C, Nicholls SJ, Channon KM, Figtree GA. Redox biomarkers in cardiovascular medicine. Eur Heart J. 2015;36(25):1576-1582.
- Passam F, Chiu J, Ju L, Pijning A, Jahan Z, Mor-Cohen R, Yeheskel A, Kolšek K, Thärichen L, Aponte-Santamaría C, Gräter F, Hogg PJ. Mechano-redox control of integrin de-adhesion. Elife. 2018, Jun 22.
- Butera D, Passam F, Ju L, Cook KM, Woon H, Aponte-Santamaría C, Gardiner E, Davis AK, Murphy DA, Bronowska A, Luken BM, Baldauf C, Jackson S, Andrews R, Gräter F, Hogg PJ. Autoregulation of von Willebrand factor function by a disulfide bond switch. Sci Adv. 2018 Feb 28.

A



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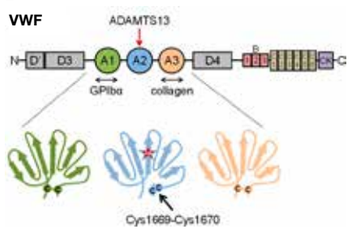


Alterations of disulphide bonds in clotting proteins that cause decreased clotting activity in (A) the platelet receptor a2bb3, bond Cys177-Cys184 and in (B) von Willebrand factor, bond Cys1669-Cys1670.

For more information contact: Dr Freda Passam, Freda.Passam@hri.org.au

HAEMATOLOGY RESEARCH GROUP
DR FREDA PASSAM

PROJECT 3 – DEVELOPING BIOCHIPS FOR THE STUDY OF HAEMOSTASIS AND THROMBOSIS

Aim: To develop microfluidic devices that can detect the thrombotic or bleeding tendency in patients with clotting problems.

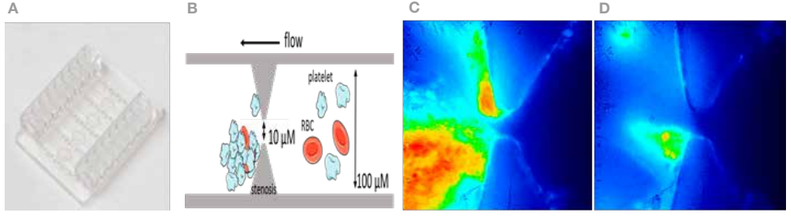
Background: Many patients with bleeding and clotting disorders go undetected by routine laboratory tests in part because the available assays do not reflect the conditions in the circulation. In our research, we use biochips in a microfluidic system that allows blood to flow through passages under controlled conditions. The passages are designed to mimic blood vessels and include features, eg, 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.

Project overview: This project will study blood cell adhesion and thrombus formation in microfluidic devices to assess for persisting thrombotic tendency in patients with a history of venous clots, who have completed treatment. Samples from patients with bleeding disorders on treatment will be assessed for haemostatic potential. A range of parameters that 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 microfluidics chips, microscopy and image analysis.

Relevant publications

- 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.



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.

For more information contact: Dr Freda Passam, Freda.Passam@hri.org.au

INFLAMMATION AND FIBROSIS GROUP
DR BEN RAYNER

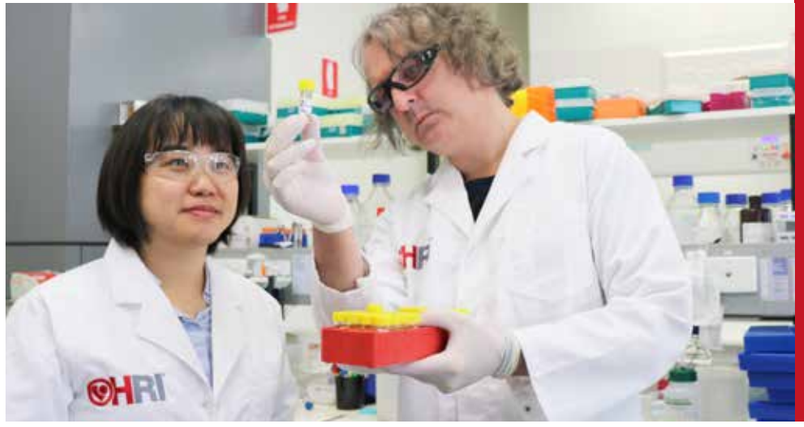
RESEARCH INTEREST

We focus on a range of causative aspects of cardiovascular disease, from the role the balance between oxidative stress and antioxidant defence systems play within cells of the vasculature and heart, to how to better combat atherosclerotic lesion development through antioxidant therapy, through to development of better targeted therapy for heart attack patients. Whilst improvements in intensive care have reduced the number of deaths attributable to heart attacks, the damage caused by an ischaemic event within the heart remains poorly managed, resulting in ongoing morbidity.

PROJECT – TARGETING THE UNDERLYING MECHANISMS OF CARDIAC FIBROSIS AND HEART FAILURE

Project overview: Cardiac remodelling following an adverse cardiac event involves instrumental changes to the extracellular matrix controlled by the transformation of resident cardiac fibroblasts towards a myofibroblast phenotype, causing tissue fibrosis and scar formation, leading to reduced heart function and progression to heart failure. To date, no effective therapeutical approach has been developed to combat these phenomena. Citrullination, the conversion of arginine to citrulline, is an irreversible protein post-translational modification that changes the biochemical and functional properties of proteins. Citrullination in vivo occurs through the activation of the calcium-dependent peptidylarginine deiminase (PAD) family of enzymes and has been shown to occur within failing hearts. We have obtained novel data demonstrating a pivotal role for PAD activation underlying the fibrosis and scar formation that is evident during heart failure.

This project will assess the effect of PAD inhibition on cardiac damage following ischaemia reperfusion injury in rats. Specifically, determination of circulating plasma fibrotic and inflammatory markers, including IL-1β, IL-6, IL-10, MCP-1 and TNFα by ELISA. Tissue fibrosis within the heart will be assessed through histological techniques, including Masson's Trichrome and Picrosirius red staining, as well as IHC for fibrosis markers αSMC actin, CTGF and the fibroblast-specific periostin, with matrix metalloproteinase (MMP) expression and activity assessed by gelatin zymography. These tissue dynamics will also be confirmed with qPCR analysis of mRNA expression within heart tissue.



For more information contact: Dr Benjamin Rayner, Benjamin.Rayner@hri.org.au

RESEARCH INTEREST

Our research is focused on the haemostatic and innate immune systems and their dysregulation in cardiovascular disease. Our main research focus is on blood cells (platelets, leukocytes), blood coagulation proteases and endothelial cells. These cell types play a fundamental role in the pathogenesis of diseases such as heart attack and ischaemic stroke, but also more broadly, in the context of inflammation, cancer metastasis and vascular development.

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.

PROJECT 1 – DISCOVERY AND DEVELOPMENT OF NEW CLASSES OF ANTIPLATELET AND ANTICOAGULANT DRUGS FOR THE TREATMENT OF STROKE

Project overview: 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 reopening 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). We have developed isoform-selective inhibitors against PI3Kβ and demonstrated that these inhibitors are highly effective at promoting and facilitating thrombus dissolution and complete vascular reperfusion, without markedly increasing tail bleeding times. Preliminary studies have

For more information contact: Associate Professor Simone Schoenwaelder, Simone.Schoenwaelder@hri.org.au

THROMBOSIS GROUP
PROFESSOR SHAUN JACKSON

revealed that PI3Kβ inhibitors lead to localised regions of thrombus instability and subsequently, the development of channels within the body of the thrombus. 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: (i) *in vivo* models of thrombosis and thrombolysis; (ii) genetic mouse models; (iii) state-of-the-art imaging systems (tissue clearing techniques, confocal microscopy, intravital microscopy, laser doppler flowmetry and laser speckle contrast imaging); and (iv) behavioural assessment to determine cerebral damage following recovery from stroke.

Relevant publications

- Samson AL 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 2 – INVESTIGATION OF A NEW THROMBOSIS AND INFLAMMATION MECHANISM TRIGGERED BY 'DEATH PATHWAYS' IN PLATELETS

Project overview: Ischaemia reperfusion (IR) injury commonly occurs in a wide range of human diseases, including acute myocardial infarction and ischaemic stroke. IR injury is characterised by poor blood flow in the micro-vasculature, exacerbating tissue ischaemic and organ injury.

Our recently published study has identified a new mechanism by which platelets and neutrophils cause microvascular obstruction. This previously unrecognised thrombotic mechanism is induced by the fragile membranes from dying platelets, that physically bridge adjacent neutrophils to facilitate neutrophil aggregation, leading to vessel occlusion.

In our most recent studies, we demonstrate that dying platelets can convert neutrophils to a hyperadhesive inflammatory state. Furthermore, neutrophils can in turn induce platelet death via production of oxidants. These new findings suggest a bidirectional communication mechanism operating between platelets and neutrophils, that may exacerbate microvasculature dysfunction and inflammation during IR injury. We are investigating this unique communication and its contribution to IR injury.

During this project, you will learn: (i) *in vitro* functional assays used to assess platelet death and neutrophil hyperadhesive function; (ii) mouse models of IR injury adopted to investigate microvascular dysfunction; (iii) real time *in vivo* imaging of platelet death and neutrophil-platelet adhesion dynamics in the microvasculature during IR injury; and (iv) gain knowledge in the relevant field.

Relevant publications

- Yuan Y 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.

For more information contact: Associate Professor Simone Schoenwaelder, Simone.Schoenwaelder@hri.org.au

PROJECT 3 – EXAMINATION OF A NEW THROMBOSIS MECHANISM LINKED TO DIABETES

Project overview: Diabetes mellitus (DM) has become one of the major healthcare challenges of the 21st century and a leading cause of cardiovascular disease worldwide. Up to 70 per cent of all diabetes-related deaths are due to cardiovascular disease, primarily related to atherothrombosis. Diabetes enhances the atherosclerotic process in large arteries, increasing the risk of acute myocardial infarction (heart attack), cerebral infarction (ischaemic stroke) and peripheral vascular disease. In addition to developing more extensive atherosclerosis, diabetic individuals also exhibit a prothrombotic phenotype that manifests as an exaggerated accumulation of platelets at sites of plaque disruption. However, the mechanisms by which diabetes causes platelet hyperactivity and a prothrombotic phenotype remain incompletely understood.

We have recently defined a new mechanism that promotes arterial thrombus formation, termed biomechanical platelet activation. We now have evidence that this aggregation mechanism is dysregulated in diabetes, leading to excessive platelet aggregation and thrombus formation. We have identified that chronic oxidative stress in diabetes plays a key role in amplifying platelet aggregation by altering the shear-sensitivity of the major platelet adhesion receptor integrin αIIbβ3 (commonly referred to as GPIIb-IIIa). We hypothesise that this biomechanical prothrombotic mechanism is associated with alterations in redox-sensitive signal pathways linked to GPIIb-IIIa. Importantly, exaggerated platelet aggregation is not inhibited by conventional antiplatelet agents such as aspirin and clopidogrel, which may partly explain reduced efficacy of antiplatelet therapies in individuals with diabetes.

This project will examine: (i) the impact of DM on the biomechanical adhesive function of GPIIb-IIIa; (ii) how redox-sensitive signalling pathways are dysregulated in DM platelets; and (iii) whether inhibiting platelet redox-sensitive signalling pathways reduces platelet hyperactivity and thrombosis in diabetes.

In order to investigate platelet mechanobiology at a cell-molecular scale, we have established this 4Ms approaches in Australia – Mechanics, Microscopy, Microfabrication & Molecular Mouse Models – by combining the live-cell dynamic force spectroscopy BFP system with other complementary technologies including the TIRF/STORM super-resolution imaging, microfluidics, and *in vivo* mouse models of thrombosis.

Relevant publications

- 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 SP and Schoenwaelder SM. Nature Reviews Drug Discovery, 2:775-789, 2003.
- Nesbitt WS et al. Nature Med. (Article) 15(6):665-673, 2009.

PROJECT 4 – UNDERSTANDING THE MECHANISMS LEADING TO MICROVASCULAR DYSFUNCTION AND POOR CEREBRAL PERFUSION IN STROKE

Project overview: 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 reopen the blocked arteries to salvage the dying ischaemic tissue. However, despite the reopening of the culprit artery, blood perfusion in surrounding microvasculature supplying the tissue can remain poor, a common complication of ischaemia reperfusion (IR) injury, known as microvascular obstruction (MVO).

MVO occurs in 60 per cent of 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. Our studies have revealed that both ischaemia and reperfusion trigger endothelial cell death, however these events are phenotypically distinct. These cell death processes are associated with the onset of distinct vessel occlusive mechanisms involving distinct blood cell subsets, including red blood cells (during ischaemia), and platelets and neutrophils (during reperfusion), which cooperatively lead to MVO in local gut and remote organs. These findings demonstrate an intimate spatiotemporal relationship between endothelial injury and vaso-occlusion mechanisms. They also help explain why existing therapies remain ineffective. Building on these studies, this project will focus on the pathways by which endothelial cells undergo cell death and the molecular mechanisms that trigger these death pathways during both ischaemia and reperfusion. Importantly, we will examine whether inhibiting endothelial cell death represents an effective approached to prevent MVO associated with IR injury.

This project will examine: (i) pathogenesis of microvascular dysfunction associated with IR injury, relevant to heart attack and stroke; (ii) cell death pathways by which endothelial cells die during IR injury; (iii) molecular mechanisms which trigger endothelial cell death *in vivo*; (iv) cutting-edge confocal microscopy on living animals; and (v) physiologically relevant cell function assays.

Relevant publications

- Yuan Y, Alwis I, Wu M et al. Sci. Trans. Med, 2017; Sep 27;9(409); doi: 10.1126/scitranslmed.aam5861.
- Jackson SP. Nature Med. 17(11):1423-1436, 2011.

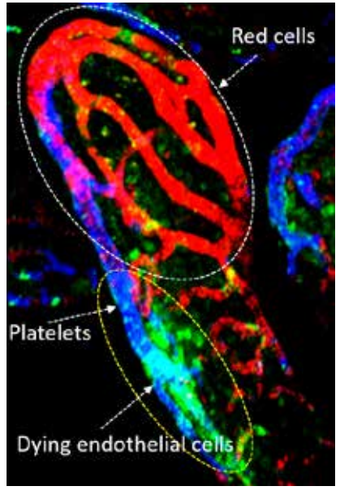


Image courtesy of Thrombosis Group.

For more information contact: Associate Professor Simone Schoenwaelder, Simone.Schoenwaelder@hri.org.au

THROMBOSIS GROUP
PROFESSOR SHAUN JACKSON

For more information contact: Associate Professor Simone Schoenwaelder, Simone.Schoenwaelder@hri.org.au

VASCULAR COMPLICATIONS GROUP
DR MARY KAVURMA

RESEARCH INTEREST

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 diabetes and cardiovascular diseases (CVDs) including atherosclerosis. In particular, the laboratory is interested in tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL). TRAIL is a TNF-ligand family member, originally identified as an inducer of cancer cell death. Our studies in CVD indicate important new functions for TRAIL that mediate survival of cells, distinct from its cell death promoting activities.

PROJECT 1 – CAN WE IMPROVE ATHEROSCLEROSIS BY TARGETING MONOCYTE/MACROPHAGES FOR THERAPY?

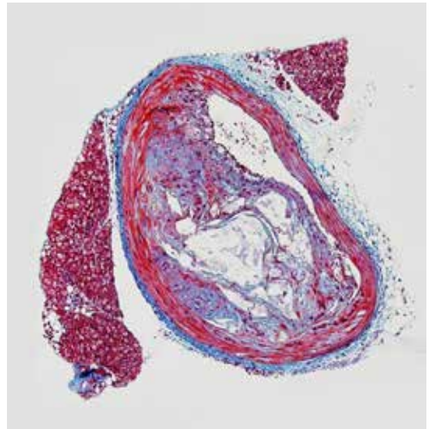
Aim: To investigate whether increasing the level of TRAIL specifically in monocyte/macrophages will improve their function and reduce atherosclerosis and therefore whether TRAIL could be a potential treatment in people.

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 a protein called TRAIL protects against 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, thus accelerating atherosclerosis. Significantly, we have shown that monocytes from people with CVD have reduced TRAIL, suggesting that its presence protects against CVD.

Project 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 (eg, cholesterol efflux, migration and inflammatory phenotype) of increasing TRAIL levels in macrophages *ex vivo*.

Relevant publications

- 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, Kavurma MM et al. TRAIL-expressing monocyte/macrophages are critical for reducing inflammation and atherosclerosis. iScience. 2019; 12:41-52.



Atherosclerotic plaque in brachiocephalic artery sections, stained with Masson's trichome, demonstrating muscle fibres (red), collagen (blue) and cell nuyclei (black). Image courtesy of Dr Siân Cartland, Vascular Complications Group.

For more information contact: Dr Mary Kavurma, Mary.Kavurma@hri.org.au or Dr Siân Cartland, Sian.Cartland@hri.org.au

VASCULAR COMPLICATIONS GROUP
DR MARY KAVURMA

PROJECT 2 – IS DR5 IMPORTANT IN ISCHAEMIA-INDUCED ANGIOGENESIS?

Aim: To investigate whether DR5 plays a role in angiogenesis in ischaemia.

Background: TNF-related apoptosis-inducing ligand (TRAIL) was discovered for its unique ability to selectively kill cancer cells. In complete contrast to its role in cancer, our research uncovered entirely novel functions of TRAIL in the vasculature, challenging general dogma. Important for this project is our discovery that TRAIL stimulates angiogenesis (growth of new blood vessels) *in vitro*, and *in vivo* in peripheral artery disease in mice, restoring blood flow to normal, and preserving tissue survival and function. How TRAIL regulates angiogenesis is unclear and whether this involves its receptor DR5 is unknown.

Project overview: 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 ischaemic injury (hindlimb ischaemia). Additional techniques include Laser doppler perfusion, histology, gene expression (PCR, Western blotting) and ELISA.

Relevant publications

- Di Bartolo BA, Cartland SP, Kavurma MM et al. 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.

For more information contact: Dr Mary Kavurma, Mary.Kavurma@hri.org.au or Dr Siân Cartland, Sian.Cartland@hri.org.au

www.hri.org.au

Heart Research Institute, 7 Eliza Street, Newtown NSW 2042 ABN 41 003 209 952
Email: research@hri.org.au

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