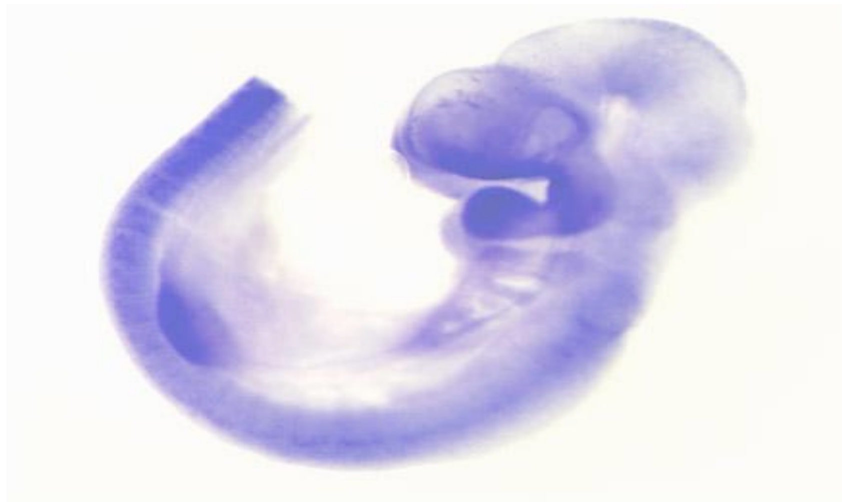
THE 6TH INTERNATIONAL JOINT SYMPOSIUM ON "DENTAL AND CRANIOFACIAL MORPHOGENESIS AND TISSUE REGENERATION" AND "ORAL HEALTH SCIENCE"

4-5th March, 2011
at Fukuoka Recent Hotel
Fukuoka, Japan



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Program & Abstracts

○ **会期：平成２３年３月４日～５日**

○ **会場：福岡リーセントホテル（下図参照）**

○ **主催：九州大学　大学院　歯学研究院**

「口腔組織の再生・再建医療」プロジェクト

「口腔健康科学」プロジェクト

文部科学省　特別教育研究経費

「歯学連携ネットワークによる口腔からQOL向上を目指す研究」

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Program

=4th March, 2011=

Opening remarks

12:50 - 12:55 Masato Hirata (*Council member of Kyushu University*)

12:55 - 13:00 Hiroshi Nakanishi (*Dean, Faculty of Dental Science, Kyushu University)*

Special Lectures (Dental and Craniofacial Morphogenesis and Tissue Regeneration)

Chairpersons: Katsumasa Maeda (*Section of Periodontology, Kyushu University)*
Ichiro Takahasi (*Section of Orthodontics and Dentofacial Orthopedics, Kyushu University)*

SL-1 13:00 - 13:50
FGF-2 stimulates Periodontal Regeneration
Shinya Murakami
Professor and Chairman, Department of Periodontology, Graduate School of Dentistry, Osaka University

SL-2 14:00 - 14:50
Periodontal Disease and Rheumatoid Arthritis
P. Mark Bartold
Professor and Director, Colgate Australian Clinical Dental Research Centre, University of Adelaide

SL-3 15:00 - 15:30
The new approaches for bone regeneration through the activation of Wnt/β-catenin signaling pathway
Etsuko Matsuzaki[1], Fumi Takahashi-Yanaga[2], Katsumasa Maeda[1]
[1]Section of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, [2]Department of Clinical Pharmacology, Faculty of Medical Sciences, Kyushu University

Break

Oral Session (Part I)

Chairperson: Hidefumi Maeda (1-1~1-3)

1-1 16:00 – 16:10

The roles of stretch loading in human periodontal ligament cells

Satoshi Monnouchi[1], Hidefumi Maeda[2], Shinsuke Fujii[2], Atsushi Tomokiyo[1], Naohisa Wada[2], Kiyomi Kono[1], and Akifumi Akamine[1, 2]

[1]Department of Endodontology and Operative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University

[2]Department of Endodontology, Kyushu University Hospital

1-2 16:15 – 16:25

Anti-Dkk1 antibody induces osteoblast differentiation through the Wnt/β-catenin signaling pathway

M. Kobayashi, E. Matsuzaki, T. Hamachi, S. Hiratsuka, Y. Aida, and K. Maeda

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

1-3 16:30 – 16:40

Sphingosine-1-phosphate activates the Wnt/β-catenin signaling pathway and increases osteoprotegerin gene expression

S. Hiratsuka, E. Matsuzaki, M. Kobayashi, T. Hamachi, Y. Aida, and K. Maeda

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

Chairperson: Haruyoshi Yamaza (1-4~1-5)

1-4 16:45 – 16:55

Functional implication of thymosin beta-4 in the morphogenesis of the tooth germ via the regulation of odontogenic gene expressions

Yukiko Ookuma[1, 2], Ieyoshi Kobayashi[1], Tamotsu Kiyoshima[1], Kengo Nagata[1], Hiroko Wada[1], Kazuaki Nonaka[2] and Hidetaka Sakai[1]

[1]Laboratory of Oral Pathology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University

[2]Section of Pediatric Dentistry, Division of Oral Health, Growth and Development, Faculty of Dental Science, Kyushu University

1-5 17:00 – 17:10

Expression pattern and possible function of thymosin beta-10 in developing tooth germ compared with thymosin beta-4

Maho Shiotsuka[1, 2], Hiroko Wada[1], Tamotsu Kiyoshima[1], Ieyoshi Kobayashi[1], Kengo Nagata[1], Hiroaki Fujiwara[1], Ichiro Takahashi[2] and Hidetaka Sakai[1]
[1] *Laboratory of Oral Pathology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University*
[2] *Section of Orthodontics and Dentofacial Orthopedics, Division of Oral Health, Growth and Development, Faculty of Dental Science, Kyushu University*

Chairperson: Hiroshi Takeuchi (1-6~1-7)

1-6 17:15 – 17:25

Involvement of PRIP in the bone formation

Koshiro Tsutsumi[1, 2], Miho Kotani[1], Ayako Murakami[1], Miho Matsuda[1] and Masato Hirata[1]
[1]*Laboratory of Molecular and Cellular Biochemistry, Faculty of Dental Science, Kyushu University*
[2]*Division of Fixed Prosthodontics, Faculty of Dental Science, Kyushu University*

1-7 17:30 – 17:40

Effects of allergic inflammation on orthodontically induced tooth root resorption

Naohisa Murata[1, 2] Hideki Ioi[2], Masahiro Ohuchi[1,2], Tomoka Takao[1], Hanako Oida[1], Takayoshi Yamaza[1], Ichiro Takahashi[2], Mizuho A. Kido[1]
[1]*Department of Molecular Cell Biology and Oral Anatomy, Graduate school of Dental Science, Kyushu University*
[2]*Department of Orthodontics, Graduate school of Dental Science, Kyushu University*

Chairperson: Yasunori Ayukawa (1-8~1-10)

1-8 17:45 – 17:55

Stress distribution of maxillary implant supported overdentures in vitro

Asuka Haruta, Yasuyuki Matsushita, Yoshihiro Tsukiyama and Kiyoshi Koyano
Section of Implant and Rehabilitative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University

1-9 18:00 – 18:10

Resistant factors of the conservative therapy to BRONJ

Goto Y[1], Kawano S [1], Hayashida J [1], Ikari T [2], Kurahara S [2], Toyoshima T [1], Minamizato T[1], Kiyosue T[1], Matsubara R[1], Oobu K[1], Takenoshita Y[2], Mori Y[2], Shiratsuchi Y[1], Nakamura S[1]
[1]*Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University*
[2]*Section of Oral and Maxillofacial Surgery, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University*

1-10 18:15 – 18:25

Ca^{2+} stimulates the expression of bone morphogenetic protein-2 through calcium-sensing receptor in fibroblasts

Yoshie Yahara

Section of Oral and Maxillofacial Surgery, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University

19:00 – 21:00

Getting Together Party

(MC: Masato Hirata, *Council member of Kyushu University*)

=5th March, 2011=

Opening remarks

9:50 - 9:55 Seiji Nakamura (*Section of Oral and Maxillofacial Oncology, Kyushu University*)

Special Lectures (Oral Health Science)

Chairperson: Yoshihisa Yamashita (*Section of Preventive and Dental Public Health, Kyushu University)*

SL-4 10:00 – 10:50

Re-Engineering of Gut Microbiota with Traditional Chinese Medicinal Foods for Preventive Management of Metabolic Diseases

Liping Zhao

Laboratory of Molecular Microbial Ecology and Ecogenomics, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai Center for Systems Biomedicine, 800 Dongchuan Road, Shanghai, 200240, CHINA

SL-5 11:00 – 11:50

Effects of butyric acid, the bacterial metabolite, on the periodontal tissue and reactivation of latent HIV-1

Kuniyasu Ochiai

Department of Microbiology, Nihon University School of Dentistry

Lunch

Oral Session (Part II)

Chairperson: Zhou Wu (2-1~2-3)

2-1 13:00 – 13:10

Phosphatidylserine containing liposomes facilitate osteogenic maturation and bone formation of rats

Hong Mei Ma[1], Zhou Wu[1], Yin Ji Li[2], Toshio Kukita[2] and Hiroshi Nakanishi[1]

[1]*Department of Oral Aging Science and Pharmacology, Faculty of Dental Science, Kyushu University*

[2]*Department of Molecular Cell Biology and Oral Anatomy, Faculty of Dental Science, Kyushu University*

2-2 13:15 – 13:25

A possible involvement of cathepsin B in nociceptive pain through the processing and secretion of Interleukin-1 family from microglia

Li Sun, Zhou Wu, Yoshinori Hayashi, Hiroshi Nakanishi
Department of Oral Aging Science and Pharmacology, Faculty of Dental Science, Kyushu University

2-3 13:30 – 13:40

Effect of PRIP deficiency in autophagy

Hisanori Umebayashi[1], Miho Matsuda[1], Takashi Kanematsu[2], and Masato Hirata[1]
[1]Laboratory of Molecular and Cellular Biochemistry, Graduate School of Dental Science, Kyushu University
[2]Department of Dental Pharmacolgy, Graduate School Hiroshima University

Chairperson: Masaharu Nakagawa (2-4~2-5)

2-4 13:45 – 13:55

Fabrication of interconnected porous calcium phosphate cement

Trung Kien Pham[1], Kanji Tsuru[1], Michito Maruta[1], Masaharu Nakagawa[1], Shigeki Matsuya[2] and Kunio Ishikawa[1]
[1]Department of Biomaterials, Faculty of Dental Science, Kyushu University
[2]Section of Bioengineering, Department of Dental Engineering, Fukuoka Dental College

2-5 14:00 – 14:10

Fabrication of bioactive polyethylen terephthalate (PET) substrates by ozone-calcium treatment

Ahmed Nafis Rashid, Masaharu Nakagawa, Kanji Tsuru, Giichiro Kawachi and Kunio Ishikawa
Department of Biomaterials, Faculty of Dental Science, Kyushu University

Break

Chairperson: Kanji Tsuru (2-6~2-8)

2-6 14:30 – 14:40

Mechanical strength improvement of carbonate apatite foam bone substitute by PLGA reinforcement

Girlie M. Munar[1], Melvin L. Munar[1], Kanji Tsuru[1], Shigeki Matsuya[2] and Kunio Ishikawa[1]
[1]Department of Biomaterials, Faculty of Dental Science, Kyushu University
[2]Section of Bioengineering, Department of Dental Engineering, Fukuoka Dental College

2-7 14:45 – 14:55

Effect of O_3 treatment on the basic properties of apatite cement

Ira Artilia, Michito Maruta, Kanji Tsuru and Kunio Ishikawa
Department of Biomaterials, Faculty of Dental Science, Kyushu University

2-8 15:00 – 15:10

Effect of surface modification on initial setting time of α-tricalcium phosphate based apatite cement

Ruslin[1], Michito Maruta[1], Kanji Tsuru[1], Giichiro Kawachi[1], Shigeki Matsuya[2] and Kunio Ishikawa[1]
[1]Department of Biomaterials, Faculty of Dental Science, Kyushu University
[2]Section of Bioengineering, Department of Dental Engineering, Section of Bioengineering, Fukuoka Dental College

Chairperson: Tsuyoshi Sugiura (2-9~2-10)

2-9 15:15 – 15:25

Association of cytokeratin 17 expression with differentiation of tumor cells in oral squamous cell carcinoma

Ryoji Kitamura, Takeshi Toyoshima, Shintaro Kawano, Kazunari Oobu,
Takahiro Kiyosue, Ryota Matsubara, Yuichi Goto, and Seiji Nakamura
Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University

2-10 15:30 – 15:40

Localization of Th subsets in salivary gland of Sjögren syndrome

Takashi Maehara, Masafumi Moriyama, Jun–nosuke Hayashida, Akihiko Tanaka, Shouichi Shinozaki, Kaori Matsumura, and Seiji Nakamura
Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University

Closing remarks

15:45 - 15:50 Seiji Nakamura
15:50 - 15:55 P. Mark Bartold
15:55 - 16:00 Liping Zhao

Abstracts

SL-1

FGF-2 stimulates Periodontal Regeneration

Shinya Murakami, D.D.S., Ph.D.
Professor and Chairman,
Department of Periodontology,
Graduate School of Dentistry, Osaka University

Periodontitis progressively destroys the periodontal tissue and no conventional periodontal treatments can ever regenerate lost periodontal tissue or normal structure and functionality. However, improving the biological potential of endogenous mesenchymal stem cells and/or progenitor cells within periodontal ligaments and stimulating the regeneration of periodontal tissue are now recognized as clinically possible. One of the most physiologically efficient methods for stimulating the cells is the use of recombinant cytokines. Basic fibroblast growth factor (FGF-2) displays strong angiogenic activity and inductivity to proliferate mesenchymal cells. We revealed that FGF-2 is efficacious in regenerating periodontal tissue in models of artificial loss of periodontal tissue in beagles and non-human primates. Furthermore, we recently conducted a double-blinded clinical trial (phase II) with patients from multidental facilities. The purpose was to both clarify the activity of FGF-2 to regenerate periodontal tissue in periodontitis patients and to confirm drug safety. Subjects underwent periodontal surgical treatment during which we also administered 200 ml of the appropriate investigational drug (FGF-2 plus 3% hydroxypropylcellulose: HPC) to sites displaying periodontal tissue defect. As a result, standardised radiographs revealed significantly increased alveolar bone mass in 0.3%-FGF-2 Group compared to Placebo Group (HPC alone). Throughout the investigation period, no emergence of any serious adverse effects was identified. This result suggests that topical application of FGF-2 can induce regeneration of periodontal tissue in periodontitis patients.
In this symposium, I would like to discuss the present status and future outlook of FGF-2 therapy in the field of periodontal therapy.

SL-2

Periodontal Disease and Rheumatoid Arthritis

P. Mark Bartold, D.D.Sc., Ph.D.
Professor and Director, Colgate Australian Clinical Dental Research Centre, University of Adelaide

Periodontitis and rheumatoid arthritis (RA) appear to share many pathologic features. In this presentation the common pathologic mechanisms of these two common chronic conditions are explored. Emerging evidence now suggests a strong relationship between the extent and severity of periodontal disease and RA. While this relationship is unlikely to be causal, it is clear that individuals suffering from advanced RA are more likely to experience more significant periodontal problems compared to their non-RA counterparts, and vice versa. A case is made that these two diseases could be very closely related through common underlying dysfunction of fundamental inflammatory mechanisms. The nature of such dysfunction is still unknown. Nonetheless, there is accruing evidence to support the notion that both conditions manifest as a result of an imbalance between pro-inflammatory and anti-inflammatory cytokines. As a result, new treatment strategies are expected to emerge for both diseases that may target the inhibition of pro-inflammatory cytokines and destructive proteases. The clinical implications of the current data dictate that patients with RA should be carefully screened for their periodontal status.

SL-3

The new approaches for bone regeneration through the activation of Wnt/β-catenin signaling pathway

Etsuko Matsuzaki[1], **Fumi Takahashi-Yanaga**[2], **Katsumasa Maeda**[1]

[1]Periodontology Section, Division of Oral Rehabilitation,
Faculty of Dental Sciences,
[2]Department of Clinical Pharmacology, Faculty of Medical Sciences,
Kyushu University

Cell signaling cascades activated by Wnt proteins (collectively the Wnt signaling pathways) have been well conserved throughout evolution. As well as regulating cellular processes including proliferation, differentiation, motility and survival/apoptosis, the Wnt signaling pathways play key roles in embryonic development and maintenance of homeostasis in mature tissues. Among the described Wnt signaling pathways, the Wnt/β-catenin signaling pathway (canonical pathway) is best characterized and it has been shown that this signaling pathway is involved in bone biology. We previously reported that differentiation-inducing factor-1 (DIF-1), a morphogen of *Dictyostelium*, altered osteoblast differentiation, including the alkaline phosphatase (ALP) expression, by suppressing the Wnt/β-catenin signaling pathway. For the development of new drugs targeting to the bone regeneration, we investigated the possible activators of Wnt/β-catenin signaling pathway on osteoblast differentiation. Dkk1 (Dickkopf-1) is a soluble inhibitor of Wnt signaling pathway by binding to the Wnt co-receptor low-density lipoprotein receptor-related protein (LRP) 5/6 and inhibits the bone formation. Sphingosine-1-phosphate (S1P) is well known for the signaling sphingolipid and it has been reported that S1P activated osteoblast and suppressed osteoclast, resulting in the inhibition of bone resorption. Therefore, we examined the effect of anti-Dkk1 antibody and S1P on osteoblast differentiation. We found that both anti-Dkk1 antibody and S1P activated the Wnt/β-catenin signaling pathway and induced ALP activation and mineralization. In this symposium, summarizing our recent findings on the possible activators of the Wnt/β-catenin signaling pathway on the osteoblast differentiation, we will show the possibility for development of bone regenerator by activating this signaling pathway.

SL-4

Re-Engineering of Gut Microbiota with Traditional Chinese Medicinal Foods for Preventive Management of Metabolic Diseases

Liping Zhao

Laboratory of Molecular Microbial Ecology and Ecogenomics, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai Center for Systems Biomedicine, 800 Dongchuan Road, Shanghai, 200240, CHINA

Gut microbiota, as the second genome for humans, must work in harmonious integration with the first genome to sustain a healthy immunity and metabolism for the host. In animal models, diet has been shown to play a dominating role in shaping gut microbiota. Long term intake of high-fat diet can override host genetics that wildtype animals with no known relevant genetic defect can develop severe obesity and insulin resistance. It has been shown that high-fat diet can disrupt gut microbiota to increase population levels of opportunistic pathogens such as sulfate-reducing bacteria and decrease the levels of gut barrier-protecting bacteria, such as bifidobacteria. This long term dysbiosis would lead to increased antigen load in host bloodstream to provoke an unresolved chronic inflammation impairing insulin receptor, vessel walls etc for development of metabolic diseases. This new understanding of the key mediating role of gut microbiota between high-fat diet and deranged host metabolism opens vast possibilities for modulating gut microbiota with designed foods in preventive management of the devastating epidemic of metabolic diseases.

Traditional Chinese medicine has a legacy of using foods as drugs to prevent chronic diseases. These "medicinal foods" are regulated as common foods but have been repeatedly demonstrated to have medicinal effects over thousands of years of "human trials". The scientific foundation has yet to be elucidated with modern methodologies. We designed a diet pattern with ingredients from TCM foods. 123 volunteers (BMI﹥30) were recruited in a self-controlled clinical trial to test the efficacy of this TCM-based diet for management of weight and metabolic syndrome. Clinical data and blood, urine and fecal samples were collected before, during and after the intervention. Preliminary data showed a significant effect of TCM foods for modulating gut microbiota, attenuating inflammation and alleviating the metabolic syndrome. Metagenomics, metabonomics and multivariate statistics are being used as whole-body

systems biology approaches to understand the dynamic associations between rebalanced architects of gut microbiota, attenuated inflammation, improved insulin sensitivity, weight reduction and global metabolic homeostasis.

SL-5

Effects of butyric acid, the bacterial metabolite, on the periodontal tissue and reactivation of latent HIV-1

Kuniyasu Ochiai

Department of Microbiology,

Nihon University School of Dentistry

Butyric acid, an extracelluar metabolite from periodontopathic bacteria, induces apoptosis in T-cells and macrophages, and exerts immuno- modulatory properties. We demonstrated that human gingival fibroblasts (HGFs) rescue butyric acid-induced T-cell apoptosis via the proinflammatory cytokines, which were produced in HGFs stimulated with butyric acid. Moreover, T-cells adhered to HGFs is enhanced by butyric acid and butyric acid-induced T-cell apoptosis is down-regulated by T-cell adhesion to HGFs. These data suggested that the cell-cell communication among T-cells and HGFs is essential for maintaining a normal gingival tissue.

Latently infected cells harbor the HIV-1 proviral DNA integrated in heterochromatins allowing the persistence of transcriptionally-silent proviruses. Hypoacetylation of histone proteins by histone deacetylases (HDACs) is involved in the maintenance of HIV-1 latency by repressing transcription from HIV-1 proviral DNA. Progression of AIDS is associated with development of severe periodontitis. Although it is known that butyric acid is involved in reactivation of the repressed chromatin, it is not known whether periodontitis is involved in progression of HIV-infection. Thus, we examined whether infection of periodontopathic bacteria could facilitate progression of AIDS by reactivating the latent HIV provirus. Our results indicated that periodontopathic bacteria produce high concentrations of butyric acid that act as a potent inhibitor of HDACs and appear to induce acetylation of histone, thus eventually leading to reactivation of HIV-1 in latently infected cells. These results suggested that periodontal diseases could act as a risk-factor for HIV reactivation in the latently infected individuals.

1-1

The roles of stretch loading in human periodontal ligament cells

Satoshi Monnouchi[1], Hidefumi Maeda[2], Shinsuke Fujii[2], Atsushi Tomokiyo[1], Naohisa Wada[2], Kiyomi Kono[1], and Akifumi Akamine[1, 2]

[1]Department of Endodontology and Operative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, and [2]Department of Endodontology, Kyushu University Hospital

The mechanical loading caused by occlusion and mastication is known to play an essential role in maintaining periodontal ligament (PDL) tissues. Our recent study demonstrated that stretch loading up-regulated transforming growth factor-β1, and alkaline phosphatase in human PDL cells (HPDLCs) in vitro, that was mediated by increased angiotensin II. Therefore, the present study purposed to detect other biological factors yielded in HPDLCs subjected to stretch loading.

HPDLCs obtained from healthy third molars were pre-cultured in flexible-bottomed culture chambers coated with type I collagen until reaching sub-confluence. HPDLCs were subjected to stretch loading (0.5 sec stretch and 0.5 sec relaxation per a cycle) for 1 hr. Gene expressions were analyzed by quantitative RT-PCR, and protein expressions were analyzed by enzyme-linked immunosorbent assay.

Here, we identified up-regulated gene and protein expressions of interleukin-11 (IL-11) in HPDLCs that were exposed to stretch loading. HPDLCs stimulated by angiotensin II also increased gene expressions of IL-11. Furthermore, immuno-localization of IL-11 was detected in rat PDL tissues, but not in pulp tissues, dentin and bone matrices. Additionally, the intense reaction was detected at the extracellular matrices in the bone side of PDL tissues.

It has been reported that IL-11 is a multi-functional cytokine involved actively in bone metabolism, and recent reports have discussed that IL-11 induced by mechanical stress enhanced osteoblastogenesis. Moreover, in PDL cells, it has been shown that IL-11 expression stimulates osteoblastic differentiation.

These results suggested that IL-11 was up-regulated via angiotensin II induced by stretch loading in HPDLCs, and that it might contribute to bone metabolism under the environment of mechanical load in PDL tissues.

1-2

Anti-Dkk1 antibody induces osteoblast differentiation through the Wnt/β-catenin signaling pathway

M. Kobayashi, E. Matsuzaki, T. Hamachi, S. Hiratsuka, Y. Aida, and K. Maeda
Section of Periodontology, Division of Oral Rehabilitation,
Faculty of Dental Science, Kyushu University

The Wnt/β-catenin signaling pathway is central to bone development and homeostasis. Dkk1 is a soluble inhibitor of this signaling pathway by binding to the Wnt co-receptor LRP5/6, and inhibits bone formation. It has been reported that anti-Dkk1 antibody was effective in preventing the development of osteolytic bone disease in multiple myeloma. However, the role of anti-Dkk1 antibody on osteoblast differentiation is still unknown. In this study, we investigated the effect of anti-Dkk1 antibody on osteoblast differentiation and the Wnt/β-catenin signaling pathway.

MC3T3-E1 and SaOS-2 cells, osteoblast-like cells, were used for this study. First, we examined the effect of anti-Dkk1 antibody on cell proliferation and alkaline phosphatate (ALP) activity, a crucial marker of osteoblast differentiation. We found that anti-Dkk1 antibody significantly increased ALP activity in both MC3T3-E1 and SaOS-2 cells without affecting cell proliferation. We also investigated the expression levels of mRNA and protein of osteoblast differentiation makers, ALP and osteocalcin (OCN). Both mRNA and protein expression levels of ALP and OCN were increased after treatment of anti-Dkk1 antibody in MC3T3-E1 cells. Next, we studied the effect of anti-Dkk1 antibody on osteoblast-mediated mineralization. Anti-Dkk1 antibody increased osteoblast-mediated mineralization.

Subsequently, we examined the effect of anti-Dkk1 antibody on the Wnt/β-catenin signaling pathway. In this signaling pathway, β-catenin is a key player. Activation of this signaling induces the stabilization of cytoplasmic β-catenin, and accumulated β-catenin enters the nucleus to regulate target gene expression with T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) transcriptional factors. The expression level of β-catenin protein was increased by treatment of anti-Dkk1 antibody and TCF transcriptional activities were also increased. Furthermore, we investigated the effect of anti-Dkk1 antibody on human ALP promoter activity mediated by the putative TCF binding site (-1023/-1017bp) and found that anti-Dkk1 antibody significantly increased ALP promoter activity.

These results suggest that anti-Dkk1 antibody induces Wnt/β-catenin signaling pathway, resulting in osteoblast differentiation. Therefore, anti-Dkk1 antibody might have a therapeutic value to induce bone regeneration.

1-3

Sphingosine-1-phosphate activates the Wnt/β-catenin signaling pathway and increases osteoprotegerin gene expression

S. Hiratsuka, E. Matsuzaki, M. Kobayashi, T. Hamachi, Y. Aida, and K. Maeda

Section of Periodontology, Division of Oral Rehabilitation,

Faculty of Dental Science, Kyushu University

[Objective]

Sphingosine-1-phosphate (S1P) is well known for the signaling sphingolipid and the bioactive lipid mediator that impacts migration, proliferation, and survival in diverse cell types. Most of the biological effects of S1P are mediated by signaling through the cell surface G-protein-coupled receptors $S1P_1$-$S1P_5$. Recently, it has been reported that S1P stimulates osteoblast migration and survival. Furthermore, S1P has been shown to inhibit osteoclast differentiation and bone resorption. However, the effects of S1P on osteoblast differentiation and bone formation are still unknown.

In this study, we investigated the effect of S1P on osteoblast differentiation. We particularly paid attention to the possible involvement of the Wnt/β-catenin signaling pathway because this signaling pathway plays an important role in osteoblast differentiation and bone metabolism.

[Materials and Methods]

Osteoblast-like cell lines MC3T3-E1 and SaOS-2 cells were used in this study. The effect of S1P on osteoblast differentiation was monitored by alkaline phosphatase (ALP) activity. mRNA and protein expressions were analyzed by northern blotting/RT-PCR and western blotting. Reporter gene assays were also carried out to examine the effect of S1P on the Wnt/β-catenin signaling pathway. Von Kossa staining and alizarin red staining were also performed to detect osteoblast-mediated mineralization.

[Results]

S1P significantly increased ALP activity compared with control. Next, we examined the effects of S1P on osteoprotegerin (OPG) and receptor activator of nuclear factor-kappa B ligand (RANKL) expressions, the crucial markers for osteoblast-osteoclast coupling. S1P increased the mRNA expression levels of both OPG and RANKL.

Wnt proteins control the expression of many genes including ALP, OPG and RANKL through the inhibition of GSK-3β and the subsequent activation of β-catenin/TCF/LEF transcriptional activity. Therefore, we investigated the effect of S1P on the Wnt/β-catenin signaling pathway. Not only phosphorylation level of Ser^9 on GSK-3β, but S1P increased TCF transcriptional activity. Furthermore, S1P elevated the OPG

promoter activity. These results indicated that S1P activated the Wnt/β-catenin signaling pathway. In addition, S1P induced the osteoblast-mediated mineralization.

1-4

Functional implication of thymosin beta-4 in the morphogenesis of the tooth germ via the regulation of odontogenic gene expressions

Yukiko Ookuma[1, 2], Ieyoshi Kobayashi[1], Tamotsu Kiyoshima[1], Kengo Nagata[1], Hiroko Wada[1], Kazuaki Nonaka[2] and Hidetaka Sakai[1]

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We previously detected *thymosin beta-4* (*Tβ4*) as a differentially expressed gene in embryonic day 12.0 (E12.0) mouse mandible. We reported that the temporal expression pattern of Tβ4 mRNA in the developing tooth germ of the mouse embryo and neonate, and demonstrated the possible association of Tβ4 with the morphogenesis and development of the tooth germ in the 3rd symposium. However, the precise functional roles of Tβ4 have not yet been clarified in the initiation and development of the tooth germ. In this study, we focused on functional implications of Tβ4 in the mouse first molar development. First, an inhibition assay using *Tβ4* antisense sulfur-substituted oligodeoxynucleotide (AS S-ODN) was performed in cultured E11.0 mouse mandibles or E15.0 tooth germs. Enamel organ formation in the cultured E11.0 mandibles at 8th day was significantly suppressed by the treatment with AS S-ODN. However, the AS S-ODN treatment was negligibly affected on morphological development in the cultured E15.0 tooth germ at 8th day. The quantitative analysis using real-time PCR revealed that the expression of type II/III Runx2 and dentin matrix protein 1 (Dmp1) mRNA was significantly suppressed in both the cultured E11.0 mandible and E15.0 tooth germ by the AS S-ODN treatment. Next, in using dental epithelial or pulpal cell line treated with AS S-ODN, we analyzed the mRNA expressions by real-time PCR, enzymatic activities of secretory matrix metalloproteinase-2/-9 (MMP-2/-9) by gelatin zymography, and cell motility. The expression of *type II/III Runx2*, *nucleolin (Ncl)*, *Mmp2/9* and *Dmp1* was decreased by the AS S-ODN treatment in both the cells, suggesting that Tβ4 could be involved in those transcriptions. Enzyme activity of MMP-2 was inhibited by the AS S-ODN treatment in both the cells. The dental epithelial cells treated with AS S-ODN significantly migrated into the acellular gap. Tβ4 may be critical for expression of Runx2, Ncl, MMP-2 and Dmp1 in both the cells, and for cell motility in the dental epithelial cell.

In conclusion, we have proven multi-functional implications of Tβ4 in the development of the mouse lower first molar, via its effects on various-gene expressions.

1-5

Expression pattern and possible function of thymosin beta-10 in developing tooth germ compared with thymosin beta-4

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[Objectives] Our previous study has shown the expression pattern and function of thymosin beta-4 (Tβ4) in developing tooth germ. Thymosin beta-10 (Tβ10), of which sequence is highly homologous to Tβ4, has been reported to play an important role in cell shaping and motility by forming a complex with G-actin similarly to Tβ4, while Tβ10 acts as a potent inhibitor of angiogenesis unlike Tβ4. So far, there is no report on the role of Tβ10 in tooth development. In this study, we investigated the expression pattern and possible function of Tβ10 in developing tooth germ compared with those of Tβ4.

[Materials and Methods] We performed *in situ* hybridization of these two genes to study their detailed expression pattern in the mouse tooth germs at embryonic day 10.5 (E10.5) to postnatal day 7.0 (P7.0), and used real-time PCR to examine the quantity of Tβ4 and Tβ10 mRNA expression levels in mandibles at E10.5 and E12.0 and in tooth germs at E15.0, E18.0, P1.0, and P5.0.

[Results] The *in situ* expression pattern for *Tβ10* in tooth development was apparently different from that of *Tβ4* at the same days. *Tβ10* mRNA was intensely expressed in the mesenchymal cells and odontoblasts. On the other hand, the *in situ* signal for *Tβ4* was detected mainly in dental epithelial cells and ameloblasts. Real-time PCR demonstrated that both genes were highly expressed in the early stage of odontogenesis and tooth matrix formation after birth.

[Conclusion] These results suggest that Tβ10 participates in differentiation of the odontogenic mesenchymal cells, differentiation of odontoblasts, and dentinogenesis in the developing tooth germ. Meanwhile, Tβ4 is associated with differentiation of the odontogenic epithelial cells, differentiation of ameloblasts, and amelogenesis.

1-6

Involvement of PRIP in the bone formation

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PRIP (phospholipase C-related, but catalytically inactive protein) is a signaling protein isolated as an inositol 1,4,5-trisphoshpate binding protein. PRIP has a domain organization similar to phospholipase C-d but lacking the enzyme activity. PRIP-deficient mice (PRIP-KO) mice showed an increased gonadotropin, but a decreased sex steroid hormone. The difference was similar to that for the cause of bone disease, such as osteoporosis. In the present study, therefore, we analyzed PRIP-KO mice with a special reference to the bone condition. We first performed three dimensional analysis of the femur of female mice. The bone mineral density and trabecular bone volume was higher in the mutant mice. We further performed histomorphometrical assay of bone formation parameters: bone formation rate, mineral apposition rate, osteoid thickness and osteoblast number were increased in the mutant, indicating that the increased bone mass is caused by the enhancement of bone formation ability. We then performed the primary culture of calvaria prepared from both genotypes. In cultures from the mutant mice, osteoblast differentiation was accelerated as assessed by the differentiation marker gene expression including alkaline phosphatase, runx2, osteonectin etc. Phosphorylation of Smad1/5/8 in response to BMP4 lasted longer, probably causing the increased expression of osteoblast differentiation markers. These results indicate that PRIP is involved in the negative regulation of the bone formation.

1-7

Effects of allergic inflammation on orthodontically induced tooth root resorption

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The success of orthodontic treatment depends on dynamic alveolar bone metabolism, which leads to tooth movement. There are variations in the time required and the amount of movement achieved, and some patients show tooth root resorption at the end of the treatment. Epidemiologic study reported that patients who suffer from allergic conditions have higher rates of tooth root resorption. In this study, we investigated the effects of allergies on tooth movement and bone/tooth root resorption. Tooth movement and bone and tooth root resorption were increased in ovalbumin (OVA)-sensitized Brown-Norway rats subjected to orthodontic force (OF). The expression levels of RANKL and pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β were increased in periodontal tissues of OVA-sensitized animals subjected to OF, compared with non sensitized rats. Furthermore, mRNA expression levels of enzymes involved in the lipid mediator biosynthetic pathway, and unique receptors for 5-lipoxygenase- derived lipid mediators were also low-dose aspirin to OVA-sensitized OF rats. Aspirin reversed the increase in tooth movement and suppressed tooth root resorption. The results of this study suggest that systemic allergies can affect bone metabolism, and that aspirin or 5-lipoxygenase-mediated lipid mediators could be potential therapeutic agents to combat tooth root resorption.

1-8

Stress distribution of maxillary implant supported overdentures in vitro

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Objectives

Maxillary implant-supported overdentures have been documented with a high implant loss relative to other implant treatment options. Some studies reported that at least four implants were necessary to support maxillary implant-supported overdentures, but there is still a lack of studies on the influence of anchorage design on force distribution. The purpose of this in vitro study is to evaluate the influence of splinted and unsplinted implant of maxillary implant-supported overdentures by measuring strain of implants.

Materials and Methods

In this study, edentulous maxilla model was used, which were simulated anatomical structure and bone quality. Four implants were inserted in right and left, canine and premolar position. The surface of the model was covered with 4mm thickness layer of impression material to simulate oral mucosa, and a palateless overdenture was fabricated. Two different types of attachment were considered; splinted Dolder bar attachment with clip and unsplinted ball attachment. The maxilla model was fixed and 50N concentrated load was applied at anterior (loading point A), first premolar (loading point B) and first molar region (loading point C) of the overdenture. Strains in abutments were measured and axial force and bending moment were calculated. Additionally, denture base movement was measured by a position sensor attached in the left first molar region.

Results

With the loading point A and B, axial forces on bar attachment were larger than that on ball attachment, while with loading point C, axial force on ball attachment were larger. With the loading point A and C, bending moment on ball attachment was smaller than that on bar attachment. With the loading point A, denture movement was the largest on both attachments. With the loading point B and C, denture movements were larger on ball attachment.

Conclusion

Within the limits of the study, unsplinted design could be advantageous for maxillary implant-supported overdentures based on the lower stress and better denture stability.

1-9

Resistant factors of the conservative therapy to BRONJ

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Objectives: Treatment strategy for bisphosphonate-related osteonecrosis of the jaws (BRONJ) is not established definitely. We have mainly provided the conservative therapy including sequestrectomy and debridement to the patients with BRONJ. The purpose of this study was to assess the resistant factors of conservative therapy.

Patients and methods: Thirty-two patients with BRONJ (2 males and 30 females; mean age, 72.0years; range 46-88years), who clinically diagnosed from 2004 to 2010 at the Department of Oral and Maxillofacial Surgery in Kyushu University Hospital, were evaluated clinical parameters (sex, age, route of administration, duration of bisphosphonates therapy, systemic factors, local risk factors, treatment methods, drug holidays, outcome of the treatment, and duration to cure completely).

Results: Oral bisphosphonates (OB) were used in 15 patients. OB combined with corticosteroids (OC) were used in 5 patients. Intravenous bisphosphonates (IB) were used in 12 patients. The treatment methods for BRONJ were local irrigation in all cases, antibiotic therapy in 29, sequestrectomy in 13, debridement in 11, and hyperbaric oxygen therapy in 6. As for outcomes of management, 15 cases (46.9%) were completely cured and 17 (53.1%) were under treatment at this time. The rates of complete cure were 10 of 15 patients (66.7%) in OB, 3 of 5 (60.0%) in OC and 2 of 12 (16.7%) in IB. The average duration of therapy to cure completely was 446.9days. The duration in the patients with corticosteroids therapy (1167.3days) was significantly longer than that in the patients without (269.0days).

Conclusion: It is difficult to cure in the cases of BRONJ caused by IB or OC, though the conservative therapy for BRONJ is useful.

1-10

Ca^{2+} stimulates the expression of bone morphogenetic protein-2 through calcium-sensing receptor in fibroblasts

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Calcium-sensing receptor (CaSR), which is a seven-transmembrane GTP-binding protein-coupled receptor, regulates various pathophysiological functions. We have shown that CaSR is expressed in the fibroblasts isolated from keratocystic odontogenic tumors (KCOTs) and gingiva, and high extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) stimulates the expression of cyclooxygenase-2 and the secretion of prostaglandin E_2 (PGE_2) in the fibroblasts through CaSR. Since PGE_2 regulates bone metabolism by regulating osteoclastogenesis and osteoblast differentiation, Ca^{2+} might affect bone metabolism through CaSR in fibroblasts. Bone morphogenetic protein (BMP)-2 affects bone metabolism via stimulation of osteoblastic cell differentiation and maturation and also direct and/or indirect regulation of osteoclastogenesis. Recently, it was reported that the activation of CaSR stimulated the expression of BMP-2 in colonic myofibroblasts. This finding leads us to speculate the released Ca^{2+} from bone might stimulate the fibroblasts around the bone and affect the bone metabolism by inducing not only PGE_2 secretion but also BMP-2 expression through CaSR. In this study, we investigated whether Ca^{2+} activates BMP-2 expression through CaSR in KCOT and gingival fibroblasts.

Five mM $[Ca^{2+}]_o$ and CaSR activator neomycin enhanced the expression of BMP-2 mRNA and protein in KCOT and gingival fibroblasts. In the KCOT fibroblasts, both phospholipase C inhibitor U-73122 and protein kinase C inhibitor staurosporin attenuated the Ca^{2+}-induced expression of BMP-2 mRNA. Furthermore, specific inhibitors for p38 mitogen-activated protein kinase (MAPK) SB203580 and nuclear factor κB (NF-κB) PDTC attenuated the Ca^{2+}-induced expression of BMP-2 mRNA. Neither PGE_2 nor indometacin affected the elevated $[Ca^{2+}]_o$-induced expression of BMP-2 mRNA in the KCOT fibroblasts.

Thus, in KCOT fibroblasts high concentration of extracellular Ca^{2+} may enhance BMP-2 expression via p38 signal transduction pathway through CaSR without PGE_2.

2-1

Phosphatdiylserine containing liposomes facilitate osteogenic maturation and bone formation of rats

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Posphatidylserine-containing liposomes (PS liposomes) can be engulfed by mononuclear phagocytes to promote their production of TGF-b1 and PGE_2. We have demonstrated that PS liposomes inhibit osteoclastogenesis and arthritis induced bone loss (Wu et al., J Immunol. 2010). In present study, we investigated the effects of PS liposomes on osteogenesis. The cultured rat calvarial osteoblast-like cells (OBs) were used to determine the effects of PS-liposomes on cell proliferation, alkaline phosphatase (ALP) activity, osteogenesis-related gene expression, and mineralized nodule formation in OBs. We found that PS-liposomes did not affect the proliferative activity of OBs at day 7 of culture. On the other hand, PS-liposomes significantly increased the ALP activity at day 7, and PS-liposomes significantly increased the expression of osteocalcin, a marker for osteogenic maturation after day 14 by real time RT-PCR. Furthermore, PS-liposomes markedly increased mineralized nodule formation after day 21 of culture. Moreover, local treatment with PS-liposomes significantly improved the bone formation in rat tooth extraction model. Dependent on about results, PS liposomes strongly promote osteogenesis. Together with the inhibitory effects on osteoclastogenes, PS liposomes are involved in regulation of bone remodeling.

2-2

A possible involvement of cathepsin B in nociceptive pain through the processing and secretion of Interleukin-1 family from microglia

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It is now evident that caspase-1, an essential enzyme for maturation of interleukin-1 (IL-1) family, can be activated through the assembly of a cytosolic protein complex that is known as the inflammasome. However, there is still evidence suggesting the existence of lysosomal-proinflammatory caspase pathways. We have recently reported that cathepsin B-deficiency abrogated the pro-IL-1β maturation and its release from microglia following stimulation with chromogranin A (Terada et al., Glia, 2010). Furthermore, the maturation of pro-caspase-1 in chromogranin-treated microglia was also significantly inhibited by CA-074Me, a specific inhibitor of cathepsin B. In the present study, we further found that the maturation and secretion of both IL-18 and IL-33 from microglia was significantly suppressed by cathepsin B-deficiency or CA-074Me treatment. These results strongly suggest that cathepsin B plays a key role in the maturation of pro-forms of IL-1 family, including IL-1β, IL-18 and IL-33, through the caspase-1 activation in chromogranin A-treated microglia. Next, we examined the effect of cathepsin B-deficiency on the nociceptive processing, because IL-1 family is known as "painful cytokine". As expected, cathepsin B-deficient mice showed a significant resistance against inflammation- and nerve injury-induced pain. Therefore, either pharmacological or genetic inhibition of cathepsin B may provide an effective therapeutic intervention in inflammatory and naturopathic pain.

2-3

Effect of PRIP deficiency in autophagy

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We isolated a novel protein as an inositol 1,4,5-triphosphate binding protein molecule similar to phospholipase C, but catalytically inactive, then we termed it PRIP (PLC-related catalytically inactive protein). We found that PRIP binds GABARAP (GABA receptor associated protein)、regulating the membrane trafficking of $GABA_A$ receptor. Recently it was reported that GABARAP family are involved in autophagy. Therefore, we studied the possible involvement of PRIP in autophagy. Autophagy is a mechanism for the degradation of cellular material either as a way to provide nutrients during times of starvation or as a quality control mechanism that eliminates unneeded proteins and/or organelles during normal growth and development. Of 34 autophagy-related proteins (ATG) identified so far, ATG8 (LC3 in mammal) is used as a specific marker to monitor the process of autophagy. ATG8/LC3 is a member of GABARAP family. We first confirmed the interaction of PRIP with GABARAP family. PRIP interacted with GABARAP, and also with LC3 and other GABARAP family molecules, albeit to a lesser extent. Immunoprecipitation assay showed that PRIP interacted with both LC3-I (cytosolic form) and LC3-II (membrane bound form). Secondly, autophagy activities were monitored by Western blotting analysis using lysates obtained from wild-type (WT) and PRIP-deficient (PRIP-KO) mouse embryonic fibroblast (MEF) starved by amino acid-deprivation along with lysosomal inhibitors. The ratio of LC3-II/LC3-I was higher, and degradation of LC3-II (Autophagic flux) was more increased in PRIP-KO MEF. Thirdly, we generated WT and PRIP-KO mice, expressing transgene of GFP-LC3 by mating and prepared MEF from the mice. Enhanced autophagic activity, as assessed by more GFP dots observed under a confocal microscopy, was observed in the PRIP-KO MEF. Finally, PRIP gene was transfected to PRIP-KO MEF. The number of GFP dots was decreased in an inverse proportion to the expression level of PRIP. These results suggest that PRIP deficiency induces more autophagy, thus indicating that PRIP plays a negative role in autophagy, probably by interacting with LC3.

2-4

Fabrication of interconnected porous calcium phosphate cement

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For bone tissue regeneration, the interconnected porous structure plays an important role since this porous structure allows cell penetration and cell revascularization. In this study, we fabricate calcium phosphate cement with fully-interconnected porous structure based on setting reaction of α-TCP microsphere with 0.2 $mol \cdot L^{-1}$ monocalcium phosphate monohydrate - 0.1 $mol \cdot L^{-1}$ acid phosphoric (MCPM-H_3PO_4) solution at 37°C. For the basic study, set two-α-TCP microsphere specimens were evaluated by scanning electron microscope (SEM) and X-ray diffraction (XRD) to understand the setting mechanism. SEM results revealed that new crystals were formed at the interface between microspheres after the reaction for a minute and then grown and interlocked together after 5 minutes. XRD results also indicated that the surface of α-TCP microsphere was transformed to brushite when treated with MCPM-H_3PO_4 solution. Based on the basic study, calcium phosphate cement with fully-interconnected porous structure was fabricated by using many α-TCP microspheres and MCPM-H_3PO_4 solution at 37°C. The interconnectivity of set porous cement was checked by μ-CT analysis. The interconnected porous calcium phosphate cement, which has in-situ setting property at 37°C, is expected to become an ideal bone substitute for bone tissue regeneration.

2-5

Fabrication of bioactive polyethylen terephthalate (PET) substrates by ozone-calcium treatment

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At present, polyethylene terephthalate (PET) has been widely used as several biomedical applications especially for ligaments and vascular grafts. Considering artificial ligaments, PET prosthesis prevents full integration with the body because it is synthetic, thus prosthesis failure is induced. PET with bioactive surface might help to overcome this problem. We previously reported that ozone-calcium chloride treatment could induce osteoconductivity to titanium and concluded that Ca ions might be immobilized with hydroxyl groups on the surface. This method might be applicable for polymers which can form hydroxyl and carboxylic groups during ozone (O_3) treatment. The present study focused on PET as a target substrate. PET films were subjected to ozone treatment in the presence of 1 mol/L $CaCl_2$ solution for 6 hours in atmospheric pressure. For comparison, PET films were treated by O_3 in distilled water (DW). Peak fitting of C1s spectra by X-ray photoelectron spectroscopy (XPS) showed increased amount of carboxyl groups and Ca2p spectra by XPS showed immobilization of Ca^{2+} onto the substrate after O_3-$CaCl_2$ treatment. Scanning electron microscope (SEM) images revealed no morphological changes based on O_3-$CaCl_2$ treatment. Water contact angle was found to be decreased after O_3-$CaCl_2$ treatment. Initial cell attachment of NIH3T3 cell-line (fibroblast cells) for 3 hours showed higher cell attachment rate in case of O_3-$CaCl_2$ treated samples than non treated and O_3-DW treated ones. Based on the results obtained, O_3-$CaCl_2$ treatment method might be applicable for surface modification of PET and to improve interactions between the polymeric implant and surrounding tissue.

2-6

Mechanical strength improvement of carbonate apatite foam bone substitute by PLGA reinforcement

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Carbonate apatite foam bioceramics is a potential bone defect filler or scaffold for bone tissue engineering due to its close similarities to the biological apatite of bone, morphology which is characterized by interconnecting open pore structure and osteoclast-induced bioresorption. In spite of these merits, the primary drawback carbonate apatite foam is low resistance to fracture. This makes handling difficult like cutting and shaping to fit the bone defect. As the organic phase of bone provides the strength and resiliency, reinforcing the carbonate apatite foam with organic material may also improve its mechanical strength for sufficient handling. In this study, an attempt to improve the mechanical strength of carbonate apatite foam by the addition of an organic phase was performed. The carbonate apatite foam was first fabricated and then reinforced with biodegradable copolymer of poly(DL-lactide-co-glycolide) (PLGA) using freeze-vacuum infiltration technique. After PLGA reinforcement, the compressive strength increased from 0.04 MPa up to 1.5 MPa while porosity remained as high as 82%. SEM observation showed the preservation of the three-dimensional interconnecting pore structure and presence of PLGA both in the internal hollow structures and external surface of the foam. X-ray diffraction and FT-IR analysis revealed no compositional transformation of the carbonate apatite phase. In conclusion, addition of organic polymer such as PLGA is a useful method to increase the mechanical strength of carbonate apatite foam without changing its morphology and composition.

2-7

Effect of O_3 treatment on the basic properties of apatite cement

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Ozone (O_3) treatment of powder phase of apatite cement (AC) was found to improve the wettability of AC's powder phase and thus result in improved handling properties without changing setting time, compositional transformation to apatitic mineral as well as mechanical strength of the set mass. At the same handling property of its paste phase, the mechanical strength of O_3 treated AC was approximately twice higher when compared to non-treated AC. Smaller amount of the liquid to perform higher handling property index and resulting reduced porosity of the set mass was a key for the improved mechanical strength of the O_3 treated AC.

2-8

Effect of surface modification on initial setting time of α-tricalcium phosphate based apatite cement

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The key advantage of apatite cement (AC) is its self-setting ability and appropriate setting time is required for its clinical application. AC should have an initial setting time that enables the clinician to fill the bone defect with the apatite having exactly the same shape with the bone defect. Otherwise, if AC fails to set and powder is released into soft tissue, it will cause an inflammatory response called crystalline inflammatory response. Typically the initial setting time is close to 10 minutes. Unfortunately, setting time of α-tricalcium phosphate (α-TCP) based AC is too long if free from additive. Therefore, chelating agent such as succinic acid or citric acid is employed for the initial setting reaction of AC. Even though, it prevents compositional transformation to apatite. In this study, effect of surface modification of αTCP powder with distilled water (DW) on basic setting reaction of AC has been investigated. Briefly, αTCP powder was soaked in DW with different temperature and periods. After the treatment, the treated powder was immersed in acetone for 3 min to stop the reaction followed by drying in an oven at 60°C. The αTCP powder thus obtained was used as powder phase of AC whereas DW was used as its liquid phase. AC was mixed with liquid to powder of 0.5 and packed in Teflon mold. The packed cements were allowed to set at 37°C and 100% relative humidity. Setting time of this cement was confirmed by a Vicat needle method. When αTCP powder was pre-treated at 50°C for 30 minutes, the setting time became much shorter, while apparently, a slight morphological changes and crystal transformation could be observed by Scanning Electron Microscope (SEM) and X-Ray Diffraction (XRD). Furthermore, when α-TCP powder was pretreated at 50°C for 90 minutes, the setting time became much shorter than that of 30 minutes pre-treatment. In this pre-treatment condition, new fine precipitations could be observed by SEM. Moreover, XRD measurement indicated that 17 % α-TCP transformed to calcium deficient apatite. Finally, after immersion at 50°C for 180 minutes of α-TCP powder, the setting time becomes 9 minutes. It was concluded that the immersion of αTCP powder in DW may be useful to reduce the initial setting time of α-TCP which clinically acceptable.

2-9

Association of cytokeratin 17 expression with differentiation of tumor cells in oral squamous cell carcinoma

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Objectives: Cytokeratin (CK) is expressed in basal cells of normal oral mucosa and squamous cell carcinoma cells, however, little is known about the CK expression pattern in oral squamous cell carcinoma (OSCC). The aim of this study was to evaluate the relevance of various CKs to clinicopathological parameters of OSCC.
Methods: In 62 samples from patients with primary OSCC prior to either chemotherapy or radiotherapy, the expression of CKs in tumor cells was immunohistochemically examined by anti-CK14, anti-CK16, anti-CK17, anti-CK18, anti-CK19, and anti-CK20 monoclonal antibodies. A comparison of CK expression patterns with clinicopathological parameters (tumor region and size, lymph node states, mode of invasion by Yamamoto-Kohama's criteria, differentiation grade, and clinical stage) was carried out, to clarify the clinically pronounced CKs from 6 kinds of CKs. The stained sections with more than 10% reactive cells in tumor cells were defined as positive.
Results: Of 62 samples, CK14 was detected in 62 samples (96.8%), CK17 in 44 (71.0%), CK16 in 36 (58.1%), CK19 in 22 (37.1%), CK18 in 15 (24.2%), and CK20 in 14 (22.6%). CK17 was significantly expressed in N0 cases ($p<0.05$) more than N-positive cases. Moreover, CK17 was significantly expressed in YK-1, YK-2, and YK-3 cases ($p<0.01$) more than YK-4C and YK-4D cases. CK20 was significantly expressed in T3 and T4 cases ($p<0.01$) more than T1 and T2 cases. There were no significant differences between other CKs (CK14, CK16, CK18, and CK19) and clinicopathological parameters.
Conclusion: The expression of CK17 was associated with metastasis of neck lymph nodes and characteristic of tumor invasion. The expression of CK20 was associated with clinical progression of OSCC.

2-10

Localization of Th subsets in salivary gland of Sjögren syndrome

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Objectives: Sjögren's syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration into the salivary and lacrimal glands with concomitant destruction of the glandular tissue. We had reported that T helper (Th) 1 / Th2 balance was associated with the initiation and progression of SS. Recently, Th17 and regulatory T cells (Treg) had been shown to have critical function in a variety of autoimmune disease. Moreover, the localization of Th subsets such as Th1, Th2, Th17, and Treg at each part of lesion has not been still poorly understood. Therefore, the aim of this study was to investigate the localization of these Th subsets in the labial salivary glands (LSGs) from SS patients.

Methods: Thirty-two patients with SS and 16 healthy controls were included in this study. Th subsets of infiltrating lymphocytes in the LSGs were examined by immunohistochemical staining. The mRNA expression of these Th type molecules including cytokines, chemokines, and transcription factors was determined by real-time polymerase chain reaction. In addition, lymphocytes without germinal center (GC) : (GC (−)) and lymphocytes with GC : (GC (+)) were extracted by using laser capture microdissection, and then, mRNA expression of these molecules in each part of lesion compared between two groups.

Results: In SS patients, the expression of Th17 type molecules was increased more than that in healthy controls. Whereas the expression of Th17 and Treg type molecules had no association with the degree of lymphocytic infiltration. In addition, Th1 and Th17 type molecules were detected strongly in the GC (−), while Th2 type molecules were in the GC (+).

Conclusion: These results suggest that SS has definitive localization of Th subsets in the lesions of LSGs. Furthermore, SS might be initiated by Th1 and Th17 cells and progress in association with Th2 cells.