
Literature

reviews

The following reviews were prepared by the postgraduate students from the University of Melbourne.

Cone-beam computed tomography airway measurements: Can we trust them?

Ryan DPO, Bianchi J, Ignácio J, Wolford LM, Gonçalves JR

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Scope: Pharyngeal airway space (PAS) assessment by the use of CBCT has been used to investigate the effects of surgical, orthopaedic and orthodontic procedures on airway dimensions. Since it is known that cranio-cervical inclination has an effect on the PAS, it is imperative that a standardised protocol for measuring airway volume is adopted. Unfortunately, it is not known how reliable CBCT assessments of PAS are, even under standardised conditions. This study investigated the reliability and reproducibility of PAS measurements taken at different time points using CBCT technology.

Materials and methods: This was a retrospective study that compared CBCT scan records of 27 patients at two time points. Patients were undergoing presurgical fixed orthodontic treatment, and scans were taken pretreatment and four to six months into treatment. The mean pretreatment patient age was 31 years. Inclusion criteria identified patients receiving presurgical fixed orthodontic treatment, female patients ≥ 15 years of age, male patients ≥ 17 , and an absence of previous surgical procedures in the craniofacial region. The exclusion criteria included the presence of syndromes, cleft lip and palate, facial trauma history, previous maxillomandibular, TMJ and oropharyngeal surgeries and current use of an orthopaedic dental appliance. The airway was segmented into three distinct regions of interest, nasopharynx, oropharynx and hypopharynx. Three-dimensional imaging software (Dolphin Imaging, CA, USA) was used to measure the volumes of the three regions. The cranio-cervical position was measured by the use of lateral cephalograms.

Results and discussion: The average difference in T0 and T1 scans for cervical and cranio-cervical flexion was found to be 0.6 and 2.7 degrees respectively, indicating very small changes. Despite this, the authors suggested that variations in measurements at different time points may be due to these differences in cranio-cervical posture, as well as breathing and swallowing patterns.

The differences in absolute PAS values at the two different timepoints were 682.1 mm³, 2255.3mm³, and 517.4mm³ for the nasopharynx, oropharynx and hypopharynx respectively. These results show that the oropharynx, which is the most common site of airway obstruction, experienced the greatest variation in measured values. This variation occurred in spite of the fact that patients were all adult patients with fixed appliances which cannot change airway dimension and were followed for a short period of four to six months, when no anatomical changes in airway dimensions would be expected. This also occurred in spite of a standardised protocol, in which patients were seated in an upright position with Frankfort plane parallel to the floor, in centric relation, and were advised not to swallow. The clinical relevance of these findings is that CBCT studies on investigations aimed at increasing airway volume should demonstrate a treatment benefit above the average airway variation of the oropharynx of -5735.3 to 5103.6 mm³. This may be problematic for appliances that may produce a smaller airway change, but may be useful for interventions such as maxillomandibular advancement, which will produce a larger airway change.

A limitation of this study is that body weight and BMI were not assessed in the assessed subjects. While unlikely, it is possible that weight variations between the time points may have affected airway volume, as it has been demonstrated that there is a negative correlation between body mass index and airway volume.

Conclusion: CBCT scans taken at different time points with identical scanning and patient protocols can result in different PAS readings. This study has shown that careful interpretation of CBCT volumetric data is important, to reach valid conclusions about clinical outcomes of any given intervention. Further research is needed to validate a more controlled and standardised patient positioning protocol to reduce cranio-cervical and volumetric differences between scans.

Joshua Ch'Ng

Alterations of the oral microbiome in patients treated with the Invisalign system or with fixed appliances

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Background: It is well known that fixed orthodontic appliances are plaque-retentive, which can lead to a change in the oral microbiota. This can cause an increased risk of caries and periodontal disease. However, Invisalign has been touted as a more hygienic appliance with better oral health outcomes due to its removable nature that enables better oral hygiene practices. While many reports have focused on clinical parameters between traditional orthodontic appliances and the Invisalign system, only a few have compared the microbiological aspect. In those studies, only selected pathogenic species were investigated using real-time PCR. As a result, a comparison of the true difference in the microbiome between the two treatment modalities remains unclear.

Aim: To determine and compare the changes in oral flora between Invisalign and fixed appliance treatment, using a high-throughput sequencing technique, to better understand possible oral health outcomes in patients undergoing orthodontic treatment with these appliances.

Materials and methods: Twenty-six subjects between the ages of 20 and 25 years were enrolled and comprised of 12 fixed appliance, seven Invisalign patients, and seven untreated control patients. All subjects were instructed on oral hygiene procedures. Saliva samples were taken six months into treatment and stored in 2 ml of pre-cooled phosphate buffered saline at -80°C. Five samples in each group were randomly selected for DNA sequencing. DNA was extracted from the saliva and the amplified 16S rRNA hypervariable V3-

V4 region was sequenced using the high-throughput sequencing Illumina MiSeq platform. Differences in the relative abundance of phyla and genera among groups were determined using the Kruskal-Wallis test.

Results: There were significant differences between the Invisalign, fixed appliance, and control groups in the composition and diversity of oral microbial population. The top 10 predominant phyla accounted for 98.7% of total sequences. The three phyla that differed significantly in abundance between the groups were Firmicutes, Bacteroidetes, and candidate division TM7. At the genus level, the top 10 taxa comprised 81.5% of the total sequences. The relative abundances were compared to determine the variation of these genera. Four genera (*Neisseria*, *Prevotella*, *Rothia*, and *Fusobacterium*) had significant differences between the groups.

Discussion: The findings show that Invisalign and fixed orthodontic appliances caused microbial dysbiosis compared to the normal oral flora in the control group, with the diversity of the oral microbiota decreasing as a result of orthodontic treatment, regardless of type. The Invisalign group was not significantly different from the fixed appliance group. However, the abundance of some types of bacteria differed between the two orthodontic treatment groups, as well as between the treatment and control groups at both the phylum and genus level. As the Invisalign group did not have statistically significant differences in microbial composition and diversity compared to the fixed appliance group, reports of improved oral health outcomes with the Invisalign appliance could not be supported from the microbial composition aspect. Any improved oral health parameters might be a result of other factors, such as ease of oral hygiene measures. A limitation in this study was the small sample size of each group, which may have limited the statistical power of the findings. As there was also only one sample collection at one timepoint for each subject, it is not known whether any differences between each subject existed before treatment. Furthermore, the oral microbiome as analysed from salivary samples was less representative of the local microbiome at the tooth level compared with dental plaque.

Wayne Ly

The role of orthodontics in the repair of gingival recessions

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Introduction: The authors discuss the risk of developing bony dehiscence by root displacement beyond the alveolus and acknowledge reported improvements in marginal bone heights and gingival recession following movement of roots into the confines of the alveolus. Also discussed were the correlations between the initial amount of gingival recession and tooth position on the prognosis of mucogingival surgery aimed at establishing complete root coverage at sites of gingival recession. The authors suggest that the association between orthodontic correction of root position and improvement of gingival recession has not been evaluated in a systematic manner. With this in mind, the aim of this study was to quantify the changes in gingival recession following orthodontic movement of displaced roots towards the centre of the alveolus.

Methodology: Twelve consecutive adult patients with one mandibular incisor presenting with either buccal or lingual gingival recession and with the root clearly positioned outside the alveolar bone were enrolled for orthodontic root correction prior to mucogingival surgery. The sample consisted of nine females and three males with a mean age of 28 years and an age range of 22–41 years. None of the patients enrolled exhibited periodontal inflammation, and no patients displayed clinical or radiological signs indicative of a history of periodontitis.

Orthodontic corrections were completed with segmental appliances consisting of a 0.019 × 0.025 inch titanium-molybdenum alloy torque arch hooked onto a 0.020 inch stainless steel base arch. The torque arch was inserted into a bracket on the displaced mandibular incisor, with the base wire above the bracket to prevent undesired tipping and vertical movements. Some patients had finishing corrections made with a continuous wire. A calibrated periodontal probe and standardised clinical photographs were used at baseline and after orthodontic treatment to measure and record recession depth and width, the area of recession and keratinised tissue height at the mid-buccal or mid-lingual aspect. Periodontal probing depths at the site of recession were also recorded, and each site was classified according to

Miller's classification. Measurements on photos were repeated after a minimum of 15 days and statistical analysis of error was also completed.

Results: An analysis of error showed excellent reliability of the measurements obtained. The width, depth and area of recession were found to have reduced in all patients. All patients showed a change in classification of recession from a Miller's Class III or IV to a Miller's Class I or II. On average, recession defects reduced by 23% in depth, 38% in width, and 63% in area, with significant variation between individuals. Some patients showed up to 0.75 mm increases in height of keratinised tissue, and no changes were noted to periodontal probing depths in all patients.

Conclusion: The authors conclude that the orthodontic correction of roots toward the centre of the alveolar envelope consistently reduced gingival recession and may improve the prognosis for successful full root coverage if subsequent mucogingival surgery is undertaken.

Critical analysis: The aim of quantifying changes in gingival recession following orthodontic root movement towards the centre of the alveolus was addressed by means of a case series study. This study was descriptive in nature, with a limited sample size consisting of more females than males. Little information was provided regarding the source of the patients (i.e. private practice, public hospital etc.) or the overall occlusal features of patients. There was also limited information provided related to the amount of tooth and root movement for each case. Therefore, the representativeness of the sample is unclear.

The process for obtaining measurements was thorough, and a strength of this study was the standardised method used for obtaining and analysing photographs. Measurements were repeated a second time after an interval of at least 15 days, and subsequent statistical analysis of error showed excellent reliability of the measurements.

The study is limited by a small sample size, the lack of a control group, a restriction to mandibular incisors, and the inherent selection bias involved with a case series. A control group was not included in this study for ethical reasons, because of the risk of progression of recession if left untreated. Nonetheless, this study provides unique information regarding a common clinical condition and paves the way for further systematic examination of the relationship between

orthodontic root movement towards the centre of the alveolus and changes in gingival recession.

Raoul Mascarenhas.

Clinical effect of a fluoride-releasing and rechargeable primer in reducing white spot lesions during orthodontic treatment

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Scope and aim: White spot lesions (WSLs) are a significant complication associated with orthodontic treatment. The prevalence of WSLs ranges from 2 to 96%. Fluoride-containing adhesives and primers are available to prevent WSL development, but do not have a significant preventive effect when used once only. A recently released primer, Opal Seal, has a fluoride recharging ability and can be applied repeatedly to increase its effectiveness; however, only short-term evidence of its effectiveness exists. Therefore, this clinical study aimed to (1) compare the WSL prevention effect of Opal Seal with a conventional primer during full fixed orthodontic treatment, and (2) compare the bond failure rate of brackets bonded with Opal Seal and a conventional primer.

Materials and methods: This study was a prospective, non-randomised clinical trial involving 60 patients divided equally into two groups: Group 1 received the Opal Seal primer (Ultradent Products, UT, USA), and Group 2 received the Transbond XT primer (3M Unitek, CA, USA). Inclusion criteria included: (1) no visible WSL or restorations on the buccal surfaces of the teeth; (2) no morphologic crown anomalies; (3) permanent dentition; and (4) no physical disability preventing effective tooth brushing. For Group 1, Opal Seal was applied to acid-etched enamel and cured for five seconds before bracket bonding. The patients were recalled every four weeks for re-application onto the tooth surface. New primer was only added when UV light fluorescence showed a lack of primer on any part of the enamel surface. For Group 2, the primer was applied to the acid-etched enamel before bracket bonding. The same adhesive-coated brackets were used for both groups.

Digital images of each tooth were taken to score and measure the area of WSLs before and after orthodontic treatment, where the presence and severity of WSLs

were recorded using a modified scoring system. After deband, the buccal surfaces of the teeth in both groups were dried and measured with a DIAGNOdent device, which provided a score that indicated normal enamel, incipient caries or dental caries. The area of WSLs was measured using the ImageJ software. Patients were recalled every four weeks to record bracket bonding failure and only first-time bracket failures were recorded.

Results: There was no significant difference between the two groups for WSL development (Opal Seal – 26.9%; Transbond – 29%) and WSL severity. However, use of the DIAGNOdent showed statistically significant fewer WSLs in the Opal Seal group compared to the Transbond group. ImageJ analysis showed no significant difference for WSL area between the two groups. There was no significant difference between the primers, dental arches, bracket types (anterior, canine and premolar), or gender in the clinical survival of brackets.

Conclusion: The study concluded that there is no benefit in using a fluoride-releasing primer over a conventional primer to reduce WSL development during the course of full fixed orthodontic treatment.

Critical appraisal: Overall, this was a well-designed study to determine if there were any advantages to using a fluoride-releasing primer to help with reducing the incidence of WSLs. Such studies are difficult to conduct given that WSL development is multifactorial. A strength of this study was that patients were followed from the beginning to the end of treatment.

However, there are several limitations worth considering. As acknowledged by the authors, a split-mouth design was not chosen due to potential cross-over from fluoride-releasing materials. They attempted to control this by dividing the subjects equally into two groups. Despite acknowledging the importance of patient education and motivation, there was no measure of oral hygiene, nor an indication of how this could have confounded the results. It also appears that the subjects were not randomised into either of the two groups, which may introduce bias. Furthermore, there was no testing for intra-examiner reliability for each WSL analysis method. Another limitation was the lack of blinding of the investigator to the study groups, and it is not clear whether the same investigator also conducted the analyses or whether the second author was involved. It may have

been worthwhile to use a cross-polarisation filter to eliminate glare as this may have affected visualisation of WSLs in the digital images.

Alan Tran

Occlusal contact area changes with different retention protocols: 1-year follow-up

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Background: The changes that occur following treatment and the challenges of retention remain an active area of interest in orthodontics. Occlusal contacts change over time and may be affected differentially by various retentive regimes. Despite being relatively commonplace, information regarding occlusal settling during and following Essix and bonded retainer use remains lacking.

Aim: The purpose of this study was to evaluate changes in occlusal contact area and a cast-radiograph evaluation (CRE) score in patients with three different retention protocols after one year.

Materials and methods: A cross sectional study of 90 patients treated in a postgraduate university setting was performed. Inclusion criteria were non-extraction, Class I molar and canine relationship at the end of treatment, compliance with retainer wear, good oral hygiene, normal growth pattern, no missing teeth (except third molars) and no prosthetically replaced teeth. Exclusion criteria removed dental or skeletal anterior open bite or deep bite patients, incomplete records, scores >27 CRE at deband, heavily filled posterior teeth and patients with symptoms of TMD.

The patients were evenly split into three treatment groups according to different retention protocols: upper bonded retainers (BR) and Essix, upper BR and Hawley/LBR and upper and lower BR. Digital laser scans of dental casts were used to examine contact area changes using 3shape Orthoanalyzer®. The contacts were divided into two regions and screenshots were transferred to ImageJ software for 2D area measurements.

Results: The Hawley and bonded retainer groups showed a significant increase in occlusal contacts in anterior and posterior regions. There was no significant difference between these two groups. The Essix group showed a statistically significant

decrease in occlusal contacts except at the incisors. The clinical variables that performed worst included rotation and buccolingual inclination in all groups. Most improvements were seen at the marginal ridges, overjet and occlusal contacts.

Discussion: The study concluded that Hawley and BRs demonstrated the greatest amount of occlusal settling following one year of retention. The Essix group showed a decrease in occlusal contacts over the same period.

The limitations of this retrospective study include a selection bias for patients for whom adequate records were available at T1, T2 and at follow up. There was no information regarding attrition rate and the authors provided little information regarding the treatment characteristics of the cohort, which may introduce some biases. It was mentioned that the Hawley groups were younger but no other information was provided on age differences between the groups. The treatments being offered were restrictive, completely excluded orthodontic extractions, and perhaps did not accurately represent clinical reality. Also excluded were unfavourable growth patterns including vertical changes such as anterior open bite and deep bite malocclusions, which may greatly impact on the findings. There was no evidence of consistency regarding the fabrication and design of the various retainers.

In addition, there was no evidence regarding the number of clinicians involved in the treatments and the criteria used for retainer selection. There was no evidence of concealment of treatments when examining occlusal contact data from each patient.

Many of the statistically measured CRE variables showed large standard deviations, greater than the magnitude of the mean values, and therefore the clinical relevance of this data may be questionable. The authors have also performed a large array of tests on various parameters, increasing the likelihood of significant findings due to random probability.

Ultimately the study provided valuable information about occlusal settling with removable and bonded retainers over one year. Further research is needed utilising randomised prospective methodologies and a longer follow-up time.

Ashley Freeman

Airway and cephalometric changes in adult orthodontic patients after premolar extractions

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Scope and aim: The relevance of extractions in orthodontic treatment and their effect on airway capacity continues to be a contemporary topic of debate. Recent trends in orthodontic treatment seem to favour less frequent extraction patterns and are more focussed toward arch expansion. These trends follow the belief that the extraction of premolars may lead to negative changes in airway capacity. Previous studies that reported negative extraction effects on airways did not assess dimensional changes using CBCT technology, hence were unable to report on changes to transverse dimension, area or volume. Instead changes in size of the airway were reported using lateral cephalograms. Some studies also failed to include a control group. The present study compared CBCT data at the beginning and end of treatment to investigate the relationship between premolar extractions and changes in airway dimensions.

Materials and methods: The study was a retrospective, single centre cohort study involving 83 non-growing, orthodontically-treated patients. The sample was matched for gender and age, and half of the cases had premolar extractions and the other had been treated non-extraction. The sample consisted of patients who had undergone orthodontic treatment in a university clinic between January 2007 and June 2018. Subjects were included if they were over 18 years of age with routinely-taken pretreatment (T1) and post-treatment (T2) CBCTs available. To be selected in the extraction group, patients had to have had at least two premolars extracted, and of the 41 subjects in the extraction group, six subjects had only upper premolar extractions, while 35 had four premolars extracted. The subjects in the non-extraction group (control) were chosen by being matched for age and gender. During the CBCT scans, subjects were instructed to bite into maximal intercuspation and remain stationary without swallowing. For the purpose of analysis, the airway measurements were performed by two assessors. Five features were analysed by dividing the airway into three regions: nasopharynx, retropalatal and retroglottal; in addition, 10 skeletal and dental features were assessed at the pretreatment (T1) and post-treatment (T2) phases. Regional airway

measurements assessed included total airway volume, calculated in cubic centimetres (cm³) and the most constricted minimum cross-sectional area (MCA) of each region, calculated in square millimetres (mm²).

Results and discussion: The results of the study showed no significant inter-group differences between the extraction and non-extraction groups related to changes over time in airway volume (cm³) and MCA (mm²) when comparing the pretreatment (T1) and post-treatment (T2) CBCT data. Although the subjects in both groups were matched for age and gender, the extraction group measured, on average, slightly larger airways at both baseline and end of treatment, with no differential effects related to extraction. A finding of significance showed a negative association, demonstrating that calculated MCA of small airways grew in size and, conversely, large airways decreased in size, when airway features were compared to baseline conditions in both groups.

Conclusion: Although the place of extractions in orthodontic treatment continues to be a debateable topic, in some cases, the removal of teeth is necessary to achieve the desired orthodontic treatment outcome. It is imperative, in order to provide patients the best treatment outcomes, that cases requiring extractions be evaluated on an individual basis, ideally with CBCT and the aid of additional diagnostic tools such as clinical evaluation and/or a sleep questionnaire in cases where airway concerns arise. Future studies comparing the effect of extractions on airway changes may warrant the use of polysomnography in addition to CBCT to further evaluate tooth extractions on airway changes.

Critical appraisal: Overall the study reported results similar to recent literature and aimed to overcome limitations of past studies by comparing airways using CBCT data. However, due to the inherent variability in measuring airway dimension at a single static time point, additional diagnostic information such as sleep questionnaires and/or a sleep study may be helpful in making clinical decisions regarding extractions in orthodontic treatment and/or the basis for future studies. In addition, the patients included within the sample were restricted to adult, non-growing patients and patient body mass index (BMI), which has a known effect on airway dimensions, was not recorded. Hence the conclusions of the study are only relevant when taking into account the included variables.

Paul Lam

A cone-beam computed tomographic evaluation of alveolar bone dimensional changes and the periodontal limits of mandibular incisor advancement in skeletal Class II patients

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Background: Skeletal Class II mandibular retrognathic patients receiving camouflage orthodontic treatment may experience lower incisor proclination as a result of Class II elastic wear.

Aim: This study utilised CBCT imaging to quantify and evaluate the changes in alveolar bone dimension and the presence of dehiscences associated with mandibular central incisors in skeletal Class II patients before and after treatment.

Materials and methods: This retrospective study sampled 48 patients (19 male and 29 female) treated with full fixed appliances with a standardised protocol for Class II correction using short Class II elastics (1/4 inch, 4 ounce). Pre- and post-treatment CBCT images (0.3 mm voxel size) of mandibular central incisors were processed and evaluated in axial and cross-sectional slices to evaluate changes in labial and lingual alveolar bone thickness, alveolar bone height and labial dehiscences. These values were correlated with various cephalometric indicators including lower incisor position (L1-NB and IMPA).

Results and discussion: This study found an increased prevalence of dehiscence following orthodontic intervention (50% vs. 27.1%). Regression analysis demonstrated that changes in L1-NB or IMPA were correlated with decreases in alveolar bone width and increased the probability of developing dehiscences. There was an estimated 50% probability of vertical bone loss at a L1-NB change of 2.00 mm or, equivalently, an IMPA change of 8.02 degrees.

Changes of L1-NB and IMPA below a threshold (L1-NB = 0.71 mm, IMPA = 3.02 degrees) had relatively minimal or no effect on alveolar bone height loss but changes beyond the threshold correlated with extensive bone loss.

Conclusion: The findings of this study provide some insight into the undesirable periodontal outcomes of mandibular lower incisor movement during orthodontic intervention in Class II subjects. Clinicians are reminded to exercise caution when

considering significant changes in lower incisor position or angulation during treatment. It is important to note that this was a retrospective study of a continuous convenience sample with no control sample. Furthermore, a sample size calculation was not described and concerns related to the power of the logistic regression performed must be acknowledged with large confidence intervals expressed for many of the outcome parameters.

Peter Tran

Vacuum-formed retainer versus bonded retainer for dental stabilization in the mandible—a randomized controlled trial. Part I: retentive capacity 6 and 18 months after orthodontic treatment

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Background: Maintaining dental alignment post-orthodontic treatment remains an ongoing challenge for any orthodontist. Numerous retention protocols have been proposed utilising fixed and/or removable options. A Cochrane review published in 2016 stated that high quality evidence regarding retention regimes was still lacking and further randomised controlled trials (RCT) were needed.

Aim: This RCT aimed to assess mandibular arch stability 6 and 18 months into retention following the use of two different retainer types.

Materials and methods: This single-centre, two-arm parallel-group RCT compared a removable vacuum-formed retainer (VFR) with a fixed canine-to-canine (CTC) retainer after 6 and 18 months of retention. One hundred and four participants were recruited and randomised into either VFR group (52 patients) or CTC group (52 patients). Patients treated with and without extractions were included. All retainers were placed and followed up by a single orthodontist. All patients received a VFR that covered all maxillary teeth. In the intervention group, participants received a lower VFR (1.0mm Essix C+ Plastic, Dentsply, FL, USA) that covered only first premolar to first premolar in the lower arch. They were instructed to wear it full-time during the first week and night-time thereafter. The active comparator group received a lower fixed

CTC retainer made from 0.8 hard Remanium® wire (Dentaurum, Ispingen, Germany) and bonded with Tetric Flow (Ivoclar, Vivadent, Schaan, Liechtenstein). Alginate impressions were taken at deband (T1), 6 months (T2) and 18 months (T3). The models were scanned and digitised. DDP-Ortho software was then used to assess post-treatment changes. The primary outcome was to assess the retainers' retaining ability by measuring post-treatment stability of overbite, overjet, arch length, inter-canine width, inter-molar width and Little's Irregularity Index (LII). Secondary outcomes were retainer survival and the impact of tooth extractions on arch stability. Correlations between gender, tooth extractions and LII were also assessed.

Results and discussion: With regards to the primary outcome of stability, no significant difference between the VFR and CTC retainers was found at either 6 or 18 months. Any relapse that did occur, arose within the first 6 months of retention but it was small overall and not clinically significant. Retainer failures were low with only three in each group. This may also account for the low post-treatment changes. Extractions during orthodontic treatment did not appear to have any significant impact on post-treatment changes. This study did stratify their randomisation for homogenous gender distribution between the groups. No gender and LII correlations were found except that males had higher LII at debond than females. However, this difference did not persist after 6 and 18 months

Conclusions: This study concluded that VFR and CTC mandibular retainers were both effective after 18 months. Statistically significant relapse, which occurred in both groups, developed within the first 6 months but was not of clinical significance. It was also concluded that part-time wear was not associated with increased rates of relapse and that shorter mandibular VFRs do not cause negative vertical effects. Future papers utilising this patient cohort will evaluate patients' perceptions during the 6–18 month retention period (Pt II) and long-term stability of maxilla and mandible after 5 years of retention (Pt III).

Bethany Cunning

Transcriptome analysis of ankylosed primary molars with infra-occlusion

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Scope and aim: Dental ankylosis, defined as the fusion of cementum or dentine with alveolar bone, is a problem that orthodontists face when treating growing patients. Primary molar ankylosis may lead to clinical consequences such as infra-occlusion, bony defects, tipping of adjacent teeth and loss of arch space, midline deviation, dental asymmetry and supra-eruption of opposing teeth. Permanent successors may also become impacted, suffer from deflected eruption paths and ectopic eruption. One issue facing the management of primary molar ankylosis is that despite a multitude of clinical and epidemiological data, genetic and molecular aetiology remains largely unknown. Evidence has demonstrated a familial inheritance of the condition, with higher prevalence in certain ethnic groups, and a relation to certain dental anomaly patterns leading to a suspected genetic basis. Potential molecular pathways of ankylosis have been examined in experimental animal settings, in rare cases of a specific genetic mutation in humans, and examination of expression of pre-selected hard-tissue regulation-related genes using ankylosed human primary molars.

While some insights may be gained from these studies, their applicability to typical human ankylosis may be limited due to its seemingly idiopathic and spontaneous nature, and that it is not typically trauma induced. These studies are also limited by the scale of known or postulated candidates for dental ankylosis, and hence a human transcriptome-wide analysis was timely to elucidate the genes involved. In this present exploratory study by Tong and colleagues, RNA sequencing (RNA-seq) was utilised to characterise and compare transcriptome profiles of primary molars with and without infra-occlusion, analyse the possible molecular pathways, and examine a small sample of differentially expressed (DE) genes for their distribution within a developing tooth germ.

Materials and methods: In this case-control study, primary mandibular second molar samples were donated by consenting parents of children who attended orthodontic clinics in Melbourne, Australia. These met the inclusion criteria which were that (1) subjects were under 15 years old, healthy and had not

yet started orthodontic treatment, and (2) all primary molars had missing permanent successors to avoid potential confounders from erupting tooth follicles. The infra-occluded group consisted of five subjects of mean age 13.5 years, providing seven infra-occluded mandibular second molars, and a non-infra-occluded group (controls), made up of eight subjects of mean age 11.5 years, providing nine non-infra-occluded mandibular second molars.

Infra-occlusion was defined as the occlusal surface of the primary second molar clinically and radiographically judged to be positioned at least 1 mm below that of the adjacent non-infra-occluded permanent molar. Bone was harvested at the time of extraction from the bifurcation of the primary second molars, stored and homogenised using a Bullet Blender. Total RNA was isolated, followed by removal of rRNA, conversion to cDNA and preparation of a gene library. Bioinformatic analysis was carried out in reference to the human genome and gene expression determined to identify differentially expressed genes. Hierarchical clustering and heatmap analysis were carried out using all differentially expressed genes with false discovery rate (FDR) < 0.05. These differentially expressed genes were studied further via Gene Set Enrichment Analysis (GSEA) with Ingenuity Pathway Analysis (IPA) performed, and significantly enriched gene ontology (GO) pathways (FDR<0.05) produced which were further analysed for size, overlap and positive/negative enrichment. Finally, fixed and sectioned mouse tooth germs (postnatal day 1) were utilised as a model to independently validate the expression of six differentially expressed genes via immunohistochemical analysis

Results and discussion: Four hundred and thirty-two genes (2.3%) were found to be differentially expressed in the furcal bone of the infra-occluded versus the non-infra-occluded groups of primary second molars, with most exhibiting a 1.5–2× fold change or more. This relatively low percentage of differentially expressed genes in ankylosed primary molars (compared to other pathological conditions such as cancers) reflects a strong degree of biological and molecular homogeneity between infra-occluded and non-infra-occluded primary molars. This further indicates that the DE genes are representative of those associated with the infra-occluded phenotype.

The RNA-seq method has many benefits, including that knowledge of pre-existing genes is not required, greater sensitivity, and decreased confounding

from contamination. A limitation of RNA-seq is the requirement for sample groups to be as similar as possible, necessitating sample groups in which permanent successors were absent (due to the potential for follicle genes to contaminate the samples). This limited the number of samples that could be collected under the time constraints.

Another limitation acknowledged in this study is that non-infra-occluded primary molars cannot be invariably excluded from being ankylosed; a small portion have been found to histologically display ankylosis. Due to the scarcity of available tissue in this study, no parallel histological exclusion of ankylosis was performed; however, this would still be unable to detect any changes in transcriptional activity in a non-infra-occluded tooth that may be in the early stages of what could become future clinical ankylosis.

Clear differences between infra-occluded and non-infra-occluded groups were found on hierarchical clustering and heatmap analysis, with greater variation between individual infra-occluded samples found, which was supported by principal component analysis (PCA). Like other pathological conditions, this leads to the idea that ankylosis is likely due to a variable dysregulation of normally tightly regulated biological functions, and hence infra-occluded samples that would have otherwise appeared as outliers were included in this study.

Pathway analyses of differentially expressed genes revealed two principle clusters relating to (1) Epithelial cell differentiation, keratin expression and cell junction activity and (2) Inflammatory response. In particular, certain types of keratins involved in epithelial cell change were some of the most highly expressed, keratins that are also consistent with those expressed in human epithelial cell rests of Malassez (ERM) undergoing proliferation and differentiation in response to inflammation. Several other genes were likewise consistent with human ERM. Additional findings included decreased expression of bone-degrading genes, increased expression of pro-mineralisation genes, and decreased expression of anti-mineralisation genes, signifying the dysregulation of mineralisation in ankylosis.

Immunohistochemical detection of proteins encoded by differentially expressed genes found that all those selected were present in developing tooth structures. The dental follicle was the most common site of protein expression, unsurprising as it is comprised of pluripotent ectomesenchymal cells that are the

precursors for the periodontal ligament, bone and cementum all involved in ankylosis. In particular, two of these proteins were found in the inner enamel epithelium, which eventually leads to formation of the ERM, a structure significant in its implication in the pathogenesis of ankylosis by many studies. A limitation of this technique is that the choice of genes was determined by commercial availability of antibodies to their proteins, as opposed to the magnitude of expression or pathway relationships.

With findings taken together, the proposed mechanism developed by the author was that ankylosis involves a heightened inflammatory response in conjunction with disruptions to the remnants of the dental follicle and epithelial rests of Malassez.

The nature of a case-control study is that only an association, not a causal relationship, may be inferred between ankylosis and the genes found to be differentially expressed; the DE genes may be the result of ankylosis rather than the cause due to the pre-existing nature of the condition at time of tissue sampling.

Conclusion: This study is a pioneer on the topic of molecular pathways involved in molar ankylosis and is the first known published study to examine the transcriptome characteristics of ankylosed primary molars. It utilises several elegant techniques to not only identify differentially expressed genes in primary molars but to also elucidate the biological pathways in which they are involved.

This study, as supported by past familial studies, found that ankylosis is not a monogenetic disease with a dominant gene, but that it instead involves a complex coordinated expression of multiple genes over multiple pathways. Pathway analysis revealed that the expression of these are consistent with the cellular inflammatory response and epithelial cell turnover, and six of these genes were further found to be expressed in the inner enamel epithelium; progenitor populations of the periodontal ligament, cementum, bone and epithelial rests of Malassez. The author has hypothesised that an increased inflammatory response and ERM dysregulation plays a role in primary molar ankylosis pathogenesis. This study has uncovered differences in gene expression of ankylosed primary molars, which may act as a basis for further research into the molecular basis of pathological ankylosis.

Sarah Roath

A comparative assessment of clinical efficiency between premium heat-activated copper nickel-titanium and superelastic nickel-titanium archwires during initial orthodontic alignment in adolescents: a randomized clinical trial

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Background and aims: Superelastic nickel-titanium (NiTi) and copper nickel-titanium (CuNiTi) archwires are commonly used as initial alignment wires during orthodontic treatment. Tanzo premium CuNiTi archwires (American Orthodontics, WI, USA) are newly developed heat-activated CuNiTi archwires. According to their manufacturers, it is claimed that they provide lower loading and more consistent unloading forces compared to regular superelastic NiTi, resulting in more efficient tooth movement. Thus, the authors of this study aimed to determine if there was a difference between two different NiTi archwires (premium heat-activated Tanzo CuNiTi and NT3 Superelastic NiTi) related to maxillary anterior alignment efficiency, maxillary arch width change at the canine and molar regions, and maxillary incisor inclination change.

Methodology: The authors conducted a single-centre, two-arm parallel group, blinded prospective randomised clinical trial (RCT). Fifty adolescent subjects were recruited from the orthodontic department of Hacettepe University and randomly allocated in one of two groups; Group 1: premium heat-activated Tanzo CuNiTi (mean age 14.76 ± 1.77 years) or Group 2: NT3 Superelastic NiTi (mean age 14.75 ± 1.52 years). Randomisation was aided by computer generation and allocated by a non-study participant via sealed envelopes. All study participants had either a Class I or Class II malocclusion in the permanent dentition, a Little's irregularity index of ≥ 7 mm in the maxillary arch, the absence of periodontal disease, and were undergoing non-extraction orthodontic treatment. Self-ligating 0.022-inch slot Empower 2 brackets were used for all study participants and all clinical work was performed by the three study authors. The archwire sequence for each group was a 0.014-inch archwire for the first eight weeks, followed by a 0.018 inch archwire for another eight weeks. Subjects were reviewed every four weeks and maxillary impressions were taken

at the beginning of treatment and every four weeks thereafter. An ANOVA with a statistical significance of $p < 0.05$ was performed to compare the means in both groups.

Results: No significant difference was observed for alignment efficiency between the CuNiTi or NiTi groups as crowding alleviation was 3.58 mm and 4.07 mm respectively over the 16-week study period. Similarly, there was no significant difference in the amount of maxillary arch expansion or incisor inclination change between the groups at the end of the study. The study did find a significant effect of archwire diameter on the amount of initial alignment, with 0.014 inch archwires in both groups reducing the irregularity index significantly more compared to 0.018 inch archwires. No statistical differences in age, gender, mandibular plane angle, initial crowding, and inter-canine/inter-molar widths were found between the groups.

Conclusion: There was no difference in maxillary anterior alignment efficiency between the superelastic NiTi and premium heat-activated CuNiTi archwires. Both archwires produced similar changes in intercanine and intermolar width, and incisor inclinations.

Critique: The findings of this study are applicable only to the maxillary arch with moderate crowding in patients undergoing non-extraction Class I or Class II treatment. Randomisation and study participant allocation was blinded but the operators were not. The potential for operator bias was, however, reduced by having a single operator conducting all the cast and cephalometric measurements on de-identified participant data. Intra-examiner reliability was calculated and within acceptable limits. The trial was not registered, and no information was provided to explain this omission. The force-type of Tanzo CuNiTi archwire used in the study was not disclosed; this archwire comes in a low and mid force variant. There was no mention of activation temperature for the Tanzo thermal CuNiTi archwires as the manufacturers do not disclose this. The limitations of the study such as the short follow-up time of 16-weeks and the lack of generalisability were addressed adequately. There was no mention as to why the authors did not conduct a convenient concurrent parallel trial looking at the mandibular arch of the same study participants. Future studies comparing initial alignment archwire should also include extraction and Class III participants, analysis of the mandibular arch, different

types of thermal CuNiTi archwires, as well as patient satisfaction and pain score outcome measures.

David Zhang

Prevalence and severity of apical root resorption during orthodontic treatment with clear aligners and fixed appliances: a cone beam computed tomography study

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Scope and aim: Mainstream orthodontic treatment has traditionally used fixed appliances for the movement of teeth. In recent years, clear aligners have become increasingly popular due to their advantage of being aesthetic and comfortable. Permanent hard tissue loss at the root apex of teeth is an undesirable side effect during orthodontic treatment. The prevalence of apical root resorption (ARR) in patients treated using clear aligners is still controversial. Previous literature has implied that clear aligners are used in simpler cases compared to fixed appliances and this is the reason for the reduced root resorption. This study intended to remove the biases of case complexity and assess both treatment modalities with the subjects of equal difficulty being treated. The stated aim of this study was to investigate and compare the prevalence and severity of ARR in patients treated with clear aligners and fixed appliances using cone beam computed tomography (CBCT).

Materials and methods: The investigation was a retrospective cohort study. A total of 373 tooth roots from 70 subjects (mean age 23.61 ± 7.03 years, 21 males and 49 females) were included from the Department of Orthodontics at Sichuan University, China. Sample sizes were calculated, ethics approval and subject informed consent were obtained prior to starting the study. Subjects were selected by the study's clearly outlined inclusion and exclusion criteria, similar baseline characteristics and American Board of Orthodontics (ABO) discrepancy index scores (i.e., treatment difficulty). The two groups included the clear aligner group (Invisalign, Align Technology, CA, USA) and a fixed appliance group (Victory Series; 3 M Unitek, CA, USA). The root length of all upper and lower anterior teeth (incisors and canines) were measured on the CBCT images by two blinded investigators using Dolphin 3D 11.9 program

(Dolphin Imaging & Management Solutions, CA, USA). The ARR on each tooth was calculated as the difference of root length (millimetres) before and after orthodontic treatment. Chi-square test and paired *t*-test was used to compare the ARR between the two groups as well as before and after orthodontic treatment.

Results and discussion: The prevalence of ARR in the clear aligner group (56.30%) was significantly lower than that in the fixed appliance group (82.11%) ($p < 0.001$). The severity of ARR in the clear aligner group (0.13 ± 0.47 mm) was significantly less than that in the fixed appliance group (1.12 ± 1.34 mm) ($p < 0.001$). The severity of ARR was also significantly less for each individual tooth in the clear aligner group than the fixed appliance group ($p < 0.05$). The most severe ARR was found associated with the maxillary canine (1.53 ± 1.92 mm) and lateral incisor (1.31 ± 1.33 mm) in the fixed appliance group; the least ARR was found on the mandibular canine (-0.06 ± 0.47 mm) and lateral incisor (0.04 ± 0.48 mm) in the clear aligner group ($p < 0.001$). The authors also evaluated the clinical significance of the severity of root resorption; however, the methodology was erroneously described in the results section of the article.

Conclusion: This study demonstrated that the prevalence and severity of ARR measured using CBCT in patients with clear aligners was less than in those with fixed appliances. Root resorption related to tooth movement is common during orthodontic treatment. However, the use of clear aligner therapy may be associated with a decreased risk of ARR, but this remains inconclusive according to the literature. Further studies are required to better understand the aetiology, prevention, and sequelae of root resorption, and the long-term management of the dentition that has had resorptive changes.

Critical appraisal: Overall, this was a well-designed and executed study. It aimed to address the discrepancy in ARR prevalence and treatment difficulty associated with aligner therapy and fixed appliances. The study is inherently at risk of bias due to its retrospective design. However, the methodology was thorough and described in detail in an attempt to reduce the risk of bias and the influence of confounding factors. It is well documented that there are many confounding factors associated with ARR including genetics, ethnicity, systemic diseases, gender, age, treatment types, treatment times as well as type and magnitude

of orthodontic forces. Therefore, this study is reflective of a portion of the contributing factors to the current limited understanding of root resorption.

There are minor concerns surrounding the methodology. Inclusion and exclusion criteria exist; however, several dental anomalies associated with increased root resorption were not mentioned in the exclusion criteria. Subjects receiving extractions were also not clearly identified. Extractions and the varying biomechanics are associated with an increased risk of root resorption. The study's baseline characteristics assessment and the ABO discrepancy index (DI) were carried out to ensure that patients' treatment difficulty using either technique was similar. The DI for the two groups was similar; however, there was a statistically significant increase and large standard deviation in the severity of the mandibular plane angle (SN-MP angle) observed within the fixed appliance subjects. The increased severity and large variation in vertical pattern could influence the length of treatment and ARR results for the fixed group.

The assessment of the root resorption of anterior teeth was used for 'practical convenience and good accuracy of ARR measurement'. Maxillary incisors have been found to be most susceptible to ARR; however, all teeth are susceptible to resorption and, in particular, the lower molars. It is a pity that the study did not investigate the root resorption on all teeth. There was no discussion of the large standard deviations within their results. In saying that, the study identified and addressed the findings of negative values of ARR (an increase in root length after treatment). Given the mean age of subjects being 23.61 ± 7.03 years, the negative values would potentially be attributed to measurement error rather than biological variability in growing individuals.

The majority of previous studies on orthodontic ARR utilised two-dimensional radiography such as panoramic and periapical radiographs. CBCT has shown to be advantageous in the accuracy and efficacy for diagnosis and measurement of root resorption, albeit at an increased radiation risk compared to traditional radiography. This study used the same CBCT machine for all subjects and stated 'good quality' of their CBCT. Unfortunately, there was no further elaboration on their assessment of the CBCT quality or voxel size, or discussion of inherent issues associated with the interpretation of the results.

Adam Wallace