Molecular microbiology

Programme Leader: Associate Professor Brian Monk

Molecular Microbiology research within the SJWRI encompasses microbiological investigations applied to a variety of disciplines relevant to Dentistry. These include endodontics, periodontics, implantology, cariology and treatment with antimicrobials, antifungal and antibacterial drug development, drug resistance, structural biology and microbial genomics.

Research is primarily conducted in the Molecular Biosciences Laboratory, which relocated in October 2018 to a temporary facility in the ground floor of the Department of Biochemistry during the redevelopment of the Walsh Building.

Key personnel

Staff

Professor Richard Cannon
Associate Professor Vincent Bennani
Dr Peter Cathro
Associate Professor Dawn Coates
Dr Gemma Cotton
Dr Nick Heng
Dr Mikhail Keniya
Dr Erwin Lamping
Professor Karl Lyons
Dr Li Mei
Dr Trudy Milne
Associate Professor Brian Monk
Dr Don Schwass
Associate Professor Geoffrey Tompkins
Dr Rajni Wilson

Postgraduate students

PhD

Dina Abdelmoneim
Minati Choudhury
Zhen Dong
Asrar Elahi
Christina Gee
Parham Hosseini
Chitra Krishnan
Golnoush Madani
Yasmeen Ruma
Amira Salem
Shaikha Al Samahi

DClinDent

Deepak Chellappa
James Millar
Nurul Thiyahuddin
Marguerite Paterson
Anumala Ram
Michael Skilbeck

Current research projects

Structure-directed discovery of next-generation antifungals

Brian Monk, Mikhail Keniya, Rajni Wilson

The emerging problem of antifungal resistance, together with a lack of structural information on existing antifungal drug targets, is a significant issue in both medicine and agriculture. We are investigating the structure and function of a number of antifungal drug targets, including the azole target lanosterol 14α-demethylase, the terbinafine target squalene monooxygenase, the echinocandin target glucan synthase and drug efflux pumps from the ATP binding cassette and major facilitator superfamilies. Overexpressing these proteins in yeast provides proteins for purification and structural resolution by X-ray crystallography, as well as enabling targeted physiological screens for antifungals and valuable tests of antifungal efficacy. This information is being used to design potent chimeric antifungals that combine the best attributes of existing antifungals, and has enabled computer-based screens of large compound libraries in efforts to discover novel antifungals.

Since 2014, our group has deposited in the Protein Data Bank over 30 crystal structures of wild type and mutant lanosterol 14α-demethylase from *Saccharomyces cerevisiae* in complex with a range of azole drugs and agrochemicals, plus the first crystal structures of full-length lanosterol 14α-demethylase from the fungal pathogens *Candida glabrata* and *Candida albicans*. We have also investigated lanosterol 14α-demethylase in other important and emerging fungal pathogens including the human pathogens *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Candida parasilosis* and the plant pathogens *Zymoseptoria tritici* and *Phakopsora pachyrhizi*. This research is providing phenotypic and structure-based insight into the intrinsic azole resistance associated with the ancient mucormycete family of fungal pathogens.

The group published 4 papers in this area in 2019-20. Our primary funding was a Health Research Council of NZ grant (2016-2019) entitled "Structure-directed discovery of next-generation antifungals" and a subsequent HRC grant (2019-2022) "Readying next-generation antifungals for drug development". These grants built on previous Marsden (2010-2015) and HRC (2013-2016) funding. The award of a Catalyst Fund grant in 2018 supported a collaboration and researcher exchange in 2019-20 with Associate Professor Michaela Lackner of the Medical University of Innsbruck.

A/Prof Michaela Lackner (right) with PhD students Yasmeen Ruma (left) and Parham Hosseini (centre).

Fungal colonisation and drug resistance

Richard Cannon, Erwin Lamping

The increased incidence of infections caused by drug resistant microorganisms is a major global health concern. While the multidrug resistance of bacteria is most prominent, drug resistance of fungi is also of great concern.The main cause of high-level azole drug resistance in the most common oral fungal pathogens, *Candida albicans* and other non-albicans *Candida species*, is the over-expression of ATP-binding cassette (ABC) transporters that protect cells from azole antifungals.

Professor Brian Monk.

We have used our patented, and further optimised, *Saccharomyces cerevisiae* system for heterologously overexpressed membrane proteins to study efflux pumps from a number of important fungal pathogens including the major efflux pumps contributing to antifungal drug resistance of *C. albicans* (Cdr1), *Candida auris* (Cdr1) and *Fusarium keratoplasticum* (Abc1).

In a project supported by the Marsden Fund, site-directed mutagenesis has been used to investigate the role of amino acids, particularly cysteines, in pump function. We have also used the expression system to study antifungal drug resistance mechanisms of the more recently emerging human fungal pathogens *Candida auris* and *Fusarium keratoplasticum*. Other projects have ii) created a new set of plasmids for the functional characterization of membrane proteins with various N- and C-terminal fluorescent (double)-tags; and ii) studied the possible homo-dimerization of Cdr1 in live yeast cells.

Before organisms can cause oral infections, they must first colonise the oral cavity. Little is known about the range of fungal species and diversity of *C. albicans* strains that colonise people's mouths. We have used rDNA sequencing and multilocus sequence typing (MLST) to identify and investigate fungi colonising people with dentures, oral cancer and older people. Surface roughness of oral surfaces can facilitate oral colonization. We have investigated how interproximal reduction of teeth affects surface roughness and microbial adherence. With our colleagues in the Tokyo Institute of Technology we have developed a novel assay to measure *C. albicans* adhesion to hydroxyapatite (the main component of enamel).

Our group published 7 papers and 1 book chapter in 2019-2020.

Key collaborators

Structure-directed discovery of next-generation antifungals

A/Prof Michaela Lackner (Medical University of Innsbruck, Austria)

A/Prof Joel Tyndall (University of Otago School of Pharmacy)

Prof Robert Stroud (University of California San Francisco, USA)

Prof Gabriele Cruciani and PhD student Lucia Cesarini (Università Degli Studi Di Perugia, Italy)

MicroCombichem (Wiesbaden, Germany)

Fungal colonisation and drug resistance

A/Prof Alok Mitra (University of Auckland)

Profs Lutz Schmitt and Holger Gohlke (Heinrich Heine University Düsseldorf, Germany)

A/Prof Jacinta Santhanam (Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia)

Prof Stefan Raunser (Max Planck Institute for Molecular Physiology, Dortmund, Germany)

A/Prof Morgan Han (Chongqing Medical University Stomatological Hospital, Chongqing, China)

Dr Masakazu Niimi, Dr Kyoko Niimi and Prof Susumu Kajiwara, (Tokyo Institute of Technology, Tokyo, Japan)

Microbial profiling, monitoring and genomics

Professor Amarila Malik (Universitas Indonesia, Depok, Indonesia)

Professor Lucio Gonçalves (University Estácio de Sá, Rio de Janeiro, Brazil)

Professor Julian Crane (University of Otago Wellington)

Dr Xochitl Morgan (University of Otago)

BLIS Technologies (Dunedin, NZ)

Key funding successes

$1,199,968. Structure-directed discovery of next-generation antifungals. Health Research Council of NZ, 2019 (Brian Monk, Mikhail Keniya, Joel Tyndall (Pharmacy), Rajni Wilson)

$14,805. Evaluation of Electrolysed Oxidising Water as a multipurpose, non-toxic and cost-effective disinfectant in dental healthcare. New Zealand Dental Research Foundation, 2019 (Richard Cannon, Karl Lyons, Geoff Tompkins, Chitra Krishnan)

$13,478. Surface modification of orthodontic elastomers to overcome biofilm formation. New Zealand Dental Research Foundation, 2019 (Li Mei, Richard Cannon, Michael Skilbeck)

$10,000. Drug resistance in the emerging fungal pathogen *Candida auris*. Maurice and Phyllis Paykel Trust, 2019 (Richard Cannon, Erwin Lamping)

$8,000. Efficacy of electrolysed oxidising water as a cost-effective dental disinfectant. Maurice and Phyllis Paykel Trust, 2019 (Geoffrey Tompkins, Richard Cannon)

$15,000. MiniG®1600 - Automated Tissue Bead Mill Homogeniser and Cell Lyser. New Zealand Dental Research Foundation, 2020 (Trudy Milne, Erwin Lamping, Richard Cannon)

$14,000. Genome analysis of bacterial strains. BLIS Technologies Limited, 2020 (Nicholas Heng)

Major funding supporting research within the Programme during 2019-2020 came from: New Zealand Dental Research Foundation, Health Research Council of NZ, Ministry of Business Innovation and Employment, Marsden Fund of the Royal Society of NZ, Sir Thomas Kay Sidey Postgraduate Visiting Fellowship of the University of Otago Faculty of Dentistry, Ministry of Higher Education Malaysia Fundamental Research Grant Scheme, Catalyst Fund of the Royal Society of NZ, Maurice and Phyllis Paykel Trust, SJWRI Colgate Research Grant, Fuller Scholarship, Return on Science/Otago Innovation Ltd, KiwiNet/Otago Innovation Ltd, Lottery Grants Board.

Microbial profiling and monitoring, and genome characterisation using molecular tools

Nick Heng, Trudy Milne

The oral cavity of each human and animal harbours its own distinctive community of microbes, termed the "oral microbiota". The human oral microbiota alone is estimated to comprise over 700 species of microbes. Many species have long been associated with disease such as *Streptococcus mutans* (dental caries) and *Porphyromonas gingivalis* (periodontal disease). Bacterial profiling of oral samples from healthy or diseased participants using next-generation DNA sequencing technology have helped identify some species that may either contribute to disease

progression or are associated with good oral health. Some bacterial species, e.g. *Streptococcus salivarius*, are potential probiotic species, i.e. they are believed to confer beneficial effects when colonising their human (or animal) hosts. This research group is not only interested in revealing the genomic secrets of cultured species such as antimicrobial-producing *S. salivarius* strains and new oral streptococcal species isolated from other animals, but also developing real-time PCR probe sets to specifically detect and monitor particular probiotic species.

In 2019-2020, our group published 5 journal articles.

Oral bacteriology

Geoffrey Tompkins, Peter Cathro

Bacteria are involved in various diseases affecting the teeth and gingival tissues. Current projects in this group include: (i) development of new antimicrobials directed at the extremely alkaline-tolerant bacteria that cause root canal treatments to fail; (ii) evaluation of lasers to remove biofilms from dental implants; (iii) the effect of various antimicrobials, including chlorhexidine, and silver-based antimicrobials on oral microbial ecology.

Developing novel antimicrobial agents for oral applications

Dawn Coates, Gemma Cotton

Antibiotic resistance has become an increasing problem in clinical medicine. This team undertakes research on the development of novel antimicrobials for oral applications and as an adjunct to bone grafting materials. Research includes both chemically synthesised compounds and those derived from New Zealand native plants. Antimicrobial action, formulation, release profiles, molecular mechanisms of action, along with in vitro and in vivo trials on efficacy and compatibility are all conducted.

Microbial biofilms

Vincent Bennani, Li Mei

Most microorganisms live within biofilms and in the mouth these biofilms can cause diseases such as dental caries, periodontitis and peri-implantitis. We are interested in how biofilms form on oral surfaces including denture acrylic, implant titanium, and orthodontic appliances – and measuring how effective methods are for removing these biofilms. We have also investigated the use of *Streptococcal salivarius* strains as probiotics to inhibit the growth of oral pathogens and improve oral health in orthodontic patients.

Oral immunology

Trudy Milne

Understanding the role the immune system plays in response to smoking and Type 2 diabetes and the effect on dental pulp vitality and healing will enable clinicians to offer personalised patient management strategies. The role cellular signaling between osteoblasts and periodontal ligament cells and the inflammatory response during orthodontic tooth movement is also under investigation.

Left (L-R): Prof Richard Cannon, Dr Trudy Milne and Dr Erwin Lamping with the MiniG®1600 Automated Tissue Bead Mill Homogeniser and Cell Lyser, funded by a grant from the NZDRF.