

University Teachers Group Abstracts

Characterization of cell cultures from the human masseter muscle:

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Aims: Isolated human masseter satellite cells recapitulate developmental processes (proliferation and fusion of single cells, and growth of multinucleated myotubes) that lead to the formation of muscle fibres. However, satellite cell populations derived from the human masseter are limited in number due to the small amounts of tissue available at biopsy. Consequently, the quantity of these cells (a heterogenous population of satellite cells and fibroblasts) needs to be increased by a number of rounds of cell culture or passage. How this amplification affects the characteristics of the cell population must be assessed prior to their use for experimental investigations. As part of this characterization, expression of the gelatinases (MMP-2 and MMP-9) will be investigated. These enzymes are members of the matrix metalloproteinase (MMP) family and are essential in the physiological regulation of extracellular matrix turnover. Although their production has been demonstrated by satellite cells and fibroblasts in other human systems, their production in human masseter muscle has not been demonstrated.

Materials and Methods: Primary populations of cells were prepared from outgrowths of masseter muscle biopsies obtained following elective oral surgery: (1) Satellite cells were identified by their immunoreactivity to an anti-desmin antibody. Their proportions (relative to the total mononuclear cell population) were determined throughout a 10 day time course that included phases of proliferation, fusion and myotube growth. (2) serial passages of three cultures investigated the stability of satellite cell proportions. (3) Expression of MMP-2 and MMP-9 (as determined by gelatin substrate gel zymography of conditioned media) was related to the proportion of satellite cells and timing of fusion.

Results: Fusion in all cultures could be identified by a decrease in satellite cell number as these cells were incorporated into myotubes. The proportions of satellite cells between 12 cultures were stable (mean = 20.0 per cent, SD = 3.2) and were not affected by passage. Although MMP-2 was expressed throughout the culture period, MMP-9 was only expressed prior to fusion.

Conclusions: Serial passage of primary cultures is an effective means of increasing the material available for experimental investigation of human masseter muscle. It appears that regulation of matrix turnover via MMP-9 is involved in the events leading to myotube formation.

Investigation of bond strength of a new moisture insensitive primer

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Objectives: A common reason for orthodontic bond failure is moisture contamination. A new moisture insensitive

primer compatible with composite resin has been developed by 3M Unitek to overcome this. The aims of this study were to investigate the *in vitro* bond strength and clinical failure rate of brackets bonded using this new primer compared with a conventional primer.

Methods—Laboratory study: 30 extracted human premolar teeth were bonded with adhesive precoated Mini Uni-Twin brackets using the new primer. Thirty controls were bonded with a conventional primer. The brackets were bonded using an accepted protocol and then the force to debond assessed using an electromechanical debonding machine, utilising jigs designed to control the direction of the debonding force.

Methods—clinical study: 35 patients had brackets bonded on one side of the mouth using the new primer and the other side with the conventional primer. Over 6 months any bond failures were recorded including the time to failure.

Results: Using the Weibull analysis and log hazard ratios, brackets bonded *in vitro* with the moisture insensitive primer were found to be almost four times as likely to fail as those bonded with a conventional primer ($P < 0.0001$). A log-rank test showed that brackets placed *in vivo* using the new primer had a reduced survival rate ($P < 0.0001$). There was a clinical failure rate of 18.8 per cent after 6 months compared to 6.77 per cent with the conventional primer.

Conclusions: Brackets bonded with the new moisture insensitive primer showed a reduced bond strength *in vitro* and an increased clinical failure rate compared with the conventional primer. It can therefore not be recommended for clinical use.

Long-term effects of neonatal intubation on palatal form

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Objectives: To determine the effect of prematurity and low birthweight on maxillary arch development, and the long-term effects of neonatal intubation and its duration on palatal form and symmetry.

Materials: An experimental group of 43, 9-12 year-old previously intubated premature and low birthweight children (25 intubated ≤ 15 days; 18 > 15 days), and a control group of 50 non-intubated normal birth weight term children age- and sex-matched.

Methods: Clinical examination: skeletal pattern, enamel hypoplasia/opacities, and height and weight. Study cast measurements: arch widths, palatal widths/width asymmetry and palatal heights/height asymmetry. Measuring instrument: Reflex Microscope (3-dimensional digitizer employing stereoscopic vision) using a Cartesian co-ordinate system with fixed reference planes for palatal width, height, and asymmetry measurements.

Results: Parametric data: one-way Analysis of Variance, Student's *t*-tests and Pearson's Correlation Coefficient. Non-parametric data: Chi-squared (with Yates' Continuity Correction), Kruskal-Wallis and Mann-Whitney *U*-tests. Clinical: higher percentage of class III malocclusions ($\chi^2 =$

0.04^{*}) and increased no. of enamel defects ($\chi^2_y = 0.048^*$) in the premature children. Premature and low birthweight children significantly shorter ($p < 0.001^{***}$) and lighter ($p = 0.003^{**}$) than controls but no association found between arch and body dimensions ($r = 0.353$; $p > 0.05$). Study casts: narrower palatal widths ($p = 0.02^*$), posterior palatal width asymmetry (left side wider than right) ($p < 0.05^*$) and steeper palatal vaults anteriorly ($p = 0.007^{**}$) in the intubated children. No differences found in the number or severity of defects with length of intubation ($p = 0.783$). **Conclusions:** The previously intubated premature and low birthweight children exhibited more Class III malocclusions, narrower palatal width dimensions, a left-sided posterior palatal width asymmetry, and steeper palatal vaults anteriorly. Length of intubation (\leq or > 15 days) had no effect on the number or severity of defects. Recommendations for orally-intubated neonates include the use of protective plates, light extra-oral tube stabilization, and longitudinal follow-up.

The expression of FHL in cultured human masseter muscle
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FHL1, 2 and 3 belong to a subgroup of the LIM-protein family, the former being specific to skeletal muscle. The recently discovered LIM proteins have been shown to play fundamental roles in cellular development and differentiation. FHL proteins may therefore play an important role in skeletal muscle development. It is well known that muscle composition varies in the extremes of vertical facial form, what determines this variation is not yet known. FHL protein may have a role to play in muscle development and phenotype determination. The aim of this research was to determine the developmental expression of FHL1, 2 and 3 in human masseter satellite cell cultures, and to relate any changes in developmental expression to changes occurring in the cell cultures.

Satellite cell cultures derived from masseter biopsy material were grown over a 3-week period, during which time RNA was isolated for quantitative analysis. These time course studies were repeated using samples from three different subjects and each time course repeated three times for each subject.

Northern analysis results were interpreted using an NIH image analysis and the data then statistically analysed using a balanced two-way ANOVA. This demonstrated an upregulation of FHL1 during cell development until the point of myotube formation. FHL3 had maximal expression during the proliferative phase and declined after myotube formation. FHL2 expression was not demonstrated.

FHL1 and 3 may play a fundamental role in early muscle development, with the expression of FHL1 increasing as that of FHL3 decreases. FHL2 expression was not demonstrated at this stage of muscle development.

Childhood facial growth in three dimensions studied by optical scanning

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Objectives: A cross-sectional study of the three-dimensional changes of the face with age, and of the three-dimensional differences between the faces of boys and girls at different ages.

Methods: 132 Caucasians aged from 5 to 10 years were measured using the optical surface scanning system. Average scans were constructed by computer for each age and sex group. The averages were superimposed by the scanning software to assess the differences between different ages and sexes. An error study was performed to assess the accuracy of scan acquisition and landmarking. This involved scanning a group of non-growing subjects twice, with a week's interval between the scans. The differences between the scans showed the error of the method. **Results:** Males were generally larger than females in all dimensions. The greatest differences were between the facial heights, and the least in the mid-facial dimensions. The face height of both sexes increased by an average of 3–4 mm each year. Mid-face prominence and width altered little, whilst the mandibular prominence and width increased with age. Mandibular width increased by 1–3 mm a year, rising to 3–5 mm in some years at the inferior areas of the mandibular region. These increases were greater than the changes reported by cephalometric studies, possibly as the optical surface scanner measured the soft tissues as well as the hard tissues. Nose height and prominence and alar base width increased by 2 mm per year on average. The width of the dorsum changed little.

Conclusions: The topical surface scanner was suitable for the three-dimensional study of the faces of children. Boys were generally larger than girls for all the age groups studied. The degree to which the face enlarged with age varied upon the region studied. Growth in facial height was greatest. Growth of the mid-face in both prominence and width was minimal at most ages. The exception to this was the nose, where prominence and alar base width increased at most of the ages studied.

Comparing pain reported during alignment using various nickel titanium wires

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Objectives: Discomfort experienced during orthodontic alignment was assessed comparing the use of three different nickel titanium wires. A total of 103 subjects with a mean age of 15.6 years were enrolled and randomly allocated to one of three groups treated with either 0.014-inch round austenitic active wire, 0.016-inch round austenitic active wire, or 0.017 × 0.025-inch rectangular martensitic active nickel titanium wire.

Methods: Prior to treatment, anxiety levels were assessed using standardised psychometric questionnaires, as anxiety has been shown to influence pain threshold in dental patients undergoing treatment. Clinicians bonded only one arch with arch wires ligated fully where possible. Subjects recorded their level of discomfort immediately following arch wire ligation on a 100-mm Visual Analogue Scale

(VAS) and used a pain diary with a Verbal Rating Scale for the following 7 days.

Results: For the different groups, no statistically significant differences were observed between VAS scores obtained following arch wire ligation. Compared to the subjects in the two austenitic round wire groups, statistically significantly ($P < 0.02$) higher levels of mean pain scores were reported by the subjects in the rectangular 0.017×0.025 -inch martensitic active wire group. There appeared to be no difference in mean pain scores reported when comparing subjects in the 0.014 and 0.016-inch diameter austenitic active wire groups. Anxiety levels of the subjects prior to treatment were not statistically significantly different from those reported for similarly aged individuals in the general population, and were similar in all three groups.

Conclusions: Immediate levels of reported pain were similar regardless of which wire was used. Significantly higher levels of pain were reported with the use of 0.017×0.025 -inch rectangular martensitic active wire ($p = 0.02$). There was no difference in pain scores comparing the use of 0.014 and 0.016-inch austenitic active wires.

Orthodontic co-operation: a parental dilemma

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Rationale: The problem of compliance has occupied many researchers over an equally large number of years. Some attempts to explain the dynamics of co-operation have employed factor analysis and other multivariate techniques.¹⁻⁴ Identifying factors with mathematical methods still requires their interpretation to allow them to contribute to professional understanding. This paper argues for the addition of qualitative methodology to augment our understanding.

Purpose of study: To extend our understanding of the dynamics of co-operation from a consumer perspective.

Data collection: 15 in-depth interviews with former patients who had discontinued orthodontic treatment as children. The subjects were currently parents of 10 year old children. The parents were identified from a random sample of parents used in an earlier study.⁵

Data analysis: Interviews were audio-recorded and the texts analysed using grounded theory,⁶ a rigorous approach to qualitative investigation widely used in Health Services Research.

Findings: The major concern of parents is balancing the need to respond to current-child-distress with the need to support the future-adult-intention. Parental orthodontic history influences the way in which parents resolve this dilemma.

References

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Frictional resistance of new technology brackets

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An *ex vivo* comparison of the static and kinetic frictional resistance of titanium brackets, titanium nitride-coated brackets, new high molybdenum stainless steel brackets with that of conventional stainless steel orthodontic brackets was devised. The friction was generated through sliding contact with round and rectangular stainless steel archwires, and also against beta titanium archwires, the latter in order to estimate the frictional resistance of a completely biocompatible system. A specially constructed jig was used to hold the bracket and archwire securely in place, with the archwire at a fixed tension, the bracket at a fixed angulation and with a fixed ligation force. The jig was clamped in an Instron testing machine to allow accurate recording of the frictional resistance.

There was a high degree of variation between the values obtained for replicate tests. This, combined with the small sample size, implied that only large differences in friction were detected. This study is the first to provide evidence of increased friction with titanium nitride-implanted brackets with increasing bracket/wire angulation. The high friction of β titanium archwires was not compounded by the coupling of pure titanium brackets and β titanium archwires, as the study was unable to show greater friction for titanium brackets. There was no significant difference between the values obtained for static and kinetic frictional resistance.

Use of a videotape for improving oral health during orthodontic treatment

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Three methods of imparting oral hygiene instruction to patients wearing fixed appliances were compared (hygienist, video, and written education) using plaque and gingival indices on mandibular buccal surfaces. The production of the video was a major part of the work is described. Change in knowledge of oral hygiene procedures were demonstrated using pre- and post-education questionnaires. All three methods failed to improve the gingival health of the subjects. Written education affected no change in plaque scores. Video education demonstrated a statistically significant change ($P < 0.05$) on buccal surfaces gingival to the bracket. Hygienist education demonstrated a statistically significant change ($P < 0.05$) on buccal surfaces gingival to the bracket, but also on the total buccal surface ($P < 0.01$). Both the video and hygienist education improved knowledge of oral hygiene procedures to a highly significant extent (video $P < 0.01$, hygienist $P < 0.001$). Whilst a video may not fully replace one to one interaction, it has been shown to aid improvement in oral hygiene and knowledge in oral hygiene procedures with minimal time and cost to the clinical.