



ASIA-PACIFIC CONFERENCE in FUKUOKA 2013

International Symposium on Oral Education and Research in Kitakyushu

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Asia-Pacific Conference in Fukuoka 2013

International Symposium on Oral Education and Research in Kitakyushu

Kyushu Dental University, Kitakyushu, Japan
Jan 26th “2013

Organizing Committee:

Tatsuji Nishihara, President
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Organized by Kyushu Dental University
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Welcome message



Tatsuji Nishihara, D.D.S., Ph.D.

President
Kyushu Dental University

Welcome to Asia-Pacific Conference in Fukuoka 2013. It is our great honor and pleasure to invite you to attend the International Symposium on Oral Education and Research in Kitakyushu, Japan, January 26th, 2013. I am inviting you to participate in an exciting opportunity to obtain valuable information on Oral Education and Research in Asian countries.

Progress in oral education and research over the last decade has been great and we have greatly contributed to this issue. At this conference, we plan to address wide-ranging themes concerning oral education on oral biology, oral care and health, and collaboration between dentistry and biotechnology, through an invigorating combination of symposia, poster presentations and discussions. It is our wish to provide an opportunity for the presentation on the forefront of oral biology, exchange information on oral health, and flash an innovative idea into your mind to build true partnership with Asian countries concerning oral education with an academic collaboration.

We thank you in advance for your interest and active participate and look forward to welcoming you to the Asia-Pacific Conference in Fukuoka 2013.

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Program

13:00 Registration

13:20 Welcome Address and Opening Remarks
Prof. Tatsuji Nishihara (President of Kyushu Dental University)

13:30 - 15:00

Symposium I

”Grand Design for Education of Dentistry”

Chairs: Yasuaki Kakinoki, Eijiro Jimi

Prof. Shwe Toe (Dean, University of Dental Medicine, Mandalay)

A case report on Ameloblastoma and Oral Submucous fibrosis

Prof. Shun-Te Huang (Dean, Dental School of Kaohsiung Medical University)

Grand Design for Education of Dentistry

Prof. Ryuji Hosokawa (Dean, Dental School of Kyushu Dental University)

Centennial History of Kyushu Dental University and Dental Education in Japan

Coffee Break

15:15 - 16:15

Symposium II

**“Basic and Clinical Study on Telomerase
for Oral Cancer Diagnosis”**

Chairs: Keisuke Nakashima, Yuji Seta

Prof. Shigeori Takenaka (Kyushu Institute of Technology)

Development of electrochemical telomerase assay aiming at a cancer diagnosis

Prof. Kazuhiro Tominaga (Kyushu Dental University)

Electrochemical telomerase assay for oral cancer detection

16:20 - 17:20

Poster Sessions

Exhibition

17:30 Closing Remarks
Prof. Shin-ichi Masumi (Vice-President of Kyushu Dental University)

18:30 Banquet

Plenary Lectures



A case report on Ameloblastoma and Oral Submucous fibrosis

Shwe Toe

Rector, University of Dental Medicine, Mandalay

Ameloblastoma is a slow growing benign tumor of the jaws consisting of proliferating odontogenic epithelium. They are notorious for their invasive growth and their tendency to recur and usually present considerable size to cause facial disfigurement, displacement of teeth and pathological fracture. It is the most commonly encountered odontogenic tumour in Africa and Asia, but the second most common odontogenic tumour in North and South America . In Myanmar, it is not usually uncommon and some patients from upper Myanmar seek for the treatment at UDM (Mandalay).

Oral Submucous fibrosis (OSMF) is insidious potentially malignant disorder affecting any part of oral cavity & sometimes the pharynx. It is characterized by inflammation, progressive subepithelial fibrosis and stiffness of deeper connective tissues leading to limitation of mouth opening. Although several agents have been implicated in etiology, conclusive evidence now exists that OSMF is caused by areca nut. Numerous countries including Myanmar, parts of Malaysia, Pacific islands and others have higher rates of areca nut use. Unfortunately, the incidence of OSMF is still higher in Myanmar regardless of public health education on areca nut usage, early detection and prompt treatment given.



Grand Design for Education of Dentistry

Shun-Te Huang

Division of Special Care Dentistry,
Department of Oral Hygiene
Dean, College of Dental Medicine,
Kaohsiung Medical University

Diverse changes in the global social environment such as economic depression and demographic structure have occurred since the end of the 20th century and it has impacted our dental society rapidly and dramatically. The evolution in dental society is an unbalanced supply of dentists and need for young dentists, high technique and advancement of the exploration of dental sciences and dental instruments, regulation in the standard of dental procedure and request for the quality of dental treatment in hospital accreditation. All of these items increase the expenditure of educational cost to universities; in contrast, tuition from students has not risen in accordance with expenditure. These problems affect the financial management of universities. On the other hand, the oral health awareness has increased the demand for the care of comprehensive oral function and esthetic restoration in which dental implantology, cosmetic dentistry and orthodontics are included. These have become main stream. For the same reasons people have begun to pay more attention to oral health care from the curative approach. Thus, demands for preventive dentistry increased. The humanistic attitude toward the minorities and indigent people stimulate the growth of special care dentistry, gerontology, and oral care for patients under long-term care and medically compromised patients. All of these newly developed fields are comprehensive care, oral medicine. Needs of advanced knowledge and skills that require professional and multi-disciplinary team work were initiated to create a new era of dentistry. In order to approach this goal, the system of dental education shall be restructured with a reflection of this evolution taking place globally.



Centennial History of Kyushu Dental University and Dental Education in Japan

Ryuji Hosokawa

Dean, Faculty of Dentistry,
Kyushu Dental University

Kyushu Dental University was founded in 1914 by Masaomi Kuninaga as Kyushu Dental School, and its purpose was to give Japanese students the opportunity to learn modern American dental medicine. Masaomi Kuninaga went to US from 1902 to 1910 and he obtained DDS from the University of Illinois College of Dentistry (Class of 1909). At the time he returned to Japan, dentistry was not yet recognized as a profession. Moreover, as Japanese government had adopted a policy to strengthen the country, little attention was paid toward the development of dental education. Despite these problems, Masaomi Kuninaga succeeded in getting his school awarded professional school status in 1907. The school was awarded full college status in 1949, making it one of the first 6 colleges of dental education in Japan. Thus, the history of our university represents Japan's modernization of society as well as development of dental education, and it has continued to pioneer dental medicine and devoted to serve the local community.

Dental education in Japan has arrived at crossroads. In recent years, the position of dental education within the university is being questioned as is its relationship to medicine and the larger health care system. In this session, I would like to discuss various aspects of issues and future prospects regarding dental education in Japan as well as in our University.



Development of electrochemical telomerase assay aiming at a cancer diagnosis

Shigeori Takenaka

Research Center for Bio-microsensing Technology
and Department of Applied Chemistry,
Kyushu Institute of Technology

Recently, telomerase is attracting attention as cancer marker and simple and quick detection of telomerase activity will be useful for cancer diagnosis. The detection of telomerase activity was established first by Kim *et al.*¹ as a TRAP assay, including the extension reaction of a TS primer carrying a sequence extendable by telomerase, PCR amplification of its product, and analysis by gel electrophoresis. When the TS primer immobilized on the electrode can be extended by telomerase, an electrochemical assay of telomerase activity will be realized without PCR. To realize this assay, we applied to the ferrocenylnaphthalene diimide (FND)-based electrochemical hybridization assay.² Spectroscopic studies revealed that FND can bind to a tetraplex DNA at high potassium ion concentration. The tetraplex DNA was stabilized by the binding of FND and this effect was larger than that of any other tetraplex stabilizers which are known as a telomerase inhibitor. Quantitative analysis with circular dichroism and a quartz crystal microbalance (QCM) strongly suggested a 3:1 binding stoichiometry of FND to the tetraplex DNA. The telomere sequence could be extended by telomerase with the TS primer on the surface of an electrode as proven by an increased current signal of FND bound to the tetraplex DNA formed on the electrode. The peak current was in proportion to the amount of cell in the range of 50-150 cells, which is the same as that for traditional methods using PCR. Therefore, it was found that FND can bind to tetraplex DNA and the telomerase activity can be electrochemically detected without PCR. Oral cancer diagnosis was successfully achieved by this technique.³ This work will be presented by Prof. Kazuhiro Tominaga, Kyushu Dental College in next presentation.

References

1) N. W. Kim *et al.*, *Science*, **266**, 2011 (1994). 2) S. Sato *et al.*, *Analytical Chemistry*, **77**, 7304 (2005). 3) K. Mori *et al.*, *Clinical Chemistry*, **59**, 289-295 (2013).



Electrochemical telomerase assay for oral cancer detection

Kazuhiro Tominaga

Division of Maxillofacial Surgery,
Department of Science of Physical Function,
Kyushu Dental University

The prevalence of oral cancer in Japan is only 3 % of whole malignant neoplasm. In south or south-east Asian, however, it is one of the most prevalent cancer (20 to 30 %). The specific risk of oral cancer in those areas is mal-habit of betel quid chewing. Primary prevention of oral cancer is to quit the mal-habit. And secondary prevention involves early detection of cancer before the appearance of symptoms (screening). The purpose of our study was to develop a novel screening system of oral cancer using electrochemical telomerase assay (ECTA). Telomerase has long been known as a cancer marker. However, the conventional detection method of telomerase activity; telomerase repeat amplification protocol assay (TRAP) is complicated and time consuming to perform. ECTA was found to be simple and provide quick results without PCR and gel electrophoresis. We compared the sensitivity and specificity for detection of oral cancer patients between TRAP and ECTA.

We tested 3 types of samples; exfoliated cells from the whole oral cavity (OEC), exfoliated cells from the mucosal lesion (LEC) and small tissue from lesion (T), which were obtained from 30 oral squamous cell carcinoma patients and 17 healthy volunteers. The sensitivities of cancer detection with ECTA in the samples of OEC, LEC and T were 93.1%, 81.3% and 93.3%, respectively, while those values with TRAP were 39.3%, 58.6% and 90%, respectively. The specificities with ECTA in the same sample groups were 81.3%, 68.2% and 83.3%, respectively while those with TRAP were 58.8%, 43.5% and 50%, respectively. Both sensitivity and specificity with ECTA were much higher than those with TRAP regardless sample types. We concluded that ECTA could be an excellent screening system for the early detection of oral cancer.

Poster Presentations

Inactivation of periodontal pathogens by *Pseudomonas aeruginosa*

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Wataru Ariyoshi², Tatsuji Nishihara²

¹Department of Biological Functions and Engineering, Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology

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A probiotic study for periodontal diseases is promising as a hopeful approach to eradicate pathogenic bacteria since antibiotic therapies often fail through the acquirement of drug resistance. In this study, we investigated the inactivation of a Gram-negative periodontal pathogen, *Aggregatibacter actinomycetemcomitans* by *Pseudomonas aeruginosa*. The bacterial growth of *A. actinomycetemcomitans* was remarkably inhibited when the secretion products from were mixed to the growth media. The impact enhanced according to the increase of cell turbidity of *P. aeruginosa*; in fact the culture fluid at a late growth stage of *P. aeruginosa* had a strong inhibition impact rather than that at an early stage. Enzyme treatments and heat treatments for the culture fluid were conducted and the treated culture fluid had the same growth inhibition as that without the treatments; hence, it indicated that the factor to act on the growth inhibition of *A. actinomycetemcomitans* is not enzymes such as protease, cellulase, and amylase and the factor is stable for heat. Finally, we figured out that one of the factors was pyocyanin, a blue, secondary metabolite produced by *P. aeruginosa* because a pure pyocyanin compound showed the growth inhibition to *A. actinomycetemcomitans*. The growth inhibition by pyocyanin may be due to the reactive oxygen species generated by this compound with a redox-active property. The impact of growth inhibition by pyocyanin was not observed in the other oral pathogens, *Porphyromonas gingivalis* and *Streptococcus mutans*.

Acknowledgements: This study is a collaborative research with Dr. Toshinori Okinaga, Dr. Wataru Ariyoshi, and Dr. Tatsuji Nishihara, Kyushu Dental College.

Classification of periodontal disease patients by FT-IR

Satoshi Fujii¹, Keisuke Fukuda², Shinobu Sato², Toshinori Okinaga³,
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Shigeori Takenaka²

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It is known that periodontal disease is caused by the propagation of abundant bacteria inside the oral cavity. Periodontal disease is a risk factor for diabetes, thus it is important to estimate the amount and the type of bacterial growth in the oral cavity for proper diagnosis of periodontal disease. Under such circumstance, we analyzed saliva samples using Fourier transform infrared (FT-IR) spectroscopy, in which case, the abundance of bacteria contained in saliva samples were estimated. We performed screening of the periodontal disease by means of the IR spectra difference.

We tested saline solutions including saliva, which are grouped into two, namely: periodontal disease patients and healthy volunteers. These samples were fractionated using a centrifuge. One μL of the supernatants were spotted with ca. 1.0 mm of diameter on CaF_2 plate. These spots were dried under atmospheric condition and IR spectra of these spots were measured using FT-IR microscopy. The magnitude of IR raw spectrum was about 10 times larger on periodontal disease patient samples as compared from the healthy volunteer samples. The shape of the 2nd derivative spectrum was clearly different between periodontal and healthy volunteer samples.

The result indicates that the amount of bacteria on periodontal saliva samples were different from those taken from healthy volunteer samples. Partial least squares discriminant analysis was used for the discrimination of periodontal samples based on second derivative spectrum having 97 % of leave one outcross validation discrimination accuracy. The result obtained in this study suggests that FT-IR technique should be considered a useful method in screening progress of periodontal disease from saliva.

New detection system for the adhesion of oral bacteria on macrophages *in vitro*

Masaki Morishita¹, Toshinori Okinaga², Wataru Ariyoshi²,
Keisuke Nakashima¹, Tatsuji Nishihara²

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²Division of Infections and Molecular Biology,
Department of Health Promotion, Kyushu Dental University

Streptococcus sanguinis is a member of the viridans group of oral streptococci which cause infective endocarditis, and its pili have been reported as potential virulence factors via adhesion to human epithelial cells. In the present study, we developed the detection system of cell-bacteria adhesion *in vitro*, and examined the possible involvement of *S. sanguinis* pili in the attachment on the macrophage cell clumps.

We cultured mouse macrophage cell line RAW 264.7 in media supplemented with 10 % fetal bovine serum, and stimulated with lipopolysaccharide (LPS) derived from *Aggregatibacter actinomycetemcomitans*. To evaluate the adhesion ratio of bacteria to macrophages, GFP-expressed *S. sanguinis* SK36 and SK36 pili-deficient-mutant (Δ pili) were detected with a fluorescence microscope using our developed microchannel chip. *S. sanguinis* SK36 strongly attached to macrophage cell clumps. Treatment of LPS remarkably enhances the adhesion of *S. sanguinis* SK36 on macrophage cell clumps. However, *S. sanguinis* SK36 Δ pili did not attach to macrophage cell clumps even when the cells were treated with LPS. In addition, quartz-crystal microbalance was employed to analyze the affinity of *S. sanguinis* pili and intercellular adhesion molecule-1 (ICAM-1). Although pili of *S. sanguinis* SK36 showed strong binding activity to ICAM-1, no binding was detected between *S. sanguinis* SK36 Δ pili and ICAM-1. *S. sanguinis* was found to adhere to macrophage cell clumps in our detection system. Our results suggest that the adhesion occurs through the interaction between *S. sanguinis* pili and ICAM-1 on macrophages.

The involvement of inflammasome in mouse macrophage infected with *Aggregatibacter actinomycetemcomitans*

Toshinori Okinaga, Wataru Ariyoshi, Tatsuji Nishihara

Division of infections and molecular biology, Kyushu Dental University

We previously reported that periodontopathic bacteria, *Aggregatibacter actinomycetemcomitans*, induced cell cycle arrest in mouse macrophages. Recent study reported that inflammasome, which are intracellular pattern recognition receptors, is required for immune response. In the present study, we demonstrated the role of inflammasome in *A. actinomycetemcomitans*-infected macrophages.

A. actinomycetemcomitans Y4 and ATCC29522 strains were used in this study. First, we investigated the internalization of bacteria in mouse macrophages using FITC-labeled *A. actinomycetemcomitans*. In immunofluorescence staining, we confirmed the invasion of *A. actinomycetemcomitans* in mouse macrophages. We showed the slightly inhibition of cell viability at MOI 50 in *A. actinomycetemcomitans* infection using MTT assay. We detected the pro-inflammatory cytokine, such as IL-1 β and IL-18, in *A. actinomycetemcomitans*-infected macrophages by ELISA and real-time RT-PCR analysis. We found that inflammasome components, NLRP3 and ASC, were upregulated by Western blotting analysis and real-time RT-PCR, indicating that *A. actinomycetemcomitans* infection activated the formation of inflammasome complex in macrophages. On the other hand, in NLRP3 knockdown macrophages, the expression of mature IL-1 β induced by *A. actinomycetemcomitans* infection was completely prevented.

These results suggest that *A. actinomycetemcomitans* infection activates the inflammasome and secretes the mature IL-1 β in mouse macrophages.

P-5 Evaluation of the Newly Created “Oral Implantology” Course for Undergraduates at Kyushu Dental College

Tetsuji Nakamoto¹, Chihiro Masaki¹, Yusuke Kondo¹, Taro Mukaibo¹,
Ikuya Miyamoto², Tetsuya Goto³, Yasuhiro Morimoto⁴, Eijiro Jimi⁵,
Ryuji Hosokawa¹

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Implant supported prosthesis treatment is becoming very common for the replacement of missing teeth, and there is a rising need for a class in “Implant Dentistry” for undergraduate education. In the present study, we evaluated the educational effects of a newly created class on implant treatment, “Oral Implantology,” which began in the 2011 school year at Kyushu Dental College. For that purpose we compared the test scores of the students who took “Oral Implantology” (4th year group: LG, n=96) and those who did not take the course (6th year group: UG, n=75). The average test score in LG (75.2%) was significantly higher than that in UG (71.3%), though the difference was small and the median value was identical (75%). Results seem to indicate that detailed instruction was effective to increase knowledge concerning implantology, but general instruction was more important for further increasing the effectiveness of the course.

The risk factor analysis of immediate-loaded implants in edentulous upper jaws.

Chihiro Masaki, Yusuke Kondo, Taro Mukaibo, Tetsuji Nakamoto,
Ryuji Hosokawa

Department of Oral and Reconstruction and Rehabilitation,
Kyushu Dental University

The success rate of dental implants has been increasing. However, there are still some failures, especially when the implants undergo immediate loading. The objectives of this retrospective study were to identify the anatomical risk factors for immediate functional loading implants in edentulous upper jaws. Twenty-nine cases that underwent immediate loading were reviewed in this retrospective study. Patients were divided into two groups; Successful Group (SG: 21 patients, 60.5 ± 1.80 years old) and Lost Group (LG: 8 patients, 55.1 ± 3.94 years old). Those who lost implants within 6 months after implant placement were classified into LG. In addition to systemic data, anatomical measurements were conducted through CT data utilizing 3D CT data analyzing software (SimPlant Pro Ver. 11.03, Materialize Japan) to compare SG and LG. Mann-Whitney U-test was used for statistical analysis. Since all patients in LG were males, females were excluded from this study. There were no significant differences in background factors (age, systemic diseases, and all data obtained from blood samples) between SG and LG. In addition, there were no significant differences in measurements of skeletal indices except length of body of maxilla. However, a significant difference was found in the width of masseter muscle (maximum width from CT axial sections): LG showed a significantly larger measurement than SG ($p < 0.01$, LG: 14.8 ± 0.68 mm, SG: 11.0 ± 0.45 mm). CT data analysis is very useful not only for implant simulation but also to diagnose risk factors for immediate loading. It seems that if the width of masseter muscle is greater than 10 mm, we must consider the potential for mechanical overloading to avoid disintegration during the early stage of implant treatment. Further studies will be required to confirm these findings and to elucidate risk factors in females.

P-7 Hyper- and hypo- osmolarity effect mice submandibular gland function

Yusuke Kondo, Taro Mukaibo, Manami Kidokoro, Atsushi Imamura,
Chihiro Masaki, Tetsuji Nakamoto, Ryuji Hosokawa

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Kyushu Dental University

Epithelial cells are sensitive to osmotic circumstances. The effect of osmotic challenge on salivation and its molecularly regulation mechanisms were explored. Perfused mice submandibular gland were used and the osmolality in the perfusion solution was changed to examine the response of submandibular gland to hypotonic or hypertonic condition. Amount of secreted saliva and ion concentrations of saliva were analyzed. For intracellular signaling measurements, dispersed cells were prepared, and then Ca^{2+} or pH sensitive fluorescence dye was used. Students' t-test or one-way ANOVA and then Tukey post hoc test was appropriately used for statistical analysis. Fluid secretion was increased at hypotonic condition with the maximum at 30% hypotonic, and fluid secretion was extensively decreased at 30mM (around 30% decrease) and 100mM (around 60% decrease) sucrose added hypertonic condition. Ca^{2+} response was unchanged in hypotonic condition, whereas significantly decreased in hypertonic conditions. In the presence of bumetanide, an inhibitor $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ co-transporter, 30% hypotonic induced fluid increase was almost completely diminished. And this was further confirmed by $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ co-transporter induced NH_4^+ transport activity under ammonium shock, where NH_4^+ transport was up-regulated by 40% at 30% hypotonic osmolarity, which was also inhibited by bumetanide. Conclusions: The increase in response to hypotonic condition is attributing to specific activation of $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ co-transporter expressed in basolateral membrane. In contrast, intracellular Ca^{2+} response has a central role in decrease during hypertonic osmolarity.

3D FEA Analysis of Post-Core Construction for Endodontically Treated Teeth

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Many dentists sometimes observe root fractures in endodontically treated teeth supported by metal posts. Recently, fiber post with composite resin, which physical property is almost same with root dentin, is often applied for the post-core construction to avoid root fractures. However, causes of root fracture of tooth supported by post-core construction are not well understood. To clarify effects of materials used in post-core construction on root fracture, we carried out 3D FEA of tooth supported by post-core construction, and analyzed stress distribution. Models of metal and fiber posts were created using modeling software Rhinoceros 4.0 (Robert McNeel and Associates), and inputted into finite-element-analysis software Abaqus 6.11 (SIMULIA). In 3D FEA analysis, concentrated static load (100 N) was applied at the tip of cusp of each model. As results, when the interface between post-core construction and root dentin was separated in each model, tensile stress distributed at the load side. This stress distribution was intensely observed in fiber post model. On the other hand, at separated condition, compression stress distributed near the junction of root dentin and the post-core construction in the wide range at the opposite side, and intensely observed in metal post model. Furthermore in the metal post model, stress concentration was observed at the tip of a post. These results suggest that the difference of physical properties between metal and fiber, as well as adhesion between post-core construction and root dentin may affect the modality of root fracture.

P-9 Effects of Platelet-Rich Plasma on Odontoblast-like Cells and Human Periodontal Ligament Stem Cells.

Miki Ichimaru-Suematsu¹, Ayako Washio¹, Sizu Hirata-Tsuchiya¹,
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In the dentistry, platelet-rich plasma (PRP) from autologous blood is known as one of useful and safe biomaterials to deliver growth factors for the induction of proper wound healing. In this study, to clarify mechanisms of wound healing and local regeneration of dentin-pulp complex and periapical periodontal tissue, we examined effects of PRP on proliferating pulp progenitor cell line (KN-3 cells) and human periodontal ligament stem/progenitor cell line (HPDLCs). After treatment with PRP, cell morphology and expression of markers of odontoblast differentiation, such as dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1) were examined on KN-3 cells were examined by phase-contrast microscopy and real-time PCR, respectively. Cell morphology and cell proliferation of HPDLCs were also examined by phase-contrast microscopy and WST-8, respectively. We found that PRP induced morphological change of KN-3 cells, as well as the expression of DSPP and DMP-1. PRP also induced the process elongation and cell proliferative suppression on HPDLCs. These results suggested that PRP may have effects on wound healing and dental pulp and periapical periodontal tissue.

P-10 Role-playing Using Treatment Leaflets for Childhood Patients

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Dental residents and clinical training students have restriction in actually gaining clinical experience through many patients from the reasons of a patient's degree of cooperation, the difficulty of a case, etc. Therefore, simulation education attracts attention in order to compensate the contents of medical examination with an insufficient clinical experience so that many clinical experiences can be gained. This time, we took up the caries which is a typical disease which encounters by pediatric dentistry clinical for the student of laboratory assignment, created the leaflet for a medical interview and scenario to guardians and childhood patients, exercised in role play form, and recorded the situation by video. Furthermore, students performed the medical interview after clinical-training start using the leaflet actually created to guardians and childhood patients. From the feedback after the end of a role play, and a questionnaire, by the role play supposing a family, we could realize each position and feeling, and interaction, and it was thought that a learning effect was expectable. Furthermore, it was thought that we were effective in the objective rating to self production or the interaction between each element, and the extraction and discussion of a spontaneous problem of the feedback by video were attained. Moreover, it was thought that we were effective also in communications skills, the improvement in establishment of a confidential relation, and an improvement of a fundamental attitude.

P-11 Pulp revascularization and root development of an immature permanent tooth with apical periodontitis: a case report

Yuko Fujita and Kenshi Maki

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We describe successful revascularization treatment of an immature mandibular left second premolar with apical periodontitis in a 10-year-old patient. The tooth was treated using coronal root irrigation with 5% sodium hypochlorite (NaOCl) and 3% hydrogen peroxide without instrumentation and then packed using calcium hydroxide paste into the coronal canal in a single visit. X-ray photographic examination showed the start of apical closure 5 months after the revascularization procedure. Thickening of the canal wall and complete apical closure was confirmed 15 months after the initial treatment. The successful outcome of this case suggests that this conservative revascularization treatment approach can preserve the vitality of dental stem cells and angiogenic factors and create new hard tissues for pulp repair, resulting in completion of root maturation. The present technique might not be applicable in all revascularization cases. However, we should consider choosing this approach first in partially necrotic pulp in which vital pulp tissue and apical papilla might still be present in the canal and at the apex.

P-12

Stimulation of bone formation in the rapid expanding inter-premaxillary suture in rat

Katsura Saeki, Masanobu Kashitani, Takahiro Saito, Kazumasa Morikawa,
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Changes of the rates of calcification, as well as bone density and were examined by expansion of inter-premaxillary sutures in rats. Wistar rats , 6 weeks of age at the beginning of the experiment, were used for the present study. A rapid expansion appliance was set on the maxillary incisors of rats in the experimental rats and no appliance was placed in the control. The rate of calcification in the inter-premaxillary suture was measured by calcein staining, while measurements of cortical bone density using peripheral quantitative computed tomography (pQCT) were performed. The amount of the calcification between the control and 2.0 mm expansion group was significant different with the rate of calcification found to be accelerated in the first week of expansion in all of the experimental groups. Measurements of bone density and mineral content by pQCT demonstrated that there were significant differences between the 2.0 mm expansion group and control groups ($p < 0.05$). The present results showed that the rate of calcification in the inter-premaxillary suture was faster than in other areas, while changes were found in the quantity of bone mineral following mechanical stimulation cause by rapid expansion.

P-13 Usefulness of ^{18}F -FDG accumulation in the evaluation of the extent of dental inflammation

Shinji Kito, Masafumi Oda, Nao Wakasugi-Sato, Shinobu Matsumoto-Takeda, Tatsuro Tanaka, Yasuhiro Morimoto

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The significance of positron emission tomography (PET)-computed tomography (CT) using fluorine-18-labeled (^{18}F) fluoro-2-deoxy-D-glucose (FDG) for oral cancers has become obvious. However, because the distribution of ^{18}F -FDG throughout the body mainly reflects glucose metabolism of individual tissues, inflammatory cells also have increased glucose metabolism. In the present study, the relationship between the extent of ^{18}F -FDG accumulation and the size of the bone resorption area or imaging findings related to periodontal or periapical inflammation was examined to evaluate the usefulness of ^{18}F -FDG for the evaluation of the extent of dental inflammation. ^{18}F -FDG accumulations on PET-CT were retrospectively compared with the size of the bone resorption areas caused by periapical, periodontal inflammation or dental caries on panoramic radiographs, CT, and magnetic resonance imaging (MRI) in 44 subjects. A significant correlation was found between the size of the bone resorption area caused by periodontal ($r=0.595$, $p<0.01$) or periapical ($r=0.560$, $p<0.01$) inflammation and the highest standardized uptake value (SUVmax) of ^{18}F -FDG accumulation. A significant correlation was found between the periodontal ($r=0.622$, $p<0.01$) or periapical ($r=0.394$, $p<0.01$) inflammatory findings on MRI and SUVmax of ^{18}F -FDG accumulation. The SUVmax of ^{18}F -FDG around most teeth with caries ranged under 1.5. ^{18}F -FDG accumulation reflects the extent of dental inflammation, not dental caries.

P-14

Identification of peripheral vessels in oral and maxillofacial regions visualized by the Fresh Blood Imaging technique

Tatsurou Tanaka, Masafumi Oda, Shinji Kito, Nao Wakasugi-Sato,
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The purpose of this study is to evaluate the three-dimensional images of thinner main peripheral vessels in oral and maxillofacial regions made without contrast medium using a new technique, fresh blood imaging (FBI). A second objective is to discern arteries from veins using the combination of FBI with the subtraction technique. Images from FBI were compared with those from three-dimensional phase-contrast magnetic resonance angiography (MRA) of blood vessels in 20 healthy subjects. All images were scored for visualization and image quality of the main blood vessels. In addition, appropriate flow-spoiled gradient pulses were applied to differentiate arteries from veins in the peripheral vasculature using a combination of FBI sequences and subtraction between systole- and diastole-triggered images. The scores of MRA using FBI for the visualization of thin blood vessels were significantly better than those using phase contrast, while scores for the visualization of main blood vessels were equal. Additionally, we succeeded in our attempt to differentiate arteries from veins with a reasonable acquisition time. Thus, our experience shows that FBI could be a very useful method to identify three-dimensional vasculature and to differentiate arteries from veins among thinner peripheral vessels in the oral and maxillofacial regions without using contrast medium.

P-15

Relationship between viable and dead cariogenic bacteria prevalence among oral specimens monitored by qPCR in combination with propidium monoazide

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Streptococcus mutans and *Streptococcus sobrinus* are associated with the development of dental caries in humans. However, previous diagnostic systems are unsuitable for monitoring viable cell numbers in oral specimens. Assessing the relationship between the number of viable and dead bacterial cells and oral status is important for understanding oral infectious diseases. Propidium monoazide (PMA) has been reported to penetrate dead cells following membrane damage and to cross-link DNA, thereby inhibiting DNA amplification. In the present study, we developed an assay for selective analysis of viable human cariogenic pathogens, namely, *S. mutans* and *S. sobrinus*, using PMA combined with real-time PCR (PMA-qPCR). In addition, we applied this assay to oral specimens and analyzed the relationship between viable cell numbers among oral specimens. Finally, we analyzed the usefulness of this assay for *in vitro* biofilm experiments. The qPCR-based enumeration assays specifically quantified the cell numbers of each pathogen. PMA treatment effectively prevented DNA amplification from dead cells. Various ratios of live/dead cells were examined, and no amplification of DNA from dead cells was observed in these organisms. We quantified the viable cells in oral specimens, and a significant correlation was found between the number of viable *S. mutans* cells in saliva and that in plaque among caries-free patients, whereas no correlation was observed between saliva and carious dentin. Additionally, we applied PMA-qPCR for monitoring viable *S. mutans* cell numbers *in vitro* in planktonic cells and biofilm treated with hydrogen peroxide (H₂O₂). In planktonic cells, viable cells decreased with increasing H₂O₂ concentration, whereas no significant

difference was observed in biofilm cells. PMA-qPCR is potentially useful for quantifying viable cariogenic pathogens in oral specimens and is applicable to oral biofilm experiments. This assay will help elucidate the relationship between viable cells among oral specimens in relation to oral status.

Regulation of taste bud cells by transcription factors

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The gustatory cells in taste buds have been identified as paraneuron, they possess characteristics of both neuronal and epithelial cells. Like neurons, they form synapses, store and release transmitters, and are capable of generating an action potential. Like epithelial cells, taste cells have a limited life span and are regularly replaced throughout life. However, little is known about the molecular mechanisms that regulate taste cell genesis and differentiation. In the present study, to begin to understand the mechanisms that regulate taste bud cell differentiation, we have investigated the role of *Mash1* in regulating taste bud cell differentiation using *Mash1* KO mice and forced expression of transcription factors in lingual epithelial cells. We found that amino acid decarboxylase-immunoreactive (AADC-IR) cells were not evident in either the circumvallate papilla epithelia or in taste buds in the soft palates of *Mash1* KO mice. In *Mash1* KO/GAD67-GFP mice, GFP-positive (GAD67 expression type III cell) cells are also missing in the taste buds of soft palate. However gustducin, a marker of type II taste bud cells, was expressed in taste buds in the soft palates of *Mash1* KO mice. Forced expression of neural-lineage-specific transcription factors in tongue epithelial cells induced neural cell marker expression and neural cell morphology. These results suggest that transcription factors could play an important role of differentiation of taste bud cells from tongue epithelial cells.

The link between bone, gut and glucose metabolism

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The uncarboxylated form (ucOC), but not the γ -carboxylated form (GlaOC), of the bone-derived protein osteocalcin stimulates insulin secretion and regulates energy metabolism in insulin target tissues. Glucagon-like peptide-1 (GLP-1) is an insulin secretagogue that is released from the gut in response to food intake. We have now found that Gprc6a, a putative ucOC receptor, is expressed in epithelial cells of the mouse small intestine as well as in STC-1 enteroendocrine cells. Secretion of GLP-1 by STC-1 cells was stimulated by ucOC but not by GlaOC. The serum GLP-1 concentration in mice was increased by intraperitoneal or oral administration of ucOC whereas GlaOC was effective in this regard only after oral application. Serum insulin levels were also increased by ucOC, and this effect was potentiated by an inhibitor of dipeptidyl peptidase IV and blocked by a GLP-1 receptor antagonist. Intravenous injection of ucOC in mice also increased the serum GLP-1 concentration in the fasted state and tended to increase the serum insulin level in the fed state. Our results suggest that ucOC acts *via* Gprc6a to induce GLP-1 release from the gut, and that the stimulatory effect of ucOC on insulin secretion is largely mediated by GLP-1.

Processing of NF- κ B2 and the nuclear localization of RelB are required for osteoclast differentiation

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The transcription factor NF- κ B regulates the expression of a wide variety of genes that are involved in immune and inflammatory responses, proliferation, tumorigenesis, and survival. NF- κ B activation pathway consists of two distinct pathways, termed the classical and alternative NF- κ B signaling pathways. The alternative NF- κ B signaling pathway is activated by NF- κ B-inducing kinase (NIK) which results in processing of the p100 precursor to p52. The processing of p100 allows nuclear translocation of RelB, which may heterodimerize with p52 for subsequent gene transcription. We previously reported that alymphoplasia (*aly/aly*) mice, which have a natural loss-of-function mutation in the *Nik* gene, show mild osteopetrosis with decreases in osteoclast differentiation, suggesting that the alternative NF- κ B pathway via the processing of p52 from p100 induces osteoclast differentiation. We show here that overexpression of RelB induced processing of p52 from p100 and restored osteoclast differentiation in *aly/aly* cells. The overexpression of RelB also induced phosphorylation of p100 by IKK α , and MG132, a proteasome inhibitor prevented these events. In contrast, in the presence of a mutant form of p100, p100 Δ GRR, which cannot be processed to p52, over expression of RelB did not induce osteoclastogenesis in *aly/aly* cells. Thus, the alternative NF- κ B pathway via the processing of p52 from p100 and translocation of RelB into nucleus play key roles in osteoclast differentiation.

Possible involvement of NF- κ B in the bone invasion by oral squamous cell carcinoma

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Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the oral cavity, head and neck. The jaw bone invasion by OSCC is a critical factor, which, because it leads to metastasis, affects the prognosis of patients. Nuclear factor- κ B (NF- κ B) is constitutively activated in many cancers, including OSCC, and is involved in the invasive characteristics of OSCC. We previously reported that a selective inhibitor of NF- κ B inhibited bone invasion by OSCC using mouse model. In this study, we examined the molecular mechanism by which BAY11-7082 or IMD-0560, selective inhibitors of NF- κ B, prevent mandibular invasion by OSCC. Pretreatment with BAY11-7082 or IMD-0560 inhibit TNF α -induced translocation of p65, a main subunit of NF- κ B, and I κ B α degradation in human OSCC cell lines, SAS, Ca9-22 and HSC-2 cells. Furthermore, BAY11-7082 or IMD-0560 prevent migration of OSCC cells, through a gelatin-coated membrane and activity of matrix metalloproteinase 9 (MMP9) of these cells induced by TNF α . These results indicated that selective inhibitors of NF- κ B prevent mandibular invasion by OSCC by suppressing of both invasion and degradation of bone matrix by MMP9 in OSCC cells. Taken together, our data clearly indicate that inhibition of NF- κ B is useful for inhibiting bone invasion by OSCC *in vitro* and *in vivo*.

Development of Dental Image Viewer and Reduction of Metal Artifacts on the CT Images Based on Frequency Analysis

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Recently, medical images with digital information can be easily obtained on medical fields and can analyze abnormalities based on visual screening. Also, many of the hospital introduced a PACS as a medical network. The PACS system can transfer the DICOM images and medical data of patients from one doctor to another doctor via the networks. Furthermore digital system requires the small hospital to use the useful medical image viewer for visual screening based on DICOM image. In this paper, we develop a CR image viewer that useful for the dental CT viewer having a CAD (computer aided diagnosis) system. Our system uses GUI (Graphical User Interface) in order to make it user-friendly for radiologist. We also implement a segmentation method by using the image processing techniques such as segmentation and reducing the metal artifacts to analyze the dental CT images.

In this paper, we introduced a function to extract the area of bone separately for teeth and mandibles automatically as addition to the system that can do a series of operation before simulating by the strike of the implant based on segmentation technique for the dental screening. Also we propose a method to reduce the artifact on dental CT image using the frequency analysis. We apply the proposed technique to five cases which obtained dental CT images and satisfactory experimental results are achieved. We also developed the MPR, VR techniques for displaying the result image in three dimensions.

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