

2270 The Effect of Swedish and American Snuff Extract on Periodontal Ligament Fibroblasts *in vitro* -- A Pilot Study. Å. WAHLIN*, G. BRATTHALL, G. ANDERSSON, and G. SODERHOLM, Center for oral health sciences, Malmö University, Sweden

Objectives: To evaluate the effect of Swedish moist snuff extract on growth and morphology of periodontal ligament fibroblasts (PDLF). American moist snuff extract was used as reference. The primary aim was to develop a suitable study design.

Methods: Human PDLF were obtained from extracted premolars of healthy individuals. Cells were seeded in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS) or 4% bovine serum albumin (BSA). Snuff extract in the concentrations 0.1%, 0.3%, 1%, 3%, 10% were tested. Photographs were taken at 0, 1, 3, 6, 9, 24 and 48h, and analysed for growth and morphology. Alkaline phosphatase activity (ALP) activity was measured.

Results: At 0.1% and 0.3% concentrations Swedish and American snuff stimulated growth of PDLF in DMEM with FBS but not with BSA. Vacuolisation was recorded after 24 and 48 hours when grown in 1% Swedish and American snuff extract in DMEM with BSA but not with DMEM and FBS. When the PDLF were exposed to 3% Swedish snuff extract in DMEM with BSA and FBS vacuolisation was recorded at 24 and 48 hours, while few remaining cells could be seen after exposure to American snuff extract. At 10% snuff extract cell death occurred after 3 hours and at 9 hours all cells were dead. Alkaline phosphatase production increased at lower snuff extract concentrations but decreased over 3%.

Conclusion: The American reference snuff extract seemed to cause a toxic reaction at lower concentrations than the Swedish snuff extract.

2271 Effects of Chlorhexidine on the Migration of Human PDL Fibroblasts. J. WINARUKWONG, T. TEPARAT, and R. SURARIT*, Mahidol University, Bangkok, Thailand

Chlorhexidine (CHX) has been used to eliminate periodontal pathogens, as an adjunct to scaling and root planing for the treatment of periodontal diseases. Objectives: The purpose of this study is to investigate the effect of CHX on the cytotoxicity and the migration of cultured periodontal ligament (PDL) cells grown on dentine slab. Methods: The PDL fibroblast cells were obtained from explanted human tissue scraped from the root of normal premolar or molar teeth extracted for orthodontic reasons. Thirty six dentin slabs were divided into 6 groups: control and 5 experimental groups of various concentrations of CHX (0.0012%, 0.002%, 0.012%, 0.12% and 0.2% (w/v)). CHX was applied on the lateral zones of each dentin slab with the central zone being occupied by PDL cells. After incubation at 37°C for 40 hours, cells migration was measured and the number of cells that migrated were counted after staining with toluidine blue O. Results: The results of this study showed that CHX at the concentration of 0.004% (w/v) resulted in almost 100% cell death. The ID50 and ID30 of CHX were 0.0015% and 0.0012% (w/v), respectively. There was a significant reduction in the migration of PDL fibroblasts in all experimental groups when compared to the control group ($p < 0.05$), except that the 0.0012% (w/v) CHX group showed no significant difference. The morphology of cells were also observed by SEM. Conclusions: The results provide additional evidence that CHX is toxic to PDL cells and could inhibit the migration of these cells on dentin surface. Supported by the Faculty of Dentistry, Mahidol University. Dtrsr@mahidol.ac.th.

2272 Fluoride Exposure of Pregnant Women Assessed on the Basis of Determination of Fluoride Levels in Urine and Blood Plasma. J. OPYDO- SZYMACZEK*, and J. CHAPOWSKA, University of Medical Sciences in Poznan, Poland

Objectives: The aim of the study was to assess the fluoride exposure of pregnant women, coming from the city of Poznan, where the level of fluoride in drinking water ranges from 0.4 to 0.82. Methods: The assessment was made on the basis of fluoride levels determination in urine and blood plasma samples. The subjects were 32 pregnant women aged 22-34. All the subjects were using fluoridated toothpaste. The samples of urine and venous blood were collected in the morning, while fasting, in the 28th and 33rd week of pregnancy. Measurements of fluoride concentration were performed using ion-selective electrode ORION 96-09. The results were statistically analysed with the use of the Student's *t*-test and the Pearson's correlation coefficient ($p=0.05$). Results: Fluoride concentrations in the blood plasma and urine samples collected in the 33rd week of pregnancy were statistically significantly higher than those determined in the 28th week of pregnancy: 0.071 ppm and 0.063 ppm in blood plasma and 0.884 ppm and 0.667 ppm in urine respectively. A positive correlation was found between fluoride levels in blood plasma and in urine, both in the 28th and 33rd week of pregnancy. Conclusions: Fluoride concentrations in body fluids did not exceed the levels quoted in literature for the areas with the optimum concentration of fluorides in drinking water. Levels of fluorides in body fluids in the 28th week of pregnancy were lower than those determined in the 33rd week of pregnancy, which suggests that the metabolism of fluorides changes with the pregnancy progress. Fluoride concentrations in the urine samples collected in the morning while fasting correlated with the values determined in blood plasma, which confirms the validity of this biomarker in assessment of fluoride exposure. Supported by Polish State Committee for Scientific Research E01922

2273 Jaw Opening, Chewing, and Bite Force in Patients with Temporomandibular Joint Pain and Matched, Healthy Control Subjects. R. HANSDOTTIR, and M. BAKKE*, School of Dentistry, University of Copenhagen, Copenhagen N, Denmark

Objective: The aim of this study was to evaluate the effect of temporomandibular arthralgia on mandibular mobility, chewing, and bite force. Methods: Twenty female patients, 19-45 yr, with unilateral temporomandibular joint (TMJ) pain as the single or overriding orofacial symptom. Inclusion criteria: TMJ tenderness at palpation, and TMJ pain during chewing and provocation. The chewing pain corresponded to 40 ± 26 mm (M \pm SD) on a 100mm visual analogue scale. The TMJ conditions were classified as 9 disc derangement disorders (6 displacements with and 3 chronic without reduction), 7 osteoarthritis cases (6 primary and 1 secondary) and 4 inflammatory disorders (3 synovitis/capsulitis and 1 polyarthritis). The patient group was compared with an age- and sex-matched control group of healthy volunteers without orofacial pain and TMJ tenderness. Exclusion criteria (both groups): <24 teeth and significant malocclusion. The following methods were used, (a) algometric assessment of pressure-pain thresholds (PPT) over the TMJ, (b) clinical and computerized, kinematic recordings of maximum unassisted jaw opening, (c) computerized, kinematic assessment of maximum velocity and cycle duration during chewing of soft gum, and (d) measurement of unilateral, molar bite force. The data were compared with *t*-tests with a significance level of $P < 0.05$. Results: The PPT (kPa) in the patients' pain side was significantly lower (68 ± 19) than in the contralateral side (76 ± 24), and also significantly lower than in asymptomatic controls (107 ± 5). Jaw opening (mm) was significantly less in patients (42 ± 8 , controls: 52 ± 3). Chewing and maximum closing velocity (mm/s) was significantly slower in patients (141 ± 46 and 948 ± 185) than in controls (175 ± 43 and 772 ± 101), and bite force (N) significantly lower in patients (237 ± 99 ; controls: 389 ± 80). Conclusion: These systematic findings supplement results from acute pain experiments, and confirm indications from unspecific patient groups; presence of chronic TMJ pain in patients is associated with marked functional impairment, probably due to a reflex avoidance mechanism.

2274 Jaw-movement-related Mirror Neuron System in Humans. Y. SHIBUKAWA*, M. SHINTANI, T. KUMAI, M. KATO, T. SUZUKI, Z. ZHANG, T. JIANG, M. SHIMONO, T. ISHIKAWA, and Y. NAKAMURA, Tokyo Dental College, Chiba, Japan, Matsumoto Dental University, Shiojiri, Japan, Keio University, Tokyo, Japan, Peking University, China, Teikyo Heisei University, Ichihara, Japan

Objectives: A group of neurons in the monkey premotor cortex discharge when the monkey is performing a given action as well as when it is observing the experimenter performing a similar action (mirror neuron system; MNS). Neuromagnetic studies have shown that the primary motor cortex is included in the human MNS. The present study was aimed to determine spatial and temporal activation patterns of the human MNS in association with jaw movements. Methods: Neuromagnetic activities of the cerebral cortex in 6 healthy adults were recorded using a 306-channel whole-head neuromagnetometer. The subjects were asked to carefully observe, on videotape, symmetrical jaw opening movements performed by another individual. Neuromagnetic responses were examined using time-varying multi-dipole analysis to estimate current dipoles which were superimposed in the individual MRI to determine the source locations. Results: The magnetic signals were averaged at the onset of jaw-opening movements. During observation of the movements, prominent magnetic fields were detected on the occipital region and the lateral parieto-frontal region bilaterally with four peak signal components. Their peak latencies were 130, 220, 340 and 440 ms. In the multi-dipole analysis, four main source areas with left hemisphere dominance were sequentially activated during observation of the movements: 1) occipito-temporal area near inferior temporal sulcus (MT/V5), 2) inferior parietal cortex (IPC), 3) inferior part of precentral gyrus (maxillofacial area of primary motor cortex; M1mx), and 4) primary visual cortex (V1). Conclusions: These results indicated that both the M1mx and IPC were activated during observation of jaw movements and involved in jaw-movement related-MNS which play important roles in orofacial motor recognition in human. (Supported by Grants (HRC 3A04, 12 and 13) for High-Tech Research Center Projects from the MEXT in Japan) yshibuka@tdc.ac.jp

2275 Electromyographic Responses to Acupuncture Point Stimulation of the Face. W.T.Y. LOO*, and L.J. JIN, University of Hong Kong, Hong Kong

The electrical stimulation by acupuncture may excite or inhibit temporalis muscle through facial cutaneous acupuncture points (acupoints), which may improve the rhythmic activity of the jaw-opening and -closing muscles and therefore may be applied to the treatment of temporomandibular joint disorders and trigeminal neuropathies as well as oral rehabilitation. Objectives: This study aimed to measure the electromyographic (EMG) responses of the temporalis muscle to stimulation of ten acupoints of the face, i.e. Shangguan, Xiaguan, Jiache, Daying, Chengjian, Dicang, Shuigou, Yingxiang, Qianiao and Taiyang. Methods: The stimulated points were selected and the relevant threshold was determined following International Standard of Acupuncture. Ten healthy adults were invited to clench two thin pieces of wood and keep a constant bite force of 80 Newtons for 30 seconds, and the left anterior temporalis muscle was chosen for EMG recording. The EMG output was fed to an amplifier then transferred to be rectified and averaged. The data were analyzed by one-way ANOVA. Results: The mean EMG responses were positive at ten points in A (early reflexes) area. In B (late reflexes) area it responded negatively at acupoints Jiache, Chengjian and Dicang. The highest responses of Taiyang and Shangguan were found at both areas by stimulation of the relevant acupoints. No statistically significant difference was observed in the responses between age and stimulation intensity for both A and B areas, but significantly correlated to the distance of acupoint to recording electrode for both areas. The longer the distance, the lower response was recorded. Conclusion: Electromyography monitors the sum of electrical signals or action potentials generated by masticatory muscles. The surface electrical current applied to facial skin acupoints may excite or inhibit the anterior temporalis muscle via reflex pathways.

2276 Neck Muscle Inhibition Induced by Intra-oral Stimulation in Humans. T. TORISU*, H. SUENAGA, R. KANAOKA, K. NOGUCHI, and H. FUJII, Nagasaki University, Japan

Several studies have been made on trigemino-cervical reflexes. Objective: To evaluate the effect of the intraoral stimulation on the neck muscle activities. Methods: The experiments were performed on 7 healthy volunteers (F=4; M=3; mean age=24.6yrs). Electrical stimuli (0.2ms duration, 0.3Hz) were applied to the maxillary attached gingiva at intensities of 1-13 x perceptive threshold. Surface EMG activities were obtained from the ipsilateral dorsal neck muscles before and after local anesthesia to the periodontal tissues by means of fine wire electrodes. The EMG responses were full-wave rectified, and then averaged from 20 ms before the stimulus to 180 ms afterward. Results: The integrated value between 40-50 ms after the stimulation (P40-50) was significantly reduced than that of pre-stimulus before anesthesia ($P=0.028$, Wilcoxon test). No significant differences were found in P40-50 after anesthesia ($P > 0.9$, Wilcoxon test). Conclusion: These results suggest that the afferent information from the intraoral receptors influences the dorsal neck muscle activities.

2277 Granulometry of Food Boli after Mastication Completion. A. MISHELLANY*, A. WODA, and M.-A. PEYRON, Dental Faculty, Clermont-Ferrand, France

Objective: The primary role of mastication is to transform the mouthful into a bolus ready to swallow by the reduction of the food into small particles. The aim of this work was to determine the size of the particles constituting a food bolus at the end of mastication, just before swallowing.

Method: Ten subjects with normal dentition aged 36.7 \pm 9.5 years were selected for the study. Six foods, 3 dry nuts and 3 moist raw vegetables, were tested (peanut, almond, pistachio, cauliflower, radish and carrot). Each subject masticated dry nuts (portions of 2.5 to 3.5g) and cylindrical samples of vegetables (3g) and then spat the sample out after a self-estimation of mastication completion. Two replicates were obtained for each food boli. Particles size distribution was measured by sieving and laser diffraction.

Results: Particles were much larger in vegetables than in nuts. 70% of vegetable particles were bigger than 2mm whilst 70% of nuts particles were less than 0.4mm. Particles size distributions were similar within nuts and within vegetables. After spitting the weights of the boli were approximately 40% of the initial food weight. This was similar within subjects and within foods. Surprisingly, no statistical inter-individual variability was observed in the particles distributions for the six foods. This contrasted with what was known for the physiological parameters of mastication and can be exemplified as follows: the masticatory frequency of the 10 subjects ranged from 1.2 to 2.5 Hz and the number of necessary strokes to complete mastication ranged from 13 to 52 cycles/sequence.

Conclusion: These results suggest that the wide variation observed between subjects in the physiological mastication parameters is caused by the need of different individuals to prepare a bolus with common and accurate physical properties.