

**3489** The Myofibroblasts in Oral Submucous Fibrosis Caused by Betel Nut Chewing. T.-L. LEE\*, C.-H. Peng, M.-D. Hsu, and M.-Y. CHOU (Chung Shan Medical and Dental College, Taichung, Taiwan, R.O.C.)

Oral submucous fibrosis (OSF) is a chronically progressive fibroconnective disorder. Patients of advanced OSF suffer from the restriction of mouth opening, reduced mobility of tongue, and eventually permanent trismus. Although the excessively accumulated and hyalinized extracellular matrix might passively contribute to the rigidity of oral mucous, we hypothesized that either constitutive or transiently-modified myofibroblasts present in the submucous foci could actively contract and cause a tetanus-like situation. To test our hypothesis, ultrastructural studies using transmission electron microscopy, immunofluorescence studies of the expression of myofibroblastic-specific differentiation markers, and contractility studies of fibroblasts from explants of both normal skin and OSF cultured in a three-dimensional histotypic culture system were conducted. Fibrotic cytokine TGF- $\beta$  and betel nut alkaloids arecoline and arecaidine treated fibroblasts were also compared. In conclusion, our data show that like in granulation tissues, the contractile, smooth-muscle-like myofibroblasts have been activated and increased in number. In the in vitro studies, cells from explants of OSF tissues retain the phenotypic characters of that of myofibroblast. Moreover, the phenotypic transition can be induced in vitro by treatment of TGF- $\beta$  and betel nut alkaloids.

**3491** Extraction of DNA suitable for RSM analysis from oral cytology samples. MJ Wilson\*, N Mellaugh, JG Cowpe\* (Cardiff and Bristol Dental Schools, UK)

Premalignant oral lesions are common and screening procedures still rely on subjective clinical assessment. Many molecular prognostic markers have been investigated including p53 mutations which have been demonstrated in a high proportion of oral carcinomas, in addition to a number of proto-oncogene lesions. A recently introduced technique, the restriction site mutation assay (RSM, Parry *et al.*, 1990), can be used to measure base changes which occur in the DNA coding for bacterial restriction enzymes. RSM is based on the differential susceptibility of the mutant and non-mutated restriction site to digestion by a chosen enzyme following PCR amplification using primers either side of the restriction site. The aim of this study was to investigate whether DNA of sufficient quantity and quality for analysis by RSM could be extracted from cells collected by exfoliative oral cytology from potentially malignant lesions. Cells were collected from lesions of leukoplakia with a range of clinical appearances by firmly rotating a Cytobrush against the lesion for 30 sec. Cells were washed twice in phosphate-buffered saline. Three methods were compared for the extraction of DNA from 30 oral smears: a standard phenol/chloroform method; a high salt precipitation method and a commercial method using resin-based purification columns. The commercial extraction was shown to most convenient and reproducible, routinely providing 1.5  $\mu$ g of DNA per sample. DNA quality was assessed by subjecting 5 samples to the RSM as follows: Genomic DNA from each sample was digested with enzymes *FokI*, *MspI*, *NcoI*, *RsaI*, *HinfI*, *BspI286*, *HhaI*. Exons 5/6, 7 and 8 were then amplified and the PCR products compared following separation by polyacrylamide gel electrophoresis. No mutations were detected in these samples. In summary, we have shown how cell populations collected by exfoliative cytology can provide DNA of sufficient quantity and quality for analysis of p53 gene mutations by RSM.

**3493** Detection of Epstein-Barr virus in Warthin's tumor by in situ hybridization. H.J.KIM\*, S.PHONG, J.LEE, S.M.PAIK, C.Y.LIM. (Department of Oral Pathology, College of Dentistry, Seoul National University, Seoul, Korea)

Epstein-Barr virus has been reported to be a causative agent of various diseases such as Burkitt's lymphoma, infectious mononucleosis, nasopharyngeal carcinoma and hairy leukoplakia, and its causative relationship with salivary gland lesions such as Sjogren's disease has been studied. EBV has been suggested to be related to Warthin's tumor by detecting viral DNA in their tissues, proposing that salivary gland, especially the parotid gland, be the harbor of EBV. In this study we investigated the presence of EBV in Warthin's tumor, various salivary lesions and normal salivary gland. We selected 25 cases of Warthin's tumor, 10 cases of chronic nonspecific sialadenitis and 10 cases of normal salivary gland from the department of oral pathology and the department of pathology, Seoul National University Hospital. In situ hybridization was performed on formalin-fixed, paraffin-embedded tissues, using hybridization solution containing biotinylated EBV-DNA probe, which consists of the BamHI-W fragment of EBV DNA specific for the viral internal repeats (IR1) and internal repeats 3 (IR3) separately. We detected positive signals in the cytoplasm of the living epithelial cells of all Warthin's tumors tested and focally positive signal in the acini of the normal and mild sialadenitis lesions. It was suggested that EBV was in the latent state in the epithelial cells in normal gland and Warthin's tumor, and especially related to the pathogenesis of the Warthin's tumor.

**3495** Ki-67 antigen expression in ameloblastomas. M. ONG\*UTI, A.T. CRUCHLEY, G.L. HOWELLS & D.M. WILLIAMS\*. St. Bartholomew's and the Royal London School of Medicine and Dentistry, UK.

The aim of this study was to assess the state of tumour cell proliferation in ameloblastomas and to examine whether cell turnover correlated with clinical features or histology. 54 ameloblastomas were collected from Kenyan patients (32 females, 22 males; ages 9-70 years) and cryostat sections reacted with a specific monoclonal antibody (Ki-67; Dako UK) and an avidin biotin immunoperoxidase technique. The labelling index (LI) for each tumour was calculated by expressing the percentage of Ki-67 positive cells in 10 adjacent high power fields in each section. Overall, follicular ameloblastomas had a significantly greater LI ( $p < 0.05$ ) than plexiform tumours ( $5.0 \pm 0.5$ ;  $3.2 \pm 0.6$  respectively; mean  $\pm$  SEM). Plexiform ameloblastomas presenting in the mandibular symphyseal region had a significantly lower LI ( $p < 0.05$ ) than those from the posterior mandible ( $1.8 \pm 0.5$ ,  $3.9 \pm 0.8$  respectively). There was no significant difference in LI in tumours from males and females. Further analysis of the histological patterns revealed that the LI was higher in squamous strands ( $6.4 \pm 3.1$ ) than in epithelial cords and cysts ( $1.4 \pm 1.3$ ;  $p < 0.001$ ). These results suggest that tumour cell proliferation in ameloblastoma may vary according to histological pattern and possibly, in the case of plexiform ameloblastoma, with site of presentation. Further studies are necessary to establish whether Ki-67 antigen expression may be a useful prognostic marker of clinical behaviour.

**3490** Immunohistochemical study on oral leukoplakia. M. Nakamura\*, K. Yui and M. Nagumo (Second Department of Oral and Maxillofacial Surgery, Showa University, Tokyo, Japan)

The correlation of epithelial dysplasia with the expression of TGF- $\alpha$ , Lewis' (Le') antigen and p53 protein was evaluated in oral leukoplakias. Changes in the expression of TGF- $\alpha$ , Le' antigen, p53 protein, and PCNA were examined immunohistochemically in 30 cases of tongue leukoplakia with epithelial dysplasia (mild dysplasia: 11 cases, moderate dysplasia: 10 cases, and severe dysplasia: 9 cases). In addition, the expressions of these molecules in normal tongue epithelia and leukoplakias without epithelial dysplasia were also investigated. The results revealed that Le' antigen was distributed throughout the epithelium except for the basal cell layer in normal epithelia, whereas in leukoplakias it was localized only in the superficial epithelium. p53 protein and PCNA appeared chiefly in the basal cell layer in normal epithelia. TGF- $\alpha$  was scarcely stained in normal epithelia, whereas in leukoplakias it was expressed in cells just above the basal cell layer. The positive rate of Le' antigen decreased with the grade of epithelial dysplasia. In contrast, TGF- $\alpha$ , p53 protein and PCNA positivities increased with the grade of epithelial dysplasia. Specifically, significant changes in the positivities of Le' antigen and p53 protein were observed in leukoplakia, when epithelial dysplasia progressed from mild to moderate. These results suggest that mutation of the p53 gene, and alterations of TGF- $\alpha$  and Le' antigen may occur in the stage at which epithelial dysplasia progresses from mild to moderate.

**3492** Differential Display Applied to Archival Oral Mucosal Tissue. T.P. McALINDEN<sup>1</sup> and J.J. MARLEY<sup>2</sup> (The Schools of Dentistry, University of Minnesota, USA<sup>1</sup> and The Queen's University, Belfast, UK<sup>2</sup>).

The use of Differential Display Hybridization (DDH) to identify differentially expressed genes in oral mucosal carcinogenesis has previously been reported by Marley *et al.* (Eur J Cancer, Oral Oncology Vol B, 30: 305-11, 1994). The technique of Differential Display RT-PCR (DDRT-PCR) originally described by Liang and Pardee (Science 257:967-971 1992) has several advantages over DDH. In that it requires only minute amounts of starting tissue and will allow the simultaneous comparison of more than one sample of RNA. These attributes make this an ideal approach to identify differential gene expression which accompanies the progression of potentially malignant oral mucosal lesions to oral squamous cell carcinoma. The recorded sequential development of these lesions is comparatively rare as described by Cowan *et al.* at the 1996 BSDR (J Dent Res (in press)) and the time for development prohibitively long for prospective studies. We reasoned that by accessing available archival material of sequential biopsies from the same site in the same patient with recorded risk factors and known outcome, we would be in much better position to eliminate some of the confounding variables which have hindered previous candidate gene expression studies based on cross sectional material. We would then obtain a more meaningful insight into the molecular pathogenesis of the disease. Following on from pilot studies reported by Cairns *et al.* at the 1996 BSDR (J Dent Res (in press)), we have examined the feasibility of applying DDRT-PCR to archival oral mucosal tissue and attempted to optimize reaction parameters for such an application. We have investigated the effects of several different methods of RNA isolation, reverse transcription using different anchored oligo-dT primers and sequential PCR amplification steps in combination with various random primer pair combinations. Readable displays have been successfully obtained from the archival material thus far examined. We conclude that DDRT-PCR may be applied to sections of archival oral tissue processed in a standard fashion.

**3494** Proliferative Activity as Detected by Immunostaining with MIB-1 and PCNA in Epithelial Lesions of Parotid Gland. QIANRU ZHU\*, FRANK H. WHITE AND GEORGE L. TIPOE (Department of Anatomy, Faculty of Medicine, University of Hong Kong, Hong Kong)

A retrospective study of a series of parotid gland lesions was designed to evaluate the diagnostic and prognostic value of the proliferation-associated antigen Ki-67 with monoclonal antibodies MIB-1 immunohistochemically. Tissue samples comprised human normal parotid gland (N, n=10), chronic sialadenitis (CS, n=8), Warthin's tumor (W, n=10), benign pleomorphic adenoma (BPA, n=8), mucoepidermoid carcinoma (MEC, n=13), carcinoma in pleomorphic adenoma (CPA, n=8) and adenoid cystic carcinoma (ACC, n=12). The results showed that the value of MIB-1 labeling index (MI), the numerical percentage of positive nuclei, increased progressively in benign lesions in comparison with the N group and in malignant neoplasm in comparison with non-neoplastic groups and benign lesions. The mean value of MI in BPA was significantly lower than those in malignant groups, suggesting that it may be used as diagnostic discriminators. Spearman rank correlation analysis showed a highly positive correlation between the MI and the severity of the lesions. Furthermore, the mean value of MI was significantly higher in patients who died of the malignant tumors than in those patients who survived. Our results indicate that MIB-1 index might be useful markers for discriminating between benign (BPA) and malignant tumors of the parotid gland and it may have prognostic applications. Similar parameter for PCNA did not differ significantly when compared with MIB-1 and showed similar trend.

**3496** Calcium Transport Protein Expression in Ameloblastoma: relationship to normal amelogenesis. PH GILCHRIST\*, JI. BORKE and BB SINGH (Medical College of Georgia, Augusta, GA, USA)

The morphological and inductive relationships among various parts of normal tooth germ are reproduced to varying degrees in odontogenic tumors. Odontogenic epithelium associated with ectomesenchyme generally produces dental hard tissue, while ameloblastoma, a tumor of proliferating odontogenic epithelium without ectomesenchyme does not, (see Typing of Odontogenic Tumors: W.H.O., 1992). Mechanisms relating calcification of enamel matrix to ameloblast function remain unknown. The process of normal amelogenesis is characterized by the progressive formation and mineralization of enamel matrix, and the parallel expression in the ameloblasts of the calcium transport proteins, plasma membrane Ca-pump (PMCA) and calbindin-28kDa. The objectives of this study were to localize these proteins in ameloblastomas by immunohistochemistry, and to compare their expression with the stages of differentiation found in normal amelogenesis. Eight cases of ameloblastoma were sectioned for localization of PMCA and calbindin-28kDa. PMCA (+++ to ++++) and calbindin-28kDa (+ to ++++) protein expression was found in all cases studied. Expression of both proteins occurred primarily in the ameloblast-like palisading peripheral cells. In normal tooth forming tissues, calbindin-28kDa is found exclusively in ameloblasts. These findings support ameloblast-like differentiation in the pathogenesis of ameloblastoma. In normal amelogenesis, PMCA and calbindin-28kDa are not expressed in presecretory ameloblasts, but are expressed later as enamel begins to mineralize. Ameloblastomas, however, do not mineralize. These data suggest that the expression of calcium transport proteins in ameloblastomas may be uncoupled from the mechanism of mineralization associated with normal ameloblast differentiation.