



<b>Title</b>	<b>Oral yeasts and coliforms in HIV-infected patients in Hong Kong</b>
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- 113** Maxillary Sinus Augmentation Using Porous Hydroxyapatite as a Grafting Material. M.B. HÜRZELER<sup>1</sup>, J.R. STRUB<sup>1</sup>, C.R. QUINONES<sup>1</sup>, A. KRISCH<sup>1</sup>, P. SCHÜPBACH<sup>1</sup>, R.C. CAFFESSE<sup>2</sup> (<sup>1</sup>Univ. of Freiburg, <sup>2</sup>Univ. of Texas, <sup>3</sup>Private Practice, Stuttgart, <sup>4</sup>Univ. of Zurich).

The aim of this study was to evaluate clinically and histologically the use of porous hydroxyapatite (Interpore-300®) as a bone grafting material for maxillary sinus augmentation procedures. In 8 adult male rhesus monkeys (*Macaca Mulatta*) all the maxillary molars on one side of the jaws were extracted. The remaining bone between the alveolar crest and the bottom of the sinus was then evaluated radiographically and reduced to 3-4 mm. After 3 months, maxillary sinus augmentation procedures were performed in each monkey, and two (MZ plasma coated implants were immediately placed (i.e. simultaneous implants-loaded group). In 4 monkeys, hydroxyapatite was used for the augmentation procedure. In the other 4 monkeys, no grafting material was used. After 4 months, two additional similar implants were placed into the previously augmented sinuses (i.e. delayed implants-loaded group). Four months later, the abutment connection was performed and all four implants were loaded with a gold-alloy bridge for 6 months (i.e. until sacrifice). The contralateral side of each monkey received the same treatment with the exception that the extractions were performed 7 months after those in the opposite side and that the implants in this side were not loaded. Thus, 2 additional study groups (i.e. simultaneous implants-unloaded group and delayed implants unloaded group) were obtained. Computer-assisted histomorphometry was performed to quantitative areas of new bone and areas of fatty marrow and/or fibrous connective tissue in the remaining bone and the augmented bone. Differences between groups were compared using student's t-test. Regarding the percentage of direct bone contact in the areas augmented with hydroxyapatite, there was no difference between the simultaneous unloaded group (46.2%) and delayed unloaded group (46.6%). The loaded groups exhibited significantly greater direct bone contact (i.e. simultaneous loaded 56.5% and delayed loaded 55.3%). In addition, the control groups showed significantly less bone apposition (i.e. simultaneous loaded 14.9% and delayed loaded 14.7%). In conclusion, hydroxyapatite appears to enhance bone formation and implant osseointegration in the augmented sinuses. Loading of the implants in the augmented sinuses seems to increase bone apposition at the implant/bone interface. (This study was supported by: IMZ-GmbH Friauf, Mannheim, Germany.)

- 115** Effect of Bone Morphogenetic Protein on Bone Healing around Endosseous Implants. A Pilot Study in Beagle Dogs. T.H. Howell<sup>1</sup>, D. Buser<sup>2</sup>, J. Fiorellini<sup>1</sup>, H. Stich<sup>2</sup> & J. Wozney<sup>3</sup> (<sup>1</sup>Harvard School of Dental Medicine, Boston, USA, <sup>2</sup>University of Bern, Switzerland & <sup>3</sup>Genetics Institute, Cambridge, USA).

Although replacement of teeth with dental implants has become an effective treatment modality, their predictability relies on successful osseointegration formed during the healing period. The purpose of this pilot study was to evaluate the effect of recombinant Bone Morphogenetic Protein-2 (rhBMP-2) on early bone formation around dental implants in the beagle dog model. Mandibular premolars were extracted from two 1 year old beagle dogs. Following a healing period of 3 months, 10 implants were placed with rhBMP-2/vehicle and 9 implants with vehicle alone using a split mouth design. After 21 days of submerged healing the beagles were sacrificed, histological block sections processed and stained with toluidine blue. The histological sections were evaluated for the presence (+) or absence (-) of new bone formation within the implant perforation and the marrow space. Results were then compared with a Sign Test. Data indicated that significantly more bone formation occurred with rhBMP-2 treated sites within the implant perforation ( $p < 0.01$ ) and the marrow space ( $p < 0.01$ ) as compared to vehicle alone. This pilot study indicates that rhBMP-2 has a positive effect on bone formation in combination with dental implants. Supported by Genetics Institute Inc., ITI Foundation and by NIDR Grants DE07010 and 5K16 DE00275.

- 117** Oral yeasts and coliforms in HIV-infected patients in Hong Kong. P.C.S. TSANG<sup>1</sup>, L.P. SAMARANAYAKE, S.S. LEE, P.L.I. R.G. NAIR (University of Hong Kong, Queen Elizabeth Hospital & Department of Health, Hong Kong)

Although there are studies which report the oral prevalence rates of yeasts and coliforms in HIV-infected patients, there is no such data available from Asia. The concentrated rinse culture technique (Samaranayake et al. J Oral Pathol 1986; 15:251-4) was therefore used to study the oral prevalence rates of these organisms in 32 HIV infected patients in Hong Kong. Patients were asked to rinse their mouth for one minute using phosphate buffered saline. The rinse was then concentrated by centrifugation and spiral plated onto appropriate culture plates. The prevalence of yeasts was 65.6% and *Candida albicans* comprised 95% of all yeasts isolates. Coliforms were isolated in 40.6% of all cases and on two thirds of the occasions they were isolated together with yeasts. *Enterobacteriaceae* comprised 55% of all coliforms while *Pseudomonas aeruginosa* was the commonest isolate (30%). No significant association was found between the prevalence rate of yeasts and coliforms with the age groups, CDC classification, CD4+ count and risk groups. In conclusion, comparison with other studies suggests the oral prevalence of yeasts and coliforms in HIV infected patients in Hong Kong may be higher than in other parts of the world. Supported by the Research Grants Council of Hong Kong (Grant No: HKU 274/92M).

- 119** Oral Gram-Negative Rods and Yeasts in Hospitalized Patients. C.M. SEDGLEY<sup>1</sup>, SAMARANAYAKE, W.H. HU<sup>1</sup> and M.T. LEB (Oral Biology Unit, <sup>1</sup>University Department of Medicine, University of Hong Kong, Hong Kong).

While oral epidemiological studies from various parts of the world report diverging point prevalence rates of aerobic facultatively anaerobic Gram-negative rods (AGNR) in hospitalized population groups, there is currently no information on hospitalized patients in Hong Kong. Saline oral rinse samples were obtained from 100 hospitalized patients in Hong Kong to determine the oral point prevalence of AGNR and yeasts. The oral prevalence of AGNR was 59% with the commonly isolated AGNR species being *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Acinetobacter spp.*. Patients over 60 yr (n=48) had a higher prevalence of AGNR than those under 60 yr ( $\chi^2 = 52; p < 0.05$ ). *Enterobacteriaceae* comprised 50% of all AGNR isolated with an overall prevalence of 32%. *Enterobacteriaceae* prevalence was high in patients taking  $\beta$ -adrenergic blocking agents ( $p < 0.01$ ), angiotensin converting enzyme inhibitors ( $p < 0.05$ ), combination of antacids and analgesics ( $p < 0.05$ ) and combinations of antacids and antibiotics ( $p < 0.05$ ). In addition, *Enterobacteriaceae* prevalence was higher in patients hospitalized for longer than 15 days than in those hospitalized for 15 days ( $p < 0.05$ ). The oral prevalence of yeasts was 53%, with *Candida albicans* comprising 75% of all yeasts isolated. Subjects over 60 yr had a higher prevalence of yeasts than those under 60 yr ( $p < 0.05$ ). Patients wearing dentures (n=48) had a higher oral yeast prevalence of 76% than those not wearing dentures (45%) ( $p < 0.01$ ). In conclusion, the prevalence of AGNR in hospitalized patients in Hong Kong may be higher than in other parts of the world. This study supported by the Hong Kong University and Polytechnics Grants Council. Grant 337/268/0002.

- 114** In Vitro and In Vivo Evaluation of Plasmaspray and Magnetron-Sputter Ca-P Coatings. J.E.G. HULSHOFF, J.A. JANSEN\*, W. KALK (Dept. Oral Function, Lab. Biomaterials, Dental School, KU Nijmegen, The Netherlands).

Recently concern has been raised regarding the viable use and prognosis of plasmaspray Ca-P coated dental implants. Therefore, in our laboratory experiments are performed to develop improved, in terms of thickness, degradation and adherence, Ca-P coatings using a magnetron-sputter technique. The purpose of the present study was to test the osteogenic potential of these coatings in *in vitro* cell culture and *in vivo* animal conditions. Two different types of sputter and two plasmaspray Ca-P coatings were used in the experiments. The coatings were characterized by XRD and FTIR. For the *in vitro* study, titanium discs provided with the coatings were used. Osteoblastic cells, using the rat bone marrow culture method, were seeded on these substrates. After incubation periods of 8 hours and 5 days, no significant differences (ANOVA) in cell attachment and proliferation rates were found between the various materials. After 18 days of culture, cells on all surfaces showed similar cell morphology. Furthermore, with SEM no severe degradation of the sputter coatings was seen. They even appeared to induce apatite formation. For the *in vivo* experiments, cylindrical titanium implants were provided with the coatings. The implants were inserted in the left and right femoral condyle of 18 rabbits. Each animal received four implants, one of each type. The animals were killed after 3, 6 and 9 weeks. Then, the implants retrieved and prepared for evaluation of the bone response. Histomorphometry revealed no significant differences (ANOVA) in bone contact for all types of coatings. All plasmasprayed implants showed after 9 weeks signs of coating loss. Supported by the results, we conclude that: (1) Ca-P sputter coatings induce the same bone behavior as plasmaspray Ca-P coatings, (2) magnetron sputtering seems to be a promising method to produce bioactive Ca-P coatings.

- 116** Alkaline and acid phosphatase activities around titanium implants. A. PIATTELLI<sup>1</sup>, A. SCARANO, M. PIATTELLI, L. CALABRESE (Universities of Chieti and Cagliari, Italy).

Alkaline phosphatase (ALP) may play a very important role in the mineralization processes of bone, while acid phosphatase (ACP) is implicated in bone resorption. The demonstration of these enzyme activities is particularly difficult in specimens embedded in a glycolmethacrylate resin (Technovit 7200 VLC, Kulzer, Germany). Aim of the present study was an histochemical characterization of ALP and ACP at the bone-implant interface in plastic-embedded specimens after the insertion of 24 smooth screw-shaped threaded c.p. titanium implants in rabbit tibia. Four implants each were retrieved after one, two, three, four, eight and twenty-four weeks, the specimens were plastic-embedded and processed to obtain thin ground sections. It was possible to observe a very strong positivity in the cytoplasm of osteoblasts near the implant surface in the first three weeks. These osteoblasts surrounded islands of soft tissue or trabeculated woven bone. In the first month a very large quantity of newly formed bone was observed arising from the periosteal and endosteal surfaces. A sharp decrease of the ALP activity was observed from the third week onwards and at two months it was possible to observe that the ALP and ACP activities were of similar entity, possibly in relation to the bone remodelling processes. From two to six months there were no significant morphological differences. This study showed that it was possible to evaluate simultaneously the presence of ALP and ACP activities in the bone growth and remodelling processes around titanium implants in plastic-embedded specimens.

- 118** HIV related oral candidiasis in England and South Africa. SJ CHALLACOMBE\*, SP SWEET, CJ COPE, P ROBINSON, MM COOGAN & C RACHANIS (Oral AIDS Research Centre, Dept. Oral Medicine & Pathology, Guy's Hospital, London, UK)

The prevalence of oral candidiasis in HIV infection is markedly increased and can be a predictor of the progression towards AIDS. However, the majority of studies have focused on homosexual populations in America and Europe. The prevalence of oral candidiasis in HIV infected populations in Africa is less clear. This study compared HIV-antibody positive and negative white homosexual and black heterosexual men attending STD clinics in London, UK and Johannesburg, South Africa, respectively. Mean CD4 counts of the HIV infected patients in the UK group was 321 compared with 290 in the SA group. Parameters studied included the prevalence of oral candidiasis, oral yeast carriage rates and the species and virulence of infecting yeasts. Saliva was collected by expectoration into sterile plastic containers. Yeasts were isolated on Sabouraud's agar, counted and selected colonies speciated using the API 20C AUX system. Oral candidiasis was clinically diagnosed in 35% of HIV infected UK men and 6% of HIV infected SA men ( $P < 0.01$ ). Less than 1% of HIV negative males in either group were diagnosed with oral candidiasis. The percentage of HIV infected SA men with >1000 yeast colony forming units per ml of saliva was not significantly different to HIV negative men (SA or UK), but significantly more HIV infected UK men carried high levels of salivary yeasts ( $P < 0.01$ ). Yeast isolates from the UK group consisted predominantly of *Candida albicans* (97%). In contrast, the SA isolates (HIV+ and HIV-) consisted of 40% *Saccharomyces cerevisiae*, 3% *C. albicans* and many isolates that could not be reliably identified ( $P < 0.01$ ). This study suggests that there is a lower prevalence of oral candidiasis in HIV infected heterosexual SA men compared with UK homosexual men. This may be related to SA men carrying less pathogenic, non-*C. albicans* yeasts and a lower prevalence of high salivary yeast counts, although factors related to race, sexual preference or geographic location may be involved.

- 120** Immunoglobulin producing cells in the gingiva from HIV-infected persons. K. ODDEN<sup>1</sup>, K. SCHENCK<sup>1</sup> and B. HURLEN<sup>2</sup>. (Depts. of Oral Biology<sup>1</sup> and Oral Surgery and Oral Medicine<sup>2</sup>, Dental Faculty, Univ. of Oslo, Norway)

B-cell abnormalities like hypergammaglobulinaemia and reduced antibody response to foreign antigens are found in persons with human immunodeficiency virus (HIV) infection. The aim of our investigations are to explain the increased susceptibility to periodontal diseases of HIV-infected persons as compared to HIV seronegative persons. The present study was undertaken to determine whether abnormalities in the number of immunoglobulin producing cells in inflamed gingival tissues could contribute to the explanation of this increased susceptibility. Gingival biopsies were taken from 12 HIV seropositive and 10 HIV seronegative persons with periodontitis. Five of the HIV seropositive persons were categorized as having advanced periodontitis, based on rapid loss of periodontal support and gingival pain. The biopsies were washed for 48 hours in cold PBS and fixed in ethanol according to the method of Saine-Marie (J Histochem Cytochem 1962; 10: 250). Sections of 5  $\mu$ m were stained with monoclonal antibodies against IgG, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgA, IgA<sub>1</sub>, IgA<sub>2</sub>, and IgM and positive staining was visualized by indirect immunoenzymatic methods. Stained cells were counted in 3 fields, outlined by an ocular grid, at  $\times 500$  magnification in the densest inflammatory infiltrate subjacent to the pocket epithelium. IgG was the most frequent isotype observed in both groups of patients, followed by IgA. Both groups had very few IgM-producing cells. The HIV seropositive patients had significantly fewer IgG<sub>2</sub> producing cells compared to the HIV seronegative patients (Student's t-test,  $p < 0.05$ ). The HIV seropositive patients with advanced periodontitis had significantly fewer IgG<sub>2</sub> producing cells than the HIV seronegative patients with moderate periodontitis ( $p < 0.05$ ). IgG<sub>2</sub> is produced in response to carbohydrate antigens and is considered important in the defence against bacterial infections. IgG<sub>2</sub> is found in long-term inflammatory reactions. The low numbers of IgG<sub>2</sub> and IgG<sub>3</sub> producing cells in the gingiva of HIV seropositive patients might therefore contribute to a reduced defensive capacity of the gingival inflammatory infiltrate.