VP-9 **Cytolethal-distending toxin gene of *Actinobacillus actinomycetemcomitans*: A self-splicing intron.** **K. S. TAN[1]*, K. P. SONG[1] and G. ONG[2] ([1]Dept of Microbiology, [2]Dept of Preventive Dentistry, National University of Singapore)**

In eukaryotic cells, genes are interrupted by non-coding DNA sequences called introns. Introns are transcribed as part of a precursor RNA. These intervening sequences are subsequently removed by splicing, to generate mature mRNA which are translated. However, introns are rarely found in bacteria. *Actinobacillus actinomycetemcomitans (A. a)* is a gram-negative bacteria, which is implicated in the etiology of aggressive periodontitis. The cytolethal-distending toxin (*cdt*) of *A. a* is one of the several complex multi-gene toxin systems, which are not found in other recognized periodontal pathogens. This cytotoxin causes cell-cycle arrest at the G_2 phase. In this study, we report a novel finding: the ability of *cdt* mRNA to undergo self-splicing. RT-PCR was used to detect self-splicing of *cdt* mRNA *in vivo* and *in vitro*. Spliced transcripts were cloned in *E. coli* and sequenced by dideoxy cycle sequencing. Site-directed and deletion mutants were created to determine sequences which are important for splicing. Transcripts of sizes 2.1 kb, 1.5 kb and 0.9 kb were obtained by RT-PCR. Similar splicing pattern was observed when *cdt* carrying plasmid was cloned into *E. coli*. Sequence analyses revealed that transcript variants differ in their 5' ends but share an identical 3' region. Mutagenesis experiments suggest that consensus sequences are required for accurate splicing. Sequence analysis revealed the presence of ribosome-binding site (RBS) upstream of the spliced transcripts. The *cdt* mRNA of *A. a* is a ribozyme with self-splicing ability. This phenomenon occurs naturally in the bacteria and produces mRNA variants from a single coding sequence. This study is supported by grants R-182-000-044-213 and R-222-000-012-112 from National Medical Research Council and National University of Singapore, respectively.

VP-10 **Expression and Role of Keratinocyte Growth Factor in Periodontal Healing.** **S. MOHD SAID*, S.J.DAS[#] and I. OLSEN[#] (Dept of Periodontology, Eastman Dental Institute, University College London, UK[#] and Universiti Kebangsaan Malaysia*)**

Keratinocyte growth factor (KGF) has been shown to play a major role in wound healing processes in many tissues as it uniquely promotes the re-epithelialisation of damaged epithelium. However, enhanced growth of the gingival epithelium resulting from over-expression of KGF could interfere with the repair and regeneration of periodontal ligament. This study was therefore undertaken to determine i) whether KGF is present in the gingival crevicular fluid (GCF) as a measure of KGF expression in normal and diseased periodontal tissue and ii) whether KGF is elevated during periodontal wound healing. Enzyme-linked immunoabsorbant assay (ELISA) was used to measure KGF in GCF, which was collected from nine healthy individuals and also from surgically-treated sites and untreated control sites of twelve patients undergoing pocket-reduction or regenerative surgery. The amount of KGF in GCF was calculated from a standard curve generated using human recombinant KGF. The results from this study indicate that KGF is detectable in the GCF of healthy as well as diseased periodontium, at (mean $\pm$ SEM) 0.33 $\pm$ 0.05 ng/ml and 0.35 $\pm$ 0.04 ng/ml, respectively. Analysis using Mann-Whitney Test showed no statistical significant difference between the means in both groups ($p = 0.02$). In addition, KGF expression was found to be up-regulated during the periodontal tissue healing. The finding of KGF up-regulation in periodontal environment during the healing process particularly in the re-epithelialisation phase suggests that this growth factor may interfere with the successful healing of damaged periodontal tissue.

VP-11 **The Effect of Topical Application of Killed *Streptococcus mutans* on Oral Mucous.** **SAMDHARU PRAMONO*, BOEDI OETOMO ROESLAN (Faculty of Dentistry, Trisakti University, Jakarta, Indonesia)**

Salivary IgAs act as the defence mechanism to dental caries. The amount of this antibody will be increased by antigent stimulation which is either locally or systemic. A single block randomized experiment was done with control to find out the effect of topical application of killed *Streptococcus mutans* INA99 at oral mucous to the amount of salivary IgAs. Twenty Winstar rats which were inoculated with *Streptococcus mutans* INA99 and feed with the addition of 20% sucrose were divided into two groups, one group was applied with killed *Streptococcus mutans* INA99, and another group was designed as control group. The application was given every two days for two weeks. The result of covariant analysis with amount of salivary IgAs before it was applied as co-variable shows a significant elevation of IgAs amount ($p<0.01$) at the groups which applied with killed *Streptococcus mutans* INA99. It was concluded that topical application of killed *Streptococcus mutans* INA99 on oral mucous will increase the amount of salivary IgAs.

VP-12 **Prognosis and Long-term Outcome of Teeth in Periodontitis Patients.** **PUNZALAN PB*, LEUNG WK, CORBET EF (The University of Hong Kong, Hong Kong SAR)**

This retrospective study aimed to evaluate the long-term (6 -19 years) outcome for teeth in periodontitis patients. Records of patients who discontinued periodontal treatment at least 6 years beforehand (mean 10 + 3.85 years) and who had been advised to seek care from general dentists were screened. Patients with unfavorable treatment response prior to treatment discontinuation, defined as $\geq$ 5% sites with $\geq$ 5mm PPD, were identified. From among these, 86 patients underwent (1) full-mouth clinical examination, (2) questionnaire interview, and (3) panoramic radiographic examination. Data from clinical and radiographic examinations obtained prior to discontinuation and at recall were used in determining individual tooth prognosis against a set of pre-defined criteria. A total of 321 teeth out of 2102 had been lost (mean 3.73 + 3.82 teeth/patient). Tooth mortality revealed a mean annual adjusted tooth loss rate of 0.28/year. Deterioration in periodontal health in the form of increased number of sites with PPD $\geq$ 5mm and increased number of teeth with $\geq$ 50% bone loss was observed ($p \leq 0.001$, paired t-test). Changes in individual tooth prognosis revealed that for teeth with good, fair and hopeless prognosis at initial determination, the periodontal condition worsened with time ($p \leq 0.000$) as tested by Stuart-Maxwell x^2-test. Regression analysis showed this deterioration to be associated with increasing age, years elapsed since discontinuation, poor periodontal conditions in terms of bone loss and number of sites with PPD $\geq$ 6mm at discontinuation and with current cardiovascular problems ($p \leq 0.04$), whereas maintenance after discontinuation was associated with greater periodontal stability ($p = 0.03$). This study showed that the periodontal condition at recall of teeth in periodontitis patients, regardless of initial prognosis, had deteriorated in the absence of supportive care. It also demonstrated high tooth mortality in discontinued patients without strict supportive periodontal care.

VP-13 **Platelet Rich Plasma in the Treatment of Periodontal Intrabony Defects.** **S. JAIN*, L.J. JIN and E.F.CORBET (Periodontology, Faculty of Dentistry, University of Hong Kong, Hong Kong**

Platelet rich plasma (PRP) has recently been used in Surgery due to its unique wound healing characteristics of promoting haemostasis and tissue adhesion, and its high content of growth factors such as PDGF and TGF-β. Objective of the present study was to evaluate the clinical effects of access surgery in combination with application of PRP gel as an adjunct in periodontal defects. The participants consisted of 12 subjects with chronic periodontitis who contributed 24 pairs of well-matched defects for this study. Just prior to the surgery, 30 ml of patient's own blood was obtained by venipuncture and separated into PRP gel and platelet poor plasma (PPP) gel by differential centrifugation. The paired defects were randomly assigned to access surgery with papilla preservation technique in combination with application of PPP and PRP gels (Test) and access surgery alone (Control). The primary outcome measures were changes in probing pocket depth (PPD), clinical attachment level (CAL) and gingival recession at 3 months. Both treatment approaches led to significant PPD reduction at 3 months with 2.0 1.2mm ($p<0.01$) in Test sites and 1.5 $\pm$ 1.5mm ($p\leq0.001$) in the Control sites. Greater CAL gain was found in Test (1.2 $\pm$ 1.2mm) than in the Control (0.4 $\pm$ 1.5mm), while no statistical significance was found. Nine out of twelve (75%) of the subjects showed greater gain of attachment in the test sites than the control sites. This study indicates that access flap surgery combined with application of PRP and PPP is effective in achieving reduction of probing depths and CAL gain in periodontal defects. Adjunctive use of PRP and PPP gels increases the likelihood of greater gain of attachment compared to access surgery alone.

VP-14 **Adjunctive effect of a low-power laser on periodontal treatment.** **S.M.L.LAI*, K.Y.ZEE and E.F.CORBET (Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China)**

The aim of this study was to evaluate the adjunctive effect of a low-power laser in non-surgical periodontal treatment of patients with moderate to advanced periodontal disease. A total of 14 patients were recruited. Clinical parameters, including presence of supragingival plaque (PI%), bleeding on probing (BOP%), probing pocket depth (PPD), probing attachment level (PAL) and gingival crevicular fluid (GCF) volume, were recorded at baseline and 3 months after treatment. A Helium-Neon (He-Ne) low-power laser operating at 632nm with an output power of 0.2mW was used. Split-mouth design was adopted, whereby the test sides were randomly selected by coin toss. Two sites on each side with PPD>5mm and presence of infrabony defects were selected, one from the incisor to canine and the other from the premolar region. Both test and control sides were treated non-surgically. Laser irradiation was applied directly to the buccal or palatal aspects of the selected sites, each time for ten minutes for a total of eight times in the first 3-month period by a trained operator. The whole-mouth PI% decreased from 83% at baseline to 16% at 3 months while the BOP% decreased from 95% to 32%. Mean PPD reductions for test and control sides were 3.9mm and 4.0mm and mean PAL gains were 2.0mm and 2.3mm, respectively. No statistically significant differences could be found between test and control sides using Wilcoxan signed rank test. The GCF volume reduced from 69.2 to 12 in test sides and 66.2 to 17.5 in control sides after 3 months and the difference in the reduction was statistically significance ($p<0.05$). In conclusion, results of the present study showed that there was no adjunctive clinical effect of this He-Ne laser irradiation on periodontal tissue healing in patients with moderate to advanced periodontal disease.

VP-15 **The wild type and "switch" phenotypes of *Candida glabrata* exhibit a high degree of genetic homogeneity.** **G. Luo*, G. Tang, B.P.K. Cheung, L.P. Samaranayake, Oral Bio-Sciences, Faculty of Dentistry, The University of Hong Kong, Hong Kong**

Phenotypic switching is believed to contribute to the virulence of *Candida* spp., and recently this phenomenon has been shown in *Candida glabrata (Cg)*, the second commonest *Candida* species. However, the mechanism of phenotypic switching of *Cg* is unknown. Hence, the aim of this study was to compare the genetic diversity, if any, in "switched" strains and wild parental strains of *Cg*. Switched strains of *Cg* were obtained by using the method described before (Luo *et al.*, *JCM*, 2001,39:2971-4). Genomic DNA was extracted and purified and subjected to Random Amplified polymorphic DNA (RAPD) with 8 custom designed primers, named OPA1 (CAGGCCCTTA), OPA2 (TGCCGAGCTG), OPA4 (AATCGGGCTG), OPA10 (GTGATCGCAG), OPA12 (TCGGCGATAG), OPA18 (GACCTGCCGA), OPE4 (GTGACATGCC), and OPE 18 (GGACTGCAGA). A standard contour-clamped homogeneous electrophoretic field electrophoresis (CHEF) procedure was also performed to characterize the genotypes of switched strains of *Cg*. After 7 days incubation, four major switched phenotypes of *Cg* termed white (Wh), light brown (LB), dark brown (DB) and very dark brown (vDB) were obtained. However, all the "switch" and wild parental strains of *Cg* yield identical genetic profiles when evaluated by CHEF and RAPD, irrespective of the primers used. This suggests that the change of the morphotype and other phenomic features of the switched strains are transient features not necessarily related to genomic alterations. Further studies at RNA level (instead of DNA level) are in need to define the possible relationship between the gene expressions and phenotypic switching in *Cg*. (Partially supported by Outstanding Researcher Award of the HKU Research Committee awarded to L.P. Samaranayake.)