ABSTRACTS

OF LECTURES AND POSTERS

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Oral Presentations

1 LOWER INCISOR LABIAL MOVEMENT AND THE POSITION OF THE GINGIVAL MARGIN

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AIM: To study the effect of labial movement of the lower incisors on the prevalence and severity of gingival recession in orthodontically treated adult patients.
MATERIALS AND METHOD: A retrospective case-control study was based on the analysis of study casts and intra-oral slides of 300 adult patients. One hundred and fifty pairs matched by age and sex were selected using simple random sampling. Recordings of gingival recession were made on the casts by direct measurements of the distance from the cemento-enamel junction (CEJ). A second set of measurements was performed on the intra-oral slides using the width of the crown for correction of the enlargement. A recession was defined as having a distance of the marginal gingiva from the CEJ of more than 0.01 mm.
RESULTS: The intra-oral slide recording of gingival recession seemed to be more accurate and more clinically valid than the cast recordings. An increase in prevalence and severity of gingival recession after labial movement of mandibular incisors was statistically significant when the analysis was based on recordings of intra-oral slides only when all four incisors were included in the analysis, but not when the calculation was undertaken for an individual tooth. The mean value of the extent of recession on the four lower incisors was 0.36 mm among cases and 0.2 mm among controls ($P > 0.10$).
CONCLUSIONS: There was a tendency towards an increased prevalence of recession amongst the proclined incisors, but the difference between cases and controls in the amount of gingival recession (on average 0.12 mm) was not significant, indicating that controlled orthodontic treatment did not result in a deterioration of the gingival margin level.

2 THE BIOLOGICAL BASIS OF ORTHODONTIC TOOTH MOVEMENT

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AIM: To investigate molecular mechanisms and to identify putative signalling pathways that are involved in the process of bone remodelling during orthodontic tooth movement.
MATERIALS AND METHODS: Human periodontal ligament (PDL) fibroblasts were isolated and cultured. Their putative osteoblast-like properties were examined biochemically and histochemically. Forces applied during orthodontic tooth movement were simulated in *in vitro* culture systems and the stimulated cultures were examined at the molecular level.
RESULTS: Electron microscopy and x-ray diffraction of PDL cell cultures revealed mineralized formations resembling immature bone. PDL cultures under stress increased their DNA synthesis without the involvement of an autocrine mechanism. To that extent, although major cytoskeletal components such as vimentin and tubulin do not appear to be implicated in the force induced signalling pathway, proteins such as the Rho and rab family members, which are effectors of signals initiated at the cell membrane, exhibit either up- or down-regulation. Modulation in the expression profiles of many other proteins further highlight the complexity of the mechanisms involved. Inside the nucleus, transcription factors such as the jun and fos are also involved leading to the induction of genes which ultimately determine the osteoblastic phenotype.
CONCLUSIONS: The above described phenomena most likely appear to play pivotal roles in a reaction initiating at the cell membrane and targeting the cell nucleus.

3 HOW DO THE CELLS OF THE PERIODONTIUM UNDERSTAND THE ORTHODONTIST?

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AIM: Orthodontic tooth movement depends on the remodelling potential of the periodontal ligament (PDL). Empirically described as tension and compression, histological observations in the PDL of treated teeth have demonstrated morphological changes in the supporting alveolar bone allowing for tooth movement to take place. Even though clinical judgement has supported the notion that similar bracket systems and wires lead to different tissue reactions (= mm/month of tooth movement) little is known about the intricacies of how the gross macroscopic forces are 'transduced' into microscopic cellular reactions. The main point of contention for the cell refers to the relative secondary role that mechanics play in tooth movement. The emphasis of this presentation will be on the nature of the applied stimuli (orthodontic forces) with an overview of the current knowledge of the signal transduction mechanisms in osteoblasts and PDL fibroblasts following application of mechanical stimuli.
An orthodontic force is usually applied via brackets on the tooth. Dependent on certain parameters (i.e. centre of resistance of the same tooth), such a force leads to a deformation in restricted contralateral areas of the PDL, referred to as pressure and tension zones. Osteoblasts and PDL fibroblasts are connected via integrin receptors to different types of extracellular matrix (ECM) components such as collagen, fibronectin or vitronectin. Through this connection the cells in question may be able to recognize either zones of tension or compression. The following four basic levels of cellular recognition based on signal transduction of mechanical stimuli will be discussed: 1. specificity of stimulation, 2. specificity of

the receptor, 3. specificity of the signal transduction pathway, and 4. specificity of genomic control. Based on these successive events, a clinical force may be initially 'understood' by the effector cells prior to initiation of the various processes of the so-called remodelling response. The interaction of forces and cells is only possible if the environment in which the cells are embedded is capable of providing the means for the necessary cellular activity. Thus, the triad 'cells-environment-forces' is the basis for clinical orthodontics.
CONCLUSION: This review demonstrates that the basic mechanisms of cellular mechano-transduction are an integral part of any clinical orthodontic procedure. Therapeutic intervention should not be solely chosen on the basis of apparent clinical success rates but on scientifically proven principles of biology and mechanisms combined.

4 ANGIOGENIC GROWTH FACTORS RELEASED IN HUMAN PULP FOLLOWING ORTHODONTIC FORCE

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AIMS: To examine the release of four angiogenic growth factors in human dental pulp during orthodontic tooth movement by using neutralizing growth factor antibodies (NAs) to block their effects.
MATERIAL AND METHOD: Following ethical committee approval, the dental pulps of 40 premolar teeth from 10 patients treated with straight wire fixed appliances for two weeks were divided vertically and each half pulp further sectioned horizontally and co-cultured with a section of rat aorta in collagen surrounded by growth media. In each patient, one of the four NAs (anti VEGF, anti hFGBb, anti hPDGF and anti TGFß) was added to the media of the co-cultures from one half of each of the pulps from the four teeth. Sections of rat aorta alone were also cultured. All cultures were examined daily by light microscopy for angiogenic changes in the form of microvessel proliferation and a count of microvessels was taken at day 5 and day 10.
RESULTS: The addition of each of the four NAs to the growth media in the co-cultures resulted in a significant reduction ($P < 0.004$ Wilcoxon signed rank test) in pulpal and rat aorta microvessels at day 5 and day 10 compared with the control half pulp co-cultures. Anti VEGF, anti hFGBb and anti hPDGF resulted in similar reductions in microvessel numbers, while anti TGFß had a more varied effect. The addition of the four NAs also showed individual patient variation in angiogenic response. There was no significant difference in the rat aorta microvessel numbers from the co-cultures with NAs and the rat aorta alone cultures.
CONCLUSIONS: The results indicate that all four growth factors examined appear to be released during orthodontic tooth movement and may play a role in the angiogenic response of the pulp. However, there is also variation in the individual patient's response.

5 THE EFFECT OF ORTHODONTIC TOOTH MOVEMENT ON PULPAL VASCULARITY: A HISTOMORPHOMETRIC STUDY IN RATS

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AIMS: Orthodontic tooth movement has been implicated in secondary changes in dental pulp. The aim of this study was to investigate the effects of well defined parameters of orthodontic force and force application time on pulpal vascularity and predentine formation in rat molars using histomorphometric parameters.
MATERIAL AND METHOD: Four groups, each consisting of 4 adult Wistar rats, were studied with differing force magnitudes (10, 20, 30 N) for 3, 6, 10 and 14 days. A sham operation group was used consisting of the same number of animals. Also the contralateral (non-orthodontically treated side) was included and used as a further control. The specimens were harvested 3, 6, 10 and 14 days after the start of the experiment. Undecalcified specimens were embedded in Technovit 7002 and stained with toluidine blue. Pulpal measurements were made with an image analyser in order to detect pulpal hyperaemia and vasodilatation.
RESULTS: The findings indicated an increase in the number of pulpal capillaries in all treated animals. The capillaries were always dilatated. However pulpal hyperaemia seemed not to stimulate predentine formation.
CONCLUSIONS: Orthodontic force application in rats may not contribute to the ageing of the pulp.

6 CHANGES IN GINGIVAL AND ADJACENT ALVEOLAR TISSUES DURING ORTHODONTIC TOOTH MOVEMENT

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AIMS: Investigation of histologic tissue reactions in the gingiva and the adjacent periodontal ligament during the first three days of orthodontic tooth movement.
MATERIALS AND METHODS: The incisors of rabbits were moved sideways with helical torsion springs for periods varying from three to 70 hours. The initial force of the springs was 50 g.
RESULTS: Comparison with control animals and animals with passive springs showed that tooth movements lead to trauma in the dentogingival area within a few hours. This trauma was characterized by tears and ulcerations in the epithelium, by tears and ruptures in the fibres, by leukocytic infiltration, and by the presence of extravascular erythrocytes. Tissue damage increased significantly with time. The histologic data of the dentogingival area in animals wearing active springs were compared with those of the underlying periodontal ligament by MANOVA. This analysis showed that there were significant differences between the tissue reaction in different areas of the periodontium. A close relationship was found between the damage to the fibres and

the occurrence of extravascular erythrocytes and leukocytes. Resorption of cementum and enamel could be observed occasionally after 24 hours and frequently after 72 hours of tooth movement. Metaplasia of the labial marginal periodontal ligament was found after 72 hours of tooth movement and showed hyperplasia and keratinization of the crevicular epithelium and replacement of the crevicular epithelium by cementum, while simultaneously the labial periodontal ligament changed from loose connective fibres to a dense connective tissue network between enamel surface and alveolar bone.
CONCLUSIONS: Local tissue damage caused by orthodontic tooth movement is characterized by rupture of fibres, leading to an inflammatory reaction in the gingiva and periodontal ligament. Moreover, orthodontic tooth movement causes metaplasia of crevicular epithelium, cementocytes and periodontal fibres.

7 TISSUE REACTIONS DURING RELAPSE

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AIMS: To evaluate relapse and concomitant tissue reactions after standardized orthodontic tooth movement in dogs.
MATERIALS AND METHODS: In 19 young adult beagles the mandibular third premolars were extracted. After healing, orthodontic appliances were placed to bodily distalize the second premolars using standardized forces of 50, 100, and 200 cN. Sixteen weeks later the forces were removed while the appliances were left in place thus allowing bodily relapse of the second premolar. Relapse was measured intraorally twice a week. Time-displacement curves were related to the applied force and the tooth displacement during active treatment. At 18 days and at the end of the relapse period histological evaluation was carried out.
RESULTS: Large individual differences were found in the duration of relapse (range 45–108 days), but no significant differences were found amongst the force groups. High correlations existed between the amount of active tooth displacement and the amount of relapse (PCC 0.86) or the duration of the relapse period (PCC = 0.62). Histological evaluation revealed that after 18 days of relapse periodontal principal fibres at the former tension side had been replaced by thin collagenous fibres parallel to the root surface and that osteoclasts were resorbing alveolar bone. In some areas relapse had caused localized root resorption at the former tension side. At the end of the relapse period the normal structure of the periodontal ligament had been restored. Eventually, some cementum was laid down on former resorption areas, but the root outline remained irregular in those areas.
CONCLUSIONS: The amount and the duration of relapse is related to the amount of orthodontic tooth displacement, but not to the force magnitude used to achieve it. Adverse side effects of orthodontic treatment, such as root resorption, also can occur during relapse.

8 BIOLOGICAL REACTION OF ALVEOLAR BONE TO ORTHODONTIC MOVEMENT

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AIM: To describe the reaction of alveolar bone to the changes in the stress strain relationship generated by orthodontic forces applied to teeth and to interpret these reactions from a bone biological point of view.
BACKGROUND: Controversy: Bone biologists generate apposition by loading; orthodontists seem to relate pressure with resorption.
MATERIAL AND METHODS: Six adult *Macaca fascicularis* monkeys had their second premolar and first molars extracted and orthodontic appliances were designed for the generation of translation of first premolar and the second molar by forces ranging from 100 to 300 cN. Superelastic springs were applied buccally and lingually to power arms. The appliance was calibrated every two weeks for eleven weeks. The tooth movement was measured by an electronic gauge and on standardized intraoral radiographs. After sacrifice, 15–20 parallel horizontal sections were produced and evaluated histomorphometrically. The results were related to the stress strain distribution of the bone adjacent to the alveolus.
RESULTS: Loading of the teeth resulted in a general increase in bone turnover around the alveolus. The relative extension of apposition was doubled in comparison with the control teeth from 7–13 to 15–20 per cent. The force level had no significant influence on this tendency, but the density of the bone was highly related to the loading of the bone confirming that the increase in loading resulted in a positive balance of the bone turnover and unloading resulted in a reduction in bone density.
CONCLUSION: Reconsidering the tissue reaction secondary to orthodontic forces from a bone biological point of view, a hypothesis solving the apparent controversy between orthopaedist and orthodontist is suggested.

9 THE EFFECT OF DIFFERENT ORTHODONTIC FORCES ON OSSEOINTEGRATED IMPLANTS

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AIM: To assess the peri-implant bone modifications occurring as a consequence of the application of different orthodontic forces for different lengths of time.
MATERIAL AND METHOD: Seventy one titanium screw implants were positioned in the calvarium of 30 rabbits. Eleven implants were used as controls. The animals were divided into 5 groups according to the type and duration of the application of the distalizing force of 150 g between the

abutments. A continuous force was applied 2.5 months after the surgical procedure, for 21 (group A), 60 (group B) and 120 days (group C). Immediate continuous force was applied in group D and immediate alternate force in group C, for 60 days in both groups. Phases of bone modification were marked with terramicine and alizarine. Ten days after the last injection the animals were sacrificed and block-sections carried out. The sections were x-rayed with a standardizing device and analysed both microscopically and with a computer program, after scanning. The peri-implant space, on both compression and tension sides, was then measured and scored. Score 0 was attributed to no resorption/apposition, score 1 equalled resorption < 0.25 mm, score 2 = 0.25–0.5 mm and score 3 > 0.5 mm.
RESULTS AND CONCLUSIONS: Good clinical stability was obtained and the distances between the implants remained unchanged. In the analysis of the radiographs, a high percentage of resorption was observed in group D (score 0 = 37 per cent) compared with group A (90 per cent), B (67 per cent), C (75 per cent) and E (80 per cent). Thus less resorption was observed after application of immediate alternate forces. Stereoscopic analysis had previously shown that mineralization in group B (60 days) was similar to normal bone, and optical microscopic examination had also shown rapid maturation of bone tissue after loading. Preliminary results of the histomorphometric analysis after the application of immediate continuous forces (group D) has now shown a higher contact between bone and implant on the tension side compared with the compression side 61–55 per cent, 50 per cent in the control. This would seem to support the hypothesis that early adequate loading might facilitate bone maturation and even improve early osseointegration.

10 PROLIFERATIVE RESPONSE OF RAT PERIODONTAL LIGAMENT CELLS TO COMPRESSION AND TENSION *IN VIVO*

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AIMS: To study the *in vivo* proliferative response in periodontal ligament areas subjected to compression and tension.
MATERIAL AND METHODS: Elastics, 0.5 mm thick, were inserted between the maxillary Ml and M2 of male rats aged 8 weeks, which were immediately labelled with ^{3}H-TdR (S.A. 25 Ci/mmol) and killed in groups together with labelled control animals, after periods ranging from 1–168 hours. Autoradiographs were prepared from two 2.5 μm mesiodistal plastic sections from each animal. Compression and tension were assessed using an image analyser by comparing the width of the ligament in both stimulated and control animals along the distal surface of M2 root and the mesial surface of M3 root in 125 μm steps in a corono-apical direction beginning at the level of alveolar bone crest. Cell proliferation was assessed by determining the percentage of ^{3}H-TdR-labelled cells (PLC) in standard size measurement areas in the middle of the ligament and in the immediate vicinity of the alveolar bone and cementum placed at the different levels in a corono-apical direction. The results were tested for statistical significance using two-way ANOVA and appropriate pairwise comparison procedures.
RESULTS: The width of the entire ligament distal to M2 was significantly ($P < 0.05$) decreased in stimulated animals for up to 24 hours of stimulation, and remained reduced ($P < 0.05$) in the coronal third for up to 72 hours. In the ligament mesial to M3 it was significantly ($P < 0.05$) increased in the coronal two-thirds for up to 24 hours and remained enhanced in the middle third for up to 120 hours ($P < 0.05$). Significantly lower PLC were measured on the pressure side compared with unstimulated controls ($P < 0.05$). On the tension side at the level of the middle third of the root, cell proliferation was increased in all measurement areas ($P < 0.05$). Immediately below the interdental bone crest on the tension side, cell proliferation was reduced in the vicinity of the bone and in the middle of the ligament ($P < 0.05$) and remained unchanged near the cementum ($P = 0.15$).
CONCLUSIONS: It appears from the results obtained that distal tipping of M2 and M3 as well as bending of interdental bone septum occurred under the conditions of the present study. Mechanical stimulation of the periodontal ligament may lead to substantial differences in proliferative response depending on the type of mechanical challenge (pressure versus tension), apico-coronal location of the stimulated tissue and, possibly, locally generated force magnitude.

11 BONE PHYSIOLOGICAL CONSIDERATIONS IN ORTHODONTIC PRACTICE

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KEYNOTE ADDRESS

The literature was reviewed relative to bone manipulative procedures associated with correction of dental and skeletal malocclusions.
Biomechanical manipulation of bone is the physiological basis of orthodontics and dentofacial orthopaedics. Bone modelling and remodelling processes are the biological vectors of bone adaptation to metabolic and mechanical demands. The physiological mediators of orthodontic therapy are the mandibular condyle, maxillary sutures, periodontal ligament and attached gingiva. Orthodontic induction of bone formation is a vascularly mediated process that is remarkably similar at all osteogenic sites. Orthodontic treatment in growing children is largely a guided eruption process, but adult treatment requires extensive bone resorption (the rate limiting step in tooth movement). The rate of tooth movement relates to the type of bone ahead of the moving roots. Post-treatment osseous maturation of the alveolar process is a fundamental aspect of retention and long-term stability. Root resorption may be related to uncontrolled episodes of occlusal trauma during active treatment and/or retention. Fatigue failure of the roots in a catabolic

environment appears to be the mechanism. Teeth with a healthy periodontium can be moved through the floor of the maxillary sinus and into atropic areas of the alveolar process. Periodontium can be augmented substantially with orthodontic tooth movement. A major problem in mechanotherapy is lack of adequate anchorage. Osseointegrated implants can be utilized as rigid anchorage for broadening the scope of clinical orthodontics.
The application of fundamental principles at the clinical level can substantially expand the scope of orthodontics and enhance treatment outcomes.

12 EFFECTS OF DENTAL MATERIALS ON MONOCELL CULTURES AND *IN VITRO* GENERATED EPITHELIA OF GINGIVAL CELLS

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AIM: To assess whether effects detected by the classical Agar-Overlay Assay also become visible in a surface epithelium. The epithelial integrity and histoarchitecture (scanning electron microscopy, SEM) was studied in comparison to histomorphology and as functional aspects patterns of proliferation and keratin expression in organotypic co-cultures of gingival cells by indirect immunofluorescence (IF).
MATERIALS: Acrylics and silicones (Orthocryl®, Erkolok®, Durabase®, Bisico®, Heraeus-Kulzer®) used in orthodontics for removable plates, as palatal plates in new-born cleft patients and as splints in TMD-patients were tested.
RESULTS: The Agar-Overlay Assay demonstrated that, irrespective of the mode of treatment, none of the tested materials caused cell lysis. However, the observed neutral red-free inhibition areas of various sizes indicated that the tested materials, although not directly cytotoxic, were classified as having potential physiological effects on cells. SEM analysis of organotypic co-cultures revealed growth of differentiated surface epithelia based on fibroblasts producing high amounts of extracellular matrix. However, none of the splint acrylics, including Durabase® which exhibited the largest inhibition area in the conventional assay, caused damage to the epithelium surface. Also methylmethacrylate, used as a positive control, had no effect on surface integrity. In addition to structural stability, IIF for MIB-1 and cytokeratins 14 and 13 also revealed maintenance of cell proliferation and differentiation as criteria of epithelial function in comparison with the negative control.
CONCLUSIONS: Since in this system the tested acrylics and silicones exhibited no direct epithelium-damaging influence, organotypic co-cultures consisting of gingival cells are a suitable model to assess basic-toxic material influences seen in monocell cultures under more tissue-relevant conditions and render them a tool for prospectively testing effects such as inflammation.

13 RECONSTITUTION OF GINGIVAL EPITHELIUM *IN VITRO*: PARALLELS WITH THE TISSUE OF ORIGIN

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AIM: For prospective testing of putative dental material effects under *in vivo* relevant conditions, oral mucosa equivalents consisting of keratinocytes and fibroblasts were generated from unkeratinized gingivae. In this model proliferative activity, site-specific keratins, integrin patterns and deposition of basement membrane (BM) components of the established epithelia closely resembled the tissue of origin.
MATERIALS AND METHOD: The sequence of events in tissue normalization was assessed on frozen sections by immunohistochemistry and *in situ* hybridization.
RESULTS: The initial activated stage (one week), compared to early wound healing, was characterized by (1) high keratinocyte proliferation, (2) extended expression of the basal cell-specific keratin K5 and a patchy pattern of the suprabasal keratins K4 and K13. After two weeks the improvement of histoarchitecture correlated to (1) predominant K5 gene expression in the basal cell compartment, (2) extension of K4 and K13 within the suprabasal cell compartment, (3) extensive expression of the keratinocyte-type integrins α6ß4 and α3ß1 (including the ligand laminin 5) in all keratinocytes at the dermo-epithelial junction zone and (4) progressive deposition of basement membrane components. Virtually complete tissue normalization (three weeks) was indicated by (1) restriction of K5 to the basal cell area, (2) suprabasal localization of the differentiation-specific keratins, (3) polarization of integrins to basal and parabasal cells and (4) linear co-distributions of collagen IV and laminin 1 and 5 underneath the basal cells.
CONCLUSIONS: These organotypic cultures thus represent relevant equivalents for the *in vivo* situation of non-keratinized oral mucosa, indicated by the typical gingival differentiation features, which render these co-cultures a suitable model for tissue compatibility testing.

14 VISCOELASTIC RESPONSE OF THE PERIODONTAL LIGAMENT TO ORTHODONTIC FORCES

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AIMS: To determine the viscoelastic behaviour of the periodontal ligament (PDL) in response to orthodontic forces. The ultimate aim of the study was to calculate changes in stress/strain distribution in the periodontium and to correlate it to the remodelling processes during tooth movement.
MATERIALS AND METHODS: Five young adult beagle dogs were used in the *in vivo* experiments. A specially

designed measurement device was placed on the left/right second premolars to determine the initial tooth displacement during 5 hours loading with forces of 50, 100 and 300 cN, respectively. In combination with a 3D finite element mesh, viscoelastic properties for the PDL were obtained by fitting results from the FE-model on the results from the experiments.
RESULTS: A two-step response of the tooth after the application of force was found in the experiments: 1) an initial rapid displacement within 4 seconds after force application, followed by 2) a more gradual displacement (creep) reaching a maximum after 5 hours. Similar results were described by Moxham *et al*. (1987), for buccolingually loaded teeth. The best FE-fit was found using a viscoelastic model for the PDL composed of two Maxwell elements (spring and damper in series) in parallel with a linear spring. For the Maxwell elements, specific creep times of 400 and 4000 seconds, respectively, were found.
CONCLUSIONS: The PDL exhibits viscoelastic behaviour. Further investigations are required to elucidate whether the initial rapid displacement is caused by elastic deformations within the bone or by the compression of blood vessels in the PDL. The creep response can be explained by the exudation of interstitial fluid.

Moxham B J *et al*. 1987 A laboratory method for studying tooth mobility of the mandibular central incisor of the sheep. Res. Vet. Sci 42: 61–64

Poster Presentations

15 MODULATION OF GROWTH OF THE MANDIBULAR CONDYLE BY MAGNETIC FIELDS

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AIM: The regulation of growth and development of the mandible is not completely understood. In functional orthodontic therapy, orthodontists try to modulate the growth of the condyle which is an important growth centre of the mandible. Cell differentiation during condylar growth resembles that during fracture healing. Pulsing magnetic fields (PMF) are used in orthopaedics to stimulate the healing of unmanageable fractures (non-unions). The aim of this study was to analyse the effects of PMF on the growth of the rat mandibular condyle *in vitro.*
METHODS: Mandibular condyles were dissected from 4-day-old Wistar rats and cultured for 3 weeks with or without PMF (1.5 Hz, 1.5 G max). Cultures were stimulated for 3 hours/day or 24 hours/day. Standardized photographs were taken three times a week to monitor growth. At the end of the experiment the glycosaminoglycan and hydroxyproline contents of the condyles were analysed. The effect of PMF on the synthesis of glycosaminoglycans (GAG) was determined from the incorporation of $^{35}SO_4$ after 1 and 5 days' stimulation.
RESULTS: In 3 weeks the area of the condyles in all groups increased from 2.5 to 8.5 mm^2. Neither of the stimulation regimes affected growth. There were also no differences in the GAG and hydroxyproline contents between the stimulated groups and the controls. The GAG-synthesis at day 5 was decreased by 50 per cent compared with day 1 in all cultures. However, it was not modified by PMF.
CONCLUSIONS: This culture model is suitable to study the growth of the mandibular condyle. Under the experimental conditions the PMF-stimulus had no effect on growth of the mandibular condyle nor on the other parameters studied. To find an effective stimulus, modification of the PMF characteristics is required.

16 RESPONSE PROPERTIES OF RAT PERIODONTAL MECHANORECEPTORS IN AN *IN VITRO* PREPARATION

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AIMS: Responses of single periodontal primary afferents to mechanical stimulation with von Frey hair were investigated in an *in vitro* jaw-nerve preparation (Toda *et al*., 1995).
MATERIALS AND METHOD: Single unit activities were recorded from the inferior alveolar nerve with the microneurographic technique. Fifty five lower incisors and 100 lower third molars were obtained which responded with spikes to mechanical stimulation of the periodontal ligament left on the surface of the alveolar cavity after tooth extraction.
RESULTS: Both rapidly adapting (RA) and slowly adapting (SA) types were found among afferent fibres from the incisor ligament, while only the RA type was found in the fibres from the molar ligament. The majority of these belonged to Aδ fibres. The response threshold of fibres innervating the incisor was 11.8 mN except for one unit, while those innervating the molar showed various threshold values (11.8, 7.8, and 2.9 mN). The response patterns of the RA type were divided into three groups (on, off, and on-off). In the molar, many single primary afferents innervated the periodontal ligament of more than one tooth, and in the on-off units innervating the molar periodontal ligament, a significant difference in mechanical threshold was found among the three groups classified by the numbers of the innervating molar.
CONCLUSIONS: These findings show that the response properties of the periodontal mechanoreceptors are different between the incisor and molar.

Toda K, Ishii N, Nakamura Y 1995 An *in vitro* jaw-nerve preparation for oral sensory study in the rat. Journal of Neuroscience Methods 61: 85–90

17 THE POSSIBILITY OF A MONO-NUCLEAR OSTEOCLAST

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AIM: We have reported a mono-nuclear osteoclast-like cell *in vitro* by 16 mm cine-micrography which contained a rod-type apparatus for bone resorption instead of the typical ruffled border and cytoplasmic vacuoles moving towards nuclei. The aim of this research was to find this mono-nuclear osteoclast-like cell in an *in vivo* experimental model.
MATERIALS AND METHODS: The first molars of Wistar strain albino rats were moved mesially by orthodontic coil springs. Tartrate resistant acid phosphates (TRAP) histochemical staining was applied for light microscopic examination of histological sections of the periodontal area during tooth movement for 1, 2, 3, 5 and 7 days.
RESULT: A few mono-nuclear osteoclast-like cells stained with TRAP could be observed adjacent not only to alveolar bone but also to molar roots in the early stage of tooth movement.
CONCLUSION: TRAP positive mono-nuclear osteoclast-like cells could be found during experimental tooth movement in rats, and they can be characterized as osteoclasts because of their staining capability and cytoplasmic vacuoles moving towards the nuclei.

18 HYALINIZATION AND ROOT RESORPTION DURING EARLY ORTHODONTIC TOOTH MOVEMENT IN ADOLESCENTS

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AIM: To elucidate hyalinization of the periodontal ligament with time and its relationship to early root resorption in orthodontically moved premolars in adolescents.
SUBJECTS AND METHODS: Fifty six maxillary premolars in 18 boys and 38 girls (mean age 13.8 years) were moved buccally with a fixed orthodontic appliance. A weekly reactivated force of 50 cN (= 50 g) was applied. The experimental periods varied from 1 to 7 weeks and concerned 8 individuals in each of 7 groups. The contralateral premolar served as a control. The teeth were extracted and subjected to histological examination.
RESULTS: Local areas of overcompression in the periodontal ligament were recorded in 33 test teeth (59 per cent) and 2 control teeth (4 per cent). Hyalinization, with cell-free or almost cell-free zones, was seen in all experimental groups (I–VII), more often after the first 4 weeks (I–IV) of force application. The hyalinized areas were recorded opposite an intact root surface (54 per cent) or close to and just apical or coronal of a root resorption (46 per cent), and were usually located bucco-cervically and linguo-apically, corresponding to expected pressure zones of the periodontal ligament.
CONCLUSION: Hyalinization of the periodontal ligament was often seen during early orthodontic tooth movement, close to a root resorption lacuna or opposite an intact root surface.

19 APICAL ROOT RESORPTION DURING EXPERIMENTAL TOOTH MOVEMENT IN RATS

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AIMS: Understanding the mechanisms of pathological root resorption during tooth movement is of major interest in orthodontics. Unrepairable severe root resorption during orthodontic treatment is usually found at the apex of the tooth root. The local mediators which initiate apical root resorption are largely unknown because of the lack of suitable study models. The aim of the present study was to determine whether premature contact plays a role in apical root resorption during experimental tooth movement in rats, and to develop a model for studying the mechanisms of apical root resorption.
MATERIALS AND METHODS: The maxillary first molar of young male Sprague-Dawley rats was moved by work-hardened Ti-Ni alloy wire that delivered light dissipating forces with or without composite resin on the occlusal surface to cause premature contact. Sham-treated control data were also included for comparison.
RESULTS: Stereomicroscopic observation revealed severe attrition of the resin added to the occlusal surface in the premature contact groups, which suggests traumatic occlusion. Histochemical examination of tartrate-resistant acid phosphatase (TRAP) activity revealed that active apical root resorption with TRAP-positive cells took place in the group with tooth movement with premature contact. In contrast, few resorptive pits and TRAP-positive cells were observed around the apex of the first molars in the group of tooth movement without premature contact and the group of premature contact-only. The body weight of rats increased steadily during the experiment.
CONCLUSIONS: The results support the hypothesis that premature contact that becomes traumatic may be involved in the initiation of apical root resorption during orthodontic tooth movement. The group with tooth movement and premature contact can be used as a model for studying the mechanism of apical root resorption in rats.

20 INFLUENCE OF SYSTEMIC BONE METABOLISM ON MAXILLOFACIAL BONE DENSITY

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AIMS: It is well-known that bone metabolism is closely related to orthodontic tooth movement and dento-maxillofacial development. However, the effect of systemic

metabolism on maxillofacial bone remodelling has not been examined quantitatively and longitudinally. The purpose of this study was to investigate the changes of bone mineral density (BMD) in total, and regional body composition using dual-energy X-ray absorptiometry (DXA) as a method for measuring body composition.
MATERIALS AND METHODS: Ovariectomized (OVX) rats were used to compare the alveolus and systemic bone metabolism. The BMD was measured by DXA method (DCS-600, Aloka Co. Ltd., Japan) in thirty 38-week-old Sprague-Dawley rats that had been divided into two groups (OVX: n = 15, sham: n = 15). Both groups were fed a diet containing 0.5 per cent calcium. BMD was measured at the skull, mandibular alveolus, left humerus, lumbar vertebrae (L2–4) and left tibia at 0, 60 and 90 days after the operation.
RESULTS:
1. OVX was associated with a 10–20 per cent reduction in humeral and tibial BMD at 90 days post-operatively. In addition, vertebral BMD decreased 35 per cent after 90 days.
2. No significant change in BMD of either skull or alveolus was seen.

CONCLUSIONS: These results demonstrate that the rate of bone metabolism varies between sites. In the results expressed, the alveolus BMD does not show a significant change. These findings suggest that the differentiated guidance is achieved by bone remodelling. The hypothesis is that the local effect of mechanical stimuli from mastication is a major contributor to maintain bone mass in OVX rats. The relative clinical importance of these results remains to be elucidated, especially in the use of some drugs administered during orthodontic treatment to control regional bone metabolism.

21 EARLY REPARATIVE PROCESS OF ORTHODONTICALLY INDUCED ROOT RESORPTIONS IN ADOLESCENTS

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AIM: To determine the pattern of repair of root resorption regarding the location and type of tissue in adolescents after application of a well-controlled force magnitude.
MATERIAL AND METHODS: In 16 adolescents (mean age 13.8 years), the maxillary first premolars were buccally moved with a weekly reactivated force of 50 cN (= 50 g) for 6 weeks following which the appliance was made passive for 2, 3, 6 and 7 weeks. The subjects were divided into two groups of 8 individuals for which the retention periods were 2 and 6 weeks (group I), or 3 and 7 weeks (group II), implying intra-individual differences of 4 weeks. The teeth were extracted and subjected to histological investigation.
RESULTS: Reparative cementum in the resorption cavities was seen in all test teeth, significantly more often after 6 and 7 weeks of retention (82 per cent) compared with 2 and 3 weeks (35 and 44 per cent, respectively). The reparative process appeared to commence in the bottom of the resorption cavity, frequently covered by a thin layer of acellular cementum. However, most of the reparative cementum was of the cellular type and always covered the initially formed acellular cementum. There were great individual variations regarding the occurrence of healing of orthodontically induced root resorption.
CONCLUSION: The reparative process seemed to start early. The initially formed repair cementum was located in the bottom of the resorption lacuna and was of an acellular type. However, later deposition was mainly of cellular cementum.

22 COMPARISON OF PROTEOGLYCANS IN CONDYLAR, COSTAL AND NASAL CARTILAGES

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AIMS: In this study the compositions of extracellular matrices of condylar, costal and nasal cartilages were compared to characterize differences in growth patterns in relation to matrix composition.
MATERIALS AND METHODS: Condylar, costal and nasal cartilages of 25- and 35-day-old rabbits were extracted and subjected to biochemical analysis to determine amounts and aggregating properties of proteoglycans and total amounts of collagen.
RESULTS: It was found that proteoglycan content and aggregate formation were greatest in nasal cartilage, and markedly lower in costal and condylar cartilage. The amount of proteoglycans increased by varying amounts in all samples with age. Collagen content was highest in costal cartilage. In 25-day-old rabbits the quantity of collagen in condylar cartilage exceeded that in nasal cartilage. In 35-day-old rabbits the quantities were nearly the same.
CONCLUSIONS: Based on the results, it seems that the amount of proteoglycans is greater in cartilages which have greater independent growth potential. It is suggested that collagen not only provides tensile strength, but counteracts forces responsible for interstitial growth, such as osmotic pressure. Variations in the increase in the amount of proteoglycans with age could reflect differences in the timing of growth of such cartilages.

23 UNILATERAL MASTICATION AND THE PROTEOGLYCAN CONTENT OF MANDIBULAR CONDYLAR CARTILAGE

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AIMS: The changes in proteoglycan content in the condylar cartilage after unilateral masticatory function were determined biochemically.
MATERIALS AND METHOD: The right side molars of 10-day-old rabbits were ground down twice a week and the

animals were killed at the ages of 25 and 35 days. Rabbits of the same age with unaltered occlusions served as the controls. The proteoglycans were extracted from the homogenized cartilage samples with 4M guanidium chloride. After centrifugation the extractant was ultracentrifuged and the highest density fraction A1 was reaggregated with exogenous hyaluronic acid, chromatographed, and each fraction was precipitated on a nitro-cellulose filter. The amounts of proteoglycans were compared quantitatively with standards by computerized digital image analysis.
RESULTS: In both experimental groups the total amount of proteoglycans of mandibular condylar cartilage, especially the quantity of aggregating proteoglycans, was higher on the left than on the right side, whereas in the control animals the difference was reversed.
CONCLUSION: The induced left side masticatory function loaded the condylar cartilage differently from the normal and reversed the natural asymmetry in proteoglycan content of the mandibular condylar cartilage.

24 BIOELECTRICAL STIMULATION IN ORTHODONIC TREATMENT

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AIM: Tooth movement acceleration in orthodontic treatment.
SUBJECTS: Ten Iranian dental students who required orthodontic treatment were selected.
METHOD: During bilateral upper canine retraction to relieve crowding, or in the treatment of subjects with Class II malocclusion, one side of the dental arch was stimulated with an electronic circuit designed and constructed to generate a weak pulsating magnetic field, mounted adjacent to the experimental side, during conventional canine retraction with a closed coil spring. The other side of the arch served as the control.
RESULTS: Retraction of the upper canines was continued until a normal canine position on both sides was achieved. Study models, radiographs and photographs taken before and after canine movement were obtained and the experimental and control sides were compared.
CONCLUSION: The rate of tooth movement in the experimental side was greater than in the control side. This finding suggests enhanced tooth movement due to the secondary effect of the magnetic field on bone remodelling.

25 INITIAL TOOTH DISPLACEMENT *IN VIVO*—A PREDICTOR OF LONG-TERM DISPLACEMENT

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AIM: Previously the dry skull has been used as a hypothetical model to test initial orthodontic and orthopaedic force systems. The question, however, as to whether this hypothetical model can be used as a predictor of long-term displacements *in vivo* remains unanswered. In this study an attempt was made to compare initial tooth displacement with the long-term effect after application of the force system for a longer period of time, in adult dogs.
MATERIAL AND METHOD: In six adult dogs tooth displacement was obtained by applying a force by means of a coil spring (push) system. After application of a force of 50 g in the first series (n = 3) and 80 g in the second series (n = 3), initial displacements were registered by means of speckle interferometry. The long-term displacement was registered in the same dog by leaving the force system in place for +5 weeks. By means of standardized cephalometry the long-term displacement was measured.
RESULTS: The mean values of the displacement vectors of the second premolars in the 6 dogs were compared and tested by the paired *t*-test. No significant differences were found between the initial and long-term displacements in any of the dogs. According to these findings, both groups of measurements belong statistically to the same sample.
CONCLUSION: Initial tooth displacement measured by means of speckle interferometry is a valuable predictor for forecasting the long-term displacement *in vivo* after 5 weeks.

26 LOCALIZATION OF ANTI-MONOCYTE/MACROPHAGE ANTIBODY-POSITIVE CELLS AFTER TOOTH MOVEMENT

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AIM: To examine the distribution of monoclonal anti-monocyte/macrophage (ED1) and macrophage (ED2) antibody-positive cells in periodontium after orthodontic tooth movement.
MATERIALS: Rat maxillary molars were moved by insertion of band materials and the immunoreaction was detected by confocal laser scanning microscopy.
RESULTS: Immunofluorescence of ED1 and ED2 antibodies were intensely detected in mononuclear and multinuclear cells of rat periodontium. The localization of ED1-positive mononuclear cells in the experimental teeth was little different from that in the controls. While ED2-positive mononuclear cells were located throughout the periodontium, as well as the ED1-positive cells, in the distal side of controls, the number of positive cells decreased in the pressure side of the treated teeth.
CONCLUSIONS: Most of the immunoreactive mononuclear cells found in the distal side of the controls were macrophages. In the pressure side of the experimental teeth, mononuclear cells expressing macrophage-associated phenotype, presumably osteoclast precursors, decreased after experimental periods, suggesting the formation of multinucleated osteoclasts.

27 THE INFLUENCE OF BONE TURNOVER ON TOOTH MOVEMENT IN A RAT MODEL

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AIM: To assess the effects of bone turnover rate on orthodontic tooth movement and associated bony reactions.
MATERIALS AND METHODS: Twenty-one 6-month-old male Wistar rats divided into 3 groups with: 1) high, 2) low and 3) normal bone turnover. Their upper left first molars were mesially moved for 21 days with a 25 gram Sentalloy® closed coil spring fixed to the molar and upper incisors. After sacrifice the maxillae were excised and the distance between the first and third molar was measured bilaterally. The maxillae were scanned in a Micro-CT machine producing 122, 35 μm thick sagittal sections per side. Linear attenuation was determined as a measure for the local bone density at four sites around the first root of the first molar: 1) mesial/cervical, 2) mesial/apical, 3) distal/apical and 4) distal/cervical. For each first molar the section with the longest root was selected and the inflation expressed as the angle between the first root and the occlusal plane.
RESULTS: The tooth movement occurred as a controlled tipping. The rate of tooth movement was reduced significantly (68.21 per cent) in the case of low turnover and a tendency towards an increase was observed in the case of high turnover (20.3 per cent). Regarding bone density, the control side showed a lower density at site 1 than at site 4 in all three groups. Compared with the control side, bone density was lower in the treated side at site 4 in groups 1 and 3, but not in group 2. On the treated side the density was lower at site 4 than at site 3 in group 1.
CONCLUSIONS: The speed of tooth movement is influenced by bone turnover, as it is directly proportional to its rate. The lower bone density at site 1 on the control side reflected the physiological distal drift of rat molars (King *et al*., 1991). Treatment inverses this phenomenon, yet is less pronounced in the low turnover group. As a clinical consequence re-activation in these patients should be less frequent.

King G J, Keeling S D, McCoy E A, Ward T H 1991 Measuring dental drift and orthodontic tooth movement in response to various initial forces in adult rats. American Journal of Orthodontics and Dentofacial Orthopedics 99: 456–465

28 VASCULAR CHANGES IN RAT GINGIVAL PAPILLA INCIDENT TO MECHANICAL STIMULATION

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AIMS: To study the vascular response in gingival connective tissue during orthodontic tooth movement.
MATERIAL AND METHODS: Elastics, 0.5 mm thick, were inserted between the maxillary Ml and M2 of male rats aged 8 weeks, which were immediately labelled with ^{3}H-TdR (S.A. 25 Ci/mmol) and killed in groups together with labelled control animals after periods ranging from 1–168 hours Autoradiographs were prepared from 2.51 μm mesio-distal plastic sections, and vascular changes were assessed using an image analyser by measuring the number of blood vessels and the total lumen area of blood vessels as well as determining the percentage of ^{3}H-TdR-labelled endothelial cells in standard sampling areas (975×0.650 μm) placed coronal to the alveolar bone crest in the transseptal fibre region between M2 and M3. The results were tested for statistical significance using two-way ANOVA and appropriate pairwise comparison procedures.
RESULTS: There was no statistically significant difference between the numbers of blood vessels per unit area in the stimulated and control animals over the time course. Total vascular lumen area in each animal was significantly increased ($P < 0.05$) in mechanically stimulated animals from 1–48 hours relative to controls but dropped to control level at 72 hours. A further increase ($P < 0.05$) occurred at 120 hours of stimulation as compared with controls. Significantly higher ($P < 0.05$) values of the percentage of ^{3}H-TdR-labelled endothelial cells were revealed from stimulated animals only at 24 hours after the initiation of mechanical stimulation.
CONCLUSIONS: The results obtained indicate that early vascular response in gingival connective tissue comprises an immediate transient vasodilatation. There was no evidence of true angiogenesis during the experimental period of the present study.