The 106th Annual Meeting of the American Academy of Periodontology
in collaboration with the Japanese Society of Periodontology,
and the Japanese Academy of Clinical Periodontology.

Abstracts of JSP/JACP Poster Session

November 6-15, 2020
Virtual

The Japanese Society of Periodontology
The Japanese Academy of Clinical Periodontology

第106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・
2020年大会

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JSP/JACP ポスターセッション抄録集

一般演題（基礎研究，臨床研究），症例報告，歯科衛生演題

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General (Basic Research, Clinical Research), Case Report, Dental Hygiene

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JSP/JACP ポスター発表・
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General
(Basic Research)

GB-01

Effects of aging in LPS-induced chronic periodontitis mouse model

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Background and objective: Alveolar bone resorption due to chronic inflammation is the main symptom of periodontitis. Conventional mouse periodontitis models require improvement as a model of chronic inflammation, particularly with respect to the method of inducing inflammation during the observation period. In this study, alveolar bone resorption was induced by a method which caused little mechanical irritation, and periodontitis progression in young and old mice was compared.
Materials and Methods: Lipopolysaccharide（LPS）from *Porphyromonas gingivalis* was administered 12 times（twice per week）on the palatal gingiva of 8- or 24-weeks old C57BL/6J mice. The LPS was injected by a microsyringe（33 G）, and the dose was 20 μg per 1 shot. Maxilla samples were collected 1 week and 4 weeks after the final administration, and micro-CT scans and tissue slices were prepared.
Results: Injection of LPS by the microsyringe did not require strong external force. Results from the micro-CT showed that bone resorption stimulated by LPS was greater in the older mice than in the young mice. In the comparison between 1 week and 4 weeks after the final administration, the aged mice demonstrated more bone resorption later than the young mice in after 4 weeks.
Conclusion: LPS administration with a micro-syringe caused alveolar bone resorption in both young and old mice. In older mice, bone resorption was observed continuously in 4 weeks after LPS administration.

GB-02

Exosomes from dental pulp cells can suppress periodontitis symptoms

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Background and objective: Extracellular vesicles, called exosomes, are secreted from various cell types and serve as a tool in intercellular communications. We have previously established and characterized human leukocyte antigen（HLA）haplotype homo（HHH）dental pulp cell lines（DPCs）from human third molar teeth. In this study, exosomes secreted from HHH-DPCs were injected into mice with periodontal disease, and their inhibitory effect for periodontal disease progression was evaluated.
Materials and Methods: For exosomes purified from HHH-DPCs, we confirmed the particle size, exosome marker, and HLA class I expression and analyzed miRNA expression profiles of both HHH-DPCs and exosomes. Furthermore, we evaluated the cell migration and proliferation potential of DPCs using HHH-DPC exosomes. We also examined the suppression effect of HHH-DPC exosomes on periodontal disease progression caused by silk ligation.
Results: HHH-DPC exosomes had a homogeneous and spherical membrane structure, and as compared to HHH-DPCs, they showed lower expression of HLA class I. HHH-DPC exosomes promoted the migration and proliferation of DPCs, and the miRNA expression profile of HHH-DPC exosomes was similar to that of the original cells. In addition, mice with periodontal disease showed an inhibitory effect on alveolar bone resorption.
Conclusion: Exosomes promote migration and proliferation of DPCs and exhibit an alveolar bone resorption inhibitory effect in mice with periodontal disease. This suggests that HHH-DPC exosomes may be used in the treatment of periodontitis without cell transplantation.

GB-03

Effects of designer self-assembling peptide hydrogels on periodontal defect healing

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Background and objective: We previously reported that the local application of self-assembling peptide（SAP）nanofiber hydrogel, RADA16, promotes periodontal healing in vivo. RADA16 can be functionalized by cell adhesion motifs. This study aimed to investigate the effects of the local application of two designer SAP hydrogels, PRG（2-unit RGD binding sequence）and PDS（laminin cell adhesion motif）, on the healing of surgical periodontal defects in rats.
Materials and Methods: In vitro, rat periodontal ligament-derived cells were cultured, seeded on each gel, and analyzed by cell proliferation assay（WST-1）. In vivo, standardized periodontal defects were surgically created mesially in the maxillary first molars of rats. Defects were treated with RADA16, PRG, PDS or left unfilled. After 2 and 4 weeks postoperatively, healing of periodontal defects was assessed by microcomputed tomography, histological and immunohistochemical analyses.
Results: Proliferation of PDL-derived cells in PRG group was significantly greater than that RADA16 and PDS groups（$p < 0.01$）at 72 hours. At 4 weeks postoperatively, the bone volume fractions in PRG group（59.1 ± 2.4%）and PDS group（56.9 ± 3.1%）were significantly greater than RADA16 group（48.5 ± 6.3%）（$p < 0.05$）. Histologically, new bone formation was more pronounced in PRG and PDS groups compared to RADA16 group at 4 weeks postoperatively. In PRG group, increased proportions of PCNA- and VEGF-positive cells were observed near the newly formed bone adjacent to the root at 4 weeks postoperatively（$p < 0.01$）.
Conclusion: The results of this study suggest that designer SAP hydrogels, especially PRG, enhanced periodontal healing by increasing cell proliferation and angiogenesis.

GB-04

Effect of CTLA-4 on bone resorption in periodontitis model mouse

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Background and objective: CTLA-4 is one of the immune checkpoint receptors expressed on the cell surface of T cells. It has been reported that CTLA-4 may suppress bone resorption in rheumatoid arthritis. However, its effect on alveolar bone resorption has not been clarified. The purpose of this study is to investigate the role of CTLA-4 in bone resorption in periodontitis.
Materials and Methods: Periodontitis was induced by silk ligature to the second molar in C57BL/6 mice, CTLA-4-Ig was administered intraperitoneally. The effect of CTLA-4-Ig on alveolar bone resorption and osteoclast differentiation was evaluated by μCT and TRAP staining. In vitro, Raw264.7 cells were treated with RANKL and CTLA-4-Ig, and the role of CTLA-4-Ig on the osteoclast differentiation was evaluated. The expression levels of osteoclast differentiation markers（c-fms, Carbonic anhydrase II, and Cathepsin K）and signaling molecule downstream of CTLA-4（PPA2）were assessed by qRT-PCR. The number of osteoclasts was counted by TRAP staining.
Results: Bone resorption and the number of osteoclast-like cells were decreased by administration of CTLA-4-Ig in periodontitis model mice. In vitro, CTLA-4-Ig treatment significantly reduced mRNA expression of osteoclast differentiation markers and the number of osteoclast-like cells. In addition, the expression level of PPA2 was increased by CTLA-4-Ig treatment in a concentration-dependent manner.
Conclusion: Our data showed that administration of CTLA-4-Ig reduced the bone resorption and the number of osteoclasts. In addition, the expression of PPA2 was increased by CTLA-4-Ig. In conclusion, it is suggested that CTLA-4-Ig reduces alveolar bone resorption through the inhibition of osteoclast differentiation via activation of PPA2 at local periodontitis site.

GB-05

Combined effects of FGF-2 and DBBM on periodontal healing: a preclinical investigation

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Background and objective: Fibroblast growth factor（FGF）-2 has been reported to promote periodontal healing and used for periodontal regeneration therapy. Deproteinized bovine bone mineral（DBBM）has osteoconductivity and has been clinically applied as a scaffold material. However, information is limited regarding the combined effects of FGF-2 and DBBM on periodontal healing. The purpose of this study is to investigate the effects of the combination of FGF-2 and DBBM on the healing of surgically created periodontal defects in rats.
Materials and Methods: Periodontal defects were created mesially to the maxillary first molars in Wistar rats. Defects were filled with FGF-2, DBBM, FGF-2+DBBM, or left unfilled. Microcomputed tomography, histological（H&E staining）, and immunohistochemical（PCNA staining）examinations were used to evaluate periodontal healing at 2 or 4 weeks postoperatively.
Results: At 2 weeks postoperatively, bone volume fraction was significantly greater in the FGF-2 and FGF-2+DBBM groups than that in the Unfilled and DBBM groups. At 4 weeks postoperatively, bone volume fraction was significantly greater in FGF-2, DBBM, and FGF-2+DBBM groups than that in the Unfilled group. Histological assessment showed that newly formed bone in the FGF-2 and FGF-2+DBBM groups appeared to be greater than in the Unfilled group. Proportions of PCNA-positive cells above newly formed bone in the FGF-2, DBBM, and FGF-2+DBBM groups were significantly greater than the Unfilled group at 2 weeks.
Conclusion: These findings suggest that the combination of FGF-2 and DBBM enhances bone formation in the treatment of periodontal defects.

GB-06

Quantitative changes of *Porphyromonas gingivalis* and EBV DNA in saliva before and after initial periodontal therapy in Japanese periodontitis patients

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Background and objective: Recently, Epstein-Barr virus（EBV）is thought to be involved in the pathogenesis of periodontitis as well as *Porphyromonas gingivalis* which is the representative periodontopathic bacteria. The purpose of initial periodontal therapy（IPT）is to enhance motivation and remove calculus, periodontopathic bacteria and their byproducts, in order to restore periodontal health. To elucidate the effects of IPT on incidence of *P. gingivalis* and EBV DNA, we used whole saliva samples from the chronic periodontitis（CP）patients.
Materials and Methods: Twenty CP patients were recruited and determined periodontal status by probing pocket depth（PD）, bleeding on probing（BOP）and intraoral radiography, and whole saliva were collected at first visit and after IPT. Saliva samples were subjected to a real-time PCR to detect *P. gingivalis* and EBV DNA.
Results: P. gingivalis and EBV DNA were detected in 20（100%）and 14（70%）saliva samples from the CP patients at first visit. After IPT, number of detections of *P. gingivalis* and EBV DNA were decreased to 17（85%）and 9（45%）saliva samples from the patients. Coexistence of *P. gingivalis* and EBV DNA were detected in 14 saliva samples from the patients at first visit, and significantly decreased to 8（40%）after IPT. Significant improvements in mean PD and BOP were observed after IPT.
Conclusion: These results suggest that the IPT was effective in improvement of clinical parameters such as PD and BOP and reducing the coexistence of *P. gingivalis* and EBV in the saliva from CP patients.

GB-07

Bone regeneration by novel combination materials using phosphorylated pullulan and triphosphate calcium

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Background and objective: Phosphorylated pullulan（PPL）is a unique material which shows a high affinity to crystalline calcium phosphate. In this study we have examined a novel component mixed with PPL and α-tricalcium phosphate（TCP）or β-TCP if it has the preferable osteoconductivity, when grafted into the bone defects.
Materials and Methods: Bone defects with a diameter of 2 mm were formed in the tibial cortical bone of ten-weeks-old Wistar rats, and then filled with PPL, β-TCP, α-TCP, PPL+β-TCP, or PPL+α-TCP. After 1-, 2-, and 4-weeks, the regenerated bones were histochemically evaluated by examining histochemistry of TRAP, ALP, osteopontin, von Kossa staining and element mapping using an electron probe micro-analyzer（EPMA）.
Results: There was no infiltration of inflammatory cells in the region of bone defects of all the groups. At 1- or 2-weeks, TRAP-reactive/cathepsin K-positive osteoclasts and ALP-positive osteoblasts were localized on the α-TCP/β-TCP granules, as well as PPL, the surface of which showed osteopontin-positivity. At 4 weeks, new bones were formed on not only the grafted α-TCP/β-TCP granules but also directly on the PPL. Interestingly, the grafted PPL allowed the invasion of vascular endothelial cells and osteoblastic cells inside, and also showed the distribution of calcium phosphate crystals when estimated by EPMA and von Kossa staining. Thus, PPL appeared to trap calcium phosphates inside.
Conclusion: It seems likely that PPL could effectively serve as an adequate scaffold for bone regeneration, and that the combined components with PPL and TCP could provide a favorable microenvironment for bone regeneration.

GB-08

Lamin A is required for mechanical stress-dependent osteoblast responses

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Background and objective: Lamin A is an intermediate filament underlying inner nuclear membrane being connected to actin cytoskeleton via LINC proteins. It controls chromatin organization, DNA duplication, and transcription. We hypothesized that cell nuclear membrane associated proteins like Lamin A play an important role to transmit mechanical stress to osteogenesis mechanism, and thus are involved in the progression of periodontal diseases. In this study, we aim to investigate the role of Lamin A in the mechanical stress response during osteoblastic differentiation.
Materials and Methods: *Lmna*$^{+/-}$ mice were purchased from the Jackson Laboratory. Calvarial-osteoblasts were isolated of newborn WT and *Lmna*$^{-/-}$ littermate mice. The calvariae were digested for 10 minutes at 37℃ with αMEM containing 0.1% collagenase and 0.2% dispase. Primary osteoblasts were seeded onto a stretch chamber at a density of $3x10^5$ cells / well and cultured in osteoblast differentiation medium under continuous cyclic stretch of 6% amplitude at a rate of 5 cycles/min for 7days using Cell Stretching System. Osteoblastic differentiation was assessed by ALP staining and quantitative RT-PCR for *Alpl*, *Col1a1*, *Runx2*, and *Bglap2*.
Results: ALP staining tended to be lower in Lmna$^{-/-}$ osteoblasts than WT osteoblasts, and both decreased under mechanical stress. The expression levels of marker genes tended to be lower in stressed Lmna$^{-/-}$ osteoblasts than in stressed WT osteoblasts. Early differentiation markers *Alp*, *Col1a1*, and *Runx2* were decreased in a stress-dependent manner in both cells.
Conclusion: It is suggested that Lamin A is involved in the mechanical stress response during osteoblastic differentiation.

GB-09

Amelogenin suppresses IFNγ-induced MHC class II expression in macrophages

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Background and objective: Enamel matrix derivatives（EMDs）-based regenerative therapy is known to promote better healing with minimal inflammatory response after periodontal surgery. We previously demonstrated that amelogenin suppressed major histocompatibility complex class II（MHC II）gene expression in monocytes using DNA microarray analysis. The aims of this study were to understand the molecular mechanism how amelogenin suppressed IFNγ-induced MHC II expression.
Materials and Methods: THP-1 macrophages were pretreated with amelogenin and stimulated with IFNγ. The cell surface expression of MHC II molecules was evaluated by flow cytometry, and signal transduction was analyzed by real-time PCR and western blotting. The uptake of labeled amelogenin into macrophages was monitored by confocal microscopy. Histone modification of class II transactivator（CIITA）promoter region was assessed by chromatin immunoprecipitation. T cell activation was evaluated by mixed leukocyte reaction（MLR）.
Results: Amelogenin down-modulated IFNγ-induced cell surface expression of MHC II, following accumulation in the nucleus at 15 min, and inhibition of subsequent CIITA expression. Reduced MHC II expression on macrophages pretreated with amelogenin down-regulated T cell activation markers, T cell proliferation, and IL-2 production in MLR. H3K27ac and H3K4me3 in CIITA promoter IV region, both of which are essential for conversion to euchromatin, were markedly suppressed by amelogenin.
Conclusion: The results show that amelogenin suppresses MHC II expression by altering chromatin structure and inhibiting CIITA transcription activity, and attenuates subsequent T cell activation. Clinically observed accelerated healing after periodontal surgery by EMDs may, at least in part, be mediated by the mechanism elucidated in this study.

GB-10

Therapeutic potential of exosomes derived from GMSC in periodontal disease

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Background and objective: Mesenchymal stem cells from gingiva（GMSCs）are easier to isolate, and appear to secrete higher amounts of exosome than other MSCs. Our preliminary data demonstrated that exosome from TNF-T-stimulated GMSCs markedly drove macrophage polarization toward anti-inflammatory（M2）phenotype, suggesting that this negative feedback loop could be beneficial for better wound healing. To further investigate the therapeutic potential of exosomes from GMSCs, we investigated its effect on alveolar bone loss from the viewpoint of exosomal miRNA.
Materials and Methods: GMSCs were isolated from gingival tissues under the approval of Institutional Board protocol（2019-374）, and the exosomes were purified from culture supernatant. To examine the protective role of GMSC-derived exosomes on periodontal tissue destruction, the ligature-induced alveolar bone loss was created in mice. Exosomes were injected into the palatal gingiva of mice using a split-mouse experimental design. The effect of exosome from GMSCs on RANKL expression in LPS-stimulated human periodontal ligament cells（hPDLCs）was examined. After TNF-. stimulation, expression profiles of exosomal miRNA in GMSCs were analyzed by miRNA array. Signaling pathways leading to LPS-induced RANKL expression were analyzed, and the involvement of exosomal miRNA in RANKL expression was investigated.
Results: Exosomes from GMSCs markedly suppressed alveolar bone resorption in mice model of periodontitis. Exosome from GMSC significantly inhibited RANKL expression in LPS-treated PDLCs, and the effect was further enhanced by prior treatment of GMSCs with TNF-,.
Conclusion: Exosomes derived from GMSCs suppressed bone loss, and TNF- -induced exosomal miRNA was essential for the suppression of RANKL expression.

GB-11

Combined effects of geranylgeranylacetone and amelogenin on periodontal tissue regeneration

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Background and objective: We previously reported that amelogenin（rM180）directly binds to glucose-regulated protein 78（GRP78）, a member of heat-shock protein family, and that cell migration activity was enhanced when GRP78-overexpressing human periodontal ligament cells（hPDLCs）were treated with rM180. Geranylgeranylacetone（GGA）is a heat-shock protein-inducing medication that is commonly used to treat gastric ulcers. In this study, therefore, we hypothesized that GGA and amelogenin exhibit synergistic effect for better healing and tested its possibility using hPDLCs.
Materials and Methods:
1. GGA-induced GRP78 expression and its effect on cell functions was evaluated.
2. Microarray analyses to identify genes upregulated by the stimulation with or without GGA or GGA+ rM180 in hPDLCs, and its verification was performed.
3. The tube formation by supernatants from hPDLCs treated with each stimulation was investigated using human umbilical vein endothelial cells（HUVECs）.

Results & Conclusion: We found that GGA increased GRP78 protein expression at 18 hours after the stimulation, and enhanced cell migration. Microarray analysis demonstrated that the increased GRP78 expression triggered the production of angptl4 and amphiregulin, both of which are involved in enhanced angiogenesis and subsequent wound healing. Moreover, the addition of rM180 further accelerated the hPDLCs cell migration and tube formation of HUVECs due to the upregulation of IL-8, MCP-1, and IL-6, all of which are known as angiogenesis-inducing factors. Neutralizing antibodies against these cytokines significantly inhibited tube formation by HUVECs. Thus, the combined application of GGA and rM180 may create a suitable environment for periodontal wound healing.

GB-12

Investigating the mechanism of environmental stress response in *Treponema denticola*

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Background and objective: Bacteria are exposed to environmental stresses in oral cavity. To adapt to environmental stresses, bacteria usually regulate gene expressions using DNA-binding proteins. *Treponema denticola* has been frequently isolated from the lesion of chronic periodontitis and must overcome environmental stresses to colonize. We have reported that the expressions of some genes coding for DNA-binding protein in *T. denticola* are regulated by environmental stress such as low nutrient conditions. The purpose of this study is to investigate the role of a novel *T. denticola* DNA-binding protein in the response to environmental stresses.
Materials and Methods: A mutant deficient in the gene coding for a DNA-binding protein was constructed by homologous recombination. Under low nutrient conditions, the growth and proteolytic activities（dentilisin and oligopeptidase B）were evaluated. The viability of the cells was measured under oxygen, osmotic downshift and heat stresses.
Results: Under low nutrient medium, the growth of the mutant strain was significantly decreased compared with wild type at 24-96 hours and the dentilisin activity of the mutant strain was significantly decreased compared with wild type. The viability of the mutant strain was significantly decreased under oxygen stress and increased under heat stress at 1, 2 hours. No significant difference was observed between the mutant and wild type strains under osmotic downshift stress.
Conclusion: It is suggested that the DNA-binding protein is involved in the growth, dentilisin activity and survival of *T. denticola* under environmental stresses such as low nutrient, oxygen, and heat.

GB-13

Roles of PDLIM5 in periodontal ligament and gingival epithelial cells

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Background and objective: During wound hearing, inhibition of gingival downgrowth play a critical role in leading to successful periodontal regeneration. Cell extensions play an important role in several processes, such as wound healing and matrix remodeling. PDLIM5 is a member of the PDZ-LIM proteins. We recently reported that PDLIM5 was required for cell extensions formation in mouse fibroblast cells. In this study, we investigated the effects of PDLIM5 on association with cell extensions formation and migration activity of human periodontal ligament（HPDL）and gingival epithelial（HGE）cells.
Materials and Methods: We used 2 types of cells；SV40T-Ag and human TERT immortalized HPDL fibroblast cell lines（1-11), and SV40T-Ag immortalized HGE cell lines（OBA-9). PDLIM5 expression in 1-11 and OBA-9 was confirmed by immunoblot analysis. To examine the roles of PDLIM5 in 1-11 and OBA9, PDLIM5 was knocked down by two different siRNAs. 1-11 and OBA-9 with PDLIM5-knockdown were planted on grid-supported floating collagen gels, and formation of cell extensions was examined. 1-11 and OBA-9 with PDLIM5-knockdown were cultured in cell culture inserts for cell migration assay.
Results: PDLIM5 expression was observed by immunoblot analysis in 1-11 and OBA-9. 1-11 with PDLIM5-knockdown reduced cell extensions formation and migration activities, compared with control cells.
OBA-9 with PDLIM5-knockdown promoted cell extensions formation and migration activities, compared with control cells.
Conclusion: These results suggested that PDLIM5 may regulate cell extensions formation and migration activity of HPDL and HGE cells during wound hearing. PDLIM5 would be used as a therapeutic agent for periodontal regeneration.

GB-14

Functional analysis of a DNA binding protein-coding gene in *Treponema denticola*

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Background and objective: Treponema denticola is one of the predominant bacteria in deep periodontal pockets. *T. denticola* has various virulence factors, such as major outer sheath protein（Msp）. However, the mechanism of the Msp expression has yet to be clarified. We previously found some genes coding for transcriptional regulators which might be implicated in the regulation of *msp* expression. Among them, we focused on a gene coding for a DNA binding protein. The objective of this study is to clarify the regulation mechanism of Msp expression through analysis of the function of the gene encoding the DNA binding protein.
Materials and Methods: We constructed the mutant strain deficient in the gene coding for the DNA binding protein by homologous recombination. Using this newly constructed mutant, we performed genetic analysis by DNA microarray and qRT-PCR. Based on the results of the genetic analysis, we also analyzed some phenotypic changes of the mutant.
Results: The expression levels of *msp* or other genes coding for virulence factors in the mutant were similar to those of wild type. However, the genes implicated in the motility of *T. denticola* were significantly upregulated. Also, the moving pattern of the mutant was considerably changed.
Conclusion: The gene coding for the DNA binding protein does not play a direct role in the regulation of *msp* or other genes coding for virulence factors. However, it is suggested that the DNA binding protein plays a certain role in the regulation of the genes implicated in the motility of *T. denticola*.

GB-15

Butyric acid modulates periodontal mechanical sensitivity in periodontitis in mice

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Background and objective: It is well known that periodontitis gradually progresses accompanied by alveolar bone loss and progressive inflammation without periodontal pain. However, the modulating mechanism of periodontal mechanical sensitivity in periodontitis is poorly understood. In this study, we examined the involvement of butyric acid in the changes in periodontal mechanical sensitivity in experimental periodontitis produced by *Porphyromonas gingivalis*（*P.g.*）in mice.
Materials and Methods: Under deep anesthesia, a 5−0 silk ligature was tied around the maxillary second molar, and *P.g.* was inoculated into the tied silk ligature to establish the murine model of experimental periodontitis. Under light anesthesia, mechanical stimulation was applied to the gingival tissue adjacent to cervical regions of the maxillary second molar by using electronic von Frey anesthesiometer and the mechanical head withdrawal threshold（MHWT）was measured for 14 days. On day 2 following the ligation, the expression of short−chain fatty acid receptor, GPR41, in trigeminal ganglion and the amount of butyric acid in the inflamed periodontal tissue. Moreover, we examined the changes in the MHWT in the experimental periodontitis model by daily intra−periodontal GPR41 antagonist, hydroxybutyric acid administration.
Results: There was no change in the MHWT in the experimental periodontitis model for 14 days. GPR41 was expressed in trigeminal ganglion neurons and butyric acid was increased on day 2 following the ligation. The daily hydroxybutyric acid administration decreased the MHWT decreased on day 8 following the ligation.
Conclusion: Butyric acid produced by *P.g.* in the periodontal tissue may contribute to periodontal mechanical sensitivity in periodontitis.

GB-16

Investigation of junctional epithelial stem/progenitor cells using the multicolor lineage tracing method.

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Background and objective: The junctional epithelium（JE）has a distinctive contribution to infection prevention, owing to its location on the front lines of defense in the oral cavity, where it directly contacts the tooth surface. However, it remains unclear whether the stem/progenitor cells exist in the JE. The aim of this study was to investigate the cell lineage and the existence of stem/progenitor cells in the JE using the multicolor lineage tracing method.
Materials and Methods: All of the cells in the body were randomly labeled with four colors（green: GFP, blue: mCerulean, orange: mOrange, red: mCherry）using Rosa26$^{CreERT2/rbw}$ mice at postnatal week 4 with tamoxifen（TAM）. The cell lineage was analyzed in the JE of Rosa26$^{CreERT2/rbw}$ mice at 3 days, 4 and 8 weeks after TAM injection by fluorescence microscopy.
Results: Labeled cells with four colors were found in the JE of Rosa26$^{CreERT2/rbw}$ mice at 3 days after TAM injection. Two regions within JE were distinguished by colors and were different from the oral epithelium in Rosa26$^{CreERT2/rbw}$ mice at 4 and 8 weeks after TAM injection. Furthermore, three−dimensional imaging indicated the existence of two clonal cells in the JE of those mice.
Conclusion: Present study possibly suggested the existence of stem/progenitor cells in the JE. The long−term multicolor lineage tracing observations will be needed to determine whether the stem/progenitor cells of JE survives in the long term and contribute to homeostasis of JE.

GB-17

Endotoxemia by *Porphyromonas gingivalis* aggravates inflammation in brown adipose tissue

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Background and objective: Adipose tissue is known as not only storage organ for energy, but also endocrine organ. Since adipocytes produce various kinds of adipocytokines, including adiponectin and inflammatory cytokines, many studies suggested the relationship between adipocytes and systemic diseases. Especially, several reports have shown that the functional decline of brown adipose tissue is related to obesity. Previously, we reported that intravenous injection of sonicated *Porphyromonas gingivalis*（*Pg*）increases body weight and visceral fat volumes in mice. However, the direct effects of bacillaemia by *Pg* on adipocytes remain unclear. This study was performed to evaluate the effect of sonicated *Pg* injection on adipocytes in short-term.
Materials and Methods: Endotoxemia was induced in male C57BL/6J mice（12-weeks-old）by intravenous injection of sonicated *Pg*. The mRNA expression level in brown adipose tissue from interscapular fat was evaluated by qPCR at 18 hours after *Pg* injection. Microarray analysis was performed in brown adipose tissue at 18 hours after *Pg* injection.
Results: The mRNA expression of inflammation-related genes in brown adipose tissue were increased, and *Adipoq* expression was decreased. Gene set enrichment analysis revealed enrichedment of several inflammation-related gene sets and downregulation of several gene sets, including PI3K AKT mTOR signaling, cholesterol homeostasis, and bile acids metabolism in brown adipose tissue at 18 hours after *Pg* injection.
Conclusion: Endotoxemia by periodontitis may elicit inflammation and change the endocrine and lipid metabolism in brown adipose tissue. These results suggested that endotoxemia by periodontitis might affect obesity through disturbance of adipocytes.

GB-18

Insulin resistance in gingival fibroblasts exacerbates experimental periodontitis in mice

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Background and objective: Periodontitis is known to be exacerbated and insulin resistance in the gingiva was shown in in obesity and diabetes. We aimed determine how and in which type of cell insulin resistance contributes to exacerbation of periodontitis in obesity and diabetes.
Materials and Methods: Smooth muscle protein 22α-Cre-$IR^{fl/fl}$（SMIRKO）mice were generated by crossbreeding $IR^{fl/fl}$ mice with SM22α-Cre mice. SMIRKO, wild-type（WT）and WT mice fed with high-fat-diet（HFD）for 8 weeks were used. Gingiva samples were stimulated with 0-100nM insulin for 10 minutes. Experimental periodontitis was induced by 7-0 silk ligation around maxillary 2nd molars for 4 and 14 days. Additionally, primary gingival fibroblasts（GFs）were stimulated with *E.coli* lipopolysaccharides（LPS）for 6 hours in the presence or absence of pretreatment of 100nM insulin pretreatment for 30 minutes.
Results: Gingival insulin receptor-beta（IRβ）expression in SMIRKO and HFD-fed mice was decreased by 70% and 50 %, respectively, with comparable reductions in insulin-induced phosphorylation of Akt. Ligature-induced alveolar bone loss in SMIRKO and HFD-fed mice was greater by 70% and 40% , respectively, as compared to regular diet（RD）-fed WT mice. Neutrophil recruitment was delayed significantly in both SMIRKO and HFD-fed mice. Similarly, decreased gene expression of the neutrophil-recruiting factor CXCL1 was seen in the gingiva at day 4. Unlike SMIRKO and HFD mouse GFs, WT GFs showed that insulin enhanced LPS-induced CXCL1 expression.
Conclusion: These data provided the first evidence that insulin action is critical for neutrophil recruitment during infection by enabling CXCL1 expression in GFs, which is inhibited in diabetes in response to bacterial LPS stimulation.

GB-19

Combination of functional water with hydroxyapatite / collagen composite accelerate bone regeneration

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Background and objective: Hydroxyapatite / collagen composite（HA/Col）can regenerative bone. Acid electrolyzed functional water（FW）generated by electrolyzing saline has a sterilizing effect. We have reported that FW accelerate the regeneration in rat mandibular bone's defect. Therefore, we evaluated the effects of these combinations.
Materials and Methods: Artificial bone defects（5.0 mm diameter）were made on the 8-week-old female Wister rats calvaria. The combinatorial group: 1 minute FW irrigation and HA/Col filling, The HA/Col group: 1 minute PBS irrigation and HA/Col filling, The FW group: 1 minute FW irrigation, HCL group: 1 minute HCL irrigation. The control group: PBS irigation. New bone was measured by micro-CT for 6 weeks after surgery. Peripheral blood was collected before and 1, 6 and 24 h after surgery and subjected to ELISA. The surgical area was observed histologically and analyzed genetically.
Results: New bone was significantly enhanced in the combinational group after 2-weeks of surgery. The enhanced bone formation was confirmed with histological examination（HE）and the TRAP staining revealed the increased accumulation of osteoclasts around the bony rim in the combinational group. Real-time PCR analysis revealed that the expression of osteogenesis- and angiogenesis-related mRNAs was increase in the combinational group. Among them, FGF2 concentration in the peripheral blood increased in the combinational group 1 and 6 hours after surgery.
Conclusion: These findings suggest that FW facilitates new bone formation by inducing angiogenesis- and bone remodeling-related genes. Taken together, FW was shown to improve the bone regeneration when used in combination with HA/Col.

GB-20

Role of quiescent osteoclast precursors in occlusal trauma in mice

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Background and objective: Traumatic occlusion promotes bone resorption at alveolar bone crest and furcation area and induces osteoclasts in a relatively short period. It was reported that cell cycle–arrested quiescent osteoclast precursors（QOP）preexist at the site of osteoclastogenesis（J Cell Biol, 2009）. The purpose of this study was to clarify the role of QOP in bone resorption caused by traumatic occlusion.
Materials and Methods: Occlusal trauma was induced in the lower left first molar of mice by bonding a wire to the upper left first molar. The mice were injected intraperitoneally with bromodeoxyuridine（BrdU）, which can be incorporated into dividing nuclei. The number of osteoclasts and percentage of BrdU-positive nuclei in total osteoclasts nuclei were counted after tartrate-resistant acid phosphatase（TRAP）and BrdU double labeling. QOP was detected as TRAP-positive and BrdU-negative cells.
Results: Following the application of traumatic occlusion for 2 days, hyalinized tissue appeared in the furcation area at the first molar in the mice. On day 3, many osteoclasts were observed at the top of the inter-radicular septum. A few TRAP-positive and BrdU-negative cells emerged in the furcation area on day 1 and increased until day 3. On day 5, TRAP-positive and BrdU-negative cells were found in the furcation area.
Conclusion: QOP accumulated in the furcation area immediately after the application of traumatic occlusion, suggesting that QOP played an important role in the rapid alveolar bone resorption caused by traumatic occlusion.

GB-21

Advanced glycation end products promote the COX2 expression of osteoblasts

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Background and objective: Periodontitis has been shown correlation with various metabolic syndrome. Particularly, an epidemiological study showed that diabetes is associated with periodontitis（Demmer et al., 2011）. Advanced glycation end products（AGEs）are formed by between the excess sugars and proteins produced from hyperglycemic disorders such as diabetes. Thus, the involvement of advanced glycation end products（AGEs）has been focused as an important factor of diabetes. However, the mechanism of AGEs effects on periodontitis are not well understood. We therefore aimed to investigate the effect of AGEs on the expression of cyclooxygenase（COX）which is the synthetase of prostaglandin E_2 is a major factor of inflammation that causes the production of RANKL.
Materials and Methods: MC3T3-E1 cells were stimulated with LPS（10 ng/ml）and AGEs（100 μg/ml）. mRNA expression of COX1, COX2, and RAGE was determined on days 3, 7, and 14 of culture using real-time PCR
Results: LPS + AGE increased mRNA levels of COX2 on day 14 of culture compared to LPS alone. LPS + AGE also increased mRNA levels of RAGE on day 3 of culture compared to LPS alone. However, LPS + AGE did not affect mRNA levels of COX1 on day 3, 7, 14 of culture.
Conclusion: These results suggest that AGEs affect LPS-induced inflammation of osteoblasts. AGEs may promote inflammatory bone resorption.

GB-22

Effect of topical PTH administration in rat calvarial GBR model

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Background and objective: Intermittent administration of PTH（1-34）is one of common treatments of osteoporosis, and not only increases the bone density but also promotes osteogenesis. In previous studies, we have shown that intraperitoneal administration of PTH（1-34）promotes vertical bone augmentation in calvarial GBR model of ovariectomized osteoporosis model rats. In this study, we investigated the effect of local administration of PTH（1-34）on the same animal model.
Materials and Methods: At 6 weeks of age, ovariectomy（OVX-PTH rats）or Sham operation（control rats）was performed. At 14 weeks of age, plastic caps were placed on calvaria of both group rats, following bone marrow penetration. In the OVX-PTH rats, PTH（1-34）（35 μl / kg）was locally administered to the calvaria three times a week, the same amount of saline was locally administered to control rats. Micro-CT images were taken every other week until week 12, and quantitative analysis of new bone-like tissue was performed. At 12 weeks, histochemical sections were prepared, and immunostained..
Results: Formation of newly bone-like tissue was observed in both the groups in micro-CT. There was no significant difference between two groups in the amount of newly formed bone-like tissue. Histological observation showed that many blood vessels, osteoblasts and osteoclasts were found in the new bone in both groups by HE staining.
Conclusion: It was showed that intermittent topical administration of PTH（1-34）to the GBR site reduced the effects of estrogen deficiency on bone augmentation and restored bone growth to healthy levels.

GB-23

Collagen membrane as GBR material in rat vertical augmentation model

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Background and objective: In GBR（Guided Bone Regeneration）procedure, a space for bone regeneration is secured by using GBR barriers. Recently, highly permeable materials such as absorbable membranes have been clinically used for GBR. In this study, the effects of collagen membrane for GBR were examined by both radiographic and histological assessments in rat calvaria.
Materials and Methods: Ten-week-old male rats（F344 / jcl）were used. The calvaria of rats were exposed, and plastic pipes were placed bilaterally. Each pipe was filled with Bio-Oss®, and collagen membranes（Bio-Gide®）were put on the half of plastic pipes as lid（BG）. The others were kept with no tops（OP）. Surgery was performed at week 0, and images were taken up to 12 weeks by using with micro-computed tomography（micro-CT）. In addition, paraffin-embedded and resin-embedded sections at 12 weeks were assessed histologically.
Results: Formation of newly bone-like tissue was observed in both the groups in micro-CT. There was no significant difference between two groups in the amount of newly formed bone-like tissue. Histological observation showed that new bone formation was observed surrounding Bio-Oss® particles at 12 weeks in both groups. However, infiltration area of fibrous connective tissue was confirmed in the upper part inside of pipes in the OP group.
Conclusion: Although infiltration of fibrous soft tissue was large in OP groups, newly formed bone tissue was confirmed in rat calvaria GBR model made with high permeable barrier.

GB-24

Proinflammatory nature of aspirated periodontopathic bacteria may cause pneumonia

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Background and objective: Aspiration pneumonia is a major health problem owing to its high mortality rate in elderly people. The secretion of proinflammatory cytokines such as IL-8 and IL-6 by respiratory epithelial cells, which is induced by infection of respiratory bacteria such as *Streptococcus pneumoniae*（*S.p*）, contributes to the onset of pneumonia. These cytokines thus play a key role in orchestrating inflammatory responses in the lower respiratory tract. Although emerging evidence has revealed an association between aspiration pneumonia and periodontitis, how periodontitis contributes to the onset of aspiration pneumonia remains unclear. Most periodontopathic bacteria are anaerobic and are therefore unlikely to survive in the lower respiratory organs of humans. Hence, we aimed to elucidate whether exposure to heat-inactivated periodontopathic bacteria induces proinflammatory cytokine production by several human respiratory epithelial cells and in the lower respiratory organs and serum in mice.
Results: Periodontopathic bacteria, especially *Fusobacterium nucleatum*（*F.n*）strongly induced IL-8 and IL-6 production by BEAS-2B bronchial epithelial cells. In addition, *F.n* induced IL-8 production by A549 alveolar epithelial cells as well as IL-8 and IL-6 production by Detroit 562 pharyngeal epithelial cells. Furthermore, *F.n* induced considerably higher cytokine production than *S.p*. This was also observed in the entire lower respiratory organs and serum in mice.
Conclusion: F.n is a powerful inflammatory stimulant for respiratory epithelial cells and can stimulate cytokine production, thereby potentially contributing to the onset of aspiration pneumonia.

GB-25

Laser irradiation decreases Sost expression in bone and osteogenic cells

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Background and objective: Er: YAG laser has been applied for bone ablation in both periodontal and peri-implant surgery. However, the effects of Er: YAG laser irradiation on bone tissue remain unclear. The purpose of this study was to evaluate comprehensive and sequential gene expression in Er: YAG laser-ablated, bur-drilled, and non-treated control bones to clarify the biological responses. Furthermore, bio-stimulation effect of Er: YAG laser on osteogenic cells was investigated.
Materials and Methods: The calvarial bone of male, 10-week-old Wistar rats was ablated by Er: YAG laser and conventional bur. Gene expression in the laser-ablated, bur-drilled, and non-treated control bones at 6, 24, and 72 h were evaluated by microarray. Osteoblast-like cells were isolated from the calvaria of 3–5-days-old Wistar rats and cultured with osteoinduction medium to induce osteogenic cells. Osteogenic cells were irradiated using Er: YAG laser at energy fluences of 1.5 and 3.1 J/cm^2.
Results: Gene expression patterns were clearly different among non-treated, bur-drilled, and laser-irradiated bone tissues at 24 hours. Gene Set Enrichment Analysis showed inflammation-related gene sets were enriched in the bur-drilled bone at 6 h, whereas these gene sets were enriched in the laser-ablated bone at 72 h compared to that of the control bone. Er: YAG laser irradiation suppressed *Sost* expression both in bone tissue and osteogenic cells. Differentially expressed genes between bur-drilled and laser-ablated bone showed that less inflammation was induced in the laser-ablated bone.
Conclusion: Our study suggests that bone ablation with Er: YAG laser may stimulate osteocytes in the bone tissue and induce advantageous responses in new bone formation.

GB-26

Involvement of CXCR4 in alveolar bone resorption in periodontitis-mice

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Background and objective: Periodontitis caused by bacterial infection progresses periodontal tissue destruction little by little. As a result, teeth lose their supporting structures, and this leads to the loss of a tooth. CXC-chemokine receptor 4（CXCR4）is known to be expressed in lymphocytes, fibroblasts, and osteoclasts in periodontal tissues, suggesting that periodontal CXCR4 signaling contributes to alveolar bone resorption at the periodontitis. However, the role of CXCR4 signaling in the pathogenesis of periodontitis has remained unknown.
Materials and Methods: We established a mouse model of periodontitis by ligating a silk thread to the upper molar and inoculating *Porphyromonas gingivalis*（*P.g.*）. In order to confirm whether this periodontitis model progress without pain, the mechanical pain threshold at periodontal tissue was compared with positive control using CFA. Furthermore, a CXCR4 neutralizing antibody was administered to periodontal tissue after *P.g.* inoculation, bone resorption was observed in the experimental period using μ-CT, and tissue staining was performed to observe cells infiltrated locally.
Results: It was indicated that this periodontitis model using *P.g.* progress with no pain increase. In this periodontitis model, administration of CXCR4 neutralizing antibody enhanced inflammatory cell infiltration in periodontal tissue on day 14 of the experimental periods and enhanced bone resorption on days 4 to 14.
Conclusion: These findings suggest that periodontal CXCR4 signaling in several type of immune cells in the situation of periodontitis induced by *P.g.* depresses alveolar bone resorption. CXCR4 signaling might be a target for therapeutic intervention to prevent alveolar bone resorption in periodontitis.

GB-27

The effects of Er: YAG laser irradiation on primary osteoblast-like cells

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Background and objective: A number of reports have shown that various lasers have favorable biological effects on cells. However, the effects of low-level Er: YAG laser irradiation on osteoblasts remain unclear. The purpose of this study was to evaluate the effects of low-level Er: YAG laser irradiation on proliferation and osteogenic differentiation of primary osteoblast-like cells.
Materials and Methods: Osteoblast-like cells isolated from the calvariae of 3–5-day-old Wistar rats were irradiated by Er: YAG laser at energy fluences of 2.2, 3.3, or 4.3 J/cm^2. Cell surface temperature was measured and cell proliferation was evaluated. Calcification was evaluated by measuring the Alizarin red S staining area after 7-day-culture with osteoinductive medium. Gene expressions in non-irradiated and laser-irradiated cells were evaluated by qPCR at 3, 6, and 12 hours after irradiation. Microarray analysis was performed to comprehensively evaluate gene expressions of non-irradiated cells and irradiated cells at 3.3 J/cm^2 at 6 hours after irradiation.
Results: No pronounced increase of cell surface temperature was induced by low-level Er: YAG laser irradiation, and the irradiation did not affect osteoblast-like cell proliferation. Calcification was significantly increased 7 days after irradiation at 3.3 J/cm^2. *Bglap* expression was significantly increased in cells irradiated at 3.3 J/cm^2 at 6 hours post-irradiation. Microarray analysis showed that irradiation at 3.3 J/cm^2 up-regulated of inflammation-related genes and down-regulated of *Wisp2*. Gene set enrichment analysis clarified enrichment of inflammation-related and Notch signaling gene sets.
Conclusion: Low-level Er: YAG laser irradiation enhanced calcification of primary osteoblast-like cells via enhanced *Bglap* expression and enriched Notch signaling.

GB-28

Effects of Porphyromonas gingivalis LPS on cardiac function in mice

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Background and objective: Periodontitis was recently reported to induce activation of sympathetic nervous systems. On the other hand, chronic activation of sympathetic activity is known to induce the development of cardiac remodeling and heart failure. We thus examined in mice treated with lipopolysaccharide derived from *Porphylomonas gingivalis*（PG-LPS）at a dose equivalent to the circulating levels in patients with periodontitis with/without ß-adrenergic receptor（ß-AR）blocker propranolol（PPL）.
Materials and Methods: Mice（C57BL6/J）were divided into four groups: 1）Control group, 2）LPS group（0.8mg/kg/day i.p.）, 3）PPL group（via the drinking water containing 1g/L）, and 4）PPL+LPS group.
Results: After 1 week, we examined the cardiac function using echocardiography. Cardiac function as exemplified by the left ventricular cardiac function was significantly decreased by the treatment of PG-LPS（Control（n=6）vs. LPS（n=6）: 66 ± 0.5 vs. 57 ± 0.9%, $P<0.001$ vs. Control）. However, co-treatment of PPL suppressed the decrease of PG-LPS-mediated cardiac dysfunction（62 ± 0.6% , n=5, $P<0.001$ vs. LPS group）. The number of apoptotic myocytes was increased（approximately 10-fold）and the area of fibrosis was increased（approximately 2-fold）. Again, these increases were abrogated by the co-treatment of PPL. The impairment of cardiac function in PG-LPS-treated mice appears to involve dysregulation of Ca^{2+} homeostasis in cardiac myocytes induced by ß-AR activation and its downstream molecules via PG-LPS.
Conclusion: Our results indicated that ß-AR signaling might be new therapeutic targets for treatment of cardiovascular disease in patients with periodontitis.

GB-29

LIPUS stimulation promotes collagen synthesis via P2X7 receptor.

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Background and objective: Low-intensity pulsed ultrasound（LIPUS）is a clinically applied medical device in the medical and dental fields. In addition, it has been reported that P2X7 receptor, a receptor for adenosine triphosphate, is involved in bone formation by mechanical signaling. Further, type I collagen（Col I）is one of the major protein components of extracellular matrix protein produced in bone tissue and is particularly involved in osteoblastic bone formation. However, the interaction between LIPUS-induced ATP and collagen metabolism at the P2X7 receptor has not been clarified. In this study, we examined the role of P2X7 receptor on collagen metabolism in LIPUS-induced bone formation by cell biological and molecular biology.
Materials and Methods: Osteoblastic MC3T3-E1 cells were seeded on a 6 well plate and cultured in the presence or absence of the selective P2X7 receptor inhibitor A438079, followed by 30 min/day of LIPUS stimulation using OSTEOTRON D^2. In addition, these cells transfected with P2X7 shRNA（P2X7 knockdown cells；shP2X7）were also prepared and stimulated with LIPUS under the same conditions. The protein expression of Col I and matrix metalloproteinases（MMPs）was determined using ELISA.
Results: In both osteoblastic MC3T3-E1 cells in the presence of A438079 and their P2X7 knockdown cells（shP2X7）, LIPUS stimulation reduced the protein expression of Col I, whereas induced the protein expression of MMP-1, 3, and 13.
Conclusion: These results suggest that LIPUS promotes osteogenesis by stimulating osteoblastic collagen metabolism to the synthetic system via the P2X7 receptor.

GB-30

The effect of sword bean extract on periodontitis and atherosclerosis in mice

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Background and objective: Recently, several studies have suggested that periodontitis accelerates atherosclerosis（AC）. Our laboratory reported that sword beans extract（SBE）has effects for anti-inflammatory and anti-bacterial in periodontal tissue. The purpose of this study was to examine the effect of SBE on AC accelerated by periodontitis.
Materials and Methods: Six-weeks-old male $ApoE^{-/-}$ mice were induced periodontitis by oral *P. gingivalis* infection（*P. g*）or 5-0 silk ligature tied around the molars（Lig）. They were randomly divided into six groups as follows: ① AC（Control）, ② *P. g*, ③ Lig, ④ SBE（added to drinking water starting at 7 weeks of age）, ⑤ *P. g*+SBE, ⑥ Lig+SBE. All the mice fed high fat diet. At the age of 17 weeks, all the mice were sacrificed. Periodontal tissue and aorta were evaluated histologically and morphologically. The gene expressions of iNOS, IL-6 and TNF-α in the heart tissue were evaluated.
Results: The amount of alveolar bone loss in the Lig+SBE was significantly lower than that of the Lig. In analysis of the cross sections in aorta, *P. g* + SBE and Lig + SBE were significantly lower in area of atherosclerosis than *P. g* and Lig. Gene expression of iNOS and TNF-α in the heart of *P. g* + SBE were significantly lower than that of *P. g*.
Conclusion: This study indicated that SBE down-regulated iNOS gene expression and inhibited progression of AC accelerated by periodontitis. It was suggested that SBE could be applied as a therapeutic agent for AC.

GB-31

LIPUS enhances rhBMP9-induced bone formation in rat calvarial bone defects

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Background and objective: It has recently been reported that bone morphogenetic protein (BMP)-9 has great osteoinductive properties in pre-clinical studies in various craniofacial/periodontal bone defects. Bone repair is also regulated by local mechanical stimulation. Therefore, the aim of this study was to examine the combined effects of Low-intensity pulsed ultrasound (LIPUS) and recombinant human (rh) BMP-9 on bone healing in rat calvarial defects.
Materials and Methods: Circular calvarial defects were surgically created in 18 wistar rats. The animals were divided into two groups (LIPUS-applied (+) and LIPUS-non-applied (-)) and the 36 defects in each group randomly received the following treatments: absorbable collagen sponge (ACS) (ϕ 2.7 mm) implantation (ACS), ACS with 1.0 μgrhBMP-9 (rhBMP9/ACS) and empty control (control). As a result, the following 6 groups (control (+) / (-), ACS (+) / (-), and rhBMP9/ACS (+) / (-)) were evaluated. The LIPUS-applied group received daily LIPUS exposure starting immediately after the surgery. At 4 weeks, the animals were sacrificed for micro-CT and histologic evaluation.
Results: Postoperative clinical healing was uneventful at all 72 sites. More new bone was observed in the LIPUS-applied (+) group compared to the LIPUS-non-applied (-) group. The bone volume ($2.46 \pm 0.65 mm^3$), newly formed bone area (NBA) ($1.25 \pm 0.31 mm^2$) and NBA/total defect area ($62.80 \pm 11.87\%$) in the rhBMP9 (+) group were significantly greater than those ($1.76 \pm 0.44 mm^3$, $0.88 \pm 0.22 mm^2$ and $42.66 \pm 7.03\%$ respectively) in the rhBMP9 (-) group ($p < 0.05$). Furthermore, the rhBMP9 (+) group showed the highest level of bone formation among all the groups.
Conclusion: The present results suggest that LIPUS enhances rhBMP-9-induced bone formation in calvarial defects in rats.

GB-32

Behavior of MC3T3-E1 cells on strontium doped titanium surface

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Background and objective: The purpose of the study was to establish a new and simple coating method on the mirror-finished Ti surfaces to enhance titanium/bone integration.
Materials and Methods: Commercially pure grade-2 titanium disks were mechanically polished using colloidal silica. The test groups were immersed in 5% NaOCl solution for 24 h and ultrasonically cleaned in distilled deionized water (DDW), dipped in 1% $Sr(NO_3)_2$ solution for 30 seconds, dried with spinning and were subsequently treated with or w/o ultrasonic DDW. The control group was as-polished titanium disks. The concentrations of various ions, doped in each surface, were evaluated with SPM, strontium ions concentration released were examined with ICP-OES and wettability was measured. Afterward, specimens were transferred to 24 well plates and MC3T3-E1 cells were seeded at $100x10^3$/well. After 2h, attached cells to each surface were counted.
Results: Sr ions released on day 1 of the NaOCl-Sr specimens was 5.69 ppm, 0.05 ppm on day 3 and within 1 week almost no Sr ions was detected. The Sr ions of the NaOCl-Sr-washed specimens, on day 1 was 0.64 ppm, which as one order of magnitude lower than that of the NaOCl-Sr. The amount of released Sr ions decreased with time. The number of the attached cells on the NaOCl-Sr was higher than on the NaOCl-Sr-washed surface. The NaOCl-Sr-washed and as-polished surfaces were less adhesive than the polystyrene-dish.
Conclusion: The Sr doped smooth titanium surface could be created by simple chemical treatments. The surface's physical properties were enhanced by Sr inducement.

GB-33

Effect of aging on junctional molecules of gingival epithelial cells

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Background and objective: Aging causes progressive dysfunction in the mechanical and biochemical function of tissues. Junctional molecules have critical role in maintaining epithelial homeostasis. Age related destruction of gingival tissues might be the consequences of altered junctional molecules. We aimed to elucidate altered expression of clauin-1, 2 and E-cadherin in young and senescence induced gingival epithelial cells with and w/o *Porphyromonas gingivalis Lipopolysaccharides*（Pg LPS）.
Materials and Methods: Human gingival epithelial cells（HGEP）were treated with hydrogen peroxide（H_2O_2）to induce senescence. 400μM of H_2O_2 was considered as optimal concentration to induce senescence, which was confirmed from the vitality test, senescence associated β-galactosidase staining and mRNA expression of senescence markers, p16, p21 and p53. The cells were divided into four groups；young cells（Control）, young+H_2O_2（Old）, young+LPS（Y+LPS）and old+LPS（O+LPS）. For LPS treatment, cells were treated with Pg LPS（1μgmL^{-1}）. The mRNA and protein expression of junctional proteins such as claudin-1, -2 and E-cadherin were compared using quantitative real time polymerase chain reaction and western blot, respectively. Mann-Whitney U test was used to test the significance.
Results: The mRNA expression of claudin-1, -2 and E-cadherin were all upregulated in the old cells as compared to young and Y+LPS cells whereas claudin-1 and E-cadherin was downregulated in O+LPS cells as compared to old cells（$p<0.05$）. The protein expression, confirmed by western blot were all in accordance with the mRNA expression.
Conclusion: Age related alteration of junctional molecules may be responsible for progressive tissue destruction in elderly gingival tissues.

GB-34

Effects of mechanical stress on cells derived from human oral mucosa

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Background and objective: The oral mucosa is exposed to mechanical stress（MS）including occlusal force via the denture. In this study, we applied MS to cells derived from human oral mucosa and evaluated its effects.
Materials and Methods: Subconfluent human oral mucosal epithelial cells（HO-1-N-1）and human gingival fibroblasts（HGF）were used to seed 1 10^5 cells/dish and, after 24 hours, subjected to MS（1 and 3 MPa, 60 min）using a hydrostatic pressurizer. Morphological changes of the cells were examined by Giemsa stain, and changes in cell activity were measured by the WST assay. mRNA was collected from the cells, and inflammatory cytokines and growth factors genes were measured by real-time RT-PCR assay and ELISA.
Results: HO-1-N-1 showed no morphological change by Giemsa stain but exhibited a significant decrease in cell activity by WST assay. In HO-1-N-1, the expression of inflammatory cytokines, and ICAM mRNA was significantly increased after MS at 1 MPa but was attenuated at 3 MPa. In HGF, the expression of IL-6 mRNA was enhanced. In HO-1-N-1 and HGF, the IL-6 and IL-8 protein levels increased significantly after MS. In HGF, the EGF and FGF-2 protein levels increased significantly by stimulation at 3 MPa.
Conclusion: MS applied to the oral mucosa caused a decrease in the activity of oral mucosal epithelial cells and induced enhancement of inflammatory cytokine production. Also, excessive MS was suggested to promote the cure of oral mucosa wounds by promoting the production of growth factors in the oral mucosa.

GB-35

Anti-inflammatory effects of β-cry on 5FU-induced cytokine production in hOMF

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Background and objective: Beta-cryptoxanthin（β-cry）, a carotenoid abundant in satsuma mandarin, has anticancer and antioxidant effects. We previously reported the anti-inflammatory effects of β-cry on the periodontal ligament. However, the effects of β-cry on oral mucositis, an adverse event due to cancer chemotherapy, remain unclear.
Fluorinated pyrimidines are frequently employed for oral cancers, with a high incidence of stomatitis. In the present study, we examined the effects of 5-fluorouracil（5-FU）and β-cry on oral mucosal fibroblasts.
Materials and Methods: Human oral mucosal fibroblasts（hOMF）were cultured with 5FU（1 Hg/mL）and/or β-cry（100 nM）. Then, a cell proliferation test was performed by WST assay and the expressions of inflammatory cytokines and apoptosis-related genes were examined by real-time RT-PCR.
Results: The proliferation of hOMF was inhibited by the addition of 5-FU, and the inhibition was suppressed with β-cry. Inflammatory cytokines, such as IL-1β, IL-6, and IL-8, and the ratio of Bax/Bcl-2 in hOMF were increased with 5-FU, and the increase was suppressed with β-cry.
Conclusion: Beta-cry may have anti-cytotoxic and anti-inflammatory effects on oral mucositis caused by 5-FU, suggesting that it may provide a novel method for preventing oral mucositis due to cancer chemotherapy.

General
(Clinical Research)

GC-01

Clinical outcomes of rhFGF-2 therapy with M-MIST for intrabony defects

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Background and objective: Modified Minimally Invasive Surgical Technique（M-MIST）（Cortellini and Tonetti 2009）was designed to improve wound stability and reduce patient morbidity. This case-series aimed to evaluate clinical outcomes of periodontal regenerative therapy using recombinant human fibroblast growth factor（rhFGF）-2 with M-MIST for the treatment of intrabony defects.
Methods: Following initial periodontal therapy, six intrabony defects（mean depth；3.6 mm, width；2.1 mm）in four patients received rhFGF-2 therapy with M-MIST. A buccal incision was placed to gain access to the defect without interdental and lingual incisions. After granulation tissues were removed by sharp dissection and root instrumentation, 0.3% rhFGF-2 was applied to the defect. Flaps were immediately closed by sutures. Probing depth（PD）, clinical attachment level（CAL）, gingival margin（GM）, and radiographic bone fill were evaluated at baseline, 3 and 6 months postoperatively.
Results: The mean surgical time was 48 ± 5 minutes. Early wound healing was uneventful: primary wound closures were obtained in all sites at 1 week postoperatively. Patients did not report any pain or swelling. At 6-months postoperatively, mean PD reduction was 3.8 ± 0.8 mm and CAL gain was 4.3 ± 0.5 mm. A minimal increase in gingival recession（-0.5 ± 0.5 mm）was recorded. An improvement in radiographic bone fill（53.3 ± 33.3%）was noted.
Conclusion: This case series showed that rhFGF-2 therapy with M-MIST yielded favorable clinical and radiographic outcomes in the treatment of intrabony defects.

GC-02

Periodontal regeneration using rhFGF-2 with DBBM: An 18-month follow-up of a randomized controlled trial

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Background and objective: In our earlier randomized controlled trial comparing use of recombinant human fibroblast growth factor（rhFGF）-2 in combination with deproteinized bovine bone mineral（DBBM）and rhFGF-2 alone in the treatment of intrabony defect, we reported no significant difference in clinical attachment level（CAL）gain between groups at 6 months. We also showed that the combination therapy yielded an enhanced radiographic outcome. The aim of this follow-up study is to evaluate the outcomes at 18 months postoperatively.
Materials and Methods: Participants in the base study（Saito et al. JCP 2019）were reevaluated at 12 and 18 months postoperatively. Original measures（clinical parameters and patient-reported outcome measure）were repeated. Radiographic bone fill was evaluated at 12 months.
Results: At 18 months postoperatively, a total of 38 sites in 30 patients were reevaluated. In both test（rhFGF-2+DBBM）and control（rhFGF-2 only）groups, there was a progressive improvement in CAL and PD values during maintenance care. No significant difference between groups was observed. At 12 months, mean value for radiographic bone fill in the test group（54.6%）was significantly greater than that in the control group（36.7%）（p = 0.003）. In both groups, no significant change in total OHRQL score was observed at 18 months.
Conclusion: In the treatment of intrabony defects using rhFGF-2, with or without DBBM, further improvement in CAL was observed at 18-month follow-up. The combination therapy yielded a significantly greater bone fill. In both groups, favorable clinical outcomes can be sustained for at least 18 months.

GC-03

Oral health behavior and the oral condition of pregnant women

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Background and objective: The association between periodontal disease and low birth weight has been previously reported. Therefore, maintaining good oral hygiene during pregnancy is important to reduce the risk of a poor pregnancy outcome. The objective of this study was to investigate oral health behavior in relation to oral condition in pregnant women.
Materials and Methods: Fukuoka City provides health services, including dental examinations, for pregnant women. Pregnant women who underwent examinations between 2018 and 2019（n = 4,275）participated in this study. The presence of decayed teeth was evaluated, and periodontal disease was assessed using the modified Community Periodontal Index（CPI）.
Results: The percentage of pregnant women with decayed teeth and deep periodontal pockets（pocket score 2 in CPI）was 49.4％ and 12.9％ , respectively. Logistic regression analysis showed that the presence of decayed teeth was associated with no regular dental visits before pregnancy（odds ratio［OR］2.22, 95％ confidence interval［CI］1.95–2.52）and eating between meals, three times and over per day（OR 1.24, 95％ CI 1.00–1.54）. The deep periodontal pockets were associated with no regular dental visits before pregnancy（OR 1.61, 95％ CI 1.31–1.98）and no use of dental floss（OR 1.26, 95％ CI 1.04–1.52）.
Conclusion: This study found that pregnant women who did not regularly visit a dental clinic before pregnancy tended to have poor oral conditions. It is suggested that pregnant women focus on their oral health behavior in order to maintain good oral health.

GC-04

Systemic parameters associated with periodontal inflamed surface area

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Background and objective: The association between periodontitis and systemic disease has been shown, and it has been reported that patients with severe periodontal disease are likely to develop systemic disease. Periodontal inflamed surface area（PISA）is the total surface area of the periodontal pocket at the sites with bleeding on probing. Although the usefulness of PISA as a periodontal indicator, information on the link between PISA and markers for systemic disease is still scarce. The purpose of the present study was to clarify systemic parameters associated with PISA.
Materials and Methods: Subjects were recruited at Medical and Dental Collaboration Center in Kanagawa Dental University Hospital from 2018 to 2020. PISA was calculated from the periodontal pocket depth and bleeding on probing, and oral function examinations, body composition analysis, and blood tests were performed. Comparative analysis was performed for PISA and markers of general condition. The Ethics Committee of Kanagawa Dental University approved the protocol of the present study（No, 553）.
Results: Totally 200 participants（136 women and 64 men）with mean age of 68.3 were enrolled in this study. The systemic markers correlated with PISA were body fat mass, percent body fat, body mass index, waist circumference, calf circumference, and serum gamma-GTP. In addition, oral markers correlated with PISA were protein, leukocyte, halitosis, salivary occult blood, and red-complex bacteria in dental plaque.
Conclusion: The results of this study revealed a link between PISA, a periodontal inflammation marker, and many systemic and oral disease markers.

GC-05

Risk factors for chemotherapy-induced febrile neutropenia in hematological cancer patients

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Background and objective: Febrile neutropenia（FN）is one of the frequent adverse events of hematological cancer chemotherapy. The aim of this study is to investigate the association between chemotherapy-induced FN and oral bacteria and/or oral immunity in patients with hematological cancer.
Materials and Methods: Thirty-two patients with hematological cancer who received their first chemotherapy at Tokushima University Hospital were enrolled in the study. The chemotherapeutic drug used, the systemic condition, the period including the presence of fever were obtained from the patient medical record. Secretory immunoglobulin A（sIgA）in saliva and the anaerobic bacteria in tongue coating of each subjects was assessed before the first chemotherapy.
Results: Eleven subjects had an onset of FN（FN subjects）and 21 subjects did not（non-FN subjects）during the observation periods. A significant difference was observed in the levels of sIgA between two groups（$p<0.05$）. The rate of *F. nucleatum* count per total bacterial count（%）showed a higher tendency in the FN subjects than that in the non-FN subjects（$p=0.088$）. Using ROC curve analysis, the optimal cut-off point based on the AUC in the *F. nucleatum* / sIgA ratio was 0.023, and the risk of FN onset was significantly higher among the group of ≥ 0.023 *F. nucleatum* / sIgA ratio compared with the group of＜0.023（HR 36.3, $p<0.01$）.
Conclusion: These results suggest that the rate of *F. nucleatum* and the levels of sIgA at baseline might be related to FN onset as risk factors.

GC-06

Efficacy of toothbrushes with titanium dioxide electrode and solar cell

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Background and objective: Effective control of dental plaque by daily brushing is critical for oral health. Toothbrushes with titanium dioxide electrode and solar cell have been reported to be efficient；this study was designed to evaluate the dental plaque removal effect of these toothbrushes.
Materials and Methods: The subjects included 8 members of the Nippon Medical School Chiba Hokusoh Hospital Dental Clinic, who had thorough knowledge regarding the techniques of maintaining oral hygiene. The six teeth selected by Ramfjord（16, 21, 24, 36, 41, and 44）were used. The toothbrushes comprised the sonic powered type（SOLADEY RHYSHM 2®, group R2）, the manual type with large solar cell（SOLADEY N4®, group N4）, and the manual type with small solar cell（SOLADEY 3®, group SO3）；all were manufactured by Shiken Co., Ltd. The Rustogi Modified Navy Plaque Index was used.
Results: The rate of dental plaque removal in the entire mouth was 77.1% for group R2, 63.8% for group N4, and 55.2% for group SO3；the same trend was noted on the surface of all six teeth.
Conclusion: In this study, toothbrushes with titanium dioxide electrode and solar cell, both sonic-powered and manual types, were effective for plaque control.

GC-07

Retrospective study of dental implant treatment for patients with periodontitis.

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Background and objective: Although the choice of rehabilitation with implant-supported fixed prostheses in periodontal therapy is increasing, the pathogenesis of peri-implant disease in the patients with periodontitis is still unclear. We performed a retrospective study to assess the outcome of implant treatment for the patients with periodontitis and the possible risk factors of peri-implant disease.
Materials and Methods: Eighty seven patients had been treated comprehensively from 2006 to 2018 in Ohu University Dental Hospital and then 342 implants were enrolled for this retrospective study. Success rates were calculated from the degree of marginal bone loss（MBL）as part of a prognostic study. Various clinical data and patients-related risk factors such as smoking habit, occlusal parafunction, bone augmentation and diabetes mellitus, were analyzed. Chi-square test was used for statistical processing.
Results: The average period after getting occlusal function was 63.8 ± 29.9 months and the survival rate was 95.9%. Compared with survival implants, the disintegrated implant had a single implant and bone augmentation with artificial bone（GBR）compared with survival implants. The success rate calculated by MBL＜3.0mm was 85.7% , while MBL ≧ 3 mm were found in the patients with high risk for periodontitis compared with others（p＜0.05）.
Conclusion: These results suggest that prostheses with a single implant and GBR could be risk for disintegration of implant and the progression of peri-implantitis is much faster for high-risk patients with periodontitis than the others.

GC-08

Carotid artery calcification and periodontal disease progression in Japanese people

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Background and objective: The purpose of this study is to evaluate the association between alveolar bone loss（ABL）detected on panoramic radiographs and carotid artery calcification（CAC）, which is the final step in the development of atherosclerosis, detected on Computed Tomography（CT）.
Materials and Methods: The subjects were 295 patients（mean age ± SD, 64.6 ± 11.8 years）who visited Matsumoto Dental University hospital between 2014 and 2018. The rate of ABL and number of present teeth were measured on panoramic radiographs. Univariate analyses with the t-test and the chi-squared test were used to evaluate the differences in age, gender, history of diseases, number of present teeth and ABL between subjects with and without CAC. Moreover, multivariate logistic regression analysis with forward selection was used. Finaly, receiver operating characteristic curve（ROC）analysis was used to clarify how degree for the identification of CAC by ABL.
Results: Number of subjects with and without CAC was 121 and 174. Univariate analyses showed that CAC was significantly associated with age, hypertension, osteoporosis, number of present teeth and ABL. Multivariate logistic regression analysis with forward selection adjusted for covariates revealed that the presence of CAC was significantly associated with age（OR=1.096, 95%CI=1.051-1.143）, hypertension（OR=3.748, 95%CI=1.748-8.037）and ABL（OR=1.233, 95 % CI=1.167-1.303）, significantly. In the ROC analysis predicting the presence of CAC, AUROC was the highest 0.932（95% CI= 0.904-0.960）for ABL, significantly.
Conclusion: It was suggested that measurement of ABL on panoramic radiographs may be an effective tool for identifying patients with an increased risk of CAC.

GC-09

The influence of periodontal disease in peripheral artery disease patients

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Background and objective: Peripheral artery disease（PAD）is a kind of arteriosclerosis that leads to ischemia in extremity. Some studies reported that patients with periodontitis are at a risk for PAD. But evidence on the relationship between these two diseases has not yet been clarified completely. Therefore, we performed a cross-sectional study to assess this relationship by comparing patients with PAD to those with arrhythmia（ARR）as a control group.
Materials and Methods: A large-scale survey was conducted on patients who received cardiovascular care at Tokyo Medical and Dental University Hospital. Clinical dental findings such as PPD, BOP, CAL and number of tooth loss were measured in all patients. Subgingival plaque, salivary samples, peripheral blood samples were also collected. Besides, we performed real-time PCR and ELISA to detect inflammatory mediator levels such as tumor necrosis factor（TNF）-alpha, interleukin（IL）-6, matrix metalloproteinase（MMP）-2, 8, 9 and bacterial counts and anti-bacterial antibody titers in the samples. All patients with PAD and one tooth at least were extracted from the overall study population. We extracted patients with ARR by matching the sample size of the group in terms of age, prevalence of hypertension, dyslipidemia, diabetes, obesity, and the smoking rate（n=20 for each）.
Results: There were significantly higher values in PAD patients than ARR in terms of serum levels of TNF-alpha, IL-6, MMP-8, 9 and tooth loss. Multivariate logistic analysis also showed that significantly higher odds ratios were observed in tooth loss and TNF-alpha of PAD than ARR.
Conclusion: This study suggested that periodontal inflammation might be correlated with PAD through systemic inflammation.

GC-10

Genetic and Environmental Factors on Periodontitis in Elderly Twins

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Background and objective: Periodontitis is caused by dental biofilms, while the genetic and environmental factors affect the onset and progression of the disease through life. In this study, we aimed to evaluate the effort ratios between genetic and environmental factors on periodontitis by comparing the clinical phenotypes of periodontitis in pairs between monozygotic（MZ）and dizygotic（DZ）twin pairs in elderly Japanese populations.
Materials and Methods: 147 pairs of twins were examined at the Twin Research Center of Osaka University Graduate School of Medicine. The following tests were performed: 1）remaining teeth, 2）probing depth（PD）, 3）percentage of bleeding on probing（BOP）sites（%）, 4）score of bone resorption, 5）Serum antibody titer for periodontopathic bacteria. dental plaque.
Results: Twin intra-class correlations for PD and % of BOP sites in MZ twins was higher than DZ twins. On the other hand, those for the number of remaining teeth and score of bone resorption in MZ twins was comparable to DZ twins. In MZ female twins, intra-class correlations for % of BOP sites were high but for PD, score of bone resorption and number of remaining teeth were low.
Conclusion: For the pathology of periodontitis, PD and BOP, which are related to the patient's inflammatory responses, are likely to be relatively influenced by genetic factors, while bone resorption and tooth retention are likely to be relatively influenced by environmental factors in elderly populations.

Case Report

CR-01

Case reports: Periodontal regeneration with FGF-2 plus β-TCP

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Background: Human recombinant fibroblast growth factor（FGF-2）was approved as a periodontal regenerative medicine in Japan and has been commercially available（Regroth®）since 2016. Several clinical reports demonstrated that FGF-2 even alone could induce periodontal regeneration efficiently. However, in cases where bone defects are large and complicated, complete recovery by FGF-2 might not be possible. In this report, successful cases treated with FGF-2 plus β-tricalcium phosphate（β-TCP）as a bone filling material will be presented.
Case: Three patients（male 54-year-old, female 50-year-old, female 65-year-old）presented with severe one-wall infrabony defects at each anterior tooth site（#23, #12, #43, respectively）.
Clinical Procedures and Outcomes: The periodontal regenerative operation with FGF-2 plus β-TCP（Cerasorb® M granulat dental, 150- 500 μm, Curasan, Germany）was performed. The incision line was avoided above the bone defect wherever possible. Full thickness buccal and lingual mucoperiosteal flaps were reflected. Residual calculus and granulation tissues were thoroughly debrided and the root surfaces were treated with 24% EDTA（Prefgel®, Straumann, USA）for 2 minutes. Then, FGF-2 plus β-TCP were applied into the infrabony defects. Buccal and lingual flaps were closed with simple sutures under tensionless. Professional cleaning of the surgical sites was performed every visit and periodontal and dental X-ray examinations were carried out after the operation carefully. 5-7 months after the operation, the new bone formation was observed at the bone defects in each patient.
Conclusion: These case reports suggest that FGF-2 plus β-TCP could be useful for periodontal regeneration in cases where bone defects are severe.

CR-02

Treatment of chronic periodontitis using rhFGF-2 in combination with DBBM

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Background: Here we report a 1.5-year follow-up of a chronic periodontitis patient who had received periodontal regenerative therapy using rhFGF-2 with deproteinized bovine bone mineral（DBBM）.
Case: The patient was a 49-year-old woman who presented with the chief complaint of swollen gums. An initial examination revealed that 33.4 % of sites with a probing depth（PD）of ≥ 4 mm and 29.5 % of sites with bleeding on probing. The O'Leary plaque control record was 34.6% . Radiographic examination revealed angular bone resorption in # 22, 33, 36, 46 and 47. A clinical diagnosis of generalized chronic periodontitis（Stage Ⅲ Grade B）was made.
Clinical Procedures and Outcomes: The patient underwent initial periodontal therapy including plaque control, scaling and root planing. After reevaluation, periodontal regenerative therapy using rhFGF-2 were performed on #22 and 46, 47. Combination therapy with rhFGF-2 + DBBM was performed on #33, 36. Following reevaluation, the patient was placed on supportive periodontal therapy. A 3.3 mm gain in mean clinical attachment level（CAL）was observed at the sites treated with rhFGF-2 at 1.5 year after surgery. In #33 and 36, rhFGF-2 + DBBM, treatment yielded CAL gains of 4.0 mm and 3.0 mm, respectively. Distinct radiographic bone fill was observed at 1.5 year following the treatment with rhFGF-2 + DBBM.
Conclusion: In the present case, periodontal regenerative therapy resulted in a decrease in PD and improvement in CAL. Combination therapy using rhFGF-2 + DBBM yielded an enhanced radiographic bone fill. This improvement has been adequately maintained over a 1.5-year period.

CR-03

Two-year follow-up case of periodontal regenerative therapy using rhFGF-2

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Background and objective: Here we report a two-year follow-up of a chronic periodontitis patient who received periodontal regenerative therapy using rhFGF-2.
Case: The patient was a 54-year-old man who requested treatment of periodontal disease. Initial examination revealed 57.4 % of sites with a probing depth（PD）of ≥ 4 mm, mean PD was 4.3 mm. The level of plaque control as assessed by the O'Leary plaque control record was 92.6%. Radiographic examination revealed angular bone resorption in #14 and 33. Patient's oral health-related QoL was assessed by the OHRQL instrument. The total OHRQL score in this patient was 21. A clinical diagnosis of generalized chronic periodontitis（Stage III, Grade B）was made.
Clinical Procedures and Outcomes: The patient underwent initial periodontal therapy including plaque control, scaling and root planing, and root canal treatment. After reevaluation, a series of surgical interventions were performed in #14-16, 26, 27, 33-37, 46, 47. Because the distal aspect of #14 and 33 had intrabony defect, regenerative therapy using rhFGF-2 was performed. Following reevaluation, then the patient was placed on supportive periodontal therapy. At 2 years postoperatively, in #14 and 33, the treatment yielded clinical attachment level（CAL）gains of 3.5 mm and 2.5 mm, respectively. Distinct radiographic bone fill was observed following the treatment with rhFGF-2. Sites with a PD of ≥ 4 mm improved to 4.9% and mean PD to 2.6 mm. The total OHRQL score was improved to 10.
Conclusion: At 2 years postoperatively, the regenerative therapy using rhFGF-2 yielded improvements in clinical parameters, radiographic bone fill and patient's oral-health related QoL.

CR-04

Treatment of chronic periodontitis using rhFGF-2 and GTR with bone graft

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Background: Here we report a 1-year follow-up of a chronic periodontist patient who had received periodontal regenerative therapy using recombinant human fibroblast growth factor（rhFGF）-2 and guided tissue regeneration（GTR）with autogenous bone graft.
Case: The patient was a 71-year-old woman who presented with the chief complaint of mobile tooth（#14）. An initial examination revealed that 53% of sites with a probing depth（PD）of ≥ 4 mm, mean PD was 3.6 mm, and 28% of sites with bleeding on probing. The O'Leary plaque control record was 62% . Radiographic examination revealed angular bone resorption（#14, 15）. Furcation involvement was Degree Ⅱ for #46. Patient's oral health-related quality of life was assessed by the OHRQL instrument. The total OHRQL score was 23. A clinical diagnosis of generalized chronic periodontitis（Stage Ⅳ, Grade B）was made.
Clinical Procedures and Outcomes: The patient underwent initial periodontal therapy. After reevaluation, periodontal regenerative therapy using rhFGF-2 was performed on #14 and 15. Combination therapy with GTR and autogenous bone graft was performed on #46. Following reevaluation, the patient was placed on supportive periodontal therapy. A 2 mm gain in clinical attachment level（CAL）was recorded at the sites treated with rhFGF-2 at 1 year after surgery. In #46, GTR + bone allograft, treatment yield a CAL gain of 4 mm. An improvement in radiographic bone fill was noted. OHRQL score was reduced to 10.
Conclusion: In the present case, periodontal regenerative therapy resulted in a decrease in PD and improvement in CAL, radiographic bone fill and OHRQL score.

CR-05

The 27-year course of generalized chronic periodontitis in a patient with posterior bite collapse

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Funakoshi Dental Office

Purpose: Posterior bite collapse can cause secondary occlusal trauma and progression of periodontitis. In the present case, combined periodontal and partial orthodontic treatment improved the occlusion and periodontal environment for easier self-care and enabled the patient to maintain a favorable condition for 27 years, despite not visiting a clinic for 12 years during that period.
Case description: A 42-year-old female presented to the author's clinic on November 14, 1992 with a chief complaint of difficulty in chewing with her back teeth. Generalized gingival redness and swelling were observed in addition to a periodontal pocket of over 4 mm. Vertical bone defect with a deep periodontal pocket was observed.
Diagnosis: Generalized moderate chronic periodontitis with secondary occlusal trauma
Treatment protocol: The treatment protocol consisted of the following: (1) initial periodontal therapy, (2) re-evaluation, (3) partial orthodontic treatment, (4) periodontal surgery, (5) re-evaluation, (6) restorative dental treatment, and (7) supportive periodontal therapy (SPT).
Treatment course: Partial orthodontic treatment was performed in the mandible to straighten teeth 37 and 47 as well as improve the position of each mandibular tooth. Flap surgery was performed on teeth 37, 42, 43, and 47. After restorative dental treatment, stabilization of the occlusion and periodontal tissues was confirmed, and treatment was transitioned to SPT.
Results and conclusions: The present case suggests the importance of creating proper occlusion and a favorable oral environment that facilitates easy self-care for the patient.

CR-06

Treatment of generalized chronic periodontitis with rhFGF-2 and GTR

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Background: For the treatment of deep angular bone defects, periodontal regenerative therapy is indicated. We report a case of generalized severe chronic periodontitis treated by periodontal regenerative therapy ; recombinant human fibroblast growth factor (rhFGF)-2 and guided tissue regeneration (GTR).
Case: The patient was a 54-year-old woman who presented with the chief complaint of swelling in maxillary right gingiva. An initial examination revealed that 30.4% of sites with a probing depth of ≥ 4 mm. The prevalence of sites with bleeding on probing was 57.7% . The plaque control record was 66.1% . Radiographic examination revealed vertical bone defects around the molars. A clinical diagnosis of generalized chronic periodontitis (Stage III, Grade C) was made.
Clinical Procedures and Outcomes: The patient underwent initial periodontal therapy. An improvement was observed in periodontal conditions at reevaluation. A series of periodontal regenerative therapy with rhFGF-2 were performed on angular bone defects in #14, 15, 25, 27. GTR was performed on #37, 47. Open flap debridement was performed on #16, 17, 26, 36, 46. Following reevaluation, the oral function was restored using all-ceramic crowns (#21, 26). Then the patient was placed on supportive periodontal therapy.
Conclusion: In the present case of generalized chronic periodontitis, periodontal regenerative therapy with rhFGF-2 and GTR resulted in a stable periodontal condition that facilitated favorable level of plaque control.

CR-07

A Case of regenerative therapy of maxillary canine with intrabony defect for periodontal prosthesis.

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Background: In patients with periodontitis, the occlusal load on the anterior teeth increases due to the missing molar teeth, along with progression of shifting of diseased teeth and occlusal collapse due to secondary occlusal trauma. In such a case, the propriety of preserving a single tooth has a notable effect on the prosthetic design. This patient underwent correction and regeneration therapy for severe periodontitis and intrabony defect due to occlusal trauma in a canine. Occlusal reconstruction was performed using prosthesis and good progress was achieved.
Case: The patient was a 58-year-old female who was first examined in September 2018 for chief complaint of swelling in #23 region, and oral examination revealed grade-2 mobility and swelling in #23. A dental radiograph showed intrabony defect extending up to 1/3rd of the root. Her medical history revealed hypertension and a non-smoker.
Clinical procedure and outcome: An old bridge prosthesis using #23 as abutment teeth was removed, and after performing primary periodontal treatment, #23 was extruded. We then performed regeneration therapy using b-FGF in order to restore the periodontal tissue of #23 that supported the maxillary prosthesis. It had a double crown bridge structure for removable design due to corresponding future washout of cement.
Conclusion: Bone formation in the anterior tooth area of #23 improved, and the tooth has remained stable. When designing prosthesis that uses periodontally affected teeth with intrabony defects as abutments, the recovery of supporting periodontal tissue by correction and regeneration therapy is extremely useful for achieving stability.

CR-08

Orthodontic treatment in two patients with severe periodontitis and high pathological mobility of the anterior teeth

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Background: Pathological tooth migration is often observed as periodontitis progresses. Here, we report two cases of severe periodontitis with significant morbid tooth migration for which extraction was considered, but where orthodontic treatment successfully led to conservation of the teeth.
Case 1: The patient was a 52-year-old woman who was a smoker. The date of initial consult was April 16, 2014. The chief complaints were gingival swelling and hemorrhage. The patient first went to another hospital and was recommended to visit a periodontist. The patient had a medical history of pediatric asthma and slight hypertension. Oral examination indicated gingival reddening and swelling, as well as deep periodontal pockets. Overbite and overjet were also observed, her #11 tooth was greatly displaced in the labial direction, and the mandibular anterior teeth showed protrusion and crowding.
Case 2: The patient was a 42-year-old woman. Her details are printed on the poster.
Details of Treatment and Results: In both cases, re-evaluation was performed after completion of basic periodontal treatment, and orthodontic treatment or definitive periodontal surgery was performed. Oral function recovery therapy was initiated, which was followed by supportive periodontal therapy（SPT）.
Conclusion: Both cases showed pathological tooth migration to the extent that extraction was considered, but the use of orthodontic treatment and attempts to conserve the teeth as much as possible greatly contributed to patient motivation and led to the teeth being retained.

CR-09

New surgical approach for the treatment of periodontal intrabony defects

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Background: In this study, we devised a new surgical approach for the treatment of periodontal intrabony defects.
Cases: Case 1 was a 65-year-old male（non-smoker）with target tooth 27 showing distal probing pocket depth（PPD）of 9 mm.
Case 2 was a 48-year-old female（non-smoker）with target tooth 47 showing distal PPD of 8 mm.
Treatment and Results: Procedure: A #390 scalpel blade was tilted to approximately 45°, and a full-thickness incision to the periosteum was made at an angle of approximately 30° from the distolingual line angle of the second molar while avoiding the bone defect. After making an incision on the gingival sulcus on the distal-most surface of the second molar, a horizontal incision was made in the bone defect from the lingual side to the buccal bone crest to form an interdental papilla-like buccal valve. After removal of infected granulation tissue, followed by debridement and application of periodontal tissue regeneration material, the bone graft material was packed. A needle was inserted into the buccal valve and lingual keratinized gingiva to perform internal cross-mattress suturing, and the thread on the lingual side was used to perform modified mattress suturing. One to two more simple sutures were applied at the distal-most end, and suturing was completed.
In the re-evaluation performed 9 months postoperatively, dental computed tomography showed the presence of bone formation and amelioration of the PPD.
Conclusion: This method is a minimally invasive procedure that allows for easy dissection, rapid completion of the procedure, and secure wound closure.

CR-10

10-year follow-up of severe chronic periodontitis with occlusal trauma

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Background: Severe chronic periodontitis causes the affected tooth loses attachment and increases mobility, resulting in occlusal trauma. We report on a periodontitis patient with occlusal trauma over a ten-year follow up.
Cases*:* A 58-year-old female presented on May , 2007 with the chief complaint of gingival swelling and pain in the maxillary left region. 44.4% of sites had a PPD ≥4 mm and 57.2% of sites exhibiting BOP. Tooth mobility 1 ～ 3 and PPD ≥6 mm in the molars with occlusal interference at the non-working side on jaw movements. #25 had a PPD 10 mm palatally, with mobility 3. #47 had a PPD 10mm mesially, with mobility 3 and a two-wall bony defect mesially detected.
Clinical Procedures and Outcomes: Full mouth disinfection was performed with azithromycin because P. gingivalis was detected by the real-time PCR（5.36%）. Three months later, BOP improved（8%）but the mobility in the molars remained. Therefore, #38 and 48 were extracted and occlusal adjustment was performed on the molars. At the reevaluation, tooth mobility improved. Regenerative therapy was performed on #15 and 47 for the intrabony defects and seven months later the regeneration was confirmed. #15 was splinted to #14
Conclusion: Periodontal treatment combined with occlusal adjustment reduced tooth mobility, indicating trauma from occlusion was involved with periodontitis progression. No significant change was observed over ten years, aside from enamel defect on #47 which was treated with a crown restoration. SPT will continue while controlling and eliminating trauma factors as well as inflammation.

CR-11

Long term observation: healing pattern after periodontal treatments

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Background: Long epithelial attachment（LEA）, which is previously known as an unstable healing condition, is considered to be a healing pattern after scaling and root planing（SRP）and open flap debridement（OFD）. However, an animal study by Shimono et al［1］ demonstrated that LEA could be eventually replaced by connective tissue attachment（CTA）. Here, I report the findings showing the replacement of LEA by CTA in a patient with severe periodontitis who has undergone periodontal maintenance（PM）for 25 years.［1］Shimono M. Pathology of Wound Healing, New Edition – Answering from Basic Science to Clinical Questions –, Ishiyaku Publishers, Tokyo,2011.
Case Report: A 43-year-old woman firstly visited our dental clinic with chief complaint of gingival swelling and pain of tooth #27 on September, 1995. Full mouth radiographs indicated alveolar bone loss（stage IV/grade C periodontitis）.
Clinical Treatment and Outcomes: Following SRP and occlusal adjustment, OFD was carried out, consequently, inflammation disappeared and a stable condition was returned in the periodontal tissues. During the PM period, the gingiva has become thick, and even the lamina dura has been radiographically apparent. Volume rendered 3D CT image showed the regeneration of alveolar bone after 25 years of the debridement, suggesting that LEA is replaced by CTA.
Conclusion: Long epithelial attachment is not an unstable periodontal healing pattern, but can be replaced by connective tissue. Continuing maintenance care for eliminating periodontal pockets and preventing a recurrence of flare-up is of extremely importance.

CR-12

A case of Root Coverage for gingival recessions with Subepithelial connective tissue and bFGF

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Background: The use of subepithelial connective tissue in root coverage evidentially presents permanent treatment results. Its combined use with the basic fibroblast growth factor（bFGF）seems more effective because it promotes the growth of periodontal ligament fibroblasts and the migration and proliferation of multiple cells forming the periodontal tissue, e.g., vascular endothelial cells, epithelial cells, and osteoblasts.
We presented a case where root coverage using subepithelial connective tissue and bFGF favorably treated the gingi-val recession of the maxillary anterior teeth.
Case: Patient: 51-year-old woman（non-smoker）
Initial visit: November 2018
Chief complaint: Cosmetic disturbance
General medical history: None
Dental history: Dental treatment at another clinic five years previously
Examination findings: Mild gingival recession of 11 and 21 and 3 mm gingival recession of 23（Class I under Miller's classification）
Treatment and Results: 1）Basic periodontal treatment, 2）Re-evaluation, 3）Periodontal surgery（root coverage）, 4）Re-evaluation, and 5）Maintenance
Periodontal surgical treatment:
23: Coronally-advanced flap using subepithelial connective tissue
11 and 21: Modified coronally-advanced tunneling technique using bFGF combined with subepithelial connective tissue
Three months postoperatively, follow-up was performed once a month along with regular maintenance.
Currently, periodontal tissue at the transplant site is stable and the postoperative course is favorable.
Conclusion: Remarkable root coverage was achieved at the gingival recession site using bFGF, a cell-growth factor having various cell growth and angiogenic effects. Thus, better results can be obtained with root coverage than with conventional methods. However, continued investigations on controlling the disease progression are necessary.

CR-13

Periodontal regenerative therapy using rhFGF-2 and DBBM for furcation defects

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Background: The key points in treatment of furcation involvements are to prevent further attachment loss and to make maintenance easier. Here we report a chronic periodontitis case with furcation involvements treated by rhFGF-2 with deproteinized bovine bone mineral（DBBM）.
Case: The patient was a 50-year-old man who requested treatment of periodontal disease. An initial examination revealed that 30.4% of sites with a probing depth（PD）of ≥4 mm and 69.6% of sites with bleeding on probing（BOP）. Radiographic examination revealed angular bone resorption in #17, 24, 26, 32, 34 and 46. Furcation involvements（Degree Ⅱ）were observed in #26 and #46. A clinical diagnosis of generalized chronic periodontitis（Stage Ⅲ Grade B）was made.
Clinical Procedures and Outcomes: The patient underwent initial periodontal therapy. After reevaluation, periodontal regenerative therapy using rhFGF-2 were performed on #32 and combination therapy with rhFGF-2 + DBBM was performed on #26, 34 and 46. Following reevaluation, oral function was restored using a fixed bridge prosthesis. At the transition to supportive periodontal therapy, 1.2% of sites presented with a PD of ≥4 mm and 9.9% of sites with BOP. At 2 years postoperatively, a 3.0 mm gain in clinical attachment level（CAL）was observed in #32 treated with rhFGF-2. Furcation involvements in #26 and 46 treated with rhFGF-2 + DBBM yielded CAL gain of 4.0 mm.
Conclusion: In the present case of chronic periodontitis with furcation involvements, the regenerative therapies resulted in a decrease in PD, improvement in CAL, and favorable osseous architecture.

CR-14

Improvement of uneven cervical line and gummy smile with periodontal surgery: A case report

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Background: Bone level, gingival level, and continuity of the dentition are important for the long-term stability of the periodontium. The gingival level can be corrected by prosthetic restoration；however, periodontal surgery is required in complicated cases. Several criteria influence the esthetic goals, including the smile line, classified as low, average, or high. The gummy smile（＞3 mm of gingival exposure）is usually considered unesthetic. Obtaining an ideal gum line requires assessment of bilateral symmetry, parallelism, gingival zenith point, and interdental papillary height. The factors affecting the gum line include bone morphology, bone level, tooth position and inclination, root shape, attached gingival width and mode of attachment, and biotype of these, bone level based on the biological width is the most important.
Case: A 26-year-old female patient presented with a chief complaint of unesthetic upper front teeth. Medical history: N.P. Non smoker
Treatment & Result: This case was assessed as type 2 subgroup B based on the altered passive eruption classification by Coslet. Periodontal surgery and prosthetic therapy were performed.
Conclusion: We achieved esthetic restoration, periodontal stability, and patient satisfaction in this case. In addition to paying attention to the shape and shade of the crowns for an aesthetic appearance during dental treatment, an optimal treatment plan based on examination and diagnosis of the relationship between the gums and teeth, i.e., the harmony of the cervical line, must be formulated. Moreover, the harmony between excessive gingival display and the lips and the relationship with the smile line must also be considered.

CR-15

Periodontal regenerative therapy with EMD: a four-year follow-up case

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Background and objective: Here we report a four-year follow-up of a chronic periodontitis patient who received periodontal regenerative therapy with enamel matrix derivative（EMD）.
Case: The patient was a 50-year-old woman who complained of mobile teeth in the molar region. Initial examination revealed 52.6 % of sites with a probing depth（PD）of ≥ 4 mm, full-mouthmean PD was 3.8 mm. The O'Leary plaque control record was 53.8% . Radiographic examination revealed angular bone resorption in #13,15,24,27,33,35,37,46,47. A clinical diagnosis of generalized chronic periodontitis（Stage III, Grade B）was made.
Clinical Procedures and Outcomes: The patient underwent initial periodontal therapy including plaque control, scaling and root planing. After reevaluation, a series of surgical interventions were performed in #13,15-17,24,27,33,35,37,46,47. Regenerative therapy using EMD was performed on the narrow intrabony defects of #13,46,47, and combination therapy using EMD and autogenous bone graft was performed on the wide and deep intrabony defects of #15,24,27,33,35,37. Following reevaluation, then the patient was placed on supportive periodontal therapy. At 4 years postoperatively, #13,46,47 yielded clinical attachment level（CAL）gains of 4 ± 2.4 mm and #15,24,27,33,35,37 yielded CAL gains of 2.5 ± 0.8 mm, respectively. Distinct radiographic bone fill was observed following the treatment with EMD. Sites with a PD of ≥ 4 mm improved to 4.5% and mean full-mouth PD was reduced to 2.2 mm.
Conclusion: At 4 years postoperatively, the regenerative therapy using EMD yielded improvements in clinical parameters and radiographic bone fill.

CR-16

Generalized-chronic periodontitis and type-2 diabetes with comprehensive periodontal therapy

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Background: Chronic periodontitis and diabetes are closely linked, and it is known that controlling periodontitis improves blood sugar level. We report the case of a patient who received periodontal and regenerative therapy. She improved with supportive periodontal therapy（SPT）and blood sugar control.
Case: A 67-year-old woman, who was diagnosed with type 2 diabetes mellitus and fatty liver, visited the hospital after experiencing a strange feeling in tooth #9. An X-ray image revealed a lesion on the apex of the symptomatic tooth（tooth #9）. Gingival swelling and deep periodontal pockets were observed in the molars due to uncontrolled diabetes. Mild horizontal bone resorption was observed throughout the jaw, especially in tooth #30, which was furcated. Teeth #2 and #31 had a cross-bite.
Clinical Procedures and Outcomes: 1.Initial periodontal treatment, instruction on oral hygiene, and tooth #9 apicectomy 2. Re-evaluation 3. Periodontal surgery（tooth #30 periodontal regenerative therapy using basic fibroblast growth factor and autologous bone）5. Re-evaluation 6. Final prosthesis 7. SPT.
Conclusion: The periodontal pockets improved, and gingival swelling was not observed. The patient's blood sugar control improved. Although pockets were observed in the molars, the tissue was stable, and bleeding on probing was not observed. Tooth #9 was in a good condition after apicectomy, and recurrence was not observed. Night guards were prescribed for the cross-bite on the left side of teeth #2 and #31 to reduce an occlusal overload with tooth #30. Interval between SPT continued for a few months for control of plaque formation.

CR-17

Restore Oral functions with Periodontal Therapy with GTR

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Background: In Dec 2010, the patient（58-year-old male）had a concern regarding his discomfort while chewing. This was due to the prosthesis being detached causing difficulty in chewing by the teeth at the posterior region, and then anterior periodontal destruction. In order to treat the masticatory function, we used the prosthetic treatment and periodontal approach.
Case: Gingival swelling and redness were observed throughout the jaw. The probing depth ≥4 mm was 34％ , bleeding on probing was 31％. Class I furcation involvement was observed in tooth #17 #18 and #27. Vertical bone resorption was observed in tooth #24, #31, #45, and #46. Severe bone resorption（up to root apex）was observed at tooth #14, #22, and #27. According to the above findings, the patient was diagnosed as Generalized Severe Chronic Periodontitis with Occlusal Trauma（Stage IV and Grade B）.
Clinical procedures and Outcomes: Initial therapy was performed between January 2011 and September 2012. This was followed up by periodontal surgery which involved a Guided Tissue Regeneration with absorbable membrane and an autogenous bone graft at tooth #46 and a flap surgery at tooth #17 and #18 until September of 2014. After the re-evaluation, oral rehabilitation was completed with the use of a fixed and removable partial denture. Maintenance therapy has been performed since 2016.
Conclusion: This case report presented that recovering masticatory functions by using a therapeutic appliance and severe intrabony defects can be treated with absorbable membrane and autografts. This method improved the periodontal parameters and radiographic bone fill.

CR-18

Management of calcium channel blocker-associated gingival overgrowth without changing medication

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Background: Drug-induced gingival overgrowth is a well-known side effect of calcium channel blocker administration for cardiovascular conditions. Although gingival overgrowth induces periodontitis, dental caries, and masticatory dysfunction, the recommendation to discontinue calcium channel blockers is controversial.
Case: We report a clinical case of severe periodontitis in a 66-year-old Japanese male with a past medical history of hypertension and type 2 diabetes mellitus. He exhibited gingival overgrowth which covered the teeth. He consulted with his physician about changing or finding an alternative for nifedipine, which was linked to gingival overgrowth. Ultimately, nifedipine was discontinued and amlodipine was started during the course of his periodontal disease treatment.
Clinical Procedures and Outcomes: Periodontal treatment consisted of absolute oral hygiene instruction and scaling/root planning. Because the patient's blood pressure was poorly controlled, nifedipine was resumed during periodontal treatment. However, his periodontal clinical scores were significantly improved without changing to another calcium channel blocker.
Conclusion: Rather than being a direct effect of calcium channel blocker administration, aggressive gingival overgrowth may instead result from poor oral hygiene. Significant improvement of gingival overgrowth was observed following basic periodontal treatment, periodontal surgery, and intensive and regular follow-ups to monitor oral hygiene without changing the calcium channel blocker.

CR-19

Ten-year follow-up of a patient who underwent comprehensive treatment for severe chronic periodontitis

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Background and Objective: Severe chronic periodontitis is an inflammatory disease in the periodontium that is characterized by progressive destruction of attachment and bone loss. We report the 10-year follow-up of a patient after periodontal regenerative and localized orthodontic therapies for functional recovery with prosthodontic implants.
Case: A 45-year-old woman had a chief complaint of tooth mobility and swelling of back teeth. She reported that the gingiva had been swollen for 3 years. She was referred by a general practitioner to our dental hospital. In the molar region, significant tooth mobility, a deep periodontal pocket（8 mm）, and infrabony defects were observed. The diagnosis was 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；and（7）supportive periodontal therapy.
Clinical Procedure and Outcomes: After basic periodontal therapy, we performed periodontal regenerative therapy and localized orthodontic therapies. A final prosthesis was chosen to serve as a dental implant for establishing stable occlusion. Consequently, the alveolar bone developed in a normal physiological form, and thorough cleansing of the oral cavity was achieved because of the shallow gingival sulci. The patient's favorable condition has been maintained in the 10 years since the treatment. Conclusion: Comprehensive treatment can enable stable occlusion and establishment of periodontal tissue with high cleansablilty, even in patients with severe periodontitis. As a result, long-term favorable prognosis can be expected.

CR-20

Aberrant occlusal trauma can cause cemental tears that induce periodontitis.

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Background: Multiple risk factors are involved in the progression of periodontal disease. Especially, local factors, such as anatomic anomaly, occlusal trauma and inadequate dental arch may induce a site-specific exacerbation of periodontal tissues. Cemental tears（CTs）has been sometimes found when periodontal surgery has been performed where acute gingival inflammation and tissue destruction had been occurred previously. We show the 3 case series of subcliagnostic CTs and discuss the possibility that occlusal trauma may induce the CTs and play a role on the progression of periodontitis.
Cases: Three patients, 55, 70 and 71-year-old, were enrolled. We observed all of them had a habitual bruxism. Clinical findings were recorded before and after treatment. CTs were not found from oral radiographs at their first visit but firstly found during periodontal surgery. During periodontal surgery, regenerative periodontal therapy with Emdogain® was performed after meticulous root debridement. After the treatment, clinical indices were improved. The probing pocket depth decreased to less than 3 mm without BOP and the radiolucency was improved and the lamina dura was observed at all the sites. In all cases, it was stable for at least 5 years under the control of occlusal dysfunction.
Conclusion: Aberrant occlusal trauma can cause CTs and then induce the rapid destruction of periodontal tissues. Treatment regimen by both regenerative periodontal therapy and the regulation of occlusal trauma may be effective.

CR-21

Non-surgical treatment and microbiome of periodontitis and peri-implantitis: seven-year follow-up.

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Background and objective: Non-surgical therapy is a standard method for periodontitis and has been reported to be ineffective for peri-implantitis. Here we report a case of successful non-surgical treatment for peri-implantitis and periodontitis with long-term stability.
Case: The patient was a 69-year-old man with chief complaints of discomfort in right maxilla. #17 implant showed probing depth（PD）8mm with bleeding on probing（BOP）, and #34 teeth showed PD 5mm with BOP. Bone loss was radiographically observed around both diseased sites. O'Leary plaque control record（PCR）was 54.8% at the first visit.
Clinical Procedures and Outcomes: An ultrasonic instrument and gracey curettes were used for decontamination of #17 and #34 after the PCR reached a level of 10% . At the 7-year follow-up, no periodontal problems were observed in both sites and good oral hygiene was maintained. Microbial analysis based on 16S rDNA sequencing was conducted before and after treatment. Periodontal pathogens including *Porphyromonas gingivalis* were predominant at #17 and #34 before treatment, whereas they were decreased at both sites after the follow-up. The bacterial composition within the microbiota after the follow-up was predicted to be similar to that of the healthy site.
Conclusion: This case report demonstrated clinically and bacteriologically that non-surgical treatment can be effective in peri-implantitis. Improvement of self-performed plaque control and low-amount horizontal bone loss would be key points for the success of treatment. Periodic inspection of the bacterial composition within the peri-implant microbiota may be useful in clinical practice.

CR-22

A case report of severe chronic periodontitis with diabetes

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Background: Patients with diabetes mellitus（DM）and severe chronic periodontitis need not only a medical but a dental approach. In such patients, surgical approach is required after evaluating initial preparation and controlling DM. We report a severe chronic periodontitis patient with DM.
Case: A 46-year-old male presented on July, 2017 with the chief complaint of tooth mobility in the mandibular left region. He had been taking antihypertensive and antidiabetic drugs. His BP and HbA1c were 130/95 and 7% , respectively. Anterior guidance was lost, 46% of sites had PPD ≧4mm and 65% of sites exhibited BOP. Molars showed tooth mobility 1 to 3, sites with PPD ≧6 mm, and furcation involvements. #13 had a palatal 10 mm PPD, with mobility 2 and a mesial 1 to 2-wall bony defect. #15 had a mesial 8mm PPD, with mobility 2 and a mesial 1 to 2-wall bony defect.
Clinical Procedures and Outcomes: 8 months after initial preparation, BOP and PPD ≧4mm improved to 18% and 30% , respectively. SRP with anesthesia and occlusal adjustment was subsequently performed, followed by resolution of the mobility in the molars. Regenerative therapy was performed in the intra-bony defects of #13 and 15. #13 and 15 were treated by a fixed partial denture.
Conclusion: Initial preparation combined with SRP and occlusal adjustment improved root furcation involvements, reduced tooth mobility and exhibited remission of diabetes. These were considered to be due to the reduction of inflammation by periodontal treatment. SPT will continue while controlling and eliminating trauma factors as well as inflammation.

CR-23

Long-term comprehensive management of generalized severe chronic periodontitis: A case report

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Background: Severe chronic periodontitis can cause pathological tooth migration or malalignment. Thus, maintaining appropriate and stable tooth alignment is essential in the management of severe chronic periodontitis. This case report presents the failure, recovery, and practical consideration in the 10-year long-term management of tooth alignment in a patient with severe chronic periodontitis.
Case: A 63-year-old female first visited on February 21, 2009, with severe periodontal disease associated with crowding of anterior teeth and occlusal trauma.
Clinical Procedures and Outcomes: First, initial periodontal treatment was initiated. After reevaluation, periodontal surgical treatment with Emdogain® and autologous bone grafting were performed. After 6 months, we performed partial orthodontic treatment. Finally, periodontal prostheses were performed on the upper and lower molars. Both the upper and lower anterior teeth remained fixed for a while and were placed in SPT. After 8 years and 6 months, flare-ups occurred at 12, 11 Out；thus, we used 12, 11, 21, and 23 abutment teeth and a 22-pontic bridge to replace them. Currently, a decade has passed since we began this treatment, and the patient's severe chronic periodontitis has been managed well.
Conclusion: The tooth position in the splint with resin and occlusal adjustment must be judiciously considered to maintain stable dentition, as a temporary splint needs repair because of the composite resin deterioration. Hence, fixed prosthesis could be preferred over temporary splint to maintain stable dentition in the management of chronic periodontitis.

CR-24

One-stage full mouth a-PDT in severe chronic periodontitis: A case report.

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Background and objective: Antimicrobial photodynamic therapy（a-PDT）has a potential to be an effective treatment adjunct in non-surgical treatment of chronic periodontitis. One-stage full mouth disinfection（Os-FMD）protocols have been proven to be a relevant treatment option. This case report shows Os-FMD combination with a-PDT in treatment of severe chronic periodontitis patient.
Case: A 51-year-old male visited my clinic in February 2019, with the chief complaint of occlusal pain. Periodontal pocket depth was 3 to 10 mm. The percentage of sites with PPD ≧4mm was 48.8％. The percentage of BOP was 34％. Mobility of most molars was second or third degree. The patient was diagnosed as generalized severe periodontitis.
Clinical Procedures and Outcomes: Before one-stage full mouth a-PDT, #17,27,37, and 47 were extracted. After meticulous supragingival scaling, the a-PDT with subgingival SRP was applied in one session as a one-stage full-mouth a-PDT on all periodontal sites. a-PDT involves toluidine blue as photosensitizer and diode laser（632-644 nm）as light source.
The result was satisfactory. The percentage of sites with PPD ≧4mm decreased to 2.8％. The percentage of BOP decreased to 0％. A significant improvement of periodontal condition was achieved.
Conclusion: The result of this treatment show that Os-FMD combination with a-PDT is an useful adjunct in non-surgical treatment to improve periodontal condition, and to shorten treatment period in chronic periodontitis.

Dental Hygiene

DH-01

The dynamic responses during maintenance phase after the regenerative surgery by FGF-2

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Background: The efficacy of human recombinant fibroblast growth factor（FGF-2）medicine（Regroth®）has been demonstrated by many clinical cases in Japan. However, there is no report on how periodontal tissue regenerated by Regroth® changes over a long period of time. We will report a case in which the social and environmental impact on the patient was observed markedly during the maintenance phase following the periodontal regenerative surgery.
Case: A 42-year-old female with a one-walled severe infrabony defect and Class III furcation involvement at tooth #36, and Class II furcation involvement at tooth #37（lingual）underwent the periodontal regenerative surgery by Regroth® without any bone filling materials.
Clinical Procedures and Outcomes: The supportive periodontal therapy（SPT）and radiographic observation have been provided for this patient following the surgery. Fifteen months after the operation, the new bone formation was observed dominantly. From the 15th month through the 18th month, however, the patient was unable to follow through the SPT due to the overwhelming load of social duties. As this caused a rapid deterioration of periodontal tissue, the immediate attention was given by the dental hygienist to reengage the patient with the SPT. This action has resulted in the dramatical improvement in the patient's periodontal condition.
Conclusion: This case suggests that the long-term SPT is very important at the periodontal regenerative surgical site. It is necessary for dental professionals to pay a close attention to difficult social situations patients may face in order to maintain the positive results of surgery.

DH-02

Non-surgical periodontal treatment of a patient with generalized severe chronic periodontitis

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Background: We reported a case of generalized severe chronic periodontitis that was ameliorated by non-surgical periodontal treatment performed by a dental hygienist.
Case: A 61-year-old male patient first visited our hospital on December 25, 1994 with a chief complaint of gingival enlargement.
Gingival redness and enlargement were observed in the entire jaw, PCR was 91.3％, bleeding on probing（BOP）was 28.8％, and PPD at ≥7 mm was 8.3％.
The patient was diagnosed with generalized severe chronic periodontitis.
Treatment and Results: Scaling and root planning（SRP）were performed by coordinating with the dentist regarding the time of removal of the defective prosthesis, etc. Cleaning tools were changed according to the changes in the oral environment during treatment, such as application of a periodontal treatment device, gingival recession after SRP, and application of an orthodontic device. The PPD of up to 12 mm improved to 2 mm. Moreover, maintaining the patient's motivation helped to reduce all the periodontal pockets to＜3 mm without periodontal surgery. It has been almost 3 years since the transition to supportive periodontal therapy, and the PPD is ≤ 3 mm for all teeth with the use of five cleaning tools and the periodontal condition has remained stable with PCR of 4.9％ and BOP of 2.8％.
Conclusion: It can be surmised that by assessing the patient's personality and degree of interest in the oral hygiene, severe chronic periodontitis can be ameliorated with non-surgical treatment by ensuring sufficient communication and building a relationship of trust.

DH-03

Response to inflammation due to changes in the general condition

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Background: We reported a case of recurrence of periodontitis associated with a change in the general condition of the patient.
Case: A 42-year-old female patient first visited our hospital in June 2015 with a chief complaint of gingival enlargement between teeth 16 and 17. Radiographic imaging revealed loss of alveolar bone between 16 and 17. A grade I furcation lesion was observed at the distal portion of 16.
Treatment and Results: Basic periodontal treatment was performed for the gingival enlargement between 16 and 17, and all PPDs became ≤4 mm and the BOP and PCR stabilized（4.8% and 4.2% , respectively）; therefore, the transition to SPT was made in May 2016. With onset of herpangina in August 2017, the patient showed decreased maintenance of oral hygiene, and the gingival enlargement between 16 and 17 recurred with a 6-mm PPD. Changes in appointments by the patient due to poor physical condition were noted, and she visited the internal medicine department after experiencing fatigue for several months. The results indicated a decrease in the values of the thyroid gland parameters and decrease in immunity strength. Considering the stamina and oral condition of the patient, the SPT interval was set to 1 month, and thorough oral cleaning guidance was given and symptoms ameliorated.
Conclusion: Because of the recurrence of inflammation due to changes in the general condition in the present case, a review of oral cleaning tools was performed and the appropriate SPT interval was continued, thereby leading to stabilization of symptoms.

DH-04

A case of 35-year-successful periodontal treatment in chronic periodontitis

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Background: Oral health is a key INDICATOR of overall health, well-being and quality of life（WHO）.
Case: In 1985, a fifty-six year-old female with chief complaint of pain in left molars visited our clinic. The narrow gingival width and 4-8mm of overall periodontal pockets were recognized. X-ray examination showed the horizontal bone absorption.
Clinical Procedures and Outcomes: The patient received a diagnosis of chronic periodontitis with crowded dental arch and started the treatments with basic periodontal treatments, orthodontic treatments, and prosthodontics treatments. In 1988, the treatment shifted to follow up phase with SPT. The SPT consists of 1. Strengthen motives, 2. Estimation of plaque control, 3. Periodontal tissue examination, 4. Interviewing about the diet, 5. Estimation of the occlusion, 6. Removal of calculus, and 7. Ordinary dental treatments if necessary. At present, the patient turned 91-year old and does not have problems to live alone. She has been showing the active attitudes toward to the good health and keeps coming for recalls. Overall probing pocket depth was kept around 2 - 3mm and the plaque control had been well performed（Plaque Score = 7.4%）.
Conclusion: Despite getting chronic periodontitis 35-years ago, this case went well probably because the patient has been highly motivated to be health. It is suggestive that dental hygienist has important roles in SPT especially during strengthen motives toward to overall health.

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