Craniofacial biology and clinical oral physiology

Programme leader: Professor Mauro Farella

The *Craniofacial Biology and Clinical Oral Physiology* research programme encompasses a diverse range of exciting fields, including the basic and molecular sciences relevant to craniofacial growth, the impact of malocclusions on oral health, jaw function, and psychological wellbeing, and the understanding of the peripheral and central mechanisms of orofacial pain and jaw dysfunction with their clinical correlates.

Several research approaches are used to study topics relevant to craniofacial biology, including cell response to mechanical loading, animal models, and clinical genetics. The latter focuses on identifying genetic markers for some dentofacial anomalies which could potentially provide us with a clinically important window of opportunity to predict abnormal growth patterns at an early age and, possibly, to provide personalized orthodontic treatments.

An additional area of active research is focusing on the development of novel treatment strategies for clinical problems such as craniofacial syndromes, jaw discrepancies and misaligned teeth. Furthermore, the impacts of craniofacial anomalies and smile problems are quantitatively and qualitatively assessed at population and individual level using survey methods including social media. Social media enables us to gather opinions from the public about the importance of smiles for individuals themselves and also the perspective of their peers.

Research in the field of clinical oral physiology examines mastication and jaw kinematics, bruxism and non-functional oral behaviours, sleep disordered breathing including snoring and sleep apnea, intra-oral tongue pressure, dysphagia, tooth wear, eating behaviour, and novel food products. We are currently using wired and wireless sensors to monitor intraoral pH, temperature, and jaw activity for the purpose of identifying and evaluating ways of overcoming orofacial pain, dental wear, jaw dysfunction, jaw clicking sounds, snoring, and obstructive sleep apnea. We also use monitoring equipment to improve the quality of sleep in New Zealand children and adults.

Highlights 2015-2016

In total 33 articles were published in the peer-reviewed scientific literature. For details, please refer to the Publications data in the 'Our Achievements' section of the 2015-16 SJWRI Research Report. The total number of conference presentations made was 32 (including 8 keynote addresses).

Total research funding (external) obtained in 2015-2016 amounted to $492,271.

Prof Farella was the recipient of the Alan Docking Award from the International Association for Dental Research (2015), and of the Sir John Walsh Award (2015), to acknowledge the excellence achieved in dental research.

Li Mei won the SJWRI Clinical Research Award 2015

Gareth Benic won the prize for the best research presentation within the Research Programme in Craniofacial Biology and Clinical Oral Physiology at the 2016 Research Day.

PhD (Theses submitted): Andrew Quick, Ghassan Idris, Erin Hutchinson.

Doctor of Clinical Dentistry (completed): Andrew Parton, Sophie Gray, Shahrzad MacAvoy, Coreen Loke, Mohamad Al-Dujaili, Lydia Meredith, Yana Itskovich, Azza Al-Ani, Catherine Carleton, Gareth Benic.

Master of Health: Austin Kang

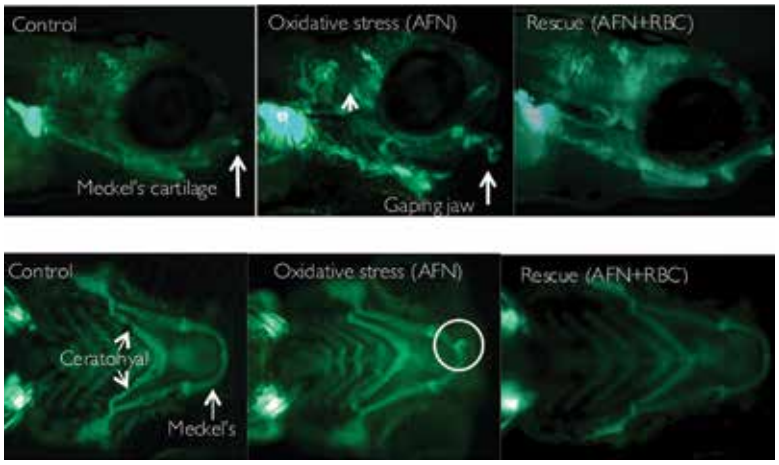
Honours Students: Erni Park, Sharifa Al Balushi, Sherry Lee.

Research projects

A novel model for exploring the causes and treatments of craniofacial birth defects

Research Team: Catherine Carleton, Julia Horsefiled, Joseph Antoun, and Mauro Farella

The aim of this research was to determine how environmental causes of craniofacial birth defects affect the growth and survival of cells contributing to the craniofacial skeleton during embryonic development. A second objective was to determine whether factors that enhance cell survival, such as antioxidant molecules, could rescue craniofacial defects. Our findings indicate that oxidative stress in a zebrafish model results in craniofacial cartilage defects that can be rescued by the antioxidant RiboCeine. These findings may have translational significance, as treatment with antioxidants may help to prevent craniofacial defects in children, especially in families where there is an identified genetic or environmental risk.

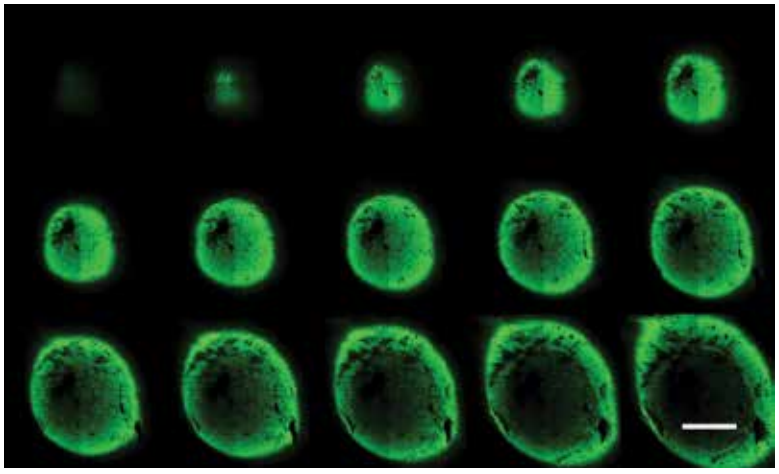


Zebrafish embryos exposed to oxidative stress (Auranofin) developed a gap jaw defect. Treatment with the antioxidant RiboCeine™ rescued the jaw defect so that the embryos had the appearance of untreated controls fish.

A new approach to engineering 3-dimensional constructs of human bone matrix in a mechanically-active environment

Research Team: Yana Itskovich, Murray Meikle, Trudy Milne, Richard Cannon, and Mauro Farella

The development of suitable alternatives to autogenous and allogeneic bone has been a goal of bone and biomaterials research for many years. An autograft is the biologic gold standard against which biocompatibility of all new materials are compared. Numerous materials have been tested, but with limited success. The present research uses a semi-synthetic hydrogel, which provides a close resemblance of the extracellular matrix, and the opportunity to modify the gel cross-linking ratios. The aim of the present study was to develop a 3-dimensional cell culture model and to validate assays that could be used to aid the engineering of an artificial mineralized bone matrix *in vitro*. We have examined the proliferation and hydroxyapatite deposition of human femoral and calvarial osteoblasts cultured in two modifications of a thiol-modified hyaluronan-gelatin-PEGDA cross-linked hydrogel. A hydrogel construct has also been cultured under intermittent compressive mechanical strain and preliminary gene expression analysis undertaken.



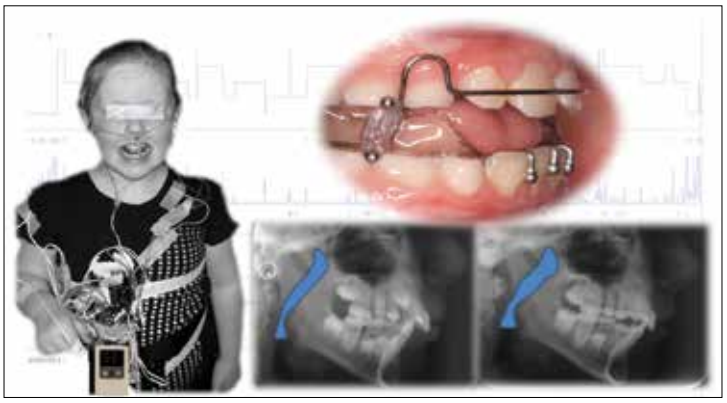
Fluorescently labelled viable human osteoblasts grown in a 3-D gel. Serial sections of gel were photographed using a confocal microscope.

Efficacy of a mandibular advancement appliance on Sleep Disordered Breathing in Children

Research Team: Ghassan Idris, Barbara Galland, Chris Robertson, and Mauro Farella

Sleep-Disordered Breathing (SDB) varies from habitual snoring to partial or complete obstruction of the upper airway, and can be found in up to 10% of children. SDB can significantly affect children's wellbeing, as it can cause growth disorders, educational and behavioural problems, and even life-threatening conditions, such as cardiorespiratory failure. I patients with craniofacial anomalies, for whom adenotonsillectomy or other treatment modalities have failed, or surgery is contraindicated, mandibular advancement splints (MAS) may represent a viable treatment option. We designed a crossover randomised controlled trial to determine the efficacy of mandibular advancement appliances (MAS) for the management of Sleep-Disordered Breathing (SDB) and related health conditions in children.

The Apnoea Hypopnoea Index (AHI) represented the main outcome variable and was assesses via home-based polysomnography. Compared to a Sham MAS, wearing an Active MAS resulted in a significant reduction in AHI and snoring time.



A patient with sleep disordered breathing wearing an oral appliance to increase oropharyngeal airway. Polysomnography is used for a home-based sleep study.

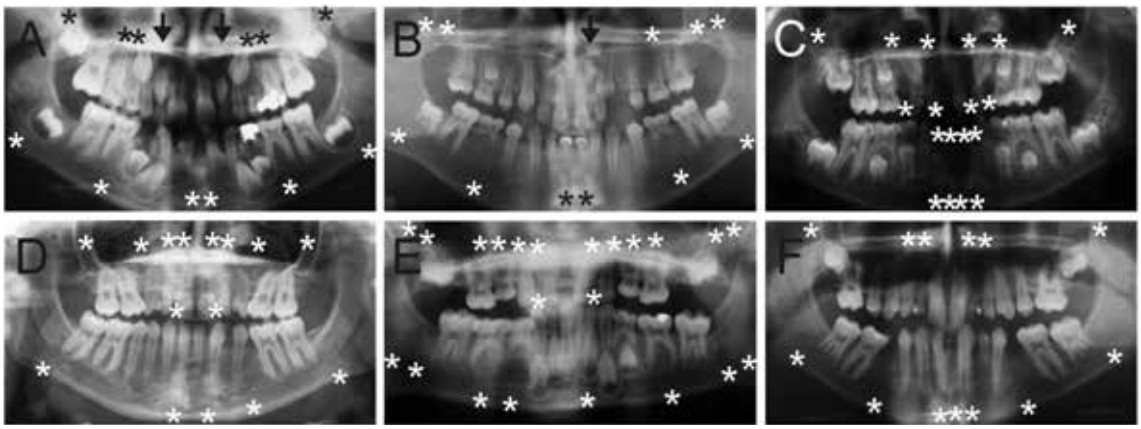
Our preliminary findings indicate that the short-term use of mandibular advancement splints significantly reduced AHI, supine AHI, and snoring time in children with SDB, and improved subjectively assessed SDB symptoms and quality of life, in addition to significant changes in children's behaviour.

Environmental and genetic factors associated with hypodontia

Research Team: Azza Al-Ani, Joseph Antoun, Mauro Farella, Murray Thomson, and Tony Merriman

Hypodontia, or tooth agenesis, is the most prevalent craniofacial malformation in humans. Both environmental and genetic factors are involved in the aetiology of hypodontia, with the latter playing a more significant role. The objectives of this study were two-fold: (1) to investigate the association between non-syndromic hypodontia and single nucleotide polymorphisms (SNPs) of candidate genes paired box *9 (PAX9), msh* homeobox 1 *(MSX1)*, axis inhibition protein 2 *(AXIN2)*, and ectodysplasin A *(EDA)*; and (2) to examine its association with environmental factors, such as exposure to smoking and alcohol during pregnancy.

Our preliminary findings reveal some evidence that polymorphisms of the *EDA* and *PAX9* genes are associated with specific phenotypes of non-syndromic hypodontia. Furthermore, this research is the first to date to test the association between maternal cigarette smoking during pregnancy and having a child with hypodontia.



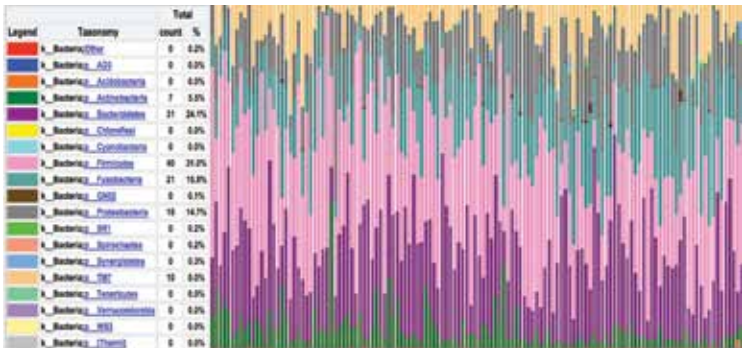
Ortopantomographs showing different patterns (see asterisks) of hypodontia and oligodontia.

Efficacy of oral probiotics in managing biofilm formation in patients wearing fixed orthodontic appliances

Research team: Gareth Benic, Mauro Farella, Patrick Biggs, Nicholas Heng, Richard Cannon, and Li Mei

This research aims to investigate the efficacy of the oral probiotic *Streptococcus salivarius* M18 on managing biofilm formation in patients wearing fixed orthodontic appliances. We have designed a prospective, randomized, triple-blind, two-arm parallel-group, placebo-controlled trial in patients undergoing fixed orthodontic treatment. The outcome measures were plaque index (PI), gingival index (GI) and halitosis-causing volatile sulphur compound (VSC) levels. Oral microflora was analysed utilising next-generation sequencing of the bacterial 16S rRNA gene.

Our preliminary findings indicate that the oral probiotic *S. salivarius* M18 reduced the VSC levels in patients with fixed appliances but did not decrease their plaque or gingival indices. The influence of probiotic *S. salivarius* M18 on oral microflora seems to be minimal. A longer intervention and follow-up period are needed.

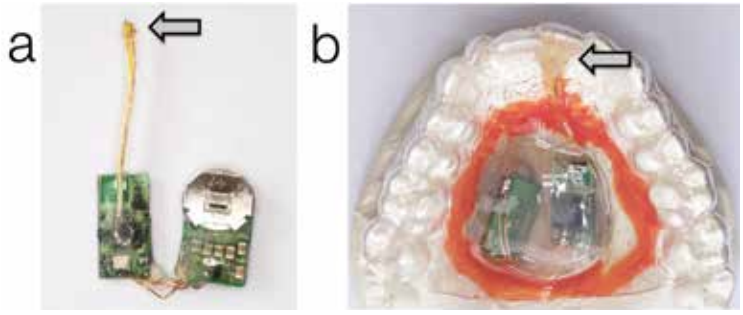


Distribution of phyla (Y-axis) from samples of oral plaque (X-axis) sequenced, in orthodontic patients.

Wireless Monitoring of Intra-oral pH

Research Team: Coreen Loke, Mauro Farella, Sylvia Sander, and Richard Cannon.

Intra-oral pH plays a significant role in the pathogenesis of tooth wear as well as white spot lesions in patients with fixed or removable orthodontic appliances. However, there is currently very little information about intra-oral pH variation over time, and in real life settings. The aims of this research were 1) to develop a wireless monitoring intra-oral pH device, which can be used to record real-time pH and temperature data in a natural environment, in which participants carry out their normal daily activities; and 2) to collect preliminary data in a sample of healthy volunteers, in a manner that is as non-invasive as possible, for over 24 hours.



Example of wireless device for measuring intraoral pH. a) Tip of antimony electrode (see arrow). b) wireless unit embedded in a vacuum-formed splint.

The wireless device consisted of a miniature pH telemeter, which included a temperature sensor and is capable of transmitting data wirelessly to a smartphone. A monitoring and calibration software application was also developed and was used to aid data input from the participants. *In vitro* tests were conducted to calibrate and validate the measurements of the device, and the measurements were tested against a gold standard reference electrode. The wireless device was then embedded into an in-mouth appliance and worn by a number of participants for at least 24 hours, while conducting regular daily activities.

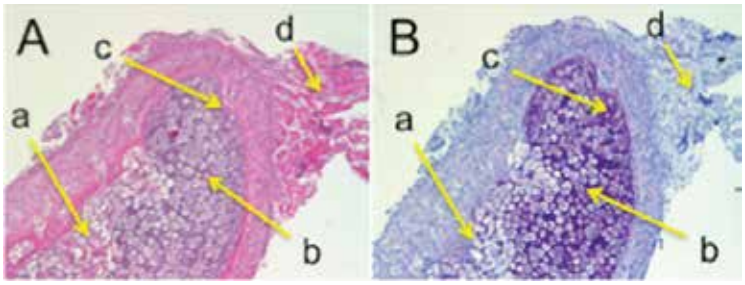
Preliminary results suggest that real-time variations in intra-oral pH and temperature can be successfully collected in participants. This wireless device is therefore capable of providing new insights into the variations of intra-oral pH over time, and its relationship with dental wear, white spot and caries.

Growth factor expression in the condylar cartilage of rats

Research Team: Mohamad Al-Dujaili, Trudy Milne, Richard Cannon, and Mauro Farella

The mandibular condylar cartilage has attracted significant interest in orthodontic research, as it is a key site of growth and development of the mandible. The aim of this research was to assess the expression of an array of growth factors and to identify changes in their regulation during early stages of growth. Condyles were extracted from rats aged 4, 10, 21 or 90 days, processed through a validated RNA extraction protocol, and subjected to histological analysis. Quantitative polymerase chain reaction (qPCR) was used to compare the relative gene expression of 28 growth factor-related genes. There were detectable changes in the regulation of genes involved in chondrogenesis and osteogenesis for each age group. However, none of the growth factor genes showed a change greater than two-fold. The present study showed that the cryogenic grinding protocol was a

valid technique for extracting RNA from rat condyles and that all the growth factors analysed were present. However, only weak evidence is provided for the regulation of the growth factors investigated at any of the selected time points.



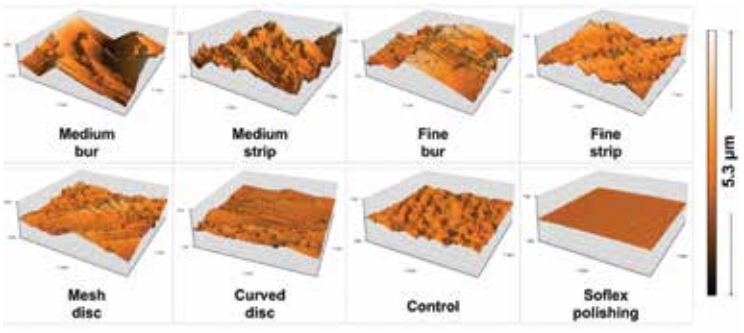
Coronal sections of the condyle of a 4-day old rat. Sections stained with H&E (A) and TB (B). 100X magnification a) erosive cell layer b) hypertrophic cell layer c) resting cell layer d) muscle tissue.

The influence of orthodontic interproximal reduction on enamel roughness and bacterial adhesion

Research Team: Lydia Meredith, Li Mei, Richard Cannon, and Mauro Farella

Interproximal reduction (IPR), also known as enamel stripping, is becoming more and more popular in orthodontics, but it leaves many grooves and furrows on the enamel surface, which may increase the risk of caries. We investigated the influence of IPR on the morphology and roughness (Ra) of enamel surfaces and the bacterial adhesion to these surfaces. The specific aims of this research were to assess the roughness of enamel surfaces (both qualitatively and quantitatively) produced by the most commonly used IPR instruments, to investigate the adhesion of bacteria to these surfaces, and to evaluate the effect of polishing after IPR on the amount of bacterial adhesion.

Extracted human premolar teeth were used to prepare enamel blocks, which were subjected to interproximal reduction. The morphology and roughness of the enamel surfaces were investigated qualitatively and quantitatively using atomic force microscopy. From the seven IPR-treated groups, the samples from the three instruments that yielded significantly different roughnesses, as well as the control group, were used for the adhesion experiments. Adhesion of Streptococcus sanguinis ATCC10556 to the enamel surfaces was assessed by counting the colony forming units that adhered to the roughened surfaces after 30 min exposure.



Examples of atomic force microscopy 3D images of enamel surfaces after using different IPR instruments.

The findings suggest that diamond burs created the roughest enamel surfaces, followed by diamond strips, followed by diamond discs. The Soflex polishing discs created the smoothest surfaces, even smoother than that of the untreated enamel. There was a positive relationship between enamel surface roughness and the number of bacteria that adhered.

Other research projects

- Relationship between sugar sweetened drinks, tooth wear and dental caries in Māori
- Jaw muscle overload as a possible cause of orofacial pain
- Assessing three-dimensional tooth movements during orthodontic activations using an E-typodont
- The effect of mechanical strain on the unfolded protein response of periodontal ligament cells in a three dimensional culture
- Predictive factors of orthodontic pain
- The influence of orthodontic therapy on mandibular motion
- Genetic aspects of the long face
- Growth factor expression in the rat condyle: implications for craniofacial development
- Intra-oral monitoring of oral pH and bruxism
- Mandibular growth in 3D: CBCT analysis in a rabbit model.
- Intraoral pressure changes upon varying the vertical facial dimension.
- Morphometric analysis of cervical vertebrae in relation to mandibular growth

Research funding

University of Otago Research Grant I just want my teeth straightened. (LF Page, JS Antoun, PW Fowler, HC Jack) $36,000

Ministry of Oral Health Reading between the lines: how do young New Zealanders from lower socio-economic backgrounds feel about not getting their teeth straightened? (LF Page, JS Antoun, HC Jack) $29,000

New Zealand Dental Research Foundation Genetic and psychological factors associated with orthodontic pain in children and adolescents (Farella M, Antoun J, Chandler N, Merriman T, Sew Hoy W) $8,441

New Zealand Dental Research Foundation Jaw muscle overload as a possible cause of orofacial pain and headache (Farella M, Ramanan D, Polonowita A, Hamilton J, Palla S). $12,350.

Lottery Health Grant "3dMD Trio Imaging System and Software for 3D imaging of the face". Supported by Lottery Health Grant (Mauro Farella, Joseph Antoun) $90,000

ERDG/FORENZAO Charitable Trust "Efficacy of a Mandibular Advancement Appliance on Sleep Disordered Breathing in Children (Ghassan Idris, Mauro Farella, Barbara Galland, Jules Kieser) $10,100.

CureKids Charitable Trust "Efficacy of a Mandibular Advancement Appliance on Sleep Disordered Breathing in Children (Mauro Farella, Ghassan Idris, Barbara Galland, Jules Kieser) $31,782

Ministry of Oral Health "Efficacy of a Mandibular Advancement Appliance on Sleep Disordered Breathing in Children (Mauro Farella, Ghassani Idris, Barbara Galland, Jules Kieser) $24,734

Health Research Council Genetics of dentofacial anomalies (Joseph Antoun, Mauro Farella, Tony Merriman, Murray Thomson) $149,462

Ministry of Oral Health Research Fund Efficacy of the oral probiotic Streptococcus salivarius in managing biofilm formation in patients wearing fixed orthodontic appliances (Li Mei, Gareth Benic, Mauro Farella, Nick Heng) $11,410

New Zealand Dental Association Research Foundation A novel model for exploring the causes and treatments of craniofacial birth defects (Julia Horsfield, Joseph Antoun, Mauro Farella, Catherine Carleton) $10,400

Otago Medical Research Foundation A novel model for exploring the causes and treatments of craniofacial birth defects (Julia Horsfield, Joseph Antoun, Mark Hampton) $30,000

New Zealand Dental Association Research Foundation A new approach to engineering 3D constructs of human bone matrix in a mechanically active environment (Murray Meikle, Trudy Milne, Yana Itskovich, Mauro Farella, Richard Cannon) $11,862

New Zealand Dental Association Research Foundation Growth factor expression in the rat condyle: Implications for craniofacial development (Trudy Milne, Mauro Farella, Li Mei, Richard Cannon, Mohamad Al-Dujaili) $14,042

Key personnel

The programme is led by Professor Mauro Farella, and includes the following SJWRI researchers:

Dr Joseph Antoun
Florence Bennani
Prof Richard Cannon
A/Prof Rohana De Silva
Professor Mauro Farella
Dr Winifred Harding
A/Prof Nick Heng
Dr Hannah Jack
Dr Carolina Loch Santos da Silva
Dr Li Mei
Dr Trudy Milne
Dr Christopher Robertson
Dr Benedict Seo
Suzan Stacknik
Prof Murray Thomson

A/Prof Geoffrey Tompkins
Dr Mike Brosnan
A/Prof Nick Chandler
Dr Harsha De Silva
Prof Warwick Duncan
A/Prof Lyndie Foster Page
Prof Karl Lyons
Dr Ajith Polonowita
A/Prof Neil Waddell

Postgraduate students

Sabarinath Prasad (2016) (PhD)
Joseph Antoun (PhD)
Erin Hutchinson (PhD)
Ghassan Idris (PhD)
Andrew Quick (PhD)
Joanne Choi (2015) (PhD)
Ana Low (DClinDent)
Caleb Lawrence (DClinDent)
Divya Ramanan (DClinDent)
Fiona Firth (DClinDent)
Will Sew Hoy (0DClinDent)
Austin Kang (DClinDent)
Azza Al-Ani (DClinDent)
Gareth Benic (DClinDent)
Catherine Carleton (DClinDent)
Yana Itskovich (DClinDent)
Mohamad Al-Dujaili (DClinDent)
Coreen Loke (DClinDent)
Lydia Meredith (DClinDent)
Austin Kang (MHealSc)

Research collaborations

The research group actively collaborates with other renowned scientific groups within the University of Otago such as:

Center for Bioengineering and Nanomedicine

Department of Anatomy

Department of Chemistry

Department of Physics

Department of Computer Science

Department of Human Nutrition

Department of Physics

Department of Zoology

D4 Network

Genetics Otago

Neuroscience Programme

Otago Zebrafish Facility

Pain@Otago

The programme also collaborates with the MedTech Centre of Research Excellence (CoRE), the Consortium for Medical Device Technologies (CMDT), the New Zealand Biomouth Research Group, and internationally works closely with the Department of Neuroscience at the University of Naples Federico II (Italy) and the Laboratory for Jaw Biomechanics at the University of Zurich (Switzerland).

University of Otago collaborators

Dr Azam Ali (Department of Applied Sciences)

Mr Hamza Bennani (Department of Computer Science)

Dr Claire Cameron (Department of Preventive & Social Medicine)

A/Prof George Dias (Department of Anatomy)

A/Prof Julia Horsfield (Department of Pathology)

A/Prof Barbara Galland (Department of Women's and Children's Health)

Dr Louise Mainvil (Department of Human Nutrition)

Prof Tony Merriman (Department of Biochemistry)

A/Prof Michael Paulin (Department of Zoology)

A/Prof Sylvia Sander (Department of Chemistry)

Prof. Steven Robertson (Department of Women's and Children's Health)

Dr Bernard Venn (Department of Human Nutrition)

Dr Louise Mainvil (Department of Human Nutrition)

Overseas collaborators

Prof Alan Brook (University of Adelaide)

Dr Iacopo Cioffi (University of Toronto)

Jie Fang (Sichuan University)

Prof Luigi Gallo (University of Zurich)

A/Prof David Healey (University of Brisbane)

Prof Beverley Kramer (University of Johannesburg)

Jialing Li (Nanjing Medical University, China)

Prof Ambra Michelotti (University of Naples, Federico II)

Em Prof Sandro Palla (University of Zurich)

Visiting scientists and students

Em Prof Sandro Palla (University of Zurich)

Prof Philip Benson (University of Sheffield)

Dr Annemarie Renkema (University of Nijmegen)

Mr Aurelio Songini (University of Cagliari)

Dr Francesca Fabiano (University of Messina)

Key publications

Hutchinson EF, Farella M, Kramer B. Variations in bone density across the body of the immature human mandible. Journal of Anatomy, 2016. Accepted for publication.

Meredith L, Mei L, Cannon RD, Farella M. Atomic force microscopy analysis of enamel nanotopography following interproximal reduction. Am J Orthod, 2016. Accepted for publication.

Antoun JS, Thomson M, Merriman T, Farella M. Impact of Facial Divergence on Oral Health-Related Quality of Life and Self-Report Jaw Function. Korean J Orthod, 2016. Accepted for Publication.

Antoun JS, Mei L, Gibbs K, Farella M Effect of Orthodontic Treatment on the periodontal tissues. Periodontology 2000. Accepted for publication, 2016.

Tan A, Bennani F, Thomson WM, Farella M, Mei L. A qualitative study of orthodontic screening and referral practices among dental therapists in New Zealand. Aust Orthod J 2016; 32: In press.

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Gray, S., Stacknik, S., & Farella, M. (2016). Space maintenance: An overview for clinicians. New Zealand Dental Journal, 112(3), 76-80.

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