

**The 16th Annual Meeting of the Society for Hard Tissue Regenerative Biology  
in Matsumoto Dental University**

*Period: September 22, 2007 Venue: Matsumoto Dental University, Shiojiri, Japan*

Special Lecture

**The morphological science of hard tissue**

**“The advancement of methodology and recent views on the morphological research of hard tissue”**

Hidehiro Ozawa

*Matsumoto Dental University Graduate School of Oral Medicine*

It is well known that “the advancement of science is commensurate with the advancement of methodology”, and the same is true of the morphological research of hard tissue. Because mineralized matrices make it difficult to get thin sections of hard tissue, several specific methodologies have been developed in morphological research of hard tissue. For example, decalcification methods introduced for thin sectioning of hard tissue have made it possible to develop fine structural observations and several histochemical studies of hard tissue. Additional improvements and ingenious advancements have been needed, however, because decalcification causes dislocation and/or diffusion of some organic and inorganic materials. I would now like to introduce several morphological methods for hard tissue, and some results from the field of hard tissue research.

Symposium

*Overview:S-1*

**A principle for hard tissue reconstruction**

Yoshinori Kuboki

*Professor Emeritus, Hokkaido University*

So much anticipation from the side of needy patients is caused by recent advance of tissue engineering technology. However, it seems that effective results can be hardly achieved unless we establish the proper principles for this technology. For this reason, we previously published a book, “Principles of Hard Tissue Reconstruction” in 1989, proposing the five factors theory, that is: (1) cells, (2) natural and artificial matrices, (3) regulators (4) vascularization and (5) mechanical dynamics, must be taken into consideration for effective bone reconstruction. We emphasized importance of their geometry, above all the concept of “optimal spaces” that guides and orients the hard tissue formation.

S-2

**Functional biomaterials for the regeneration of hard tissue**

Junzo Tanaka

*Tokyo Institute of Technology*

Biological tissues are composed of many compounds, e.g. proteins, polysaccharides, nucleotides and calcium phosphates, etc. These compounds in the tissues generally form well-organized structure build up from nanometer-scale. In order to reproduce such nanostructure or to create novel high functional biomaterials that induce cell responses similar to or better than autologous tissues, interfacial interactions between compounds in the biomaterials are important. In the present paper, an artificial bone material composed of hydroxyapatite/collagen self-organized nano-composite is introduced for one good example for novel highly functional biomaterials synthesized by soft-nanotechnology. In near future, such well-controlled functional nano-materials will be applied to the treatment of osteoporosis and cancer, etc.

S-3

**Industrialization of growth factors for hard tissue reconstruction**

Shigeki Hijikata

*FGF Strategic Planning, Kaken Pharmaceutical Co. Ltd.*

Major growth factors were discovered from 1960s to 70s and mass-production methods of them were established with development of biotechnology in 1980s. As a result, many health science companies tried to develop the drugs or

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medical devices for hard tissue reconstruction using growth factors. BMP-2, OP-1 (BMP-7) and PDGF have already  
been launched as medical devices for hard tissue reconstruction in the US and Europe. In Japan, although hard tissue  
reconstruction drug has not been launched yet, two clinical trials using bFGF for fracture healing and regeneration of  
periodontal tissue including alveolar bone are ongoing.

S-4

**History and problems of medical ceramic materials**  
**Summary of ceramics in medicine and advanced materials**

Toshiyuki Akazawa

*Section of Materials Chemistry, Department of Materials Technology,  
Hokkaido Industrial Research Institute*

In graving society, for regenerative therapy for patients, ceramics in medicine are important biomaterials and  
collaboration among the educational-industrial-administrative complex is an effective action. After returning the transition  
of bioceramics, functionally graded apatites (fg-HAp) originated from natural bone will be outlined as an interdisciplinary  
study focusing on biomimetic materials. The role and scheme of regenerative therapy-studies that are useful out there  
will be discussed. Since fg-HAp has excellent degradation-absorption, rhBMP-2-adsorption and release characteristics  
due to body fluid permeation and blood permeability, rhBMP-2/fg-HAp is an osteoinductive bioceramic with bone-  
remodeling. The fg-HAp can be designed and controlled by the calcination and dissolution-precipitation and it might  
be applied to higher advanced medical care by strong connection with medical and dental teams.

S-5

**New medical system using autogenous tissues -Biorecycle medical treatment-**

Masaru Murata

*Oral and Maxillofacial Surgery, School of Dentistry, Health Sciences University of Hokkaido*

The osteoinductive property of rabbit demineralized dentin matrix (DDM) was discovered in 1967. A surprising  
operation for recovering female's eyesight, osteo-odonto-keratoprosthesis therapy, was first done in 2003, Japan. This  
symposium introduces the basic researches of human DDM and the pioneering clinical trials of the autogenous DDM  
prepared by our developed automatic machine. Several DDM autograft cases for local bone augmentation will be  
discussed.

2006 Award Special Lecture

**Gene expression patterns of modulators for collagen mineralization during initial mineralization by  
osteoblasts cultured on titanium implant material**

Takashi Matsuura

*Department of Oral Rehabilitation, Fukuoka Dental College*

Titanium implants create a unique collagenous ultrastructure with thinner fibril morphology at the bone-implant  
interface. This study investigated the temporal mRNA expression patterns of collagen-binding small leucine-rich  
proteoglycans (SLRPs), lysyl hydroxylases (LHs), and matrix metalloproteinase (MMP)-3 and -13 during mineralization  
by MC3T3-E1 cells cultured on titanium. The members of SLRPs showed the distinct patterns from one another during  
mineralization, likewise, the members of LHs and MMPs showing the different. Although remaining to be investigated,  
SLRPs and LHs, modulators for collagen fibrillogenesis, and MMP-3 and -13, which degrade SLRPs and collagens,  
may play actual but distinct roles on the formation of a unique ultrastructure at the bone-implant interface.

Oral Session

**Osteoclast survival is enhanced by p38 MAPK signaling**

Teruhito Yamashita<sup>1)</sup>, Yasuhiro Kobayashi<sup>1)</sup>, Sakae Tanaka<sup>3)</sup>,  
Nobuyuki Udagawa<sup>2)</sup> and Naoyuki Takahashi<sup>1)</sup>

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<sup>3)</sup> Department of Orthopedic Surgery, University of Tokyo

To clarify the role of p38 MAPK in the survival and activation of  
osteoclasts, we examined whether forced-phosphorylation of p38  
MAPK modulates osteoclast function. Phosphorylation of p38  
MAPK in osteoclasts was not induced by LPS, although JNK and

ERK were activated. Adenoviral expression of a constitutively active form of MKK6 (MKK6CA) in osteoclasts resulted in phosphorylation of p38 MAPK. MKK6CA expression enhanced the survival of osteoclasts. Dentine-resorbing activity of MKK6CA-expressing osteoclasts was not altered. These findings suggest that activation of p38 MAPK signaling in osteoclasts enhances their survival.

### **Effects of far-infrared radiation (FIR) on osteoblast-like cells (MC3T3-E1) and bone formation**

Tatsuo Ishikawa<sup>1)</sup>, Kikuji Yamashita<sup>1)</sup>, Jun Ishibashi<sup>2)</sup>, Kaori Sumida<sup>1)</sup>, Takafumi Masui<sup>2)</sup> and Seiichiro Kitamura<sup>1)</sup>

<sup>1)</sup> Department of Oral and Maxillofacial Anatomy, Graduate School of Health Biosciences, University of Tokushima

<sup>2)</sup> Buranka Dental Office

To investigate the effects of FIR, we cultured osteoblast-like cells, MC3T3-E1 in with and without FIR incubator we had developed. Histological observation, comprehensive genetic analysis with PCR, and microarray methods were performed. Proliferation of osteoblasts were suppressed, expression of calcified nodules were upregulated by 29 per cent. Alkaline phosphatase, osteocalcin and expression of related-genes of interferon were facilitated. In vivo, though mice with FIR lost body weight, they gained bone weight compared with non-irradiated control. These results indicate promoter effects of bone formation and induction of expression of IFN.

### **$\beta$ -alanyl-L-histidinato zinc decreases M-CSF expression by osteoblast**

Noato Suzuki<sup>1,3)</sup>, Natsuko Tanabe<sup>2,3)</sup>, Masao Maeno<sup>2,3)</sup> and Kichibe Otsuka<sup>1,3)</sup>

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We examined the effect of  $\beta$ -alanyl-L-histidinato zinc (AHZ) on expression of M-CSF by osteoblastst (MC3T3-E1). AHZ suppressed both mRNA and protein expression of M-CSF induced by TNF Rel/NF- $\kappa$ B activation in response to TNF- $\alpha$  was down-regulated by AHZ. These results suggest that AHZ may inhibit osteoclast differentiation via decreasing Rel/NF- $\kappa$ B activation and subsequent expression of M-CSF by osteoblast. Moreover, when osteoclast precursor cells (RAW264.7) were cultured with soluble RANKL, number of TRAP-positive cells was decreased by the addition of AHZ.

### **A basic research of CaTiO<sub>3</sub>-C as a coating material for implants**

HaiLong Hu<sup>1)</sup>, Mika Okauchi<sup>1)</sup>, Ryo Tamamura<sup>1)</sup>, Andrea Paola

Rodriguez<sup>1)</sup>, Miho Inoue<sup>1)</sup>, Keisuke Nakano<sup>2)</sup>, Toshiro Sagae<sup>3)</sup>, Hitoshi Nagatsuka<sup>1)</sup>, Toru Takagi<sup>1)</sup> and Noriyuki Nagai<sup>1)</sup>

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A binding material called calcium titanate with amorphous carbon (CaTiO<sub>3</sub>-C) has been developed to enhance the bond strength and stability without increasing the interface between hydroxyapatite (HA) and titanium. Modified thermal decomposition method was used to increase Ca/P and Ca/Ti ratios and to decrease sintering temperature. Results revealed that a thin and homogenous coating was created between HA and titanium enhancing the bond strength as well as the stability of the implant. CaTiO<sub>3</sub>-C was negatively charged same with HA but had lower solubility than HA. The results suggest that CaTiO<sub>3</sub>-C induce long-term bone formation and possibly a coating material for implants.

### **Evaluation of CaTiO<sub>3</sub>-C *in vitro* study**

Miho Inoue<sup>1)</sup>, Andrea P. Rodriguez<sup>1)</sup>, Mika Okauchi<sup>1)</sup>, Ryo Tamamura<sup>1)</sup>, Hailong Hu<sup>1)</sup>, Masahisa Inoue<sup>2)</sup>, Hitoshi Nagatsuka<sup>1)</sup>, Tohru Takagi<sup>1)</sup> and Noriyuki Nagai<sup>1)</sup>

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Nowadays, titanium and hydroxyapatite (HA) are widely used as biomaterial for dental and medical applications. HA-coated titanium implants has excellent biocompatibility and mechanical properties. However, the adherence of HA film formed on titanium substrate is weak, because of the lack of chemical interaction between HA and titanium. A solution to this problem is to form an intermediate film on titanium substrate, which provide excellent adherence to both titanium substrate and HA. Because of this, we have developed a novel biomaterial called Calcium Titanium Oxide –Amorphous Carbon (CaTiO<sub>3</sub>-C; patent: 2005-269868; 2005-269869). The purpose of this study was identification of cellular proliferation and differentiation, response of osteogenic cells, and cytotoxicity. Osteoblastic cell line MC3T3-E1 were growth with powder of CaTiO<sub>3</sub>-C and HA, and on Ti-etching plate coating with CaTiO<sub>3</sub>-C, HA. CaTiO<sub>3</sub>-C has high cellular proliferation and no cell toxicity compared to HA.

### **Properties of osteoinduction and BMP-2 release in $\beta$ -TCP from spongy bone**

Junichi Tazaki<sup>1)</sup> Masaya Yamamoto<sup>2)</sup>, Yasuhiko Tabata<sup>2)</sup>, Toshiyuki Akazawa<sup>3)</sup>, Jun Hino<sup>4)</sup>, Masaru Murata<sup>4)</sup>, Makoto Arisue<sup>4)</sup> and Takanori Shibata<sup>1)</sup>

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<sup>4)</sup> Oral and Maxillofacial Region, School of Dentistry, Health Sciences University of Hokkaido

The aim of this study is to investigate *in vivo* local BMP-2 PK and bone induction in two bioceramics blocks, based on different composition and surface structures. Spongy bones in bovine femur were calcined at 800 °C by the step-wise calcinations to obtain bulk HAp (b-HAp). The  $\beta$ -TCP from spongy bone ( $\beta$ -TCP/ HAp) was designed by the partial dissolution-precipitation method. We estimated the *in vivo* release profile of <sup>125</sup>I-labeled BMP-2 and induction of hard tissues histologically. The  $\beta$ -TCP/ HAp is more effective for both BMP-2 retention and bone induction, compared to b-HAp.

### Experimental study on regeneration of the temporomandibular joint (TMJ) disc using *in situ* tissue engineering

Eizaburo Kobayashi<sup>1)</sup>, Tatsuo Nakamura<sup>2)</sup>, Kazuhisa Mori<sup>1)</sup> and Izumi Mataga<sup>1)</sup>

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The purpose of this study is to induce the regeneration of the TMJ disc and to evaluate it histologically. Japanese white rabbits were used. The TMJ was surgically exposed and a round full-thickness perforation was made in the center of the disc. In group A, a collagen sponge was placed in the perforation. In group B, a collagen sponge mixed with autologous bone marrow was placed in the same methods. In group A, after 12 weeks, the perforation was still not completely closed. In group B, after 2 weeks, regeneration of the disc was seen. This result may indicate that the collagen sponge with autologous bone marrow has a potential to induce the regeneration of the damaged TMJ disc.

### Involvement of CD81 in abnormal growth of RA synovial cells

Hiroki Mori<sup>1)</sup>, Hitoshi Nagatsuka<sup>2)</sup>, Ryo Tamamura<sup>2)</sup>, Keiichiro Nishida<sup>2)</sup>, Toshihiro Nakajima<sup>3)</sup>, Noriyuki Nagai<sup>2)</sup> and Tohru Nakanishi<sup>1)</sup>

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Synovial fibroblasts abnormally proliferate and generate osteoclasts under stimulated condition in rheumatoid arthritis (RA). We focused on the abnormal proliferation of RA synovial fibroblasts, and characterized these RA synovial fibroblasts by

DNA chip profiling of gene expression. The results showed that CD81 mRNA is highly expressed in RA synovial fibroblasts. Furthermore, stimulation of a synovial cell line with monoclonal antibodies against CD81 molecule promotes the expression of Synoviolin, a causative factor for RA with anti-apoptotic effect on synovial cells. These results suggest that CD81 is an upstream regulator of synoviolin gene, and is involved in the initiation and progression of RA.

### Calcification of the basement membrane under the proximal convoluted renal tubule after intravenous injections of lanthanum

Tateo Daimon<sup>1)</sup>, Makoto Miyagawa<sup>2)</sup> and Yoh Katoh<sup>3)</sup>

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The present study was undertaken to determine the effects of Lanthanum (La) on the kidney of the rat using an analytical electron microscope equipped with EDX analyzer. The animals were treated with five intravenous injections of La at 5mg La/kg BW per a week. The kidney was frozen and freeze-substituted in acetone using a freezing device. X-ray microanalysis defined the accumulation of La in the lysosomes of the mesangial cells and the matrix of the basement membrane of the tubular cells. A high amount of Ca and P was also detected in the La precipitates on the basement membrane indicating the calcification.

### Biological analysis of a candidate stem cell –KUSA/A1 cell– for bone tissue engineering

Andrea Paola Rodriguez, Tsujigiwa Hidetsugu, Nagatsuka Hitoshi, Borkosky Silvia, Takagi Tohru, Hailong Hu, Tamamura Ryo and Nagai Noriyuki

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KUSA/A1 cell is a bone marrow stromal cell, capable of differentiating into osteoblasts under inducing conditions. We demonstrated that KUSA/A1 cells with non-osteogenic potential seeded in diffusion chamber differentiated into osteoblasts and produced mineralized bone-like tissue. Finally, we evaluated the effect of honeycomb scaffold to produce abundant bone formation using KUSA/A1 cells implanted in subcutaneous tissues of SCID mice. 1x10<sup>6</sup> KUSA/A1 cells with honeycomb scaffold showed abundant new bone formation. While, 5x10<sup>6</sup> KUSA/A1 cells alone showed only few small islands of new bone. This study support that KUSA/A1 cell is a good candidate for basic research in bone tissue engineering.

### **An investigation of the ability to differentiate to dental tissue cells of the bone marrow derived mesenchymal stem cells**

Hidetsugu Tsujigiwa<sup>1)</sup>, Hitoshi Nagatsuka<sup>2)</sup>, Andrea Paola Rodriguez<sup>2)</sup>, Hailong Hu<sup>2)</sup>, Masao Yamada<sup>1)</sup> and Noriyuki Nagai<sup>2)</sup>

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We investigated the differentiation ability of the bone marrow derived stem cells, using GFP transgenic mice and rats. Wild-type mice/rats were irradiated (totally 10G) and transplanted GFP mice/rats derived bone marrow cells from caudal vein. After the transplantation, mice/rats were sacrificed and the dental tissues of them were analyzed histologically and biochemically. Immunohistochemically, GFP positive cells were observed in periodontal tissue and dental pulp. In the primary cell culture from these tissues, dominantly proliferated cells were GFP positive and possess calcifying ability. These results suggested that bone marrow derived stem cells may possess differentiation ability to periodontal tissue and odontoblasts.

### **The role of Cbfa1 on endochondral ossification in fetal bone implantation**

Masahisa Inoue, Kojun Setsu, Masahiko Akita and Tomomi Abe

Laboratory for Structure and Function, Faculty of Pharmaceutical Sciences, Tokushima Bunri University

We investigate the role of Cbfa1 on endochondral ossification using the fetal femur transplant mouse. Femurs on embryonic day 17 extirpated from Cbfa1 KO and wild type mouse were transplanted to the dorsal subcutaneous of normal mice. Specimens at 1 to 3 weeks after transplantation were examined histologically using H.E. staining and immunohistochemistry for osteocalcin and type II collagen. On the transplanted bone of Cbfa1 KO mouse, although differentiation of the chondrocytes was seen, destruction of the chondrocyte lacunae was not observed, and endochondral ossification had not taken place. Destruction of chondrocyte lacunae is essential for the progress of endochondral ossification, and Cbfa1 may play an important role for destruction of chondrocyte lacunae.

### **Diphenylhydantoin (DPH) suppressed osteoclast function at local**

Masanori Koide<sup>1)</sup>, Tadashi Ninomiya<sup>1)</sup>, Yasuhiro Kobayashi<sup>1)</sup>, Hiroaki Nakamura<sup>2)</sup>, Nobuyuki Udagawa<sup>1,3)</sup> and Naoyuki Takahashi<sup>1)</sup>

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DPH is often used as a drug for anticonvulsant. DPH has been reported to promote bone resorption. However, the effects of DPH on local bone metabolism are still unclear. The purpose of this study is to determine the effect of DPH in osteoclast (OC) function. Mouse OC cultured on dentin slices formed many resorption pits on the slices. DPH (200  $\mu$ M) completely inhibited pit-forming activity. Transgenic mice overexpressing soluble RANKL (sRANKL-Tg) show advanced bone resorption. DPH (2 mg/kg body) administered to the calvaria inhibited locally advanced bone resorption. These results suggest that DPH is a potent anti-bone resorbing drug in local bone diseases.

### **The effect of Sho-saiko-to (TJ-9) on human gingival fibroblasts**

Toshiaki Ara<sup>1,2)</sup>, Yoshihiro Maeda<sup>2)</sup>, Joji Itai<sup>2)</sup>, Yasuhiro Imamura<sup>1,2)</sup> and Pao-Li Wang<sup>1)</sup>

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The anti-inflammatory effects of TJ-9 were examined using human gingival fibroblasts (HGFs). HGFs were treated with the combination of LPS derived from *P. gingivalis* (10 ng/ml) and TJ-9 (0-0.2%w/v) for 24 h, and the amount of PGE<sub>2</sub> in culture supernatants was measured by ELISA. LPS-induced PGE<sub>2</sub> production was significantly decreased by TJ-9. The LPS-induced COX-2 expression was decreased by 0.1 and 0.2%w/v, whereas no apparent difference in PLA<sub>2</sub> expression was observed. These results suggest that TJ-9 suppressed PGE<sub>2</sub> production by the inhibition of COX-2 activity and therefore that TJ-9 has anti-inflammatory effect on periodontal disease.

### **Notch signaling in malignant transforming of odontogenic tumor**

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Notch signaling is responsible for cytological regulation of cell fate, morphogenesis and/or development. In this research, we examined Notch1 peptides and its genes (mRNA) in both ameloblastoma and ameloblastic carcinoma. After histopathological examination (hematoxylin and eosin: H&E), the

distribution of Notch1 intracellular domain (NICD) was examined by immunohistochemistry (IHC) using anti-human NICD monoclonal antibody. The IHC and ISH examination results suggest that Notch signaling plays some role in cytological differentiation or acquisition of tissue specific characteristics in neoplastic cells of tooth enamel organ-derived neoplasms, including benign and malignant neoplasms, ameloblastoma and ameloblastic carcinoma.

Poster Session

**Periodontal tissue reaction to mechanical stress in mice**

Takehiro Watanabe<sup>1)</sup>, Rina Muraoka<sup>1)</sup>, Takako Shimizu<sup>2)</sup>, Keisuke Nakano<sup>1,2)</sup>, Norimasa Okafuji<sup>1)</sup>, Saburo Kurihara<sup>2)</sup>, Kazuhiro Yamada<sup>1)</sup> and Toshiyuki Kawakami<sup>1,2)</sup>

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We examined the periodontal tissue reaction course of mice to mechanical stress according to the Waldo method. In the examination group, the arrangement of the periodontal ligament was irregular on specimen day 1. The extension and compression sites were observed at the opposite side of the roots. In day 1 and 3 specimens, the osteoclasts appeared in the compression sites. Immunohistochemical examination revealed that the expression patterns of Runx2 and Msx2 were clearly dynamic changed compared to the control specimens. These results suggest that the appearance of transcription factors related to cell differentiation of periodontal ligament, happened within 24 hours.

**The effect of local bFGF administration for growth of mouse mandiblar head**

Yuki Aoyama<sup>1)</sup>, Yosinori Arai<sup>2)</sup>, Fa-Chih Shen<sup>3)</sup>, Akihiro Kimura<sup>1,3)</sup>, Saburo Kurihara<sup>4)</sup>, Kazuhiro Yamada<sup>1)</sup> and Hasegawa Hiromasa<sup>1,3)</sup>

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Twenty-three in FGF family have various physiological activities, and bFGF is now being applied for wound healing. So we studied bFGF on the effect of mandibular condyle growth. The solution (0.1µl) of bFGF was injected around the mandibular condyle of ddY mice (1-3 days after birth). Morphometrical analyses of both 3DµCT images and histology showed that the size of experiments were larger than that of controls. However, there was no hyperplastic change in experiments. The proliferating index of experiments was significantly higher than that of controls.

Local application of bFGF might be useful for facilitating the growth of mandibular head.

**SNPs analysis of the enamelin gene in severe dental caries of primary dentition period**

Ken Ouryouji<sup>1)</sup>, Yasuhiro Imamura<sup>2,3)</sup>, Yoshihisa Fujigaki<sup>3)</sup> Hiroo Miyazawa<sup>1,4)</sup> and Pao-Li Wang<sup>2,3)</sup>

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An inherited factor is one of the cause of dental caries. We examined the relationship between SNPs of the enamelin gene and severe dental caries of primary dentition period. The subjects were 120 unrelated individuals of which 75 were severe dental caries patients (dmft•†10) who visited Matsumoto Dental University hospital, and 45 were healthy volunteers serving as controls. The lingual cells from subjects were harvested with tooth brush and DNAs were extracted. SNPs analysis of the enamelin gene at the position+2452 was carried out a PCR-RFLP method using DNAs and the specific primers. It is suggested that no significant difference was observed between healthy and severe caries subjects. The other candidate genes should be examined.

**Increase of intracellular calcium concentration in MC3T3-E1 cells by stannous compound**

Toshimi Hattori and Pao-Li Wang

Department of Dental Pharmacology, Matsumoto Dental University

SnF<sub>2</sub> is used as one of prophylactics of dental caries and Sn<sup>2+</sup> may be involved in its action. MC3T3-E1 cells were used as the material. SnCl<sub>2</sub> was adopted as the stannous compound. To determine which types of Ca<sup>2+</sup> channels these cells possess and to examine the effects of SnCl<sub>2</sub> on the intracellular calcium concentration ([Ca<sup>2+</sup>]<sub>i</sub>). High K<sup>+</sup> medium-induced rise in [Ca<sup>2+</sup>]<sub>i</sub> was significantly inhibited by nifedipine and verapamil, but not by fÖ-conotoxin GVIA or NiCl<sub>2</sub>. These findings lead us to the conclusion that SnCl<sub>2</sub> elevates the [Ca<sup>2+</sup>]<sub>i</sub> in MC3T3-E1 cells by enhancing Ca<sup>2+</sup> entry through the L-type Ca<sup>2+</sup> channels.

**Study of the effect of porous titanium on proliferation and differentiation of osteoblast-like cell MC3T3-E1**

Yoshihiko Sugita<sup>1)</sup>, Katsutoshi Kubo<sup>1)</sup>, Emiko Sato<sup>1,3)</sup>, Tatsushi Kawai<sup>2,3)</sup> and Hatushiko Maeda<sup>1,3)</sup>

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The objective of this study was to evaluate the effects of porous titanium on proliferation and differentiation of osteoblast-like cell (MC3T3-E1). In this study, the mRNA expressions of alkaline phosphatase, osteopontin and osteocalcin were investigated using real-time PCR. These mRNA expressions were not controlled. These results suggested that the porous titanium was useful scaffolds for tissue engineering applications of bone formation. In addition, we conclude that real-time PCR is useful to assess biocompatibility of biomaterials.

### **Cell type specific expression of the salivary histatin 3 gene**

Yasuhiro Imamura<sup>1,2)</sup>, Yoshihisa Fujigaki<sup>2)</sup> and Pao-Li Wang<sup>1,2)</sup>

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Salivary histatins are histidine-rich polypeptides, which have an anti-microbial activity and inhibit proteases from oral bacteria. Histatins are also specifically expressed in salivary glands. However, it has not been clarified the expression mechanisms of those genes. We have cloned the histatin 3 gene promoter and its various deletion mutants were constructed luciferase reporter plasmids. The -2108 ~ -2082 region of the promoter worked as a positive transcriptional element in HSG cells (salivary gland). In contrast, their expressions were not observed in the other cell types. A protein bound to the region was 100 kDa by UV-cross linking analysis. Taken together, the expression of the histatin 3 gene may have cell type specificity.

### **Two dimensional analysis of the degree of calcification of new bones around implants**

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To clarify new bone tissues, radiography with a newly developed tunable wavelength and highly parallel Parametric X-ray, PXR, was applied. Coated or grit-blasted Ti-alloy implants modified with coating or blasting were implanted in surgically created defects in rabbit tibia. Undecalcified polished thin sections were prepared from the implant/bone areas 1, 2 and 4 weeks after implantation. LEBRA-PXR allows quantitative of initial stage of

new bones.

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### **Examination on microstructure of acid-etched titanium-tissue interface**

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This study presents the first observation of the interface between acid-etched titanium and cultured osteoblastic tissue at micro- and ultra-structural levels. Rat bone marrow stromal cells were cultured on the thin titanium disk for 14 days. The cultured tissue was examined by SEM and EDS elemental mapping. EDS line scan from the outer surface of tissue to the titanium interface revealed that elemental peaks of Ca and P existed in an identical level of vicinity, while the element of oxygen was distributed widely in the tissue with its peak at 3 mm from the titanium surface. EDS area mapping confirmed a uniform Ca spread within the 2 mm range of tissue. We have succeeded cross-sectional imaging of the acid-etched titanium and cultured mineralized tissue interface. The interface at day 14 of culture was characterized by the intense localization of calcium.

### **Basic research for crystal composition of sialolith**

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Sialolith is an ectopic calcification of high frequency in oral region but the details were unclear. In this study, histo-pathological stainings, Micro-XRD and Micro-FTIR were carried out to investigate structure and chemical composition of sialolith. Micro-XRD showed that amount and orientation of biological apatite varied in the samples. Micro-FTIR analysis showed the variations in strength and ratio of absorption bands due to phosphate ions and carbonate ions. Histo-pathological observation revealed the inner core and the outer shell. From these results, it was suggested that these sialoliths were formed in complex manner reflecting the variation in circumstances.

### **Alveolar bone regeneration by periodontal cells**

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We examined the process of alveolar bone regeneration after subcutaneous implantation of rat molars in order to clarify the bone formation ability of periodontal ligament cells. At 5 days after implantation, BrdU-positive cells appeared adjacent to tooth root. Alpha-SMA and/or BMP4- positive reactivity were detected in several fibroblastic cells. After 10 days, new bone formation was seen in the furcation area of molar. Osteoblast-lineage cells surrounding bone matrix showed Runx2- and Osterix-positive reactivity. Additionally, osteopontin and bone sialoprotein were localized in bone matrix. These results indicate that periodontal cells possess the ability of proliferation and differentiation into osteoblasts through BMP signaling. Periodontal cells could be useful for periodontal tissue regeneration.

#### **Effects of carbonic anhydrase inhibitor acetazolamide (AZ) on osteoclasts and bone texture**

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We investigated the effects of carbonic anhydrase inhibitor acetazolamide (AZ) on osteoclasts using culture system of rat bone marrow. The number of osteoclasts decreased by AZ in a dose dependent manner, showing morphological apoptotic characteristics. We also examined histologically the changes of trabecular bone and osteoclasts on tibiae of rats subcutaneously injected with AZ for 21 days. The area of trabecular bone of AZ treated group increased compared with control group. It was also found the density of osteoclasts of AZ treated group was statistically lower than that of control group at the epiphyseal plates.

#### **Effect of Byakko-ka-ninjin-to and Gorei-san in STZ-induced diabetic saliva flow disorders mouse**

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Diabetes mellitus (DM) often accompany the mouth dryness symptom. Recently, the therapy of DM with the Japanese traditional medicine is noted and the research is continued. In this study, the effects of Byakko-ka-ninjin-to and Gorei-san on salivary flow were examined in streptozocin (STZ)-induced diabetic mice. The blood glucose level was maintained at 300 mg/dL from 7 to 21 days after administration of STZ. At day 21, salivary flow was significantly decreased in mice administered with STZ compared with control group. Administration of Byakko-ka-ninjin-to or Gorei-san to the STZ-induced DM model mice was recovered from hyperglycemia and saliva flow disorders.

#### **Search for chronic periodontitis-related gene by SNPs analysis**

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Periodontal disease is the effect of its variety and environmental condition. It has not been clarified the relation between this disease and hereditary factor. In this study, polymorphisms of the antimicrobial peptide genes, histatin3 (from saliva) and -defensin 1 (from epithelial cells), were analyzed. Adult healthy and chronic periodontal subjects were 100 persons, respectively. SNPs analysis was carried out a PCR-RFLP method using DNAs from the subjects and specific primers. The results indicated that the polymorphisms of both genes were no significant difference statistically. The other candidate genes should be examined.

#### **Notch signaling in mandibular condylar cartilage development**

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To investigate the expression pattern of Notch signaling, we fixed mandibular condyles of ddY mice from embryonic days E14 to just after birth (E19). In IHC at E14, expression of Notch peptide was observed in the nuclei of coagulating mesenchymal cells. After E15, Notch peptide appeared in the nuclei and the cytoplasm of cells. In ISH at E14 and E15 expressions of Notch

mRNA appeared in cytoplasm of proliferating chondrocytes. From E16 to E19, Notch 1 mRNA was detected throughout almost all layers. These results suggest that Notch signaling plays an essential role for mandibular condylar cartilage development.

**Jagged1 peptide appearing in mandibular condylar cartilage development**

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We investigated the expression pattern of Jagged1 peptide in mandibular condylar cartilage. Mandibular condyle of ddY mice were fixed from embryonic day 15 (E15) through just after birth (E19). At E15, the proliferating cells had positive products of Jagged1 in their cytoplasm and cell membrane of almost all coagulating cells. At E17, cytoplasmic and membranous reactions of Jagged1 factors appeared strongly in the cells just inside the condylar cartilage sheath. At just after birth, Jagged1 was observed in a portion of almost all layer cells. These results suggest that Jagged1 plays an essential role for mandibular condylar cartilage morphogenesis and development.