Beffect of Some Antifungal Drugs on Adherence of Candida albicans.

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The aim of this study was to compare the effect of 4 antifungal drugs; miconazole, fluconazole, ketoconazole and clotrimazole on adherence of Candida albicans to human buccal apithelial cells (BEC) in witro. Epithelial cells were collected from the buccal mucosa of 5 healthy male volunteers, free of oral candidosis and not taking drugs. Cells, washed with prosphate buffered saline(PBS) and resuspended at (2 x 10' cells/ml), were mixed and incubated with drugs at minimum inhibitory concentrations on a shaker at 100 rpm at 37 °C for 1 h. Three series of experiments were performed involving: preincubation of BECs with drugs; preincubation of C. albicans with drugs; direct addition of drugs to a mixture of BECs and C. albicans. Cells were harvested on 12µm-pore polycarbonate. Filters (Millipore, U.S.A.) and washed with PBS to remove unattached yeast. The washed cells were air-dried and Gram-stained. The number of yeast attached to 50 BECs was counted. All results were statistically analysed using ANOVA. The results showed that all drugs: miconazole, keltoconazole and clotimazole significantly reduced (P<0.01) the adherence of C. albicans to BECs (by more than 50%). Overall, preincubation of BECs with drugs or yeast with drugs gave greater inhibition of adherence than direct mixing, and no significant interfaces were found between different drugs. However, with direct addition of drugs to the mixture of BECs and C. albicans, some differences in drug efficacles were observed, possibly due to a time requirement in the interaction of drug to cell. In conclusion, imidazole drugs decrease the adherence of C. albicans to buccal epithelial cells with the effectiveness of inhibition of miconazole, ketoconazole and clotrimazole being similar.

Role of denture pellicles in Candida albicans biofilm development invitro.
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There is little data on the long term activity of Candida albicans biofilm on mucinous pellicles once the initial attachment process is complete. Thus the effect of either a salivary or serum pellicle on initial and subsequent stages of C. albicans biofilm formation on denture acrylic was investigated by the luminescent ATP (adenosine triphosphate) analysis (Antimicrob Agent Chemother 37: 2618-21, 1993) and scanning electron microscopy (SEM). Calbicans biofilm was allowed to form on the salivar, serum-cated and protein-free control acrylics according to our previous study (Archs Oral Biol 38:631-4,1993). When the biofilm formation on saliva-coated acrylic strips was examined, the yeast initially colonized this surface at a slower rate than the control (ANOVA, p-0.01), although with increasing incubation time, at 72 hrs, the ATP content was almost ten-fold higher than the control (p-0.01). Biofilm activity of the serum-coated specimen was almost 100-fold greater within 48 hrs incubation than that of control (p-0.01). SEM and immunocytochemical observation revealed that multilayered blastospore-blastospore co-adhesion, germ tube, hyphal and pseudohyphal emergence and blastospore-hyphal co-adherence were involved in the process and that Con-A binding material, fibrone-ctin, mannan-binding protein and/or protein-A binding materials (IgG and IgM) contributed to this phenomena, which were not observed in the control. These results taken together suggest that the candidal binding formation on acrylic strips is multifaceted in nature and is related to the growth phase, hyphal emergence, coadhesion and pellicle proteins, and most importantly perhaps, the milleu in which the biofilm develops.

Antifungal activity of lactoferrin and lysozyme against Candlda species. Y. H. SAMARANAYAKE\*, P.C.WU AND L. P. SAMARANAYAKE (Department of Pathology & Oral Biology Unit, University of Hong Kong)

Lactoferrin and lysozyme are non-immune defence factors present in polymorphonuclear leucocytes and various exocrine secretions including saliva. Previous studies have shown that both proteins either singly, or in combination are bactericidal in nature and their combined activity is synergistic. Few workers, however, have studied these interactions with Candida species and therefore we evaluated the susceptibility of 20 oral isolates of C. krusei and 5 isolates of C. albicans to both lactoferrin and sysozyme; the combined activity of the two proteins was assessed against one isolate from each species. To study the effect of each protein, standard concentrations of yeast suspensions grown for 18h in Brain Heart Infusion Broth were exposed to  $20~\mu g/ml$  of human lactoferrin or  $30~\mu g/ml$  of hen-egg-white lysoxyme (Sigma, USA) at  $37^{\circ}$ C for 150 min. The combined fungicidal effect of 20, 40 and  $80~\mu g/ml$  of two proteins was assessed using appropriate dilutions of the latter. Afterwards the viable yeasts were assessed by culturing  $50~\mu l$  of suspension on Sabourauds agar, incubating at  $37^{\circ}$ C and quantifying the resultant growth (CFU). The two Candida species exhibited significant interspecies differences in their susceptibility to lactoferrin (p < 0.05), but not for lysozyme; C. kruseibeing more sensitive to lactoferrin than C. albicans. Both species revealed significant intra-species difference in their susceptibility to lysozyme (p < 0.05), but not for lactoferrin. No synergistic antifungal activity of the two proteins on either Candida species was noted. These results imply that both lactoferrin and iysozyme may act variably on Candida species and modulate the oral carriage of yeasts in a very complex manner.

Clinical comparison between a manual and an electronic periodontal probe.

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Pocket depth is an important clinical aspect in the diagnosis of periodontitis. The degree of accuracy has a strong influence on the therapy and therefore the prognosis of the disease. Electronic probes are supposed to give more constant and reproducible values because there is no influence of the examiner. In the present in vivo study the results of manual (Hu-Friedy) and electronic (Peri-Probe, Vivadent) propring should be compared.

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30 adults with periodonitiis took part in the study. The measurements were carried out on six sites of each tooth manually and then with the electronic probe. The same procedure was done twice again after four weeks, respectively. All the data obtained and especially the differences between the same teeth of the patients and similar pocket depth values were evaluated using the program EXCEL 6.0. The mean pocket depth gained by manual measurement were 2.9 mm ± 1.2 mm with a reproducibility of 0.1 mm. Regarding the electronic device the mean values were 2.4 mm ± 1.3 mm and the reproducibility 0.1 mm. They varied for the incisors: 2.4 mm ± 1.0 mm and for the molars 3.5 mm ± 1.5 mm measuring manually. Electronically the incisors showed mean values of 1.9 mm ± 1.1 mm and the molars 2.8 mm ± 1.6 mm. The differences between manual and electronic

1.1 mm and the molars 2.8 mm ± 1.6 mm. The differences between manual and electronic measurements were statistically significant (MILCOXON-TEST). The results obtained electronically were in general 0.5 mm lower. The differences turned out to be the same regarding deeper pockets. Concluding, the results of the study indicate that both probes can be considered in clinical evaluation, but the electronic probe has no diagnostic advantage.

370 Humoral immune responses in Candida-associated denture stomatitis. D.H. FELIX\*,¹ V. BISSELL,² D. WRAY¹ (¹Department of Oral Medicine, Glasgow Dental Hospital and School, ²Department of Restorative Dentistry, Edinburgh Dental Hospital, U.K.)

Previous studies have demonstrated an increased incidence of Candida carriage and infection among cigarette smokers when compared to non-smokers. However the mechanisms underlying this difference remain unclear. The aim of the present study was to investigate the effects of cigarette smoking on the immune response in patients with Candida-associated denture stomatitis. Fifty two patients (24 smokers, 28 non-smokers) were investigated. Samples of serum and whole saliva were obtained before the start of treatment and at one, four and twelve weeks thereafter. Total serum and salivary IgG and IgA concentrations were measured by a commercially available radial unodiffusion assay (Behringwerke AG, Marburg, West Germany); serum and salivary anti-Candida IgG and IgA levels were measured using a double sandwich ELISA technique. Data was analysed using Mann-Whitney and Wilcoxon rank sum non-parametric tests. Before the start of treatment serum anti-Candida IgG levels in non-smokers (476 ± 98 [S.E.]) were significantly higher than in smokers (170 ± 30); p = 0.001. A similar difference was observed at all other times throughout the study. Total serum IgG levels were higher among non-smokers at baseline and week one (p < 0.05). Total serum IgA concentrations were higher among non-smokers at weeks one, four and 12 (p < 0.05). No significant differences were observed in salivary immunoglobulin concentrations. This study provides evidence of the effects of cigarette smoking on the immune response and may help to explain the higher incidence of Candida carriage and infection seen in

A Model to Study Relationships between Candida albicans and Oral Bacteria.

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A breakdown of the microbial homeostasis in the oral cavity may lead to the proliferation and overgrowth by *C. albicans* causing oral candidosis. The purpose of this study was to establish and identify a mixed community of oral bacteria that will control the growth of *C. albicans* in the chemostat and that can be used to investigate cause-and-effect relationships between the oral bacteria and *C. albicans*. A chemostat was operated under glucose-limiting conditions and at a dilution rate of  $D = 0.05 \, h^{-1}$ , a temperature of  $37^{\circ}C$ , a pH of 7 and under a gas phase of 5% CO, +95% N<sub>2</sub>. The chemostat was inoculated with a suspension of a tongue scraping on three different occasions. After steady-state conditions have been reached it was inoculated with *C. albicans* on two separate occasions in order to establish the yeast and determine if yeast growth will be contained. The steady-state communities of three separate experiments always consisted of the species *Streptococcus sanguis*, *Streptococcus sobrinus*, *Streptococcus minis*, *Lactobacillus casti*, *Eubacterium saburreum* and *Veillonella dispar*. *Fusobacterium nucleatum* and *Haemophilus segnis* were also present in two of the experiments. Yeast growth was suppressed in all three experiments. The yeast cells were lost at the same rate as the theoretical washout rate and after 6 days the yeast counts fell to 10 cfu/ml. This mixed community of oral bacteria can be used in the chemostat as basis for further studies to determine the parameters of oral significance that influence the relationships between the oral bacteria and *C. albicans*.

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Reproducibility and validity of furcation measurements using a pressure-calibrated probe.

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The furcation involvement of 100 molars in 25 patients suffering from moderate to advanced periodontitis was investigated. The horizontal probing attachment level (PAL-H) within the furcations was assessed twice within 2 weeks using the pressure-alibrated (0.25 N) flexible plastic universal explorer version of the TPS Probe (TPS). To determine the measurement error of PAL-H assessments, the standard deviation of single measurements was calculated. The measurements were repeated using a color-coded Nabers Probe and compared to the TPS assessments. 253 furcations were evaluated (100 buccal, 47 lingual, 53 mesiolingual and 33 distolingual, respectively). For buccal, lingual, mesiolingual and distolingual furcations, the standard deviations were 0.486 mm, 0.598 mm, 0.846 mm, 1.039 mm, respectively. Measurement error was less in buccal and lingual furcation and lingual furcation in mesiolingual and distolingual site (p < 0.005). The agreement of replicate measurements of furcation degrees was excellent for buccal and lingual furcations (weighted kappa (6.88 [0.096] and 0.749 [0.011], respectively). Only in distolingual sites there was a significant (p < 0.025; paired t-Test) underestimation of PAL-H by the TPS as compared to the Nabers Probe. At all locations the TPS underestimated furcation degrees significantly (p < 0.1; Stuart-Maxwell's  $\chi^2$ ) as compared to Nabers Probe. Measurement error of mesiolingual and distolingual furcations is significantly higher than of buccal or lingual sites. The reproducibility of PAL-H measurements in buccal and lingual furcations is comparable to data published for vertical PAL measurements (e. g. GOODSON 1986). Through and through furcations are likely to be underestimated with the flexible plastic version of the TPS Probe, whereas for degree 0 to II furcation invasions it provides valid information as compared to a color-coded Nabers Probe.

A 11-Year Periodontal Examination Using CPITN in Young Females.
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Periodontal conditions of the female students have been examined at a high school for 11 years, using the CPITN probes. Examination were carried out on 6 index teeth (11, 16, 26, 31, 36 and 46) by dentists in our department who underwent the training on the criteria of WHO in advance. The total subjects examined were 15,003. The sum of the CPITN code in each sextants of a subject was named as the T-value. Subjects were classified into 3 groups according to the T-values, i.e. the slight group (  $0 \le T$ -value  $\le 6$  ), the moderate group (  $7 \le T$ -value  $\le 12$  ), the severe group ( 13≤ T-value ). The prevalence rates were expressed by the T-values. The subjects grouped to the severe were recalled and treated at our bospital. At the end of this 11 year examination, the number of subjects in the slight and the severe groups decreased to approximately 50% and the moderate group increased approximately 3 times of the first year level. The number of sextants having Code 0 and Code 3 decreased approximately a half of the initial level. Decrease in Code 1 and Code 4 was observed. While, the number of sextants having Code 2 increased twice or more. Drastic decrease of the severe group and the number of subjects having Code 3 and Code 4 was brought about our recall system. In conclusion, the subjects having calculus and slight gingival inflammation occupied a large part of this teenage population. Screening and taking care of the subjects suffering from severe periodontitis at the early stage is effective to prevent the progress of the diseases