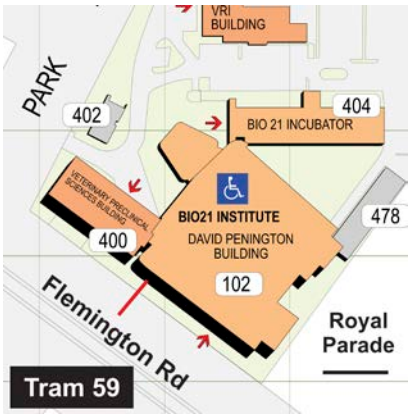
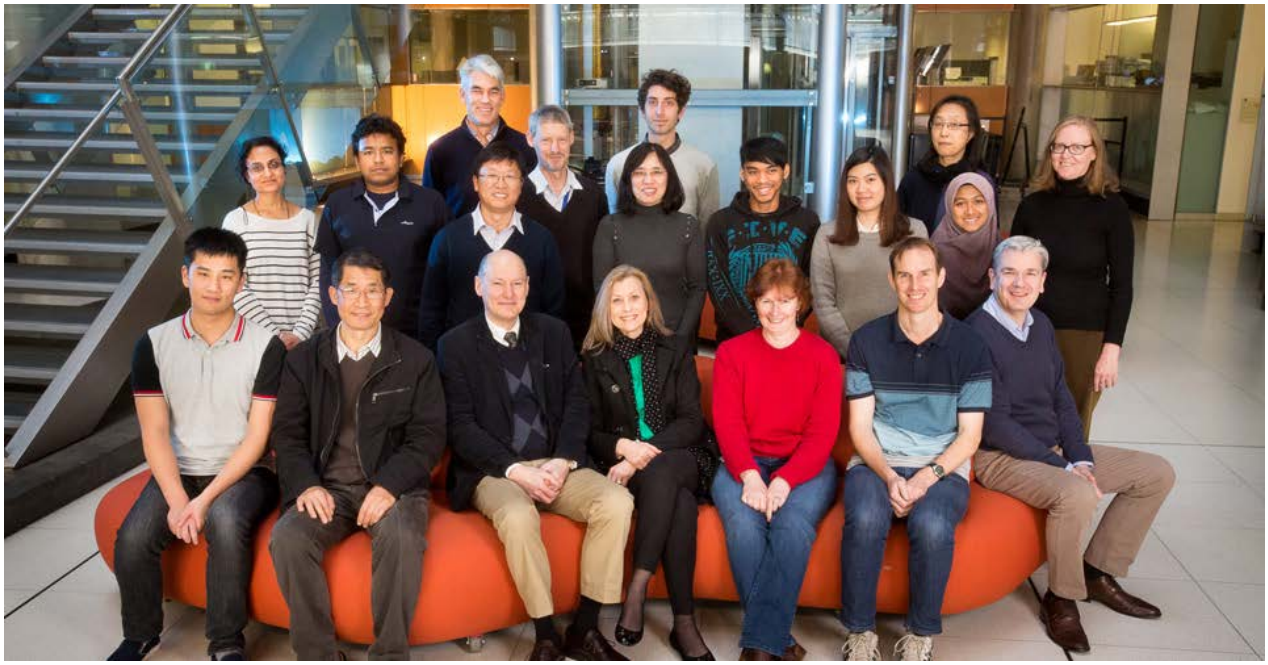


BSc Hons/PhD at MDS Bio21

Ask about our scholarships and
Tours of the Bio21 and MDS laboratories



Come and meet our current students in this photo

Bacterial pathogenesis, vaccine, and antimicrobial peptide development

1.A.11 Proteomics of oral bacteria
Dr Paul Veith, Dr Yu-Yen Chen

We have a very well equipped proteomics lab, housing two MALDI TOF/TOF mass spectrometers as well as two LC-MS systems, and robotics for sample preparation. The honours student who takes on this project will receive in-depth training in proteomics while making an important contribution to our proteomic studies of dental pathogens and their virulence factors. A variety of specific projects can be tailored to suit your interests.

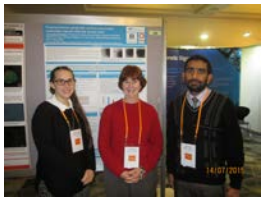
1.A.1.2 Protein Modification and Bacterial Pathogenicity
Dr Catherine Butler, Dr Paul Veith

Lysine acetylation is an important regulatory post-translational protein modification found in eukaryotes and more recently, in some prokaryotes. In eukaryotes this modification regulates diverse protein properties including DNA-protein interactions, subcellular localization, transcriptional activity and protein stability. Dysregulation of lysine acetylation and its regulatory enzymes is associated with aging and several major diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. Although lysine acetylation in Escherichia coli has recently been determined this area of research is still in its infancy in prokaryotes. We have confirmed that lysine acetylation does occur in the oral pathogen Porphyromonas gingivalis and we would like to identify the proteins which are affected by this important modification.

1.A.2.1 Polymicrobial Biofilms and Disease
A/Prof Stuart Dashper

The objective of this project is to characterise the metabolic interactions that underpin symbioses of three pathogenic oral bacterial species, *P. gingivalis*, *T. denticola* and *T. forsythia*, which grow as a polymicrobial biofilm. The proposed investigation will characterise the roles of specific bacterial proteins in the formation and development of symbiotic polymicrobial biofilms. We have developed novel methodologies for the growth and analyses of these bacteria as polymicrobial biofilms. We will use continuous culture flow cells to grow the polymicrobial biofilms which will be analysed using fluorescent *in situ* hybridization with species specific probes that allow the individual species in the biofilms to be imaged in 3D on a confocal scanning microscrope. Metabolomic and transcriptomic analyses will be used to identify how these bacteria interact with each other in these polymicrobial biofilms. This will identify metabolites and metabolic pathways.

1.A.2.2 The Influence of Oral Care Additives on the Antimicrobial Resistance of Plaque Bacteria
Dr Christine Seers



Multiple antimicrobial drug resistance of bacteria is a continually growing concern in health care. Dental plaque can serve as a reservoir for species that can cause pulmonary and systemic infections. This project will determine the antimicrobial resistance profiles of dental plaque species (1) in people who do not use oral care products with antimicrobial additives (2) in people who regularly use oral care products containing specific antimicrobial additives.

1.A.2.3 The Relationship of Exposure to Triclosan and Chlorhexidine to the Antimicrobial Resistance of Bacteria
Dr Christine Seers

Chlorhexidine and triclosan are used routinely in antiseptic solutions and oral care products to inhibit bacterial growth. Resistance to chlorhexidine and triclosan is being identified in bacteria, including pathogens of clinical importance. This project will investigate (1) the prevalence of chlorhexidine and/or triclosan resistant species in dental plaque (2) antimicrobial resistance profiles of dental plaque species (3) if the environmental pressure of exposure of bacteria to triclosan or chlorhexidine will select for resistant species.

1.A.3.1 Characterisation of a novel bacterial protein secretion system

Dr Christine Seers, Dr Paul Veith, Dr Nada Slakeski, Dr Michelle Glew, Dr Yu-Yen Chen

Bacteria secrete proteins to the extracellular environment through many different systems. Many pathogens use the recently reported type 9 secretion system (T9SS) to export their pathogenic determinants which enables them to cause disease. There are various projects on offer that aim to investigate the function of the T9SS. Students undertaking these projects will learn an array of techniques that will develop gene manipulation, gene expression, and protein analysis skills. Skills developed will be microbial cell culture, DNA cloning, gene targeting, recombinant protein expression/antibody production, protein purification and mass spectrometry amongst others.

1.A.3.2 Characterisation of a novel bacterial protein from a system essential for bacterial virulence.

Dr Christine Seers

Many proteins have been implicated as required for function of the bacterial Type 9 protein secetion system (T9SS). The T9SS is essential for presentation of virulence proteins on the bacterial surface. We have identified a novel protein essential for effective T9SS protein secretion by Porphyromonas gingivalis. The aim of this project is to characterise the function of this protein within the secretion system.

1.A.3.3 Bacterial Type 9 Secretion System Proteins - Promiscuous or Specific for Protein Partners?

Dr Christine Seers

Some components of the Type 9 Protein Secretion System (T9SS) of Bacteroidetes bacteria have multiple copies within some species. These species include some that are emerging as multidrug resistant pathogens in hospital and veterinary settings. This project aims to use genetic/protein manipulation methods to understand the function(s) of these paralogues. Ultimately we will improve our understanding of pathogenic processes in these bacteria.

1.A.3.4 Bacterial Regulation of Gene Expression

Dr Catherine Butler, Dr Nada Slakeski, Prof Stuart Dashper

Bacteria are continually exposed to environmental challenges, such as changes in temperature, nutrient availability, oxidative stress, etc. It is the ability of bacteria to adapt to these challenges which enable them to survive, colonise and cause disease. This adaptation is possible due to gene regulation; the bacteria switch on (or off) subsets of genes which express proteins required for survival. We study different forms of gene regulation that utilise sRNAs, riboswitches, or transcriptional regulators in the oral pathogen *Porphyromonas gingivalis*. Elucidation of the mechanism of action of these regulators in P. gingivalis will provide us with a better understanding of how this organism causes disease, and may provide a target for small molecule inhibitors. Numerous techniques will be used including RNA extraction and quantitation using state of the art RNA-seq, recombinant protein production, protein-DNA and protein-protein interaction studies, site directed mutagenesis, etc.

1.A.3.5 *Porphyromonas gingivalis* outer membrane vesicles
Prof Stuart Dashper, Dr Paul Veith, Dr Nada Slakeski

Gram-negative bacteria constitutively secrete outer membrane vesicles (OMVs) into the extracellular environment and recent research has shown that OMVs enhance bacterial survival and virulence. Nevertheless, the mechanisms of vesicle formation and the roles of OMVs have not yet been clearly defined. Using differential proteomic approaches we have identified ten novel *Porphyromonas gingivalis* proteins that are either only found on OMVs or are highly enriched on OMVs relative to the cell surface. The overall aim of this project is to determine the roles that these specific P. gingivalis OMV proteins play in vesicle biogenesis and structure, pathogenic biofilm formation and bacterial pathogenesis. Students undertaking this project will use state of the art equipment to perform techniques in the broad fields of molecular biology, microbiology, and proteomics.

1.A.3.6 Characterisation of the role of PorQ in the novel type 9 secretion system (T9SS) of *Porphyromonas gingivalis*
Dr Michelle Glew & Dr Paul Veith

The major virulence factors of *Porphyromonas gingivalis*, the gingipains (RgpA, RgpB and Kgp), are three of more than 30 proteins secreted by the novel type 9 secretion system (T9SS) of *P. gingivalis*. The secretion machinery recognises a conserved C-terminal signal domain (CTD) that all secreted CTD-proteins possess. Secretion of the gingipains and other CTD-proteins involves 5 critical steps: (1) inner membrane translocation, presumed to involve the SEC translocon, (2) outer membrane translocation, (3) maturation by proteolytic cleavage of pro and adhesin domains, if present, (4) CTD cleavage, and (5) covalent attachment of A-LPS/glycolipid at the cell surface. PorQ has recently been proposed to be part of this T9SS. PorQ is predicted to be an outer membrane beta-barrel protein and when mutated was shown to significantly affect the secretion of the major virulence factor gingipains. PorQ has been shown to interact with three other T9SS components involved in the covalent linkage to glycolipid. This project will identify the precise interaction sites between PorQ and its cognate partners in order to better understand this reaction process. The project will provide experience in various molecular biology and protein analysis techniques including the construction of plasmid vectors for introducing site directed gene mutations, PCR, DNA purification, DNA gel electrophoresis, SDS-PAGE, protein purification, His-tag protein purification, protein cross-linking, outer membrane vesicle preparation, 2D Blue-Native PAGE analysis and proteomics.

1.A.3.7 Characterisation of the modification of CTD proteins in *Porphyromonas gingivalis*
Dr Dhana Gorasia & Dr Paul Veith

The major virulence factors of *P. gingivalis*, called gingipains, and other CTD proteins are secreted via the type IX secretion system. The CTD proteins are then proteolytically processed and conjugated to a novel glycolipid that presumably anchors them to the outer membrane. The glycolipid is comprised of glycan units that are similar to those present in anionic lipopolysaccharide (A-LPS), as well as novel components to allow protein linkage and membrane attachment. A number of proteins have been identified to be involved in the both the synthesis of A-LPS and the novel anchor but genomic analysis suggests that there are additional proteins involved. This project aims to identify these additional proteins by the deletion of their respective genes in both W50 and 33277 strains and analysis of the mutants to see if the A-LPS and anchor are affected by the mutation. The affected mutants will be further analysed to investigate the effect on the proteolytic processing of CTD proteins and the formation of the electron dense surface layer. You will gain experience in a variety of techniques such as western blot, mass spectrometry, PCR and DNA cloning, anaerobic cell culture, subcellular fractionation, silver staining and electron microscopy.

1.A.3.8 Characterisation of the role of PorQ in the novel type 9 secretion system (T9SS) of *Porphyromonas gingivalis*

Dr Michelle Glew & Dr Paul Veith

The major virulence factors of *Porphyromonas gingivalis*, the gingipains (RgpA, RgpB and Kgp), are three of more than 30 proteins secreted by the novel type 9 secretion system (T9SS) of *P. gingivalis*. The secretion machinery recognises a conserved C-terminal signal domain (CTD) that all secreted CTD-proteins possess. Secretion of the gingipains and other CTD-proteins involves 5 critical steps: (1) inner membrane translocation, presumed to involve the SEC translocon, (2) outer membrane translocation, (3) maturation by proteolytic cleavage of pro and adhesin domains, if present, (4) CTD cleavage, and (5) covalent attachment of A-LPS/glycolipid at the cell surface. An outer membrane beta-barrel protein, LptO, shown to be essential for T9 secretion and modification has been shown to directly interact with the T9 secretion substrates. This project will identify the precise interaction sites between LptO with the conserved domain of the T9 secretion substrate partners in order to better understand how the substrates are recruited for secretion. The project will provide experience in various molecular biology and protein analysis techniques including the construction of plasmid vectors for introducing site directed gene mutations, PCR, DNA purification, DNA gel electrophoresis, SDS-PAGE, protein purification, His-tag protein purification, protein cross-linking, outer membrane vesicle preparation, 2D Blue-Native PAGE analysis and proteomics.

1.A.4.1 IMMUNITY: Mucosal and systemic immune response to bacteria

A/Prof Neil O'Brien-Simpson, Dr Jason Lenzo, Dr James Holden

We are offering a number of projects investigating the mucosal and systemic immune responses to single and multi-bacterial species colonization and infection. We have already found that there is pathogenic synergy between pathogenic and non-pathogenic bacteria and their ability to cause disease and immunopathology. These projects will use a number of techniques and in-house equipment such as Flow cytometry, ELISPOT, T-cell proliferation, real-time PCR, and cytokine DNA microarray.

1.A.4.2 IMMUNITY: Cytotoxic T cell based vaccine design and development

A/Prof Neil O'Brien-Simpson, Dr Jason Lenzo, Dr James Holden

We are offering a number of projects investigating the mucosal and systemic immune responses to single and multi-bacterial species colonization and infection. We have already found that there is pathogenic synergy between pathogenic and non-pathogenic bacteria and their ability to cause disease and immunopathology. These projects will use a number of techniques and in-house equipment such as Flow cytometry, ELISPOT, T-cell proliferation, real-time PCR, and cytokine DNA microarray.

1.A.5.1 VACCINE & FUTURE THERAPEUTICS : Vaccine development and delivery and peptide therapeutics

A/Prof Neil O'Brien-Simpson

We currently are offering a number of projects investigating the synthesis of peptides and proteins for the development of an oral vaccine for periodontitis as well as designing peptide inhibitors to bacterial enzymes. These projects will involve the chemical synthesis of peptide vaccines or the production of recombinant protein vaccines and evaluating their efficacy using a number of immunological techniques. These projects will use a number of techniques and in-house equipment such as peptide synthesis, HPLC, FPLC, Mass spectrometry, polymer chemistry, Flow cytometry, ELISPOT, T-cell proliferation, real-time PCR and cytokine DNA microarray. Areas in which projects are available are:

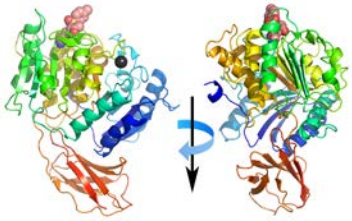
1.A.5.2 Novel Antibiotic Peptides

A/Prof Neil O'Brien-Simpson, Dr Jason Lenzo,

Synthesis of antimicrobial peptide and peptide mimetics. The use of specific antimicrobial peptides for the delivery of chemical antibiotics.

1.A.5.3 FUTURE THERAPEUTICS: Bioactive Peptides and Natural Inhibitors

Dr Laila Huq, Dr. Christine Seers, Prof Stuart Dashper



Novel delivery mechanisms of antimicrobial peptides./Natural inhibitors of bacterial pathogen proteases.

1.A.5.3.1 FUTURE THERAPEUTICS: Biacore 3000 Kinetic and affinity analysis of protease and inhibitor

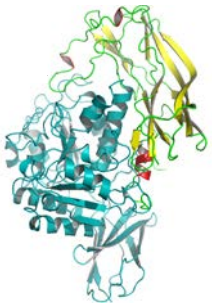
Dr Laila Huq, Dr Christine Seers, Dr Keith Cross

Projects are available to study the interaction of proteins using the Biacore 3000.

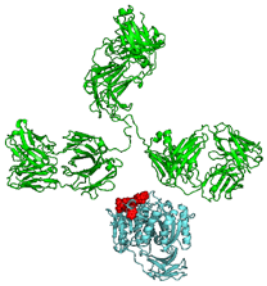
1.A.5.3.2 FUTURE THERAPEUTICS: Structure-Function Studies of the Propeptide-Protease Relationship

Dr Laila Huq, Dr Lianyi Zhang

Some proteases have evolved to use an inhibitory propeptide to prevent premature proteolytic activation. This project will investigate the protease-propeptide relationship using a variety of structure-function studies. Students will learn various biochemical and analytical techniques and have access to the facilities at Bio21.



1.A.5.3.3 FUTURE THERAPEUTICS: Structure-Function Studies of the Protein Domains



Dr Lianyi Zhang, Dr Laila Huq

Many bacterial proteases are translated as a large polypeptide with multiple domains adjacent to the catalytic domain. The precise roles of these domains and any influence on catalysis will be investigated in a series of structure-function studies. Students will learn to use various biochemical and analytical techniques for characterisation and will have access to the facilities at Bio21.

NANOTECHNOLOGY

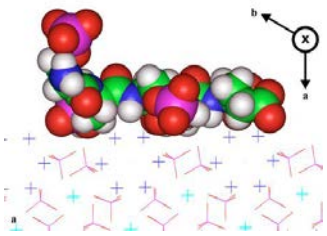
1.B.1 Bioinspired approach for the development of novel biomimetic nanoparticle materials with applications in bone/enamel tissue engineering

Dr Keith Cross

Many organisms form mineralised structures by the process of biomineralisation. Several multiple phosphoseryl-containing proteins have been identified in mineralising tissue or associated *in vivo* with calcium phosphate phases. We have the expertise and facilities to investigate novel, non-toxic, self assembling, injectable, nanoparticle biomaterials for potential applications in bone/enamel tissue engineering. Recaldent™ is a non-toxic self-assembling delivery vehicle consisting of tryptic peptides of milk caseins encapsulating the mineral calcium phosphate for targeted release at the enamel surface. This project involves exploring the interactions between casein peptides and calcium and phosphate ions in different conditions. Students will learn various biochemical and analytical techniques and have access to the facilities at Bio21.

1.B.3 Molecular modelling of the binding of multi-phosphorylated peptides to hydroxyapatite

Dr Laila Huq, Dr Keith Cross



Many organisms form mineralised structures by the process of biomineralisation. Several multiple phosphoseryl-containing proteins have been identified in mineralising tissue or associated *in vivo* with calcium phosphate phases. We are investigating the binding of multi-phosphorylated peptides to hydroxyapatite using computer-based simulations. This project involves the use of molecular modelling software run on SGI Octane and Tezro workstations.

1.B.4 Diffusion model for mineralization

Dr Keith Cross, Dr Laila Huq

Casein phosphopeptides amorphous calcium phosphate (CPP-ACP™), marketed as Recaldent™, have been demonstrated to have anticariogenic activity in laboratory, animal and human in situ experiments. The aim of this project is to mathematically model the remineralization of enamel by CPP-ACP™ in the oral cavity. The project involves the development of a finite element diffusion model for the processes involved in demineralization of enamel by acid challenge and remineralization by the casein phosphopeptides.

1.B.5 Biacore 3000 Kinetics and affinity analysis of nanotechnology therapeutics

Dr Keith Cross, Dr Laila Huq

Projects are available to examine the kinetics and affinity of the self-assembling peptides that encapsulate calcium, phosphate, and fluoride forming a delivery vehicle that is targetted to release at the enamel surface.

1.C.1 CARIOLOGY: Novel Preventive Products/Functional Foods

Dr Glenn Walker, Dr Peiyan Shen

Projects are available to test novel products and functional foods designed to assist in the prevention of dental caries and/or dental erosion. The products may include toothpastes, dental cremes, mouthrinse solutions, gels, and varnishes for topical application. The preventive products will contain anti-caries/erosion agents. Alternatively, commonly consumed foods and beverages will be modified to minimize their potential to cause loss of mineral from teeth such as during dental caries and dental erosion or modified to provide a positive health effect. Projects may include laboratory and/or *in situ* studies.

1.C.2 Enhancement of remineralization

Dr Keith Cross, Dr. Peiyan Shen

Projects are available to study the process of enamel remineralization of early tooth decay. Enamel remineralization is the process of net mineral uptake into partially demineralized tooth structure. Projects will study ways of enhancing remineralization and quantify the effects of these treatments using a number of state of the art quantification methods. These projects may include laboratory and/or *in situ* studies.

INNATE IMMUNITY & HOST DEFENCE

1.D.1 Subversion of Immune Receptor Pathways by a Human Bacterial Pathogen

A/Prof Glen Scholz

Innate immune cells (e.g. macrophages) use pattern recognition receptors, such as the toll-like receptors (TLRs) and nod-like receptors (NLRs), to detect and respond to pathogens. However, some pathogens express proteins that subvert the signalling pathways used by the receptors to activate the host immune response. In this project you will investigate the ability of a novel

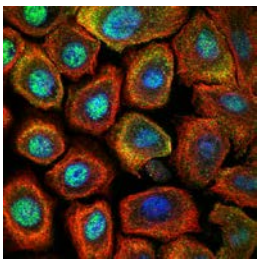
bacterial protein to subvert specific immune receptor pathways. You will gain expertise in a variety of techniques including mammalian and bacterial cell culture, PCR-based cloning, site-directed mutagenesis, gene transfection, Real-Time PCR, immunoprecipitation assays, Western blotting, and confocal microscopy.

1.D.2 Regulation of Innate Immune Cells by Protease-Activated Receptors
A/Prof Glen Scholz

Some bacterial pathogens attempt to suppress host immunity by secreting proteases capable of degrading immune receptors and cytokines. However, the expression of protease-activated receptors by immune cells allows them to detect such bacteria and respond accordingly. In this project you will investigate how a particular protease-activated receptor regulates the response of innate immune cells to a specific human pathogen. You will gain expertise in a variety of techniques including mammalian and bacterial cell culture, bacterial infection assays, gene transfection and gene silencing, Real-Time PCR, ELISA assays, Western blotting, and confocal microscopy.

1.D.3 Regulation of the Host Defence Functions of Keratinocytes by the IRF6 Transcription Factor
A/Prof Glen Scholz

Keratinocytes are highly specialised epithelial cells and integral to host defence. They not only act as a physical barrier, but also use pattern recognition receptors, such as the toll-like receptors (TLRs), to detect and respond to pathogens. In this project you will investigate how the IRF6 transcription factor regulates the response of keratinocytes to a particular human bacterial pathogen. You will gain expertise in a variety of techniques including mammalian and bacterial cell culture, bacterial infection assays, gene transfection and gene silencing, Real-Time PCR, ELISA assays, Western blotting, and confocal microscopy.



CANCER

1.E.1 Tumour-Suppressor Function of the IRF6 Transcription Factor
A/Prof Glen Scholz

Keratinocytes are highly specialised epithelial cells that protect the body against trauma, chemicals, and infection. Their proliferation and differentiation are tightly regulated to maintain tissue homeostasis. Significantly, the IRF6 transcription factor, which is important for keratinocyte proliferation and differentiation, is mutated in squamous cell cancer. In this project you will investigate how IRF6 regulates keratinocyte proliferation and differentiation. You will gain expertise in a variety of techniques including mammalian cell culture, gene transfection and gene silencing, site-directed mutagenesis, Real-Time PCR, Western blotting, confocal microscopy, and tissue processing and immunohistochemistry.

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