33 Effect of moist and dry bonding on interfacial failure modes after microtensile bond-testing.

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This study tested the null hypothesis that the application of acetone-and ethanol-based, simplified-step adhesives with a moist or a dry bonding technique produced the same pattern of interfacial failure following a "non-trimming" microtensile bond-testing method (Shono *et al.*, 1999). Eight extracted, caries-free, human third molars were divided into four groups. The occlusal enamel was removed, leaving a flat dentin surface for bonding. Resin composite buildups were made after the acid-conditioned dentin surfaces were bonded with either one of the two adhesives, Single Bond (SB) or One-Step (OS), and using either one of the two bonding techniques (moist bonding or air-drying for 5 s). After being stored in water at 37°C for one day, the teeth were vertically sectioned into 0.9mm x 0.9mm composite-dentin beams. Two teeth from each group yielded between 42 – 48 beams for bond testing. Each beam was assigned an x-y coordinate and tested for tensile bond strength. Regional mapping of the tensile bond strength was performed for each tooth. Following initial classification of the failure modes with a stereoscopic microscope, fractured dentin and composite sides of eight representative beams from each group were prepared for scanning and transmission electron microscopic examination. Results: microtensile bond strength for SB moist; 60.75±12.03 MPa; SB dry:25.74 ± 6.66 MPa; OS moist: 57.21 ± 12.30 MPa; OS dry: 27.10 ± 8.45 MPa. A two-way ANOVA based on ranks showed a statistically significant difference in the effect of the bonding technique (moist *vs.* dry; $p < 0.001$) but not of the adhesives (Single Bond *vs* One-Step; $p = 0.547$) on tensile bond strength, and that the effect of different techniques was independent of the adhesives used ($p = 0.201$). Cohesive failure within the incompletely infiltrated hybrid layer occurred in both dry bonding groups. The surface of the fractured hybrid layer was retained together with the dentin adhesive on the composite side of the fractured beam. In both moist bonding groups, the full thickness of the hybrid layer was found on the dentin side of the fractured beam, with remnants of the dentin adhesive and resin composite. Cohesive failures within the more optimally infiltrated hybrid layer and/or the underlying dentin were seen in specimens that yielded high bond strength. Conclusion: the results of the present study suggest that moist bonding produce optimally infiltrated hybrid layer with high microtensile bond strength.

34 A Comparative Study Of Caries Inhibition By Some Fluoride Releasing Materials

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Secondary caries is a continuing problem in restorative dentistry and the major reason for restoration replacement. This laboratory study was designed to evaluate the caries inhibition effect of fluoride releasing restorative materials. Class V cavities were prepared on the buccal surface of 120 caries free extracted premolars. The occlusal cavosurface margin of each preparation was on the enamel and the gingival cavosurface margin was on the root surface. Fifteen teeth were randomly assigned to one of eight test groups. The materials used were grouped as control group (Z100), fluoride releasing composite resin group (Heliomolar RO), conventional glass ionomer group (Glasionomer & Fuji II), resin modified glass ionomer group (Fuji II LC (Improved) & Vitremer), and compomer group (Dyract AP & F2000). All the surfaces of the tooth, except for a 2 mm zone adjacent to cavosurface margin, were covered with nail varnish. The teeth were placed in acidified gel at pH 4.5 for three weeks. The teeth were subsequently sectioned longitudinally to obtain three sections. Each section was made 100 ± 20 µm thick. The sections were imbibed in quinoline and examined under polarised light microscope. The surface and wall lesion of both enamel and dentine margins were measured. One-way ANOVA showed that there was a statistically significant difference between the mean lesion depth for the restorative materials at the different test sites. Post-hoc Scheffe's analysis showed that the mean lesion depth at the different test sites for F2000 and Heliomolar RO were significantly different from the other materials except with Vitremer and F2000. The mean enamel wall lesion depth for Vitremer and F2000 were not significantly different from F2000 and Heliomolar RO. Conventional glass ionomer cement, resin modified-glass ionomer cement and compomer had significantly smaller lesions as compared to fluoride releasing composite resin and non-fluoride releasing composite resin. The difference in lesion depth for conventional glass ionomer cement, resin-modified glass ionomer cement and compomer were not statistically significant. All statistical testing were performed at $P > 0.05$.

35 Correlation Between the Cyclin A and p53 Gene Expression in Oral Carcinogenesis.

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Cyclins and wild-type p53 protein are prime cell cycle regulators and may be involved in tumorigenesis. Cyclin A is a late S cyclin and its abnormalities have been reported in several cancers, but not in oral squamous cell carcinomas. In order to investigate the cyclin A expression and its correlation with p53 gene in oral premalignant lesions (OPLs) and oral squamous cell carcinomas (OSCCs), a total of 39 samples were evaluated for the expression of cyclin A and p53 gene by an immunohistochemical method using a labelled polymer assay. These samples comprised two hyperkeratotic and three oral premalignant lesions (two moderate and one severe dysplastic lesions), and 27 OSCCs, together with seven healthy controls. In the second part of the study, the Tca8113 squamous cell line with abnormality in p53 gene was transfected by vectors carrying i) wild-type p53 gene, ii) mutant p53 gene, and iii) no additional gene (control) using electroporation techniques to further confirm the correlations between the cyclin A expression and p53 gene. The cell line with no vector transfer served as the blank control. Then, the cells were ex-planted into four groups of naked mice with four mice in each group. After tumor development, the mice were sacrificed, the tumors collected and processed using routine histopathological techniques. The aforementioned immunohistochemistry assay was used to study the expression of cyclin A. The results demonstrated that the cyclin A was localized and highly expressed in the nuclei of the tumor cells of OSCCs as well as in OPLs. Although there was no correlation between cyclin detection and the local lymph node involvement, a positive correlation was noted between cyclin A and the expression of p53 gene ($P<0.05$). Tumors from naked mice revealed significantly decreased level of cyclin A expression in the wild-type p53 gene group, as compared with the mutant p53 gene group or the no gene transfer group ($P<0.05$), which were similar to the blank controls ($P>0.05$). Taken together, the foregoing suggest that cyclin A may contribute to the progression of oral cancer and correlates to some degree with that of the p53 gene activity. This work was supported partially by a grant from the Committee on Research and Conference Grants, the University of Hong Kong.Grant No. 10201937/30713/08011/301/01

36 Detection of *Candida albicans* by Polymerase Chain Reaction from paraffin embedded tissue – A Preliminary Study

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Candida albicans play a role in promoting oral neoplasia. The aim of this study was to obtain rapid identification of *Candida albicans* using Polymerase Chain Reaction (PCR). The samples consist of biopsy specimens fixed with formalin and embedded in paraffin taken from 4 cases clinically identified with fungal infection (Table 1, Case no. 1-4). Biopsy specimens from a case of aspergillosis and leukoplakia were used as control (Table 1, Case no. 5-6). All specimens were cut to 4 µm thick and stained with H & E and eosin, PAS and Grocott method. Immunohisto chemical analysis was also performed using anti-*Candida albicans* mouse monoclonal antibody (Chemicon International MAB806). Three paraffin sections of 10 µm thick were used for DNA extraction using DEXPAT (Takara) kit for PCR analysis. PCR was performed with a combination of specimens specific primers. The PCR products were then subjected to electrophoresis and the electrophoretically separated DNA fragments were transferred to N+ nylon membrane (Amersham) and subjected to southern hybridization following the manufacturer's protocol with 32dCTP labeled random primed *Candida albicans* by PCR and confirmed by DNA sequencing. The results were as follows:

Case No.	Age	Sex	Site	Diagnosis	PCR (fungi)	PCR (*Candida albicans*)	Southern blot (*Candida albicans*)	Immunohisto-chemistry	PAS	Grocott
1.	61	M	Palate	Ulcer	(+)	(+)	(+)	(+)	(+)	(+)
2.	55	M	Tongue	Median R.G.	(+)	(−)	(+)	(-)	(+)	(−)
3.	67	M	Tongue	Moderate dysplasia	(+)	(-)	(-)	(-)	(+)	(−)
4.	71	M	Buccal	Leukoplakia	(+)	(−)	(+)	(+)	(+)	(−)
5.	67	F	Tongue	Leukoplakia	(+)	(-)	(-)	(-)	(+)	(+)
6.	32	M	Maxillary Sinus	Aspergillosis	(+)	(-)	(-)	(-)	(+)	(+)

In conclusion, this study supported the usefulness and effectiveness of using PCR in rapid identification of *Candida albicans*.

37 Lactic Acid Bacteria is Beneficial For Oral Aphthous Ulcerations.

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Lactic acid bacteria stimulates the immune system in animals and humans, and enhances resistance to infections, tumours, phagocytosis and serum IgA production. To determine whether it is beneficial in the treatment of oral aphthous ulcerations which has an immunopathic basis, we studied the responses of patients with the disease on treatment with OMX capsules which contain twelve strains of lactic acid bacteria. Twenty-five patients with oral aphthous ulcerations were each prescribed six OMX capsules daily. At the first appointment the number of ulcers, locations, diameters and the degree of pain (severe, moderate, slight, no pain) were recorded. Their differential blood counts, haemoglobin content, serum iron, serum ferritin, serum and red-cell folate and Vitamin B12 values were determined. Each patient was instructed to record daily the number of ulcers present, their sizes, locations and the degree of pain experienced , and to record the four parameters of any new ulcers that might developed. We re-examined each patient at two-weekly interval for six months. No control group was used. Seventeen patients (73.9%) became free of the disease and six patients (26.1%) had very dramatic improvements six months later. Two patient dropped out of the trial. We conclude that lactic acid bacteria (OMX capsules) is beneficial in the treatment of oral aphthous ulcerations.

38 Cytotoxicity of *Jatropha curcas* (Euphorbiaceae) latex on fibroblast by MTT assay.

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The latex of *J. curcas* (getah jarak, Indonesian local name) has been used as traditional plant medicine among others to alleviate toothache (in Indonesia and Philippine). The aim of this study was to evaluate the cytotoxicity of this latex to fibroblast L929 cell line and human gingival fibroblast primary cell culture. Cells were cultured with DMEM-FCS in 96-well microplates. At confluency the media were changed and added by 7 diluted freeze-dried latex with the concentration ranging from 0 to 2,500 µg/ml in medium. All measurement were done in triplicate. After an exposure of 1-3 days, the cytotoxicity was assessed by MTT assay. 20 µl of 5 mg/ml MTT in distilled water was added to each well and incubated for 4 hours. At the end of incubation time 50 µl of 20% SDS was added to each well and left overnight. Absorbance of converted dye was measured by microplate reader at 540 nm with background substraction at 690 nm. Higher value indicates more living cells. Because the latex had its own OD at 540 nm, the obtained values were then substracted by the OD of latex. The result showed that the number of Fib-L929 cells was half of that of control at 625 µg/ml latex. After 2 days the ODs at 0 µg/ml was 0,992 (± 0.016, SD) and at 625 µg/ml was 0.473 (± 0.039). No living cells were observed at 2.5 mg/ml latex. Lower concentration of latex was needed to yield similar effect to human gingival fibroblast primary cells. After 2 days the ODs of the 0 and 300 µg/ml solutions were 0.599 (± 0.191) and 0.108 (± 0.038), respectively. It is concluded that, the latex of *J. curcas* was cytotoxic to fibroblast cells. This study was funded by Risbin Iptekdok 1997/1998.

39 Orofacial Manifestations of HIV. A Review

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Orofacial manifestations of human immune deficiency virus(HIV)/ acquired immune deficiency syndrome(AIDS) are of great importance from the point of view of diagnosis of these cases since they may be the earliest presenting signs. These manifestations include cervical lymphadenopathy (95%), opportunistic infections (85%), advanced periodontitis (90%), recurrent aphthae (70%), and neoplasms (50%). This paper reviews the various orofacial manifestations of HIV/AIDS and reports findings of a study that was done to evaluate orofacial manifestations among 20 HIV seropositive patients both male and female in the 20-45 years age group. The patients were mainly referral cases to the district government hospital. Of 20 such patients, 16 were with orofacial manifestations and 4 without orofacial manifestations. The orofacial manifestations were candidiasis (100%), cervical lymphadenopathy (95%), angular cheilitis (80%), atrophic glossitis (70%) and periodontitis (70%). The results of the present study also showed that the most common mode of transmission of HIV was sexual exposure and most commonly affected were the young and middle aged group, males and lower socio-economic group.

In conclusion this study shows that oral candidiasis particularly pseudomembranous type is seen invariably in HIV/AIDS patients. However, hairy leukoplakia and Kaposi's sarcoma are not the common lesions among the Indian HIV patients.

40 Diclofenac combined with paracetamol ± codeine after oral surgery.

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The purpose of the study was to investigate whether a single oral dose of diclofenac enteric-coated tablets when combined with paracetamol with or without codeine for pain after oral surgery enhances pain relief compared with each single drug. The trial comprised 120 patients experiencing pain intensity above 50 on a 100 mm Visual Analogue Scale following surgical removal of at least one impacted 3rd molar. The patients received in a randomised and double blind manner either

① diclofenac 100 mg (DI),
② paracetamol 1g (P),
③ paracetamol 1g + codeine 60 mg (P+C),
④ diclofenac 100 mg + paracetamol 1g (DI+P), or
⑤ diclofenac 100 mg + paracetamol 1 g + codeine 60 mg (DI+P+C) in a single oral dose.

Pain intensity and Pain relief were rated in home-diaries by the patients every 30 minutes for 8 hours. Efficacy variables were analysed with a general linear model for repeated measures or one-way ANOVA as appropriate. Significance was set at $P<0.05$. Upside assay sensitivity was confirmed as the active standard drugs; P+C was superior to P alone ($P<0.05$). DI+P with or without C was superior to DI alone, P alone, and P+C alone ($p<0.05$). Adding 60 mg of C increased the degree of experienced side effects significantly ($P=0.037$).

Thus, co-administration of diclofenac enteric-coated tablets and paracetamol in a single oral dose gives excellent and long lasting pain relief after oral surgery, superior to diclofenac alone or paracetamol with or without codeine.