

953 Characterization of the immunodominant antigens of *Porphyromonas gingivalis* 381. E.A. BOUTSI*, T. NISHIHARA, K. NAKASHIMA, H. WATANABE, I. ISHIKAWA. (Dept. of Perio., Tokyo Med. & Dental Univ. and N.I.H., Japan).

The purpose of this study was to detect and characterize the immunodominant antigens of *P. gingivalis* 381. Whole cells, phenol-water extract and fimbriae from *P. gingivalis* 381 were used. Sera were obtained from 17 patients with adult periodontitis, rapidly progressive periodontitis or juvenile periodontitis which had high antibody titers to *P. gingivalis* sonicated extracts as well as from three healthy donors. All subjects' sera were adsorbed with the phenol-water extract of *P. gingivalis* 381 and analyzed by immunoblotting. The ELISA was performed using whole cells and phenol-water extract of *P. gingivalis* 381 reacting with unadsorbed and adsorbed patients' and healthy sera. Also, immunoblotting was performed using the adsorbed sera in dilutions of 1:1,000, 1:5,000, 1:10,000 and 1:50,000. At last, whole cells were treated with papan (100 µg/ml), proteinase K (100 µg/ml) and trypsin (100 µg/ml) and then reacted with patients' sera in dilution of 1:10,000. After the immunoblot analysis, three types of patterns were obtained: the first type presented a smear between 94 and 41 kDa and gave positive reaction for the 41 kDa band, which had the same molecular weight as the fibrillin band. The second pattern showed no smear and had positive reaction for the 41 kDa band and the third type presented neither the smear nor the band. The ELISA titers against whole cells remained high after adsorption of the patients' sera with the phenol-water extract, suggesting that the phenol-water extract (consisting mainly of lipopolysaccharides) is not an immunodominant antigen. The immunoblot analysis at the lowest dilution of serum showed two protein bands at 41 and 43 kDa. The 41 kDa band was resistant to digestion of serum showed two protein bands at 41 and 43 kDa. The 43 kDa band was digested only by proteinase K. These results suggest that the immunodominant antigens of *P. gingivalis* 381 in high responder patients appear to be the fibrillin and the 43 kDa proteins.

955 Antibody Reactivity of Necrotizing Ulcerative Periodontitis Patients with Eukaryotic and Prokaryotic Heat Shock Proteins and Host Tissue Antigens. S.L. LOMELI* and T.H. BRAMANTI (Periodontology, University of California, San Francisco).

Heat shock proteins (HSPs) are produced by prokaryotes and eukaryotes under environmental stress. Prokaryotic HSPs have been shown to be immunodominant antigens in bacterial infections and immunoreactivity of infected patients to these HSPs has been implicated as a major etiologic factor in autoimmune destruction of host tissues. The purpose of this study was to characterize the antibody reactivity of healthy and periodontitis patients to HSPs of *P. gingivalis*, human gingival fibroblast (HGF) HSPs, and human collagens to determine if autoimmune reactions occur which might contribute to periodontitis. Serum was collected from patients with periodontal health (H, n=14), advanced adult periodontitis (AP, n=19), rapidly progressive periodontitis (RPP, n=10), HIV-positive periodontal health (HIV-H, n=11), and HIV-related necrotizing ulcerative periodontitis (NUP, n=17). Immunoblot analyses examined patient IgG serum reactivity to established classes of HSPs at 27, 32, 60, GroEL, 70, DnaK, and 90 kDa, to *P. gingivalis* HSP antigens, HGF HSP antigens, and human collagen types I, III, IV, and V. IgG reactivity was observed in 16 of 17 NUP patients against *P. gingivalis* HSP 26, 60, and 90 kDa antigens, against purified human HSP 60 and 90 kDa antigens, against HGF HSP 45, 60, and 70 kDa antigens, as well as against human collagen types I, III, and V. Reactivity against human HSP 60 and 90 kDa antigens was observed in 6 of 10 RPP patients. No reactivity was seen against *P. gingivalis*, purified HSPs, HGF, or collagen antigens with the other patient groups. These findings suggest that NUP patients may possess deleterious antibody responses which could result in autoimmune reactions, and that these antibodies might be stimulated by HSP antigens produced by the resident microflora. This study was supported by UCSE School of Dentistry Committee on Research and UCSE AIDS Clinical Research Center.

957 Food-induced Elevation of Gingival Temperature and Neutrophil Emigration. J. ZHANG*, B. KASHKET and R. NIEDERMAN (Forsyth Dental Center, Boston, MA, USA).

Dental plaque accumulation is accompanied by a buildup of short-chain carboxylic acids (SCCA) and can be associated with gingival inflammation. Retention of certain foods on the dentition also results in the accumulation of SCCA at the gingival margin (Kashket et al., this meeting). This study examined the effects of the ingestion of such foods on gingival inflammation. Temperature measurements (T) were made on the buccal surfaces of all bicuspids and first molars with the use of the PeriTemp™ System. Five subjects chewed 15 g of plain doughnut, 15g oatmeal cookie, 1 g of wax, or nothing, and T was measured up to 90 min. Following ingestion of doughnut (high SCCA content in retained particles), maxillary T rose by 1.3 ± 0.8 °C within 5 min, and was still 1.0 ± 0.2 °C at 60 min, compared to 0.2 ± 0.3 °C for the control without food ($p < 0.02$). Oatmeal cookies (low SCCA content) elicited a short-term elevation of T (max = 0.8 ± 0.2 °C). Wax gave a similar response, indicating that T elevations were due in part to mastication. Parallel measurements of gingival crevicular fluid revealed increases in flow rate and neutrophil emigration after ingestion of doughnuts or rinses with SCCA. These responses are consistent with an inflammatory response related to the SCCA in retained foods. It is concluded that certain foods that become entrapped on the dentition can induce inflammatory responses in healthy gingival tissue. Supported by NIDR/NIH Research Grants DE-05253 and DE-08415 and Mars, Inc., McLean, VA.

959 Comparison of measurement systems for volume assessment in soft tissue augmentation procedures. S. STUDER*, W. BUCHER, J. YELLEN AND P. SCHÄRER (Dept. of Prosthodontics & Dental Materials, Dental School, Zurich).

Different soft tissue augmentation procedures for the correction of localized alveolar ridge defects can be evaluated in a quantitative manner. The mechanical 3-D coordinate measuring machine (CMM), with an accuracy of 1.5 µm/m (Leitz PMM 864) and which calculates the volume integral by a mathematical software (Mathcad), was used as standard method. The purpose of this study was the comparison of three measurement methods with the CMM. One pair of aluminium specimens was fabricated having an identical rectangular solid form. The preoperative ridge defect was simulated by a concave, segmental sphere form, whereas the postoperative ridge defect was defined by the unchanged rectangular solid. The volume difference of these two specimens was determined fifteen times (1) by the direct measurement (DM) of the specimen's volume and weight, (2) by the Archimedes method (AM), which weighed the specimens at air and in distilled water (AG 204 Mettler-Toledo) with known temperature and specific density, and (3) by the optical Moiré-technique (MT), (Newport Instruments). Results were analyzed by ANOVA (repeated meas., $p < 0.05$). Results: The CMM determined a volume defect of $217.349 \text{ mm}^3 (\pm 0.002)$ which was defined as true volume. The other three methods obtained following mean volume differences (\pm standard deviation): DM = $215.7 \text{ mm}^3 (\pm 0.8)$, AM = $215.5 \text{ mm}^3 (\pm 1.8)$, MT = $218.5 \text{ mm}^3 (\pm 5.2)$. Mean-volume differences of DM, AM and MT differed less than 1% of the CMM result in this *in vitro* set-up. The MT revealed the highest standard deviation. All three methods, DM, AM and MT, may be regarded as precise enough to measure volume changes in soft tissue augmentation procedures. The optical Moiré-technique may be more advantageous because of its relative ease and flexibility.

954 Antigenic Cross-reactivity between *Porphyromonas gingivalis* and *Bacteroides forsythus*. D. VASEL*, T. SIMS and R.C. PAGE (Clinical Dental Research Center, School of Dentistry, University of Washington, Seattle, WA 98126).

We investigated the possibility that lipopolysaccharides (LPS) of *P. gingivalis* (Pg) and *B. forsythus* (Bf) share antigenic epitopes. Ten *Macaca fascicularis* primates were immunized with a vaccine containing killed Pg (monkey isolate #5083) and it was demonstrated that this inhibited the progression of ligature-induced periodontitis. Since the animals were colonized by several other putative periodontal pathogens, we suspected that antibodies to antigenic epitopes shared among gram-negative pathogens could account in part for the observed protection. Pre- and postimmune sera from the 5 animals manifesting the highest serum antibody response to Pg were pooled and evaluated by cross-adsorption enzyme linked immunosorbent assay (ELISA), Western Blots and Immunodot Blots against LPS, Lipid A and whole cell sonicates from Pg and Bf, human isolate #9610. The antibody titers of immune monkey sera sham adsorbed and tested on Pg LPS- and Bf LPS-coated plates were enhanced 11.8- and 5.9-fold respectively over preimmune sera. Adsorption with Pg LPS abrogated the increase in titer to Bf and Pg LPS completely. Adsorbing with Bf LPS reduced the enhancement to Pg LPS by 50% to 6.1-fold while the increase to Bf LPS was completely abrogated. The immunodot blots semiquantitatively and in good agreement with the ELISA data show an increase in titer reactive with both Lipid A and LPS from Bf in the range of 4- to 8-fold. The respective values for purified Lipid A and LPS from Pg fall into the 8- to 16-fold range. We tested the same antigens and also whole cell sonicates of both bacteria in Western Blots developed with pre- and postimmune sera. While all of the preparations showed some staining with the preimmune sera, for both Pg and Bf LPS an intensively stained distinct ladder pattern was observed with the postimmune serum. Very similar results were obtained with Lipid A from both bacteria. Thus, LPS from *P. gingivalis* and *B. forsythus* have a high degree of cross-reactive antigenic epitopes with a major portion residing in Lipid A. We suggest that shared epitopes have a great potential for use in an effective vaccine as a single vaccine could target a range of pathogens. This study was supported by NIH grants DE08555 and DE09743 and Deutsche Forschungsgemeinschaft (DFG).

956 Short Chain Carboxylic Acids in GCF, Clinical and Inflammatory Relationships. R. NIDDERMAN*, Y. BOYLE-BODIN, B.-Y. LU, C. NALEWAY and P. ROBINSON (Forsyth, Boston, Northwestern Univ. and A.D.A. Chicago).

Propionic and butyric acids are metabolic byproducts of bacterial metabolism which can alter eukaryotic gene expression. We therefore determined: 1. their concentration in gingival crevicular fluid, and 2. their correlation with clinical, microbial load, and inflammatory parameters in periodontally healthy and diseased subjects. The results indicated that there was a > 10 fold difference between healthy and diseased subjects for both propionic and butyric acid (propionic: health = 0.81 ± 0.25 , disease = 9.48 ± 1.84 ; butyric: health = 0.182 ± 0.036 , disease = 2.57 ± 0.42). These differences (mean \pm S.E.) were significant ($p < 0.0001$). The acid concentrations correlated significantly with clinical parameters (pocket depth, bleeding on probing, and attachment level), total microbial load, and subgingival temperature (all $r > 0.59$; all $p < 0.01$). These results indicate that the molecular effects of these acids must be accounted for in understanding periodontal disease pathogenesis. Supported by DE 08415.

958 Changes of Enzyme Activities and Periopathogens in Gingival Crevicular Fluid (GCF) during Experimental Gingivitis. P.-Ö. SÖDER*, B. SÖDER and L.J. JIN (Karolinska Inst. Stockholm, Sweden).

The purposes of the investigation were to study changes in enzyme activities and presence of suspected periopathogens in gingival crevicular fluid (GCF) during experimental gingivitis in young healthy subjects. Eight male students, aged 20-31 years participated in the study. After the screening examination, they refrained from any oral hygiene for 14 days. The subjects were scored with Quigley-Hein plaque index (Q-H index) and gingival bleeding index (BI). GCF was collected from the distal surfaces of 12 and 22 using a washing method and from mesial surfaces of 26 and 36 with paperstrips (Periopaper®) at day 0 and day 14. The washing samples were immediately transferred to the laboratory for biochemical analysis, microbial cultivation and microscopic examination. The glycosidases were assayed spectrofluorimetrically and expressed as pmol/µl/hg protein and elastase activity was measured in a Multistat® III-Fluorescence/light scatter Micro Centrifugal Analyzer and presented as mAbs/µl x 2h. The washing samples were cultured for *Actinobacillus actinomycetemcomitans* (A.a.), *Porphyromonas gingivalis* (P.g.) and *Prevotella intermedia* (P.i.). Student's paired t-test was used for the statistical analysis. The mean of Q-H index for whole mouth increased from 0.09 ± 0.09 at baseline to 3.42 ± 0.65 ($p < 0.001$) and BI from 0.02 ± 0.03 to 0.08 ± 0.08 ($p < 0.029$) during the experimental period. The glycosidases and the elastase activity increased significantly during the experimental period. A.a. or P.g. were not found in any subject. P.i. was always present at the same subjects and sites through the study. No significant change existed for granulocyte count during the whole study ($p > 0.05$). Conclusions: the significant increase of the glycosidase activities reflects pronounced degradation of glycoproteins in established gingivitis. *Prevotella intermedia* seems to be a suspected periopathogen present in the initiation of gingivitis.

960 Mercury Release During Ultrasonic Scaling of Amalgam. K.W. HARELMAR*, P.S. SCHULLER, H. NGUYEN, D.M. COLLINSON & G.W. THOMPSON (Faculty of Dentistry, University of Alberta, Edmonton, Alberta Canada).

Ultra-sonic scalers are routinely used in the initial phase of the scaling and root planing procedure, and may contact amalgam surfaces during use. Research has shown that polishing and cutting of amalgam results in increased Hg emissions. The purpose of this *in vitro* study was to investigate the effect of ultra-sonic scaling on Hg vapour release from amalgam. Dispersalloy was condensed into a split die to make 110 standardized Ag cylinders. Set cylinders were stored in distilled H₂O at 37°C for 24 hours. Closed one litre chambers were constructed for controlled experimental environments. Chambers had air-tight access cuffs for insertion of scalers and air extraction tubing. Extracted air samples were analyzed for Hg vapour with a JIC 511 Gold Film Hg analyzer. Ag cylinders were randomly divided into groups. The first group was scaled with a Cavitrone® unit, the second with a Titan® handpiece scaler and the third served as controls. Each cylinder was placed in a chamber and the scaler tip, at normal reciprocation frequency and H₂O spray, was moved across the surface for 20 seconds. Five seconds were allowed for spray and Hg vapour dispersion then chamber air was extracted and again analyzed. Scaler tips did not contact the Ag surfaces of controls. Results were analyzed using Student's t-test. Significantly greater vapour release occurred in the experimental groups than in the control ($p < 0.001$), with the Titan® causing significantly more release ($p < 0.001$) than the Cavitrone®. None of the vapour levels approached the NIOSH safety limit. Ultrasonic scaling on Ag releases Hg vapour. The concentrations vary between instrument types and adjustment.