

The 102nd Annual Meeting of the American Academy of  
Periodontology in collaboration with  
the Japanese Society of Periodontology and Japanese  
Academy of Clinical Periodontology

## **Abstracts of JSP/JACP Poster Session**



September 10-13, 2016  
San Diego Convention Center, San Diego, CA, USA

The Japanese Society of Periodontology  
The Japanese Academy of Clinical Periodontology



**第102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2016年大会**

**The 102nd Annual Meeting of the American Academy of  
Periodontology in collaboration with the Japanese Society of  
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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，歯科衛生士演題

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

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JSP/JACP ポスター発表・若手研究者支援  
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(50音順)

# General (Basic Research)



GB-01

### Bone regeneration using adipose-derived stem cells and platelet-rich plasma

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**Background and objective:** A key goal for successful bone regeneration is to bridge a bone defect using healing procedures that are stable and durable. Adipose-derived stem cells (ASCs) with/without several scaffolds can differentiate into osteogenic cells. Meanwhile, platelet-rich plasma (PRP) is an interesting biological means to repair tissue by inducing chemotactic, proliferative, and anabolic cellular responses.

**Materials and Methods:** This study evaluated bone regeneration using a combination of ASCs and PRP in a rat calvarial defect model. ASCs were isolated from inguinal fat pads of F344 inbred rats, while PRP was prepared from these rats. ASCs were cultured in control medium supplemented with 10% fetal bovine serum or 5% PRP in vitro. After 1 week, levels of growth factors including insulin-like growth factor-1, transforming growth factor- $\beta$ 1, hepatocyte growth factor, and vascular endothelial growth factor in the culture supernatant were measured by enzyme-linked immunosorbent assays. Moreover, the ASC/PRP admixture was transplanted into the rat calvarial defect. Micro-computed tomography, histological, and immunohistochemical (osteopontin and osteocalcin) analyses were performed at 4 and 8 weeks after transplantation.

**Results:** The in vitro study showed that the levels of growth factors secreted by ASCs were significantly increased by the addition of PRP. Transplantation of the ASC/PRP admixture had dramatic effects on bone regeneration overtime in comparison with rats that received other transplants. Furthermore, some ASCs directly differentiated into osteogenic cells in vivo.

**Conclusion:** These findings suggest that the combination of ASCs and PRP has augmentative effects on bone regeneration. The ASC/PRP admixture may be a promising source for the clinical treatment of cranial defects.

GB-02

### Low-level blue laser irradiation enhances osteoblast proliferation and differentiation

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**Background and objective:** Low-level laser irradiation (LLLI) has various biostimulation effects such as promotion of wound healing and bone regeneration. However, there are few studies which examined the effects of LLLI with a blue laser. The purpose of this study was to investigate the effects of a high-frequency ultra-short pulsed blue laser on osteoblasts.

**Materials and Methods:** Osteoblast cell lines (MC3T3-E1 and ST2, Riken Bioresource Center) and mouse primary osteoblasts were cultured in a 96-well plate with osteoinducing medium, and were irradiated for 1 minute with a high-frequency ultra-short pulsed blue laser (405 nm, pulse width 3 ps, Spectra-Physics) at 0 (control), 31.3, 93.8, 156.3 mW/cm<sup>2</sup> in a single session. During 7 days of cell culture after LLLI, cell proliferation assay (Cell Counting Kit-8, Dojindo) and alkaline phosphatase (ALP) activity assay were performed to determine the optimum power for irradiation. Then, mRNA expression of osteoblast differentiation markers such as *Runx2*, *Osterix* (*Osx*), *Alkaline phosphatase* (*Alp*), *Osteopontin* (*Opn*) and *Osteocalcin* (*Ocn*) were measured by real-time PCR.

**Results:** LLLI with the blue laser significantly and dose-dependently enhanced cell proliferation and significantly increased ALP activity of MC3T3-E1, ST2 and primary osteoblasts compared with control ( $p < 0.05$ ). The highest ALP activity was observed at 93.8 mW/cm<sup>2</sup>. mRNA expression of *Osx*, *Runx2*, *Alp* and *Opn* was significantly increased following LLLI at 93.8 mW/cm<sup>2</sup> compared with control on 3 to 7 days after LLLI.

**Conclusion:** The results suggest that LLLI with a high-frequency ultra-short pulsed blue laser enhances osteoblast proliferation and differentiation *in vitro*.

GB-03

**Resorption of deproteized cancellous bovine bone (DPBB) by osteoclasts**

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**Background and objective:** DPBB has been reported as non-absorbed bone filling material. Hydroxyapatite (HA) in bone is calcium-deficient and carbonate-rich apatite. Our previous study indicated that chemically equivalent HA was not resorbed by osteoclasts while octacalcium converted into calcium-deficient and carbonate-rich apatite (ODA), resorbed by osteoclasts. In this study, we analyzed the chemical composition of DPBB and compared the tissue reactions around DPBB and ODA after the implantation into mouse bone marrow.

**Materials and Methods:** X-ray diffraction analysis (XDA) and Fourier transform infrared spectroscopy (FTIS) were performed to analyze the chemical composition of DPBB. DPBB and ODA were implanted into the bone marrow of mouse tibiae and examined by micro-CT, histological and ultrastructural methods.

**Results:** XDA and FTIS showed that DPBB was carbonate-rich apatite. Micro-CT analysis indicated the massive bone formation on DPBB and ODA at 2 weeks after the implantation. The volume of the bone was gradually decreased to 12 weeks after the implantation. Histological examination indicated many columnar osteoblasts were aligned along the newly formed bone surface at 2 weeks after the implantation. Tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells were also detected on the surface of newly formed bone. At 12 weeks, most of bone on the materials was disappeared and TRAP-positive multinucleated cells were directly attached to implanted DPBB and ODA. Ultrastructural examination showed that these multinuclear cells had well developed ruffled border and attached directly DPBB and ODA by clear zone.

**Conclusion:** In this study, we clearly indicated that DPBB was the absorbed bone filling material.

GB-04

***Porphyromonas gingivalis* induced periodontitis exacerbates NASH progression in rat**

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**Background and objective:** In recent years, epidemiological and animal studies have reported that *Porphyromonas gingivalis* (*P.gingivalis*), known as periodontopathic bacteria, is closely related to non-alcoholic steatohepatitis (NASH). However, previous studies could not demonstrate a direct relationship between periodontitis, *P.gingivalis* infection, and NASH. The purpose of the present study was to examine the impact of *P.gingivalis*-associated periodontitis on the onset and progression of NASH.

**Materials and Methods:** Male Wistar rats were fed a high-fat diet (HFD) for 12 weeks in order to induce fatty liver. Furthermore, test animals were given a *P.gingivalis* slurry around the silk sutures ligating the maxillary first molar tooth in order to induce experimental periodontitis after 4 weeks (HFD-feeding and *P.gingivalis*-infection group; HFD/*P.g*-group). Controls were given slurry without *P.gingivalis* after ligation using the same protocol (HFD/cont-group).

**Results:** Significant increases in alveolar bone resorption and inflammation in periodontal tissue around the molar tooth in the HFD/*P.g*-group were observed when compared with the HFD/cont-group. Moreover, histological images showing NASH characterized by perivenular lipid deposition including big fatty drops, ballooning degeneration, and focal necrosis with inflammatory cells were confirmed in the liver of rats in the HFD/*P.g*-group. Significant increases of alanine aminotransaminase (ALT), aspartate aminotransferase (AST) and C-reactive protein (CRP) levels were observed in the HFD/*P.g*-group. Furthermore, endotoxin levels in serum in the HFD/*P.g*-group were significantly higher than those in the HFD/cont-group.

**Conclusion:** The present study demonstrated that experimental periodontitis induced by *P.gingivalis* led to the onset and progression of NASH in rats with fatty liver.



GB-05

**Inhibition of periodontal tissue-related transcription factors induces differentiation of chondrocyte**Hideki Takai<sup>\*1,2</sup>, Yorimasa Ogata<sup>1,2</sup><sup>1</sup>Department of Periodontology and <sup>2</sup>Research Institute of Oral Science, Nihon University School of Dentistry at Matsudo

**Background and objective:** Periodontal tissue is consisted by gingiva, periodontal ligament, cementum and alveolar bone. Stem cells present in periodontal ligament can undergo differentiation into osteoblasts, chondrocytes, myoblasts and adipocyte. Moreover, specific transcription factors (TFs) are need for differentiation of stem cells into different nature of cells. Therefore, it has been suggested that the target cells could induce different nature cells by controlling the expression of transcription factors. In this study, we investigated the specific TFs in periodontal tissues.

**Materials and Methods:** Human gingival fibroblasts (HGF) and human periodontal ligament cells (HPDL) were cultured by D-MEM, and osteoblast-like Saos2 cells were cultured by  $\alpha$ -MEM supplemented with 10% FCS. HPDL were transfected by siRNA which targeted TFs expressed in periodontal tissue cells. After 72 h transfection, cells were harvested, and real-time PCR and Western blot were performed to investigate the mRNA and protein levels of TFs expressed in mesenchymal cells.

**Results:** Runx2, Runx3 and Sox5 (osteogenic and chondrogenic TFs) mRNAs are expressed much more in Saos2 cells compared with HGF and HPDL. Whereas, Twist2, KLF12 and Pax9 mRNA are expressed much more by HGF and HPDL compared to Saos2 cells. When we inhibited three kinds of TFs (Twist2, KLF12 and Pax9) in HPDL, Sox5 mRNA and protein levels were increased in HPDL.

**Conclusion:** These results suggest that KLF12, Twist2 and Pax9 are crucial TFs to maintain the character of periodontal tissue cells compared to osteoblast-like cells. Furthermore, periodontal ligament cells could differentiate into chondrocytes by inhibiting KLF12, Twist2 and Pax9.

GB-06

**Bactericidal effects of 310 nm ultraviolet light-emitting-diode irradiation on oral bacteria**Ayuko Takada<sup>\*1,2</sup>, Kenji Matsushita<sup>2</sup>, Satoru Horioka<sup>3</sup>, Yasushi Furuichi<sup>1</sup>, Yasunori Sumi<sup>4</sup><sup>1</sup>Division of Periodontology and Endodontology, Department of Oral Rehabilitation, School of Dentistry, Health Sciences University of Hokkaido, <sup>2</sup>Department of Oral Disease Research, National Center for Geriatrics and Gerontology, <sup>3</sup>NIKISO CO., LTD, <sup>4</sup>Department of Center for Development of Advanced Medicine for Dental Diseases, National Center for Geriatrics and Gerontology

**Background and objective:** Ultraviolet light is used for phototherapy in dermatology, and UVB (around 310 nm) is effective for treatment of psoriasis and atopic dermatitis. In addition, it is known that UVC (around 265 nm) has a bactericidal effect, but little is known about the bactericidal effect of UVB. Here, we examined the bactericidal effects of UVB-LED irradiation on oral bacteria to explore the possibility of using a 310 nm UVB-LED irradiation device for treatment of oral infectious diseases.

**Materials and Methods:** We prepared a UVB (310nm) LED device for intra-oral use to examine bactericidal effects on *Streptococcus mutans*, *Streptococcus sauguinis*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* and also to examine the cytotoxicity to a human oral epithelial cell line (Ca9-22). We also examined the production of NO and H<sub>2</sub>O<sub>2</sub> from Ca9-22 cells after irradiation with UVB-LED.

**Results:** Irradiation with 310 nm UVB-LED at 105 mJ/cm<sup>2</sup> showed 30-50% bactericidal activity to oral bacteria, though 17.1 mJ/cm<sup>2</sup> irradiation with 265 nm UVC-LED completely killed the bacteria. Ca9-22 cells were strongly injured by irradiation of 265 nm UVC-LED but were not harm by irradiation of 310 nm UVB-LED. NO and H<sub>2</sub>O<sub>2</sub> were produced by Ca9-22 cells with irradiation of 310 nm UVB-LED. *P. gingivalis* was killed by applying small amounts of those ROS in culture, but other bacteria showed low sensitivity to them.

**Conclusion:** Narrowband UVB-LED irradiation exhibited a weak bactericidal effect on oral bacteria but showed low toxicity to gingival epithelial cells. Its irradiation also induces the production of ROS and may enhance bactericidal activity to anaerobic oral bacteria.

## GB-07

**Influence of horizontal load application on microgaps between fixture–abutment interfaces**

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*Background and objective:* Marginal sealing properties of the fixture–abutment interface (FAI) may be adversely affected by horizontal load application. Inadequate marginal sealing at the FAI allows pathogenic bacterial invasion, which is the main cause of peri-implantitis. This study compared the adverse effects of horizontal load application in several types of implants with a conical abutment.

*Materials and Methods:* Several implants (Ti-6Al-4V Aadva (Group A), pure titanium Aadva (Group B), Ankylos (Group C), NobelActive (Group D), and Osseospeed (Group E)) were inserted into a jig and abutments were fixed with a screw at torques recommended by the manufacturers. After applying a horizontal load (23–225 N) against the abutment, the size of the gap at the FAI was measured using scanning electron microscopy (SEM). Furthermore, the diameter (X axis; axis parallel to the horizontal load, Y axis; axis vertical to the horizontal load) of each implant platform was measured using a high-precision non-contact depth measuring microscope to determine the permanent deformation before and after horizontal load application.

*Results:* Microgaps at the FAI were significantly wider in pure titanium implants than the Ti-6Al-4V implant ( $p < 0.05$ ). Permanent deformation before and after horizontal load application was greater in pure titanium implants than the alloy implant. The mean FAI distance changed in all groups when  $\geq 114$  N horizontal load was applied. Moreover, at  $\geq 225$  N, significant differences were observed among Group A, C, D, E and Group B.

*Conclusion:* These findings suggest that bacterial invasion through FAI may occur under a horizontal load application of  $\geq 114$  N.

## GB-08

**Histological analysis of implantation to augmented bone using “Casing Method”**

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*Background and objective:* To place the implants to the severely resorbed alveolar ridge, establishing a great-sized ridge augmentation technique is important; furthermore, confirming that the integration between the augmented bone and the implant is done similarly as with the original bone, is also essential. In this study, the great amount of bone has been augmented to the dog's alveolar ridge, using “Casing Method”<sup>1)</sup> which we have previously reported; the implant was placed on the border of the original and the regenerated bone, and compared how the implant is integrated with each bone.

*Materials and Methods:* The mixture of 150–500  $\mu$ m hydroxyapatite (APACERAM-G<sup>®</sup>) and  $\beta$ -tricalcium phosphate (Osferion60<sup>®</sup>) granules impregnated into the suspension of autologous super fine bone powder and plasma was filled in a polyethylene terephthalate-made case, fixed to buccal side of the alveolar ridge. Sixteen weeks later, the case was removed, and the implant (diameter: 4.6mm, length: 8.0mm, Eight-Lobe<sup>®</sup>) was placed at the border of the original and the augmented bone. Collecting the samples 2–16 weeks after the implantation, the bone surrounding the implant was analyzed histologically.

*Results:* We were able to confirm the good bone formation in the augmented area. New bone was filled in the space between the implant and the augmented bone. What's more, the bone–implant contact was higher with the augmented bone area than with the original bone area.

*Conclusion:* It was suggested that it is positively effective to implant to the augmented bone using “Casing Method”.

1) M.Ogiso et al. ,In Trans.8th World Biomaterials Congress, 2008, No.1988.

GB-09

### The histological analysis of great-sized ridge augmentation applying the “Casing Method”

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**Background and objective:** To place dental implants to the severely resorbed alveolar ridge, establishing a great-sized ridge augmentation technique is important. We have reported that the mixture of hydroxyapatite (HA) granule and the micro-sized autogenous bone covered by the polyethylene terephthalate (PET)-made case resulted in ectopic bone formation, and bone formation fitting the shape of the case on the bone surface (*Casing Method*).<sup>1)</sup> This study aimed at evaluating whether *Casing Method* brings a great-sized ridge augmentation.

**Materials and Methods:** The mixture of the same amount of 150–500 $\mu$ m HA (APACERAM-G<sup>®</sup>) and  $\beta$ -tricalciumphosphate ( $\beta$ -TCP) (Osferion60<sup>®</sup>) granules impregnated into the suspension of autogenous super fine bone powder and plasma (bone concentration: approximately 1/100V%) was filled in 0.8mm thick PET-made case (thickness: 6mm, height: 10mm, mesial-distal length: 20mm, with  $\phi$  0.5mm nutrient foramina placed every 2mm), fixed on buccal side of the alveolar ridge. Collecting the samples 4, 8, 16 weeks after the operation, the tissues were analyzed histologically.

**Results:** Despite individual difference, new bone formation has started in about a half of the case area in four weeks, and the calcified bone around the granule became thick and were interconnected within eight weeks. In sixteen weeks, a part of the trabeculae became thicker and the bone marrow were widened. Also, the density of the granule inside the case was decreasing, and the reduction of  $\beta$ -TCP was obvious.

**Conclusion:** It was suggested that *Casing Method* has an ability of bringing a great-sized ridge augmentation.

1) M. Ogiso, et al., In Trans. 8th World Biomaterials Congress, 2008, No. 1988.

GB-10

### Availability of *in vitro*-cultured immunosuppressive macrophages for periodontitis treatment

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**Background and Aims:** The availability of immunosuppressive macrophages for the therapy of inflammatory/autoimmune diseases has been shown in recent years. We have established a bone marrow-derived mesenchymal stem cell (MSC) culture system in which MSCs and blood cells proliferate together. We found that the blood cells were differentiated to CD206-positive immunosuppressive macrophages (M2-macrophages) during the co-culture with MSCs. In this study, we investigated how these cells interact with each other in the primary co-culture and the resulting M2-macrophage polarization occurs.

**Materials and Methods:** MSCs and blood cells were separated by using Lineage (Lin)-Cell Depletion Kit from *td*-Tomato mouse bone marrow cells, and the separated cells were examined for growth, differentiation and markers expression. Moreover, two separated cells were reconstitutively co-cultured cell contact-dependent- and independently, and determined for M2-macrophage polarization.

**Results:** The growth of blood cells (Lin+) was sustained by a soluble factor secreted from MSCs (Lin-) into the culture medium. Flow cytometry revealed that 70–80% of Lin+ cells were CD206 (M2-marker)-positive, indicating that Lin+ cells were already polarized to M2-macrophages. The reconstitutive co-culture experiment of Lin+ and Lin- revealed that the mRNA expression levels of CD206 and the immunosuppressive cytokine IL10 and IL10 secretion level were higher in Lin+ cells adhered directly with Lin- than those cultured non-adherently.

**Conclusion:** It is suggested that our co-culture system seems to be beneficial as a supplier of a number of immunosuppressive macrophages, which are clinically applicable to regenerating or restoring the injured tissues with high efficiency and improving inflammatory diseases such as periodontitis.

## GB-11

**rhFGF-2/gelatin- $\beta$ TCP in the treatment of gingival recessions in dogs**

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**Background and objective:** Connective tissue graft and coronally advanced flap result mainly in periodontal repair through a long junctional epithelium and connective tissue adhesion when utilized to treat gingival recessions. Tissue engineering using recombinant growth factor technology may provide a new paradigm in root coverage. In this study we investigated the effect of recombinant human fibroblast growth factor-2 (rhFGF-2) combined with gelatin incorporating  $\beta$ TCP (gelatin- $\beta$ TCP sponge) on root coverage in dogs.

**Materials and Methods:** This study was approved by the institutional animal care and use committee of Tokyo Medical and Dental University (0160319 A). In six adult beagle dogs, artificial gingival recession defects were created bilaterally in the maxillary canines. The defects were randomly assigned to the test (rhFGF-2/gelatin- $\beta$ TCP) or the control group (gelatin- $\beta$ TCP). Width of keratinized gingiva (wKG) was measured at baseline right after surgery and before sacrifice (8 weeks). Biopsy specimens were obtained and subjected to micro-CT and histological evaluation.

**Results:** Clinically healing was uneventful and complete root coverage was achieved in both the test and control groups. There was no statistical significant difference in wKG between the test and control sites. The new bone volume was significantly greater at the test sites than control sites. Histologically the scaffolds were completely resorbed with no signs of inflammation in both groups. Histomorphometrically, the test group attained more new bone and periodontal attachment than the control group.

**Conclusion:** The combined use of rhFGF-2 and gelatin- $\beta$ TCP sponge enhances more formation of new bone and cementum than the scaffold alone in artificially created gingival recession defects in beagle dogs.

## GB-12

**Bone Metabolic Microarray Analysis of Ligature-Induced-Periodontitis in Streptozotocin-Induced Diabetic Mice**

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**Background and objective:** Many epidemiological studies have reported the progression of periodontitis in patients with diabetes; however, the associated mechanism remains unclear. We comprehensively investigated how diabetes affect the periodontal tissue and alveolar bone loss using a ligature-induced-periodontitis model in streptozotocin-induced diabetic (STZ) mice.

**Materials and Methods:** Diabetes was induced by intraperitoneal injection with streptozotocin in 6-week-old C57/BL6J male mice. A silk ligature was tied around the maxillary left second molar in 9-week-old wild type (WT) and STZ mice. Bone loss was evaluated at 3 and 7 days after ligation. mRNA expressions in the gingiva between the two groups was examined by DNA microarray and quantitative PCR (qPCR). Tartrate-resistant acid phosphatase (TRAP) and alkaline phosphatase (ALP) staining of the periodontal tissue was performed for evaluation of osteoclasts and osteoblasts in histological analysis.

**Results:** In the ligated gingiva, *TNF- $\alpha$*  mRNA expression was up-regulated at 1 day post-ligation in STZ mice but not in WT mice. At 3 days post-ligation, alveolar bone loss was observed only in STZ mice. More severe alveolar bone loss was observed in STZ mice at 7 days post-ligation compared to WT mice. Bone metabolic analysis using DNA microarray showed significant downregulation in the mRNA expression of glioma-associated oncogene homologue 1 (Gli1) and collagen type VI alpha 1 (Col6a1) at the gingiva of the ligated sites in STZ mice. Histological analysis showed increased number of TRAP-positive multinucleated cells at the ligated sites in STZ mice.

**Conclusion:** Alveolar bone loss could occur at an early phase under diabetic condition.

## GB-13

**Functional keystone species enable discrimination of peri-implantitis and periodontitis**

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*Background and objective:* Periodontitis and peri-implantitis are polymicrobial diseases with similar symptoms and occur in oral cavities. The effectiveness of clinical treatments for peri-implantitis is often unsatisfactory, even when using a treatment strategy similar to that used for periodontal disease. One possible reason for the failure might be the dissimilarity of microbial species that serve as etiological agents in each disease. Here, we performed metatranscriptomic analysis to dissect the microbiological similarities and dissimilarities related to the disease etiologies of both diseases.

*Materials and Methods:* Twelve subjects at the Tokyo Medical and Dental University Hospital (2012–2013) were recruited to analyze microbiota in subgingival plaque samples of one peri-implantitis and one periodontitis site.

*Results:* Both diseases were dissimilar in terms of 16S rRNA-based taxonomic profiles. Although disease-specific functional gene such as phage-related virulence factors were detected, no significant difference in mRNA abundance was observed for any gene between both diseases suggesting the functional composition including metabolic pathways were similar. However, the dissimilarities between the diseases were in not only network structures of co-occurrence patterns of taxonomic mRNA profiles but also the functional keystone species, which are of significantly higher mRNA (functional genes) than 16S rRNA (viable bacteria) in abundance.

*Conclusion:* Our approaches provide way to determine the potential causative agents of diseases in microbiota. Our findings lead to develop new clinical treatment approaches specific to peri-implantitis or periodontitis. Furthermore, the approaches can be used to clarify the etiologies of other polymicrobial diseases.

## GB-14

**Effect of intermittent administration of parathyroid hormone in rat cranial bone defects**

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*Background and objective:* Intermittent administration of parathyroid hormone (PTH) increases bone mass for the treatment of osteoporosis. This study was to evaluate the local intermittent administration of PTH for bone regeneration in rat cranial bone defects.

*Materials and Methods:* Eight-week-old male Wistar rats were divided into four groups. Two groups (PTH-3 and PTH-1 groups) underwent creation of two cranial bone defects using a trephine bur ( $\phi$  4.3 mm). These groups received PTH at 14.1  $\mu$ g/kg in absorbable collagen sponge placed in bone defects. The collagen group received saline at 14.1  $\mu$ g/kg in absorbable collagen sponge in bone defects. The control group underwent sham-surgery. After surgery, the PTH-3 group received subcutaneous injection of PTH (14.1  $\mu$ g/kg) twice at the experimental sites. The PTH-1 group was administered saline twice. The collagen group subcutaneously was used saline. The control group was injected subcutaneous with saline. All animals were sacrificed 21 days after surgery. BMC/TV at the experimental sites was evaluated using  $\mu$ -CT. Tissue sections were stained with HE. Anti-ALP antibody was used for immunohistochemical staining.

*Results:* BMC/TV was significantly higher in the PTH-3 group and PTH-1 group than in collagen group. Ratio of new bone area to defect area was significantly higher in PTH-3 than in controls. ALP-positive area was more widely distributed in new bone in PTH-3 than in PTH-1, collagen and controls.

*Conclusion:* These findings suggest that local intermittent administration of PTH enhanced bone regeneration in rat cranial bone defects. Local intermittent administration of PTH may be effective for periodontal regeneration.



## GB-15

**Comprehensive analysis of gene expression in the junctional epithelium from bioengineered tooth**

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**Background and objective:** Periodontal disease caused by bacterial infection shows symptom of inflammation in the periodontal tissue including junctional epithelium (JE). It has been reported that JE has several unique features to prevent bacterial infection. However, those molecular mechanisms have remained to be completely elucidated. We can isolate JE cells expressed green fluorescent protein (GFP) by using a bioengineered tooth technique in mice. The aim of this study was to perform a comprehensive analysis of gene expression in JE using a bioengineered tooth germ method.

**Materials and Methods:** Bioengineered tooth germs, which consisted of GFP-transgenic mouse-derived epithelial cells and normal mouse-derived mesenchymal cells, were cultured for 4 days. They were transplanted into alveolar bone in normal mice. GFP-positive JE cells around erupted bioengineered teeth were isolated by flow cytometry and analyzed by RNA sequencing and real-time PCR.

**Results:** The expression of secretory leukocyte protease inhibitor (*Slpi*) and keratin 17 (*Krt17*) were increased in JE cells compared with palatal gingival epithelial cells. Furthermore, immunohistochemical analysis indicated that *Slpi* protein is highly expressed in JE cells. Also, the expression of anti-oxidative stress response genes were increased in JE cells.

**Conclusion:** We determined that *Slpi* is characteristically expressed in JE cells. Gene expression patterns of JE cells from bioengineered tooth germs showed similar results as our previous reports. These results indicate that JE cells have anti-oxidative stress potential.

## GB-16

**Elucidation of the adhesion domain of *Porphyromonas gingivalis* Hgp44 for *Treponema denticola***

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**Background and objective:** Coaggregation between the two major periodontal pathogens, *Porphyromonas gingivalis* and *Treponema denticola*, is important in the biofilm formation and pathogenicity. It has been shown that the gingipain adhesion/hemagglutinin domain of *P. gingivalis* (Hgp44) is involved in its coaggregation with *T. denticola*.

The purpose of this study is to elucidate the adhesion domain of *P. gingivalis* Hgp44 for *T. denticola*.

**Materials and Methods:** The Hgp44 sequence encoding amino acid residues 720 to 1136 of Hgp44, which was C-terminal hemagglutinin/adhesion domain of *P. gingivalis* ATCC 33277, were amplified by PCR. The amplification genes were inserted in expression vector and the recombinant proteins (r-Hgp44) were induced. SDS-PAGE and Western blotting using anti-*P. gingivalis* antibody were used to confirm the production. Enzyme-linked immunosorbent assay (ELISA) was used to evaluate the adhesion to *T. denticola* ATCC 35405.

**Results:** The productions of r-Hgp44 (residues 720-1136) and the variant (residues 720-1032; r-Hgp44<sub>1</sub>) were confirmed by SDS-PAGE and Western blotting. Compared with control, a significantly greater level of *T. denticola* adherence was observed with r-Hgp44 and r-Hgp44<sub>1</sub>. However, no significant difference in the adherence was observed between r-Hgp44 and r-Hgp44<sub>1</sub>.

**Conclusion:** It was suggested that *P. gingivalis* Hgp44 domain responsible for adhesion to *T. denticola* resides in residues 720 to 1032. Further clarification of the specific Hgp44 domain is underway. Inhibition of such domain may prove to be a novel strategy to control periodontitis.

## GB-17

**Effects of vitamin B-complex supplementation on bone regeneration in rat**

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**Background and objective:** Among the nutrients necessary for normal physiological functions, vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, and folic acid play important roles in the immune response and wound healing, although the underlying mechanisms are unclear. This study examined the effect of vitamin B-complex supplementation on bone regeneration in non-critical-sized rat calvarial bone defects.

**Materials and Methods:** We used 4-week-old male Wistar rats (n=10) in this study. The drinking water of the rats in the experimental group contained 1 g/L vitamin B<sub>6</sub>, 1.25 mg/L vitamin B<sub>12</sub>, and 62.5 mg/L folic acid. Non-critical-sized calvarial bone (defect diameter 2.7 mm) were trephined into the dorsal parietal bone on both sides of the midsagittal suture. Using in vivo micro focus computed tomography (micro-CT) and tissue sectioning, we evaluated bone regeneration. Micro-CT imaging was performed every week for 6 weeks after surgery. The defect sites were removed along with surrounding bone and soft tissues and stained with hematoxylin-eosin.

**Results:** Histomorphometric and histological analysis showed that the amount of bone augmentation was significantly increased in the group supplemented with B-complex vitamins compared to controls at 6 weeks. Moreover, histological analyses revealed that the group that received vitamin B-complex supplementation showed more osteoblast-like cells than did controls.

**Conclusion:** These results obtained from micro-CT and histological analysis showed that vitamin B complex supplementation accelerated bone regeneration in non-critical-sized bone defects. This effect might be beneficial in periodontal therapy, particularly in regeneration of bone lost to periodontal disease. Further studies are required to clarify the mechanism underlying these supplementation effects.

## GB-18

**Use of BMSCs prevents alveolar bone resorption on experimental periodontitis**

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**Background and objective:** Mesenchymal stem cells are known to exert potent immunosuppression and anti-inflammatory effects. There is growing interest in their use for immunotherapy for controlling inflammation. In this study, we examine whether the transplantation of bone marrow stromal cells (BMSCs) would prevent alveolar bone resorption on experimental periodontitis in mice.

**Materials and Methods:** Bone resorption was induced by using the 5-0 silk around the maxillary second molar. The BMSCs, including mesenchymal stem cells were isolated from the femurs and tibiae of 8-week-old C57BL/6 mice. BMSCs (1 × 10<sup>6</sup> cells in 5 μL) were administered to gingiva around the ligatured maxillary right second molar using needle. At 5 days after cell administration, we evaluated the bone resorption levels around second molar by micro CT and histological examination.

**Results:** Micro-CT analysis showed that mice from the untreated group had significant bone loss compared to the cell administration group. Histologically, severe inflammation was shown in untreated mice, while inflammatory infiltration and bone resorption appeared suppressed in mouse periodontal tissue injected with BMSCs.

**Conclusion:** The findings in this *in vivo* study suggested that the local administration of BMSCs is an effective approach as a treatment for periodontitis.

## GB-19

**Dedifferentiated fat cells for tissue engineering in rat periodontal defects**

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**Background and objective:** Lipid droplet-free fibroblast-like cells, known as dedifferentiated fat (DFAT) cells, can be generated from mature adipocytes. DFAT cells can restore their proliferation activity and differentiate into various cell types under appropriate conditions in culture. The objective of this study was to assess the regenerative potential of DFAT cells combined with a solid scaffold in periodontal tissue regeneration.

**Materials and Methods:** Preparation of DFAT cells from mature adipocytes was conducted with a previously-reported modified method (Matsumoto et al., 116 2008). The PLGA-based solid scaffolds (LA:GA = 75:25, Mw. 25 kDa; PLGA-scaffold, GC Dental Product Co. Ltd., Japan), with a porosity of 80%, were used. DFAT cells were seeded onto PLGA scaffolds (DFAT/PLGA), and the scaffolds without cells were served as control (empty PLGA). Three-wall bone defects were created bilaterally at the mesial side of first molars of rats maxilla. DFAT/PLGA or empty PLGA were placed in the defects. Imaging of micro-computed tomography (CT) was performed every week for 4 weeks, and then, decalcified and processed for histomorphometrical observation.

**Results:** Micro-CT analysis showed significantly higher bone growth in DFAT/PLGA groups compared with controls at 4 weeks after surgery. Histomorphometry demonstrated thicker cementum and periodontal ligament in DFAT/PLGA groups compared with controls.

**Conclusion:** These findings suggest that the in vivo graft of DFAT cells combined with PLGA scaffolds is effective in promoting the tissue regeneration in periodontal bone defects.

## GB-20

**Fundamental research on the mechanism underlying cornification in oral mucosa**

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**Background and objective:** The cornification of gingival epithelia is essential for maintaining its barrier function and healthy periodontal homeostasis. Filaggrin, an intermediate filament-associated protein, is known to contribute to epidermal barrier function and degraded into the small molecules called natural moisturizing factors during the cornification process. However, filaggrin expression pattern and its role in the oral epithelial cornification are still elusive. In this study, we examined the expression of filaggrin and its degradation products in the stratified squamous epithelium of skin and oral mucosa.

**Materials and Methods:** The skin, palate and buccal mucosa were taken from a rat. Tissue sections were subjected to immunofluorescence using anti-filaggrin antiserum, and anti-peptidyl arginine deiminase (PAD) 1 and anti-citrullinated protein antibodies. Protein extracts were subjected to western blot analysis.

**Results:** Immunofluorescence showed that filaggrin is highly expressed from the granular layer to the lower cornified layer of skin. In the oral mucosa, filaggrin was detected in the keratohyalin granule-like structures of the granular layer. PAD1 and citrullinated protein were highly expressed in the lower cornified layer of skin, but not in the oral mucosa. Western blotting showed that the filaggrin monomer is degraded in skin but not the oral mucosa.

**Conclusion:** Our findings suggest that the different patterns of filaggrin degradation observed here may be due to differences in PAD1-mediated filaggrin citrullination in the skin and oral mucosa.



## GB-21

**LIPUS inhibits LPS-induced IL-1 $\alpha$  mediated NF- $\kappa$ B activations via AT1-PLC $\beta$  pathway**

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**Background and objective:** Periodontal disease destroys alveolar bone, is stimulated by lipopolysaccharide (LPS) -induced inflammatory cytokine IL-1 $\alpha$ . Low-intensity pulsed ultrasound (LIPUS) is used for bone healing in orthopedics and dental treatments. Previous reports have shown that mechanical stimulation by LIPUS inhibits inflammatory chemokines that are activated in response to LPS (Nakao et al., Bone 2014). However, the mechanism underlying the effects of LIPUS on LPS-induced inflammatory cytokine has not been elucidated. We aimed to investigate the role of LIPUS on LPS-induced IL-1 $\alpha$  production including its signaling pathway.

**Materials and Methods:** MC3T3-E1 cells were plated on a 6-well plate, incubated in the presence or absence of competitive LPS, and then stimulated with LIPUS for 30 min using OSTEOTRON D2. To clarify the role of LIPUS-effect, we determined the expression of IL-1 $\alpha$  stimulated with LIPUS and treated with angiotensin receptor type 1 (AT1) antagonist Losartan. The expression of IL-1 $\alpha$  and AT1 receptor was determined using real-time PCR or ELISA. The nuclear translocation of NF- $\kappa$ B was examined by fluorescent immunostaining.

**Results:** LIPUS inhibited mRNA and protein expression of LPS-induced IL-1 $\alpha$ . LIPUS reduced the nuclear translocation of NF- $\kappa$ B. Losartan and si RNA silencing AT1 cells blocked all the stimulatory effects of LIPUS on production NF- $\kappa$ B translocation. Furthermore, PLC $\beta$  inhibitor U73122 recovered NF- $\kappa$ B translocation.

**Conclusion:** These result suggest that LIPUS inhibits IL-1 $\alpha$ -mediated the activation of NF- $\kappa$ B induced by LPS in osteoblasts via AT1-PLC $\beta$  pathway. This is the first demonstration of the molecular mechanism underlying the positive effect of LIPUS on periodontitis which involved IL-1 $\alpha$ .

## GB-22

**Invasion mechanism of 'red complex species' across gingival epithelial barrier**

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**Background and objective:** It was demonstrated that periodontopathic bacteria including 'red complex species' can invade into the apical gingiva of periodontitis patients. The precise invasion mechanism, however, remains to be elucidated. In this study using the quantitation assays of bacteria and FITC-dextran, we assessed the invasion mechanism of 'red complex species' across gingival epithelial barrier through two putative routes (transcellular and paracellular routes).

**Materials and Methods:** After preculture of a human gingival epithelial cell line (Ca9-22) on 3- $\mu$ m-pore-size filters of Transwell culture insert, bacterial suspensions of freshly harvested 'red complex species' (*Porphyromonas gingivalis* ATCC33277, *Treponema denticola* ATCC33520 and *Tannerella forsythia* ATCC43037) were added to the upper compartment on the layered cells in an antibiotic-free media. At appropriate times after incubation, the translocated bacteria across epithelial cells in the lower compartment were quantified by real-time quantitative PCR (q-PCR). Then, the disruption of cell-cell junctions in Ca9-22 was examined by the translocation of FITC-dextran. The invaded and persisted bacteria within Ca9-22 were also assessed by q-PCR.

**Results:** The quantitation assays of bacteria and FITC-dextran indicated that *P. gingivalis* could invade significantly across gingival epithelial barrier at 6 hours of incubation through both transcellular and paracellular routes. *T. forsythia* could also possess the invasive ability, although *T. forsythia* utilized only transcellular route. In contrast, *T. denticola* could scarcely invade across gingival epithelial barrier.

**Conclusion:** Among 'red complex species', *P. gingivalis* and *T. forsythia*, but not *T. denticola*, could invade significantly across gingival epithelial barrier, whereas the translocation route may differ between the organisms.

## GB-23

**Effect of cavitating jet for cleaning biofilm from implant fixture**

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*Background and objective:* Although some cleaning devices have been developed, more effective methods are desired to remove biofilm from implant surface. The aim of the present study was to demonstrate the effectiveness of the cavitating jet in removing biofilms from rough surface of screw type implant.

*Materials and Methods:* The optimal nozzle dimensions and injection conditions were identified by cavitation impact measurements. Plaque biofilm was formed on the fixtures mounted on custom-made stents intraorally for 72h in four volunteers. The stained fixtures were assigned to different experimental groups. One comparison was performed between the cavitating jet and the water jet at 60 seconds. Additional comparisons were conducted among the time course experiments at 30, 60, and 180 seconds. After injection, the residual plaque biofilm (RPB) area was measured using digital microscope.

*Results:* The total RPB of the cavitating jet was significantly lower than that of the water jet. The total RPB areas of the cavitating jet at 30 seconds, at 60 seconds and at 180 seconds were significantly lower than the initial plaque biofilm. A significant decrease was detected between 60 seconds and 180 seconds. The RPB on the dedendum sector was significantly lower than that on the addendum sector at 60 seconds and 180 seconds.

*Conclusion:* This study demonstrated that cavitating jet could be one of the effective methods for removal of biofilm from screw type implant.

## GB-24

**P2X7 receptor mediates collagen synthesis induced by LIPUS**

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*Background and objective:* In orthopedics, low-intensity pulsed ultrasound (LIPUS) is commonly used for the treatment or diagnosis of fracture. In addition, LIPUS is also used to enhance the alveolar bone regeneration after the placement of implants. Adenosine triphosphate (ATP) is a key regulator of osteoblast response to mechanical stimuli. Furthermore, the ATP receptor of P2X7 is related to the skeletal remodeling by mechanotransduction. Type I collagen (Col1) is one of the major protein constituents of the bone ECM; it also functions as a scaffold for the nucleation of hydroxyapatite crystals during the calcification. However, the interaction between LIPUS-induced ATP and P2X7 receptor during collagen synthesis is poorly understood. In this study, we investigated the role of P2X7 receptor in LIPUS induced osteogenesis during collagen synthesis.

*Materials and Methods:* MC3T3-E1 cells were plated on a 6 well plate, incubated in the presence or absence of competitive P2X7 antagonist A438079, and subsequently stimulated with LIPUS for 30 min/day using OSTEOTRON D<sup>2</sup>. In addition, these cells transfected with P2X7 shRNA were also incubated and stimulated with LIPUS under the same condition. The expression of Col1, matrix metalloproteinase (MMP), and tissue inhibitor of metalloproteinase (TIMP) was determined using real-time PCR.

*Results:* LIPUS induced the gene expression of Col1, whereas it reduced MMP-1, 3, and 13. However, LIPUS didn't affect the expression of TIMPs.

*Conclusion:* These results suggest that LIPUS promotes osteogenesis through the P2X7 receptors in osteoblasts during collagen synthesis.

GB-25

### Bone augmentation using bone graft material derived from a rat GBA model

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**Background and objective:** Autogenous bone grafts are ideal, but the quantity of bone collected can limit the ability to perform effective bone augmentation. This study evaluated the effects of newly augmented tissue derived from a rat guided bone augmentation (GBA) model as bone graft material.

**Materials and Methods:** Thirty 8-week-old male Fischer rats were used. In an initial operation, a standardized plastic cap was put in place, and bone augmentation was performed. In a second operation 12 weeks later, a 5-mm-diameter bone defect was formed in the right parietal bone as the recipient site. The plastic cap was removed. In the experimental group, the recipient site was filled with newly augmented bone tissue as autotransplant bone. The recipient site was not filled in the negative control group. In the positive controls, a sham operation was performed using autogenous bone from the left parietal bone to fill the bone defect. Repeated micro-computed tomography (CT) imaging was performed from 1 to 12 weeks after the second surgery. The defect sites were removed along with the surrounding bone and soft tissues, stained with hematoxylin and eosin.

**Results:** More bone regeneration was observed in the experimental and positive control groups than in the negative control group using micro-CT. Histologically, more osteoblast-like cells were observed around the bony rim in the experimental and positive control groups than in the negative control group.

**Conclusion:** The newly augmented tissue derived from a rat GBA model was an effective bone graft material.

GB-26

### *Porphyromonas gingivalis* LPS induced the production of Angiopoietin-like protein2 in gingival epithelial cell

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**Background and objective:** Angiopoietin-like protein2 (ANGPTL2) plays pivotal roles in various inflammatory diseases such as vascular inflammation, obesity, insulin resistance, cancer and rheumatoid arthritis. However, there is no report about the association with periodontal disease. The aim of present study is to investigate the role of ANGPTL2 in human gingival epithelial cells.

**Materials and Methods:** We obtained gingival crevicular fluid (GCF) samples from patients with chronic periodontitis and healthy and then determined ANGPTL2 protein production. Human gingival epithelial cells (Ca9-22 cells) were stimulated with *Porphyromonas gingivalis* LPS and recombinant human ANGPTL2. Expression levels of ANGPTL2 and various inflammatory cytokines were detected by respectively enzyme-linked immunosorbent assay (ELISA), western blot (WB) and quantitative polymerase chain reaction (qPCR).

**Results:** We found that the production of ANGPTL2 was increased in GCF from patients with chronic periodontitis compared with it from healthy. The stimulation of *P.gingivalis* LPS clearly increased the mRNA expression and protein production of ANGPTL2 in Ca9-22 cells. Inflammatory cytokines production was increased by addition of recombinant human ANGPTL2 in Ca9-22 cells.

**Conclusion:** It is suggested that ANGPTL2 is increased by *P.gingivalis* LPS stimulation in human gingival epithelial cells and might associate with chronic inflammatory disease like periodontal disease.

GB-27

**LNGFR<sup>+</sup>THY-1<sup>+</sup> neural crest like cells have potential of mesenchymal stem cells**Takehito Ouchi<sup>\*</sup>, Satoru Morikawa, Taneaki Nakagawa

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*Background and objective:* Periodontal tissues originate from cranial neural crest derived mesenchyme. Recent reports have shown that mesenchymal stem cells (MSCs) are efficient for periodontal tissues regeneration. MSCs are classically identified by adherent culture and multipotent differentiation. The drawback leads contamination of other lineages cells. Our purpose of this study is to generate purified-human MSCs, which are developmentally derived from cranial neural crest cells for periodontal regeneration.

*Materials and Methods:* Human induced pluripotent stem (iPS) cells were induced into cranial neural crest like cells (NCLCs). To confirm the existence of highly potent purified-human MSCs that expressed LNGFR and THY-1, we analyzed cell surface markers using flow cytometry. Sorted LNGFR<sup>+</sup>THY-1<sup>+</sup> cells were analyzed for their potentials as MSCs.

*Results:* Induced LNGFR<sup>+</sup>THY-1<sup>+</sup> cells from human iPS cells, have potentials of mobilization and self-renewal. And, they expressed neural crest markers, Nestin, Sox10, AP2 $\alpha$ , and HNK-1. Therefore, we designated those cells as LNGFR<sup>+</sup>THY-1<sup>+</sup> neural crest like cells (LT-NCLCs). LT-NCLCs vigorously survived, and had multipotent differentiation into mesenchymal and neural crest lineages.

*Conclusion:* To advance human periodontal stem cell research, additional clarification is needed. Previously, we reported highly potent purified human MSCs that express LNGFR and THY-1. Here, we demonstrate a method, which provides a simple way to generate a large amount of developmentally selected MSCs that express LNGFR and THY-1. They will be promising candidates for periodontal regenerative medicine.

GB-28

**Effect of osteoporosis on bone augmentation in ovariectomized rat calvarium**Tatsuya Kubota<sup>\*1</sup>, Katsuyoshi Tsunori<sup>2</sup>, Yasumasa Ozawa<sup>1</sup>, Takanobu Yamamoto<sup>1</sup>, Naoya Tsukune<sup>1</sup>, Akira Hasuike<sup>2</sup>, Sanae Ezawa<sup>2</sup>, Kenji Tanaka<sup>2</sup>, Yoshinori Arai<sup>4</sup>, Shuichi Sato<sup>2,3</sup>

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*Background and objective:* An ovariectomized (OVX) rat model is used to mimic osteoporosis in postmenopausal women. This study investigated the effect of osteoporosis on augmented bone using a rat calvarial model.

*Materials and Methods:* The study divided 12 6-week-old female Wistar rats into two groups. OVX surgery (experimental group) or a sham operation (control group) was performed in the 6-week-old rats. The rats were bred at 8 weeks. In each rat, the calvarial bone was exposed and a circular groove was made on each side of the midline using a trephine drill with an inner diameter of 5 mm. Five small holes were drilled with a No.2 round burr to induce bleeding within each circle. A cylindrical plastic cap was placed over the grooves on both sides of the midline. The day of surgery was designated day 0. Repeated micro-computed tomography (CT) imaging was performed 1 to 12 weeks after surgery. Micro-CT and histological sections were used to measure the amount of bone augmentation within the plastic caps.

*Results:* Analysis of the micro-CT images indicated that the radiopaque contrast increased in both the OVX and control groups from 2 to 12 weeks after surgery. The difference was significant at 8 and 12 weeks.

*Conclusion:* The OVX rat model showed inhibited bone augmentation using a rat calvarial model, as confirmed by micro-CT and tissue sections. Therefore, osteoporosis may inhibit bone augmentation.

GB-29

**Apical bud transplantation model of rodent teeth for studying epithelial-mesenchymal transition**Ryuji Sakagami<sup>\*1</sup>, Naoki Maruo<sup>1</sup>, Yasunori Yoshinaga<sup>1</sup>, Kazuhiko Okamura<sup>2</sup>, Yoshihiko Sawa<sup>3</sup><sup>1</sup>Section of Periodontology, Department of Odontology, Fukuoka Dental College, <sup>2</sup>Section of Pathology, Department of Morphological Biology, Fukuoka Dental College, <sup>3</sup>Section of Functional Structure, Department of Morphological Biology, Fukuoka Dental College

*Background and objective:* Rodent mandibular incisors grow throughout the lifetime of the animal. The labial and lingual sides of an incisor are regarded as analogs of the crown and root of human teeth, respectively. This report presents a novel transplantation technique of the apical bud tissue of rodent mandibular incisors for establishing a model for investigating the cementoblasts/cementocytes formation through epithelial-mesenchymal transition.

*Materials and Methods:* Mandibular incisal apical end tissue with green fluorescent protein from transgenic mouse was transplanted to wild type mice. The cryosections were prepared without decalcification process and the development of the transplanted cells were immunohistologically observed at 2, 4, and 12 weeks after the transplantation. Podoplanin was used for investigating the characteristics of the transplanted and migrating cells.

*Results:* Results indicate that the green fluorescent apical end tissue replaced the original tissue, and cells from the apical bud differentiated and extended toward the incisal edge direction. The immunostaining with podoplanin also showed that the characteristics of the transplanted tissue were identical to those of the original. The green fluorescent cells were only found in the labial side up to 4 weeks. After 12 weeks, however, they were also found in the lingual side. The green fluorescent cementocyte-like cells were found in the cementum close to the dentin surface.

*Conclusion:* This study indicated that some of the cells from apical bud became the cementocyte-like cells in the cementum of rodent incisors. Epithelial-origin cells in the apical bud might have become cementoblast/cementocyte-like cells through epithelial-mesenchymal transition.

GB-30

**Effects of hyperglycaemia in human periodontal ligament stem cells**Makoto Umeda<sup>\*</sup>, Hirohito Kato, Yoichiro Taguchi, Isao Yamawaki, Nobuhiro Yamauchi, Masahiro Noguchi

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*Background and objective:* Diabetes mellitus is a major risk factor for periodontal disease and affects various cellular functions. Periodontal ligament stem cells (PDLSCs) play an important role in periodontal tissue regeneration. However, the effect of hyperglycaemia on PDLSCs is unclear. The aim of this study was to investigate whether hyperglycaemia affects periodontal tissue regeneration using human PDLSCs and high glucose medium as a model of diabetes.

*Materials and Methods:* PDLSCs were obtained from healthy adult human mandibular third molars. Cell proliferation and osteoblastic differentiation were investigated by culturing PDLSCs in media supplemented with four different glucose concentrations representative of normal patients (5.5 mM; control), postprandial or controlled-diabetes patients (8.0 mM), and high values similar to those seen in uncontrolled diabetic patients (12.0 and 24.0 mM). We then examined the molecular effects of hyperglycemia on PDLSC physiology with a focus on the nuclear factor-kappa B (NF-κB) signaling pathway. We also investigated the involvement of NF-κB in PDLSCs under hyperglycemic conditions with a specific NF-κB inhibitor.

*Results:* High glucose levels inhibited the PDLSC proliferation and differentiation into osteoblasts. Treatment with a NF-κB inhibitor rescued the defects in cell proliferation and osteoblastic differentiation caused by the high glucose culture environment.

*Conclusion:* The results of this study demonstrated that hyperglycemia inhibits human PDLSC proliferation and osteoblastic differentiation.



GB-31

**IL-15 and RANKL play a synergistically important role in osteoclastogenesis**

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*Background and objective:* Interleukin-15 (IL-15), a cytokine secreted by several cell types, has important physiological roles in the activity, proliferation, and viability of immune cells. IL-15 has both chemoattractant and proinflammatory properties and may promote bone destruction. A previous study has shown that IL-15 alone has no effect on osteoclastogenesis. Therefore, the current study addressed the synergistic effect of IL-15 on osteoclast formation using RAW264.7 (RAW) cells by co-stimulation with receptor activator of NF- $\kappa$ B ligand (RANKL) that has an important role in osteoclastogenesis involving the pathogenesis of rheumatoid arthritis (RA) and periodontal disease (PD).

*Materials and Methods:* Murine macrophage RAW264.7 cells were used for all experiment. Cells were stimulated with 25 ng/ml soluble recombinant RANKL and/or 100 ng/ml soluble recombinant IL-15.

*Results:* IL-15 synergistically increased the number of RANKL-induced TRAP-positive multinucleated cells.

It also synergistically increased the mRNA and protein production of osteoclast's specific markers.

We also found that the ERK signaling pathway is a critical regulator of IL-15/RANKL-mediated osteoclastogenesis.

*Conclusion:* The IL-15 and RANKL's synergistic role has been suggested to play an important role in disease with an inflammatory bone resorption such as RA and PD.

GB-32

**Attachment of human gingival epithelial cell on titanium and zirconia**

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*Background and objective:* Zirconia has attracted attention as a substitute material for titanium in implant treatments. In such cases, conferring protection at the junction of the implant body and the epithelium is important. The objective of the present study is to examine the state of adhesion between epithelial precursor cells and zirconia. After epithelial precursor cells were cultured on zirconia and titanium disks, we sequentially observed changes in cell morphology due to attachment under a scanning electron microscope.

*Materials and Methods:* The present study employs three materials in total: two zirconia disks and a titanium disk. Surface roughness and wettability were measured for all samples. Human gingival epithelial progenitor cells were obtained from CELLnTEC, which were later passaged three times and seeded onto the disks. After incubation periods of 1, 12, 24, and 48 h in the culture medium in an incubator, they were washed with water and dehydrated for fixation. Subsequently, the samples were freeze-dried, and the adherent cells on the disk were coated and examined using a scanning electron microscope.

*Results:* The measurement of surface wettability showed that the contact angle of zirconia disk was smaller than that of titanium disk. The examination under a scanning electron microscope revealed that cells were capable of attaching, extending, and proliferating over time on both titanium and zirconia disks. The composition ratio of cell morphology changed over time.

*Conclusion:* It was suggested that human gingival epithelial progenitor cells adhere to the zirconia and titanium disks as epithelial cells.

## GB-33

**Inflammatory cytokines regulate human amelotin gene transcription**

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**Background and objective:** Amelotin (AMTN) is a secreted enamel matrix protein that is localized in the basal lamina of maturation stage ameloblasts and junctional epithelium. To elucidate the transcriptional regulation of human AMTN gene in inflamed gingiva, we have analyzed the effects of inflammatory cytokines on the expression of AMTN gene in Ca9-22 human gingival epithelial-like cells.

**Materials and Methods:** Total RNA, total and nuclear proteins were extracted from Ca9-22 cells after stimulation by TNF- $\alpha$  (10 ng/ml) or IL-1 $\beta$  (1ng/ml). Real-time PCR, Western blotting, luciferase assays using constructs ligated with various length of human AMTN gene promoter and gel mobility shift assays were performed.

**Results:** TNF- $\alpha$  and IL-1 $\beta$  increased AMTN mRNA and protein levels at 6 h and reached maximal at 12h. TNF- $\alpha$  induced luciferase activities of -211 and -353AMTN constructs, and IL-1 $\beta$  increased luciferase activities of -211, -353 and -501AMTN constructs. Induction of luciferase activities by TNF- $\alpha$  and IL-1 $\beta$  were partially inhibited in the -353AMTN construct which included mutations in C/EBP (1), C/EBP (2) and YY1 elements. Transcriptional activities induced by TNF- $\alpha$  and IL-1 $\beta$  were inhibited by tyrosine kinase, ERK1/2 and PI3-kinase inhibitors. Gel shift assays showed that TNF- $\alpha$  and IL-1 $\beta$  increased nuclear proteins binding to C/EBP (2) and YY1 elements.

**Conclusion:** These results demonstrate that TNF- $\alpha$  and IL-1 $\beta$  induce AMTN gene transcription via tyrosine kinase, ERK1/2 and PI3-kinase pathways in human gingival epithelial cells by targeting the C/EBP (1), C/EBP (2) and YY1 elements in the human AMTN gene promoter.

## GB-34

**Study on regeneration of periodontal tissue microvasculature using dedifferentiated fat cells**

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**Background and objective:** Human dedifferentiated fat cells (HDFATs) may be a new cell type suitable for regenerative therapies. The aim of this study is to assess the potential of HDFATs for vascular regeneration of periodontal tissue. To do this, HDFATs and human gingival endothelial cells (HGEs) were cocultivated, and vascular regeneration was examined in vitro.

**Materials and Methods:** HDFATs were isolated from subcutaneous adipose tissue, and HGEs were isolated from gingival cells using anti-cluster of differentiation 31 antibody-coated magnetic beads. HDFATs were cocultured with HGEs in microvascular endothelial cell growth medium-2 (EGM-2MV) for 7 days. Expression of endothelial cell (EC) markers, the formation of capillary-like tubes, and the expression of pericyte markers were determined.

**Results:** HDFATs, cultured in EGM-2MV or cocultured with HGEs, expressed EC markers. HDFATs in both conditions initiated tube formation within 5 hours of seeding and formed extensive capillary-like structures within 12 hours. These structures disintegrated within 24 hours when cells were cultured in EGM-2MV alone, whereas cocultured HDFATs maintained tubes for >24 hours. Cocultured HDFATs significantly increased expression of pericyte markers, a cell type associated with microvasculature.

**Conclusion:** HDFATs possess the ability to express EC markers, and coculture with HGEs promotes differentiation into pericytes involved in the maturation and stabilization of the microvasculature.

GB-35

**Effect of high-glucose conditions on human periodontal ligament endothelial cells**Kosuke Maruyama<sup>\*1</sup>, Soh Sato<sup>1,2</sup>

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**Background and objective:** Endothelial cells participate in key aspects of vascular biology, such as maintenance of capillary permeability and regulation of inflammation. According to previous reports, endothelial cells have revealed highly specific characteristics depending on the organs and tissues. In particular periodontal endothelial cells have a higher permeability than vascular endothelial cells of other types of tissue. Periodontal disease is not only a chronic disease in oral but also affect the entire body. Diabetes and periodontal disease are closely related, with periodontal disease even been referred to as the sixth complication of disease. However, no reports have investigated the pathophysiology of microvascular in periodontal tissue once diabetes has developed. Therefore, the aim of the present study is to investigate changes in the properties of human periodontal endothelial cells (HPDLECs) that were cultured under high-glucose conditions.

**Materials and Methods:** We isolated human periodontal ligament cells from extracted teeth (males:3, females:5, age:18-56 years). We isolated HPDLECs from human periodontal ligament cells. HPDLECs were cultured under high-glucose (5.5mM, 11.0mM, 22.0mM) and investigated proliferation, apoptosis, tube formation and the expression of cell adhesion molecules.

**Results:** HPDLECs stimulated with high glucose concentration exhibited suppression of cell proliferation and an increased percentage of apoptosis-positive cells. The expression levels cell adhesion molecules increased

**Conclusion:** Thus, the results of this study demonstrate that diabetes exacerbates periodontal disease.

GB-36

**LPS reduced nicotine-mediated IL-8 secretion**Mariko Ohtsu<sup>\*1</sup>, Masatake Asano<sup>2,3</sup>, Kou Tsunoda<sup>1</sup>, Ryosuke Koshi<sup>4,5</sup>, Masahiro Eda<sup>4</sup>, Shinji Arai<sup>4</sup>, Shuichi Sato<sup>4,5</sup>

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**Background and objective:** Cigarette smoking is an important environmental risk factor for the development of periodontal disease. In an attempt to verify the role of nicotine on development of periodontitis, OSCC was cultured with *P.gingivalis*-derived LPS in the presence or absence of nicotine. Contrary to our expectation, LPS reduced nicotine-mediated IL-8 secretion. The aim of the present study was to elucidate the mechanisms underlying this phenomenon.

**Materials and Methods:** Ca9-22 was used for the experiments. Cells were stimulated by nicotine with or without *P.gingivalis*-derived LPS for 48 h. The culture supernatants were harvested and subjected to IL-8 ELISA. For acetylcholine (ACh) production, cells were cultured with PBS for 10, 30 and 60 min and ACh concentration was measured by ELISA.

**Results:** Ca9-22 was cultured with or without nicotine and LPS for 48 h. Nicotine-mediated IL-8 secretion was reduced significantly by LPS. To elucidate the mechanisms of LPS-mediated IL-8 reduction, Ca9-22 was cultured in the presence or absence of LPS and ACh concentration was measured. In the absence of LPS, ACh was accumulated in the culture supernatant in a time-dependent manner. In contrast, LPS reduced ACh accumulation. Reduction of nicotine-mediated IL-8 secretion by LPS might be attributed to LPS-mediated ACh inhibition.

**Conclusion:** The results indicated the constitutive production of ACh by Ca9-22. Although further experiments should be required, LPS might influence nicotine-mediated IL-8 secretion by reducing ACh production.



GB-37

**Osteogenic effects of high glucose concentration on nano-modified titanium surface**

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*Background and objective:* A lot of cases of an peri-implantitis have been reported, However, it is also fact that many diabetic patients have the implant prostheses in recent years. The purpose of this study was to determine the effect of glucose concentrations on the osteogenic differentiation of bone marrow mesenchymal stem cells (BMMSCs) on a nanonetwork-modified titanium surface.

*Materials and Methods:* Titanium disks treated with 10 M NaOH solution and untreated control disks were incubated with BMMSCs and then exposed to high glucose concentrations (5.5, 8, 12 or 24mM). The effects of the modified nanonetwork structure on osteogenic differentiation of BMMSCs were evaluated at four different glucose concentrations.

*Results:* High glucose levels inhibited the osteogenic differentiation of BMMSCs. The amounts of osteocalcin production and calcium extraction after 3 or 4 weeks culture in 24 mM medium was higher than in physiological medium, but those in 8 and 12mM medium were lower. The Ca extraction crystal is reduced by the rise of the glucose concentration, and it is thought that it influences the calcified condition.

*Conclusion:* These results suggests that hyperglycemia due to diabetes influence calcification and hard tissue formation of BMMSCs that play an important role to the periodontal tissue regeneration on the nano-modified titanium surface.



# General (Clinical Research)



## GC-01

**Platelet-rich fibrin with beta-tricalcium phosphate for treatment of periodontal defects**

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*Background and objective:* It has been reported that platelet-rich fibrin (PRF) membranes contain numerous platelets and angiogenic growth factors. These findings suggested that PRF membranes exhibit the characteristics of a promising therapeutic grafting material for the treatment of periodontal osseous defects.

*Materials and Methods:* To prepare the PRF, blood samples were collected from peripheral blood of patients, and centrifuged by a Medifuge system. After eliminating the red thrombus, the resulting PRF preparations were compressed by a PRF compression device, which resulted in a PRF membrane suitable for use in grafting. Thirteen interproximal infrabony osseous defects in 13 healthy, non-smoking subjects diagnosed with chronic periodontitis were included in this study. The periodontal surgery exposed the osseous defects, and allowed access for debridement and root planing. The beta-tricalcium phosphate (TCP) was placed into the osseous defects, and then they were covered with a PRF membrane before suturing. Clinical and radiographic measurements were determined at baseline and at the 3, 6, and 12-month post-surgery.

*Results:* When compared to baseline, the 12-month results demonstrated the treatment procedures resulted in statistically significant favorable changes in probing depth ( $7.9 \pm 1.6$  mm versus  $3.1 \pm 0.3$  mm;  $P < 0.01$ ), clinical attachment level gain ( $9.5 \pm 1.9$  mm versus  $6.6 \pm 1.3$  mm;  $P < 0.01$ ) and radiographic infrabony defect depth decrease ( $5.1 \pm 1.9$  mm versus  $1.9 \pm 1.0$  mm;  $P < 0.01$ ).

*Conclusion:* The present study demonstrated that grafting using a combination of a PRF membrane and beta-TCP was successful in the treatment of human infrabony periodontal defects.

## GC-02

**GTR using Xenogeneic Bone-Mineral with Collagen Membrane: A Multicenter Study**

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*Background and objective:* This multicenter prospective clinical study aimed to evaluate the outcome of GTR using a deproteinized bovine bone mineral (DBBM) in combination with a collagen barrier (CB) in the treatment of intrabony defects.

*Materials and Methods:* Patients with chronic periodontitis who have completed initial periodontal therapy were recruited in 5 centers in Japan. Participants had at least one 2- or 3-wall intrabony defect of  $\geq 3$  mm. Surgical procedures included access for debridement using the modified or simplified papilla preservation flap. Defects were filled with DBBM and covered with CB. Periodontal parameters were re-evaluated at 6 months after surgery.

*Results:* Thirty-six patients were enrolled and completed the study (mean age 53.6 years old, 18 females and 18 males). At baseline, mean clinical attachment level (CAL) of the treated site was  $8.08 \pm 1.58$  mm and mean probing depth (PD) was  $7.47 \pm 1.29$  mm. Intrabony component of the defect, measured during surgery, was  $4.96 \pm 1.83$  mm. Throughout the study, none of the participants underwent any adverse event. At 6 months after surgery, the mean gain in CAL (primary outcome) was  $3.42 \pm 1.87$  mm. Significant improvements were also noted in the secondary outcomes such as PD, Gingival Index and tooth mobility. A significant positive correlation was found between CAL gain and baseline CAL value.

*Conclusion:* This study showed non-inferiority of the GTR with DBBM and CB when compared with already published literature data. Clinical evidence after 6 months supported the effectiveness of the combination therapy in the treatment of intrabony defects.

## GC-03

**Biological effects of a-PDT and LDDS in chronic periodontitis**

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**Background and objective:** Antimicrobial photodynamic therapy (a-PDT) is a new treatment method for the removal of infectious pathogens using a photosensitizer and light of a specific wavelength. We investigated the microbiological and clinical effect of a-PDT and local drug delivery system (LDDS) in chronic periodontitis patients.

**Materials and Methods:** Twenty-three patients with chronic periodontitis were randomly assigned to two groups: a-PDT and LDDS. Subjects were received a-PDT (Periowave<sup>TM</sup>) or LDDS (minocycline) twice in periodontal pockets of 2 selected teeth. Sampling of subgingival plaque and gingival crevicular fluid (GCF), and periodontal examination were performed at baseline, 1 and 4 weeks after. Periodontopathic bacteria were quantified using the modified Invader PLUS assay. Multiplex Array was performed for inflammatory biomarkers from GCF. The data was calculated by Friedman test and Mann-Whitney's U test.

**Results:** Counts of *P. gingivalis* and *T. forsythia*, and the ratio of those bacteria (to total bacteria) in the LDDS group were significantly decreased after 1 week compared to the baseline ( $P < 0.05$ ). GCF levels of IFN- $\gamma$ , IL-1 $\beta$  in the LDDS group were also significantly decreased after 1 week ( $P < 0.05$ ). Significant reduction of probing pocket depth, clinical attachment level and bleeding on probing in the both group were indicated after 4 weeks ( $P < 0.05$ ).

**Conclusion:** It is suggested that a-PDT and LDDS may have clinical effects in periodontal treatment. In particular, LDDS may be effective on periodontal pathogen and inflammatory markers from GCF.

## GC-04

**Expression of neprilysin in periodontitis-affected gingival tissues**

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**Background and objective:** The pathogenesis between Alzheimer's diseases (AD) and periodontal diseases have some common factors including ageing and chronic inflammation but still unclear. To explore the pathogenesis of periodontitis, a comprehensive gene expression/transcriptomes in periodontitis-affected gingival tissues found that the AD pathway was significantly up-regulated in periodontitis-affected gingival tissues. AD-related genes, Amyloid beta precursor protein (APP), interleukin-1 beta and complement 1QA were significantly elevated in periodontitis. In the present study, Balance between APP and a potent amyloid degradation enzyme, neprilysin (NEP) mRNA expression levels, as well as the protein localisation of APP and NEP were analysed.

**Materials and Methods:** Eighteen periodontitis-affected and 18 clinically healthy control gingival tissues were taken from patients with severe chronic periodontitis or with tooth extraction needs. Total RNA was purified and used for quantitative reverse transcription real-time polymerase chain reaction (qRT-PCR). The localisation of APP was analysed by immunohistochemistry (IHC).

**Results:** The genes both APP and NEP were up-regulated in periodontitis-affected gingival tissues. APP-expressing macrophages and NEP expression in neutrophils and fibroblasts depends on inflammatory stages were detected in inflamed gingival tissues by IHC

**Conclusion:** The up-regulation of APP and NEP mRNA levels in periodontitis-affected gingival tissues compared with healthy controls was confirmed by qRT-PCR analyses. Thinking that, NEP is one of the primary enzymes degrading amyloid beta, the increased NEP mRNA in periodontitis may acts as an inhibitor for amyloid beta in gingival tissues, balancing increased APP mRNA. However, NEP has several effects including degradation of vasoactive substances, further research are needed.

## GC-05

### Combinational perio-regenerative therapy with/without collagen membrane –A split mouth study–

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**Background and objective:** To compare the clinical outcome in the treatment of periodontal intrabony defects using deproteinised bovine bone mineral (DBBM), enamel matrix derivative (EMD), with or without collagen membrane (CM).

**Materials and Methods:** Nine informed consented patients with chronic periodontitis were enrolled. Two defects per patient with probing depth  $\geq 6$  mm were assigned to the treatment with either EMD + DBBM + CM (CM site) or EMD + DBBM (no-CM site) (n=9 vs. 9). At baseline and after six months, clinical parameters including probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP) and the bone fill % measured by corn beam CT (CBCT) were recorded. Statistical analyses were done with Wilcoxon S.R. test.

**Results:** Significant decrease in PPD, CAL gain, absence of BOP and increased bone fill were observed at six month after both regenerative combinations ( $p < 0.05$ ). Changes in PPD ( $p = 0.03$ ) and CAL ( $p = 0.02$ ) decrease were significantly greater in CM sites compared to no-CM sites at six month examination. The other parameters including the bone fill %, no significant differences were observed between two groups.

**Conclusion:** Within the limitation of the study, combinations of periodontal regenerative therapy using EMD + DBBM + CM or EMD + DBBM for deep periodontal intrabony defects both improved clinical outcomes. Advantages in PPD decrease and CAL gain were expected with the use of CM. Further analyses assessing individual periodontal biotype, smoking status, types of defects and longer prognosis are needed and ongoing.

## GC-06

### Effectiveness of collagen membrane in combinational periodontal regenerative therapy

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**Background and objective:** Aim: To examine the clinical benefit in treatment of periodontal intrabony defects using deproteinised bovine bone mineral (DBBM), enamel matrix derivative (EMD), with or without collagen membrane (CM).

**Materials and Methods:** Forty informed consented patients with chronic periodontitis were enrolled. An intrabony defect per a patient with probing depth  $\geq 6$  mm were assigned to the treatment with either EMD + DBBM + CM (CM) or EMD + DBBM (no-CM) (n=21 vs. 19). At baseline and after six months, clinical parameters including probing pocket depth (PPD), clinical attachment level (CAL), and bleeding on probing (BOP) and the bone fill % measured by corn beam CT were recorded. Statistical analyses were done with Wilcoxon S.R. test.

**Results:** Significant decrease in PPD, CAL gain, absence of BOP and increased bone fill were observed at six month after both regenerative combinations ( $p < 0.05$ ). Changes in PD ( $p = 0.04$ ) and CAL ( $p = 0.02$ ) were significantly greater in CM compared to no-CM at six month, especially in patients with thick periodontal biotype, with smoking habit and with contained defects. The other parameters including the bone fill %, no significant differences were observed.

**Conclusion:** Within the limitation of the study, combinations of periodontal regenerative therapy using EMD + DBBM + CM or EMD + DBBM for deep periodontal intrabony defects both showed good clinical outcomes. Advantages in PPD decrease and CAL gain were expected with the use of CM, however longer prognosis needs to be examined.

GC-07

### **A retrospective study on the effects of Konus-telescope dentures for periodontal patients on the prognosis of abutment teeth**

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*Background and objective:* The use of prosthetics during treatment for oral rehabilitation in patients with advanced periodontitis is often challenging because of worsening of the crown-to-root ratio due to bone resorption, movement of the remaining teeth, gingival recession and tooth loss. Following treatment, masticatory dysfunction can occur as a result of secondary occlusal trauma. Konus-telescope dentures, a type of denture with rigid support, have many superior clinical features; however, very few reports have examined the effect of dentures on prognosis and periodontal tissue surrounding abutment teeth suffering from periodontitis from the perspective of periodontal disease. Therefore, the usefulness and applicability of Konus-telescope dentures in periodontal prosthetics must be examined. This study was conducted to examine the effects of treatment for oral rehabilitation on the prognosis of abutment teeth and their surrounding tissue in patients with periodontitis using Konus-telescope dentures.

*Materials and Methods:* Thirty-two dentures and 217 abutment teeth were examined and compared for prosthetic parameters and periodontal disease parameters during the initial examination, at insertion of dentures and maintenance.

*Results:* Each periodontal disease parameter showed a significant tendency toward improvement while wearing the dentures and during maintenance as compared with the initial examination. The survival rate of abutment teeth during the observation period was 96.3%.

*Conclusion:* These results suggest that good periodontal tissue can be maintained over the long-term with Konus-telescope dentures through continued, regular maintenance even if the amount of supporting tissue has decreased due to chronic periodontitis and the abutment teeth are difficult to treat with prosthetics.

GC-08

### **Periodontal evaluation using sIL-6R/calprotectin in GCF during SPT phase**

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*Background and objective:* Periodontitis is a chronic inflammatory disease, and the supportive periodontal therapy (SPT) is known as an integral part of periodontal treatment. In the present study, we propose a new method for periodontal evaluation during SPT phase by determining the cut-off values of soluble interleukin-6 receptor (sIL-6R) and calprotectin levels in gingival crevicular fluid (GCF).

*Materials and Methods:* A total of 16 periodontitis patients during SPT phase were selected and GCF samples were collected from each healthy and inflamed site of periodontal pockets in the patients. The relationship between periodontal condition and sIL-6R/calprotectin levels in GCF was investigated in a cross-sectional study. Cut-off values of sIL-6R and calprotectin levels in GCF to diagnose periodontitis were determined using a receiver operating characteristic (ROC) curve.

*Results:* Both sIL-6R and calprotectin levels in GCF were higher in inflamed sites than in healthy sites. When the patients were divided by gingival index (GI), sIL-6R and calprotectin levels were higher in GI:1 and GI:2 groups than in GI:0 group. The sIL-6R level was also higher in BOP positive group than in BOP negative group, although calprotectin level showed no change between BOP positive and negative group. Cut-off values of sIL-6R and calprotectin levels in GCF to distinguish the presence or absence of periodontal inflammation were as follows: sIL-6R: 43.7 pg/ml; calprotectin: 193.7 ng/ml.

*Conclusion:* Both sIL-6R and calprotectin in GCF may be useful bio-markers to evaluate the progression of periodontitis in patients during SPT phase.



GC-09

### Analysis of the relationship between periodontal disease and diabetes mellitus

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**Background and objective:** The purpose of this study was to clarify the relationship between the alveolar bone resorption (ABR) rate, a high-sensitivity C-reactive protein (hs-CRP) level and diabetes mellitus (DM), used as the indexes of the severity classification of Japanese Society of Periodontology (JSP). In addition, we investigated whether the ABR rate and the hs-CRP level could predict DM.

**Materials and Methods:** A total of 374 patients (253 men and 121 women), including 50 already diagnosed DM who visited medical examination center and department of periodontology, Matsumoto Dental University Hospital and underwent radiography and the examination of hs-CRP comprised the participants. Then, each subject was classified following the degree of the severity classification of JSP. Logistic regression analysis, adjusted for gender, age, body mass index (BMI), the number of present teeth, history of smoking was used. In addition, receiver operating characteristic (ROC) curve analysis was used to clarify whether the presence of DM was accurately identified using the ABR rate and hs-CRP level. The Ethics Committee of Matsumoto Dental University reviewed and approved the study protocol (approved number, No.0151).

**Results:** It was found that the factors associated with DM were age, BMI, ABR rate, hs-CRP values, and risk of DM became higher than the ABR rate, hs-CRP level are expensive. Furthermore, the ABR rate and hs-CRP value is useful as a screening indicator of DM was found from the ROC analysis.

**Conclusion:** It is suggested that the radiograph or hs-CRP examination at dental or medical examinations could lead early detection, early treatment of DM.

GC-10

### Repeating subgingival debridement of periodontal pockets by air polishing

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**Background and objective:** The purpose of the study was to evaluate clinical effects of repeating subgingival debridement by air polishing during supportive periodontal therapy.

**Materials and Methods:** The double blind, randomized controlled trial of 3 month duration was conducted on 18 recall patients previously treated for chronic periodontitis. Three sites with probing pocket depth (PPD) of 4-9mm and bleeding on probing in each patients were randomly assigned to treatment by (1) Repeating glycine-powder/air polishing every 4 weeks (group 1), (2) glycine-powder/air polishing once at baseline (group 2), or (3) water irrigation (group 3). Clinical parameter was recorded at baseline and 3 month post-treatment.

**Results:** Group 1 and 2 results in significant reduction in BoP, PPD and clinical attachment level at 3 months. There were statistically significant differences in change in PPD between the group 1 and 3 at 3 month examination.

**Conclusion:** This study revealed significant clinical effects of repeating subgingival air polishing in moderate to deep pockets in SPT patients.

GC-11

### Guided Bone Regeneration using Bone Enhancing Cover Screw (BECS)

Mitsuharu Inoko

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*Background and objective:* BECS (Bone Enhancing Cover Screw) system is developed to locate and fix titanium mesh (Ti-Mesh) firmly for bone augmentation around Implant. It enables to make easy environment for bone augmentation by steady bone graft and eliminating micro-movement of Ti-mesh. BECS have several kinds for each purpose. One of them is for the bone augmentation to 1 mm above the platform of an implant used in simultaneous approach.

*Materials and Methods:* BECS system is developed to locate and fix Ti-Mesh firmly for bone augmentation around Implant. The BECS simultaneously used with the Implant placement has an anchorage at the top of the bone crest where bone is required absolutely so that it can help achieve ideal bone augmentation.

*Results:* Conventional fixation of Ti-mesh was mostly done by mini-screw. Because of the mechanism of fixation by mini-screw, bone substitute material and/or Ti-mesh were easy to expose, and it required much attention to prevent complications. The GBR for horizontal bone defect of mandible, which has relatively large number of indicated cases, expedient titanium mesh extension should be avoided because of thin lingual gingiva especially for mandible. In such cases, BECS can achieve safe and ideal vertical bone augmentation, and can avoid dangerous titanium mesh extension to the lingual side because it does not need to use mini-screw.

*Conclusion:* BECS enables to make easy environment for bone augmentation by steady bone graft and eliminating micro-movement of Ti-mesh.

GC-12

### Periodontal tissue regeneration by adipose tissue-derived multi-lineage progenitor cell transplantation

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*Background and objective:* Several stem and progenitor cells are currently under investigation for their application in cell-based therapy for periodontal tissue regeneration. We have been revealed the efficacy of periodontal tissue regeneration by transplantation of adipose tissue-derived multi-lineage progenitor cells (ADMPCs) with experimental periodontitis models on beagle dogs. The objective of this study was to evaluate the efficacy and safety of transplantation of ADMPCs for periodontal tissue regeneration in a clinical study.

*Materials and Methods:* Subcutaneous adipose tissue was harvested by liposuction from a couple of patients with periodontitis who provided written informed consent. ADMPCs were isolated from the tissue and cultured in a cell processing isolator. Each patient underwent flap operation during which we transplanted autologous ADMPCs with a fibrin carrier material (Bolheal<sup>®</sup>) to the bone defect. Before and for 36 weeks after transplantation, patients underwent periodontal tissue inspection, standardized dental radiographic analysis and a variety of clinical examinations.

*Results:* 36-week follow up indicated that ADMPCs transplantation resulted in reduction of probing pocket depth, clinical attachment gain and improvement of radiolucency. This therapeutic efficiency was observed not only in 2- or 3-walled vertical bone defects but also in more severe periodontal bone defects. No transplantation-related adverse events were observed.

*Conclusion:* These results suggest that ADMPCs transplantation can induce periodontal regeneration. We expect that the accumulation of this clinical data will provide the accuracy about not only the efficacy and safety but also the indication in this ongoing clinical study.

GC-13

**Interleukin (IL)-35 and IL-17 may play important role in periodontitis**Akio Mitani<sup>\*1</sup>, Kosuke Okada<sup>1</sup>, Hisashi Goto<sup>1</sup>, Yosuke Kamiya<sup>1</sup>, Takeki Fujimura<sup>1</sup>, Makio Mogi<sup>2</sup>, Makoto Aino<sup>1</sup>, Toshimitsu Hishikawa<sup>1</sup>, Toshihide Noguchi<sup>1</sup>, Takeshi Kikuchi<sup>1</sup><sup>1</sup>Department of Periodontology, School of Dentistry, and <sup>2</sup>Department of Medical Biochemistry, School of Pharmacy, Aichi Gakuin University

**Background and objective:** Interleukin (IL)-35 plays an important role in immune regulation through the suppression of effector-T cell populations, especially T helper 17 (Th17) cells. While IL-17 and Th17 cells are involved in the pathogenesis of periodontitis, the role of IL-35 in periodontal tissues is unclear. We investigated IL-35 and IL-17 expression in gingival crevicular fluid (GCF) and human gingival tissue, and tested the effect of IL-35 on Th17 cells.

**Materials and Methods:** GCF samples were collected from patients with periodontitis and healthy controls, and were analyzed by ELISA for IL-35 and IL-17. Gingival tissue was collected from 10 healthy participants and 10 patients with periodontitis, and were analyzed by qPCR for *EBI3*, *IL12A*, *IL27p28* and *IL17A*. CD4<sup>+</sup>T cells were sorted by auto-MACS from a healthy volunteer, and then Th17 cells were induced by cytokines cocktail. Th17 cells were incubated with or without rIL-35, and analyzed by qPCR for *RORA*, *RORC* and *IL17A*.

**Results:** IL-35 and IL-17 were significantly higher in GCF from patients with periodontitis than healthy participants ( $p < 0.05$ ). We found a positive correlation between IL-35 and probing pocket depth and clinical attachment level (CAL), and a positive correlation between IL-17 and CAL. *EBI3*, *IL12A* and *IL17A* mRNA expression levels were significantly higher in inflamed gingival tissue than in healthy control tissues ( $p < 0.05$ ), but *IL27p28* was not detected in any sample. *RORA* and *IL17A* mRNA expressions were significantly reduced by addition of rIL-35 in T cells ( $p < 0.05$ ).

**Conclusion:** IL-35 and IL-17 may play important roles in the pathogenesis of periodontitis.

GC-14

**Association of salivary *P.gingivalis* with serum antioxidants in hyperlipidemia patients**Hiroyuki Watanabe<sup>\*1</sup>, Toshiyuki Nagasawa<sup>2</sup>, Shintaro Shimizu<sup>1</sup>, Yutaka Terada<sup>3</sup>, Satsuki Kato<sup>1</sup>, Yumiko Konishi<sup>4</sup>, Mari Mori<sup>4</sup>, Takeo Maida<sup>5</sup>, Tomofumi Kawakami<sup>4</sup>, Hajime Ide<sup>6</sup>, Masahiro Tsuji<sup>6</sup>, Yasushi Furuichi<sup>1</sup>

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**Background and objective:** Hyperlipidemia and type 2 diabetes mellitus (T2DM) are major risk factors for atherosclerosis. Oxygen radicals are generated by the inflammation, and oxidative stress is known to enhance atherosclerosis. Purpose of the present study was to examine the effects of periodontitis on serum antioxidants in hyperlipidemia patients with T2DM.

**Materials and Methods:** 110 hyperlipidemia patients with (48 patients) or without T2DM were enrolled in the present study. Periodontal parameters, including probing pocket depth (PPD) and bleeding on probing (BoP), were examined. Serum total cholesterol, HDL, neutral fat, super oxide dismutase (SOD) and uric acid were measured. Salivary periodontopathic bacteria, including *Porphyromonas gingivalis* (*P.gingivalis*) and *Aggregatibacter actinomycetemcomitans* (*A.actinomycetemcomitans*), were quantitated using real time PCR. Spearman's rank correlation test was used to evaluate the relationship between 2 parameters. Logistic regression analysis was performed using uric acid or SOD as a dependent variable.

**Results:** Number of *P.gingivalis* in saliva was positively correlated with SOD, but negatively correlated with uric acid in hyperlipidemia patients with diabetes. Logistic regression analysis showed that SOD and uric acid had significant positive and negative association with the number of *P.gingivalis* in T2DM patients, respectively.

**Conclusion:** Present study suggested that increase of *P.gingivalis* might enhance the consumption of antioxidants in hyperlipidemia patients with T2DM.



# Case Report



R-01

**Anti-infective periodontal surgical therapy for peri-implantitis. A 24-month clinical follow-up**Yoshihide Shirai<sup>\*1</sup>, Tomoo Kono<sup>2</sup>, Tsurayuki Takahashi<sup>2</sup>, Hajime Tabata<sup>2</sup>, Makoto Umeda<sup>1,2</sup><sup>1</sup>Osaka Dental University Hospital, Periodontology, <sup>2</sup>Department of Periodontology, Osaka Dental University

**Background:** The incidence of peri-implantitis has risen with the increasing use of implants. A number of treatments have previously been reported for peri-implantitis. This case report describes the basic periodontal surgery for peri-implantitis.

**Case:** On April 12, 2013, a 67-year-old woman was admitted to Osaka Dental University Hospital with the chief complaint of pus discharge and bleeding around the implant. There were no remarkable findings in her medical history. Upon admission, the patient exhibited pus discharge, bleeding from the left maxilla molar implant, PD was 9mm, CAL was 9mm. Furthermore, X-ray indicated about 1/3 bone resorption. The findings of the initial examination and diagnosis, as well as the subsequent general periodontal surgery, and the bleeding on probing were fully described to the patient to help her to understand the importance of establishment of plaque control.

**Clinical Procedures and Outcomes:** This case involved basic access flap and debridement (scaling, lubricated mechanically surface, irrigated with saline). The preoperative PD of 9mm was reduced to 3mm during the 24 months after surgery. Moreover, CAL also improved from 9mm to 4mm. X-Ray at 24 months, compared with the preoperative examination showed clear improvement in the alveolar bone.

**Conclusion:** Surgical treatment for one implant improved the outcome for all four alveolar bones. From the above, but the treatment for peri-implantitis has been tried non-surgical, surgical methods do peri-implantitis, committed to the establishment of edge on the plaque control modified sulcus bleeding index (mBI) (–) is believed to be is that a good result maintenance is obtained.

R-02

**A case of severe chronic periodontitis treated by comprehensive therapy including adjunct antimicrobial therapy**Yuichiro Ihara<sup>\*1</sup>, Akihiko Katayama<sup>1,2</sup>, Taneaki Nakagawa<sup>1</sup><sup>1</sup>Department of Dentistry and Oral Surgery, Keio University School of Medicine, <sup>2</sup>Yurakucho Dental Office, Tokyo

**Background:** Scaling and root planing adjunctive with antimicrobial therapy is the basic approach to control the amount of periodontal pathogens in the oral cavity. Moreover, periodontal surgery and an orthodontic approach are effective treatments to eliminate deep periodontal pockets and maintain stable periodontal tissue which leads to an improved prognosis for severe chronic periodontitis.

**Case:** A 38-year-old female visited in February 2012 with a chief complaint of a loose lower anterior tooth. She had received periodontal treatment, which included #2,15 extraction and #19 root separation in 2005. However, as the complaints had not improved, she visited our hospital.

**Clinical Procedures and Outcomes:** We harvested GCF from #12, which was the deepest pocket at her first visit. The percentage of *P.gingivalis* and *T.forsythia* in the sample were 81% and 8.6%, respectively. Therefore, we performed full mouth disinfection adjunctive with oral administration of azithromycin (500 mg once a day for 3 days). *P.gingivalis* and *T.forsythia* were not detected at the time of re-evaluation. After initial periodontal therapy, #14 root resection and apically positioned flap with free gingival graft were performed. Then the patient was treated orthodontically and final prosthodontic therapy was completed.

At 4-years after initial treatment and antimicrobial therapy, no periodontal pathogen was detected, and the shallow periodontal pocket maintained stable periodontal tissue.

**Conclusion:** The result suggests that comprehensive treatment, including periodontal surgery and antimicrobial therapy was effective to maintain good periodontal tissue and save the teeth in the treatment of severe chronic periodontitis.

R-03

### Treatment of periodontal intrabony defect in the molar region

Takamasa Ohyagi\*

Dental Ohyagi

*Aim:* This case discusses the treatment of periodontal intrabony defect in the molar region by means of initial preparation, orthodontic treatment and periodontal regeneration.

*Patient:* A 46-year-old female first visited our clinic in October 2010 with periodontal problems. The last time she visited a dentist was a decade ago. She smoked 10–15 cigarettes a day but started quitting smoking at the time.

*Examination:* The percentage of sites with PPD  $\geq$  4mm was 20.6%. The percentage of BOP was 31.3%. PPD in the molar region was  $\geq$  5mm. PPD at the distal of 17 and 26 were 7mm (both with Grade 2 mobility). 18 was impacted. PPD at the mesial of 27 was 8mm (Grade 3 mobility). 27 was extruded by the presence of 28. Two-three wall intrabony defect was found at the distal of 36 (7mm PPD, Grade 1 mobility).

*Treatment:* 1) extracted 18, 27 and 38, 2) initial preparation 3) with emdogain® regenerated one-three wall intrabony defect (17 distal), three wall intrabony defect (36 distal) and one wall intrabony defect (26 distal). 26 was done after orthodontically moving 28 to the 27 position, 4) Conducted FGG on 17 as the amount of keratinized gingiva was insufficient after the surgery and 5) SPT was introduced when the recovery of the tissues was confirmed.

*Conclusion:* Bone resorption in the molar region was treated effectively by moving 28 forward and creating one wall defect at the distal of 26 before conducting regeneration. SPT is required to maintain a good prognosis.

R-04

### Case report: Occlusal reconstruction of missing maxillary molar teeth using implants

Wataru Atsuta\*, Yuka Atsuta

Blossom dental office

*Background:* Cases of missing maxillary teeth due to reduced periodontal support by periodontitis can lead to rapid tooth loss. It can become necessary to administer treatments including periodontal therapy and occlusal reconstruction. We report that it was possible to obtain a good follow through these treatments.

*Case:* This 61 year-old man first visited in April 2010, with the chief complaint of occlusal pain at the maxillary molar site. #17 exhibited mobility (+++), swelling and pus discharge. #26 and #27 exhibited root fracture. It exhibited vertical bone resorption and gingival recession in the anterior teeth. Medical History: Sinusitis, Non-smoker.

*Clinical Procedures and Outcomes:* The initial treatment plan included periodontal therapy, #26 and #27 extraction, followed by medical treatment of the maxillary sinusitis by an otolaryngologist. After healing of the disease, dental implants were inserted into the maxillary molars missing teeth area with socketlift and GBR. After treatment of the vertical occlusion of the posterior teeth, gingival flap surgery was performed on the maxillary anterior teeth. After confirming stability of the occlusion, it was determined that the final prosthesis would include porcelain fused to zirconia crown as the final prosthesis.

*Conclusion:* Up to 2 years 10 months of maintenance follow-up, there has been no problem with the implant site or the anterior bone formation. In this case of maxillary missing teeth by periodontitis, periodontal therapy and implant treatment appeared to be very effective.



R-05

### **Long-term management of occlusal plane for generalized severe chronic periodontitis patient with occlusal trauma**

Jiro Hirano\*, Tatsuo Hirano, Naomasa Yasuda, Yuichiro Togashi

Hirano Dental Office

*Background:* Here we report a case involving periodontal treatment and occlusal management administered for a patient with generalized severe chronic periodontitis associated with occlusal trauma.

*Case:* A 48-year-old woman came to our hospital on May, 1988. During her thirties, she had experienced gingival swelling. At the age of 40, her right lower gums grew swollen and painful, hampering her ability to chew and eventually prompting her visit.

*Clinical Procedures and Outcomes:* The marginal gingiva were red and swollen. Additionally, numerous periodontal pockets of 4 mm or deeper were observed. Vertical bone loss was observed at the distal of teeth 15, 25, and 34 and at the mesial of teeth 16 and 26. Furcation involvement was present in the molar region. Movement was noted in the maxillary teeth, prompting a diagnosis of generalized severe chronic periodontitis associated with occlusal trauma. Malocclusion was Angle class-II.

Initial preparation was performed to improve her disposition and to stabilize periodontal tissue. After re-examination, periodontal surgery was performed. To change the tooth axis to a normal direction, increase arch length, and enhance occlusal support, MTM was applied, followed by periodontal surgery, including osteoplasty and rehabilitation of masticatory functions. Two years and five months after the first visit, the patient was transitioned to supportive periodontal therapy.

*Conclusion:* After the treatment for severe periodontitis patients depending on individual differences, and to control inflammation are essential for a good outcome over extended periods. In addition to reevaluations of periodontal tissue and maxillomandibular position, appropriate management involves measures monitoring of the occlusal plane and the vertical dimension.

R-06

### **Regenerative procedures for root canal perforations and bone resorption**

Kotaro Tsuchida\*

Tsuchida Dental Office

*Background and objective:* Perforation, which is one of the possible consequences of root canal treatment, may result in bacterial leakage from the root canal, which can lead to alveolar bone destruction and make it difficult to preserve the tooth. Recently, a mineral trioxide aggregate (MTA) has been used for the treatment of perforations that occur during or after endodontic therapy, and excellent outcomes have been observed in clinical settings. However, in the case of a perforation on the labial side of the anterior tooth, the repair using MTA may not be successful in maintaining the esthetic cervical line due to the severity of the bone defect. I report here on both the regenerative procedures for the treatment of bone resorption associated with a perforation on the labial side of the maxillary anterior teeth and the therapeutic course.

*Materials and Methods:* A 37-year-old female. There were two perforations and significant bone resorption on the labial side the tooth. An MTA (Pro root®) was used for perforation repair, and Xenograft (Bio-oss®) and resorbable membrane (Bio-guide®) were used for the regenerative procedures.

*Results:* After 6 years, the inflammation is controlled. There are no gingival recession and esthetic problems.

According to the CBCT, taken five years after these procedures, the regenerative procedures in the labial side maintained the newly formed bone.

*Conclusion:* This case suggests that biocompatibility between the MTA and newly formed bone after regenerative procedures is excellent.

R-07

### One-stage full-mouth disinfection for patients with severe intellectual disability

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**Background and objective:** It is difficult to manage periodontitis in intellectually disabled person, because of limited ability of self-care and maladaptation to dental treatment. Currently, methods of effective periodontal treatment for the disabled has not been established in Japan. We report a case of intensive non-surgical periodontal treatment and thorough supportive periodontal therapy (SPT) implemented for patients with severe intellectual disability.

**Materials and methods:** Patients were siblings with severe intellectual disability; a 28-year-old female and a 24-year-old male. They were not able to perform self-care. Because of severe maladaptive behavior toward dental treatment, non-traumatic physical restraint was necessary. They exhibited poor plaque control and severe gingival inflammation. The clinical diagnosis was generalized aggressive periodontitis. We performed one-stage full-mouth disinfection (Os-FMD) in combination with antimicrobial therapy using azithromycin, under general anesthesia. After the treatment, they were placed under monthly SPT, which included behavior modification.

**Results:** Although tooth brushing instruction was also given to their mother, their level of plaque control showed no improvement. Systemic azithromycin was administered two days before Os-FMD. After Os-FMD their periodontal conditions showed improvement and they have been well maintained for two years.

**Conclusion:** We have demonstrated that Os-FMD combination with antimicrobial therapy is effective in managing the periodontal condition of individuals with intellectual disability and compromised oral hygiene. Careful SPT with short intervals to supplement the home care is essential in order to maintain a good periodontal condition for the disabled.

R-08

### Interdisciplinary dental treatment of aggressive periodontitis after oral antimicrobial therapy

Masaru Shimizu<sup>\*</sup>

Alpha dental clinic, Hiroshima Japan

**Background and objective:** This case report shows the effectiveness of interdisciplinary dental treatment, involving antimicrobial therapy, periodontal regenerative therapy, implant placement and orthodontic therapy, on aggressive periodontitis patient.

**Case:** Age: 33 year old female, non-smoker, healthy

**Chief complaint:** difficulty of mastication

**First visit:** September 2009

**Dental history:** the patient has tooth mobility with occlusal pain from 20 year old.

**Family history:** Grandmother had periodontitis

**Periodontal pocket depth** was from 3 to 12 mm, BOP was 80%, Mobility of molar was second or third degree

**Diagnosis:** Aggressive periodontitis with secondary occlusal trauma

**Clinical Procedures and Outcomes:**

- ① Full mouth disinfection in conjunction with systemically administered Azithromycin (FMD)
- ② Periodontal regenerative therapy
- ③ Implant placement (#16,26)
- ④ Orthodontic therapy
- ⑤ Building shortened dental arch

**Conclusion:** The keys in this case are FMD with Azithromycin, qualitative control of bacteria in periodontal pockets, implant placement as an anchor, regenerative therapy, obtain thick and flat alveolar bone, and continuous follow up.

R-09

### A Case Report of Chronic Periodontitis with Diabetes Treated by Regenerative Therapy

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**Background and objective:** Periodontal disease has been recognized as the sixth complication of diabetes mellitus. Studies have shown that treatment for periodontal disease can improve diabetes, and diabetes treatment improves periodontal condition. We report a case of generalized chronic periodontitis and type 2 diabetes mellitus requiring periodontal treatment including regenerative therapy.

**Case:** The patient was a 66-year-old man who presented with the chief complaint of gingival inflammation and mobile teeth in the molar region. He had been receiving treatment for type 2 diabetes mellitus since 1999. His glycated hemoglobin (HbA1c) level was 7.8%. An initial examination revealed sites with a probing depth of  $\geq 7$  mm in the molar region, and radiography revealed angular bone defects in this area. A clinical diagnosis of generalized chronic periodontitis was made according to the classification of the American Academy of Periodontology (1999).

**Clinical Procedures and Outcomes:** The patient underwent initial periodontal therapy. An improvement was observed in periodontal conditions on reevaluation, and his HbA1c level showed a reduction to 6.9%. Periodontal regenerative therapies with enamel matrix derivative (EMD) were performed in #26 and 27. Autogenous bone graft was applied with EMD in #16. Following another reevaluation, a removable partial denture was fabricated for #47 and the patient placed on supportive periodontal therapy (SPT).

**Conclusion:** The results suggest the potential improvement of HbA1c levels by periodontal therapy, indicating the importance of collaboration between dentist and physician in managing periodontal and diabetic conditions.

R-10

### Multiple procedures (orthodontic treatment, hard and soft tissue management around the implant) were carried out to improve peri-implant environment: a case report

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**Background:** This case report describes improvement of the peri-implant environment by localized orthodontic treatment (LOT), implant with bone augmentation, and free gingival graft (FGG) for a patient with tooth malalignment due to loss of teeth.

**Case:** This 44-year-old male with moderate periodontitis underwent LOT for mesially inclined #37 after initial preparation for periodontitis, and implant placement for the lost part of #36 with simultaneous guided bone regeneration (GBR). Six months after the placement, FGG was performed to increase the peri-implant keratinized mucosa and cover the root surface of the adjacent tooth with gingival recession.

**Clinical Procedures and Outcomes:** Correction of #37 by LOT secured the space for implant placement to #36, and simultaneous GBR was performed due to augment a narrow residual ridge. Since keratinized mucosa was insufficient, FGG was performed to ensure proper oral hygiene. Through these procedures, keratinized mucosa could be obtained around the implant. After 3.5 years the surgery, no peri-implant inflammation is observed, and plaque control remains favorable. In addition, the adjacent tooth sensitivity was diminished by covering the root surface.

**Conclusion:** Peri-implantitis is a multi-factorial disease. To prevent this disease, great caution has to be paid to develop the site adequately with periodontal therapy for residual teeth, orthodontic treatment for alignment and bone augmentation if they are needed. Also superstructure design is essential.

Although the necessity of peri-implant keratinized mucosa remains controversial, periodontally susceptible patients may require keratinized mucosa. However, since the treatment is highly invasive, careful consideration should be paid to avoid overtreatment.

R-11

### **Treatment of a patient with generalized advanced chronic periodontitis utilized periodontal regenerative therapy and localized orthodontic treatment: 7.5- year follow up**

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**Background:** We report a case of a patient who presented with advanced periodontitis. #37 had vertical bony defect which was treated by periodontal regenerative therapy, followed by localized orthodontic treatment (LOT). After active therapy, the patient has been placed in supportive periodontal therapy (SPT) for 7.5 years.

**Treatment:** A 61-year-old male initially visited on February 28, 2006 with the chief complaint of pain on mastication on left maxillary molars. His medical history revealed unremarkable and he had quitted smoking 12 years ago.

Poor oral hygiene, gingival enlargement and redness were observed throughout the whole mouth with plaque control record (PCR): 88.0%, bleeding on probing (BOP): 81.5% and average probing pocket depth (PPD): 4.2 mm. X-ray findings revealed that horizontal bone loss was observed for most of teeth, associated with vertical bone loss for #24, 37 and #38. Also #37 and 38 were mesially inclined.

**Clinical Procedure and Outcomes:** 1. Initial preparation (oral hygiene instruction (OHI), scaling/root planing (Sc/Rp), extraction of #17, 24 and 38), 2. Re-evaluation, 3. Surgical periodontal treatment (open flap debridement (F.op) for #13-16 and #25-27, and enamel matrix derivative application a autogenous bone graft for #37), 4. Re-evaluation, 5. LOT (upright of #37), 6. Implant placement, 7. Re-evaluation, 8. Prosthodontics, 9. SPT (PCR: 8.0%, BOP: 8.0%, average PPD: 3.0 mm).

**Conclusion:** #37 could be successfully treated with periodontal regenerative therapy and LOT. At present, 7.5 years have passed since the patient has placed in SPT, We will continue to provide SPT to maintain his dentition and an implant.

R-12

### **Periodontal Treatment in Chronic Periodontitis Involved Occlusal Trauma: A Case Report**

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**Background and objective:** Excessive occlusal force has been known as the factor enhancing the degree of periodontal destruction of periodontally compromised tooth. Occlusal adjustment as a part of periodontal therapy has been controversial for years. The purpose of this case report is to demonstrate the effects of occlusal adjustment, associated with periodontal therapy, on periodontal parameters.

**Materials and Methods:** A 60-year-old female patient presented with discomfort of #24 and 46. #17 has been extracted 7-8 years ago due to poor prognosis. Class III furcation involvement, mobility class II as well as vertical bone loss were observed on multiple teeth. Patient was diagnosed as localized chronic moderate periodontitis.

**Results:** Following initial therapy, #26 was removed due to poor prognosis, but #27 was preserved and well functioned. The periodontal regeneration, using Enamel Matrix Derivative (EMD) has been applied to vertical bone loss on the mesial of #36 as well as the root separation was performed for the furcation involvement of #46. #48 was transplanted to maxillary right partial edentulous area and #35 and 45 were moved orthodontically for occlusal force distribution. A significant improvement of periodontal condition was achieved after therapy.

**Conclusion:** This case demonstrated the significance of periodontal treatment in conjunction with occlusal adjustment to reduce the progression of periodontal attachment loss. A success to key to treat chronic periodontitis involved occlusal trauma, especially for patients with multiple missing teeth was to adjust occlusion to increase the number of tooth supporting occlusion following inflammation control.

R-13

### **Generalized severe chronic periodontitis treated with regenerative therapy**

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*Background:* In persons with type 2 diabetes mellitus, control of generalized severe periodontitis is considered to be more difficult than that of chronic periodontitis. This is due to the complex etiology of generalized severe periodontitis, which is characterized by poor wound healing and increased susceptibility to infection. In this report, regenerative treatment with an enamel matrix derivative and autologous bone graft was used to control generalized severe periodontitis and regenerate lost periodontal tissues.

*Case:* The patient presented with generalized severe periodontitis, type 2 diabetes mellitus, high blood pressure and hyperlipidemia. Glycemic control was assessed using glycated hemoglobin (HbA1c) concentrations.

*Clinical Procedures and Outcomes:* After evaluation, additional diagnostic measures and consultation, an aggressive treatment regimen was developed to eliminate pathogens, regenerate lost tissues and help maintain oral health. After initial periodontal therapy, periodontal lesions were treated with regenerative therapy using an enamel matrix derivative and autologous bone graft. Patient response was favorable, marked by an elimination of the multiple osseous defects. This result was maintained for 2 years.

*Conclusion:* This case report presents the successful management of a 58-year-old male with type 2 diabetes mellitus and generalized severe periodontitis. The treatment approach combined a regenerative technique employing an enamel matrix derivative with conventional therapies. Through effective maintenance approaches, favourable results have persisted for 2 years.

R-14

### **Treatment for Moderate chronic periodontitis with occlusal trauma**

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*Background:* Traumatic occlusion is one of the risk factors for the progression of periodontal disease. In this report occlusal treatments, *i.e.*, occlusal adjustment and instruction to stop bruxism were provided to a moderate periodontitis patient at the time of the initial treatment. The patient received conventional periodontal treatment including periodontal surgery, and successful results were obtained in this case report.

*Case:* 39 year old female. Chief complaint of "mobile back teeth". She came to our clinic because her general dentist pointed out the progression of periodontitis. Oral hygiene status was good. Moderate swelling and redness of gingiva were noted. In the upper molar area, more than 6 mm of probing pocket depths were observed. Vertical bone defects were noted in the upper molars and upper incisors.

*Diagnosis:* Moderate chronic periodontitis; Occlusal traumatism

*Clinical Procedures and Outcomes:* Occlusal interferences during anterior/lateral jaw movement were adjusted, and fremitus was removed. After the reevaluation, flap surgery was performed to the area with the pocket depth of more than 5 mm. Periodontal condition was reevaluated 6 months after the surgery, and it revealed that the mobility of the teeth reduced from grade 2 to grade 1 by the treatment. It was also confirmed that the periodontal pocket depth became less than 4 mm after the treatment. The patient was put in the supportive periodontal therapy.

*Conclusion:* Achievement of good oral hygiene with definitive occlusal treatment at the time of the initial treatment showed successful treatment result.

R-15

### Treatment of severe periodontal defects with EMD

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*Background and objective:* We report regenerative procedure for severe periodontal defects with enamel matrix derivative (EMD)

*Materials and Methods:* A 38-year old female complained of severe pain of gum on the lower anterior teeth. The patient is in excellent health and reports no history of any systemic disease or any problem, which would preclude dental treatment. Tooth #5, #12, #21, #28 are missing due to the orthodontic treatment from in the past. Upon perio-examination of the patient, we formulated a diagnosis of generalized severe periodontitis. Pocket depth ranging from 3 to 9 mm with some gingival recession. Radiographically, we observed severe horizontal and vertical bone loss around all teeth. After obtaining informed consent and phase 1 therapy, we began a regimen of regenerative treatment with EMD. Prior to the regenerative treatment, mobile tooth were splinted.

*Results:* Pocket depth ranging from 2 to 4 mm with some additional gingival recession. Some clinical attachment gain found most of defective sites.

*Conclusion:* Regenerative therapy with EMD might be an effective treatment for severe periodontal defects.

R-16

### A case report of Periodontal Therapy for moderate chronic periodontitis with Occlusal Trauma

Hiroyuki Suzuki\*

Soar-bld. dental clinic

*Background:* Persistent inflammation, often due to bacteria, destroys periodontal tissue and is accelerated by trauma. Inflammation suppression by plaque control, easy-to-clean periodontal environment, and bite force dispersion maintain and stabilize the periodontal tissue.

*Case:* A 45-year-old female presenting with a chief complaint of pain in the upper-left teeth was diagnosed with moderate chronic periodontitis with occlusal trauma and underwent periodontal and orthodontic treatment. At the initial visit, probing depth was  $\geq 6$  mm for #16, 17, 26, 27, 36, 37, 46 and 47; tooth mobility was Grade I; and vertical bone resorption reaching to the root tip and widening of periodontal space were observed by X-ray. Moreover, #36 and #46 had Grade II and Grade III furcation area lesions, respectively.

*Clinical Procedures and Outcomes:* Plaque control, scaling and root planing of the entire mouth, and gingival flap operation of #14-17, 25-27 and 35-37 were followed by orthodontic treatment. Apically positioned flap surgery including surgical procedure of the bone was conducted for #46. The periodontal tissue was improved by prosthesis of #13-23, 25-27, 35, 36 and 46; therefore, treatment was changed to SPT.

*Conclusion:* The inflammation was improved by the periodontal treatment, and the anterior guidance was ensured by anterior teeth prosthesis after the orthodontic treatment. Moreover, the bone level was planarized by the root separation of #46, and an easy-to-control oral environment was established. Inflammation control, confirmation of the occlusal relationship, and continuous SPT should be implemented in the future.



R-17

### Osteonecrosis of the jaw associated with oral bisphosphonate and implant

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**Background:** Since there are few reports about medication-related osteonecrosis of the jaw (MRONJ) associated with oral bisphosphonate (BP) and dental implant removal, the non-exposed osteonecrosis (NE-MRONJ) for such a case was reported.

**Case: Clinical procedure and outcomes:** An 83-year-old male received twelve implants from 2005 to 2007, including one at the position of 17 in June 2007, at a private practice in Japan. Installation of the superstructures was completed in March 2008. BP (Fosamax<sup>®</sup>) had been administrated orally for osteoporosis treatment since November 2006 till July 2011.

Although the implants had functioned uneventfully, the 17-implant observed mobility was removed with small amount of bony tissue in October 2010. A cavity was observed in the removal site, seven weeks after the loss, with  $\phi$  1mm of the entrance,  $\phi$  3-4mm wide, 10mm deep, and roughness like bone surface inside, without any sensation. Infectious signs, such as suppuration, were observed 13 weeks after the loss. The cavity had remained with a little change at least for 10 months after the loss.

The retrospective radiological observation revealed that sequestered dense woven bone expanded from the mesial site of the implant in Oct 2007 to the all-around in Oct 2010, and that unremodeled bone had been persistent after the implant removal. We, therefore, diagnosed this case as NE-MRONJ.

**Conclusion:** Although MRONJ is reported a rare complication with the risk of dose/time dependent, this case reported that even oral-low-dose administration of BP for the relatively short duration caused NE-MRONJ associated with dental implant removal.

R-18

### A case report of guided bone regeneration therapy for generalized chronic periodontitis

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OZAKI DENTAL OFFICE

**Background and objective:** I present here a comparative report of clinical outcome of guided bone regeneration (GBR) therapy at 1-and 2-year follow-up points for a patient diagnosed with generalized moderate chronic periodontitis with occlusal trauma. A 53-year-old man came to our hospital on February 21, 2013 presenting with the chief complaints of gingival swelling, pain, and tooth mobility for tooth 41. Periodontal probing depths were <3 mm (54.8%), 4-5 mm (22.1%), and >6 mm (23.1%). Vertical osseous defects were observed in the areas of teeth 36 and 45. Premature contact was observed for teeth 27 and 36.

**Clinical Procedures:** The patient underwent the following course of treatment: 1) basic periodontal therapy; 2) re-examination; 3) GBR therapy (for teeth 36 and 45) using EMDOGAIN<sup>®</sup> and Bio-Oss<sup>®</sup>; 4) implant treatment for tooth 46; 5) rehabilitation of oral function; 6) re-examination; and 7) SPT

**Results:** The periodontal tissue of tooth 41, for which the patient had reported the chief complaints were stabilized. GBR therapy applied to teeth 36 and 45 for vertical osseous defects improved the stability of the corresponding alveolar bone.

**Conclusion:** Basic periodontal therapy improved the condition of the periodontal tissue, premature contact was eliminated, and occlusal support was established at the right and left molar regions to allow anterior guidance to be achieved, thereby stabilizing the periodontal tissue throughout the jaw. We believe that the most important factors in this case were the cleaning and occlusal examination and adjustment performed at SPT every 4 months, and maintaining patient motivation to perform daily home care.



R-19

### Non-surgical management of drug-induced gingival hyperplasia: a case report

Satoshi Ozaki\*

Ozaki Dental Clinic

*Case:* A 55-year-old woman visited us with the main complaint of gingival swelling and bleeding in July 2011. In 2009, she had experienced subarachnoid bleeding and had been taking a calcium antagonist (ADALAT) since then. Bleeding on Probing (+) 72%, 4 mm  $\geq$  periodontal pocket (62%), and significant swelling of gingiva throughout both jaws were noted.

Moreover, a prosthetic appliance was installed in the maxillary connecting from #17 through to #24. This installation was suspected as the cause of dislocation of the lower jaw to the left.

*Diagnosis:* Localized severe chronic periodontitis associated with drug-induced gingival hyperplasia

*Treatment strategy:* Through basic treatment for the periodontal area including a change of medication, inflammation and swelling were expected to be reduced. For the lower jaw, a stabilization splint was implanted to maintain a relaxed position and to re-constitute articulation at a stable jaw position.

*Clinical Procedures and Outcomes:* A standard periodontal treatment was provided. As a result, without medication change, the gingiva contracted with only plaque control, and the periodontal pocket reduced to 3 mm or less throughout the jaw.

Thereafter, the jaw position was evaluated and articulation adjustment was repeated. The jaw position changed and the upper anterior teeth with fragile periodontal tissue demonstrated significant improvement on dental X-ray.

Prosthesis treatment was provided to the entire jaw. Three years after the shift to supportive periodontal therapy, periodontal tissue was stable.

*Conclusion:* This case suggested that through extensive plaque control, drug-induced gingival hyperplasia could be improved without medication change. Moreover, the dislocated jaws might have accelerated the destruction of the periodontal tissue.

R-20

### Esthetic correction of maxillary anterior ridge deformity, caused by failed implant

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When peri-implantitis becomes advanced, removal of implants is needed. However, it causes loss of peri-implant tissue. Especially if this is the case in anterior esthetic zone, it creates major esthetic problem. In this case presentation, a failed implant in maxillary incisor area was removed and its esthetic and functional problem has been recovered with alveolar ridge augmentation, utilized connective tissue graft.

*Case:* Patient: 55-year-old female, First visit: June, 2012, Chief complaint: discharge of pus from peri-implant pocket of #21. Radiographic finding: #21 implant was located far apically from the alveolar ridge with significant resorption of the surrounding bone.

*Diagnosis:* #21 Peri-implantitis, generalized moderate chronic periodontitis,

*Treatment plan:*

1) Initial preparation (OHI, scaling/root-planing), #21: implant removal, 2) re-evaluation, 3) alveolar ridge augmentation with connective tissue graft, 4) re-evaluation, 5) occlusal functional recovery treatment, 6) SPT

*Clinical procedures and outcome:* After initial preparation, we decided to remove #21 implant, caused tremendous amount of loss of tissue in terms of both apico-coronal and labio-palatal dimension (i.e. Seibert Class III). We performed a combination onlay-interpositional graft to recover the ridge both esthetically and functionally. After it was healed, fixed partial denture has been placed. Three years post-operation, the periodontal tissue has been well maintained.

*Conclusion:* The implant removal, which had been placed far apically, caused major ridge deformity (Seibert Class III) in maxillary anterior area. Surgical correction of the Seibert Class III ridge defects is still a clinical challenge, however, we were able to obtain good result with a single connective tissue graft.

R-21

### Treatment with EMD in conjunction with bovine bone grafts

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**Background:** Although several reports have investigated the clinical effectiveness of enamel matrix derivative (EMD) in conjunction with bone grafts in the treatment of intrabony defects, this is not an established approach. We report 3 cases of large intrabony defects that showed good prognosis after treatment with EMD in conjunction with bovine porous bone mineral (BPBM).

**Case:** Case 1: A 54-year-old woman received treatment for the medial aspect of #3. Baseline probing depth (PD) was 10 mm, attachment loss (AL) was 11 mm, bone defect angle (BDA) was 40° and class II furcation involvement was noted. Case 2: A 47-year-old woman received treatment for the distal aspect of #18. Baseline PD was 12 mm, AL was 12 mm and BDA was 40°. Case 3: A 47-year-old woman received treatment for the distal aspect of #25. Baseline PD was 7 mm, AL was 7 mm, and BDA was 45°.

**Clinical Procedures and Outcomes:** After initial preparation, periodontal regenerative therapy using EMD in conjunction with BPBM was performed. Case 1: The MMPT incision technique and horizontal cross-mattress sutures and 2 simple sutures were used. Case 2: Distal wedge lined incision and simple sutures were used. Case 3: The SPPF incision technique and modified vertical mattress sutures were used.

At the time of re-evaluation, PD and CALgain were 4, 3, and 2 mm and 6, 7, and 5 mm, respectively.

**Conclusion:** The combined use of EMD and BPBM may produce good clinical improvement in the treatment of large intrabony defects.

R-22

### A case of localized severe chronic periodontitis

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**Background:** Patients with severe periodontitis often struggle to restore and stabilize their periodontal tissue to a state in which management by supportive periodontal therapy (SPT) can be continued. We report a case of a patient with severe periodontitis in whom periodontal tissue was successfully stabilized.

**Case:** The patient was a 28-year-old woman with a history of smoking. Her condition was initially diagnosed in February 2010, with chief complaints of gingival swelling and bleeding during brushing. The patient had not visited a dentist in 10 years.

The patient was confirmed to have marked localized progression of periodontal disease. Observation of a large amount of subgingival tartar deposits led to a diagnosis of localized severe chronic periodontitis.

**Treatment and Results:** We first waited before performing basic periodontal therapy. A clenching habit was noted at this time, for which autosuggestion was encouraged as a treatment for para-function. As a result, recovery was seen at sites with a deep probing depth on probing and X-ray examinations. To achieve further recovery, periodontal surgery was performed in the molar region, where probing depths remained. Thereafter, stabilization of periodontal tissue was observed, despite residual deep probing depths. The patient was subsequently switched to SPT.

**Conclusions:** The good results in the present case were likely the result of the patient's young age, the good responsiveness of the periodontal tissue to removal of bacterial factors and associated improvement in the patient's motivation, as well as the patient's cooperation in her treatment.

R-23

### Treatment of periodontal intrabony defects and furcation involvement in molars

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Nakano Station South Dental Clinic

*Objective:* This poster discusses a case where intrabony defect and furcation involvement in molars were treated by initial preparation, root separation, root extraction and periodontal regeneration.

*Patient:* A 48-year-old male visited our clinic in August 2014 because of pain in the upper left teeth that had been causing difficulty in eating. He smoked 10–15 cigarettes a day and had not been to a dentist for a decade.

*Examination:* The percentage of sites with PPD  $\geq$  4mm was 32.1%. The percentage of BOP was 46.9%. Vertical bone defects on molars were found. The pain was coming from 26 where PPD was 10mm with grade 2 mobility. PPD was 10mm at the distal of 17 with grade 3 furcation involvement. PPD at the center of 37 was 12mm, with grade 3 furcation involvement. Both 24 and 25 had 12mm PPD with grade 2 mobility.

*Treatment plan:* initial preparation with plaque control, root canal treatment, natural shifting of loose teeth, periodontal surgical treatment, temporary splint, provisional restoration, prosthetic treatment and SPT

*Treatment:* Initial preparation including plaque control was performed. The distal root of 17 was extracted. Root separation of 37 was conducted. Two wall bone defect (26 distal and mesial) and one-three wall bone defect (27 mesial) were regenerated with emdogain®. SPT was introduced when the recovery of the tissues was confirmed.

*Conclusion:* Bone resorption in molars was confirmed. Periodontal tissues had been recovered by having 27 and 28 naturally shift and regenerating the defects. SPT is necessary to monitor the prognosis.

R-24

### Case report: Extensive severe chronic periodontitis in a diabetes patient

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*Objective:* We report a case of improved periodontal tissues in a diabetic patient with extensive severe chronic periodontitis through basic periodontal therapy to improve his lifestyle habits.

*Materials and Methods:* A 54-year-old male patient first visited in November 2011.

Chief complaint: Bleeding from the alveolar ridge

History: Diabetes

Dental history: Visited our clinic 10 years ago

*Exploratory/test findings:* Ten years ago, the patient developed diabetes, which remained untreated until today. His oral cavity was unclean with reddened and swollen gingiva, deep probing depth, and many mobile teeth throughout the jaw. Dental X-ray showed loss of alveolar crest lamina dura, bone absorption reaching the apex of the tooth root, and vertical bone defects in many teeth. Although no abnormal findings were observed in the temporomandibular joint, flare out of maxillary anterior teeth, and minimal intercuspal positioning with the left molar region were confirmed, suggesting difficulty with strong biting.

*Diagnosis:* Extensive severe chronic periodontitis

*Therapeutic plan:* 1) basic periodontal treatment, 2) preparation of therapeutic dentures, 3) reevaluation, 4) prosthesis treatment, 5) reevaluation, 6) supportive periodontal therapy (SPT)

*Results:* Dental X-ray in SPT showed clear alveolar crest lamina dura, trabecular bone, and no enlarged periodontal ligament space, suggesting stable periodontal tissues and occlusion. However, HbA1c increased recently from 6.5 to 8.5, suggesting unstable diabetes and the potential for periodontitis recurrence.

*Conclusion:* At present the patient visits us once every 4 months for maintenance. In future, the physician should promote aggressive diabetes treatment, and we will continue to support the patient with plaque control and maintenance of healthy lifestyle habits.

R-25

### Follow-up of patient with generalized severe chronic periodontitis over a 21-year period

Mitsutaka Funakoshi\*

Funakoshi Dental Office

*Background:* We report herein on a 21-year follow-up of a case of generalized severe chronic periodontitis with furcation involvement, which was treated with supportive periodontal therapy (SPT) after initial periodontal and prosthetic therapies.

*Case:* A 46-year-old woman December 1, 1995 presenting with a complaint of loose back teeth. History of the present illness: The patient had visited another hospital due to a one-year history of movement in the molar region and was informed that five teeth needed to be removed. Prompted by her anxiety about this recommendation, she came to our hospital after being referred by her acquaintance.

Inflammation of the marginal gingiva, deep periodontal pockets, interproximal open contacts, tooth motility and furcation involvement were observed throughout the jaw.

*Diagnosis:* Generalized severe chronic periodontitis.

*Treatment plan:* The following procedures were performed: 1) basic periodontal therapy; 2) re-examination; 3) periodontal surgery; 4) re-examination; 5) rehabilitation of oral function; 6) re-examination; 7) SPT.

*Treatment Procedures and Outcomes:* Following the re-examination, the patient underwent periodontal surgery, including removal of the root. A final prosthesis was subsequently delivered to establish stable occlusion and to stabilize the periodontal tissue, after which the patient was again transitioned to SPT.

*Conclusion:* In the present case, reconstruction of occlusion improved strength control and the periodontal environment. Additionally, the patient's improved dietary habits, thorough plaque control, and SPT have continuously suppressed gingival inflammation, thereby stabilizing periodontal tissue, including the furcation region, for a significant period (21 years).

R-26

### A Case Report; 14 years after treatment of chronic periodontitis, which has a number of short-root teeth

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*Background and objective:* It report about a case of periodontal treatment, localized orthodontic treatment, prosthetic treatment, performs maintenance, and on the case was held for 14 years to generalized chronic periodontitis patients with a lot of short root teeth.

*Case:* Patient; 50-year-old, male, Non-smoker First visit; 2000 July 15th Chief complaint; Smarting in the lower right back teeth both cold things and warm things

Problems; ① Deep periodontal pockets and bleeding ② Significant tartar deposits ③ Canine relationship Class II both left and right ④ Many short root teeth

Diagnosis; Generalized chronic periodontitis with secondary occlusal trauma

*Clinical procedures and Outcomes:* ① upper left molars; #12 tooth extraction, #14 palatal root resection, and resective therapy

② lower right molars; #30 distal root resection and resective therapy, localized orthodontic treatment for #30 mesial root

③ lower left molars; regenerative therapy (GTR and bonegraft)

#14 buccal root was extracted by poor prognosis, and bridge were set to left upper, right upper, and left lower.

*Conclusion:* This case was appeared to be a large influence of occlusal trauma cause have many short-root teeth. It has gotten a relatively good course by firmly carry out the management of the occlusion from the basic treatment to maintenance. In addition, the prognosis of reliably performed resective therapy seems to be good. I conducted a careful maintenance without missing the subtle changes in the oral cavity future.

R-27

### Aggressive Periodontitis Associated with Palmoplantar Pustulos

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**Background and objective:** Palmoplantar pustulosis (PPP) is a skin disease characterized by pustule formation, erythema, and scaling of the affected skin of the palms and soles. It has been suggested that PPP is caused by metal allergy and focal infection of tonsils and/or periodontium. However, its etiology remains unclear. The aim of this study is to highlight the pathogenesis of a patient with generalized aggressive periodontitis (gAp) and PPP from the standpoint of periodontal medicine and comprehensive periodontal treatment performed.

**Materials and Methods:** A 36-year-old man who had been suffering from gAp and PPP. He has not been suffered from tonsillitis and no metal restoration was done in his mouth. We have examined 1) presence of putative periodontal bacteria by 16S rRNA-based polymerase chain reaction, 2) inflammatory markers and 3) serological type of HLA class II antigens. Comprehensive treatments including periodontal, orthodontic and dental implant were successfully performed.

**Results:** Mean pocket depth, Schei's bone loss index and gingival index were 5.2 mm, 57 % and 1.7, respectively. *Bacteroides forsythus* was detected from many sites, while neither *Porphyromonas gingivalis* nor *Actinobacillus actinomycetemcomitans* was detected at all. High values of CRP,  $\alpha$ -1 anti-trypsin, C3, IgG and IgA but no autoantibodies in serum were found. HLA-A11, A24, B52, B62, HLA-DR4, 15, DQ1, 3 were detected. Although periodontal treatment was successfully performed, the values of inflammatory markers and clinical findings of this patient were not improved.

**Conclusion:** Further studies focusing the association of periodontal infection with refractory dermal diseases including PPP will be required.

R-28

### 15 years follow-up of patient with generalized severe chronic periodontitis

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IKEDA DENTAL OFFICE

**Background and Objective:** We report herein on a 15-year follow-up of a case of generalized severe chronic periodontitis with furcation involvement, which was treated with supportive periodontal therapy (SPT) after periodontal regeneration and prosthetic therapies.

**Case:** A 38-year-old woman May 9, 1998 presenting with a complaint of loose back teeth and back teeth of the gingiva is swollen many times. History of the present illness: The patient noticed that the gum is swollen from two years ago. She came been introduced by her friend to our office.

Inflammation of the marginal gingiva, deep periodontal pockets, interproximal open contacts, tooth motility and furcation involvement were observed throughout the jaw.

**Diagnosis:** Generalized severe chronic periodontitis.

**Treatment plan:** The following procedures were performed: 1) basic periodontal therapy; 2) re-examination; 3) periodontal surgery including regeneration therapy; 4) re-examination; 5) rehabilitation of oral function; 6) re-examination; 7) SPT.

**Treatment Procedures and Outcomes:** Following the re-examination, the patient underwent periodontal surgery, including periodontal regeneration therapies and removal of the roots. A final prosthesis was subsequently delivered to establish stable occlusion and to stabilize the periodontal tissue, after which the patient was again transitioned to SPT.

**Conclusions:** In the present case, reconstruction of occlusion improved strength control and the periodontal environment. Additionally, the patient's improved thorough plaque control, and SPT have continuously suppressed gingival inflammation, thereby stabilizing periodontal tissue, including the furcation region, for a significant period (15 years).

R-29

### Five-year prognosis of a patient with malocclusion who underwent comprehensive treatment for severe periodontitis

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**Background:** This was a case of a patient with severe periodontitis who exhibited many infrabony defects in the molar region due to malocclusion-induced trauma. Herein, we report the 5-year prognosis of the patient after periodontal regenerative and comprehensive orthodontic therapies for functional recovery with implant prosthodontics.

**Case:** A 54-year-old female patient presented with the chief complaint of masticatory disturbance. In the molar region, significant tooth mobility, a deep periodontal pocket (10 mm), and infrabony defects were observed. She had lost frontal teeth occlusion due to significant maxillary protrusion. Malocclusion could be an exacerbating factor of infrabony defects. The diagnosis was generalized severe chronic periodontitis and malocclusion.

**Clinical Procedure and Outcomes:** After initial preparation, we performed periodontal regenerative therapy for the mandibular molar. We placed the implants after using a setup model to determine the maxillary molar position that would ensure appropriate frontal teeth occlusion after orthodontic treatment. Comprehensive orthodontic treatment was subsequently performed with the implants used as the anchoring unit. After the orthodontic treatment, a definitive surgical procedure was performed for the mandibular molar in order to place the final prosthesis. Consequently, the alveolar bone developed in a normal physiological form, and high cleansability was achieved due to the shallow gingival sulci. Stable occlusion was established with anterior guidance. A favorable prognosis has been maintained up to 5 years after the treatment.

**Conclusion:** Comprehensive treatment can enable stable occlusion and establishment of periodontal tissue and peri-implant tissues with high cleansability, even in patients with severe periodontitis and malocclusion. Long-term favorable prognosis can be expected.

R-30

### Treatment strategies for periodontal regenerative therapy of non-contained intrabony defect utilizing CBCT

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Ono Dental Clinic

**Background:** The indication of periodontal regenerative therapy for 1-wall or dehiscence intrabony defect remains difficult, and can lead to undesirable results even when therapy has been provided, i.e., complications of interdental papilla necrosis, and unsuccessful periodontal regeneration. We performed surgery after close preoperative examination with CBCT and discussed the surgical methods. Herein, we report a case in which a favorable prognosis was achieved after re-entry surgery to confirm bone regeneration.

**Case:** The female patient was 62 years old at her first visit, and did not smoke. She was diagnosed as having moderate to severe chronic periodontitis in the full mouth. Vertical bone defect and a furcation involvement were confirmed around the mesial root of tooth No.30. The patient provided informed consent for the utilization of the unapproved material described herein.

**Clinical Procedures and Outcomes:** Based on the CBCT images, it was estimated that the complete debridement was possible even into the degree 2 furcation area of tooth No.30. It was planned to make an incision in the interdental papilla on the buccal side. Since the bone defect was 1-wall type, i.e., a non-contained lesion, we determined to use EMD, autogenous bone, FDBA, and an absorbable membrane. When re-entry surgery was performed 1 year after the regenerative therapy, the bone defect was almost completely repaired with bone-like hard tissues. The patient remains under follow-up observation after final prosthesis.

**Conclusion:** Close preoperative examination with CBCT could enable successful periodontal regenerative therapy.





# Dental Hygiene



H-01

## The potential of periodontal treatment for improving problematic behavior of autistic spectrum patient

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**Background:** The patients with autistic spectrum disorder (ASD) often have difficulty in expressing oral and systemic symptoms or problems. Therefore, oral problems may significantly influence their behaviors and the quality of life (QoL) including emotional aspect. We report a case of chronic periodontitis and ASD with problematic behavior requiring periodontal treatment.

**Case:** A patient was a 34-year-old woman with ASD and severe intellectual disability, who presented with the chief complaint of gingival swelling of the lower right quadrant. She displayed symptoms and problematic behaviors including speech disappearance, compulsive hair-pulling, decrease in variation of diet and not being able to go to her care facility. She received medical examination such as brain CT, but no definite cause was identified. She had deep periodontal pockets and was diagnosed as having generalized severe chronic periodontitis.

**Clinical Procedures and Outcomes:** We performed one-stage full-mouth disinfection (Os-FMD) in combination with antimicrobial therapy using azithromycin under general anesthesia. Following Os-FMD, we performed monthly supportive periodontal therapy with behavior control method. Problematic behaviors related to oral function disappeared two weeks after the Os-FMD, and other problematic behaviors disappeared after two months. Results from interviews with their family members and caregivers suggest an improvement in QoL.

**Conclusion:** In this case of chronic periodontitis and ASD, an improvement was observed, following periodontal treatment, not only in periodontal condition but in problematic behaviors related to oral function. It is also suggested that periodontal treatment may enhance QoL of such patients.

H-02

## The Efficacy of Non-Surgical Treatment on Severe Gingival Recession

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Ikeda Dental Clinic

**Background and objective:** Severe gingival recession leads to root exposure that causes various problems such as caries, hypersensitivity and aesthetic problems. Normally severe gingival recession is an indication of periodontal surgery. However, using non-surgical methods, we improved Miller class II gingival recession and obtained favorable outcomes. We report on two severe gingival recession cases with follow-up periods ranging from 22 to 24 years.

**Materials and Methods:** Two patients were included in this study (One male, aged 31; and one female, aged 22). They had Miller class II recession defects on the labial surfaces of the anterior teeth (41,43). In these areas, attached gingivae were lost and slight inflammation was observed. Pocket depths were 2-3mm. Patients were instructed in an oral hygiene technique involving wiping plaque once a day from the surfaces of the teeth using toothpicks wrapped with cotton. Patients were instructed to discontinue brushing.

**Results:** Gingival creeping was found on the labial surfaces of the two teeth approximately two years after the first examination. Despite the fact that they had started regular brushing methods after gingival creeping had been found, they did not have recurrence of gingival recession. The effects of the treatment has been maintained for periods of 20 to 21 years.

**Conclusion:** The severe gingival recession of two patients improved remarkably by a method of plaque control that does not exert excess pressure on the gingiva. It is suggested that non-surgical treatment using this method is treatment option for gingival recession.

## H-03

**A case of chronic periodontitis improved by comprehensive periodontal therapy**Masako Sakai<sup>\*1</sup>, Koichi Ito<sup>2</sup><sup>1</sup>Dental Hygienist Section, Nihon University School of Dentistry Dental Hospital, <sup>2</sup>Nihon University

*Background:* We report a long-term case (29 years) of chronic periodontitis that was improved by comprehensive periodontal therapy.

*Case:* A 39-year-old female presented with the chief complaint of painful, swollen gums. Clinical examination at the first visit revealed 49% of sites with a probing depth  $\geq 4$ mm and 74% of sites with bleeding on probing. Plaque control record was 85%.

*Clinical Procedures and Outcomes:* Basic periodontal treatment was implemented based on a clinical diagnosis of severe chronic periodontitis. The patient was instructed to brush three times daily using the Bass method and an interdental toothbrush. Surgical periodontal treatment was subsequently performed at selected probing depths over 4mm. Occlusal adjustment and perio-orthodontic treatment was carried out as oral rehabilitation. We recalled the patient every 4 months.

Clinical examination at the re-evaluation of all sites with a probing depth 1~3mm showed no bleeding on probing and plaque control records were 3~6%.

*Conclusion:* Early extraction of hopeless teeth and comprehensive periodontal treatment for remaining teeth made it easy to manage the periodontal tissue. During 29 years of supportive periodontal therapy, the periodontal condition remained uneventful in most of the teeth. Therefore, it is important to create an adequate periodontal environment for the care of healed periodontal tissue by patients and dental hygienists before supportive periodontal therapy.

## H-04

**Case report: supportive periodontal therapy for severe localized periodontitis**Akina Tsuchikura<sup>\*1</sup>, Chiharu Kawaguchi<sup>1</sup>, Toshiaki Shibutani<sup>2</sup><sup>1</sup>Division of oral hygiene in Asahi University Hospital, <sup>2</sup>Department of Periodontology, Division of Oral Infection and Disease, Asahi University School of Dentistry

*Case:* The patient, a 40-year-old female, presented complaining of pus discharge from the lower anterior teeth. Periodontal examination revealed gingival recession, tooth mobility in lower anterior lesion; 21.1% of sites bled on probing, 21.1% of the sites had a periodontal pocket depth  $\geq 4$  mm. In particular #32 and #41 exhibited a probing pocket depths of 7mm. There was grade II mobility in #32, and grade I in #41. Based on the clinical and radiographic findings, the patient was diagnosed with localized chronic periodontitis.

*Clinical procedure and outcome:* Periodontal treatment was initiated with mechanical therapy; including systematic scaling and planning of all accessible root surfaces and the introduction of meticulous oral hygiene. After the thorough initial phase of mechanical therapy, the patient was motivated to achieve better plaque control. Flap surgery was performed including the application of enamel matrix protein to limited lesions. Reevaluation revealed decreased sites of bleeding on probing, plaque control and improvement in the probing pocket depth. A postoperative radiograph 12 months later showed significant bone formation. However, the gingiva between the interdental embrasure is shaped irregularly, which requires careful self-care and professional treatment. The patient was put on regular recall appointments for supportive periodontal therapy. The oral hygiene maintenance and compliance of the patient was excellent, and there were no signs of recurrence of the disease throughout the maintenance period of more than two years.

*Conclusion:* This case suggests that collaboration between the patient and dental professionals can maintain irregular gingival shape conditions.

H-05

**A case report of generalized chronic periodontitis evaluated by PRA**

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*Background and objective:* The primary etiology of the periodontal disease is a bacterial plaque. Various risk factors, however, may give significant influence on the disease progression. PRA (Periodontal risk assessment) is one of the tool to evaluate the risk of the progression. This study reports a case of generalized chronic periodontitis which underwent periodontal treatment followed by periodontal maintenance and the risk was evaluated utilizing PRA.

*Materials and Methods:* The patient is a 58 years old, Asian male. The smoking status was current smoker. The diagnosis is generalized severe chronic periodontitis. First, OHI was performed utilizing PRA. Then, non-surgical therapy was performed. After that, regenerative therapies were applied to five sites where deep periodontal pockets remained. Finally, every 3-month periodontal maintenance was regularly done for 5 years.

*Results:* As a result of OHI, smoking cessation and definitive treatment with the regenerative therapy, the periodontal condition was significantly improved. Followed by every 3-month periodontal maintenance, the periodontal condition at 5 years post surgery was more stable. PRA was used to assess the risk at the time of the initial examination then it was high. Even after non-surgical therapy, the risk was still high therefore regenerative therapy as a definitive treatment was chosen. After surgical treatment, the risk changed to medium and it was maintained well at the periodontal maintenance even for five years.

*Conclusion:* Long-term success was achieved by the periodontal treatment based on the risk assessment. PRA may help to evaluate and motivate a patient and make proper treatment planning.

H-06

**A case report of localized aggressive periodontitis followed up for 5 years**

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*Background and objective:* Management of furcation involvement is challenging. Management of aggressive periodontitis is challenging as well. This case report is focused on prognosis of class3 furcation involvement of maxillary molar of aggressive periodontitis patient who exhibited excellent oral hygiene status.

*Materials and Methods:* The patient is 43 years old Asian male, former smoker over 10years ago. Clinical findings suggested generalized deep periodontal pocket depth and localized radiographic vertical bone loss. Also, class3 furcation involvement was found on #26. After phase 1 therapy, regenerative therapy was performed to improve vertical bone loss and furcation involvement. After that, stable periodontal condition was confirmed then periodontal maintenance was done regularly at every 3month.

*Results:* The class 3 furcation involvement on #26 was present even after periodontal regenerative therapy and vertical bone loss was improved. After the surgical therapy, every 3 month periodontal maintenance was performed in order to maintain residual pockets. The patient's oral hygiene status has been improved then PCR significantly changed to less than 10%, which was maintained at 7 years post the surgery and it was originally 40% at the initial examination. Class 3 furcation involvement on #26 has been maintained by the careful self- brushing and subgingival ultrasonic instrumentation at periodontal maintenance.

*Conclusion:* Class 3 furcation involvement of aggressive periodontitis is expected to have poor prognosis because the patient is genetically susceptible to the periodontal disease. It may be however possible to achieve long-term success by the surgical approach and excellent plaque control level.

H-07

### Systemic Antibiotics and Regenerative Therapy for Generalized Severe Chronic Periodontitis

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Mizukami Dental Clinic

**Background:** A case with generalized severe chronic periodontitis was dramatically improved by medication of azithromycin accompanied by the single-time full-mouth scaling and root planning (FM-SRP) followed by periodontal regenerative therapy. The good prognosis in the maintenance phase is reported.

**Case:** A 51 year-old female patient presented on 2008/9/2 with the chief complaint of pain at #36 (mandibular left first molar). She was not a smoker without any particular medical history. Clinical and radiographic examinations revealed generalized gingivitis with periodontal abscess at #36 and severe bone loss. Original data were PCR 70.1%, 75.9% of deeper pockets than 4mm, BOP 100%, and suppuration upon probing on multiple sites. *P.gingivalis*, *A.actinomycetemcomitans*, *T.forsythia*, *P.intermedia*, and *T.denticola* were found by PCR-Invaser bacterial test. Diagnosis was generalized severe chronic periodontitis.

**Clinical Procedures and Outcomes:**

**Clinical Procedures:**

Initial periodontal treatment such as oral hygiene instructions, scaling, medication of azithromycin with single-time FM-SRP, and hopeless-teeth extraction were performed. Regenerative therapy before and after re-examinations, the final restoration and maintenance were applied.

**Outcomes:** After the FM-SRP, previously detected 5 bacteria were not detected and data after treatment were as follows; PCR 9%, PPD>4mm 0%, BOP 0% and bone defect showed notable radiographic improvement. Maintenance therapy was performed every 2 months. Latest data in January 2015 were PCR 6.8%, PPD>4mm 0% and BOP was 0%.

**Conclusion:** Patient's motivation and treatment efficacy have been increased by the rapid remission of inflammation because of the single-time FM-SRP with systemic azithromycin medication in the initial treatment phase.

H-08

### Management of a patient with chronic periodontal and peri-implant disease: A case report

Yuki Enomoto\*, Yukari Hashizaki, Masahiko Nikaido

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**Background and objective:** Not only periodontal diseases, but also peri-implant diseases are becoming major issues in our daily practice. In this presentation, we report the patient, who had both periodontal and peri-implant disease.

**Case:** 63 year-old female patient referred from a general practitioner to us. She had had swollen gingiva frequently, although she had been seeing by a dentist and a dental hygienist every 3 months. She also received dental implants 5 year ago which had had bleeding around implants.

Periodontal examination revealed that many teeth had  $\geq 6$ mm pockets and  $\geq 2$  mobility. Plaque index 33% and BOP: 42%.

#16 had bone loss to the apex, so that the tooth was extracted. Peri-implant condition revealed that both implant had  $\geq 7$ mm pockets,  $\geq 25$ % bone loss and mucous membrane was swollen.

**Clinical Procedures and Outcomes:** After OHI and SRP, her periodontal condition were much improved (PI: 20%, BOP: 28%) However, improvement of peri-implant condition was limited. We performed surgical approach for them, i.e. surface decontamination and implant plasty. After those approaches, improvement of peri-implant condition has been observed so that patient has been placed in SPT program.

**Conclusion:** In the periodontal therapy, patient's compliance is very essential. In this case, patient become motivated as treatment progressed. She accepted advanced surgical therapy, such as implant plasty. We continue to provide supportive therapy to maintain healthy periodontal and per-implant tissue.

H-09

### Impact of preoperative periodontal treatment on postoperative infection in cardiac valve replacement patients

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**Background and objective:** Oral infection control is important for patients undergoing cardiac valve replacement (CVR) as prophylaxis of infective endocarditis and the other post-operative complications. In the present study, we examined the effect of preoperative periodontal treatment on prevention of post-operative infection in CVR patients.

**Materials and Methods:** We prospectively recruited 55 patients who underwent CVR from August, 2014 to December, 2015 in our hospital as dental intervention group. We also retrospectively reviewed the medical records of 35 patients who had undergone CVR surgery without dental intervention from April 2012 to March 2014 as control. For the intervention group, intensive periodontal treatments were conducted before the surgery. Plaque control record (PCR), bleeding on probing (BOP) and probing pocket depth (PPD) were measured at the first visit to our hospital dental office, one day before surgery, and more than 7 days after surgery. Days of fever (over 37.5 degree), days of antibiotics use, and hospital stay after surgery were counted for both control and intervention groups, and compared statistically between them.

**Results:** In the intervention group, PPD and BOP significantly decreased prior to the CVR surgery, but PCR did not. Days of fever, and days of antibiotics use were significantly shorter in the intervention group than control, but hospital stay after surgery did not differ between them.

**Conclusion:** These findings suggest that preoperative periodontal treatment can improve oral health condition prior to the CVR surgery, and would decrease the risk of postoperative infection in those patients.

H-10

### Periodontal management of Down syndrome with hypersensitivity: A 22-year case report

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**Background and objective:** Severe periodontal disease with early onset is a common clinical feature of patients with Down syndrome (DS). In cases with sensory modulation disorder (hypersensitivity) of peri-oral and intraoral area, treatment of periodontal diseases can be challenging. We report a case of DS patient with gingivitis, who has been treated and maintained for 22 years.

**Case:** The patient was a 7-year-old boy with DS accompanying serious intellectual disabilities. The chief complaint (from his family) was difficulty in tooth brushing. He had sensory modulation disorder with hypersensitivity around his mouth. The patient presented with compromised oral condition, with marked plaque accumulation, gingival swelling and halitosis. He had eating disorder with deviated food habit and speech disorder. At the time of tooth brushing by mother, he exhibited injuring behavior to self and others.

**Clinical Procedures and Outcomes:** A systematic desensitization technique was introduced in home care, and we performed scaling and professional mechanical tooth cleaning. After problems regarding hypersensitivity and gingival inflammation were improved, we started support of self-care, functional training for food intake and speech therapy. Thereafter, we continued bimonthly supportive periodontal therapy, and this improvement has been adequately maintained over a 22-year period.

**Conclusion:** Although DS patients have inherent risk for periodontal disease, the appropriate behavior management and periodontal care from early stage could yield favorable oral condition, which can be maintained for long periods.



H-11

**25year Case: Long term prognosis of periodontal maintenance in patient with rheumatoid arthritis**Makiko Hirose<sup>\*</sup>, Taishi Hirose, Tetsushi Hirose

Yokohama Kannai perio-Implant Institute (Yuraku Dental Office)

*Background and objective:* We report long term maintenance in perio-patient with rheumatoid arthritis*Materials and Methods:* A 26years old female complained of discomfort with gum.

The patient has rheumatoid arthritis, however scientific research relation between periodontitis and rheumatoid arthritis are seldom at the time in 1991. She used to be a smoker and she quit smoke 2005.

After performed Phase1 and Phase2 periodontal treatment, recall maintenance was started.

During the past 25years every 4month recall has been performed.

Range of PCR is 0.64% to 36.3%.

She has some clinical attachment loss but any loss of teeth between the period.

*Results:* Pocket depth ranging from 2 to 4 mm with some additional gingival recession.

Some clinical attachment loss are found but all tooth are well functioned.

*Conclusion:* We could keep stable periodontal condition in the precisely maintained patient with rheumatoid arthritis.

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