



Title	Quantitative assessment of early healing of autogenous bone grafts using micro-computed tomography and Q-win image analyser
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54 *C. glabrata* exhibits superior relative cell surface hydrophobicity and adhesion to denture acrylic surfaces compared with *C. albicans*. ²G. LUO* and ¹L.P. SAMARANAYAKE (University of Hong Kong, Hong Kong, ²West China University of Medical Science, Chengdu, China)

Oral candidosis is a common opportunistic infection in debilitated individuals and *Candida glabrata* is the second most frequently isolated species from this condition, after *Candida albicans*. Candidal adherence to various biological or non-biological surfaces is considered a prerequisite for colonization, and pathogenesis of candidal infections, and their relative cell surface hydrophobicity (CSH) is likely to be a possible contributory force involved in this process. Whereas a large body of data on the latter features of *C. albicans* is available, there is surprisingly little information on *C. glabrata*. As a comprehensive database on the relative adhesion and CSH of *Candida spp.* is instructive and useful, we investigated *in vitro* the latter attributes of 34 oral isolates of *C. glabrata* and 15 isolates of *C. albicans*. There were remarkable intra-species differences in both the CSH and the adhesive ability of *C. glabrata* strains ($P < 0.001$). Compared with *C. albicans*, *C. glabrata* demonstrated a four fold greater CSII value (30.63 11.20% vs. 7.23 3.56%, $P < 0.0001$) and a two fold greater tendency to adhere to denture acrylic surfaces (75.18 39.96 vs. 30.36 9.21, $P < 0.0001$). A significant positive correlation between CSH and adhesion was also noted for both *C. glabrata* ($r = 0.674$, $P < 0.0001$), and *C. albicans* ($r = 0.636$, $P < 0.05$). When the effect of different incubation conditions on the relative CSH and adherence of *C. glabrata* was examined, CSH and the adherence to acrylic surfaces of four of six *C. glabrata* isolates were significantly affected by a reduction of the culture temperature (from 37°C to 25°C). A positive relationship also emerged when the temperature induced variation in the adherence values were correlated with their relative CSII. These data provide hitherto unavailable archival information on important pathogenic attributes of the two most common oral *Candida* species that may help explain their predominance in this milieu.

55 Relationship between caries in primary teeth and defects in permanent teeth. C.G. ZHENG*, E.C.M. LO and N.M. KING (Faculty of Dentistry, Hong Kong University)

The objective of the study was to investigate the relationship between the presence of developmental defects on enamel (DDE) on permanent teeth and the caries status of their predecessor primary teeth in a cohort of Chinese children in a non-fluoridated area. The target population was 288 children who had been involved in a previous longitudinal study. The caries status of their primary teeth from age 3 to 7 years was recorded annually. Almost 85% of these children had caries in their primary teeth and over 80% of the caries were untreated. This study was carried out in 2001 and 250 children at the age of 11-12 years were followed up. Presence and the type of DDE was determined by consensus of two trained dentists and recorded according to the modified DDE Index for each surface of the permanent incisors, canines and premolars. The results showed that 14.6% of the teeth had one or more DDE, with diffuse opacities being more commonly found than demarcated opacities, on 9.7% and 5.7% of the teeth respectively. The tooth prevalence of hypoplasia was only 0.8%. Caries in primary teeth, was found to be related to the presence of DDE in their permanent successors. For example, if the upper primary incisors had a large caries lesion at the age of 4 years, then DDE was found on 39% of their permanent successor teeth, while DDE was found on 23% of the permanent incisors if their primary predecessor teeth had no caries ($P < 0.001$).

56 Development of Novel Oligonucleotide Probes for Seven *Actinomyces* Species and Their Utility in Supragingival Plaque Analysis. G. TANG*, H.K. YIP, L.P. SAMARANAYAKE, G. LUO, B.P.K. CHEUNG and S. SIEN (University of Hong Kong, Hong Kong)

The traditional, biochemical and enzymatic methods of identifying *Actinomyces* species are frequently confounded by the similar phenotypic characteristics shared by the different members of this genus. Therefore, we developed novel species-specific oligonucleotide probes to accurately speciate seven, common pathogenic *Actinomyces* species; namely, *A. bovis*, *A. gerencseriae*, *A. israelii*, *A. meyeri*, *A. naeshlundii*, *A. odontolyticus* and *A. viscosus*. A pair of universal primers and seven, 15- to 19-base oligonucleotide probes with a tail of 20 thymidines on the 5' end were developed. The variable regions of 16S ribosomal DNA of 34 strains of *Actinomyces* belonging to the above species were amplified and labeled with digoxigenin and, an oligonucleotide-DNA hybridization assay was performed to examine the specificity and sensitivity of these probes. All seven, newly developed probes were specific and sensitive, and accurately detected 34 wild type and reference laboratory strains belonging to *Actinomyces* species, without cross-reactors. The new probes were then field tested using supragingival plaque samples from 28 healthy pre-school children. Whilst *A. odontolyticus* was detected in almost all samples (96.4%), *A. gerencseriae*, *A. meyeri*, *A. naeshlundii* and *A. viscosus* were detected in less than 50% samples. We conclude that the developed oligonucleotide probes, complementary to the variable regions of 16S rDNA, would be of potential value for differentiating *Actinomyces spp.* in clinical samples from the oral cavity and other ecosystems where such species may abound. This study was supported by RGC grant of Hong Kong (10202943) and CRCG of Hong Kong University (10203286).

57 The mechanism of apoptosis in bone cell after implant particle induction. J.M. LIU*, T.M. WANG, M.S. LEE, S.K. LIN, J.S. WANG, L.D. LIN and C.Y. HONG (Department of Dentistry, National Taiwan University, Taiwan, R.O.C.)

Biomaterial implant are widely used in dental and orthopedic fields. Despite their widespread success, implant failure due to infection or aseptic loosening remains a major clinical problem. Although longitudinal observations include that implant wear is inevitable, there are few studies focusing on bone cell response after implant particle induction. The purposes of this study were to clarify particles-induced cellular responses and to explore the possible mechanism behind the action of particles on cells. Four kinds of particles were used in this study-commercially pure titanium (cpTi), titanium nitride (TiN), titanium alloy (Ti-6Al-4V), and Co-Cr-Mo. Two human osteoblast cell lines (MG-63 and U-2 OS) were employed in the experiments. Particle characterization was done by SEM observation and Auger electron analysis. TdT-mediated dUTP Nick End Labeling (TUNEL) staining was used for observation cell apoptosis. Besides, we used western blot to evaluate the apoptotic-pathway protein expression level after particle induction. Results indicated that particles were varied in size and distribution under SEM observations. Auger electron analysis showed that particles surface element impurity. TUNEL staining indicates that only cpTi and TiN induce bone cell apoptosis. The apoptosis may *via* p53 independent Fas pathway. This study was supported by National Science Council R.O.C., grant NSC-90-2314-B-002-348

58 Molecular Mechanism of Hanatoxin Binding-Modified Gating in Voltage-Gated K⁺-Channels Revealed by Docking Simulation. P. T. Huang*, Y. Y. Shiau, K. L. Lou, (Graduate Institute of Oral Biology, Medical College, National Taiwan University, Taiwan)

Voltage-gated potassium (Kv) channels respond to the membrane excitation and allow repolarization of an action potential for muscle contraction. While S4 is known as the voltage sensor in Kv channels, the carboxyl terminus of S3 (S3_c) is of particular interest concerning the site for gating modifier toxins like Hanatoxin (HaTx). In order to have better understanding of such details, we have previously examined the putative three-dimensional structure of S3_c and illustrated the residues required for HaTx binding by performing the molecular simulation and docking. Analysis on the detailed binding residues and appropriate orientations regarding hydrophobic/philic environments has been leading to a hypothesis suggesting a specific pocket allowing HaTx to bind onto channels in proximity of the external crevice. In addition, superposition of the structures of *drk1* S3_c and HaTx before and after docking revealed a significant movement of S3_c in the direction presumably towards S4. It was indicated that both hydrophobic and electrostatic interactions are utilized to stabilize the toxin binding. This was then comprehended as a possible factor to interfere S4 translocation during gating.

59 Quantitative assessment of early healing of autogenous bone grafts using micro-computed tomography and Q-win image analyser. M. LU* and A.B. RABIE (Hard Tissue Lab., Faculty of Dentistry, the University of Hong Kong).

Micro-computed tomography (μCT) is a new tool to image and quantify trabecular bone. The aim of this study is to evaluate the early healing of intramembranous and endochondral autogenous bone grafts with μCT and Q-win image analyser, and to compare the results from both techniques using descriptive statistics and correlation analysis. Twelve critical size (15×10mm) defects were created in rabbit mandibles bilaterally. Six defects were grafted with autogenous EC bone; six defects were grafted with autogenous IM bone. Three weeks after surgery, the defects were retrieved for μCT imaging analysis and histological evaluation. Results showed a significant correlation ($r = 0.96$, $p < 0.0001$) between μCT and Q-win image analyser to measure the volume of new bone and graft bone in the mandibular defects after 3 weeks of early healing. The volume of new bone in IM bone group was proven to be more than that in EC bone group by both μCT (55.3%, $p < 0.0001$) and Q-win image analyser (62.4%, $p < 0.0001$). The graft bone volume maintenance in IM bone group was proven to be better than that in EC bone group by both μCT and Q-win. Our results indicated that IM bone was superior to EC bone with a clearly 2D and 3D inlay graft bone survival in the defects. μCT imaging is a non-destructive, fast and very precise procedure that allows for qualitative and quantitative evaluation of the early healing of IM and EC autogenous bone grafts in membranous bone defects. This study was supported by CRCG-HKU, HKSAR, No.10117-10203285-12767-08002-323-01.

60 Epigallocatechin-3-gallate suppresses oncostatin M-induced chemokine expression in MG-63. T.C. YEH* AND S.K. LIN (National Taiwan University, Taiwan)

Monocyte chemoattractant protein-1 (MCP-1) is essential to inflammatory bone resorption. Previous studies have demonstrated that MCP-1 is produced primarily by osteoblasts in periapical lesions. The purpose of this study was to investigate the signaling pathway involved in OSM-induced MCP expression and the modulating effects of epigallocatechin-3-gallate (EGCG) in this process. Northern analysis showed that OSM stimulated MCP-1 expression in MG-63 (a human osteoblast cell line) and EGCG inhibited this effect. Western analysis showed that OSM induced phosphorylation of ERK1/2 and MEK1/2 as well as dephosphorylation of Raf-1 (Ser 259), whereas EGCG inhibited these effects. Immunoprecipitation/Western blot showed that OSM induced phosphorylation of Raf-1 (Ser 338) and EGCG abolished this effect. OSM also stimulated c-Fos expression, and EGCG and PD-98059 (MEK specific inhibitor) abolished this effect. Electrophoretic mobility shift assay revealed that OSM stimulated activator protein-1 (AP-1)/DNA binding in MG-63, whereas EGCG and PD-98059 inhibited the formation of AP-1/DNA complex. In conclusion, EGCG inhibits OSM-induced AP-1/DNA binding by modulating the phosphorylation of Raf-1 (Ser 259, Ser 338) and the MEK/ERK signaling pathways, resulting in the down-regulation of MCP-1 gene expression in MG-63.

61 Saliva Profile of Irradiated Cancer Survivors and Sjögren's syndrome patients. E.H.N. POW*, K.C.M. LEUNG, A.S. McMILLAN, W.K. LEUNG, M.C.M. WONG, D.L.W. KWONG, C.S. LAU, T.M.Y. MOK. (University of Hong Kong, Hong Kong, SAR)

Radiotherapy is commonly used in the treatment of head and neck cancer. Sjögren's syndrome (SS) is an autoimmune disorder characterized by progressive lymphocytic infiltration of exocrine glands particularly the salivary and lacrimal glands. Both conditions induce salivary gland damage resulting in xerostomia and associated increased oral disease. We aimed to investigate saliva characteristics in irradiated nasopharyngeal carcinoma (NPC) survivors and SS patients. 38 NPC survivors (disease-free for >1 year), 51 SS patients (diagnosed for >1 year) and 60 age and gender matched controls took part. Stimulated whole saliva (SWS) flow, pH and buffer capacity were measured. Data were compared using Kruskal-Wallis and Chi-squared tests. SWS flow was least in NPC survivors and greatest in controls ($p < 0.01$). NPC survivors had lower pH and poorer buffer capacity than SS patients and controls ($p < 0.01$). No difference in pH and buffer capacity was found between the SS patients and controls. Radiotherapy for NPC seems to induce greater qualitative and quantitative damage to salivary glands than Sjögren's syndrome. In SS patients, the change in saliva appears to be predominantly quantitative. Supported by CRCG-HKU