国際セッション

（A会場）

5月23日（金） A会場　9：00～10：20

A-01
0900

Development of "Periodontal biosensor": A pilot study

Hidetomo Onishi

Keywords: Periodontal biosensor, FO-SPR sensor, FDF

Objectives: Fiber-optic surface plasmon resonance sensor (FO-SPR sensor) is one of the biosensor which is utilized in non-labeling and rapid detection of antigen-antibody reaction.
In this study, we constructed quantitative method for measurements of periodontopathic bacteria-derived virulence factor by using FO-SPR sensor.

Material & Methods: Two methods for the generation of self-assembling monolayers were used: (A) ORLA 91 fusion protein method, which consist of scaffold protein with antibody-binding domains and (B) ORLA 9 fusion protein method, which consist of scaffold protein without antibody-binding domains. We selected *Tannerella forsythia* Forsythia Detaching Factor (FDF) as analyte and rabbit anti-FDF polyclonal antibody was used as capture antibody. Binding events at the surface cause changes in resonance wavelength. rFDF solution was standardized to 1 to 200 μg/ml and antigen-antibody reaction was detected by FO-SPR sensor. A standard curve was developed using a series of rFDF standards.

Results: Within the concentration range of rFDF solution (1 - 200 μg/ml), the shift of resonance wavelength was detected in the concentration-dependent manner by using ORLA 91 protein ($y = 0.0105 x + 0.326$, $R^2 = 0.9873$) and not detected by using ORLA 9 protein.

Conclusions: It was suggested the possibility that FO-SPR sensor may serve as "periodontal biosensor" for clinical diagnosis of periodontitis.

A-02
0910

TGF-β1 regulates osteoblast differentiation via PI3K/Akt signaling pathway

Eiichi Suzuki

Keywords: TGF-β1, PI3K/Akt signaling pathway, osteoblast differentiation

Objective: Transforming growth factor-beta 1 (TGF-β1) produced in the presence of inflammation such as periodontitis exerts biphasic effects on osteoblast differentiation. We previously reported that TGF-β1 inhibited osteoblast differentiation via IGF-1/PI3K. However, the mechanism remains unknown. In this study, we examined the role of Akt, down-stream target of PI3K, in this process.

Materials and Methods: The degree of osteoblast differentiation in Murine preosteoblast (MC3T3-E1) cells was analyzed by ALP activity and mRNA expression of osteoblast differentiation markers. We also used MC3T3-E1 cells transfected with active Akt to overexpress phosphorylated Akt.

Results: The ALP activity levels were significantly decreased by repeated TGF-β1 administration as compared with those of control. Under the condition of Akt activation, the ALP activity levels were increased and osteocalcin mRNA levels were decreased by repeated TGF-β1 administration.

Conclusions: TGF-β1 promotes ALP expression and regulates osteoblast differentiation through Akt phosphorylation.

(Non-member corroborators: Shoko Onodera, Akiko Saito, Department of Biochemistry, Tokyo Dental College)

A-03
0920

Effect of hypoxia and *P. gingivalis*-lipopolysaccharide on the expression of inflammation-related molecules in human oral keratinocytes

Yukiko Nakajima

Keywords: hypoxia, *P. gingivalis*-LPS, cytokine, gene expression, keratinocyte

Objective: Periodontitis is caused by periodontopathic bacteria in periodontal pockets under low oxygen levels (hypoxia). Hypoxia induces expression of hypoxia-inducible factor (HIF) and HIF response is associated with inflammation. *P. gingivalis*-lipopolysaccharide (P-LPS) regulates expression of inflammatory cytokines through Toll-like receptor (TLR). In this study, we investigated the effects of hypoxia and P-LPS on the expression of molecules including inflammatory cytokines, antimicrobial peptides and growth factors in human oral keratinocytes.

Material and methods: Human oral keratinocyte cell line, TR146, was cultured under hypoxic condition (1% O_2), and RNA and protein fractions were extracted. RT-PCR and quantitative PCR were performed for gene expression analysis and Western blotting and ELISA for protein analysis.

Results: Hypoxia increased mRNA expression of TLR2 and protein level of HIF-1α. P-LPS increased mRNA expression of TLR2 under hypoxic conditions. Expressions of TNF-α, IL-8, adrenomedullin (ADM), VEGF and angiopoietin-like 4 increased under hypoxic conditions. ADM expression was inhibited by a HIF binding blocker, chetomin. Expressions of TNF-α and ADM were synergistically elevated by hypoxia and P-LPS. In contrast, hypoxia inhibited IL-6 and S100A8/S100A9 expressions, whereas it did not affect MMPs expression.

Conclusions: Hypoxia and P-LPS may synergistically aggravate periodontitis through the regulation of inflammation-related factors.

A-04
0930

A Critical Role of Toll-like Receptor in Periodontal Pathogen-Induced Pressure Overload Myocardial Hypertrophy in Mice

Makoto Kaneko

Keywords: *P. gingivalis*, Cardiac hypertrophy, Toll-like receptor

Objectives: The aim of this research was to investigate whether periodontal pathogen influenced on cardiac hypertrophy.

Methods: Male C57BL/6 mice and Toll-like receptor-2 knockout (TLR2KO) mice were used in this study. Transverse aortic constriction (TAC) operation was performed to induce cardiac hypertrophy. Mice were injected once a week with *P. gingivalis* (test) or vehicle containing phosphate-buffered saline (control). Echocardiography was performed one week after TAC operation. Four weeks after TAC operation, heart sample of each mouse was obtained.

Results: In wild-type mice, fractional shortening, which indicates cardiac function, in the test group significantly decreased a week after TAC operation, although that in the control group did not decrease. Enhanced cardiac fibrosis was shown in the test group four weeks after TAC operation, although that in the control group was slightly observed. Fractional shortening was comparable after TAC operation in the test and control groups of TLR2KO mice. Moreover, there was no significant

Conclusion: Infection with *P. gingivalis* deteriorated cardiac function and myocardial fibrosis in pressure overload-induced myocardial hypertrophy via TLR-2 signaling pathway.

A-05
0940

Basic considerations for vertical alveolar ridge augmentation

Hyun-Chang Lim

Vertical ridge deficiency cause inadequate space from the ridge crest to vital structures, such as inferior alveolar nerve, maxillary sinus and etc. Vertical alveolar bone loss has been stated as a major challenge generally to most of clinicians. Vertical deficiency can be considered as a zero wall defect, which is short of osteogenic potential and vascularity. Besides, bone has to be reconstructed three dimensionally, which requires significant amount of graft material, space-creating devices and flap extension (i.e. technique-sensitive). Therefore, vertical deficiency may be a very distressing clinical situation in an everyday clinical setting.

In some cases, short implant can be an alternative to avoid demanding vertical augmentation procedure and some authors has reported positive outcome of short implant. However, short implant can cause infra-position of abutment-fixture junction to adjacent tooth or implant, which may cause biologic problem, such as significant marginal bone loss, peri-implantitis and etc. In addition, short implant length may jeopardize implant survival in poor quality of bone.

Vertical defect has to be thoroughly inspected prior to selecting biomaterials, space maintenance, healing time and etc. The considerations include bone envelope, relationship with adjacent tooth, bone base and anatomical position. Depending on defect morphology, the entire surgical plan should be determined.

In this presentation, the basic considerations for restoring vertical ridge deficiency and related cases are presented.

A-06
0950

Inconsistency of gingival biotype assessment using the transparency of the periodontal probe: verification of actual gingival thickness with an ultrasonic device.

Jeong-Heon Son

Keywords: gingival thickness, ultrasonic device, probe visibility, gingival biotype

Aims: (1) To examine whether the assessment of biotype by visibility of periodontal probe provides accurate information on the gingival thickness; and (2) To identify factors that affect probe transparency using cluster analysis and multivariate analysis.

Material and Methods: The clinical parameters of maxillary central incisors were examined in 90 subjects. Clinical photographs, gender of patient, probe visibility (PV) during probing, probing depth (PD), gingiva width (GW), papilla height (PH), gingival thickness (GT) measured with an ultrasonic device, and the ratio of crown width and crown length (CW/CL) were recorded. Statistical analyses were performed to reveal factors that affect probe visibility and gingival thickness. Cluster analysis, based on morphological characters, was used to identify gingival biotypes.

Results: Gender was found to have a significant influence on the probe visibility score (P=0.003). However, compared with the PV score, no morphologic variables (PD, PH, GW, and CW/CL) were found to be significantly different. There was also no significant difference in the GT relative to PV score (Oneway ANOVA, $p=0.152$). Gender differences were also not significant for GT (student t-test, $p=0.138$). Multivariate analysis revealed that gender was the only significant predictor of probe visibility score (odds ratio; 6.48; 95% CI, 1.71 to 24.56, $p<0.05$). Based on morphometric parameters, two clusters were created as a result of clustering procedures. Subjects classified as cluster A showed a more slender tooth form, larger papilla height, shallower PD and narrower gingiva than those belonging to cluster B. The distribution of GT for cluster A subjects was significantly lower in comparison to cluster B subjects ($p<0.05$). However, PV score showed no significant association between subjects in cluster A and B ($p>0.05$).

Conclusions: Our present findings show that the visibility of the periodontal probe is not related to the GT directly measured with an ultrasonic device. Gender was found to be the only highly significant predictor of the transparency of the periodontal probe. GT has a statistically significant correlation with morphologic characteristics of the periodontium.

A-07
1000

Vertical Ridge Augmentation: Three different techniques

Jung-Hoon Kim

Purpose: The aim of this case report is to assess the clinical outcome of implant placement in conjunction with vertical hard tissue augmentation following tooth extraction with particular focus in the severely atrophied mandibular posterior zone.

Methods: Case 1: Following extraction of periodontally compromised mandibular left and right posterior teeth, the alveolar ridge of the extraction area underwent marked bone resorption. Guided bone regeneration(GBR) was performed in both sites and delayed implant fixtures were placed after 6 months after GBR. Free gingival graft(FGG) was performed in both sites to compensate for the shortened vestibule and keratinized mucosa.

Case 2: In the severely atrophied left mandibular area, GBR was performed and implant fixtures were placed simultaneously. FGG was performed after 6 months for the same reason as mentioned.

Case 3: GBR was performed immediately after extraction of compromised left mandibular posterior teeth. Implant fixtures were placed 3 months later according following the rationale of this technique and no additional soft tissue graft was needed.

Results: All the three cases with vertical ridge augmentation technique healed uneventfully with adequate ridge contour and volume. Three different techniques required different healing intervals. Nevertheless, additional soft tissue management was required after the healing period in cases 1 and 2. In case 3, the soft tissue level was harmonized with the adjacent soft tissue component of the implant even without the use of soft tissue graft.

Conclusion: Within the limitations of the present short term examination, vertical ridge augmentation and implant placement following extraction of compromised teeth may provide successful outcome in the reconstruction of the mandibular posterior area.

A-08
1010

Carbodiimide crosslinked simvastatin-collagen complex enhances bone regeneration in rabbit calvarial models.

Jung-Soo Park

Keywords: Simvastatin, collagen, EDC/NHS, bone regeneration

Objectives: The ideal drug delivery system for topical administration of simvastatin (SIM) has not been found yet. The aim of this study was to evaluate the efficacy of a new method of loading the SIM onto the collagen carriers, using *in vitro* and in vivo models.

Methods: 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride with N-hydroxy-sulfosuccinimide (EDC/NHS) builds chemical bonds between SIM and collagen by working as a catalyst. Cytotoxicity, collagen degradation time, and SIM release profiles of the EDC/NHS treated SIM-collagen complex were evaluated by in vitro methods. In rabbit calvarial exophytic models, 0.5, 2.5, and 5.0 mg/ml of SIM were applied with collagen sponges to box shaped Titanium-reinforced expanded polytetrafluoroethylene (TR-ePTFE) chambers (8 × 5 × 4 mm). Animals were sacrificed at 8 and 16 weeks postoperatively. Histologic and histomorphometic evaluations were performed.

Results: *In vitro* results demonstrated that collagen degradation time was delayed by the reaction of EDC/NHS to the collagen carrier itself. Histologies showed that the collagen carriers remained unabsorbed until 8 weeks, sustainably releasing SIM. Histomorphometric analysis revealed that new bone formation was enhanced in SIM-collagen groups compared to collagen only controls especially at 16 weeks.

Conclusion: EDC/NHS treated SIM-collagen complex might be a novel method of delivering SIM locally to the target defects.