The Sir John Walsh Research Institute Research Day 2019

Research Day, highlighting the research achievements of the University of Otago's Sir John Walsh Research Institute and Faculty of Dentistry, was held on Wednesday 11 September at the Dunedin Public Art Gallery. The event was made possible by the generous support of 3M Oral Care. In addition to oral presentations there was also a display of posters.

ABSTRACTS

Keynote presentation
Posterior composite restorations – friend or foe?

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While there is no question about the use of tooth-colored resin-based materials in the anterior segment, there exist still some doubts about their use in load-bearing areas, especially with larger cavities. In Switzerland, amalgam has been almost completely replaced by composite resin materials for almost 30 years and the so-called "Swiss Quality Guidelines" have been established and published to define distinct parameters for success and failure. The minimal longevity, which should be achieved in routine dental practice, has thereby been set at eight years for direct restorations and at ten years for indirect ones. Since the introduction of these guidelines, students do not learn to place amalgam at University. This pioneering work has found followers in many countries of Europe and similar standards, evaluation and teaching protocols have been established. However, several countries still rely on good old amalgam. The decision for a global phase-down of amalgam according to the "Minamata Convention on Mercury" will restrict the future use of amalgam despite its indisputable clinical indications and advantages.

In countries where amalgam is still the material of choice to restore posterior teeth with proximal caries, great concerns, mainly about marginal long-term adaptation, wear rates and related failures of posterior composite restoration remain and have to be overcome. Current materials and technical concepts allow for high quality fillings, but composite materials require specific application techniques. Sensitive aspects include preparation steps, etching and priming, material insertion using incremental techniques and light curing under strict isolation. Postoperative aspects like finishing and polishing and maintenance are also of utmost importance. Considering all this, clinical data show that modern composite resin materials can allow for adequate and excellent long-term results, which are at least comparable to amalgam under the above-mentioned premises. Wear can be regarded as a shortcoming, but modification and changes in material characteristics have also led to significant improvements. Noteworthy, some of the suggested biomechanical weaknesses of composite can also be regarded as advantages. Less stiff and brittle materials prevent the restored teeth and their antagonists from damage, which may be beneficial, especially when treating specific patient groups at risk (e.g. bruxism, implants etc.).

Nowadays, we are able to treat almost every case and situation with direct composite resin materials under the premise of strict tissue preservation and protection (sealing), the question is mainly if the manual efforts required for this are justified given the pre-existing defect size. But everything is possible, which can be elucidated by the direct full-arch restoration of severe cases of tooth wear using composite only. The implementation of such concepts requires a good knowledge of material properties and technical skills. These questions can only be answered and these doubts resolved by knowledge of the literature and filling of gaps by conducting respective additional research.

In vitro *corrosion resistance of glazes for monolithic zirconia crowns*

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Objective: The purpose of the study was to evaluate the *in vitro* corrosion behaviour of three glazes available for use with monolithic zirconia restorations. This was done through calculation of mass loss and SEM analysis across a range of clinically equivalent ageing times.
Methods: Zirconia specimens were prepared and glazed with one of three glazes according to the manufacturers' recommendations. Then aged in 4% acetic acid at 80°C as per ISO6872 Sec 7.6 Chemical Solubility for clinically equivalent times (0, 2, 4, 6, 8 and 10 years) *in vivo*. Mass loss per volume ($\mu g/mm^3$) was calculated and the initiation and propagation of corrosion was analysed via scanning electron microscopy (SEM).
Results: Differences in average mass loss for all glazes across all ageing times was not significant. However, SEM analysis showed evidence of corrosion and

increased surface roughness after 2 years' equivalent of ageing and the number and size of corrosion sites increased with time. Evidence of low temperature degradation phase transformation in the zirconia was also observed in some specimens.

Conclusion: Corrosion initiated soon after placement of the specimens at 2 years equivalent time and increased with ageing time. Loss of glaze integrity may have future implications on the survival of glazed zirconia restorations, their aesthetic stability and potential for increased biofilm adhesion.

Eating big down under: enamel microstructure and isotopic data on an extinct sperm whale from the Atacama Region, northern Chile

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Livyatan was an extinct genus of sperm whale found in Peru, Chile and Australia, dating back from 10 to 5 Ma. While modern sperm whales only have lower teeth, *Livyatan* had rather large functional upper and lower teeth, suggesting feeding on large prey. We examined the morphology, enamel microstructure, and isotopic composition of a lower Livyatan tooth from northern Chile. The subcylindrical tooth is 322 mm long and 109 mm in diameter. The crown is curved lingually, approximately 30 degrees from the main axis. The root apex is open and a thick cementum layer covers the root. A rugose enamel tip covers part of the crown. Enamel fragments were removed from the crown base for scanning electron microscopy (SEM) and isotopic analyses. SEM images revealed a moderately thick enamel (600 µm) with prominent Hunter-Schreger bands (HSB) throughout the whole thickness, with both open and closed prisms present. HSB are implicated in resisting and limiting enamel crack propagation, being considered a biomechanical response to increased occlusal loads during feeding. The carbon and oxygen isotopic compositions of structural carbonate from enamel and dentine were analysed to elucidate diet and habitat of the species. We observed an extremely low $\delta13C$ value in enamel ($\delta13C = -16.5‰$), which is consistent with feeding at latitudes greater than 40° S. Based on isotopic analysis of other fossil whales from the same area and age, it seems unlikely this specimen was feeding exclusively on large prey. The presence of HSB in *Livyatan* suggests there was a reduction in enamel complexity in sperm whales over time, as modern sperm whales have a thin layer of prismless enamel that is often worn away. As more fossil teeth are discovered and studied, new analyses considering more specimens will help elucidate the past lives of these rare whales.

Tooth wear, diet and dental properties: The interaction between occlusal wear and enamel fractures

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Aims: Tooth wear is the loss of dental tissue not caused directly by trauma or pathology. Dental wear can be used in different ways to infer diet and behaviour in past populations. However, few studies have compared how different types of tooth wear interact and how differences in these patterns may be influenced by dental properties on a species level. This research compares different types of wear across tooth crowns in a primate comparative study.

Methods: Teeth of different species of extant primates and fossil hominins were recorded and compared for different types of wear, in particular overall occlusal macrowear and for the presence of fractures. The overall wear of each tooth was scored and the location, severity and number of fractures were also recorded.

Results: Dental wear patterns were variable among species, including wear characteristics (angle, extent) and position/prevalence of fractures. While some of this variation relates to dietary and behavioural differences, dental characteristics may also play a role. In all species studied here, the molar surface (buccal/lingual) with less occlusal wear was also the one with more fractures, but there is variation in terms of fracture size and prevalence.

Conclusion: Dental properties such as enamel density, microstructure and tooth morphology likely influence the interaction between different types of wear. For example, species that commonly eat mechanically demanding foods may have evolved 'stronger' enamel on certain parts of the crown. Thus, the surface most susceptible to overall occlusal wear may be mechanically reinforced and less likely to fracture. If true, this would be another example of differences in wear patterns between species relating directly to dental properties. In sum, although broad dietary conclusions can be made based on tooth wear patterns, more refined differences between species should also incorporate dental properties and phylogenetics into the analysis.

Gingival displacement, retraction cord versus retraction paste: A clinical study

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Aims: This clinical study measured the change in opening, height and angle (geometry) of the displaced gingiva using paste and cord retraction materials and assessed if they were comparable and clinically acceptable.

Material and methods: Impressions of the participants'

maxilla were taken using a split–mouth protocol of 4 premolars from 10 participants. All participants were free of periodontal disease, had a thick biotype and gingival sulci depths of 2mm. The bleeding index (BI), gingival index (GI) plaque index (PI), sulcular depth, level of attachment and tooth sensitivity were recorded at baseline, just after retraction, at 24 hours and at 2 weeks. Impressions were made and models were poured and then cross-sectioned to allow measurement of the gingival height and displacement sizes. The gingival displacement angles were measured and the gingival geometrical shape was quantified.

Results: The paste produced a slightly smaller gap compared to the cord (0.041mm less, p=0.014) whilst the mean displacement for the cord was 0.282mm and paste were 0.213mm respectively. Gingival height with the paste was 0.047mm lower than that achieved by the cord (p=0.208). The mean gingival height for the paste was -0.013mm and -0.06mm for the cord. The mean gingiva displacement angle for the cord was 37.8 degrees and for the paste it was 33.9 degrees.

Conclusions: The cord and paste retraction materials produced comparable clinically acceptable gingival gaps, with the cord producing a statistically larger gap size. Both the cord and paste had minimal gingival height change and similar mean gingival displacement angles.

Characterisation of bacteriocin resistance exhibited by Streptococcus australis *strain I18*

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Aims: Members of the genus *Streptococcus* inhabit a variety of sites in the oral cavity and many species also produce proteinaceous antibiotics (bacteriocins). Whilst much is known about bacteriocins and their genetic determinants, less is understood about the mechanisms of bacteriocin resistance. Streptocin STH2, produced by the oral bacterium *S. gordonii*, is a competence-induced, narrow-spectrum bacteriocin that targets closely-related species including strains of *S. mitis* and *S. oralis*. The primary objective of this study was to understand the proteomic and genetic basis of resistance exhibited by *S. mitis* I18 to STH2. Methods: Total protein extracts from S. mitis I18 and its STH2-resistant derivative I18** were analysed by polyacrylamide gel electrophoresis, protein bands of interest excised, and their N-terminal amino acid sequences compared to entries in protein databases. The genomes of I18 and I18** were sequenced using Nanopore DNA sequencing technology. Results: Expression of several proteins was increased in strain I18** of which four were characterised further. Whereas two proteins (12- and 35-kDa) matched cytoplasmic "housekeeping" proteins (histone-like protein HU and ribosomal protein L6, respectively),

the other two (20- and 45-kDa) exhibited similarity to putative cell surface components (oligopeptide permease and cellobiose phosphotransferase IIC, respectively). As sugar phosphotransferases are targets of some peptide bacteriocins, overexpression of proteins such as cellobiose phosphotransferase could constitute a novel bacteriocin resistance mechanism. At time of writing, the genomes of *S. mitis* I18 and I18** have been sequenced fully. Interestingly, analysis of the 16S ribosomal RNA gene reveals I18 to be a strain of *S. australis*. Conclusions: Bacteriocin resistance in strain I18 is associated with overexpression of certain cell surface proteins. Mutational studies are required to confirm their involvement, and direct comparison of the wild-type and mutant genomes may reveal more STH2-related resistance factors. This presentation will focus on the 'hot off the press' genome analyses.

It's a matter of time: Supporting effective feedback processes in the clinical learning environment

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Feedback plays an important role in dental students' learning. The clinical dental teaching and learning environment is challenging, as clinical tutors are supervising students who are undertaking irreversible procedures on patients. Situations can arise where engaging in feedback and other 'good' teaching practices takes second place to ensuring patient, student, and tutor safety. This research investigated the specificfactors that impact clinical tutors' desires and ability to give effective feedback to students in the clinical learning environment. Twelve clinical teachers at the Faculty of Dentistry, University of Otago were interviewed. The data were analysed inductively, and four themes were identified: Feedback as a teaching moment, where tutors used the terms feedback and teaching interchangeably and viewed feedback as their primary mode of teaching in the clinical environment; time, where tutors spoke about time constraints in the clinic that made it difficult to engage with students in the depth they would like; empathy and respect where tutors were mindful that the students were emerging professionals and should be treated as the tutors wished to be treated themselves; and self-reflection where the tutors reflected on their own teaching practices in order to improve them. The results of the research provide a basis for exploring how tutors might be supported to engage in feedback practices that encourage students' learning whilst maintaining patient, student and staff safety.

Barriers to, and facilitators for, older people who receive home support accessing dental care

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This presentation reports findings of a study that aimed to explore the structural, social and individual barriers to, and facilitators for, accessing dental services and remaining dentate, among a purposeful sample of dentate older people who receive home support (henceforth, OPHS). Studies on the oral health of older people have largely been quantitative, while fewer studies have specifically explored the dental services utilisation patterns of dentate OPHS especially in a New Zealand context.

In early 2019, individual interviews were conducted with a purposeful sample of 40 dentate OPHS from Otago. A parallel coding and thematic analysis of this data was undertaken. Barriers to accessing dental services included the cost of dental treatment, negotiating transport issues, social isolation, traumatic childhood dental experiences, lack of role modelling for good oral health and hygiene, self-aging and drawing upon discourses of older people, and health conditions impacting on mobility and access to oral health care. Facilitators for accessing dental services included having a support network, parental role models, healthy diet, knowledge of the link between oral and general health, dental clearances for surgery, having a strong dislike of dentures and not wanting them, and agency to resist dentists' advice to have a full extraction.The cost of dental care is a major barrier to OPHS accessing dental care and so is social isolation.

PRESENTATIONS BY POSTGRADUATE STUDENTS

Bond strength of a direct composite resin to hybrid ceramic materials with four surface treatments

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Purpose: To evaluate the bond strength of composite resin to different CAD/CAM resin-matrix ceramic materials following different surface treatment methods using a three-point bend fracture toughness test method. Materials and Methods: A total of 240 beam shaped specimens (5mm x 5mm x 17mm) were prepared from four types of CAD/ CAM resin-matrix ceramics, Vita Enamic (VE), Lava Ultimate (LU), Cerasmart (CS) and Shofu Block HC (SB). Sixty beam specimens were prepared for each material and then subdivided into four groups (n=15) according to the surface treatment method,

group G: grinding only; group GF: grinding + 4.5% hydrofluoric acid; group GBF: grinding + airborne-particle abrasion using 50 μm aluminium oxide + 4.5% hydrofluoric acid; group GBP: grinding + airborne-particle abrasion 50 μm aluminium oxide + 37% phosphoric acid. A nanocomposite material was packed onto the treated surfaces following the application of universal adhesive material according to manufacturers' instructions. The bond strength was tested using a fracture toughness 3-point bend test. Specimens were examined under SEM to determine mode of failure. Data for groups CS and SB were analysed using a one-way ANOVA to evaluate statistical significance (P<0.05) while groups VE and LU, which showed non-parametric data, were analysed using a Kruskal Wallis test.
Results: Statistical analysis showed a significant difference between the different surface treatment within groups VE, CS and SB (P< 0.05). LU groups showed no interaction between surface treatment method and bond strength (P= 0.629). VE-G group showed statistically higher bond strength than LU-G, CS- GSBP and SB- GSBF.
Conclusion: No single surface treatment method can be generalized for all resin-matrix ceramic materials to achieve a reliable bond strength. The process of roughening the resin-matrix ceramic materials via grinding, air-borne abrasion, HF acid etching or combination of these methods produces an increase in the surface area and consequently improves the bond strength.

Is parity associated with periodontal attachment loss and other oral conditions?

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Background & Aim: Many believe women's oral health deteriorates as a result of having children. This research aimed to investigate whether social and behavioural risk factors common to both parity (number of times a woman has given birth) and dental disease (periodontal attachment loss (AL), and caries) may account for any association between oral health and parity.
Methods: The Dunedin Multidisciplinary Health and Development study is a longitudinal study of 1037 individuals (48.4% female) born from April 1972 to March 1973 in Dunedin, New Zealand. Logistic and negative binomial regression models were used to examine associations between the number of children born to female participants and their periodontal AL, dental caries experience, and tooth loss. Models controlled for confounders, including educational achievement, oral hygiene, dental service use and smoking.
Results: Data were available for 433 women (96.4% dentally assessed, aged 45). Of these, 76.2% had given birth to one or more children. Low educational attainment was significantly associated with having more children at all ages assessed. Parity by age 38 was not associated with periodontal AL, untreated dental caries, or prevalence of missing at least one tooth, but was associated with

mean tooth loss and DMFS. Women who had children by age 26 experienced poorer dental health outcomes by age 45 than nulliparous women, or women entering motherhood later in life. Parity by age 26 was associated with the number of tooth surfaces with untreated caries at age 45 (IRR 1.69, 95% CI 1.22-2.35) and teeth missing due to caries (IRR 1.62, 95% CI 1.27- 2.08).

Conclusion: The biological effects of pregnancy appear less important for the development of dental disease than the social factors associated with having children. Level of education attained appeared to influence both a woman's reproductive patterns and health behaviours, which may in turn influence the risk of dental disease and how it is managed.

Occlusal features during adolescence and temporomandibular joint sounds thirty years later

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It has been suggested that significant deviations from the 'ideal occlusion' can increase risk of temporomandibular disorders, including clicking and crepitus sounds.
Aim: To investigate whether occlusal anomalies during adolescence, such as high/low overjet, high/low overbite, and posterior crossbite, are associated with temporomandibular joint sounds 30 years later.
Methods: Participants were members of the Dunedin Multidisciplinary Health and Development Study, a longitudinal birth cohort of 1,037 children born in Dunedin between 1972 and 31 March 1973. Baseline occlusal data were collected during a dental examination at age 15. Follow-up data on self-reported and examiner-detected TMJ outcomes, self-reported parafunctional habits, and orthodontic treatment history were collected at age 45. The Multidimensional Personality Questionnaire (MPQ) was used at age 26 to characterise three personality characteristics (MPQ superfactors: positive emotionality, negative emotionality, and constraint). Associations were tested by multiple logistic regression analysis.

Results: Posterior cross-bite and overjet at age 15 were not associated with any of the self-reported and examiner-detected TMJ outcomes at age 45. However, greater odds of self-reported TMJ clicking was associated with Z-score of negative emotionality (OR = 1.3, 95% CI 1.1-1.6) and self- reported history of tooth clenching (OR = 2.6, 95% CI 1.5-4.5), while positive emotionality was associated with lower odds (OR = 0.7, 95% CI 0.6-0.9). Study members with high overbite (deep bite) at age 15 had lower odds for both self-reported and examiner-detected TMJ sounds at age 45. No associations were found between history of orthodontic treatment by age 45 and any of the TMJ outcomes.

Conclusion: Deep bite may reduce risk for examiner-detected TMJ clicking, while personality is an important source of bias that should be considered in studies that rely on self-reported measures of TMJ clicking.

Factors associated with orthodontic pain

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Introduction: The amount of pain experienced during orthodontic treatment varies largely over time and between individuals and can affect patient's compliance, ability to chew, wellbeing, and sleep quality. The reason for the inter-individual variability in pain are largely unknown: clinical activations, demographic psychological characteristics, and genetical polymorphisms of candidate genes factors are putative factors that may account to explain this variability.
The aim of this study was to investigate the effect of clinical, demographic, psychological, and genetical factors on pain levels experienced during fixed orthodontic treatment.

Method: A convenience sample of 183 patients undergoing full fixed orthodontic treatment at the University of Otago, Discipline of Orthodontics was recruited for this study. Participant's pain levels were assessed seven times over a three-day period via a smartphone App on an issued research phone. Clinical, demographic, and psychological data were collected via questionnaire. This included the Pain Catastrophising Scale (Child Version); the Corah Dental Anxiety Scale; and the State and Trait Anxiety Inventory. Participants provided a DNA sample either in the form of blood or saliva, which were used for genotyping COMT gene rs6269, rs4680, rs4646310, NR3C1 gene rs2963155 and the HTR2A gene rs9316233.

Results: Bond ups had the greatest influence on perceived levels of orthodontic pain, accounting for 20% of total variance in pain response. High pain responders had higher scores on pain catastrophising (magnification subscale). Self-reported pain during fixed orthodontic treatment was not influenced by gender, age, time into treatment, anxiety, nor by polymorphisms of HTR2A or NR3C1 gene. AA genotype of COMT rs4646310 had higher pain levels compared to the GG and AG genotypes (p=0.048).

Conclusions: Orthodontic pain is stronger during bonds-up and in patients with high catastrophizing scores. Demographics, type of clinical activations, and the genetic polymorphisms investigated in this research had little impact on perceived pain levels.

Eating fast and body mass index in young adolescents: is there a relationship?

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Behavioural aspects of chewing may influence food intake, nutritional status, and in turn body weight. Obesity is highly prevalent among New Zealander adolescents, and of a global concern, as it impacts negatively on children health.

The aims of the current study were 1) to study the chewing features in a group of adolescents, as they naturally occur in home-based settings, and 2) to test a possible association between chewing features and body weight. Forty-two participants (20 females and 22 males) aged 15.3 ± 1.3 year were recruited for this study. Based on Z-score for Body Mass Index, half of the study participants (n=21) were classified as healthy weight, while the other half as overweight-to-obese. Participants chewing features were assessed for one evening, directly in home-based settings, using a smartphone-assisted wearable electromyographic (EMG) device and a wearable camera, which always included the main meal. The outcome variables included the chewing pace, the chewing time, the number, and the power (intensity) of chewing strokes.

Eating episodes could be accurately detected by both the EMG device and the wearable camera, with accuracy values ranging from 0.8 to 0.92. The EMG device, however, could identify chewing episodes not detected by the camera. The chewing features (mean ± SD) as evaluated by EMG showed a chewing pace of 1.53 ± 0.22 Hz, a chewing time of 11.0 ± 7.7 min, a chewing episodes number of 63.1± 36.7 per evening, and a chewing power of 30.1 ± 4.8 %. There was a negative correlation between BMI and chewing pace (R = -0.42 ;p<0.001) and between the BMI and chewing time (R = -0.32; p = 0.026). The results of the current study indicate that overweight-to-obese adolescents tend to eat in a shorter time and at a slower pace than their healthy weight counterparts.

Efficacy of intravenous and submucosal dexamethasone after third molar surgery

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Objective: To compare the efficacy of submucosal (SM) dexamethasone and intravenous (IV) dexamethasone in reducing facial swelling, pain and trismus after third molar surgery, and its impact on quality of life.
Methods: This trial was designed as a randomised, controlled, surgeon- and participant-blinded single-centre trial with two parallel groups. There were 61 participants in the IV group and 64 participants in SM group. The IV group received 8mg/2ml IV dexamethasone and 2ml saline SM injection. The SM group received 8mg/2ml dexamethasone SM injection and 2 ml IV saline. Facial swelling was measured using a contactless 3-dimensional facial camera (3dMD Inc, Atlanta, GA). Pain was measured using a 100mm visual analogue scale. Maximum incisal distances were measured using a linear calliper. All measurements were taken immediately before the surgery and on postoperative days 2 and 7. Data were collected from participants by means of the oral health impact profile (OHIP)-14 to assess changes to quality of life. Data were cross-tabulated and analysed by ANOVA or Pearson Chi-Square, as appropriate. P values of <0.05 were considered statistically significant.
Results: On day 2, the IV and SM group had a mean facial swelling of 7.3 cm^3 and 7.8 cm^3, respectively. On day 7, the swelling had reduced to 2.9 cm^3 in the IV group, and 2.6 cm^3 in the SM group. Mean pain scores and limitation to mouth opening did not differ between treatment groups on either postoperative day. Both groups experienced poorer quality of life, and were affected to a similar extent. There were no statistically significant differences between the two groups.
Conclusions: There are no differences in postoperative adverse outcomes between submucosal and intravenous administration of dexamethasone in third molar surgery. Submucosal dexamethasone is an easy route of steroid administration in patients who undergo third molar surgery under local anaesthesia only.

BRAF mutations in ameloblastoma: Correlation with clinical and histopathological features

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Background: The invasive nature of ameloblastoma necessitates tumour- free surgical margins to minimize recurrence. Surgical management results in significant loss of structure and often requires extensive reconstruction. Recently it has been shown that the MAP kinase pathway plays an important role in the pathogenesis of ameloblastoma; BRAF is a key protein in this pathway. To assess the expression of BRAF V600E mutation using immunohistochemistry in ameloblastoma and to correlate these results with stromal vascularity and lesional proliferation. The results were then contextualized in relation to clinico-pathologic parameters.
Methods: Formalin-fixed paraffin-embedded samples of ameloblastoma (n=44) were stained with antibodies against BRAFV600E, CD34 and Ki67. IgG isotype was used as a negative control. Three hotspots in the epithelium of each specimen were selected for semi-quantitative analysis. BRAF intensity and proportion scores were used to calculate the immunoreactive score (IRS). The stromal CD34 mean vessel density (MVD) and the Ki-67 proliferation index (PI) were determined. Kruskal- Wallis tests were performed with p <0.05 denoting statistical significance.

Results: The mean age at diagnosis was 36 years (range of 10-73). The male to female ratio was 1.2: 1 and 89% of cases were from the mandible. The neoplastic epithelium in 93% of cases was BRAF+ve with 27% of these displaying high reactivity (IRS>5). 81.8% of mandibular lesions were BRAF+ve. CD34 MVD and Ki-67 PI corresponded with higher BRAF IRS.

Conclusion: Most ameloblastomas in this cohort were BRAF+ve. The possible association between vascularity and PI with BRAF expression should be explored further. The use of anti-BRAF therapy in the management of selected ameloblastomas may reduce morbidity.

Special Needs Dentistry, how well do you know it?

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Aims: The aim of this study was to determine the current understanding of Special Needs Dentistry (SND) among general dentists in New Zealand, and to define the role of general dentists in the management of patients with special needs, in the community setting.

Methods: All general dental practitioners in New Zealand registered under the Dental Council (New Zealand) were invited to complete an online survey. The exclusion criterion were Special Needs dentists and other dental specialists registered with the Dental Council. An analysis of the data was performed using the SPSS window version 25.

Results: Findings were that 82.8% of the survey respondents reported have treated people with special needs, but only 7.7% of them reported they felt very confident in treating people with special needs. New Zealand general dentists who had undergraduate dental training with an SND component was 49.2% of respondents. Lack of experience in treating people with special needs was the highest barrier to care for those who did not treat people with special needs.

Conclusion: This study is the first to report on perceptions of SND amongst general dentists in New Zealand. Results can be used to inform improvements to how SND is taught in New Zealand. Initiatives to encourage more general dentist to develop an interest in SND and have a positive attitude towards people with special needs and special needs dentistry would be beneficial to the community, hospitals and government authorities, and policy makers. Such initiatives would lead to improvements in the oral health care delivery for people with special needs.

Characteristics of older people presenting to Waikato Hospital Dental Department: A case series analysis

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The population of dentate older people in New Zealand is increasing, with a concomitant increase in oral disease experience. We undertook an 8-month clinical audit at Waikato hospital dental department to determine:
1. The demographic characteristics, source and reasons for referral, dentition status of the older people
2. The nature of their dental problems, the treatment received and the follow up care

Information on patients aged 65 or more was abstracted from the clinical database. Data analyzed included demographic characteristics, dentition status, source of referral, medical history, treating clinician, treatment delivered and whether there was follow-up care.

Results: Among the 203 identified patients, there was a male-to-female ratio of almost 3:2, and 90% were European. Overall, 80% of the patients were living in their own homes; 80% were dentate. Some 30% presented with at least two medical conditions, and those living in a rest home had a higher mean number of medical conditions. General dental practitioners had referred more than 40% of patients in the 85+ age group whereas general medical practitioners (GP) had referred 33% of those in that age group. More than 50% of rest home patient referrals were from their GP. Some 33% were oncology referrals (internal), while 33% were acute referrals and 60% had presented for elective dental treatment. Swollen face, odontogenic infection and irreversible pulpitis were the most likely reasons for acute referrals. Around 10% of patients had teeth extracted. Two-thirds of patients were referred to their general dental practitioner for follow-up.

Conclusion: Around 25 older patients per month are referred for care from diverse referral sources, and the proportion of acute referrals is relatively high, suggesting that poor oral health among older adults is an important problem.

Yeast species in the oral cavities of older people

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Oral candidiasis is prevalent among older people due to predisposing factors such as impaired immune defences, medications and denture use. An increasing number of older people live in rest home facilities and it is unclear how this institutionalised living affects the quantity and type of fungi colonizing these people's oral cavities. Smears and swabs of the palate and tongue, and saliva samples were taken from participants residing in rest homes (RH; n = 25) and older people living in their own homes (OH; n = 25). Yeast in samples were quantified

and presumptively identified by culturing on CHROMagar *Candida* agar. Sequencing of the ITS2 region of rDNA was carried out to confirm yeast species. Multilocus sequence typing (MLST) of 7 housekeeping genes was used to compare *Candida albicans* strains. A higher proportion of RH residents had *Candida* hyphae present in smears compared to OH participants (35% vs. 30%) although this difference was not statistically significant (p = 0.74). RH residents had, on average, 23 times as many yeast per mL saliva as OH participants (p = 0.01). Seven yeast species were identified in OH samples and only five in RH samples, with *C. albicans* and *C. glabrata* being the most common species isolated from both participant groups. All *C. albicans* strains were similar within the same participant but very different between participants. Nine strains, found in 4 rest home participants, appeared to be *C. albicans/ Candida dubliniensis* chimeras (hybrid strains). The results indicate that communal living for those who reside in an age care facility has an impact on the abundance of yeast species and the prevalence of chimera strains. This may be due to morbidities which led to the need for residential care and/or related to the rest home environment.

Neuropilin-1 expression in the tumour microenvironment of oral squamous cell carcinoma

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Neuropilin-1 (Nrp-1) is a transmembrane glycoprotein involved in multiple biologic and pathologic processes including carcinogenesis. It is widely expressed in cancers and it has been correlated with poorer prognosis. Nevertheless its expression profiles and significance in oral squamous cell carcinoma (OSCC) remain elusive. To investigate the expression of Nrp-1 in OSCC and oral epithelial dysplasia (OED), using immunohistochemistry (IHC), and to examine its co-localisation with markers of angiogenesis (VEGFR2) and immune modulation (FoxP3) using double-labelling immunofluorescence (DLIF). Specimens derived from OSCC, OED and non-specifically inflamed mucosa (NIM- control) (n=23, 17 and 14 respectively) were stained with anti-Nrp-1 antibody. Hotspot locations in the epithelium and connective tissue (n=3 each) from each specimen were photographed and semi-quantitatively analysed. Intensity and proportion scores were used to calculate the immunoreactive score (IRS). Kruskal-Wallis tests were performed with p <0.05 denoting significance. DLIF results were qualitatively examined to determine the co-localisation of Nrp-1 with VEGFR2 and FoxP3.
The OED epithelium showed significantly higher expression of Nrp-1 than NIM and OSCC. No other significant quantitative differences were observed in the epithelium or connective tissue between the groups. Qualitatively Nrp-1 was highly expressed on the stromal mononuclear cells in the OSCC and OED tissues.

Furthermore, Nrp-1+/FoxP3+ mononuclear cells were detected in the OED and OSCC but was not in NIM. There was no evidence of Nrp-1/VEGFR2 co-localisation in any of the tissue samples examined.
The epithelial overexpression of Nrp-1 in OED suggests that it may be important in the initial dysplastic transformation, but not necessarily in the malignant transformation. The Nrp-1 expression and the Nrp-1/FoxP3 co-localisation on the stromal mononuclear cell in the OED and OSCC suggest that Nrp-1 may modulate microenvironment conducive to disease progression. VEGFR2 does not appear to be a meaningful co-receptor for Nrp-1 in OSCC and thus Nrp-1-associated angiogenesis may involve alternative mechanisms.

Vital bleaching and oral-health-related quality of life in adults: a systematic review and meta-analysis

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Objectives: To carry out a systematic review and meta-analysis of studies that investigated changes in perceived quality of life following vital tooth bleaching.
Methods: Online searches (Medline, PubMed, the Cochrane database of systemic reviews and Google scholar), bibliographic, and manual searches were carried out. Two authors independently screened the 313 articles identified from the searches and two authors extracted data, including risk of bias using the Cochrane data collection form. Random effects meta-analysis was used to estimate the pooled standardized mean difference (with 95% CI) and the 95% prediction interval.
Results: Only four studies met the inclusion criteria, two showing statistically significant improvement, one worsening, and one inconclusive. The pooled standardized estimate for change in quality of life after bleaching was 0.04 (95% CI -0.15, 0.24) with substantial heterogeneity (I-squared 82.1%). Within these studies, there was a pattern of improvement in aesthetic-related domains (e.g. smiling and psychological discomfort) and deterioration in function-related domains (e.g. hygiene and pain).
Conclusions: Vital bleaching was not associated with improvements in overall Oral Health Related Quality of Life (OHRQoL) in these heterogeneous populations. Vital bleaching appeared to impact some domains of OHRQoL positively and some negatively, indicating the need for clinicians to treat patients receiving bleaching treatment so as to obtain the best improvement in aesthetics with minimal side effects. Clinicians should be aware of the potential impact caused by tooth sensitivity and offer proper instruction to prevent it or treatment to reduce its impact.
Clinical Significance: Bleaching treatment produces positive changes in young participants' OHRQoL in

aesthetic areas such as smiling, laughing, and showing teeth without embarrassment. Tooth sensitivity, the main side-effect of vital bleaching treatment, can affect quality of life, and so oral health professionals should offer evidence-based advice to prevent and manage sensitivity.

Characterization of Cryptococcus neoformans lanosterol 14α-demethylase

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Cryptococcus neoformans is an opportunistic fungal pathogen of humans. It is estimated to cause 1 million cases of meningoencephalitis worldwide in HIV-infected patients. These infections result in about 625,000 deaths, mainly in the sub-Saharan Africa, where it is a leading cause of death. *C. neoformans* infections can be treated with azole antifungals, which inhibit lanosterol 14α-demethylase (CYP51), an enzyme that is essential for the synthesis of the fungal-specific sterol ergosterol. NADPH-cytochrome P450 reductase (CPR) is the redox partner which provides electrons for CYP51 activity. Azole prophylaxis and/or long-term use of azole drugs have led to the acquisition of azole resistance in *C. neoformans*. In order to develop a platform for the discovery of more effective antifungals, we have constitutively overexpressed recombinant full-length, codon optimised and C-terminalhexahistidine-tagged CYP51 of *C. neoformans* (CnCYP51) from the *PDR5* locus of a *Saccharomyces cerevisiae* host strain deleted of 7 drug efflux pumps. In addition, the promoter of the native CYP51 was replaced with the galactose-regulated Gal1 promoter. A codon optimised cognate CPR (CnCPR) was also constitutively co-expressed from the *PDR15* locus. Agarose diffusion assays and MIC80 determinations were used to evaluate the susceptibility of the *S. cerevisiae* host and recombinant strains to various azole antifungals. Western blots were used to assess the expression levels of recombinant proteins. Expression of CnCYP51 was relatively low but co-expression with CnCPR enhanced its function ~2-fold in response to the short-tailed (fluconazole and voriconazole) and long-tailed (itraconazole and posaconazole) triazoles and a medium-tailed tetrazole (VT-1161). Structural and functional analysis of recombinant CYP51 from *C. neoformans* will aid in understanding the susceptibility of this fungal pathogen to azole drugs and will significantly advance our goal of structure-directed antifungal discovery.

Protein profiling analysis of neural crest-derived stem cells

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Neural crest derived stem cells are known to have higher differentiation capacity when compared to stem cells from ectoderm, endoderm or mesoderm. Deer antler grows at up to 3 cm a day and is the only known mammalian model of stem cell-based full organ regeneration. Deer antler and dental pulp have recently been defined as neural crest derived. Antler may thus provide a good model to study activation, maintenance and control neural crest stem cells.

The aims of this research were to investigate the osteogenic potential of human dental pulp and deer antler stem cells, the regulation of pleiotrophin as a key stem cell protein and to identify new regulated proteins/pathways during neural crest-derived stem cell control. Bone growth was investigated after osteogenic induction of both deer antler and human dental pulp stem cells.

Antler tissues included: dormant pedicle periosteum (DPP), growth centre (GC), post-active stem cells from mid-beam periosteum (MAP), and deer facial periosteum (FP) as a control (N=3, biological replication). The expression levels of pleiotrophin (PTN) were measured in antler stem cells under different stages of activation. Label-free quantification used LC-MS/MS to detect the protein profiles and PEAKS and Ingenuity Pathway Analysis software with multivariate analysis. High expression levels of PTN and receptors in the growth centre showed its potential importance in endochondrial bone regeneration. Proteomics detected that most unique proteins (eighty-seven) were found from growth centre tissue.

Protein profiles between DPP, MAP and FP indicated that antler stem cells may use similar mechanisms to maintain dormancy within a stem cell niche. Activation of antler stem cells was mainly associated with up-regulation of a number of canonical pathways and molecular/cellular functions such as PI3K/AKT signalling. This work investigates antler stem cells protein expression in different activation states and sheds new light on neural crest stem cell control.

A novel method for exosome isolation

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Exosomes are membrane bound vesicles released by cells into their extracellular environment. Cancer cells exploit this to modulate their local and/or distant environments. To study these vesicles it is essential to determine a method which is not only cost effective but also practical and yields enough exosomes to produce results. The gold standard for exosome isolation is ultracentrifugation (UC) but it is very laborious and time consuming. This study compared exosome isolation using UC and an Exoquick TC plus exosome isolation kit (kit). Exosomes were isolated from a concentrated cell culture supernatant of SCC4 cell line using established UC and kit protocols. Total RNA was extracted from exosomes and mRNA of several oncogenes was quantified using RT-qPCR.

The total RNA obtained from exosomes isolated by UC was 173.88 ng whereas that from the kit was 111.25 ng (p value < 0.01). Subsequently the RNA was reverse transcribed, preamplified and qPCR was performed. All six genes selected for RT-qPCR were detected in samples obtained from both methods. The Ct values of the mRNAs from UC were less as compared to the kit method indicating a higher concentration of individual genes with UC. Exosome characterisation revealed the sizes of the vesicles from both methods to be between 50 and 150 nm. In conclusion, the exosome isolation kit can replace the UC method as the time and effort required to isolate exosomes is much less and reliable results from qPCR are obtained. However, UC remains the gold standard since it is more cost effective and yields a higher total RNA. A concentration step makes the kit more practical by reducing the cost per sample but it is still $139.2 more per sample compared to UC.

Cooling efficiency of different coolant port designs on high-speed handpieces

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High-speed handpieces (HSH) are the most commonly-used equipment in today's dental practice, with new designs constantly being developed. Water coolant ports are important features of HSH, as water is required to prevent damage to the tooth from frictional heat produced by the handpieces. A temperature increase of 5.5oC is used as the reference threshold above which is considered damaging. This study investigated the cooling efficiency of different numbers of water coolant ports on (HSH) under cooling conditions used in the dental practice. Thermocouples were placed in the pulp chambers and temperature changes were recorded with 1-, 3- and 4-coolant port handpieces. The cooling rate was calculated for each coolant port design system and temperature changes were statistically analysed with Kruskal-Wallis Test. All three sample groups resulted in a net temperature decrease during the cutting period with water turned on. There was a pattern of increased cooling rate with increasing number of coolant ports (1- port: -4.27(0.94) °C, 3-port: -4.66(2.90) °C, 4-port: -5.03(1.08) °C). The difference was not statistically significant (p=0.681). Calculations of cooling rate showed a higher cooling rate with an increase in the number of ports (1-port: $46.13 \times 10\text{-}4$ K-1, 3-port: $51.36 \times 10\text{-}4$ K-1, 4-port: $56.32 \times 10\text{-}4$ K-1). All three water coolant port configurations showed effective cooling of the tooth during cutting and decreased pulpal temperature with no statistical difference. The results from this study can help clinicians choose which high-speed handpiece to use, as the number of coolant ports on high-speed handpiece does not significantly affect the cooling during teeth grinding.

Can a smartphone app be used for shade matching in dentistry? – an *in vitro* study

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Aims: The aim of this *in vitro* study was to evaluate the shade matching performance of two smartphone apps in comparison to a spectrophotometer used in dentistry.
Methods: Smartphone applications, Chromatcher for iOS (CRM) and ColorMatch for Android (CMX) were tested for their shade matching performance in comparison to an intraoral spectrophotometer, VITA Easyshade V (VES) by determining the shade of VITA Classical A1-D4 shade tabs. Accuracy of each shade matching tool was evaluated by percentage of exact match and colour difference from the true shade by using the CIEDE2000 formula (ΔE'). The results were categorised as imperceptible (ΔE'<0.8), acceptable (0.8<ΔE'<1.8) or unacceptable (ΔE'>1.8).
Results: The VITA Easyshade V (VES) had the highest exact match (29%). The spectrophotometer also had the best results in the shade matching exercise that were either imperceptible or acceptable (imperceptible=17%, acceptable=27%, unacceptable=56%), followed by CMX (imperceptible 2%; acceptable 10%; unacceptable 88%) and CRM (imperceptible 6%; acceptable 0%; unacceptable 94%).
Conclusions: The spectrophotometer remains as the gold standard for instrumental shade matching. While smartphone apps may be a simple and quick alternative tool, they are currently not accurate enough to replace the use of spectrophotometers.

Reliability of using digital shade matching tools in dentistry

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Aims: The aim of this clinical study was to assess the reliability of using conventional, visual tooth shade determination in comparison to currently available digital shade matching tools.

Methods: Sixty-two participants (44 females, 18 males; age range: 18 to 63 years old) with vital, intact, unrestored natural maxillary incisors were recruited for the study. One hundred and eighty-four maxillary incisors were included for shade determination by trained dental students using three methods: VITA Classical A1-D4® shade guide, a spectrophotometer (VITA Easyshade® V) and Cerec Omnicam intraoral scanner. The measurements were converted into L*C*H* (L=lightness, C=chroma, H=hue) and L*a*b* coordinates. The data were subsequently compared and analysed using the formula $\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)}$ to determine the colour difference.

Results: Digital tools were relatively quick and easy to use in shade matching compared to the conventional visual method. However, it required some steep learning curve to use the devices effectively. There was a greater correlation in the shade matching results obtained by the visual shade determination and the VITA Easyshade® V. It appeared that the shade matching results determined by the Cerec Omnicam intraoral scanner were notably restricted.

Conclusion: The use of digital instruments appears to be a good alternative to the current conventional visual method of tooth colour determination in a clinical setting. However, the accuracy of using intraoral scanners for shade matching should be further evaluated.