

The 110th Annual Meeting of the American Academy of Periodontology  
in collaboration with the Japanese Society of Periodontology,  
and the Japanese Academy of Clinical Periodontology.

## Abstracts of JSP/JACP Poster Session



**110<sup>th</sup> Annual Meeting**

**SAN DIEGO**

**October 31 - November 3, 2024**

October 31 - November 3, 2024  
San Diego Convention Center, San Diego, CA, U.S.A.

The Japanese Society of Periodontology  
The Japanese Academy of Clinical Periodontology



第110回アメリカ歯周病学会共催  
日本歯周病学会・日本臨床歯周病学会  
2024年大会

**The 110th Annual Meeting of the American Academy of Periodontology  
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**JSP/JACP ポスターセッション抄録集**  
一般演題（基礎研究，臨床研究），症例報告，歯科衛生演題

**Abstracts of JSP/JACP Poster Session**  
**General (Basic Research, Clinical Research), Case Report, Dental Hygiene**

会期：2024年10月31日～11月3日  
会場：サンディエゴ・コンベンションセンター

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JSP/JACP ポスター発表・若手研究者支援協賛企業

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# General (Basic Research)



GB-01

### Effect of heat-not-burn product smoke extract on periodontal tissues

Kengo Doya<sup>\*1</sup>, Kentaro Imamura<sup>1,2</sup>, Yurie Nakazawa-Kitamura<sup>1</sup>, Shinta Mori<sup>1,2</sup>,  
Saki Nakane-Koyachi<sup>1</sup>, Kazuyuki Ishihara<sup>2,3</sup>, Atsushi Saito<sup>1,2</sup>

<sup>1</sup>Department of Periodontology, <sup>2</sup>Oral Health Science Center, <sup>3</sup>Department of Microbiology, Tokyo Dental College, Tokyo, JAPAN

**Background and objective:** Smoking is one of the important environmental risk factors for periodontal disease. In recent years, new types of cigarettes, such as e-cigarettes and heat-not-burn products (HTPs), have emerged. However, the effects of HTPs on periodontal tissues remain unclear in many respects. Therefore, the aim of this study is to investigate the effects of HTP smoke extract (HTPE) on alveolar bone resorption in mice and on cell function in human gingival fibroblasts (HGF).

**Materials and Methods:** We prepared HTPE and conventional cigarette smoke extract (CSE). *In vivo*, C57BL/6 mice were divided into HTPE, CSE, and control (PBS) groups. Each solution was intraperitoneally administered once a day for three days. Periodontitis was induced by silk ligature. Alveolar bone resorption was evaluated by  $\mu$ -CT at 7 days later. *In vitro*, HGFs were cultured with various concentrations of HTPE or CSE. Cell proliferation, migration, and cell morphology were evaluated using WST-8, wound healing assay and confocal laser microscopy, respectively.

**Results:** HTPE and CSE induced significantly greater bone resorption compared with control. However, there was no significant difference between HTPE and CSE. HTPE had no significant impact on proliferation of HGF-1, but CSE significantly suppressed it. Cell migration was significantly inhibited by HTPE and CSE. In HTPE group, greater prevalence of actin filaments was observed compared with the control.

**Conclusion:** HTPE exerted less effect on cell migration compared with CSE, but induced a similar level of bone resorption. These results suggested that HTPs could inhibit wound healing ability and increase the risk for periodontal disease.

GB-02

### The effect of FGF-2 on periodontal healing in osteoporotic rat

Shinta Mori<sup>\*1,2</sup>, Kengo Doya<sup>1,2</sup>, Naoki Miyata<sup>1,2</sup>, Yurie Kitamura-Nakazawa<sup>1</sup>, Tasuku Murakami<sup>1</sup>,  
Daisuke Matsugami<sup>1</sup>, Wataru Yoshida<sup>1</sup>, Takahiro Bizenjima<sup>3</sup>, Kentaro Imamura<sup>1,2</sup>, Fumi Seshima<sup>1</sup>,  
Atsushi Saito<sup>1,2</sup>

<sup>1</sup>Department of Periodontology, <sup>2</sup>Oral Health Science Center, <sup>3</sup>Chiba Dental Center, Tokyo Dental College, Tokyo, JAPAN

**Background and objective:** Osteoporotic patients have significantly greater clinical attachment loss than healthy individuals. Fibroblast growth factor (FGF-2) has been shown to have effects on periodontal healing. However, the effects of FGF-2 on periodontal healing in osteoporotic patients remain unclear. Therefore, we aim to investigate the effects of local application of FGF-2 on periodontal healing using an osteoporotic rat model.

**Materials and Methods:** Wistar rats were assigned to ovariectomy (OVX)-induced osteoporosis group or control group. The control group underwent a sham operation. At 8 weeks post-OVX, standardized periodontal defects were surgically created in maxilla. The defects in each group received hydroxypropyl cellulose (HPC) only or FGF-2+HPC. At 2 weeks and 4 weeks post-periodontal surgery, we evaluated periodontal healing through histological observations (H-E staining) and micro-CT analysis.

**Results:** In histological analysis of at 2 and 4 weeks post-surgery, FGF-2-treated defects showed a greater level of newly formed bone compared with HPC only, in both OVX and control groups. Bone volume fraction of FGF-2-treated defects was significantly greater than HPC only. Trabecular number of FGF-2 treated defects tended to be greater than non-treated defects. Regardless of FGF-2 use, in Control and OVX groups exhibited coronal extension of new bone at 2 weeks and 4 weeks.

**Conclusion:** The application of FGF-2 resulted in increased bone volume fraction, trabecular number, and formation of newly formed bone in the defects of OVX and control groups at 2 and 4 weeks. FGF-2 seems to enhance periodontal healing, even under osteoporotic condition.

GB-03

### Lantibiotic Nisin Prevents Gut-liver Dysbiosis and NAFLD-Steatosis following Polymicrobial-Periodontal Infection

Ryutaro Kuraji<sup>\*1,2</sup>, Changchang Ye<sup>2,3</sup>, Chuanjiang Zhao<sup>2,4</sup>, Li Gao<sup>2,4</sup>, April Martinez<sup>2</sup>, Yukihiro Miyashita<sup>1</sup>, Allan Radaic<sup>2,5</sup>, Pachiyappan Kamarajan<sup>2,5</sup>, Charles Le<sup>2</sup>, Ling Zhan<sup>2</sup>, Helene Range<sup>2,6</sup>, Masataka Sunohara<sup>7</sup>, Yukihiro Numabe<sup>1</sup>, Yvonne L. Kapila<sup>2,5</sup>

<sup>1</sup>Department of Periodontology, The Nippon Dental University School of Life Dentistry at Tokyo, <sup>2</sup>Orofacial Sciences Department, School of Dentistry, University of California San Francisco, <sup>3</sup>State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral Diseases, Department of Periodontology, West China School of Stomatology, Sichuan University, <sup>4</sup>Department of Periodontology, Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University, <sup>5</sup>Sections of Biosystems and Function and Periodontics, School of Dentistry, University of California Los Angeles, <sup>6</sup>Department of Periodontology, University of Rennes, UFR of Odontology; Service d'Odontologie, CHU de Rennes and INSERM CHU Rennes, Institut NUMECAN (Nutrition Metabolisms and Cancer); CIC 1414, <sup>7</sup>Department of Anatomy, The Nippon Dental University School of Life Dentistry at Tokyo

**Background and objective:** Periodontal disease mediates gut microbial dysbiosis and mucosal barrier dysfunction that leads to non-alcoholic fatty liver disease (NAFLD) via the enterohepatic circulation. Therefore, treatment with antibiotics or probiotics to shift this dysbiosis towards health may improve liver disease. The aim of the present study was to evaluate the potential for nisin, a lantibiotic antimicrobial peptide produced by *Lactococcus lactis*, to counteract the periodontitis-associated gut dysbiosis and to modulate the glycolipid-metabolism and inflammation in the liver.

**Materials and Methods:** Periodontal pathogens, namely *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* and *Fusobacterium nucleatum*, were administered topically into the oral cavity to establish polymicrobial periodontal disease in mice. Microbial composition as assessed by 16S r-DNA sequencing, histological findings, and NAFLD-related gene expression as determined by RT-PCR and RNA sequencing were evaluated in feces, small intestine, and liver.

**Results:** In the context of disease, nisin treatment significantly shifted the gut and liver microbiome towards a new composition, commensurate with health while preventing the harmful inflammation in the small intestine concomitant with decreased villi structural integrity, and heightened hepatic exposure to bacteria and lipid and malondialdehyde accumulation in the liver. Validation with RNA Seq analyses, confirmed the significant infection-related alteration of several genes involved in mitochondrial dysregulation, oxidative phosphorylation, and metal/iron binding and their restitution following nisin treatment.

**Conclusion:** Nisin's ability to shift the gut and liver microbiome towards a new state commensurate with health while mitigating enteritis, represents a novel approach to treating periodontal disease-associated NAFLD.

GB-04

### Application of Delayed-Absorption Membranes in Guided Bone Regeneration

Taito Watanabe<sup>\*1</sup>, Akira Hasuike<sup>1</sup>, Eiji Goke<sup>1</sup>, Hirotaka Akiyama<sup>1</sup>, Shuichi Sato<sup>1</sup>

<sup>1</sup>Department of Periodontology, Nihon University School of Dentistry

**Background and objective:** The study investigates Guided Bone Regeneration (GBR) in dental practice, focusing on the limitations of existing membranes, such as rapid absorption and inadequate space maintenance. The aim is to compare a newly developed resorbable bilayer membrane (PLACL) with a collagen membrane in a rat GBR model.

**Materials and Methods:** An experimental model involving bilateral perforation of rat skull parietal bones was created. A cylindrical plastic device filled with hydroxyapatite (CO<sub>3</sub>AP) and demineralized bovine bone mineral (DBBM) was inserted, along with a tube-shaped L-lactide-ε-caprolactone copolymer (LA/CL) membrane or pig type 1 and type 3 collagen membrane on the top, followed by reduction and suturing. After 24 weeks of micro-CT observation in animal experiments, tissue sections were prepared and compared.

**Results:** Significant bone regeneration was observed across all groups, but after 24 weeks, the PLACL membrane exhibited notable resilience and sporadic partial degradation. This prolonged preservation of the barrier effect suggests potential benefits for optimal bone regeneration.

**Conclusion:** the PLACL membrane emerges as a promising alternative to GBR, offering a durable barrier and sustained support for bone regeneration over an extended period. The resorbable bilayer membrane shows promise in addressing current membrane limitations. However, further studies and clinical trials are imperative to validate the efficacy and safety of the PLACL membrane in human applications. This research underscores the potential of the PLACL membrane to enhance GBR procedures in dental implant placement.



## GB-05

**Investigation of a novel protein in *Treponema denticola* implicated in oxygen stress response**

Yurie Kitamura-Nakazawa<sup>\*1</sup>, Keiko Yamashita<sup>1</sup>, Rio Hisanaga<sup>1</sup>, Kengo Doya<sup>1,2</sup>, Shinta Mori<sup>1,2</sup>, Kentaro Imamura<sup>1,2</sup>, Kazuhiro Umehara<sup>4</sup>, Tomoko Kobayashi<sup>5</sup>, Kazuyuki Ishihara<sup>2,3</sup>, Atsushi Saito<sup>1,2</sup>

<sup>1</sup> Department of Periodontology, Tokyo Dental College, Tokyo, Japan, <sup>2</sup> Oral Health Science Center, Tokyo Dental College, Tokyo, Japan, <sup>3</sup> Department of Microbiology, Tokyo Dental College, Tokyo, Japan, <sup>4</sup> Umehara Dental Clinic, Aomori, Japan, <sup>5</sup> T.Y Dental Clinic, Chiba, Japan

**Background and objective:** Oxygen is a fatal stress for periodontal pathogens which are composed mainly of obligate anaerobes. *Treponema denticola* is one of the major pathogens in periodontitis. It remains unclear how this bacterium responds to oxygen stress. Using the newly constructed mutant, we investigated the roles of a novel protein focusing on the response to oxygen stress in *T. denticola*.

**Materials and Methods:** The mutant deficient in the DNA binding protein was constructed by homologous recombination. The viable cell proportion under aerobic condition was evaluated by ATP-based assay. Gene expression profiles were evaluated by RNA-seq. Protein expressions were evaluated by western blotting. Invasions were observed using a laser scanning confocal microscope.

**Results:** Under oxygen exposure, the proportion of viable cells in the mutant strain was significantly reduced (by approximately 40%) as compared to wild type strain ( $p < 0.05$ ). In the RNA-seq analysis, the gene coding for leucine-rich repeat (Lrr) protein coding gene was upregulated in the mutant strain as compared to wild type strain. When the band of Lrr protein in Western blot was analyzed by the image software, the intensity in the mutant strain was approximately 2.5-fold, compared to wild type strain. The proportion of cells in Lrr protein deficient mutant attached to epithelial cells was reduced by approximately 40% as compared to wild type strain ( $p < 0.05$ ).

**Conclusion:** It is suggested that a novel DNA binding protein of *T. denticola* is involved in the response to oxygen stress, and it regulates the expression of Lrr protein.

## GB-06

**Hydroxyapatite nanoparticles accelerates alveolar bone resorption in rat periodontitis model**

Yukio Ozaki<sup>\*</sup>, Ralph Jacob M. Elazegui, Megumi Mae, Eijiro Sakamoto, Atsutoshi Yoshimura

Department of Periodontology and Endodontology, Nagasaki University Graduate School of Biomedical Sciences

**Background and objective:** Macrophages stimulated with hydroxyapatite nanoparticles induce NLRP3 inflammasome activation and produce IL-1 $\beta$ , which can promote bone resorption. The purpose of this study was to investigate whether the administration of hydroxyapatite (HA) nanoparticles promotes alveolar bone resorption in a rat periodontitis model.

**Materials and Methods:** The maxillary right and left second molars of male Lewis rats (n=19) were ligated with silk sutures. HA nanoparticles suspended in 3% hydroxymethylcellulose were administered into the palatal gingival sulcus of the right second molar daily for 7 (n=9) or 14 days (n=10). Hydroxymethylcellulose alone was administered to the palatal gingival sulcus of the left second molar as a control. Then, the rats were sacrificed and the alveolar bone levels were measured by  $\mu$ CT. Alveolar bone resorption was also examined histopathologically after H&E and TRAP staining.

**Results:** The  $\mu$ CT images showed that bone resorption at HA-administered sites was greater than that at control sites in both the 7- and 14-days group. The number of TRAP-positive cells at HA-administered sites was also greater than that at control sites in both the 7- and 14-days group. Vertical bone resorption was often observed at HA-administered sites in the 7-days group and both vertical and horizontal bone resorption was observed at HA-administered sites in the 14-days group.

**Conclusion:** In this rat periodontitis model, alveolar bone resorption was accelerated by the administration of HA nanoparticles. It would be revealed whether dental calculus nanoparticles accelerate alveolar bone resorption as HA nanoparticles in future experiments.

GB-07

### Amelogenin prolongs the survival of skin allograft in mice.

Miyu Shida<sup>\*</sup>, Terukazu Sanui, Karen Yotsumoto, Yuki Nishimura, Chikako Hayashi,  
Masaaki Toyoda, Kentaro Kawakami, Takao Fukuda, Fusanori Nishimura

Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University

**Background and objective:** Enamel matrix proteins appear to exhibit minimal post-surgical inflammation and favorable healing outcomes. Previous studies reported that amelogenin inhibits the expression of major histocompatibility complex class II (MHC II) through histone H3 euchromatin suppression with reduced antigen presentation. However, the physiological significance of lowered MHC II expression remains unclear. This study aimed to investigate the influence of amelogenin on skin graft rejection among the mice with different haplotype antigens.

**Methods and Results:** Recombinant amelogenin was applied to the skin from C57BL/6J mice, followed by the transplantation onto recipient sites of BALB/c mice. The median survival period of the grafts was extended by up to 6 days compared to a PBS control group, accompanied by a reduction in necrotic area. Amelogenin group showed lower splenic weight and cell counts than control. Histological analysis of skin tissues at day 7 after transplantation showed significantly decreased inflammatory cell infiltration and MHC II<sup>+</sup> cells in test group, while massive immune cell infiltration was observed in the control group. Furthermore, a decrease in CD4<sup>+</sup> and CD19<sup>+</sup> cells was observed with significant reduction in IFN- $\gamma$ +CD4<sup>+</sup> cells. However, CD25+Fox3+CD4<sup>+</sup> cells increased. Serum IFN- $\gamma$  and IL-2 levels in test group were significantly lower than control.

**Conclusion:** The results may provide insights into the mechanisms by which amelogenin accelerates post-surgical wound healing during flap surgery and its potential application for additional surgery such as gingival graft for root coverage. These findings may also suggest potential use of amelogenin for the prevention of rejection after organ transplantation.

GB-08

### Oral-Brain-Axis-Association between Periodontal Pathogens in Brain and Alzheimer's Disease

Yoshimasa Taketani<sup>\*1,2</sup>, Allan Radaic<sup>2</sup>, Pachiyappan Kamarajan<sup>2</sup>, Christopher K. Williams<sup>3</sup>,  
Shino Magaki<sup>3</sup> and Yvonne L. Hernandez-Kapila<sup>2</sup>

<sup>1</sup>Division of Periodontology, Department of Oral Biology and Tissue Engineering, Meikai University School of Dentistry, <sup>2</sup>Department of Biosystems and Function and Periodontics, School of Dentistry, University of California Los Angeles, <sup>3</sup>Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California Los Angeles

**Background and objective:** Alzheimer's disease (AD), a complex/multifactorial disease of unknown etiology affecting >50 million people, is characterized by accumulation of hyperphosphorylated tau-positive-neurofibrillary tangles and insoluble amyloid  $\beta$  plaques in the brain. These changes stimulate glial cell activation and a local immune response, which damage neurons. Furthermore, systemic inflammation associated with chronic inflammatory diseases, like periodontitis, may induce neuroinflammation. To explore this oral-brain axis, we investigated the presence/quantity of periodontal pathogens in human brain specimens from individuals with and without AD.

**Materials and Methods:** DNA was extracted from brain autopsy samples (hippocampal, frontal, occipital) from individuals with severe AD (n=10) and without (n=3; AD-negative; IRB#24-000223) and evaluated for *Treponema denticola*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Tannarella forsythia* presence and quantity via qPCR.

**Results:** *T. denticola* was detected in all brain regions (hippocampal, frontal, occipital) of all AD specimens but not all normal specimens. *P. gingivalis*, *F. nucleatum*, and *T. forsythia* weren't detected. *T. denticola* copy number was 2.6-fold higher in frontal regions of severe AD ( $14.77 \pm 4.70$ ) compared to normal ( $5.58 \pm 4.15$ ) samples and 3-fold higher in occipital regions of normal ( $35.29 \pm 17.48$ ) compared to severe AD ( $10.78 \pm 5.20$ ) samples. In AD samples, *T. denticola* copy number decreased sequentially from hippocampal, to frontal, and occipital regions. These data suggest that *T. denticola* may preferentially reside in occipital regions of the brain in "health" then shift/spread to other brain regions (frontal and hippocampal) with AD.

**Conclusion:** Detection of *T. denticola* in human brains further links this microbe and periodontal disease to AD.

GB-09

### Fibroblast growth factor-2 promotes experimental gingival wound healing in rats

Yasunori Yoshinaga<sup>\*1,2</sup>, Naoki Maruo<sup>1</sup>, Kimiko Ohgi<sup>1</sup>, Hiroaki Yamato<sup>1</sup>, Nanako Tsuchimochi<sup>1</sup>,  
Masanobu Nakagami<sup>1</sup>, Ryuji Sakagami<sup>1</sup>

<sup>1</sup>Section of Periodontology, Department of Odontology, Fukuoka Dental College, <sup>2</sup>Oral Medicine Research Center, Fukuoka Dental College

**Background and objective:** Fibroblast growth factor (FGF)-2 has been shown to promote periodontal tissue regeneration. However, there are few reports on the effect of FGF-2 on wound healing after periodontal surgery. This study aimed to investigate the effect of FGF-2 on gingival wound healing in rats.

**Materials and Methods:** Seven-week-old male Sprague Dawley rats were divided into the following three groups. Control group: Incision was made in gingiva adjacent to maxillary first molar and a full-thickness flap was raised, followed by suturing. FGF-2 group and Enamel matrix derivative (EMD) group: FGF-2 or EMD, respectively was applied before suturing. All groups were euthanized 1, 3, and 7 days after treatment, the maxilla was resected, and paraffin sections were prepared. Paraffin sections were H.E. stained, and reepithelialization, gingival thickness, inflammation and angiogenesis were evaluated.

**Results:** The reepithelialization was significantly promoted in the FGF-2 and EMD groups compared to the control group at day 1. Gingival thickness was significantly greater in the FGF-2 group than in the other groups. Inflammation tended to be decreased in the FGF-2 and EMD groups compared to the control group at day 1 but increased in the EMD group at day 3. Angiogenesis tended to be increased in the FGF-2 group compared to the other groups from day 3.

**Conclusion:** FGF-2 and EMD promoted reepithelialization, and FGF-2 increased gingival thickness and angiogenesis. Additionally, EMD was suggested to have an effect on inflammatory cell infiltration.

GB-10

### The expression of neutrophil extracellular traps induced by *Porphyromonas gingivalis* lipopolysaccharide

Hiroshi Ito<sup>\*</sup>, Yukihiro Numabe, Tomohisa Sakayori

The Nippon Dental University, Graduate School of Life Dentistry at Tokyo, Department of Periodontology

**Background and objective:** A new function of PMNs, neutrophil extracellular traps (NETs), which appears to contribute to defense responses, has been reported. However, the relationship between periodontal pathogens and NET expression remains controversial. The aim of this study was to reveal the NET expression induced by *P.gingivalis* lipopolysaccharide (PG-LPS).

**Materials and Methods:** PMNs were isolated from peripheral blood collected from healthy subjects with healthy periodontal tissues and stimulated with PMA, PG-LPS, or *E.coli* LPS (EC-LPS). Peripheral blood PMNs were also stimulated after Toll-like receptors (TLRs) were blocked using specific antibodies. NETs were observed using SEM and fluorescence immunostaining of NET component DNA, histone, and neutrophil elastase (NE). In addition, gingival crevicular fluid (GCF) PMNs were isolated from the same subject and stimulated with PG-LPS, followed by observation by fluorescent immunostaining and measurement of extracellular DNA.

**Results:** Fluorescence immunostaining images showed that the filamentous structures stained positive for DNA, histone, and NE under PMA, PG-LPS, and EC-LPS stimulation, confirming that the structures were NETs. A decrease in NET expression was observed in the PG-LPS group when TLR2 and TLR4 were inhibited. On the other hand, fluorescent immunostaining showed that extracellular DNA was significantly higher in GCF-PMNs than in peripheral blood PMNs with each stimulus.

**Conclusion:** PG-LPS induced NETs expression through TLR2 and TLR4, and GCF-PMNs had higher NETs expression than peripheral blood PMNs. This study was supported by Grants-in-Aid from the Ministry of Education and Science Research Funds [JSPS (C) JP20K09981, JP23K09189].

## GB-11

**Role of HSP47 on collagen synthesis in aged periodontal ligament**

Arisa Nishikawa<sup>\*1,2</sup>, Motozo Yamashita<sup>1</sup>, Tomomi Nakamura<sup>1</sup>, Kuniko Ikegami<sup>1</sup>, Mio Suzuki<sup>1</sup>, Masahiro Kitamura<sup>1</sup>, Shinya Murakami<sup>1</sup>

<sup>1</sup>Department of Periodontology, Osaka University Graduate School of Dentistry, <sup>2</sup>Department of Periodontics and Dental Hygiene, the University of Texas Health Science Center at Houston School of Dentistry

**Background and objective:** Heat-shock protein (HSP) 47 has been revealed to regulate the triple helix structure of collagen as a chaperone. The quality control of type I collagen which is the most abundant extracellular matrix protein, is crucial for Periodontal ligament (PDL) homeostasis. However, the physiological roles of HSP47 in aged PDL are not fully understood. We aimed to determine the effects of HSP 47 on the proteostasis of type I collagen and endoplasmic reticulum (ER) stress in aged PDL.

**Materials and Methods:** Expressions of denatured collagen in aged (103 weeks) mice PDL (AMPDL) and aged human PDL (AHPDL) cells were analyzed with collagen hybridizing peptides; CHP that binds unfolded collagen. Co-localizations of type I collagen, ER, Golgi apparatus, and HSP47 were examined by confocal microscopy. Geranylgeranylacetone (GGA) was used as an inducer of HSP47. Col003 and siHSP47 were used for inhibitions of HSP47. Mature type I collagen was quantified by ELISA. Expressions of ER stress protein; IRE1, ATF6, and PERK were determined by western blotting.

**Results:** HSP47 was downregulated, and the denatured collagen was highly expressed in AHPDL cells and AMPDL. GGA ameliorated the accumulation of denatured collagen in ER, the induction of ER stress by Col003 or siHSP47, and the secretion of mature type I collagen in AHPDL.

**Conclusion:** This study suggests that stabilization of collagen structure by HSP47 is important for proper type I collagen synthesis in PDL. Therefore, the development of targeted therapy for impaired HSP47 is desired to promote periodontal health in elderly populations.

## GB-12

**miR-1260b promotes M2 macrophage polarization by targeting NFAT5**

Chikako Hayashi<sup>\*</sup>, Takao Fukuda, Miyu Shida, Masaaki Toyoda, Kentaro Kawakami, Takanori Shinjo, Terukazu Sanui, Fusanori Nishimura

Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental science, Kyushu University

**Background and objective:** Our previous study demonstrated that exosome from TNF- $\alpha$  treated GMSCs contained increased amount of miR-1260b and this exosome converted macrophage phenotype from proinflammatory M1 to an anti-inflammatory M2 (Nakao Y, *et al.*, *Acta Biomater*, 2021). However, potential target genes of this miR-1260b still remains unclear. Here, we screened novel target genes by database analysis (miRDIP) and picked up one such candidate gene product as NFAT5. Overexpression of NFAT5 is reported to activate NLRP3-inflammasome and to increase the IL-1 $\beta$  production (Ma P *et al.*, *Cell Commun Signal*. 2019). Therefore, we aimed to investigate the role of NFAT5 on macrophage polarization.

**Materials and Methods:** Human CD14<sup>+</sup> peripheral blood monocytes (PBMCs), THP-1 human monocytic cell line, and RAW264.7 murine macrophage cell line were transfected with miR-1260b mimics, followed by the stimulation of these cells with LPS. Quantitative real-time PCR analysis and western blotting analysis were performed to confirm the inhibition of NFAT5 and NLRP3. Furthermore, knock-down experiments were performed by using NFAT5 siRNA and the expression of NLRP3 were examined by qRT-PCR. The M1/M2 macrophage polarization in si-NFAT5- transfected THP-1 cells PBMCs were analyzed by Flow cytometry.

**Results:** Transfection of miR-1260b mimics inhibited the expression of NFAT5 in macrophages. Knock-down of NFAT5 decreased the expression of NLRP3 and promote M2 macrophage polarization in THP-1 cells and PBMCs.

**Conclusion:** These results suggest that miR-1260b promotes M2 macrophage polarization by targeting NFAT5, and, thus, miR-1260b-containing vesicles can be used as novel therapeutic tool against inflammatory periodontal disease.

GB-13

### Effects of maternal ligature-induced periodontitis on brain in offsprings

Chihiro Imai<sup>\*1</sup>, Sayaka Katagiri<sup>1</sup>, Yujin Ohsugi<sup>1</sup>, Peiya Lin<sup>1</sup>, Keita Toyoshima<sup>1</sup>, Tomomitsu Hirota<sup>2</sup>,  
Takahiko Shiba<sup>1</sup>, Naofumi Uesaka<sup>3</sup>, Takanori Iwata<sup>1</sup>

<sup>1</sup>Department of Periodontology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo,

<sup>2</sup>Division of Molecular Genetics, Research Center for Medical Science, The Jikei University School of Medicine,

<sup>3</sup>Department of Cellular Neurobiology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo

**Background and objective:** Previous studies suggest a connection between maternal periodontal disease and brain function in offspring, though the underlying mechanisms remain unclear. This study aimed to elucidate the effects of maternal periodontitis on offspring brain function and their mechanisms through ligature-induced periodontitis in mice.

**Materials and Methods:** Twelve-week-old female C57BL/6J mice were subjected to induction of periodontitis through silk ligatures tied around both sides of the maxillary second molars. After periodontitis induction, these females were bred with healthy nine-week-old male mice to produce offsprings. Their eight-week-old male offspring were subjected to behavioral tests. Prefrontal cortex and cerebellum in offspring were harvested for RNA-sequencing. Oral ligatures and feces were collected for microbiome analysis based on 16S rRNA gene sequencing.

**Results:** Offspring born from the maternal mice with ligature-induced periodontitis showed reduced sociability in the sociality test and impaired motor function on the first day of the rotarod test. RNA-sequencing analyses revealed altered gene expression patterns in the cerebellum through principal component analysis. Significant shifts were also observed in the maternal oral microbiome, primarily characterized by a reduced diversity, and increased prevalence of genus *Klebsiella*, without any alteration in gut microbiome. Offsprings born from maternal mice with ligature-induced periodontitis showed a significant decrease of diversity in the gut microbiome, marked by an enhanced presence of genus *Staphylococcus*. These microbiome alterations suggest an association with the observed behavioral deficits.

**Conclusion:** The findings support the hypothesis that maternal periodontitis can adversely affect brain function in offspring, potentially mediated by changes in the microbiome.

GB-14

### Insulin sensitizing reagents suppress inflammation-inducing VCAM-1 expression on endothelial cells

Tatsuro Zeze<sup>\*</sup>, Takanori Shinjo, Shuang Chen, Naoaki Ryo, Al-kafee Ahmed, Honoka Otsuka,  
Kohei Sato, Mio Imagawa, Yuki Nishimura, Akiko Yamashita, Fusanori Nishimura

Section of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University

**Background and objective:** People with diabetes are less likely to respond to periodontal treatment than healthy individuals due possibly to exaggerated inflammatory responses and impaired wound healing. Recent studies demonstrated that not only systemic but also local insulin resistance played an important role in the pathogenesis of diabetic complications. In this study, we therefore investigated the effects of insulin sensitizing reagents, metformin and imeglimin, on diabetes-related periodontitis by local administration.

**Materials and Methods:** The effect of metformin and imeglimin on *E. coli* LPS and TNF $\alpha$  (10ng/ml, respectively)-induced VCAM-1 expression and cellular adhesion with human leukocyte cell line (THP-1) were assessed using murine vascular endothelial cell line (TKD2) under hyperglycemia-induced insulin resistant conditions. Furthermore, the effect of local metformin administration on bone resorption induced by 7-0 silk ligation in 60% high-fat diet (60%HFD) fed C57BL/6J mice were evaluated.

**Results:** *E. coli* LPS and TNF $\alpha$ -induced VCAM-1 expression was significantly suppressed by pretreatment of TKD2 cells with metformin and imeglimin under hyperglycemic conditions for 48 hours, and these results were confirmed by cell adhesion test with THP-1 cells. Bone resorption appeared to be suppressed in HFD-fed mice treated with local, gingival administration of metformin as compared with those with vehicle administration.

**Conclusion:** Insulin sensitizing reagents metformin and imeglimin could suppress inflammation-induced VCAM-1 expression on endothelial cells and the cellular adhesion with leukocytes under insulin resistant conditions. Therefore, local application of these insulin sensitizing reagents may have beneficial effects against inflammatory periodontal tissue breakdown.



## GB-15

**Effect of 5-fluorouracil on periodontal tissue: an animal study**

Takayuki Maruyama<sup>\*1,2</sup>, Momoko Nakahara<sup>3</sup>, Daiki Fukuhara<sup>4</sup>, Yixuan Zhang<sup>4</sup>,  
 Mohammad Nurhamim<sup>4</sup>, Manabu Morita<sup>5</sup>, Daisuke Ekuni<sup>1</sup>

<sup>1</sup>Department of Preventive Dentistry, Faculty of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, <sup>2</sup>Advanced Research Center for Oral and Craniofacial Sciences, Okayama University Dental School, <sup>3</sup>Department of Preventive Dentistry, Okayama University Hospital, <sup>4</sup>Department of Preventive Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, <sup>5</sup>Department of Oral Health Sciences, Takarazuka University of Medical and Health Care

**Background and objective:** The purpose of this study was to investigate the mechanism by which 5-FU administration deteriorates periodontal disease in a rat model from the perspective of oxidative stress.

**Materials and Methods:** Thirty-two Wistar male rats (8 weeks old) were divided into four groups (C: control group, F: 5-FU administration group, P: periodontal disease group, and PF: periodontal disease + 5-FU administration group). Silk thread was placed around the maxillary first molar to induce periodontal disease for 8 weeks. Also, 5-FU was intraperitoneally administered twice at a dose of 80mg/kg body weight. After experimental period, alveolar bone loss and gingival oxidative stress (8-hydroxy-2'-deoxyguanosine: 8-OHdG) were measured. Tukey method was used for comparisons between groups, and the significance level was set at 5%.

**Results:** Alveolar bone loss in the C, F, P and PF group was  $0.21 \pm 0.01$ mm,  $0.25 \pm 0.08$ mm,  $0.56 \pm 0.06$ mm and  $0.71 \pm 0.08$ mm, respectively. Alveolar bone loss in the P and PF groups were significantly higher than in the C group. Also, alveolar bone loss in the PF group was significantly higher than in the P group. Level of gingival 8-OHdG in the C, F, P and PF group was  $0.43 \pm 0.01$ ng/mg mtDNA,  $0.54 \pm 0.17$ ng/mg mtDNA,  $0.92 \pm 0.09$ ng/mg mtDNA and  $1.11 \pm 0.14$ ng/mg mtDNA, respectively. Levels of gingival 8-OHdG in the P and PF groups were significantly higher than in the C group. Also, the level of gingival 8-OHdG in the PF group was significantly higher than in the P group.

**Conclusion:** Administration of 5-FU may deteriorate periodontal bone loss by production of gingival oxidative stress.

## GB-16

**AGEs reduce the barrier function in the gingival epithelial carcinoma-derived cell line of Ca9-22 cells**

Risa Ichikawa<sup>\*1</sup>, Natsuko Tanabe<sup>2</sup>, Keiko Tomita<sup>1</sup>, Misae Ono<sup>1</sup>, Yuma Masai<sup>1</sup>, Hideaki Seki<sup>1</sup>,  
 Soichiro Manaka<sup>1</sup>, Tadahiro Takayama<sup>1</sup>, Masatoshi Takane<sup>1</sup>, Kazunari Ando<sup>1</sup>, Naoto Suzuki<sup>2</sup>,  
 Shuichi Sato<sup>1</sup>

<sup>1</sup> Department of Periodontology, Nihon University Graduate School of Dentistry, Tokyo, Japan, <sup>2</sup> Department of Biochemistry, Nihon University Graduate School of Dentistry, Tokyo, Japan

**Background and objective:** In previous epidemiological studies the severity of periodontal disease is significantly higher in diabetic patients, but the mechanism is unclear. Recently, it has been reported that Advanced Glycation Endproducts (AGEs), which are produced during uncontrolled hyperglycemia, might be one of the factors causing diabetic complications. Epithelial cells have intercellular adhesion mechanisms, and tight junctions are at the forefront of bacterial invasion in the epithelium. Tight junctions such as Claudin (CLDN) and JAM-A relate to the barrier function in epithelial cells. The present study was designed to investigate the effects of AGEs on the barrier function of the gingival epithelium in Ca9-22 cells.

**Materials and Methods:** Ca9-22 cells stimulated with or without AGEs (100 µg/ml) and RAGE inhibitor (FPS-ZM1) for up to 72 hours—the mRNA expression of CLDN7, ZO-2, 3, and JAM-A using real-time PCR. In CLDN7, protein expression was examined by Western blotting, localization by immunostaining, and the effects on the epithelial barrier function on AGEs stimulation by Trans-epithelial electrical resistance (TEER).

**Results:** AGEs decreased mRNA levels of CLDN7 on hour 72 of culture compared to untreated control and BSA. In addition, FPS-ZM1 inhibited the stimulatory effects of AGEs on CLDN7, ZO-3, and JAM-A mRNA levels. AGEs also reduced the protein expression, the accumulation of CLDN7 on the cell membrane, and TEER at 72 hours of culture; in contrast, FPS-ZM1 abolished the stimulatory effect of AGEs.

**Conclusion:** These results suggest that AGEs disrupt the barrier function of the gingival epithelium by reducing CLDN7 and ZO-3 expressions.

GB-17

### Genetic Analysis of impaired Healing Responses after Periodontal Therapy in Diabetes

Keita Nakagawa<sup>\*1</sup>, Kazuki Watanabe<sup>2</sup>, Koji Mizutani<sup>1</sup>, Kohei Takeda<sup>1</sup>, Shu Takemura<sup>1</sup>,  
Eri Sakaniwa<sup>1</sup>, Risako Mikami<sup>1</sup>, Daisuke Kido<sup>3</sup>, Natsumi Saito<sup>1</sup>, Hiromi Kominato<sup>1</sup>,  
Atsuhiko Hattori<sup>4</sup>, Takanori Iwata<sup>1</sup>

<sup>1</sup> Department of Periodontology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo,

<sup>2</sup> Department of Biology, College of Liberal Arts and Sciences, Institute of Science Tokyo, <sup>3</sup> Department of General Dentistry, Institute of Science Tokyo, <sup>4</sup> Department of Sport and Wellness, Rikkyo University Niiza,

**Background and objective:** This study aims to investigate the mechanisms underlying the impaired healing response by diabetes after periodontal therapy. Outcomes of periodontal therapy in patients with diabetes are impaired compared with those in patients without diabetes. However, the mechanisms underlying impaired healing response to periodontal therapy have not been sufficiently investigated.

**Materials and Methods:** Zucker diabetic fatty (ZDF) and lean (ZL) rats underwent experimental periodontitis by ligating the mandibular molars for one week. The gingiva at the ligated sites was harvested one day after ligature removal, and gene expression was comprehensively analyzed using RNA-Seq. In patients with and without type-2 diabetes (T2D), the corresponding gene expression was quantified in the gingiva of the shallow sulcus and residual periodontal pocket after non-surgical periodontal therapy.

**Results:** Ligation-induced bone resorption and its recovery after ligature removal were significantly impaired in the ZDF group than in the ZL group. The RNA-Seq analysis revealed 252 differentially expressed genes. Pathway analysis demonstrated an enrichment of downregulated genes involved in peroxisome proliferator-activated receptor (PPAR) signaling pathway. PPAR $\alpha$  and PPAR $\gamma$  were decreased in mRNA level and immunohistochemistry in the ZDF group than in the ZL group. In clinical, probing depth reduction was significantly less in the T2D group than control. Significantly downregulated expression of PPAR $\alpha$  and PPAR $\gamma$  were detected in the residual periodontal pocket of the T2D group compared with those of the control group, but not in the shallow sulcus.

**Conclusion:** Downregulated PPAR subtypes expression may involve the impaired healing of periodontal tissues by diabetes.

GB-18

### Enhanced periodontal tissue healing following low-level Er:YAG laser irradiation

Shu Takemura<sup>\*1</sup>, Koji Mizutani<sup>1</sup>, Risako Mikami<sup>1</sup>, Keita Nakagawa<sup>1</sup>, Masahiro Hakariya<sup>1</sup>,  
Eri Sakaniwa<sup>1</sup>, Natsumi Saito<sup>1</sup>, Hiromi Kominato<sup>1</sup>, Daisuke Kido<sup>2</sup>, Kohei Takeda<sup>1</sup>,  
Akira Aoki<sup>1</sup>, Takanori Iwata<sup>1</sup>

<sup>1</sup>Department of Periodontology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, <sup>2</sup>Oral Diagnosis and General Dentistry, Institute of Science Tokyo Hospital

**Background and objective:** This study aimed to investigate the effects of low-level erbium-doped: yttrium, aluminum, and garnet (Er:YAG) laser irradiation on periodontal tissue healing and regeneration through angiogenesis in vivo and in vitro studies.

**Materials and Methods:** Intrabony defects were surgically created in the bilateral maxilla molar of rats. The defects were treated by open flap debridement (OFD) with Er:YAG laser, including low-level laser irradiation (LLLI) to bone and blood clot surfaces, or conventional procedures. The mRNA expression of vascular endothelial growth factor (VEGF) in the surgical sites was quantified using real-time polymerase chain reaction. The decalcified specimens were prepared for histometric analysis. Also, LLLI was performed on human umbilical vein endothelial cells to evaluate the effects on angiogenesis. Cell proliferation, VEGF expression, and tube formation were assessed. In addition, capsazepine (CPZ), a selective inhibitor of transient receptor potential vanilloid 1 (TRPV1), treatment was performed before LLLI for the same assays.

**Results:** Postoperative healing was sound in all animals in both groups. VEGF expression and bone formation were significantly increased in the laser-treated group compared to those in the conventional treatment group. In vitro, cell proliferation and VEGF expression were significantly increased in the LLLI group compared to the control group. Tube-formation assays showed that LLLI significantly promoted angiogenesis. CPZ treatment significantly suppressed VEGF expression and tube formation following LLLI.

**Conclusion:** This study suggests that Er:YAG laser irradiation may promote periodontal tissue healing by enhancing angiogenic effect of endothelial cells via TRPV1.

## GB-19

**Senolytic effects on periodontitis, type 2 diabetes mellitus, and atherosclerosis**Naoto Koyama<sup>\*1</sup>, Tzu Yang Kuo<sup>1,2</sup>, Yuki Ohtani<sup>1,2</sup>, Mikoto Hara<sup>1</sup>, Yuki Ozaki<sup>1</sup>, Nobuo Yoshinari<sup>1,2</sup><sup>1</sup>Department of Cariology, Endodontology and Periodontology, Matsumoto Dental University,<sup>2</sup>Oral Health Science, Department of Oral Health Promotion, Oral Science Course, Matsumoto Dental University  
Graduate School of Oral Medicine

**Background and objective:** Aging has been reported as a common etiological factor in lifestyle-related diseases such as periodontitis (P), diabetes mellitus (DM), and atherosclerosis (AT). Aging is closely related to chronic inflammation caused by cellular senescence-associated secretion phenomena induced by senescent cells. Therefore, we used aging mice and observed the effect of removing senescent cells on experimental models of P, DM, and AT.

**Materials and Methods:** One hundred and twenty-seven C57BL/6 and ApoE<sup>(-/-)</sup> mice were divided into eight groups: control, P, DM, AT, P+DM, P+AT, DM+AT, and P+DM+AT. Each group was divided further into senolytic-treated and non-treated groups and kept until 72 weeks of age. The P group was induced by ligating molars with silk threads, the DM group was induced with streptozotocin and nicotinamide, and the AT group consisted of ApoE<sup>(-/-)</sup> mice. Dasatinib and quercetin (D+Q) were administered transgastrically as senolytics. Evaluations were made to determine the SA- $\beta$ -gal-stained gingival areas, serum IL-6 concentrations, alveolar bone resorption by  $\mu$ -CT analysis, fasting blood glucose levels, and arteriolar luminal fat deposition areas.

**Results:** The percentage of SA- $\beta$ -gal staining positive areas, serum IL-6 concentrations, and Sudan IV staining positive areas in the AT group were significantly decreased in the D+Q group, alveolar bone resorption tended to decrease in the P group, and there was no significant decrease in fasting blood glucose in the DM group.

**Conclusion:** D+Q administration removed senescent cells and improved the pathological conditions of each disease except DM,

## GB-20

***Porphyromonas gingivalis* Outer Membrane Vesicles Promotes Alveolar Bone Resorption**Daisuke Nakamura<sup>\*1</sup>, Yuta Uemura<sup>1</sup>, Yoshimune Iwata<sup>1</sup>, Yuji Inagaki<sup>1</sup>, Hiromichi Yumoto<sup>1</sup><sup>1</sup>Department of Periodontology and Endodontology, Tokushima University Graduate School of Biomedical Sciences

**Background and objective:** Outer membrane vesicles (OMVs) released from bacteria contain bacterial-specific nucleic acids and proteins. It has been considered that *Porphyromonas gingivalis* (Pg)-OMVs have various effects on periodontal tissue component cells, however their involvements in alveolar bone resorption have not yet well clarified. Therefore, the aim of this study was to investigate the effects of Pg-OMVs on osteoblasts and osteoclasts *in vitro* and furthermore to elucidate their involvement in alveolar bone resorption using *in vivo* rat model.

**Materials and Methods:** The expression levels of RANKL and OPG in MC3T3-E1 cells cultured with Pg-OMVs under calcification-inducing medium were analyzed. Regarding their effects on mineralization, ALP activity and bone-like nodules were determined. RAW264.7 cells treated with sRANKL to induce osteoclast differentiation were stimulated with Pg-OMVs. The number of multinucleated cells was measured by TRAP staining, and the expression of osteoclast differentiation markers were determined by Western blotting. Furthermore, rats were divided into following four groups: healthy control group, Pg-OMVs administration group, ligature-induced periodontitis group, and Pg-OMVs administration-ligature-induced periodontitis group, and the level of alveolar bone resorption was measured using  $\mu$ CT.

**Results:** Pg-OMVs increased RANKL expression and decreased OPG expression in MC3T3-E1 cells. Furthermore, Pg-OMVs reduced ALP activity and the formation of bone-like nodules in a dose-dependent manner. In RAW264.7 cells, Pg-OMVs promoted osteoclast differentiation and increased the expression of osteoclast differentiation markers. In rat model, Pg-OMVs administration significantly enhanced alveolar bone resorption.

**Conclusion:** These results suggested that Pg-OMVs involve in alveolar bone resorption via suppressing osteoblast mineralization and promoting osteoclast differentiation.



GB-21

### Characteristics of Bacterial Communities among Healthy, Peri-implant Mucositis, and Peri-implantitis Statuses

Takahiko Nagai<sup>\*1</sup>, Takahiko Shiba<sup>1</sup>, Keiji Komatsu<sup>2,3</sup>, Shunsuke Matsumura<sup>1</sup>, Tatsuro Koyanagi<sup>1</sup>, Takashi Nemoto<sup>1</sup>, Ryota Kobayashi<sup>1</sup>, Koki Takada<sup>1</sup>, Yasuo Takeuchi<sup>3</sup>, Takanori Iwata<sup>1</sup>

<sup>1</sup>Department of Periodontology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo,

<sup>2</sup>Department of Lifetime Oral Health Care Science, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, <sup>3</sup>Weintraub Center for Reconstructive Biotechnology, UCLA School of Dentistry, Los Angeles, CA.

**Background and objective:** Dental implants are crucial for treating missing teeth, while a dysbiotic microbiome often causes biological implant complications leading to implant loss. This study aimed to clarify the characteristics of the healthy implants (HI), peri-implant mucositis (MI), and peri-implantitis (PI) microbiomes using 16S rRNA gene amplicon sequencing.

**Materials and Methods:** A total of 75 subgingival plaques, which included 25 sites from each HI, MI, and PI condition, were collected from 51 subjects. After bacterial DNA extraction from the samples, the V3-V4 region of the 16S rRNA gene was sequenced and analyzed using QIIME2 and the Human Oral Microbiome Database to elucidate the bacterial composition.

**Results:** Bacterial diversity at the MI and PI sites was higher than that at the HI site, while no statistical difference was observed between MI and PI sites. *Desulfomicrobium* and *Saccharibacteria* (TM7) [G-1] *bacterium* HMT 349 showed a significantly higher abundance at the MI site than at the HI site, suggesting that these bacterial taxa might be associated with the induction of peri-implant inflammation. Well-known disease-associated bacteria such as *Treponema*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* subsp. *vincentii* exhibited significantly higher abundance at the PI site than at the HI site. Furthermore, *Capnocytophaga granulosa* and *Prevotella* sp. HMT 304 were more abundant at the PI site than at the MI site.

**Conclusion:** Our study highlighted the bacterial characteristics in healthy implants and peri-implant diseases and suggested the bacteria that appear to be involved in the pathogenic state of dental implants, especially peri-implantitis.

GB-22

### Transcriptome analysis for the mechanisms of occlusal trauma in mice

Yosuke Tsuchiya<sup>\*1</sup>, Yujin Ohsugi<sup>1</sup>, Sayaka Katagiri<sup>1</sup>, Tomomitsu Hirota<sup>2</sup>, Takanori Iwata<sup>1</sup>

<sup>1</sup>Department of Periodontology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental

University, <sup>2</sup>Division of Molecular Genetics, Research Center for Medical Science, The Jikei University School of Medicine

**Background and objective:** Occlusal trauma is an important risk factor for the exacerbation of periodontitis, however the regulatory mechanisms remain unclear. The purpose of this study was to elucidate the molecular mechanisms by performing RNA-sequencing of periodontal tissues of periodontitis model and occlusal trauma model in mice.

**Materials and Methods:** Ligature-induced periodontitis model (Li) was created by ligating the ligature at the maxillary left second molar of 9-week-old C57BL/6J male mice, and occlusal trauma model (Tra) was created by building composite resin. Four groups were divided as follows: Li, Tra, LiTra and Control. Four groups were evaluated for bone resorption by micro-CT analysis before and 8 days after treatment. For long-term evaluation, Tra underwent micro-CT analysis after 8 weeks. Three days after the treatment, the periapical bone and gingiva of the maxillary left second molar were collected for RNA-seq.

**Results:** Micro-CT analysis showed no bone resorption in Tra, however, significantly increased bone resorption in LiTra compared to Li. No bone resorption was observed in Tra in long-term evaluation. Principal component analysis showed that gene expression patterns were different in each group, especially in bone. RNA-seq results for bone from Li and LiTra showed 167 differential expressed genes ( $|FC| > 2$ ,  $q < 0.1$ ), and *Il11*, *Il17f* expression was significantly increased in LiTra.

**Conclusion:** These results suggested that occlusal trauma exacerbates bone resorption with periodontitis but not without periodontitis. Transcriptome analysis suggested that occlusal trauma induces *Il11*, which regulates bone metabolism via mechanical stress, and *Il17f*, related to inflammation.

GB-23

### Lysosome-mitochondria interaction regulates mineralization *in vivo*

Phan Bhongsatiern<sup>\*</sup>, Tomoaki Iwayama, Yusaku Yoshida, Yuto Koketsu, Shuji Matsumoto, Masahide Takedachi, Shinya Murakami

Department of Periodontology and Regenerative Dentistry, Osaka University Graduate School of Dentistry

**Background and objective:** Understanding the mechanism of osteogenesis and cementogenesis is essential to establish better periodontal regenerative therapy. Matrix vesicle (MV)-mediated mineralization is the fundamental process for osteogenesis and cementogenesis. We previously found that MVs are formed in osteoblastic lysosomes *in vitro*. The purpose of the present study was to develop a tool for studying MV-mediated mineralization *in vivo* and identify molecules involved in the process.

**Materials and Methods:** To evaluate the cellular lineage in mouse calvaria, Twist2-Cre; Rosa26-lox-stop-lox-tdTomato mice were generated and analyzed histologically. Then, Twist2-Cre; Rosa26-lox-stop-lox-Tmem192-3xHA (Twist2-LysoTag) mice were generated to isolate osteoblastic lysosomes *in vivo*. Intact lysosomes were immunoprecipitated from mouse calvaria and lung using anti-HA magnetic beads (LysoIP). The isolated lysosomes were analyzed by electron microscopy, western blot, and proteome analysis.

**Results:** Lineage tracing results showed Twist2-Cre targeted osteoblast lineage in mouse calvaria and fibroblasts in lung. Lysosomes were successfully isolated from the mouse osteoblast of Twist2-LysoTag mice. Electron microscopic observation revealed the presence of vesicle-like structures within the isolated lysosomes, indicative of MVs. Western blot and proteomic profiling showed mitochondrial proteins were enriched in osteoblastic lysosomes compared to the lysosomes in lung fibroblasts.

**Conclusion:** Twist2-LysoTag mice for *in vivo* LysoIP demonstrated a potential tool for elucidating the mechanisms underlying osteogenesis and cementogenesis. The results suggested that lysosome-mitochondria interaction plays an important role in MV-mediated mineralization.

GB-24

### Carbonate apatite and poly (lactic acid/caprolactone) bilayer membrane in periodontal regeneration

Daichi Yamaki<sup>\*1</sup>, Munehiro Okada<sup>1</sup>, Shunsuke Takeuchi<sup>2</sup>, Shu Hoshi<sup>1,3</sup>, Shunsuke Fukuba<sup>1</sup>, Patrick R Schmidlin<sup>4</sup>, Takanori Iwata<sup>1</sup>

<sup>1</sup>Department of Periodontology, Institute of Science Tokyo, <sup>2</sup>Saida dental clinic, Saitama, <sup>3</sup>Hoshi dental clinic, Niigata, <sup>4</sup>Clinic of Preventive Dentistry, Periodontology and Cariology, Center of Dental Medicine, University of Zurich

**Background and objective:** Poly (lactic acid/caprolactone) (PLCL) membranes have been clinically utilized for guided bone regeneration. However, no study has assessed the efficacy of these novel membranes for periodontal regeneration. This preclinical study aimed to evaluate the regenerative capacities of PLCL membranes with carbonate apatite (CO<sub>3</sub>Ap) in one-wall intrabony defects.

**Materials and Methods:** In five beagle dogs, standardized one-wall defects (5 mm in depth and 6 mm in width) were surgically created on the mesial and distal roots of the bilateral third premolars in mandible. The defects were randomly assigned to the following groups; i) the experimental group: PLCL and CO<sub>3</sub>Ap, positive control groups: ii) only CO<sub>3</sub>Ap, iii) xenograft (DBBM) and collagen membrane, iv) only DBBM, the negative control group: v) empty. Images of micro-CT, histologic and histomorphometric characteristics were evaluated 8 weeks after surgery.

**Results:** No infectious complications were detected at any sites. The experimental group and positive control groups exhibited a greater height and volume of the newly formed bone than the negative control group. They also showed a greater height of the newly formed cementum than the negative control group. However, the results were not statistically significant. The newly formed periodontal ligaments were inserted into newly formed bone and cementum in the experimental and the positive control groups.

**Conclusion:** The combine use of PLCL membrane and CO<sub>3</sub>Ap demonstrated comparable performance for periodontal tissue regeneration in one-wall intrabony defects compared to conventional therapies.

GB-25

**Shifting the Microbiome Following Non-Surgical Treatment for Each Periodontal Status**

Ryota Kobayashi<sup>\*1</sup>, Takahiko Shiba<sup>1</sup>, Keiji Komatsu<sup>2,3</sup>, Takashi Nemoto<sup>1</sup>, Takahiko Nagai<sup>1</sup>,  
Shunsuke Matsumura<sup>1</sup>, Koki Takada<sup>1</sup>, Yasuo Takeuchi<sup>2</sup>, Takanori Iwata<sup>1</sup>

<sup>1</sup>Department of Periodontology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo,

<sup>2</sup>Department of Lifetime Oral Health Care Science, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, <sup>3</sup>Weintraub Center for Reconstructive Biotechnology, UCLA School of Dentistry, Los Angeles, CA.

**Background and objective:** The mechanism of microbiome shifts in the healing process at periodontal tissue remains unclear. This study aimed to identify bacteria contributing to periodontal tissue healing and maintaining periodontal health by discriminating microbiome changes at healthy, gingivitis, and periodontitis sites after non-surgical treatment.

**Materials and Methods:** Metatranscriptomic analysis was performed on subgingival plaque samples collected from 45 patients with healthy, gingivitis, and periodontitis sites in the anterior teeth before and two months after the non-surgical treatment.

**Results:** Beta diversity analyses revealed distinct differences pre- and post-treatment at periodontitis sites but not at gingivitis or healthy sites. However, after treatment, the number of bacterial species with significant changes in abundance was 33, 35, and 51 at healthy, gingivitis, and periodontitis sites, respectively. Notably, decreases in *Streptococcus* sp., *Treponema denticola*, and *Tannerella forsythia* were observed at periodontitis sites after treatment. Additionally, *Tannerella forsythia* showed a decrease after treatment regardless of the pre-treatment periodontal conditions.

**Conclusion:** Bacterial species that varied post-treatment at gingivitis and periodontitis sites may contribute to the healing of periodontal tissues and the maintenance of a healthy state. Remarkably, even healthy sites showed a reduction in *Tannerella forsythia*, a member of the red complex, indicating that microbiome dysbiosis had already occurred in these sites before treatment, potentially increasing their susceptibility to periodontal disease. Treatment led to changes in the microbiome throughout the entire oral cavity, affecting not only gingivitis and periodontitis sites but also healthy sites.



# General (Clinical Research)



## GC-01

**Correlation between MMP/TIMP expression in gingival crevicular fluid and clinical parameters in the classification (2017) of periodontal disease**

Yuta Shimizu<sup>\*1</sup>, Hirotsugu Morinaga<sup>1</sup>, Takumi Sato<sup>1</sup>, Hirotaka Ikami<sup>1</sup>, Haruna Nagase<sup>2</sup>,  
Masanori Kashimata<sup>2</sup>, Junichi Tatsumi<sup>1</sup>

<sup>1</sup>Division of Oral Infections and Health Sciences, Department of Periodontology, Asahi University School of Dentistry,

<sup>2</sup>Division of Oral Infections and Health Sciences, Department of Pharmacology, Asahi University School of Dentistry

**Background and objective:** Gingival crevicular fluid (GCF) contains bioactive substances such as tissue-destructive factors and has attracted attention as one of the diagnostic materials. This study aims to assess the correlation between GCF amount, total protein concentration, MMP/TIMP expression, BOP rate, and PISA across severity stages and progression grades in the new periodontal disease classification. The goal is improve the accuracy of diagnosis of periodontal pathology based on tissue destructive factors in GCF.

**Materials and Methods:** About 200 patients with chronic periodontitis who visited Asahi University Medical and Dental Center were included in the study, and were classified according to their initial radiographs, periodontal histology, diabetes mellitus, and smoking status. Components in GCF were eluted and evaluated for correlation with stage grade for total protein concentration and cytokine expression by membrane antibody array and ELISA. Correlations with BOP rate and PISA were also evaluated.

**Results:** GCF and total protein levels increased with stage severity and grade progression. The expression of MMP-8 and MMP-9 increased after stage III and IV and grade B and C, while the expression of TIMP-1 and TIMP-2 showed a decreasing trend with increasing stage severity and decreased after grade B and C. When comparing the correlation with BOP rate and PISA, MMP-8 and MMP-9 showed positive correlation and TIMP-1 showed negative correlations were observed.

**Conclusion:** Periodontitis triggers elevated GCF volume, disrupting MMP/TIMP balance, leading to tissue destruction and compromised homeostasis. Evaluating GCF parameters could serve as an objective measure in the classification (2017) of periodontal disease.

## GC-02

**Relationships among cognitive function, periodontitis, oral microflora and oral frailty**

Nanae Dewake<sup>\*1</sup>, Yuki Hayashi<sup>2</sup>, Ryuichi Mizutani<sup>1</sup>, Suguru Nakamura<sup>1</sup>, Keiichi Uchida<sup>3</sup>,  
Nobuo Yoshinari<sup>1,2</sup>

<sup>1</sup>Department of Cariology, Endodontology and Periodontology, School of Dentistry, Matsumoto Dental University,

<sup>2</sup>Department Oral Health Promotion, Graduate School of Oral Medicine, Matsumoto Dental University,

<sup>3</sup>Department of Oral Diagnostics and Comprehensive Dentistry, Matsumoto Dental University Hospital.

**Background and objective:** The purpose of this study was to clarify the relationships among cognitive function, periodontitis, oral microflora and oral frailty in independent elderly people.

**Materials and Methods:** Twenty-three independent elderly people aged 60 years or older, with more than 20 present teeth and no systemic history associated with dementia or periodontal disease were included. The Mini-Mental State Examination was used to examine cognitive function. The oral examination included the number of present teeth, periodontal inflamed surface area (PISA), the stage of the diagnostic classification of periodontitis, and oral diadochokinesis. The microflora of the saliva was analyzed by amplicon sequencing analysis: MiSeq and predictive metagenomic analysis using PICRUST2. The study was approved by the Ethics Committee of Matsumoto Dental University (No. 0301).

**Results:** The cognitively impaired group (N = 11) had fewer present teeth, a more advanced stage of periodontitis, and fewer "pa" and "ka" sounds in oral diadochokinesis compared with the normal group (N = 12) (all  $P < 0.05$ ). The bacterial flora analysis showed that alpha diversity (Simpson index) was significantly higher in the cognitively impaired group than in the normal group. Additionally, the genus of *Tannerella* was detected in the cognitively impaired group ( $P < 0.05$ ). In predictive metagenomic analysis, the levels of COX11 and soluble cytochrome b562 proteins produced by oral bacteria, were significantly lower in the cognitively impaired group than in normal group ( $P < 0.05$ ).

**Conclusion:** Our findings suggest that cognitively impaired elderly people have a more advanced stage of periodontitis, together with changes in the oral microflora and mitochondria-associated proteins.

GC-03

Withdrawal of presentation

GC-04

**Periodontal Disease at Five Long-Term Care Welfare Facilities in Tokyo**

Shunsuke Takeya<sup>\*1</sup>, Yuta Izumi<sup>1</sup>, Masami Saotome<sup>1</sup>, Satoshi Sekino<sup>1</sup>,  
Yukihiro Numabe<sup>1</sup>, Fumiyo Tamura<sup>2</sup>, Takeshi Kikutani<sup>2</sup>

<sup>1</sup>Department of Periodontology, School of Life Dentistry, at Tokyo, The Nippon Dental University

<sup>2</sup>Rehabilitation Clinic For Speech and Swallowing Disorders, The Nippon Dental University Hospital

*Background and objective:* During the worldwide epidemic of COVID-19, nursing homes in Japan implemented visitor restrictions to prevent infection spread. Consequently, oral care by dental care workers was also limited. The purpose of this study was to conduct and analyze a survey on the prevalence of periodontal disease at long-term care welfare facilities for the elderly in Tokyo.

*Materials and Methods:* A total of 102 edentulous residents from five facilities in Taito Ward, Tokyo, were included in this study. Patient background factors were collected through questionnaires. The number of teeth, clinical attachment level (CAL), probing pocket depth (PPD), plaque index (PII), and bleeding on probing (BOP) of the subjects were also recorded. The target teeth were selected based on six representative teeth of Ramfjörð.

*Results:* Among all subjects, 14.7% had a Barthel Index (BI) of  $\geq 60$ . 32.4% had a "Youkaigodo" of 5, 35.3% had 4, and 29.4% had a score of 3. The mean PII, PPD, CAL and BOP were  $1.5 \pm 0.6$ ,  $2.7 \pm 0.6$  mm,  $3.8 \pm 1.1$  mm and  $45.1 \pm 28.6\%$ , respectively. When the subjects were divided into 4 age groups, no significant difference were found in CAL, PPD, PII, and BOP. However, significant difference in the number of teeth was observed between the youngest group and oldest groups.

*Conclusion:* Periodontitis was prevalent in the majority of the subjects, and the number of teeth was lower in the older age group. Moreover, many residents exhibited poor oral hygiene. Thus, efforts should be made to balance infection control and oral care.



GC-05

### Periodontitis exacerbates renal function via renal fibrosis in diabetic conditions

Eri Sakaniwa<sup>\*1</sup>, Risako Mikami<sup>1</sup>, Koji Mizutani<sup>1</sup>, Kohei Takeda<sup>1</sup>, Hiromi Kominato<sup>1</sup>, Natsumi Saito<sup>1</sup>, Masahiro Hakariya<sup>1</sup>, Keita Nakagawa<sup>1</sup>, Shu Takemura<sup>1</sup>, Mari Sugimoto<sup>1</sup>, Ayu Sugiyama<sup>1</sup>, Daisuke Kido<sup>2</sup>, Akira Mima<sup>3</sup>, Takanori Iwata<sup>1</sup>

<sup>1</sup>Department of Periodontology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, Tokyo, Japan, <sup>2</sup>Oral Diagnosis and General Dentistry, Dental Hospital, Institute of Science Tokyo, Tokyo, Japan, <sup>3</sup>Department of Nephrology, Osaka Medical and Pharmaceutical University, Osaka, Japa

**Background and objective:** This study aimed to investigate the relationship between periodontal disease and renal function in diabetes. **Materials and Methods:** 73 Japanese patients with type 2 diabetes who underwent intensive diabetes care were followed up for six months. The association of the change in renal function during the follow-up period with periodontitis was evaluated with multivariate regression analysis. For in vitro experiments, mice mesangial cells were stimulated with Lipopolysaccharide from *Porphyromonas gingivalis* (PgLPS) at 1000 ng/ml in control or high glucose (HG) medium (5 or 25 mM). Transforming growth factor (TGF)- $\beta$ 1, collagen (col) 1a2, and alpha-smooth muscle actin ( $\alpha$ SMA) were analyzed for fibrosis and transformation.

**Results:** A significant decline was shown in the estimated glomerular filtration rate (eGFR) from  $76.8 \pm 23.7$  to  $71.8 \pm 21.6$  ml/min/1.73m<sup>2</sup> at 6 months ( $p < 0.001$ ). The decline in eGFR was significantly associated with severity of periodontitis (coefficient: -6.0, 95% confidence interval (CI) :-11.2, -0.8,  $p = 0.025$ ), periodontal epithelial surface area (PESA) (/100mm<sup>2</sup>) (coefficient: -0.8, 95% CI: -1.4, -0.2,  $p = 0.013$ ), and periodontal inflamed surface area (PISA) (/100mm<sup>2</sup>) (coefficient: -1.0, 95% CI: -1.9, -0.03,  $p = 0.043$ ) after adjusting for confounders. In vitro experiments showed that IL-6 was significantly increased in HG and further significantly increased by LPS stimulation. TGF- $\beta$ 1, Col1a2, and  $\alpha$ SMA were also significantly increased in the HG + LPS group compared to the HG group. The TGF $\beta$ 1 inhibitor (SB-525334) significantly reduced Col1a2 expression in the HG group.

**Conclusion:** In diabetes, severe periodontitis may exacerbate the decline of renal function. PgLPS promoted the fibrosis of mesangial cells via TGF- $\beta$  signaling pathway in vitro.

GC-06

### Development and evaluation a novel immunochromatographic device for detection of *Porphyromonas gingivalis*

Michihiko Usui<sup>\*1</sup>, Rieko Yamanaka<sup>1</sup>, Kaoru Kobayashi<sup>2</sup>, Satoru Onizuka<sup>1</sup>, Tsuyoshi Sato<sup>3</sup>, Wataru Fujii<sup>3</sup>, Wataru Ariyoshi<sup>2</sup>, Keisuke Nakashima<sup>1</sup>, Tatsuji Nishihara<sup>2</sup>

<sup>1</sup>Division of Periodontology, <sup>2</sup>Division of Infections and Molecular Biology, and <sup>3</sup>School of Oral Health Sciences, Kyushu Dental University

**Background and objective:** *Porphyromonas gingivalis* (P.g) is the most pathogenic periodontal bacterium. Recently, P.g has also been considered a responsible role of dysbiosis in the development of periodontitis. The aim of our study was to evaluate a novel immunochromatographic device with monoclonal antibodies for P.g in subgingival plaque.

**Materials and Methods:** Gold colloid-labeled antibody solution against P.g was used in the immunochromatography kit. The detection limit of number of P.g by the immunochromatographic device was validated using the cultured JCM12257, P.g bacterial strain. Seventy-two patients with chronic periodontitis and 53 periodontally healthy volunteers were subjected to clinical and microbiological examinations. Subgingival plaque samples were analyzed for the presence of P.g and compared with real-time PCR method.

**Results:** The novel immunochromatographic device successfully detected cultured  $10^4$  P.g bacteria strains. In the periodontitis group, a significant positive correlation in detection results was found between the test device score and the real time PCR method. The specificity, positive predictive value, negative predictive value, and accuracy of a test device for the presence of P.g determined by real-time PCR were 98%, 94%, 89%, and 90%, respectively. There were significant differences in the bacterial counts by real-time PCR method among groups with different ranges of device scores. There was a significant positive correlation between device scores for P.g and periodontal parameters including probing pocket depth and clinical attachment level.

**Conclusion:** The results suggested that the novel immunochromatographic device can be effectively used for rapid detection and semi-quantification of P.g in subgingival plaque.

GC-07

### Longitudinal Changes in Periodontal Conditions in Residents in Takahagi City

Satoshi Sekino<sup>\*1,2</sup>, Ryoichi Takahashi<sup>1</sup>, Ryutaro Kuraji<sup>1</sup>, Hiroaki Michael Ishii<sup>1</sup>, Yukihiro Numabe<sup>1</sup>,  
Yuichi Sugita<sup>3</sup>

<sup>1</sup>Department of Periodontology, School of Life Dentistry, at Tokyo, The Nippon Dental University, <sup>2</sup>The Nippon Dental University Hosipital, General Dentistry, <sup>3</sup>Sugita Dental Clinic

*Background and objective:* There has been limited research on the longitudinal changes in periodontal condition within the general population of Japan. The purpose of this study was to observe longitudinal changes in periodontal status among the general public living in Takahagi City, Ibaraki Prefecture.

*Materials and Methods:* From 2010 to 2013, baseline (BL) examination was conducted included a questionnaire on patient backgrounds, general dental examination, and periodontal assessment on 582 residents of Takahagi city. These patients were recalled after 2 years and same examinations were performed.

*Results:* 189 residents aged 34 to 84 years were returned for the follow-up. The mean number of teeth at BL was  $26.4 \pm 4.7$  and  $25.9 \pm 4.7$  at 2 years. 15.4% of all respondents lost one or more teeth in two years. The mean PPD was  $2.27 \pm 0.31$  mm at BL and  $2.29 \pm 0.37$  mm after 2 years. The mean CAL was  $2.66 \pm 0.54$  at BL and  $2.75 \pm 0.58$  mm after 2 years. And attachment loss of  $>2$  mm occurred in 39% of all subjects.

*Conclusion:* Considering the older age range of the patients who underwent follow-up examinations, it can be concluded that more teeth were preserved and fewer sites showed attachment loss compared to the results of a longitudinal study in Japan in the 1980s (Lindhe et al., 1989), but multiple tooth loss and attachment loss were also observed in some patients, suggesting the need for further improvement in periodontal treatment and prevention programs in community.

GC-08

### The impact of socio-economic status on obesity and periodontitis

Natsumi Saito<sup>\*1</sup>, Risako Mikami<sup>1</sup>, Koji Mizutani<sup>1</sup>, Norio Aoyama<sup>2</sup>, Takanori Matsuura<sup>3</sup>,  
Tomonari Suda<sup>4</sup>, Kohei Takeda<sup>5</sup>, Jun Aida<sup>6</sup>, Yuichi Izumi<sup>1,7</sup>, Takanori Iwata<sup>1</sup>

<sup>1</sup>Department of Periodontology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, Tokyo, Japan, <sup>2</sup>Department of Education Planning, Kanagawa Dental University, Kanagawa Dental University, Kanagawa, Japan, <sup>3</sup>Weintraub Center for Reconstructive Biotechnology, Division of Advanced Prosthodontics, UCLA School of Dentistry, California, Los Angeles, USA, <sup>4</sup>Department of Oral Surgery, Secomedic Hospital, Chiba, Japan, <sup>5</sup>Higashisakura dental clinic, Aichi, Japan., <sup>6</sup>Department of Oral Health Promotion, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, Tokyo, Japan, <sup>7</sup>Oral Care and Periodontics Center, Southern Tohoku Research Institute for Neuroscience, Southern Tohoku General Hospital, Fukushima, Japan.

*Background and objective:* Obesity is a risk factor for periodontal disease and is also known to be associated with socioeconomic status (SES). However, the impact of SES on the association between obesity and periodontal disease remains unclear. In this study, we aimed to investigate the influence of SES on the relationship between obesity and periodontal disease.

*Materials and Methods:* The social background, body mass index (BMI), and dental examinations were analyzed in 962 participants living in the Tokyo Metropolitan District. The proportion of teeth with probing depth (PD)  $\geq 4$  mm was analyzed as the primary outcome through multilevel Poisson regression analysis, adjusting for confounding factors such as the average income and education level of the residential area.

*Results:* Obesity was significantly associated with the ratio of PD  $\geq 4$  mm (ratio of means (RM): 1.25, 95% confidence interval [CI]: 1.15, 1.38,  $p < 0.001$ ), while higher-income groups showed a significantly lower ratio (RM: 0.86, 95% CI: 0.77, 0.96,  $p = 0.007$ ). Interaction analysis indicated a significant interaction in obesity and highest-income group towards the proportion of teeth with PD  $\geq 4$  mm ( $p = 0.033$ ). Subgroup analysis demonstrated that the RM of obesity for the proportion of teeth with PD  $\geq 4$  mm was higher among females as 1.33 compared to males as 1.13.

*Conclusion:* Inequality in socio-economic status has an impact on the association between obesity and periodontal disease. Patients with obesity in the low- to middle-income group might have a higher risk of periodontal disease than those with obesity in the high-income group.

# Case Report



R-01

### Case report of improved cleanability by periodontal surgery

Tetsuaki Hirano<sup>\*</sup>, Jiro Hirano, Akihiro Tanaka

Hirano Dental Office

*Background and objective:* Lifestyle modification is essential for the prognosis for full-mouth caries in young patients. In addition, it is important to establish periodontal tissues that can maintain cleanliness. Periodontal surgery may be an effective means of achieving a long-term prognosis.

*Materials and Methods:* A 30-year-old male patient presented to the clinic with a chief complaint of esthetic and masticatory dysfunction. PPD was 4mm or more in 45% of the areas, BOP 97%, and Severe caries (C4) was seen on radiographs at #18, #15, #13, #12, #23, #27, #38, #37, #36, #35, #44, #47, #48, which were considered to be unsalvageable. In addition, #38, #47, and #48 had degree II mobility, and #37 had degree I mobility. After examination, a diagnosis of masticatory dysfunction due to severe caries was made.

*Results:* The results of reevaluation after basic periodontal treatment showed significant improvement in BOP and PPD.

MTM was performed for #15 C4 caries. Crown lengthening was performed for #12, #13, #26, #28, #35, #36, and #37. In #44, a tooth graft was performed to the #47 area. After MTM and periodontal surgery, ferrule and clearance were secured and significant improvement in cleanability was achieved.

*Conclusion:* In this case, periodontal surgery was performed to improve periodontal disease and cleanability in a young patient with severe extensive caries. In addition, esthetic and masticatory disorders were also improved. In addition, by performing MTM and tooth transplantation to preserve teeth with uncertain prognosis, we were able to give hope to the patients and improve their motivation in life.

R-02

### A case in which periodontal regenerative therapy was performed using different materials for each site

Tomoaki Kariya<sup>\*</sup>

Hakusankai Medical corporation Espoir dental clinic

*Background:* In performing periodontal regeneration therapy, selecting the appropriate flap design and materials in the context of bone defect configuration is important. In this report, I describe a case in which periodontal regenerative therapy was performed using different flap designs and materials for each site, and good results were obtained.

*Case:* The case report is of a 56-year old female. Her past medical history is insignificant. She had visited a dental clinic for caries treatment about 5 years ago, but she had stopped visiting until recently. For the first time in 5 years, She resumed caries treatment and was diagnosed with periodontal disease.

*Clinical Procedures and Outcomes:* Initially, I explained the cause of periodontal disease because the patient had never been diagnosed with periodontal disease. After explaining the examination results, the patient seemed to understand her periodontal condition and became cooperative in the treatment. After reevaluation, periodontal regeneration therapy was performed for 26, 27, 44, 46. After confirming periodontal tissue stability at the reevaluation, the patient underwent prosthetic treatment and move on to SPT.

*Conclusion:* In this case, the favorable results of periodontal regenerative therapy can be attributed to the patient's good oral hygiene. In addition, selecting the appropriate flap design and materials in the context of bone defect configuration was also considered to have led to a good outcome. I will continue to explore the selection criteria of FGF-2 and emdogain based on insights gained from my experience with clinical case.

R-03

### **The Last molar– Entire Pad Preservation Technique (L-EPPT) as A new Regenerative Surgical Approach for Combined Distal Intrabony Defects and Furcation Involvement: Case Reports**

Dai Kawanabe\*

Kawanabe Dental Clinic

*Background and objective:* In the distal region of the last molar, there is concern that direct surgical access to an intraosseous defect may impair the blood supply, and make healing difficult. The Last molar–entire pad preservation technique (L-EPPT) is a new surgical technique designed to preserve the gingiva distal to the last molar and define the surgical field at the root furcation and distal bone defect. The aim is to provide an optimal environment for wound healing in regenerative therapy. I report three cases in which L-EPPT was performed for a bone defect and furcation lesion in the distal part of the last molar.

*Materials and Methods:* This case series involves three systemically healthy patients with a chief complaint of gingival bleeding and slight tooth mobility due to generalized periodontitis. At the re-evaluation session, one patient was found to have a three-wall bone defect in the distal part of the last molar, and two patients had a 2–3 wall bone defect combined with a buccal grade II furcation lesion, so I performed L-EPPT. EMD and A bovine-derived xenograft bone (Bio-Oss, Geistlich) was then filled into the intrabony and sutured.

*Results:* In all three cases, the probing depth at all sites was less than 3 mm without BOP. A buccal grade II furcation lesion showed complete closure of the defect with periodontal tissue regeneration therapy. In addition, the depth of the cuneiform bone defect distal to the last molar evaluated on CBCT was significantly reduced, confirming sealing of the intrabony defect.

*Conclusion:* The L-EPPT aims to improve the wound healing process in cases of deep intrabony defects in the distal site of the last molar combined with buccal furcation defects, where direct access to the bone defects is provided by a single continuous incision line. The application of this technique effectively supports the use of EMDs and bone substitutes and may improve clinical outcomes after periodontal regenerative therapy.

R-04

### **A case of Stage IV Grade C localized chronic periodontitis in a young patient with dentition preservation**

Masaaki Ando\*

Ando Dental Clinic

*Background:* In the treatment of young patients, it is important to preserve natural teeth and avoid dental decay in consideration of their life stages. In this report, we describe a case of a young patient with Stage IV Grade C localized chronic periodontitis in which efforts were made to preserve the natural teeth and the dentition.

*Case:* Patient: 38-year-old male, non-smoker. First visit: March 2016.

Chief complaint: Unable to bite due to tooth mobility.

Oral findings: Periodontal tissue destruction in the maxillary molar region was remarkable, especially in case #15, bone resorption extending to the root apex was observed, and in case #16, a class III furcation involvement was observed.

*Treatment and results;* we planned to transplant #38 to the position of #37, provide an implant in the area corresponding to #24 after improving the mesial tipping of #25, and orthodontically move #48 to the position of #47. #15 showed improvement initial periodontal therapy. Periodontal tissue regeneration therapy is not indicated for #16, but considering the anatomical form, the condition of the adjacent teeth, I chose tissue regeneration therapy. After that, we tried to improve the occlusal condition through orthodontic treatment. After surgery, the class III furcation involvement remained in #16, but the periodontal tissue and occlusion were stable.

*Conclusion:* In addition to the high tissue regeneration ability of young patients, we believe that the reinforcement of occlusal support and orthodontic treatment to disperse the traumatic force are factors that led to good treatment results. We consider that efforts to preserve teeth and maintain dentition were effective at the patient's life stage.

R-05

### A case of severe chronic periodontitis treated with periodontal prosthesis

Yasunobu Iwai\*

Iwai Dental Office

*Background and objective:* The treatment of severe periodontitis in middle age involves a full range of treatment options, and the patient's needs must be carefully considered and incorporated into the treatment plan. This case reports on a patient with extensive chronic periodontitis Stage IV Grade C who underwent periodontal treatment and occlusal reconstruction to restore oral function, while preserving natural teeth as much as possible.

*Materials and Methods:* Patient: 48-year-old male First visit: November 2019 Complaint: bad breath . Unable to chew due to tooth movement. General history: nothing of note. Smoking. Dental history: The patient visited a local dentist to resolve his chief complaint, but the dentist explained that it was difficult to save the teeth and that all the teeth would have to be extracted. Oral examination revealed that the patient had a low awareness of oral care, and despite his young age of 48 years due to smoking, he had gingival inflammation, tartar, ill-fitting restorations, and defects on 14.35.44. All teeth had a PPD of 6 mm or more. All teeth have a PPD of 6 mm or more, and radiographs show horizontal and vertical bone defects. Dental malposition was also observed due to periodontitis, and the occlusal head was unstable.

*Results:* Initial periodontal treatment was successful in helping the patient quit smoking while encouraging behavioral changes. The patient's movement was managed by T-fix and spontaneous paraprofessionals while taking care not to decrease the height of the occlusion. 25 was shifted to a provisional restoration within the consolidation range sought by T-fix after correcting the tooth axis by MTM because the tooth had shifted palatally. Next, periodontal tissue regeneration therapy was performed on the remaining bone defects in an attempt to further restore and stabilize the periodontal tissues. After correcting the deviated jaw position in the central position, the patient was shifted to a final restoration after 4 months of follow-up. The patient's condition is now stable 4 years after the initial examination and 2 years after SPT.

*Conclusion:* In cases of severe periodontitis with defects, it is important to consider prosthetic design during initial periodontal treatment in order to achieve a defect prosthesis after periodontal treatment. The prognosis of periodontal tissues is not only dependent on the patient's plaque control to control inflammation, but also on force control to manage the patient's sway to withstand occlusal forces. However, since it is difficult to obtain a good prognosis without the cooperation of the patient because of the difficulty of plaque control in the case of a connected prosthesis, we should continue to monitor the patient's progress with ongoing maintenance.

R-06

### Free gingival graft around dental implants: case report

Ryo Nishibayashi\*

NISHIBAYASHI Dental Clinic

*Background:* The importance of peri-implant attached gingiva is well known, but some literature suggests that it is not essential in patients with good plaque control. The present study presents a case of a patient who complained of pain and discomfort during brushing due to a lack of attached gingiva, and in whom a free gingival graft was performed to improve plaque control, and importance of the attached gingiva should be reaffirmed by presenting a case.

*Case:* 40-year-old female, presented in July 2022 with a chief complaint of implant treatment of the lower right defects. After implant placement at #45 and 46 and placement of a provisional crown, the patient complained of poor plaque control and pain and discomfort during brushing, so a free gingival graft was performed.

*Clinical Procedures and Outcomes:* Implants were placed in #45 and #46 in September 2022. A second operation was performed and a provisional crown was placed in February 2023. A free gingival graft was performed in April. Six months later, the final superstructure of the implant was placed. After that, more than 2 mm of attached gingiva was gained. Pain and discomfort during brushing were eliminated and plaque control improved.

*Conclusion:* Attached gingiva around implants is not always necessary. However, in which the patient complained of pain and discomfort during brushing due to a lack of attached gingiva, free gingival grafting was performed to obtain attached gingiva, which improved pain and discomfort during brushing and contributed to the long-term prognosis of the implants.

R-07

**A case report of root coverage procedures for multiple gingival recessions**Shuji Imura<sup>\*</sup>, Tomohiro Ishikawa

Ishikawa Dental Clinic

*Background:* Root coverage procedures benefit from Connective Tissue Grafts (CTG), but multiple adjacent recessions treated with CTG and coronally advanced flaps do not achieve full coverage comparable to single-tooth cases. This report details a case using TCAF and VISTA techniques.

*Case:* A 52-year-old male sought treatment in April 2021 to improve the appearance of elongated anterior teeth. His medical and family histories were unremarkable, with no smoking history. Oral exam showed gingival recession from the right maxillary first molar to the left second premolar and uneven gingival lines. Clinical findings indicated PCR of 57.4% and BOP of 66.6%.

*Clinical Procedures and Outcomes:* After improving to PCR of 25.2% and BOP of 12.9% with initial periodontal therapy, the patient underwent periodontal plastic surgery. Surgical approaches, including TCAF for teeth 16 to 13 and VISTA technique for teeth 21 to 25, were tailored based on recession depth, attached gingival width, and the number of affected teeth. Epithelialized connective tissue was harvested from the palate, trimmed, and used for bilateral recession, with repositioned trimmed epithelium at donor sites. Post-surgery, effective root coverage and favorable progress were noted.

*Conclusion:* In this case, considering the patient's hygiene and aesthetic concerns regarding gingival recession, attached gingival width, and the number of affected teeth, periodontal plastic surgery using TCAF and VISTA techniques were performed. This approach resulted in improved gingival phenotype and enhanced aesthetic appearance, promoting a cleaner periodontal environment. Future monitoring will focus on maintaining plaque control and vigilance for any recurrence of gingival recession.

R-08

**A case of sinus floor elevation with less than 4 mm of existing bone volume using a minimally invasive crestal approach and octacalcium phosphate/collagen composite as a bone graft material**Ryotaro Nakano<sup>\*</sup>

Child Dental Clinic, Tokyo, Japan

*Background:* Recently, the crestal approach in sinus floor elevation surgery has gained attention for being minimally invasive. Treatment guidelines require 4–5 mm of bone height, but the importance is reported to be the acquisition of initial implant stability. Additionally, the octacalcium phosphate/collagen composite (OCP/Collagen), launched in Japan in June 2022, has been reported to result in a higher proportion of new bone and a lower proportion of residual material. This case report presents the use of OCP/Collagen as a bone graft material in sinus floor elevation surgery with less than 4 mm of existing bone height, using a minimally invasive crestal approach.

*Case:* The patient was a 51-year-old female. In February 2023, she visited our dental clinic for implant treatment after the extraction of the maxillary left first molar due to root fracture. CBCT images showed the existing bone height was only 3.6 mm, indicating a vertical bone deficiency.

*Clinical Procedures and Outcomes:* After consultation with the patient, a minimally invasive crestal approach was performed, using OCP/Collagen as the bone graft material. An implant (S, 4.1 × 8 mm, Straumann) was simultaneously placed, achieving good initial stability. Postoperatively, the patient had minimal pain and swelling. CBCT images showed the elevated sinus membrane and the radiolucent OCP/Collagen image, which changed to a radiopaque image of new bone after 6 months.

*Conclusion:* The crestal approach is feasible with an existing bone height of 3 mm or more, achieving good initial implant stability. Furthermore, the use of OCP/Collagen showed potential for replacement with new bone.



R-09

### A Novel Regenerative Therapy for Periodontally Compromised Abutment Tooth

Yoshitaka Nara<sup>\*1</sup>, Shogo Maekawa<sup>2</sup>

<sup>1</sup>Chigasaki Nara Dental Clinic, <sup>2</sup>Department of Periodontology, Institute of Science Tokyo

**Background:** In recent years, surgical techniques that do not involve any incision at the interdental papillae in the target area of periodontal regeneration have been developed, and their successful clinical outcomes have been reported. However, these techniques have also disadvantages such as a limited surgical view and access only from buccal side, which often encounter difficulty in debridement. In this case report, we introduce a novel regenerative surgical approach "Flexible Tunnel Technique" for an endo-periodontal lesion.

**Cases:** The patient was 66 years old, female. An infrabony defect extending to the root apex was found on the palatal side of tooth #14. Since #14 had pulp necrosis, root canal treatment was performed during initial periodontal treatment. At reevaluation, 6 mm of probing depth (PD) still remained on #14.

**Clinical Procedures and Outcomes:** Periodontal regenerative therapy was performed for the infrabony defect of #14. By making a total of four vertical incisions, which are two buccal and two palatal incisions, and sufficient periosteal releasing incisions, the interdental papillae of #14 were able to be shifted coronally and even across over the abutment teeth. After debridement, enamel matrix derivatives and bone grafts were applied. Simple interrupted sutures were performed to obtain primary closure. One year after the surgery, all PDs improved to within 3 mm.

**Conclusion:** This surgical technique can be used to access infrabony defects extending to the root apex on the palatal side, without any incisions in the interdental papillae, which may have the potential to achieve successful periodontal tissue regeneration.

R-10

### Periodontal regenerative therapy using rhFGF-2 in combination with $\beta$ -TCP

Takeshi Futagami<sup>\*1,2</sup>, Yoshihiro Shibukawa<sup>3</sup>

<sup>1</sup>JGSDF Camp Kamifurano Dispensary, <sup>2</sup>JGSDF Camp Asaka Dispensary, <sup>3</sup>Shibukawa Dental Clinic

**Background:** Periodontal regenerative therapy using recombinant human fibroblast growth factor (rhFGF-2) might be more difficult to regenerate when used alone for 1- or 2-wall intrabony defects because it is difficult to remain there. And here we report a case of bone grafting with rhFGF-2 and  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) for severe vertical bone resorption.

**Case:** The patient was a 44-year-old man who presented with the chief complaint of gingival swelling on the whole. An initial examination revealed that 47.2% of sites with a probing depth (PD)  $\geq$  4 mm, mean PD was 4.6 mm, 80.6% of sites with bleeding on probing (BOP). The O'Leary plaque control record (PCR) was 78.1%. Radiographic examination revealed angular bone resorption in #11, 15, 33, 34, 43. A clinical diagnosis of generalized chronic periodontitis (Stage III Grade C) with Occlusal Trauma was made.

**Clinical Procedures and Outcomes:** Initial periodontal therapy such as improvement of occlusal contact habit, smoking cessation counseling, oral hygiene instruction, scaling and root planing were performed. After re-evaluation, periodontal regenerative therapy using rhFGF-2 in combination with  $\beta$ -TCP were performed on #11, 15, 33, 34, 43. At the latest re-evaluation after transition to SPT, approximately 3 years after initial visit, sites with PD of  $\geq$  4 mm improved to 0.7% and mean PD to 1.7 mm, BOP to 1.4%, PCR to 18.5%.

**Conclusion:** Periodontal regenerative therapy with rhFGF-2 in combination with  $\beta$ -TCP can be expected to achieve sufficient bone regeneration even for severe vertical bone resorption of 1- or 2-wall intrabony defects.

R-11

### Periodontal surgery for the compromised healing following surgically assisted rapid palatal expansion

Kentaro Imamura<sup>\*1</sup>, Yurie Kitamura-Nakazawa<sup>1</sup>, Kengo Doya<sup>1</sup>, Shinta Mori<sup>1</sup>, Yoshihiro Fujimoto<sup>2</sup>, Kazuya Yamanouchi<sup>3</sup>, Atsushi Saito<sup>1</sup>

<sup>1</sup>Department of Periodontology, Tokyo Dental College, Tokyo, Japan, <sup>2</sup>Fujimoto Dental Office, Tokyo, Japan, <sup>3</sup>Green Hill Dental Clinic, Chiba, Japan

**Background:** Surgically Assisted Rapid Palatal Expansion (SARPE) is a procedure for managing the constricted maxillary dental arches. We report a case of compromised healing post-SARPE, which was treated by connective tissue graft (CTG) and periodontal regeneration using deproteinized bovine bone mineral (DBBM) and fibroblast growth factor-2 (FGF-2).

**Case:** A 50-year-old woman presented in October 2019, expressing aesthetic concerns. Initial examination revealed the gingival depression in the maxillary midline. A diastema of 1.5 mm was present between #11, 21. The probing depth was 2 mm, and gingival recession (GR) was 3.5 mm at the mesial aspect of #21. CBCT revealed alveolar bone deficiency. The patient started orthodontic treatment in 2015, and SARPE was performed to improve the constricted dental arch. Then, orthodontic treatment using multibrackets was performed until 2018. Unfortunately, median diastema did not improve. A clinical diagnosis of localized chronic periodontitis (Stage II, Grade B) was made.

**Clinical Procedures and Outcomes:** The patient underwent initial periodontal therapy including plaque control and scaling. After reevaluation, a series of CTG was performed to enhance the horizontal volume of soft tissues between #11, 21. Four months later, periodontal regenerative therapy was conducted using DBBM and FGF-2. At 12 months post-surgery, the soft tissue has been flattened, and the GR has improved to 0.5 mm. Morphological correction of #11, 21 was restored with composite resin.

**Conclusion:** CTG and periodontal regenerative therapy with DBBM and FGF-2 effectively addressed the compromised healing following SARPE, providing aesthetically pleasing and easily maintainable periodontal tissues.

R-12

### A 6-year follow-up case of surgical treatment of peri-implantitis

Kazuki Ohtake<sup>\*</sup>, Koichiro Shiota, Risako Kanaya, Makiko Ishii, Kitetsu Shin, Joichiro Hayashi

Devision of Periodontology, Department of Oral Biology and Tissue Engineering, Meikai University School of Dentistry

**Background:** We report a case of peri-implantitis due to maintenance failure, poor plaque control, and morphological abnormalities of the superstructure.

**Case:** A 62-year-old female presented to the Department of Periodontology, Meikai University Hospital, with a chief complaint of bleeding from a left mandibular molar implant. After periodontal treatment at our department 5 years ago, implants were placed at #46, and #47, secondary surgery with free gingival grafting was performed, and a cemented superstructure was placed. The patient did well prior to stopping follow-up 2 years ago. The distal surface of the #46 implant superstructure was an over-contoured restoration with adhering plaque. The distal pocket depth (PD) was 6mm, bleeding on probing (BOP) was observed, and bone resorption was seen.

**Clinical Procedures and Outcomes:** After oral cleaning and professional mechanical tooth cleaning (PMTTC), the superstructure was removed and cleaned with PMTTC and medication. After reevaluation, bone grafting was performed using artificial bone. At the 6-month postoperative visit, there was no BOP, the PD was 3 mm, and bone resorption improved. Since then, the patient has followed up regularly and is doing well. 5 years after surgery, a new superstructure was fabricated to correct the morphological defects, and the patient continues maintenance.

**Conclusion:** Although treatment of peri-implantitis has not yet been established, this case has been well maintained over a long period of time by eliminating risk factors and surgical treatment. In addition, the presence of sufficient keratinized gingiva may have contributed to the successful surgical treatment.

R-13

### Periodontal Regenerative Therapy for Severe Bone Defect Beyond Root Apex: 4-Year Follow-Up

Yuichiro Ihara<sup>\*1</sup>, Taneaki Nakagawa<sup>2</sup>

<sup>1</sup>Ihara Dental Clinic, Tokyo, <sup>2</sup>Department of Dentistry and Oral Surgery, Keio University School of Medicine

**Background:** When periodontal regenerative therapy is performed for a tooth that extends beyond the root apex, root canal treatment is often performed regardless of whether the tooth is vital or not. However, there is no conclusive evidence suggesting that root canal treatment is necessary before surgery for a vital tooth. Our report shows that the periodontal condition remained stable with a vital tooth for four years after periodontal regenerative therapy was performed for a severe bone defect extending beyond the root apex.

**Case:** A 65-year-old man visited my private practice with a chief complaint of periodontal disease in January 2018. He has Hypertension and has been taking antihypertensive medication. A 10 mm periodontal pocket with bleeding on probing was observed at the lingual aspect of tooth #42. Tooth mobility was grade I.

**Clinical Procedure and Outcomes:** In 2018, the patient underwent periodontal initial therapy. The periodontal pocket on this tooth had worsened to over 10mm, and the bone resorption was enlarged on a radiographic image in December 2019. There was a pulp reaction and no difference between this tooth and the contralateral homonymous tooth. In January 2020, periodontal regenerative therapy was performed on tooth #42, which yielded outstanding results that have persisted for four years since the surgery. Furthermore, the same pulp reaction was observed in this tooth as in the contralateral homonymous tooth.

**Conclusion:** Periodontal regenerative therapy may be possible to regenerate periodontal tissue without root canal treatment before surgery for severe periodontitis extending beyond the root apex.

R-14

### A case of comprehensive treatment for a patient with Stage III-Grade C periodontitis

Takuya Takano

Takano Dental Clinic

**Background and objective:** Young patients with periodontitis need to be more cautious about tooth extraction compared to treatment of adult patients with chronic periodontitis. I feel that it is very meaningful to delay tooth extraction that are judged to be difficult to preserve by using periodontal tissue regeneration therapy.

We present a case in which periodontal tissue regeneration therapy, periodontal prosthesis, and periodontal orthodontic treatment achieved good results.

**Case:** This is a case of a 39-year-old female who diagnosed with Stage III-Grade C periodontitis. Her chief complaint was to treat periodontal disease. She also complained of pathological tooth movement and aesthetic disturbance. Vertical bone defects in #15, #14, #13, #11, #36, #37, #46, #47 were observed by radiography. Root fracture #26 Diagnosis of extensive aggressive periodontitis, Stage III-Grade C.

**Clinical Procedures and Outcomes:** First, initial periodontal treatment was performed, and then periodontal tissue regeneration therapy was performed on the site of the vertical bone defects. (#15, #14, #13, #11, #36, #37, #46, #47) After trisection of only fractured #26 MB root, the remaining tooth was extracted because it was considered difficult to preserve. In the diagnosis of orthodontics, mandibular ramus osteotomy was diagnosed, but the patient did not desire it. So the orthodontic treatment was performed with the aim of improving the crowding of the mandibular anterior teeth and adjusting the root paralleling and midline of the maxillary anterior teeth. After that, periodontal prosthetic treatment was performed for the purpose of secondary fixation. Deep periodontal pockets were also improved, and overall attachment gain was also achieved. Two year has passed after the treatment without any problems

**Conclusion:** This case suggests that Orthodontic treatment is useful treatment to improve functionality and aesthetics for pathological tooth migration

R-15

### a Case demonstrating the importance of regular maintenance

Daisuke Kido <sup>\*1</sup>, Koji Mizutani<sup>2</sup>, Hiroshi Nitta<sup>1</sup>

<sup>1</sup>General Dentistry Tokyo Medical and Dental University Hospital, <sup>2</sup>Periodontology, TMDU

**Background:** The importance of maintenance/supportive periodontal therapy (SPT) is widely recognized. This case demonstrated the effectiveness of regenerative therapy for recurrent intrabony defect due to interruption of maintenance.

**Case:** The patient was a 53-year-old female with a chief complaint of bleeding from her gum in July 2017. She had no notable medical and dental histories, and no experience of smoking. Preoperatively, she had 24 remaining teeth. Oral hygiene, particularly in the interproximally area, was poor with PCR 39.6%. The clinical diagnosis was generalized severe chronic periodontitis, Stage III, Grade B (pocket depth (PD)  $\geq$  4mm, 36.9% of sites; PD  $\geq$  6mm, 5.6%; BOP positive 49.3%; PISA 970.7 mm<sup>2</sup>; and PESA 1636.6 mm<sup>2</sup>).

**Clinical Procedures and Outcomes:** After non-surgical therapy, regenerative therapy using a basic fibroblast growth factor-2 (Regroth<sup>®</sup>) was performed on tooth #14 with 7mm PD. Maintenance had been interrupted for 9 months at 3 years of maintenance, then recurrent intrabony defect was detected in the mesial side of tooth #14. The second regenerative therapy using Regroth<sup>®</sup> was performed. Following the re-surgery, the outcome has been maintained in favorable with less than 3mm PD and without BOP for 2 years postoperatively.

**Conclusion:** This case suggested the necessity of regular maintenance/SPT at 3-month intervals following periodontal regenerative therapy. Furthermore, regenerative therapy has been shown to achieve comparable clinical outcomes to the initial surgery for recurrent intrabony defect.

R-16

### A case report of periodontal regenerative therapy using rhFGF-2

Daisuke Narita <sup>\*1</sup>, Tsutomu Kidate<sup>1</sup>, Naoka Wakabayashi<sup>1</sup>, Shiori Yamamoto<sup>1</sup>, Masahiro Ito<sup>2</sup>,  
Nobuyuki Shiomi<sup>2</sup>

<sup>1</sup>Narita Dental and Orthodontics, <sup>2</sup>Shiomi Dental Clinic

**Background and objective:** REGROTH<sup>®</sup> (rhFGF-2) has been shown to be effective in promoting CAL and bone gain. However, there is limited evidence of its regenerative effect on root furcation involvement. A combination therapy of rhFGF-2, DBBM, and CO<sub>3</sub>Ap was performed in patients with periodontitis including furcation involvement, and the results were satisfactory.

**Materials and Methods:** 54-year-old non-smoking female patient with spontaneous pain of 46. No medical history. Probing pocket depth (PPD) measured at 168 sites with 58.9% PPD  $\geq$  4 mm, 14.3% PPD  $\geq$  6 mm, and furcation involvement observed in 16 and 26. X-rays showed horizontal bone resorption in maxillary anterior and vertical bone resorption in 13, 14, 15, 16, 24, 26, and 47. Clinical diagnosis: generalized periodontitis Stage III Grade C.

**Results:** During the transition to SPT, out of 168 sites with six-point measurements, improvement was seen in 5 sites (3%) with PPD  $\geq$  4 mm, and 0 sites (0%) with PPD  $\geq$  6 mm. The furcation involvement (Class II) of 16 and 26 were improved. Periodontal regenerative therapy using rhFGF-2 showed a mean CAL gain of 3.0 mm and a mean bone increase rate of 80.6% in the DBBM combined site. The CO<sub>3</sub>Ap combined site also showed a mean CAL gain of 3.0 mm and a mean bone increase rate of 81.5%.

**Conclusion:** Combining rhFGF-2, DBBM, and CO<sub>3</sub>Ap may be effective in regenerative therapy for Class II furcation involvement and non-contained bone defects.

R-17

## A case report with 5-years of follow-up: Subepithelial Connective Tissue Graft for Root Coverage in Multiple Class III Gingival Recessions with Enamel Matrix Derivative

Sho Takaya

TAKAYA DENTAL CLINIC

**Background:** Gingival recession has been defined as the apical displacement of gingival margin related to CEJ. The exposed root surface in Miller Class III/IV or Cairo Class RT 2/3, has been a therapeutic challenge for root coverage. The most frequent etiologic factors are inflammatory periodontal disease, traumatic improper tooth brushing, teeth alignment with thin gingival phenotype, orthodontic treatment and submarginal defective restoration. In this case report, sub-epithelium connective tissue grafting procedure with enamel matrix derivative was applied to recover multiple Miller class III gingival recessions with over 5-years outcome.

**Case Description:** A 34-year-old female presented with chief complaint of generalized mandibular gingival recession with significant teeth sensitivity to cold temperature. No systemic or significant medical history was recorded. Initial examination revealed that none of the sites showed periodontal probing 4mm or greater than 4mm, bleeding of probing with overall well controlled oral hygiene. #20-29 presented multiple gingival recession with teeth mal-positioning with thin periodontal phenotype. No association with inter alveolar bone loss was noted. Despite no sign of interdental bone loss, gingival recession type was classified as Miller Class III / Cairo RT2 due to the teeth mal-position. A decision was made to perform root coverage procedure with connective tissue graft adjusted with enamel matrix derivative application.

**Treatment and Outcomes:** Type of gingival recessions on #20-29 were over all classified into Miller Class I / Cairo RT 1 except #26 which is classified into Miller Class III/ Cairo RT2 due to the buccally positioned tooth position. Root coverage procedures were executed in two different timing. Coronally positioned flap (CPF) with connective tissue graft (CTG) and the application of Enamel Matrix Derivative (EMD), was performed on the area of #21-24, and same procedure was performed on the opposite side which is #25-28. Hypersensitivity was dissolved after root coverage procedure completed. At 2-years post-operative visit, recurrence of gingival recession on #26 was observed, and double pedicle flap with CTG and EMD was rendered to restore the gingival recession as 2<sup>nd</sup> attempt. Successfully 80% on #26 and 90-100% of root coverage was accomplished with 3-4 months interval supportive periodontal therapy for 5 years with stable periodontal condition.

**Conclusion:** The stability of periodontal tissue integrity and gingival level/health have been maintained as 5-years' post-operative outcome. The reason why #26 was only site that underwent second root coverage procedure, was speculated as buccally prominent tooth position classified in Miller Class III/ Cairo RT 2. Therefore, better approach in this case was, if our treatment could be modified in similar type of situation, to improve teeth alignment with some orthodontic treatment modality initially to shift Miller Class III/ Cairo RT 2 to Class I/ RT 1 respectively. For future consideration, risk management of etiology and proper diagnosis of gingival recession type should be taken into consideration prior to active treatment plan for further predictable outcome and longevity.

R-18

## Periodontal Regenerative Therapy for Intrabony Defect and Gingival Recession in the Esthetic Zone

Takahiro Naito\*, Shunsuke Fukuba, Takanori Iwata.

Department of Periodontology, Institute of Science Tokyo

**Background:** In recent decades, advancements in biomaterials and flap designs have led to predictable periodontal regenerative therapy. However, while improvements in bone defects are achievable, restoring lost soft tissue remains challenging. In this case, a patient with intrabony defect accompanied by gingival recession in the anterior esthetic zone was received periodontal regenerative therapy aimed to simultaneously improve both hard and soft tissues.

**Case:** Patient: 59-year-old female, non-smoker

First visit: 2021/11

Chief complaint: Noted with periodontal disease at a previous dental clinic.

Systemic medical history: No significant medical history

Dental history: Underwent restorative treatment since her late 20s. Received implant therapy for tooth #46 ten years ago, which was removed six months ago due to peri-implantitis.

Diagnostic findings: Deepest PPD of 9mm, CAL of 11mm, BOP (+), REC of 2mm at tooth #11

**Clinical Procedures and Outcomes:** Performed initial periodontal treatment (OHI, Sc/PP, extraction of tooth #15, #26), followed by periodontal surgical procedures (regenerative therapy for teeth #14, #11, #23) upon re-evaluation. For intrabony defects with gingival recession at tooth #11, a combination of rhFGF-2, carbonate apatite, resorbable collagen membrane and subepithelial connective tissue graft were used for periodontal regenerative therapy. Flap design considerations for the esthetic zone avoided a vertical incision on the labial side and an incision on the interdental papillae. Favorable clinical outcomes were maintained over six months postoperatively.

**Conclusion:** This case demonstrated successful periodontal regenerative therapy addressing both bone defects and gingival recession simultaneously, with effective flap design and connective tissue grafting.

R-19

**Tooth Replantation in conjunction with Regeneration Therapy for Endo-Perio Lesions**Nobuaki Sato<sup>\*1</sup>, Ryo Sakai<sup>2</sup>, Koshi Ryosuke<sup>2</sup>, Seiko Min<sup>3</sup>, Shuichi Sato<sup>2</sup><sup>1</sup>Sato Dental Clinic, <sup>2</sup>Department of Periodontology, Nihon University School of Dentistry, <sup>3</sup>Department of Periodontics and Dental Hygiene, University of Texas Health Science Center at Houston

*Background and objective:* Drug-induced gingival hyperplasia complicates periodontal treatment, necessitating comprehensive approaches. While non-surgical methods address periodontitis, endodontically compromised teeth with deep pockets remain challenges. This case study investigates the efficacy of tooth replantation in combination with regeneration therapy, utilizing xenograft and enamel matrix derivative, for managing endo-perio lesions.

*Case:* A 48-year-old male, diagnosed with periodontitis compounded by drug-induced gingival hyperplasia, exhibited periodontal pockets extending to the root apex and tooth mobility. With a medical history of hypertension and long-term calcium channel blocker use, comprehensive periodontal intervention was initiated.

*Clinical Procedures and Outcomes:* Alternative medication, thorough nonsurgical periodontal therapy, and extraction of severely compromised teeth were undertaken. Previous failed root canal treatment necessitated tooth replantation with root apicoectomy. Following extraction of tooth #30, reverse root canal filling with mineral trioxide aggregate (MTA) was performed during apicoectomy. Regenerative therapy was carried out with enamel matrix derivative (EMD) and a 50/50 mixture of autologous bone and Bio-Oss applied to the replantation tooth's root surface. Careful replantation ensured proper positioning without socket wall contact. Substantial improvements in periodontal parameters, including reduced pocket depths and tooth mobility, were observed. Radiographic assessment revealed bone regeneration around the replanted tooth.

*Conclusion:* This case highlights the effectiveness of combination therapy of tooth replantation and regeneration therapy for endo-perio lesions. The synergistic effect of tooth replantation and regenerative therapy enhances periodontal regeneration capabilities, offering a promising treatment modality for complex periodontal cases associated with apical periodontitis.

# Dental Hygiene





H-01

### Control of periodontitis during orthodontic and implant therapy treatment in Stage 4 periodontitis: Case series

Ami Asari\*, Kotaro Tsuchida

Tsuchida Dental Clinic

*Objective:* Patients with stage IV periodontitis may require orthodontic or implants treatment to improve oral function and esthetics. Periodontal disease control is important for these patients throughout the end of active periodontal treatment. This case series presents the control of periodontal disease in patients undergoing orthodontic or implant treatment.

*Case series:* In this case series, three patients (all female, ages 45, 52, and 74) were treated for severe periodontal disease. All patients had stage IV periodontitis tooth loss and pathologic tooth migration. No specific systemic disease. 2:1 for smokers and nonsmokers. First, initial treatment was performed. Periodontal surgery and periodontal tissue regenerative therapy were then performed as needed. After reevaluation, either implant or orthodontic treatment, or both, was initiated only when inflammation was under control.

*Result:* Clinical parameters were improved after periodontal active treatment, and periodontal tissues were stabilized, so implants and orthodontic treatment were started. The periodontal tissues were stabilized by the monthly supportive periodontal care (SPC) while performing orthodontic and implant treatment. If inflammation could be controlled by periodontal treatment and SPC, orthodontic treatment and implant treatment were possible.

*Conclusion:* Patients with stage IV periodontitis may require treatment for tooth loss or pathologic tooth migration (PTM) to improve oral function. These treatments should be performed only after periodontitis is under control. However, oral hygiene and oral function can easily deteriorate during orthodontic treatment and implant therapy. Therefore, it is important to improve patient self-care and motivation in initial treatment, implant, and orthodontic treatment, as well as to ensure supragingival and subgingival plaque control through professional care.

H-02

### A case report of maintenance for a patient originally with a grade II furcation lesion

Minami Hiram\*, Shimizu Hiroyasu

Shimizu Dental Clinic, Tokyo, Japan

*Background and objective:* When performing periodontal treatment, supra and sub gingival plaque control by patient self-care and professional care are important to maintain the postoperative condition as good as possible. In this report, a case was described in which furcation lesions were improved after periodontal treatment and maintained with improving motivation through oral hygiene instruction.

*Materials and Methods:* The patient was a 60-year-old man who came to our clinic with a chief complaint of discomfort in his upper left back teeth. Clinical findings were BOP 49%, PPD 4 mm  $\geq$ 44%. Dental history included #37MT. The diagnosis was generalized periodontitis stage III grade C. Treatment was OHI, SRP, and #24 extraction. Subsequent reevaluation revealed residual PPD  $\geq$ 4 mm in the left and right upper and lower molars and a residual grade II furcation lesion in #16#36, therefore regenerative therapy was performed. After the surgery, the patient's condition was stabilized and transferred to SPT.

*Results:* The changes in shape of the periodontal tissues of #16,#36, both of which had degree I furcation lesions after regenerative therapy, were different. The two different furcation lesions were maintained thorough oral hygiene instruction. As a result, periodontal pockets of  $\geq$ 4 mm was 12.7% and BOP was 4%, then the patient was shifted to SPT. 4 years have passed since the surgery, and his condition is stable. Through communications, understanding the patient's personality and dexterity, and setting goals, correct self-care was established.

*Conclusion:* To maintain a good postoperative condition, it is necessary to learn correct self-care and control inflammation with SPT. To successfully achieve these goals, it is important to provide oral hygiene instruction that is easy for patients to continue, to communicate well with them through regular SPT so that they do not lose motivation, and to control subgingival plaque with professional care.

## H-03

**Equol testing and supplementation for female patients with desquamative gingivitis**

Aki Kawamoto<sup>\*1</sup>, Naoyuki Sugano<sup>2,3</sup>, Midori Fujimoto<sup>1</sup>, Chizuko Shimizu<sup>1</sup>, Masako Sakai<sup>1</sup>,  
Keisuke Seki<sup>4</sup>, Soichiro Manaka<sup>2,3</sup>, Naoto Yoshinuma<sup>2,3</sup>, Shuichi Sato<sup>2,3</sup>

<sup>1</sup>Dental Hygienist Section, Nihon University School of Dentistry Dental Hospital, Tokyo, Japan, <sup>2</sup>Department of Periodontology, Nihon University School of Dentistry, Tokyo, Japan, <sup>3</sup>Division of Advanced Dental Treatment, Dental Research Center, Nihon University School of Dentistry, Tokyo, Japan, <sup>4</sup>Department of Comprehensive Dentistry and Clinical Education, Nihon University School of Dentistry, Tokyo, Japan

**Background and objective:** Desquamative gingivitis (DG) is characterized by desquamative erosion, edematous erythema, and vesicles formation on the gingiva. The main treatment options focus on plaque control and topical corticosteroid application to manage lesions, alleviate symptoms, and prevent disease progression. Because of its prevalence in women during the pre- and postmenopausal period, its potential association with female hormones was suggested. This research explored the relationship between DG and equol, a soy isoflavone metabolite bearing a chemical structure similar to estrogen. Evaluating the effect of a 12-month equol supplementation to alleviate the symptoms of DG is another significant aspect of this study.

**Materials and Methods:** Between two groups, urinary equol levels, periodontal tissue examination, O'Leary's plaque control record, and gingival pain-related questionnaires were compared: 16 women with DG who regularly visited Nihon University School of Dentistry Dental Hospital (DG group) and 20 women with healthy gingiva and no symptoms of DG (H group). Additionally, these parameters were evaluated before and after a 12-month daily intake of 10 mg equol supplement in the DG group.

**Results:** About 14 out of 20 (70%) and 3 out of 16 (18.75%) study participants were equol producers in the H group and DG groups, respectively. In the DG group, the equol supplementation showed a statistically significant improvement in bleeding on probing and visual findings, and the frequency and severity of gingival pain were also reduced.

**Conclusion:** These results suggest that urinary equol testing and equol supplementation may serve as a novel treatment option for DG patients.

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Secretariat : Ms. Takako Crabb  
e-mail : jacp.jsp24@gmail.com

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