

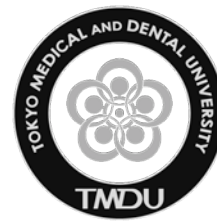


Chulalongkorn University
Peking University
Tokyo Medical and Dental University

The 3rd Tri-University Consortium on Oral Science and Education

November 6 (Wed) – 7 (Thu), 2013

*Faculty of Dentistry, Tokyo Medical and Dental University (TMDU),
Tokyo, Japan*



The 3rd Tri-University Consortium on Oral Science and Education

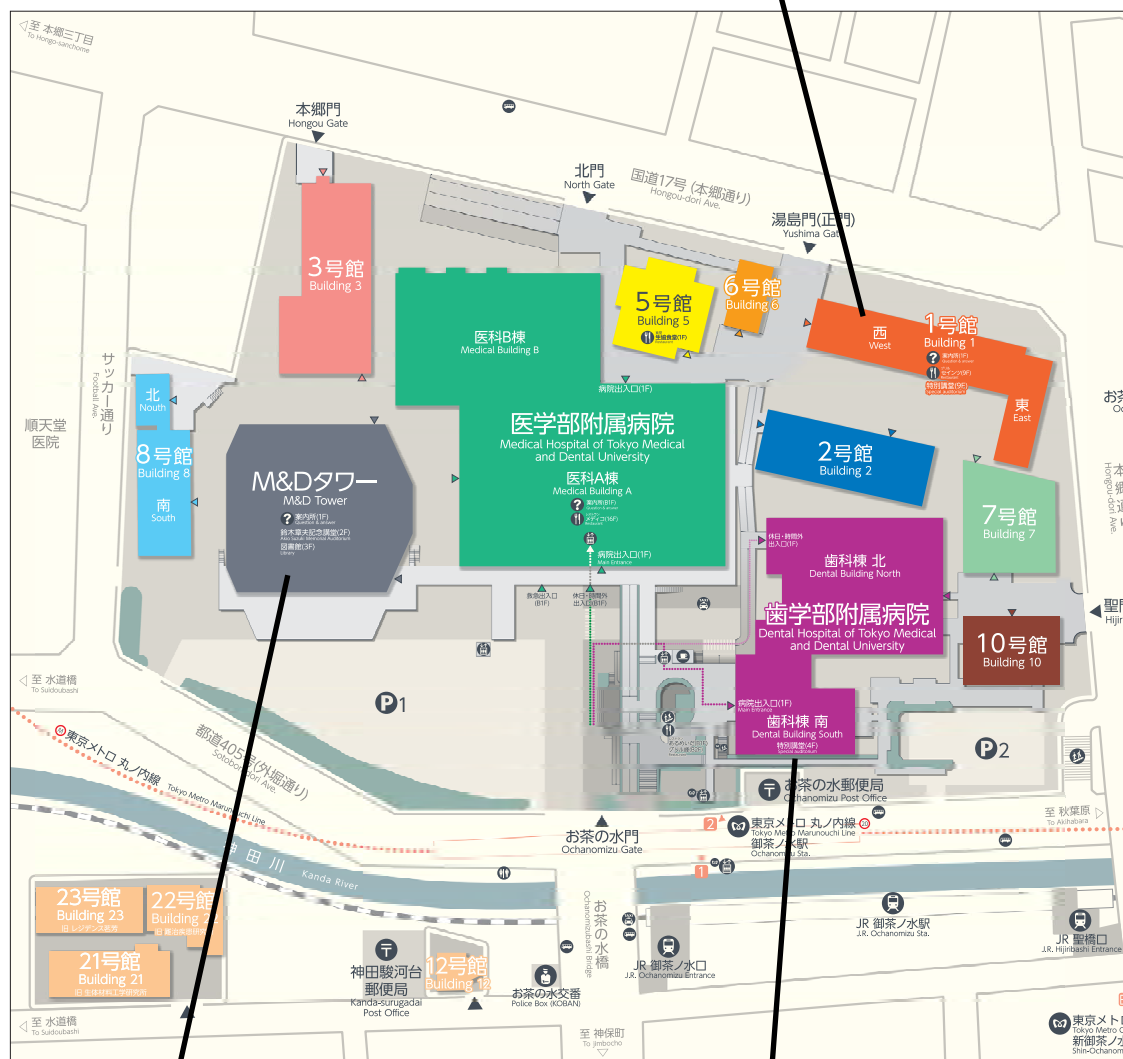
Chulalongkorn University
Peking University
Tokyo Medical and Dental University

Faculty of Dentistry, Tokyo Medical and Dental University (TMDU), Tokyo, Japan

November 6-7, 2013

Campus Map

Welcome Dinner (Nov 6):
Building 1 West 9F
Restaurant “Grill Saints”



Secondary Venue:
M&D Tower 26F
Faculty Lounge

Main Venue:
Dental Building South 4F
Special Lecture Hall

Message of Welcome



On behalf of the Organizing Committee, I would like to express my great pleasure in welcoming you to the 3rd Tri-University Consortium on Oral Science and Education, which will be held here in Tokyo, November 6th and 7th, 2013.

The Tri-University Consortium was formed in 2010, with the aim of facilitating international exchanges between young researchers from the Chulalongkorn University (CU) Faculty of Dentistry, the Peking University (PKU) Faculty of Stomatology, and the Tokyo Medical and Dental University (TMDU) Faculty of Dentistry, and we meet each year to realize our mission.

I am sure that a great deal of information and many opinions regarding current dental research will be exchanged in a relaxed atmosphere at this consortium, as was the case at the 1st and 2nd Consortiums, which were held in the great cities of Bangkok and Beijing.

I would like to note a few points of pride that we have at the TMDU Faculty of Dentistry. First of all, we are the oldest dental school among the national university corporations in Japan, and, as TMDU is the leading university in Asia regarding journal article citations, it is clear that many brilliant researchers gather here at our university, and that our research activity is highly evaluated by our peers. We are also very fortunate to enjoy a very favorable location in the center of Tokyo.

In closing, please accept my very best wishes for a fruitful time at this session, and for the Consortium's complete and continued success. It is also my hope that you will enjoy your time here in Tokyo, a cosmopolitan city which is not only active in research, culture, and commerce, but also in sports, as the Summer Olympic games will be held here in 2020.

Again, on behalf of the Organizing Committee, thank you very much for attending the Consortium.

Professor Junji Tagami, D.D.S., Ph.D.

Dean, Faculty of Dentistry

Vice Dean, Graduate School of Medical and Dental Sciences

Tokyo Medical and Dental University (TMDU)



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	09:00–09:15	Current Research Status of the Faculty of Dentistry, Chulalongkorn University Suchit Poolthong (Dean, Faculty of Dentistry, Chulalongkorn University)
	09:15–09:30	A Brief Introduction of Peking University School of Stomatology Tao Xu (Dean, Peking University School of Stomatology)
	09:30–09:45	Promotion Interdisciplinary International Projects and Educating Research-oriented Health Care Professionals Junji Tagami (Dean, Faculty of Dentistry, Tokyo Medical and Dental University)
	09:45–10:00	Group Photo, Break
Session 2	10:00–11:30	Research Highlights
	10:00–10:30	Integrating Measures of OHRQoL into the National Oral Health Services of Thailand Sudaduang Krisdapong (Chulalongkorn University)
	10:30–11:00	Cellular Damage of Oral Mucosa Cells following Dental X-ray Examinations Gang Li (Peking University)
	11:00–11:30	Management and Treatment in the Partially-edentulous Restored Dentition Noriyuki Wakabayashi (Tokyo Medical and Dental University)
	11:30–12:15	move to Faculty Lounge (M&D Tower 26F), Lunch Break
	12:15–14:00	Poster Session
	12:15–13:00	Poster Viewing
	13:00–14:00	Poster Discussion Moderators: Sudaduang Krisdapong, Thantrira Pornaveetus (Chulalongkorn University) Gang Li, Lingfei Jia (Peking University) Keiichi Yoshida, Kou Kayamori (Tokyo Medical and Dental University)
	14:00–14:30	Poster Removal, move to Special Lecture Hall (Dental Building South 4F)

Session 3	14:30–16:10	Interdisciplinary Approach in Dentistry
	14:30–14:50	Longitudinal Associations between Oral Health Impacts and Quality of Life among a National Cohort of Thai Adults Tewarit Somkotra (Chulalongkorn University)
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	15:30–15:40	Break
Session 4	15:40–17:00	Clinical Research
	15:40–16:00	Dentin Strain Produced by Root-end Cavity Preparation using Er:YAG Laser Satoshi Watanabe (Tokyo Medical and Dental University)
	16:00–16:20	Study on the Current Status of Primary Tooth Pulpotomy among Dentists in China Jiajia Zheng (Peking University)
	16:20–16:40	Increased Anti- <i>Porphyromonas gingivalis</i> Antibody in Coronary Heart Disease Patients Norio Aoyama (Tokyo Medical and Dental University)
	16:40–17:00	Research Activities at Chulalongkorn Periodontal Department Rangsini Mahanonda (Chulalongkorn University)
	18:00–20:00	Reception



Itinerary - Thursday November 7, 2013

Session 5	09:00–10:40	Basic Research
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	09:20–09:40	The Mechanism of Bone Destruction by Oral Squamous Cell Carcinoma Kou Kayamori (Tokyo Medical and Dental University)
	09:40–10:00	The Role of R-Spondin and Lgr in Tooth Development Thantrira Porntaveetus (Chulalongkorn University)
	10:00–10:20	GNAS Mutational Analysis in Differentiating Fibrous Dysplasia and Ossifying Fibroma of The Jaws Rui-Rui Shi (Peking University)
	10:20–10:40	Bone Resorption Inhibitor Peptides Work as a Bone Formation Stimulator Kazuhiro Aoki (Tokyo Medical and Dental University)
	10:40–10:50	Break
Session 6	10:50–11:50	Presentations by PhD Students
	10:50–11:10	Interleukin 12 Increased RANKL/OPG Expression Ratio in Human PDL Cells Benjar Issaranggun Na Ayuthaya (Chulalongkorn University)
	11:10–11:30	Sleep Disturbance and Psychological Distress as Possible Risk Indicators for the Development of TMD Myofascial Pain Jie Lei (Peking University)
	11:30–11:50	Oral Malodor Treatment Improves the Patients' Psychological Condition and QOL Yuri Uraoka (Tokyo Medical and Dental University)
	11:50–12:00	Closing Address
	12:00–13:00	Lunch Break
	13:00–	Optional Tour
		TMDU Tour
		GC Co. Visit

Session 1

Current Research Status of the Three Universities



Current Research Status of the Faculty of Dentistry, Chulalongkorn University

Suchit Poolthong

Dean, Faculty of Dentistry, Chulalongkorn University

The Faculty of Dentistry at Chulalongkorn University recognizes the importance of a robust research program in order to achieve academic and innovative goals for both students and staff of the University. Locally, we have a record of high performance, receiving an award for Excellence in Research Activities among the Faculties of Dentistry in Thailand. However, we still have room to develop and strengthen our research to promote global oral health. The first step in the evolution of the research program at our Faculty was the quantification of research activities aimed at gaining increased funding and publications. We have achieved our goal domestically and begun the second step of targeting the quality of our research outcomes. Our overall plan is aimed towards three areas of research: Innovation, Translational Research, and also Educational Research, which is important to develop our students to be the “Pillar of the Kingdom” in terms of oral health improvement through prevention and treatment. Currently, we have more than 50 research projects annually for undergraduate investigators and many more research projects at the post-graduate level. The research projects of our faculty and staff are also important and they are strongly supported by our core research facilities. Educational research is being given particular attention in the enhancement of our research program since curriculum development is guided by internal educational research. With the continued efforts of our four outstanding research units at the Faculty, our ultimate goal is the establishment of centers of excellence to maintain our status as the Pillar of the Kingdom.

A Brief Introduction of Peking University School of Stomatology

Tao Xu

Dean, Peking University School of Stomatology, Beijing, China

First of all, I would like to send my congratulation to “the Third Tri-University Consortium on Oral Science and Education”, and extend my greetings to our friends and colleagues from Chulalongkorn University and Tokyo Medical and Dental University. In the past two years, Tri-University Consortium provided an excellent platform for young dental researchers, clinicians and academicians to build relationships with academic and professional colleagues and enhance the research collaborations, and last but not least, enjoy the friendship.

Peking University School of Stomatology (PKUSS) is one of the leading oral health educational institutions in China, PKUSS has been devoted to promoting Chinese dental education by providing a broad-spectrum dental and medical education for new generations of oral health professions. Also, with the aim of developing the school into a premier world-class institution, PKUSS has been pioneering and continuing leading new initiatives domestically as a key leading institution, which contains 1 national engineering research laboratory, 13 school level laboratories, 9 interdisciplinary study centers. The basic research fields include molecular and genetic mechanisms in oral and maxillofacial diseases, functional and pathological mechanisms of oral and maxillofacial organs and clinical-translational researches, stem cell biology and tissue regeneration, development and evaluation of new dental biomaterials and technology with intellectual property rights, and digital technology in dentistry, whereas clinical research areas include functional reconstruction and repair on oral and maxillofacial malformation and defect with application of digital surgery, bone lengthening, oral implantation and team approach for cleft palate, pathology, etiology, prevention and treatment of common oral diseases and the relationship with systemic diseases, non-surgical treatment of skeletal Class III malocclusion and development of transmission appliance in orthodontic treatment, integrated subject comprised of multiple new diagnostic and treatment technology of oral radiology, prosthodontic treatment with integrated digital devices, pediatric dentistry treatment under anesthetized conditions, treatment of vascularized autogeneous submandibular gland transfer for KCS and application of 125I on oral and maxillofacial malignant tumors, and multidisciplinary research of stomatology with engineering, medicine to highlight the pathology, etiology, prevention and treatment of oral diseases. In addition, with the history of over 70 years, the school has manifested itself in international communication and gained its reputation worldwide. After being authorized as the first international cooperation base in stomatology field, PKUSS has collaborated with institutions worldwide in academic research and opportunities are given to young dental researchers to establish their roles in research programs. Together, we could indeed make more difference for the oral health education, clinical service, advanced research and oral care public health awareness in the future.



Promotion interdisciplinary international projects and educating research-oriented health care professionals

Junji Tagami

Dean, Faculty of Dentistry

Vice Dean, Graduate School for Medical and Dental Science, Tokyo Medical and Dental University

It is our great pleasure to be able to host the 3rd Tri-University Consortium on Oral Science and Education at Tokyo Medical and Dental University. We would like to extend a warm appreciation to everyone for the great effort to have continuously supported this event. We believe that we can promote the friendship among our schools, and internationality as well as the activity and quality of education and research at each school. Particularly, program for young researchers and students is valuable for our future collaborations and developments. The Tri-University Consortium must become the core network for promoting dental research and education in Asia, hopefully in worldwide in future. We wish we have more collaborated research and educational projects among the 3 schools.

The Faculty of Dentistry at Tokyo Medical and Dental University, which consists of School of Dentistry, School of Oral Health Care Sciences is an integrated institute for the dental education and research. In 2008, we celebrate its 80th anniversary. Our university with the longest history of national dental university has contributed to the developments of dental education and dental science by throwing up a number of world leading graduates in the field of dental science and dental practice. In 2000, our faculty was reorganized as the graduate school to establish the center of education, research and clinic in the dental field, aiming the further contribution to the society. Recent development of science and technology has brought about great sophistication and more interdisciplinary characteristics in research in dentistry. People demand more sophisticated care with the advancement of aged society and changes of the disease structure. Against this backdrop, our school serves better to our society and to the world by promotion interdisciplinary international projects and by educating research oriented health care professionals.

Session 2

Research Highlights

Integrating measures of OHRQoL into the national oral health services of Thailand.

Sudaduang Krisdapong^{1*}, Piyada Prasertsom², Khanit Rattananarangsima², Supreda Adulyanon³, Aubrey Sheiham⁴

¹ Department of Community Dentistry, Chulalongkorn University, Bangkok, Thailand;

² Dental Health Division, Ministry of Public Health, Nontaburi, Thailand;

³ Thai Health Promotion Foundation, Bangkok, Thailand;

⁴ Department of Epidemiology and Public Health, University College London, London, United Kingdom.

Background: Despite the fact that the importance of socio-dental or Oral Health-Related Quality of Life (OHRQoL) measures of oral health has been highlighted since 35 years ago because “The greatest contribution of dentistry is to the improvement of quality of life”, national oral health services generally do not include such measures in setting oral health goals for the nation. **Methods:** This research series were to assess the associations between oral diseases and OHRQoL of a nationally representative sample of 12- and 15-year-old Thais, and to apply the findings to formulate proposals for oral health goals for these two age groups. The study was a part of the Thailand National Oral Health Survey, including half of the national survey’s sample, 1,100 12- and 871 15-year-olds. The Oral Impacts on Daily Performances (OIDP) and Child-Oral Impacts on Daily Performances (Child-OIDP) were used for collecting OHRQoL data of 12- and 15-year-olds respectively. Analyses on OHRQoL and its associations with oral diseases were performed using various approaches, namely, any level as well as moderate or severe oral impacts, overall impacts as well as Condition-Specific impacts (CS-impacts). Hierarchical technique was applied for multivariate analyses. **Results:** Oral impacts in Thai children and adolescents were prevalent, 85.1% of 12- and 83.2% of 15-year-olds, however, only one-third experienced impacts at moderate or severe degree (35.1% of 12- and 39.0% of 15-year-olds). Impacts were related more to dental caries, particularly untreated and severe untreated decay, than to periodontal diseases. Gingivitis, particularly when coexisted with calculus in the same sextant, was related to moderate/higher oral impacts, while calculus had minor impacts on quality of life. Periodontal diseases in terms of gingivitis and calculus would cause moderate/higher oral impacts only if they occurred at a great extent (3 or more sextants). Based on the findings, we proposed national oral goals for Thai children and adolescents aiming at reductions of 1) those with untreated decayed teeth, 2) those with extensive periodontal diseases in any form, and 3) those with extensive gingivitis coexisting with calculus. **Conclusions:** By integrating measures of OHRQoL into the system of oral health services, we proposed oral health goals which are different from the traditionally defined goals.

sudaduang@hotmail.com **Keywords:** oral health survey, goals, quality of life

Cellular damage of oral mucosa cells following dental X-ray examinations

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Background: With the introduction of 3-dimensional imaging technique, such as computed tomography (CT) and cone beam computed tomography (CBCT) for dental and Maxillofacial radiology, the diagnostic radiation dose to patients has been increased dramatically. For those patients who undergoing orthodontic or orthognathic treatment, usually a series of radiographs including a panoramic radiograph, a lateral cephalometric radiograph, a posteroanterior cephalometric radiograph and temporomandibular joint CBCT images are acquired within a very short period of time for the purpose of diagnosis, treatment planning and prognosis evaluation. It is well known that X-ray is one of carcinogen to human beings. Will the radiation dose from such a series X-ray examinations do some harmful effect on human bodies? With this question bearing in mind, the present study was to evaluate genetic damage and cellular death in exfoliated buccal mucosa cells from patients undergoing dental X-ray examinations.

Materials and Methods: Buccal cells were collected from 48 patients (21 males and 27 females) before and 10 days after they underwent different series of X-ray examinations for orthodontic or orthognathic treatment. Cytological preparations were successively dyed with methods of Feulgen and fast-green, and analyzed under a light microscope at x 400 magnification. Micronucleated cells and other type of cells (basal cells, binucleated cells, condensed chromatin cells, karyorrhectic cells, pyknotic cells ,karyolytic cells and cells with nuclear buds) were scored in 1000 cells.

Results: There was a statistically significant difference ($P=0.026$) between the frequency of micronucleated cells before and after dental X-ray examinations. The frequency of micronucleus was not significantly different between the patients aged under 18 and those over 18 years old. Large dose exposure was related to increased karyolytic cells ($P=0.008$) .

Conclusion: X-ray dose was directly related to increased cell apoptosis. The patients younger than 18 years old were not more susceptible to X-ray than those over 18.

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Keywords: Dental X-ray, Buccal mucosa cell, Micronucleus

Management and treatment in the partially-edentulous restored dentition.

Wakabayashi N*

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The removable partial prosthodontic has always been a special challenge in dentistry. Important and continuing demographic changes of the partial denture population and the increasing availability of alternative treatments are now significant influences on their use. Despite these discouraging reality, it is believed that the future need for partial dentures to restore aesthetic and functional limitations will substantially increase. In terms of materials and techniques, very little new treatment option has been accepted by academics in recent years for the clinical provision of partial dentures. The recent achievements of our research team have been focused on the potential of candidate materials for the next generation removable prostheses, as well as on the effects of tooth loss and subsequent treatment on the related oral tissues. In this presentation, the conventional processes of the removable partial prosthodontics will be shown for the baseline consensus. Clinical factors that may be crucial for a decision making process will be shown in a variety of clinical cases, where the long-term function of a partial denture is compromised by variety of events that may occur during function. The etiological analysis and the management of the failures will be featured for following categories: (1) Abutment complications such as root caries and periodontal disease; (2) Denture complications such as fracture, distortion and wear; (3) Soft tissue and edentulous problems such as denture stomatitis including generalized and localized inflammation of mucous membrane, and excessive bone resorption. Careful designing and provision of the prostheses that embrace effective support, clearance of gingival margins, simplicity, and rigid connector are essential for minimizing the potential risk of the failures. Most importantly, the long-term success will be determined by the success in establishing a healthy oral environment prior to denture construction, and to the maintenance of the oral health once the denture has been fitted. This presentation will provide ideas as to why current complications occur and what needs to be conducted in order to improve the oral health in the partially-edentulous patients. The role of the dental profession on diagnosing and managing the failures in the partially-edentulous restored dentition will be emphasized. wakabayashi.rpro@tmd.ac.jp

Session 3

Interdisciplinary Approach in Dentistry

Longitudinal associations between oral health impacts and quality of life among a national cohort of Thai adults

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³School of Human Ecology, Sukhothai Thammathirat Open University, Nonthaburi, Thailand

Background: Oral health has been of interest in many low and middle income countries due to its impact on general health and quality of life. But there are very few population-based reports of adult Oral health impacts in developing countries and its association with health-related quality of life. This study aimed to report evidence on the association between oral health impacts and health-related quality of life among national cohort Thai adults residing throughout Thailand. **Materials and Methods:** In 2005, and 2009, a comprehensive health questionnaire was returned by distance learning cohort members recruited through Sukhothai Thammathirat Open University (N= 87134, and 60569, respectively). Oral health impacts at the baseline included were pain, difficulty chewing, swallowing, speaking, discomfort with social interaction. Measured health and quality of life outcomes used the SF-8 questionnaire (Medical Outcomes Study Short-Form 8). Mean scores for each SF-8 domain were compared between various oral health groups with the statistical significance of any differences determined using t-tests, and multivariate linear regressions to examine the longitudinal links between baseline oral health and 4-year follow-up physical (PCS) and mental (MCS) scores. **Results:** In 2005, 16.4% had difficulty chewing and/or swallowing, 13.4% reported difficulty speaking and/or discomfort with social interaction, and 10.8% of the cohort reported having pain associated with teeth or dentures. Cohort members reporting one or more oral health impacts had lower SF-8 mean scores. In particular, monotonic dose-response gradients in 2005-2009 associations were found between an increase in number of oral health impacts (0, 1, 2, 3) and a decline in SF-8 Physical Component Summary scores (mean of 50.2, 49.0, 48.3, 47.6) as well as SF-8 Mental Component Summary scores (mean of 48.1, 45.9, 45.2, 43.6) in younger cohort members. Similar dose response gradients were found in older cohort members. **Conclusion:** This study addresses the association between oral health problems and adverse health and quality of life among Thai adults. This finding supports oral health as an important part of overall health and well-being. tewarit.s@chula.ac.th **Keywords:** oral health, longitudinal survey, quality of life, Thailand

Inflammation Aggravates Iodoacetate-induced Temporomandibular Joint Osteoarthritis.

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Background: Osteoarthritis (OA) is a common degenerative joint disease that affects cartilage, subchondral bone and synovium in the temporomandibular joint (TMJ). But whether synovitis is one of the etiological factors of OA or secondary to the cartilage degradation is still unclear.

Materials and Methods: 60 Female Sprague-Dawley rats (180–200 g) were randomly assigned to four groups, including control, CFA (Complete Freund's Adjuvant), MIA (Monosodium Iodoacetate) and MIA+CFA group. Experimental OA and synovial inflammation was induced by intraarticular injection of MIA or CFA into bilateral TMJs, respectively. Enhanced synovitis in OA group was induced by injection of MIA combined with CFA. Control group received saline injection. Immunohistochemical (IHC) staining of Collagen II, CD68, and iNOS were analysis. Osteoclasts in subchondral bone were analysis by Tartrate-resistant acid phosphatase (TRAP) activity. Subchondral bone resorption was evaluated by Micro CT.

Results: CFA injection induced evident inflammatory changes of in the TMJ, including inflammatory cells infiltration, increased expression of iNOS and CD68, but there was no evident cartilage or bone destruction in the CFA group. MIA injection induced evident cartilage degradation. However, compared with the MIA group, synovial inflammation induced by CFA enhanced the osteoarthritic-like destruction induced by MIA in the CFA+MIA group, including obvious loss of Toluidine blue and Collagen II staining, disc perforation and subchondral bone erosion. There were more TRAP positive osteoclasts in the subchondral bone of CFA+MIA group than the MIA or CFA group.

Conclusion: Synovial inflammation aggravates the progression of OA rather than initiating the degradation of cartilage.

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Keywords: osteoarthritis, inflammation, temporomandibular joint.

Estimation of patient-specific cancer risk from cone-beam CT exposures

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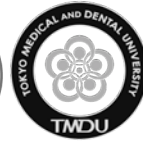
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Background: In recent years, cone beam computed tomography (CBCT) has become a commonly used imaging modality for a wide range of clinical indications in dentistry and otolaryngology. Several studies have investigated the radiation dose of CBCT exposures, but the effect of patient-specific factors (*e.g.* age, gender, size) on radiation dose and radiation-induced cancer risk has not yet been investigated. The aim of this study was to measure entrance skin doses on patients undergoing CBCT examinations, to establish conversion factors between skin and organ doses, and to estimate individual patient risk from CBCT exposures. **Materials and Methods:** 269 patients (age 8-83 years) were included. CBCT scans were acquired using the SCANORA 3D (Soredex, Tuusula, Finland) and NewTom 9000 (QR, Verona, Italy). Eight thermoluminescent dosimeters were attached to the patient's skin at standardized locations. Using previously published organ dose estimations on various CBCTs with an anthropomorphic phantom, the correlation between skin dose and organ doses was investigated and conversion factors were calculated to estimate individual organ doses based on skin measurements. An age- and gender-dependent risk model defined in the Biological Effects of Ionizing Radiation (BEIR) VII report was applied to estimate the lifetime attributable risk (LAR) of mortal radiation-induced cancer for each patient. **Results:** For the SCANORA 3D, average skin doses over the eight locations varied between 484 and 1788 μGy . For the NewTom 9000 the range was between 821 and 1686 μGy for Centre 1 and between 292 and 2325 μGy for Centre 2. The average LAR for all patients was 0.00066% or $\sim 1:150\,000$. Individual risks varied between 0.000057% (83 year old male) and 0.0028% (11 year old female). On average, the risk for female patients was 54% higher. **Conclusion:** Entrance skin dose measurements demonstrated the combined effect of exposure and patient factors on dose uptake. The actual radiation risk was primarily influenced by the age at exposure and gender, pointing out the continuing need for justification and optimisation of CBCT exposures, with a specific focus on children and female patients. pauwelsruben@hotmail.com **Keywords:** Cone-beam Computed Tomography, Radiation Dose, Radiation-induced Cancer

Session 4

Clinical Research



Dentin strain produced by root-end cavity preparation using Er:YAG laser

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Background: To investigate the kinetics of dentin strain and micro-fractures produced by root-end cavity preparation using Er:YAG laser and ultrasonics.

Materials and Methods: Forty roots of extracted human anterior teeth were used. After the tooth crown was removed with a diamond disc and the length of the root was adjusted to 9 mm, the root-end 3 mm was resected. Then, the root canal was instrumented to apical size 25 or 80 using the step-back technique with Gates-Glidden drills and K-files. The root canal was laterally condensed with gutta-percha and root canal sealer (Sealapex, Sybron Endo, USA). Strain of dentin was measured by strain gauges (Kyowa Electronic Instrument, Japan) mounted on the apical third root surface during root-end cavity preparation using an ultrasonic device (Piezon Master 400, EMS, Switzerland, highest power setting), or Er:YAG laser (Erwin AdvErL, Morita, Japan, 140 mJ 10 pps) with water cooling. After the root-end cavity preparation, presence or absence of root fracture and the root surface morphology were observed under a microscope using Indian ink to help visualization.

Results: When the root canal was prepared to apical size 25, the mean maximum strain produced by Er:YAG laser preparation of retro-cavities was significantly lower than that by ultrasonic preparation ($p < 0.05$). Preparation of root end cavities using a ultrasonic device showed a significantly higher strain for root canals prepared to apical size 25, compared to those prepared to apical size 80 ($p < 0.05$), while that using Er:YAG laser showed no significant difference between the two apical sizes ($p > 0.05$). In the microscopic observation, micro-fractures were found only in the ultrasonic groups.

Conclusion: When the final apical size of the prepared root canal is narrow, a lower dentin strain may be produced by Er:YAG laser preparation of retro-cavities compared to that by ultrasonics.

s.watanabe.endo@tmd.ac.jp **Keywords:** dentin strain, micro fracture, Er:YAG laser

Study on the current status of primary tooth pulpotomy among dentists in China

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Background: Pulpotomy is the universal treatment for deciduous teeth with deep caries and reversible pulpitis. The purpose of this study is to evaluate the clinical decision of Chinese dentists on primary tooth pulpotomy in patients with pulp exposure by deep caries.

Materials and Methods: A total of 103 dentists from 44 different hospitals in China completed the same questionnaire. The questionnaire consisted of two parts, doctors who performed pulpotomy on primary tooth filled out “part A”, the rest were asked to fill out “part B”. The questionnaire assessed the decision on performing direct pulp capping, pulpotomy or pulpectomy when the pulp was exposed, as well as the material for pulp capping. Chi-square test was used for the analysis of data.

Results: 48 dentists from 22 hospitals answered part A, primary tooth pulpotomy was indicated by 16.67% of dentists, whereas primary tooth pulpectomy was recommended by 41.67%. 97.72% of the pulp capping agent used was Calcium Hydroxide. Only 8.33% dentists used rubber dam to isolate the tooth, and 43.75% used a special sterilized package. 55 dentists from 22 hospitals answered part B, primary tooth pulpectomy was indicated by 47.27% of dentists, whereas pulpectomy was recommended by 30.91%. There was difference on the knowledge of the importance of vita pulp of primary teeth between 2 groups of dentists. The usages of primary tooth pulpotomy technique were highly significant different in 5 kinds of dental facilities.

Conclusion: Pulpotomy for primary teeth is not widely used in China. For pulpotomy, most pediatric dentists use calcium hydroxide as pulp-capping agent. The results of this survey also suggest that there is need for relevant continuing professional development courses for pediatric dentists in China.

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Keywords: Pulpotomy, Primary tooth, Pulp-capping agent

Increased anti-*Porphyromonas gingivalis* antibody in Coronary Heart Disease Patients

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Background: Although observational studies showed an association between periodontal disease and coronary heart disease (CHD), a causative relationship of these diseases has not been clarified. From periodontitis lesions, many periodontopathic bacteria, such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*, have been found and it is well known that inflammatory reaction varies widely between individuals. The purpose of the present study was to assess the relationship between periodontal bacterial burden and CHD. **Materials and Methods:** We studied 506 patients with circulative diseases in Tokyo Medical and Dental University Hospital from May 2012 to April 2013. The subjects were divided into six groups according to age and existence of CHD (31-60 years with CHD (n = 27): Group YC, 61-70 years with CHD (n = 61): Group MC, over 70 years with CHD (n = 94): Group EC, 31-60 years without CHD (n = 129): Group YN, 61-70 years without CHD (n = 108): Group MN, and over 70 years without CHD (n = 87): Group EN). The number of teeth and probing pocket depth (PPD) as a periodontal disease parameter were measured. The serum level of anti-periodontal bacterial antibodies and the number of periodontal bacteria in saliva were determined. **Results:** The number of missing teeth was the least in Group YN (5.6 +/- 0.7) and the most in Group EC (16.7 +/- 0.8, p < 0.05). Average PPD in Group YN (2.30 +/- 0.05) was significantly lower than that in Group EC (2.55 +/- 0.07, p < 0.05), while there was no statistical difference between Group YC (2.45 +/- 0.11) and EC. Anti-*Porphyromonas gingivalis* antibody in Group YC (median: 87,000 unit/ml) was significantly higher than that in Group YN (median: 40,000 unit/ml, p < 0.05). The numbers of *Porphyromonas gingivalis* in saliva were comparable between Group YN (median: 1,000 /ml) and YC (median: 3,900 /ml). There was no statistical difference of antibodies and numbers of *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* between groups in same age categories. **Conclusion:** In conclusion, the CHD patients had a high level of anti-*Porphyromonas gingivalis* antibody. Continuous inflammatory reaction with *Porphyromonas gingivalis* from earlier years may influence on CHD events. aoyama.peri@tmd.ac.jp

Keywords: Coronary heart disease, *Porphyromonas gingivalis*, Inflammatory reaction

Research activities at Chulalongkorn Periodontal Department

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Research activities in the areas of periodontal immunology and epidemiology at the Department of Periodontology, Chulalongkorn University will be presented. We have recently investigated the innate immune response in periodontal tissues. Periodontal cells and mediators will be discussed for their possible role in the innate anti-bacterial as well as innate anti-viral immune response in periodontal tissues. Our Periodontology Department has also conducted a longitudinal periodontal epidemiology study to investigate the association between periodontal disease and cardiovascular disease (CVD). It is a collaborative research with a medical team from Ramathibodee Hospital, Mahidol University. The study cohort was 1,530 older Thai adults. Age range was 50 to 73 (mean=60). In the studied population at baseline, there were 16% with no periodontitis, 45% with mild periodontitis, and 39% with moderate to severe periodontitis. During a 7 year period, there were 89 CVD events (4.6%). Results from a 7 year-longitudinal study suggest a trend towards the association between periodontal disease and CVD, but it is not statistically significant. More studies with larger population and longer periods are needed. (This work was supported by grants from The Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (RES560530242-AS), The National Research Council of Thailand (นน./2553-138), Chulalongkorn University Centenary Academic Development, and Thailand Research Fund (BRG5380011).

rangsini.m@chula.ac.th **Keywords:** periodontal tissues, innate immune response, cardiovascular disease

Session 5

Basic Research

Expression, Regulation and Roles of MiR-26a and MEG3 in Tongue Squamous Cell Carcinoma

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Background: miR-26a and MEG3 are aberrantly expressed in multiple types of disease. But little is known about the dysregulation of miR-26a and MEG3 in tongue squamous cell carcinoma (TSCC). In this study, we investigated the expression, regulation and roles of miR-26a and MEG3 in TSCC.

Materials and Methods: Using quantitative reverse transcription polymerase chain reaction, we evaluated miR-26a and MEG3 expression in TSCC samples from 76 patients. Correlation between the expression of miR-26a and MEG3 was analyzed using Pearson's rank correlation coefficient analysis. Overall survival of these patients was examined using Kaplan–Meier curves with log-rank tests and the Cox proportional hazards model. miR-26a effects on the expression of DNA-methyltransferase-3B (DNMT3B) were examined by luciferase reporter assays and Western blot. The effects of miR-26a and MEG3 overexpression on cell proliferation, cycle progression and apoptosis were examined in transfected TSCC cell line using Cell Counting Kit-8 (CCK-8) assays and fluorescence-activated cell sorting assays. **Results:** miR-26a and MEG3 expression were both reduced in TSCC and miR-26a expression was positively correlated with the expression of MEG3 in clinical specimens. Kaplan–Meier survival analysis indicated that TSCC patients with combined low levels of miR-26a and MEG3 had poor overall survival and in multivariable analyses this combined low expression levels emerged as an independent prognostic factor for this poor clinical outcome. Overexpression of miR-26a reduced DNMT3B expression and enhanced the expression levels of MEG3 in TSCC cell line. Overexpression of miR-26a or MEG3 inhibited TSCC cell proliferation, cycle progression and promoted cell apoptosis. **Conclusion:** Combination of the expression levels of miR-26a and MEG3 may have potential applications as a prognostic factor for TSCC patients.

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Keywords: lncRNA, miR-26a, MEG3

The mechanism of bone destruction by oral squamous cell carcinoma

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Background: Although oral squamous cell carcinomas (OSCCs) frequently invade the jaws and this leads patients to poor prognosis, the molecular mechanism of bone destruction by OSCCs has not been well elucidated. The purpose of this study is to clarify the mechanism.

Materials and Methods: Histopathological and immunohistochemical study was conducted by using primary human gingival SCC samples. Microarray analysis was performed by using primary OSCC specimens obtained from 43 anonymous patients. We conducted in vitro study using human OSCC cell lines and mouse bone derived stromal cells, ST2. Furthermore, xenograft experiments of OSCC cell line to the athymic mice were performed.

Results: Histopathological study revealed that varying amounts of fibrous stroma intervened between cancer cells and resorbing bone tissues in all cases. Immunohistochemical study demonstrated that the stromal fibroblastic cells expressed RANKL at bone resorbing region close to cancer cells. In in vitro experiments, conditioned medium (CM) isolated from OSCC cell lines induced the RANKL expression in mouse ST2 cells. Moreover, CM derived from OSCC cell lines induced osteoclast formation in co-culture of ST2 and mouse bone marrow cells in the presence of 1,25-(OH)₂D₃ and dexamethasone. To explore the soluble factors produced by OSCCs which induced RANKL expression in ST2, we focused on two factors, PTHrP and IL-6. Microarray analysis revealed that many of the cancer cells overexpressed PTHrP, but a few overexpressed IL-6. Immunohistochemical study demonstrated that IL-6 was expressed not only cancer cells but also in fibroblasts at the tumor-bone interface. Antibodies against both human PTHrP and mouse IL-6 receptor suppressed Rankl in ST2 cells, and osteoclast formation induced by CM derived from OSCC cell lines by in vitro experiments. The inhibitory effects of IL-6 antibody were greater than those of PTHrP antibody. These CM effectively induced IL-6 expression in ST2 cells. Xenograft athymic mouse models presented similar histology and expression profiles of RANKL and IL-6 to those observed in bone-invasive front in human OSCCs specimens.

Conclusion: These results indicated that the fibroblastic cells locating at the bone-cancer interface plays an important role in bone resorption. And IL-6 synthesized by stromal fibroblastic cells in response to cancer cells are important factors inducing the RANKL expression and osteoclastogenic bone resorption.

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Keywords: Oral Cancers, Fibrous stroma, Osteoclast

The role of R-Spondin and Lgr in tooth development

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Background: Tooth is an organ that develops as a result of sequential and reciprocal interactions between oral epithelium and neural crest-derived mesenchyme. Multiple signaling pathways including Tnf, Bmp, Fgf, Wnt, and Shh play roles in regulating tooth development. It is widely accepted that modern placental mammals have evolved from a common ancestor with three incisors, one canine, four premolars and three molars. Mice have only one incisor and three molars in each jaw quadrant that are divided by a toothless region, the diastema. Although mice lost teeth in the diastema during evolution, the remnants of the evolutionary lost teeth are observed as transient epithelial buds in the wild-type diastema during early stages of development. The R-Spondin family of secreted glycoproteins, R-Spondin1 – R-Spondin4, plays vital roles in craniofacial development. Recently, Leucine-rich repeat-containing G-protein-coupled receptor (Lgr)4, Lgr5 and Lgr6, have been identified as receptors for R-Spondins. Although craniofacial malformations have been shown in R-spondin2 mutant mice, the role of R-spondin2 in tooth development has not been studied. **Materials and Methods:** The expression of R-spondins and Lgrs in developing tooth germs was examined. R-spondin2 mutant mice were analyzed by micro CT scanning, histology, immunohistochemistry and in situ hybridization. **Results:** R-spondins and Lgrs were expressed in a dynamic spatio-temporal pattern during murine odontogenesis. Supernumerary teeth were found in the diastema of R-spondin2 mutants. Correspondingly, Shh signaling was upregulated in the mutants. **Conclusion:** R-spondin2 is involved with Shh signaling and plays a role in regulating tooth development in the diastema. (This research has been supported by the Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (RES560530246-AS) and Grants for Development of New Faculty Staff, the Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University).
Thantrira.p@chula.ac.th. **Keywords:** R-Spondin, Lgr, Tooth Development

GNAS Mutational Analysis in Differentiating Fibrous Dysplasia and Ossifying Fibroma of The Jaws

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Background: Differential diagnosis of fibrous dysplasia and ossifying fibroma of the jaws may often pose problems for pathologists. The purpose of this study was to evaluate the role of GNAS mutations in differentiating these two conditions. **Materials and Methods:** DNA samples from patients with fibrous dysplasia (n=30) and ossifying fibroma (n=21) were collected to analyze the presence of GNAS mutations in exon 8 and 9, two previously reported hotspot regions, using polymerase chain reaction and direct sequencing. In addition, a meta-analysis of previously published reports on GNAS mutations in fibrous dysplasia and ossifying fibroma was also performed to substantiate our findings. **Results:** Missense mutations of arginine at codon 201 (R201) in exon 8 of the GNAS gene were found in 90.0% (27/30) cases of fibrous dysplasia, with 19 cases of G-A transitions in the second place at codon 201 and 8 cases of C-T transitions in the first position corresponding to the previously reported Arg-to-His (R201H) and Arg-to-Cys (R201C) substitutions respectively, while no mutation was detected in exon 9. No mutation was found in all 21 cases of ossifying fibroma. In meta-analysis, 24 reports including 307 cases of fibrous dysplasia and 23 cases of ossifying fibroma were evaluated. The overall incidence of GNAS mutations in fibrous dysplasia was 86.0% (264/307), and the major types of mutation were R201H (52.8%) and R201C (45.2%). No GNAS mutation was detected in all patients with ossifying fibroma. To validate the availability of GNAS mutational analysis in differentiating fibrous dysplasia and ossifying fibroma, we also reported a case with uncertain diagnosis due to overlapping clinicopathological features of fibrous dysplasia and ossifying fibroma. A R201H mutation was detected in this case thus confirming a diagnosis of fibrous dysplasia. **Conclusion:** Taken together, our findings as well as other reports indicate that mutational analysis of GNAS gene is a reliable adjunct to differentiate fibrous dysplasia and ossifying fibroma of the jaws. litiejun22@vip.sina.com

Keywords: Fibrous Dysplasia, Ossifying Fibroma, Differential Diagnosis

Bone resorption inhibitor peptides work as a bone formation stimulator.

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Background:

We have involved in developing the peptide drugs for preventing inflammatory bone diseases. We previously showed that the tumor necrosis factor (TNF)- α antagonist peptide W9, which was designed from the critical contact site of TNF- α on the TNF type 1 receptor, works as a receptor activator of NF- κ B ligand (RANKL) antagonist, and inhibits osteoclastogenesis. Recently we found that W9 can stimulate bone formation. In this study, we investigated whether the other RANKL binding peptide OP3-4, could stimulate bone formation, comparing with the effects of W9.

Materials and Methods:

The OP3-4, an osteoprotegerin mimetic peptide, was provided by Dr. Murali. An ectopic bone formation study at the back of the muscle and a local bone formation study at the calvarial bone were performed by using 5 week-old male C57BL/6J mice. One μ g of BMP-2 was used as a positive control in both studies. The ST-2 cells were used for the RANKL-reverse signaling studies.

Results:

OP3-4 (0.56 mg) promoted the BMP-2-induced ectopic bone formation more than W9 (0.56 mg). OP3-4 (0.56 mg) also stimulated local bone formation more than W9 (0.56 mg) without BMP-2. The phosphorylation of Akt and S6K1, the downstream of the RANKL reverse signaling, was increased upon the stimulation of OP3-4 more than that of W9.

Conclusion:

The RANKL-binding peptides might promote bone formation through the RANKL-reverse signaling, and the difference of the anabolic effects of W9 and OP3-4 could be explained by the difference of the RANKL-reverse-signaling intensity.

Acknowledgement: The authors thank Dr. Masashi Honma (Department of Pharmacy, The University of Tokyo Hospital, Tokyo 113-8655, Japan) for performing the RANKL-reverse signaling.

kazu.hpha@tmd.ac.jp **Keywords:** bone anabolic peptide, RANKL-binding peptide, the RANKL-reverse signaling

Session 6

Presentations by PhD Students

Interleukin 12 increased RANKL/OPG expression ratio in human PDL cells

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Background: Interleukin 12 (IL-12) is a multifunctional pro-inflammatory cytokines that involved in Th1 differentiation and also play a role in osteoclastogenesis. **Objective:** The aim of this study was to investigate the osteoimmunology effect of IL-12 on human periodontal ligament cells (hPDLs). **Methods:** Human PDLs were cultured with 0-20ng/ml of IL-12, range for 24-120 hours. The effect of IL-12 on RANKL and OPG mRNA expression was performed by quantitative PCR. The signaling pathway that involved was examined by means of chemical inhibitors. **Results:** IL-12 increased RANKL/OPG ratio of mRNA expression in a dose dependent manner. This effect could be observed form 8 hours after IL-12 treatment. Addition of STAT4 and NF-kB inhibitors, but not indomethacin, suppressed the inductive effect of IL-12 on RANKL expression, suggesting the involvement of STAT4/NF-kB signaling pathway. However, these inhibitors didn't show any significant effect on OPG expression. From immunofluorescence analysis, IL-12 could induce NF-kB nuclear translocation. Moreover, application of STAT4 inhibitor could not inhibit the nuclear translocation, suggesting that STAT4 might function downstream of NF-kB. **Conclusion:** IL-12 regulated RANKL/OPG ratio via STAT4/NF-kB signaling pathway. The result indicated the role of IL-12 in periodontal tissue homeostasis.

Belle_wlovely@hotmail.com **Key words:** Interleukin 12, human PDL cells, RANKL/OPG ratio

Sleep disturbance and psychological distress as possible risk indicators for the development of TMD myofascial pain

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Background: We conduct this study to explore the relationship between neuropsychological distress (sleep disturbance and psychological distress) among patients with temporomandibular disorders (TMD) and to discuss the possibility of sleep disturbance and psychological distress as risk indicators in relation to the development of TMD. **Materials and Methods:** A batch of standardized and validated self-reported questionnaires including Self-Rating Scale of Sleep (SRSS) and the short form of Depression, Anxiety and Stress Scales (DASS-21) are used to measure the neuropsychological distress (sleep disturbance, depression, anxiety and stress). Information about signs and symptoms of TMD and sociodemographic variables is also collected. Total 510 TMD patients (123 males and 387 females), with an average age of 31.06 years (SD=14.38) are included in the study and analysis is conducted among different subgroups. **Results:** 510 TMD patients are grouped into 7 diagnoses according to RDC/TMD diagnostic criteria, I, II, III, I+II, I+III, II+III, I+II+III. Overall, prevalences of moderate to severe neuropsychological distress in patients with I (myofascial pain, MFP) or comorbidity of I (MFP), are significantly higher than those of other diagnoses without MFP. Based on the preliminary results, 510 TMD patients are further grouped into two subgroups, with MFP subgroup (128 patients) and without MFP subgroup (382 patients). Prevalences of moderate to severe neuropsychological distress with MFP subgroup are still significantly higher than those without MFP subgroup. Stepwise logistic regression analyses demonstrate that sleep disturbance and psychological distress (anxiety and anxiety comorbid with depression) are possible risk indicators for MFP with the odds ratio (OR) 2.532, 3.616, 2.054, respectively, and the P values are still significant even after controlling for 18 other confounders. **Conclusion:** This study supports the frequent comorbidity of self-reported sleep disturbance and psychological distress in TMD patients. Self-reported sleep disturbance and psychological distress are demonstrated to be possible risk indicators for the development of myofascial pain compared with disc displacement and degenerative joint disease. kqkyfu@bjmu.edu.cn

Keywords: Temporomandibular disorders; Myofascial pain; neuropsychological distress

Oral malodor treatment improves the patients' psychological condition and QOL

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Background: Oral malodor is a common problem affecting a lot of people. The main causes of oral malodor are Volatile Sulfur Compounds (VSCs), such as Hydrogen sulfide (H_2S), Methyl mercaptan (CH_3SH) and Dimethyl sulfide ($(\text{CH}_3)_2\text{S}$). People worry about oral malodor because it negatively impacts on social life. The objective of this study was to assess the changes of Volatile Sulfur Compounds (VSCs) level, oral malodor anxiety, self-perceived oral malodor and quality of life (QOL) before and after treatment at initial visit.

Materials and Methods: The subjects were 520 oral malodor patients who visited the Fresh Breath Clinic, Dental Hospital of Tokyo Medical and Dental University. Oral malodor was measured by the gas chromatography. The outcomes were shown as concentrations of H_2S , CH_3SH and $(\text{CH}_3)_2\text{S}$ (ng/10 mL). The level of oral malodor anxiety and self-perceived oral malodor were evaluated by the Visual Analogue Scale (VAS:1-100) and QOL by the Face Scales (FS:1-5).

Results: The mean age of all participants was 52.0 ± 14.7 . Male accounted for 38.3% of the samples. The mean concentrations of H_2S , CH_3SH , and $(\text{CH}_3)_2\text{S}$ before treatment were 5.4 ± 5.3 , 2.3 ± 2.9 and 0.8 ± 0.7 . After oral malodor treatment, the mean concentrations significantly decreased to 0.3 ± 0.5 , 0.1 ± 0.2 and 0.1 ± 0.1 , respectively ($p < 0.01$).

The mean VAS score of oral malodor anxiety was significantly reduced from 69.6 ± 26.3 to 27.1 ± 23.2 ($p < 0.001$). The mean VAS score of self-perceived oral malodor also significantly declined from 77.2 ± 21.0 to 23.0 ± 20.1 ($p < 0.01$). There was a significant decrease in mean FS score after (1.4 ± 0.8) the treatment compared to before (3.4 ± 0.9) ($p < 0.01$).

Conclusion: Oral malodor treatment improved not only the oral malodor level of patients but their psychological condition. Therefore, it can be concluded that oral malodor treatment may help in relieving patients' anxiety and contributing to the promotion of their QOL.

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Keywords: Oral malodor, QOL, VSCs

Session 7

Posters

P1) Effect of remineralization paste on enamel erosion In Vitro

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Objective: The present study aimed to evaluate remineralization effect of Multi-Oral RemiDent paste and artificial saliva on hardness of intact enamel after eroded by a Cola drink. **Method:** Forty extracted human premolars were used and baseline Vickers micro-hardness measurements were carried out with a force of 100 g for 15 seconds (average of 5 indentations at buccal surface on each tooth specimen). The prepared specimens were immersed in a cola-drink for 5 seconds and then in artificial saliva for another 5 seconds. Ten cycles of the immersion process were conducted at room temperature. This protocol was repeated 2 times at 6 hours interval. Between each interval, samples were kept in artificial saliva at room temperature. After the erosion process was completed samples were washed with deionized water and blotted dry. The hardness values of enamel surface after exposed to a cola drink were remeasured. The eroded samples were randomly divided into 2 groups. The first group (n=20) was applied by Multi-Oral RemiDent paste (BioClin, Amsterdam, the Netherlands) following the manufacturer's instruction and the other group (n=20) was stored in artificial saliva (Chulalongkorn University, Bangkok, Thailand). The hardness values of each sub-group (n=5) were measured after 30 min, 1h, 3h and 6h. Statistical analysis was conducted by Paired-Sample T-Test ($\alpha = 0.05$). **Result:** The hardness values of enamel (353.6 ± 14.4) decreased significantly after exposed to a cola drink (186.7 ± 14.3). When applied with Multi-Oral RemiDent paste, the hardness values of enamel (268.1 ± 24.7) increased significantly. There was no significant increase of enamel hardness (178.5 ± 6.2) when stored in artificial saliva. The hardness values of enamel significantly increased from 241.8 at 30 min after applied with the paste to 301.5 at 6 hour application. **Conclusion:** The remineralization paste significantly increased the microhardness of the enamel eroded by a cola drink in vitro.

Suchit.P@chula.ac.th **Keywords:** Remineralization, Enamel, Cola

P2) Compatibility of newly introduced one-step self-etch adhesive with dual-curing resin cement with or without dentin substrate

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Background: This study was to investigate the compatibility of newly introduced one-step self-etch adhesive containing new initiation system with dual-curing resin cement with or without dentin substrate, compared to conventional adhesive. **Materials and Methods:** Two one-step self-etch adhesives and one resin cement were selected: Tri-S Bond (TS, Kuraray Noritake Corp. Japan), SE One Bond (SO, Kuraray Noritake Corp. Japan) and DC Core plus (DC, Kuraray Noritake Corp. Japan). After thorough solvent evaporation, the adhesives were poured into silicone molds (8mm in diameter and 4mm in depth), and incrementally light-cured into twenty-four blocks. Twenty-four human molar were selected, of which superficial dentin was exposed and flattened. The dentin surfaces and resin blocks were all subjected to three bonding treatment protocol as substrates: a) a layer of adhesive was applied on substrate according to manufacturer's instruction and light cured for 10s, on which DC were applied and light cured for 20s; b) a layer of adhesive was applied on substrate according to manufacturer's instruction and light cured for 10s, on which DC were applied and self-cured for 10min; c) a layer of adhesive was applied on substrate according to manufacturer's instruction and left uncured, on which DC were applied and self-cured for 10min. Twelve groups were got by different adhesive, substrate and curing protocol (with four teeth or adhesive blocks for each treatment group). The cement-substrate blocks were cut into beams with 1mm*1mm cross sectional area and subjected to microtensile bond strength testing (MTBS). Statistical analysis was carried out with two-way ANOVA in SPSS 17.0 software. **Results:** Statistical results showed that both adhesive and treatment protocol had significant influence on MTBS. SO got significant higher strength ($81.40 \pm 10.87 \text{ MPa}$) than TS ($50.0 \pm 19.23 \text{ MPa}$) on adhesive substrate for self-curing mode. The strengths of the two adhesives were similar for the other treatment protocols on adhesive substrate. Bond strengths were significantly lower on dentin surface than adhesive surface within the same treatment group. Strength values were lower for TS ($18.72 \pm 8.11 \text{ MPa}$) than SO ($24.55 \pm 8.16 \text{ MPa}$) on dentin substrate but the difference was insignificant. **Conclusion:** Within the limitation of this study, SO showed better compatibility with DC than TS, especially when adhesive and cement were neither light cured. Dentin substrate possibly contributed to the adverse reaction between one-step self-etch adhesive with dual-curing resin cement and thereafter the incompatibility.

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Keywords: one-step self-etch adhesive; dual-cure resin cement, compatibility

P3) Remineralization Assessment of Adhesive with Simulated Pulpal Fluid Using Micro-CT

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Background: Artificial caries methods for *in vitro* studies have been created and used to analyze the remineralizing potential of different materials. The purpose of this study was to evaluate the efficacy of the adhesive systems containing different phosphoric acid monomers, MDP and Phenyl-P, on remineralization potential of an artificial caries-affected dentin (ACAD) model under the simulated pulpal fluid pressure.

Materials and Methods: An artificial caries-affected dentin (ACAD) model was created from crowns of human molars. The root was cut and remaining pulpal tissue was carefully removed. Crown segments were attached to a pulpal pressure device of 15cm simulated pulpal fluid. A MDP-containing two-step self-etch adhesive system, Clearfil SE Bond (MDP) (Kuraray Noritake Dental) and one experimental with Phenyl-P (PP) was applied to the surfaces of ACAD according to the manufacturer's instructions. Each tooth was built up with resin composite (GC Gradia Direct, Tokyo, Japan) using a layer of 1mm height. After immediate, 24 h and 1 month incubation of the specimens, the internal angle values calculated from the slope of the mineral profiles obtained by micro-CT were calculated. **Results:** Two-way ANOVA revealed that both factors, "storage time" ($p=.035$) and "type of adhesive" ($p=0.43$) had effect on the angle values ($p=.048$). Moreover, MDP group resulted in statistically significant different with respect to PP group after 1 month. **Conclusion:** MDP adhesive demonstrated change of the slope angles from the mineral profiles in the ACAD model after 1 m storage, however, the PP adhesive did not change over time. The MDP-containing self-etch adhesive system has positive effects on remineralization of caries-affected dentin.

Corresponding author: inoue.ope@tmd.ac.jp **Keywords:** artificial caries-affected dentin, MDP, Phenyl-P

P4) Influence of Artificial Tooth Thickness on Fracture Resistance of Acrylic Denture Base

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Background: Lack of interarch distance is one of the significant factors of failures in the prosthodontics. The aim of this study was to investigate the influence of material and thickness of the artificial denture tooth on the fracture resistance of acrylic denture base that replaces edentulous space of reduced interarch distance.

Materials and Methods: Each test specimen consisted of a mandibular right first molar denture tooth embedded in a denture base resin block (Acron). A commercial artificial tooth of an acrylic resin (Wearless Acrylic (AC)) or a composite resin (Surpass (CO)) was embedded in the center of the denture base resin block (20 mm × 12 mm × 2.0 mm). Prior to the denture base polymerization, the base of each tooth was ground so that the tooth thickness became one of the following: 0.5 mm, 1.0 mm, 2.0 mm, or 2.5 mm (n=7). Each specimen was subjected to a three-point flexural test and loaded at the support span of 8 mm or 12 mm until fracture occurred. The maximum fracture loads were statistically analyzed ($p=.05$). **Results:** With the 8 mm support span, all specimens fractured at the center of the specimen base (center mode). The mean fracture loads of the CO were significantly higher than those of the AC ($p<.05$), but the mean loads were insensitive to the tooth thickness ($p>.05$). With the 12 mm support span, the mean fracture load of the AC was also insensitive to the tooth thickness with the center mode fractures, except for the specimens of 2.5 mm-thickness that showed a significantly lower value than the other specimens ($p<.05$) with the fractures at the edge of the tooth (edge mode). The mean fracture load of the CO significantly decreased as the tooth thickness increased from 0.5 mm to 2.5 mm. The mean fracture load of the 2.5 mm-thick CO tooth specimens that showed the edge mode was significantly lower than those with the center mode ($p<.05$). As for the mean fracture load, there were no significant differences between the AC and CO specimens ($p>.05$). **Conclusion:** The denture base embedding the composite resin tooth was more resistance to fracture in comparison with the acrylic tooth if used in a shorter interarch distance. The artificial teeth should be embedded so that they are sufficiently secure distance between bottom of artificial teeth and denture base.

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Keywords: denture base, artificial tooth, fracture resistance

P5) 3T MRI Movie: A new detective tool for articulation

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Background: MRI movie is a method that can be used to observe articulation in human. Although, other methods can also use but MRI movie technique has more advantages than others. Because of the effectiveness and non-invasive property, MRI movie has been used to observe articulation in cleft lip and palate patients in previous study. MRI scanner is same as other technologies that always have evolution. Recently, 3T MRI scanner becomes more widely used instead of 1.5T MRI scanner. However, research has been used 3T MRI scanner to reveal articulation. The aim of this study was to apply MRI movie in 3T MRI scanner, which may reveal clear articulation with only few artifacts.

Materials and Methods: Two female healthy subjects with and without anterior open bite were participated in this study. Custom-made circuitry was connected to 3T MRI scanner that used to observe articulation. T1-weighted gradient echo (FLASH) with segmented cine and parallel imaging technique (GRAPPA) pulse sequence was used. The mid-sagittal plane image was obtained with time resolution of 9ms, matrix size of 128x128, pixel size of 1x2 mm, slice thickness of 4 mm and acceleration factor of 2. The subject was in the supine position and produced fricative sound (/asa/) synchronized with the external trigger pulse. The images were analyzed by ImageJ software.

Results: Continuous 116 images during fricative sound (/asa/) with 9-ms time resolution were obtained. Compared with 1.5T, the number of pronunciation repetition was decreased (42 times in 1.5T, 35 times in 3T) and MRI image quality was significantly improved, the soft tissue articulators were clearly visible with few artifacts. Moreover, this method revealed differences during articulation between the two subjects.

Conclusion: 3T MRI movie is effective and non-invasive method that can clearly reveal articulation. Because of the higher time resolution than normal video, the variation of speech function can be diagnosed. This method might be useful to perform in patients that have articulation problem in the future.

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Keywords: 3T MRI, MRI Movie, Articulation

P6) Trigeminal neuralgia at the Faculty of Dentistry, Chulalongkorn University

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Background: Orofacial pain is a complicated oral health problem that results in a low quality of life for the patients and is discouraging for clinicians. The development of pain in orofacial locations stems from a variety of disorders. While dental pain and temporomandibular disorders are the leading causes of orofacial pain, neuropathic pain disorders, primary headache disorders or disorders of associated structures are also quite common. The most common neuropathic pain condition in the orofacial area is trigeminal neuralgia.

Materials and Methods: This study retrospectively examined 149 trigeminal neuralgia patients at the Department of Oral Medicine, Faculty of Dentistry, Chulalongkorn University for the period of 20 years.

Results: The patients' age range was 20-85 years old. The ratio of females to males was two to one. The pain occurred mainly on the right side of the face (65%). The most common trigger zone was located at the lower right posterior teeth (16%). One third of the patients had dental treatments performed in the area of chief complaint prior to their visits to the school. The dental treatments included extraction and root canal treatment. Carbamazepine was mostly used for this disorder. The lowest dose that effectively controlled the pain was 200 mg per day. The side effects of the drug were vertigo, nausea, vomiting and decreased white blood cell counts. **Conclusion:** Carbamazepine was the drug of choice for trigeminal neuralgia.

Kanokporn.Bh@chula.ac.th **Keywords:** Trigeminal Neuralgia, Carbamazepine, Orofacial pain

P7) Evaluation of oral health promotion program in Huay-Hang subdistrict

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Background: The effectiveness of oral health promotion (OHP) has been emphasizing on individual dental status change. However, a healthier environment through policy development and implement are the goal of oral health promotion interventions. The aim of this study was to evaluate the community OHP program by measure healthy public policies and healthy environments.

Materials and Methods: This project started with community engagement by discuss with community leaders and key persons. The OHP team was formed by volunteer local people under supervise from the main researcher. Community needs assessment and OHP program was developed by OHP team. 40 volunteer health workers were empowered and trained by OHP team, then call as a “community dentist”. Community dentists acted as advocator of dental issue in this community. One community dentist take care dental health of 10 household, totally 637 people. The principal of primary school set up tooth brushing system at lunch under supervision of community dentists. OHP team and community dentists implement several activities in community such as brushing before go to bed campaign, apply silver diamine fluoride and fluoride varnish, good teeth competition and lobbying for subdistrict healthy public policy.

Results: The population of Huay-Hang subdistrict has 7,705 living in 12 villages. The main oral health problem in this area was early childhood caries. The caries in 3 year old children was 86.54 at dmft 6.81 and higher in 5 years old children. After 2 years with multi-tasks interventions, healthy public policies and healthy environment, with community participation, were shown in policy statement of subdistrict and school level. Eighty one percent of village merchants (57 merchants) join the policy of “no sale of candy and jelly” in their shop. All primary school and kindergarten announce the policy of less sweet and no soft drink sale inside schools. Community dentists and OHP team play a major role in enabling, mediating and advocacy. Subdistrict Administrative Organization, as local government, participate and support this program by grant addition budget to this project. Subdistrict Headman, a part of provincial administration, joined as a member of OHP team.

Conclusion: Healthy public policy and environment create by community dentists and OHP team can be useful to evaluate OHP program.

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P8) Inequality in dental care utilization among Thai elderly despite universal coverage

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Background: This study aimed to assess the inequality of dental care utilization and the unmet perceived need for dental care among Thai elderly. In addition to determine the association of underlying determinants and the inequality of dental care utilization. **Materials and Methods:** The data of 11826 elderly Thais (over 60 years of age) from the national representative Thailand Health and Welfare Survey in 2011 were analyzed. Descriptive analyses and chi-square test were performed dental care utilization and the unmet perceived need of dental care among Thai elderly. Multivariate logistic regression was used to determine the determinants of inequality in dental care utilization. **Results:** Analyses indicate that there are socioeconomic (SES)-, and geographic-related inequality in dental care utilization among Thai elderly. It significantly tends to favor those of high-SES and residents of urban area than their peer counterparts ($p < .05$). However, the reported unmet-perceived need of dental care among Thai elderly is less than 1%. The main reasons are physical barrier and transportation. Multivariate regression reveals that socioeconomic-, geographic-, demographic characteristics are associated with the inequality in dental care utilization among Thai elderly. **Conclusion:** This study highlights the socioeconomic inequality in dental care utilization among elderly in Thailand where universal coverage has recently been achieved. Despite persistent inequalities, mitigating such inequalities in the delivery of dental care to the less well-off elderly through emphasizing the delivery of care on primary health care system, provides lesson that may be relevant to other countries, particularly those developing countries.

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P9) Infant oral health audio-visual educational aid for pregnant women

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Objectives: The objective of this study were to develop and test the infant oral health educational audio-visual aid for pregnant women.

Methods: The audio-visual aid containing evidence-based information about infant oral health care and early childhood caries prevention has been developed and test in 91 pregnant women having prenatal care at U-Thong hospital, Suphanburi province during October 1, 2008 to November 30, 2008. A questionnaire was developed to test knowledge and attitude of expectant mother before and after viewing the audio-visual aid. The efficiency and effectiveness index of the audio-visual aid were determined. The differences of knowledge score were tested with Wilcoxon match-pair signed-ranks test. Kruskal-Wallis test and Mann-Whitney test were used to determine factors related to knowledge score.

Results: The efficiency of an audio-visual aid was 87/70 and effectiveness index was 0.64. The pregnant women significantly improved their infant oral health knowledge ($p < 0.05$). The pre-test and post-test knowledge scores were statistically significantly related to educational level ($p < 0.05$). The differences between pre-test and post-test knowledge scores were statistically significant related to number of children ($p < 0.05$). Approximately or more than 80 percent of the participants rated proper timing, speed and contents of audiovisual aid, and highly satisfaction on narration, illustration, easy understanding, interesting presentation, and useful.

Conclusions: This infant oral health educational audio-visual aid promises to be an effective tool for pregnant women in providing anticipatory guidance regarding infant oral health care to prevent early childhood caries.

Key words: audiovisual aid; effectiveness; efficiency; infant oral health education; pregnant women

P10) Analyses of physiological responses associated with emotional changes induced by viewing video images of dental treatments

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Background: The understanding of emotional changes induced by dental treatments is important for dentists to provide a safe and comfortable dental treatment, particularly in pediatric dentistry, since it is difficult for young child patients to express their feeling in words. Thus, we analyzed electroencephalogram (EEG), electrocardiogram (ECG) and facial electromyogram (EMG) during watching video images of dental treatments to search for the best objective indices reflecting emotional changes in this study.

Materials and Methods: Fourteen healthy subjects (six males and eight females) with the mean age of 22.6 ± 3.2 (SD) years voluntarily participated in the present study. EEG, ECG and EMG of the corrugator muscle was recorded during watching video images of intra-oral examination, infiltration anesthesia and cavity excavation. By analyzing EEG and ECG, the emotional changes and autonomic nervous activities were estimated, respectively. The subjective discomfort level was acquired by Visual Analog Scale method. This study was approved by the Ethics Committee of our university.

Results: Analysis of EEG and ECG demonstrated that watching the image of infiltration anesthesia and cavity excavation induced a lowering of relaxation level of emotion and an increase in sympathetic nervous activity which elevates with mental stress. The peak activity of corrugator muscle was increased by watching all three examined images. The subjective discomfort level was highest during watching infiltration anesthesia image.

Conclusion: These results suggest that subjective discomfort can be assessed to some extent by analyzing EEG, ECG and EMG, but the close relationship between subjective feelings and these indices has not been obtained yet. Further investigation is required to find the most suitable index to understand the emotional states of patients during dental treatments, which is especially applicable for pediatric patients.

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P11) The use of oral rinse in reducing the number of microorganisms in oral cavities of patients with fixed orthodontic appliance

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Objective: To evaluate the effectiveness of 0.2% chlorhexidine mouth rinse in reducing the number of microorganisms in oral cavities of patients with fixed orthodontic appliance prior to dental treatment and to compare the effectiveness of 0.2% chlorhexidine mouth rinse with 0.9% normal saline solution.

Materials and methods: Collection of saliva samples from 2 groups of 30 patients each with fixed orthodontic appliance using oral rinse method with phosphate-buffered saline solution before and after rinsing with 0.2% chlorhexidine mouth rinse or 0.9% normal saline solution. Saliva samples were cultured on 5% blood agar. The recovered microorganisms were counted in colony forming units. The data were statistically analyzed. **Results:** 0.2% chlorhexidine mouth rinse was effective in reducing the number of microorganisms by 56.5 fold. When compared to 0.9% normal saline solution, which could reduce by 3.6 fold, the difference was statistically significant. ($P < 0.05$) **Conclusion:** 0.2% chlorhexidine mouth rinse was effective in reducing the number of microorganisms in oral cavities of patients with fixed orthodontic appliance. It was also more effective than 0.9% normal saline solution.

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P12) Plant extracts inhibit *Streptococcus mutans* adhesiveness to saliva-coated hydroxyapatite powder.

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Background: One of the strategies to fight against dental caries is to inhibit the viability and virulence properties of the cariogenic bacteria. The objective of this study is to investigate the inhibitory concentration of seven plant extracts on the adhesion of *Streptococcus mutans* to saliva-coated hydroxyapatite powder (SCHP). **Methods:** Extracts were prepared from seven dried plants, *Curcuma xanthorrhiza* (root), *Dahlia pinnata* (whole plant), *Momordica charantia* (whole plant), *Ocimum gratissimum* (fruit), *Oxalis repens* (whole plant), *Stemona tuberosa* (root) and *Zingiber cassumunar* (root) using 95% methanol, evaporated, dried, and dissolved in 10% DMSO. The SCHP adhesion assay was performed in a 96-well plate by incubating *Streptococcus mutans* ATCC25175 (106 cfu/ml) at 37°C for 2 h with SCHP in either 10% DMSO or plant extract at concentrations of 0.25, 0.75, 1.25, 2.5, or 5.0 mg/ml. The powder was then washed twice with phosphate buffer and incubated with alamarBlue^R dye at 37°C for 60 min. Adhered bacteria reduced the dye to its fluorescent product, which was measured using a spectrofluorometer at excitation and emission wavelengths of 570 nm and 585 nm, respectively. **Results:** All of the extracts at a concentration of 0.25 mg/ml reduced the adhesion of *Streptococcus mutans* to 54-69% of the control level. *Curcuma xanthorrhiza* and *Oxalis repens* at a concentration of 5.0 mg/ml had the highest inhibitory effects, reducing the adhesion to 34.9 and 38.7%, respectively, of the control levels. **Conclusions:** All of the tested plant extracts at 0.25 mg/ml inhibited the adhesion of *Streptococcus mutans* and could be used for the development of new caries prevention agent.

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P13) Measurement of the remaining dentin thickness using optical coherence tomography for crown preparation

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Background: Optical coherence tomography (OCT) is a well-known laser technique for providing noninvasive, high spatial resolution images of the biological microstructure. It works similarly to ultrasound but simply uses light waves instead of sound. It is possible to decrease excessive tooth preparation and the pulpal injury if the remaining dentin thickness (RDT) is measured with OCT. This study aimed to investigate the refractive index of the dentin near the pulp and to explore the possibility of measuring the remaining dentin thickness (RDT) using OCT.

Materials and Methods: Human third molars were used in this study. In experiment 1, the cuspal dentin blocks (thickness of 0.50 mm, 0.75 mm, 1.00 mm, 1.25 mm, 1.50 mm and 1.75 mm) were prepared. Each specimen was scanned using OCT. In experiment 2, the teeth were embedded in an epoxy resin and sliced in parallel along the long axis of the teeth. The sliced blocks were trimmed perpendicular to the long axis of the teeth until the remaining occlusal dentin was 0.50-mm or 1.00-mm thick. After that, each specimen was scanned using OCT and micro-CT, and the refractive index was measured.

Results: In experiment 1, OCT images could be obtained for all specimens that had thicknesses of 1.00 mm or less. In experiment 2, the resulting refractive indices of the 0.50-mm RDTs were significantly lower than that of the 1.00-mm RDTs.

Conclusion: Within the limitations of this study, OCT was effective for measuring RDT in a clinical setting and prevented pulpal injury when the refractive index was carefully set.

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Keywords: Optical coherence tomography, Remaining dentin thickness, Refractive index

P14) Assessment of Remaining Dentin Thickness (RDT) During Caries Excavation by Swept Source Optical Coherence Tomography (SS-OCT)

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Background: Excavation of deep dentine caries might lead to iatrogenic or unwanted exposure of the dental pulp. This accidental damage to the dentin structure over the pulp will shift the treatment strategy from restorative to endodontic and endanger the longevity of tooth. Estimation of remaining distance from the pulp horn/chamber is necessary for conservative treatment of dental caries. Usually radiographs are taken for the diagnosis and to see the extension of the caries. Since radiograph produces harmful x-radiations and superimposition of the structures results in limited diagnostic accuracy, it is not a suitable option at the time of treatment. It seems that non-destructive and non-invasive imaging devices are necessary during the treatment of caries. This study was carried out to evaluate the efficacy of Dental SS-OCT in visualizing the remaining dentin thickness during excavation of deep dentine caries. **Materials and Methods:** Human molars with deep dentine caries were selected. The most superficial soft debris was removed with spoon excavator. Roots were flattened parallel to the occlusal surface during OCT scanning at 1330 nm center wavelength (Dental SS-OCT Prototype 2, Panasonic Healthcare Co., Ltd, Japan) 2D and 3D OCT scans were obtained before and after the application of Caries Check (Nishika institution, Japan). Pulpal horns and pulp chamber were observed. Afterwards specimens were sectioned using diamond saw (Isomet, Buehler, USA) at the same sections observed under OCT. Specimens were not grind intentionally to mimic the clinical situation. CLSM (Lasertec Co.) images were taken at magnification level of 5x to confirm and compare the remaining dentin thickness measurements between SS-OCT and CLSM. Thickness measurement of the RDT was done on samples section by image analysis software (ImageJ version 1.45). From each image RDT was measured at 5 regions and average value was calculated. For the confirmation of OCT findings CLSM images were taken at the same cross-section observed under OCT. **Results:** The remaining dentine thickness (RDT) visualized under OCT corresponded to those of CLSM considering the refractive index of dentine (n=1.55). Nevertheless, in case of infected dentin, it was difficult to determined strong attenuation through the carious dentine. OCT with near infrared light can act as a reliable tool to visualize the RDT due to optical contrast between the dentine and the pulp. Further study will comprise using simulated pulpal fluid followed by confirmatory tests with X-ray microfocus computed tomography (micro-CT).

Conclusion: This study was a trial to visualize RDT over the pulp chamber using SS-OCT and it was found that it is possible to use SS-OCT during deep caries excavation for the prevention of perforation of the vital dental pulp. OCT with near infrared light can act as a reliable tool to visualize the RDT because of the optical contrast between the dentine and pulp chamber. Further study will be done by using simulated pulpal fluid followed by confirmatory tests using X-ray microfocus computed tomography (micro-CT). majkut.ope@tmd.ac.jp Keywords: RDT, Caries Excavation, SS-OCT

P15) The effect of Capsaicin cream and Carbarcole on Prevention of Duct Obstruction of Transplanted Submandibular Gland

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Background: Autologous microvascular transplantation of the submandibular gland (SMG) is an effective treatment for severe keratoconjunctivitis sicca (KCS). However, the transplanted gland may suffer from duct obstruction after transplantation. The affected gland swells repeatedly and produces very little and viscous excretion, which result in insufficient ocular lubrication and even treatment failure. The objective of this study was to evaluate the effect of capsaicin cream and cabachole on prevention of duct obstruction of transplanted SMG.

Materials and Methods: 154 patients (166 glands) who underwent successful SMG transplantation were included. 120 glands (group 1) were treated with topical application of capsaicin cream (4 to 6 times a day) or subcutaneous injection of carbachol (once every 2 or 4 weeks) in the first three months post-operation (latent period). 46 glands (group 2) received neither of the two drugs. Secretion of the transplanted gland was evaluated by Schirmer's test. The effects of the two drugs were observed and obstruction of the duct was recorded.

Results: Both of capsaicin cream and cabachole had significant promotion functions to transplanted SMG secretion. Schirmer's test increased from $1.64 \pm 2.26\text{mm}$ to $4.46 \pm 3.87\text{mm}$ ($P < 0.05$), and from $1.67 \pm 1.71\text{mm}$ to $1067 \pm 888\text{mm}$ ($P < 0.05$) after application of capsaicin cream and cabachole, respectively. The rate of duct obstructions in group 1 (5%) was lower than that in group 2 (15.2%) ($P < 0.05$). No serious adverse reactions were observed.

Conclusion: Daily use of capsaicin cream could maintain a basic secretion of the transplanted SMG, while large amount of secretion could be achieved by intermittent injection of carbachol to wash out the deposited substances in the duct. Combination of these two drugs could effectively prevent the duct obstruction of transplanted SMG.

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Keywords: keratoconjunctivitis sicca, submandibular gland transplantation, capsaicin and cabachole

P16) Condylar remodeling accompanying splint therapy: A cone-beam computerized tomography study of patients with temporomandibular joint disk displacement

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Background: Anterior repositioning splint(ARS) therapy is effective for the management of temporomandibular joint(TMJ) disk displacement with reduction. It was reported that ARS therapy not only repositions displaced articular disks but also leads to condylar bone remodeling that is manifested as a “double contour” on magnetic resonance imaging (MRI) of the condylar heads. Cone-beam CT (CBCT) has been recommended for TMJ evaluation and is the preferred TMJ imaging method when bony conditions are involved. The use of CBCT for analyzing osseous condylar changes after splint therapy, however, has not been reported. The aim of this study was to evaluate osseous changes accompanying ARS therapy in patients with temporomandibular joint disk displacement. **Materials and Methods:** CBCT data of 36 patients with intermittent or permanent closed-lock were used; 23 patients with permanent closed-lock had their displaced disks physically reduced by mandibular manipulation before ARS therapy. CBCT was performed before and ~6 months after ARS therapy. The presence and location of “double contour” images suggesting condylar bone remodeling were statistically analyzed. **Results:** The “double contour” images after ARS therapy were observed in ~80% of patients, more frequently in joints with signs of displaced disks. The “double contour” appeared more often on the posterior bevel as well as the medial and middle part of condyles ($P < 0.01$). **Conclusion:** ARS therapy can facilitate regenerative remodeling of condyles. CBCT is a useful tool for monitoring osseous changes in condyles.

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Keywords: Condylar remodeling; Splint therapy; Cone-beam computed tomography

P17) MRI characteristics of rheumatoid arthritis in the temporomandibular joints

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Background: Rheumatoid arthritis (RA) is an autoimmune disease that develops frequently in the elderly. Although RA commonly develops in joints of the hands, legs and shoulders, it can also occur in the temporomandibular joints (TMJs). TMJ damage severely reduces the quality of life of patients. Accordingly, the importance of imaging diagnosis of RA in the TMJs should be emphasized. The purpose of this study was to investigate characteristic MRI findings of RA in the TMJs, and to compare them with MRI findings of temporomandibular disorder (TMD).

Materials and Methods: Sixty-one patients (122 TMJs) with RA in the TMJ diagnosed by the criteria of the American Rheumatism Association (ARA)¹ and 50 patients (100 TMJs) with temporomandibular disorder (TMD) were included in this study. MR images of these patients were assessed by two oral radiologists for the presence or absence of osseous changes, disk displacement, joint effusion and synovial proliferation. These findings were compared between the two patient groups.

Results: Osseous changes in the condyle and articular eminence / fossa in the RA patient group were significantly more frequent than in the TMD patient group. The two thirds of the TMJs in the RA group have very severe bone absorption. Joint effusion was also significantly more frequent in the RA patient group. Synovial proliferation was found in all TMJs in the RA patient group, while it was uncommon in the TMD patient group.

Conclusion: Severe osseous changes in the condyle and synovial proliferation were considered characteristic MRI findings of RA in the TMJs.

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Keywords: rheumatoid arthritis, temporomandibular joints, MRI diagnosis

P18) Malignancy - Mimicking Ameloblastoma: The Unusual Radiographic Findings

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Objective: The aims of this report are to present and to discuss the unusual radiographic findings of intraosseous ameloblastoma presenting as exophytic growth with ill-defined, invasive margin and extensive periosteal reaction. **Case report:** A 48-year-old Thai male came for a chief complaint of painless swelling at the left posterior mandibular area adjacent to the extraction site over the last year. Early clinical sign and symptom shows on and off swelling with clear fluid discharge. Subsequently, the swelling steadily increased in size without fluid secretion. He had no history of pain, numbness or other abnormal sensations. Physical examination indicates normal mucosal coverage and clinical impressions included cystic lesion or benign tumor. Subsequently, this patient was referred for conventional radiographs, where additional Cone-Beam Computed Tomography was then prescribed. Unusual radiographic findings from these images can be concluded as follows: (1) ill-defined margin of lesion surrounded by large sclerotic zone; (2) obvious bucco-crestal origin with minimal lingual involvement in crestal region; and (3) several clear triangular shaped (Codman's) periosteal reaction. Based on the above-mentioned information, the differential diagnosis of this lesion was malignant or benign tumor with chronic infection. Biopsies were performed and diagnosed as acanthomatous variant of ameloblastoma. **Conclusion:** This particular case shows unusual radiographic findings of intraosseous ameloblastoma mimicking malignant lesion which can be appreciated thoroughly through Cone-Beam Computed Tomography. These unusual findings have rarely been reported, and collecting them might be primary steps to revolutionize old concept of radiographic interpretation in the future.

kiwi_dent@hotmail.com **Keywords:** Cone-Beam Computed Tomography, ameloblastoma, malignant, infection, periosteal reaction

P19) ARID3B Plays a Critical Role in Proapoptotic Gene Expression and Cell Death following DNA damage

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Background: ARID3B/DRIL2/Bright-like is a member of the ARID (AT-rich interaction domain) family of DNA-binding proteins. Proteins of this family have been shown to be involved in regulation of cell cycle, gene expression, differentiation, embryonic development, chromatin remodelling, and transcriptional regulation. ARID3B and closely related ARID3A share additional conserved regions that extend outside the ARID domain and bind specific AT-rich DNA sequences. We have reported that ARID3A cooperates with p53 to activate *p21WAF1*, a p53-target gene involved in cell-cycle arrest, but not the proapoptotic target genes. While ARID3B has been shown to be overexpressed in neuroblastoma and ovarian cancer, the function of ARID3B in cancer has not been fully investigated. Here, we examined the roles of ARID3B in proapoptotic gene transcription and cell death after DNA damage. **Materials and Methods:** *Cell culture and drug treatment;* U2OS human osteosarcoma cell line was maintained in Dulbecco's modified Eagles's medium supplemented with 10% fetal bovine serum. To induce DNA damage, cells were treated with 0.2 mg/ml doxorubicin for 24 h. *Small interfering RNA (siRNA);* cells were reverse transfected with siRNAs that target ARID3A, ARID3B and p53 using Lipofectamine RNAiMAX according to the manufacturer's instructions, and incubated for 48 h prior to doxorubicin treatment. *Quantitative real-time reverse transcription (RT)-PCR analysis;* was performed on the LightCycler 480 instrument in triplicate using THUNDERBIRD SYBR qPCR Mix, according to manufacturer's instructions. The relative expression of mRNA, normalized to 18sRNA, was calculated using the $2^{-\Delta\text{Ct}}$ method. *Cell viability assay:* Cell viability was determined by trypan blue exclusion test 48 h after DNA damage. **Results:** siRNA-based ARID3B knockdown suppressed the expression of proapoptotic genes, including *PUMA*, *PIG3*, *DINP1*, and *TNG-R2*, after DNA damage, whereas ARID3A suppressed the expression of *p21WAF1*, but not that proapoptotic genes. In line with this, ARID3B, but not ARID3A, siRNA suppressed cell death induced by DNA damage. **Conclusion:** These results suggest critical roles for ARID3B in proapoptotic gene expression and cell death following DNA damage.

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Keywords: p53, ARID3B, Cell Death

P20) Bmi-1 expression predicts prognosis in salivary adenoid cystic carcinoma and correlates with EMT-related factors

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Background: Salivary adenoid cystic carcinoma (SACC) is known for high propensity to invade and metastasize. Bmi-1 acts as an oncogene by controlling cell cycle and self-renewal of adult stem cells, and its overexpression correlates with metastasis and poor prognosis in several cancers. Epithelial-mesenchymal transition (EMT) plays a central role in cancer metastasis. A key step in EMT is the down-regulation of E-cadherin that can be repressed by the transcriptional factors, such as Snail and Slug.

Materials and Methods: We investigated Bmi-1, Snail, Slug and E-cadherin expression immunohistochemically in 102 patients with SACC and analyzed statistically whether their expression correlated with clinicopathologic factors and prognosis. RT-PCR was also performed in 22 tumor tissues and the adjacent non-cancerous tissues to confirm Bmi-1 status in SACCs.

Results: Our data demonstrated significant associations between the tumor metastasis and the expression of Bmi-1, Snail, Slug and E-cadherin. Furthermore, a high level of Bmi-1 was not only correlated with the overexpression of Snail and Slug but also indicated an unfavorable metastasis-free survival and served as a high risk marker for SACC. In addition, Bmi-1 mRNA level was found much higher in SACC tissues than in the adjacent non-cancerous salivary gland tissues.

Conclusion: Our results suggest that Bmi-1 may play a crucial role in SACC metastasis by interaction with EMT-related markers and predict poor survival.

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Keywords: Bmi-1, epithelial-mesenchymal transition, salivary adenoid cystic carcinoma

P21) Notch1 expression is downregulated in chemically-induced oral epithelial dysplasia.

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Background: Notch is a transmembrane receptor that regulates proliferation and differentiation of various cell types. We previously found that Notch1 expression was downregulated in epithelial dysplasia and squamous cell carcinoma of the oral cavity. This study aimed to clarify the role of Notch1 downregulation in oral epithelial tumor pathogenesis by analyzing 4-Nitroquinoline-1-Oxide (4-NQO)-induced lesions in mice.

Materials and Methods: Six-week-old ICR mice were divided into treatment and control group. Treatment group was administered drinking water containing 4-NQO. Formalin-fixed-paraffin-embedded tongue specimens were analyzed histologically and immunohistologically using anti-Notch1 (EP1238Y, Epitomics), Keratin (K)13 (ab16112, Abcam), K14 (LL002, Neomarkers), K15 (EP14, Epitomics) antibodies. **Results:** All mice in 4-NQO treatment group developed epithelial dysplasia in tongue and none was observed in the control group. In normal epithelium, Notch1, K14 and K15 were expressed in the basal layer while K13 was expressed in the suprabasal layer. In epithelial dysplasia, downregulation of Notch1, K13 and K15 were observed. Notch1 expression was downregulated more frequently and broadly compared to K13 and K15. Notch1 downregulation was also observed in the epithelia with minimum histological changes. Downregulation of K15 coincided with that of Notch1, but observed in narrower regions. K13 was least downregulated in the lesions. K14 expression was diffusely observed in both basal and suprabasal layer of the lesions. **Conclusion:** Disturbance of squamous epithelium differentiation which was revealed by the altered expression of keratins appeared to be related with the preceding downregulation of Notch1, suggesting its essential role in the initial stage of oral neoplasia.

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Keywords: Notch1, oral epithelial dysplasia (OED), 4-NQO

P22) Epithelial-derived Mediators Suppressed *Porphyromonas gingivalis* LPS-induced RANKL Expression in Human Primary Bone Cells

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Background: Oral epithelial cells formed the protective barrier of the oral cavity and continuously encountered with pathogens. While invading deep tissue, *Porphyromonas gingivalis*, one of the important periodontal pathogens, could stimulate destructive host immune responses. From our preliminary data, the lipopolysaccharides of *P.gingivalis*, or PgLPS, might play a role in the inflammatory-mediated bone loss by increasing RANKL expression in primary bone cells. However, it remains unclear whether oral epithelial cells provide protective mechanism to control PgLPS-mediated periodontal inflammation and bone loss.

Objective: To investigate whether epithelial-derived mediators could modulate PgLPS-induced RANKL expression in primary bone cells *in vitro*. **Methods:** Human primary bone cells were cultured from the excess bone tissue obtained from mandibular third molar extraction sites in DMEM supplemented with 15% FBS and 1% L-glutamine. Bone cells were challenged with 2.5µg/ml PgLPS for 24h to stimulate RANKL expression. To test the effect of epithelial-derived mediators, the supernatant collected from epithelial cultures was added to PgLPS-stimulated bone cells. Total RNA was extracted using TRIzol[®] reagent. RANKL-, OPG-, IL6- and GAPDH-specific mRNA was amplified using reverse transcription PCR. Amplicons were analyzed using Image Bio1D software (Viber Lourmat), and relative expression of genes was compared to untreated cells. **Results:** RANKL-specific mRNA expression was significantly increased in human primary bone cells in response to PgLPS stimulation while OPG-specific mRNA was unchanged. The inflammatory cytokines-specific mRNA, including IL-6, was upregulated. When PgLPS-stimulated bone cells were treated with epithelial supernatant, the RANKL-specific mRNA expression was substantially suppressed to the baseline level. **Conclusions:** PgLPS could induce primary bone cells to up-regulate RANKL-specific mRNA expression along with IL-6. This response may lead to bone resorption process. However, epithelial-derived supernatant demonstrated suppressive effect on PgLPS-induced RANKL expression in primary bone cells. The mechanism of RANKL suppression remains to be investigated.

Key words: Human primary bone cells, *Porphyromonas gingivalis* Lipopolysaccharide: PgLPS, Oral epithelium-derived mediators

P23) 17-Beta-Estradiol Enhanced Hyperalgesia of Inflammatory Temporomandibular Joint through Upregulation of Trigeminal Ganglionic Nav1.7 in Ovariectomized Rats

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Background: Temporomandibular disorders (TMD) are an assorted set of clinical conditions characterized by pain in the temporomandibular joint (TMJ). TMD has the highest prevalence in women of childbearing age, approximately twice than in men, which suggest that estrogen may play an important role in TMD pain processing. The mechanism of TMD pain and its sex differences remains unknown. Nav1.7 sodium channel plays a prominent role in pain perceptions, and our previous study has found that Nav1.7 in trigeminal ganglion (TG) has an important function in the hyperalgesia of the inflamed TMJ. Whether estrogen affects trigeminal ganglionic Nav1.7 and then involved in hyperalgesia of inflammatory TMJ remains to be elucidated. The aim of this study was to examine whether estrogen enhanced hyperalgesia of inflammatory TMJ through trigeminal ganglionic Nav1.7.

Materials and Methods: Female SD rats (200-220 g) were divided into 5 groups (8 rats per group), including the control group, sham-ovariectomized group, and three groups of ovariectomized rats dosed with 0, 20 and 80 µg/day of 17β-estradiol for 10 days, respectively. The sham-ovariectomized and ovariectomized groups were intra-articularly injected with complete Freund's adjuvant to induce TMJ inflammation. Head withdrawal threshold and food intake were used to evaluate pain behavior. Real-time PCR and Western blot were used to evaluate Nav1.7 mRNA and protein expression in TG. Then, Estrogen receptor antagonist ICI 182,780 was applied to further examine whether the effects of estradiol on Nav1.7 expression were through the estrogen receptor.

Results: Estradiol further aggravated TMJ inflammation-induced decrease in head withdrawal threshold and food intake; meanwhile estradiol potentiated TMJ inflammation-induced upregulation of Nav1.7 expression in TG. Estrogen receptors blocker partially reversed inflammation-induced decrease in head withdrawal thresholds and food intake and completely reversed inflammation-induced upregulation of Nav1.7 expression in TG.

Conclusion: Estrogen further aggravated TMJ inflammation induced pain sensitive and upregulation of Nav1.7 expression in TG depending on estrogen receptors, indicating that estrogen may also be involved in the sex difference of TMJ inflammatory pain. yunding666@126.com or kqyehuagan@bjmu.edu.cn

Keywords: Temporomandibular joint, Nav1.7, Estradiol.

P24) An Osteoprotegerin-like Peptide Prevents Bone Loss in Collagen-Induced Murine Arthritis.

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Background: The cyclic peptide OP3-4 (YCEIEFCYLIR) was designed to mimic the most critical RANKL (receptor activator of NF- κ B ligand) contact site on osteoprotegerin (OPG), and proven to bind to RANKL, thereby inhibiting osteoclastogenesis. Recently we found that the other RANKL binding peptide W9 could accelerate bone formation. Given the significant reduction of bone formation and severe bone destruction in the inflammatory bone diseases, the bone anabolic reagents other than parathyroid hormone, a sole anabolic reagent in the clinical use, need to be developed for the treatment of inflammatory bone diseases. Here we show the effects of the RANKL-binding peptide OP3-4 on bone formation and bone loss in the rheumatoid arthritis model.

Materials and Methods: The human rheumatoid arthritis model, collagen type II-induced murine arthritis (CIA) was used in this study. Osmotic mini-pumps were implanted in the backs of all mice after the onset (day 28), and vehicle or OP3-4 (9 mg/kg/day or 18 mg/kg/day) was continuously infused for 3 weeks. The vehicle was also infused in the naive mice (7-week-old male DBA/1J mice). The arthritis-score assessment had been performed until the mice were killed (day 49). Thereafter, radiographic, histological and biochemical analyses were performed.

Results: The OP3-4 treatment did not inhibit CIA-induced increases in the arthritis score significantly. Micro CT images revealed that 18 mg/kg/day OP3-4 prevented CIA-induced bone loss at both epiphysis and metaphysis of femurs and tibiae. Quantitative measurements of bone mineral density confirmed above observations. As expected, OP3-4 significantly reduced the CIA-induced serum CTX levels, the bone resorption marker. Interestingly, the bone histomorphometric analyses by using the double labeling technique showed OP3-4 prevented the reduction of bone formation-related parameters induced in CIA model.

Conclusion: The OPG mimetic peptide could be a useful template for the development of small molecules to prevent inflammatory bone loss by inhibiting bone resorption and stimulating bone formation.
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Keywords: Inflammatory bone loss, RANKL binding peptide, bone formation

P25) *PTCH1* Gene Mutations in Keratocystic Odontogenic Tumors: a study of 43 Chinese patients and a Systematic Review

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Background: The keratocystic odontogenic tumor (KCOT) is a locally aggressive cystic jaw lesion that occurs sporadically or in association with nevoid basal cell carcinoma syndrome (NBCCS). *PTCH1*, the gene responsible for NBCCS, may play an important role in sporadic KCOTs. In this study, we analyzed and compared the distribution pattern of *PTCH1* mutations in patients with sporadic and NBCCS-associated KCOTs. **Materials and Methods:** We detected *PTCH1* mutations in 14 patients with NBCCS-associated KCOTs and 29 patients with sporadic KCOTs by direct sequencing. In addition, five electronic databases were searched for studies detecting *PTCH1* mutations in individuals with NBCCS-associated or sporadic KCOTs, published between January 1996 and June 2013 in English language.

Results: We identified 15 mutations in 11 cases with NBCCS-associated KCOTs and 19 mutations in 13 cases with sporadic KCOTs. In addition, a total of 204 *PTCH1* mutations (187 mutations from 210 cases with NBCCS-associated and 17 mutations from 57 cases with sporadic KCOTs) were compiled from 78 published papers. **Conclusion:** Our study indicates that mutations in transmembrane 2 (TM2) are closely related to the development of sporadic KCOTs. Moreover, for the early diagnosis of NBCCS, a genetic analysis of the *PTCH1* gene should be included in the new diagnostic criteria.

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P26) Retrospective study and immunohistochemical study of ameloblastic carcinoma

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Objective: To review all the cases of Ameloblastic Carcinoma at the Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University and to evaluate the expression of survivin in these cases. **Material and Methods:** In this study, 13 cases of Ameloblastic Carcinoma between 1962-2011 were retrieved and analysed for age, sex, clinical feature, location and radiographic feature. A total of 11 cases were examined immunohistochemically with antibody against survivin. **Results:** A male to female ratio of 11:2 were found. The majority of patients were in their third and fourth decades, ranged from 20 to 56 year of age. Most of the cases involved in the posterior region of mandible with a painless swelling of the jaw. Multilocular radiolucent lesions were found more than unilocular lesions. Immunohistochemical reactivity for survivin was observed in ameloblast cells and squamous cells of all cases. Whereas, only some stellate reticulum cells showed positive staining. **Conclusion:** Expression of survivin in Ameloblastic Carcinoma confirmed that survivin played an important role in inhibiting apoptosis of Ameloblastic Carcinoma.

vvichitra@yahoo.com **Key Words** Ameloblastic Carcinoma, Immunohistochemical Study, Retrospective Study, Survivin

P27) Leptin and its receptor expression in dental and periodontal tissues of primates

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Background: Leptin has been reported to play central roles in energy balance, immune-inflammatory response and bone metabolism by activating its specific receptor-OBR. The short and soluble forms of OBR mediate the transport and degradation of leptin, while the long form (OBRb) mediates most of leptin's functions. Evidence showed that leptin and OBR might have some relationship with inflammatory diseases of dental and periodontal tissues. To prove it, the first step should be to determine their expression in these tissues.

Materials and Methods: Specimens of mandibular posterior teeth with surrounding soft and hard tissues were obtained from three adult male *Macaca fascicularis* monkeys. Then mesio-distal serial sections of these dental and periodontal tissues were prepared. Leptin and its receptor expression were examined by immunohistochemistry. For further confirmation, major cell components of dental and periodontal tissues, including dental pulp cells, gingival epithelial cells, gingival fibroblasts and periodontal ligament cells, were isolated and cultured from human dental and periodontal tissues. The expression of leptin and OBR was detected by reverse-transcription PCR (RT-PCR) and immunocytochemistry.

Results: Leptin and OBR were predominantly expressed and widely distributed in dental and periodontal tissues of primates. Their immunoreaction was especially strong in junctional epithelium and mineralizing areas of dental pulp and periodontal ligament. And the expression levels of leptin in periodontal ligament and dental pulp were equivalent to or even more than that in bone marrow adipose tissues, the most potential local source of leptin production and secretion. Expression and distribution of OBR were generally the same as that of leptin. And impressingly, OBRb mRNA was expressed by all major cell components of dental and periodontal tissues.

Conclusion: Our data for the first time systematically demonstrated that dental and periodontal tissue could be a new important source of leptin and an active place for leptin transport and degradation. The expression of OBRb indicated direct effects for leptin on major cell components of dental and periodontal tissues, thus taking active participation in local metabolism, defense and regeneration.

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Keywords: leptin, OBR, dental and periodontal tissues

P28) Restoration of gingival blood flow during curcumin administration in streptozotocin-induced diabetic rats

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Background: Endothelium dysfunction characterized by impairment of vasorelaxation has been widely documented as an underlying cause of both macro- and micro-angiopathy in diabetes mellitus (DM). In the present study, we investigated gingival microcirculation in diabetic rats. Focus was put on the effect of curcumin, an extract of turmeric root which have been identified to possess antioxidant hypoglycemic and hypolipidemic effects, on gingival blood flow. **Methods:** Wistar Furth rats were divided into three groups: control (CON) (single injection of citrate buffer), diabetes (DM) (streptozotocin, 50 mg/kg.BW, *i.v.*), diabetes treated with curcumin (DM+cur) (daily oral feeding of 300 mg/kg.BW curcumin). On 8th week after streptozotocin injection, laser Doppler flowmetry was used for gingival blood flow (GBF) measurement. Blood glucose (BG), glycosylated hemoglobin (HbA_{1C}), and lipid profile were measured in blood samples collected at the end of each experiment. The contents of liver malondialdehyde (MDA) were also quantified for each group. **Results:** Gingival blood flow in DM rats decreased significantly compared to the corresponding level of CON rats. Treatment with curcumin could restore the decrease of GBF. Compared to DM rats, the diabetic rats with curcumin (DM+cur) demonstrated a significant reduction of BG, HbA_{1C}, dyslipidemia, and MDA. **Conclusion:** curcumin might have the effect in protecting the abnormality in gingival microcirculation of streptozotocin-induced diabetic rats through its antioxidant with hypoglycemic and hypolipidemic actions.

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P29) Periostin inhibits hypoxia-induced periodontal ligament cell apoptosis via TGF- β signaling

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Background: Periostin is an extracellular matrix protein predominantly expressed in periodontal ligament (PDL) cells. Periostin promotes cellular tolerance against stress and inhibits cell death in PDL and many cancers. Little is known about the effects of periostin in PDL cells under hypoxic conditions which can occur in the initial stage of orthodontic tooth movement. **Materials and Methods:** Human PDL cells were cultured under hypoxic condition (~1% O₂). Cell apoptosis, protein and gene expression were measured in the presence or absence of recombinant periostin peptide (rhPOSTN), periostin over-expression, and periostin gene silencing. TUNEL staining was performed after 48 hrs of hypoxic culture for the detection of cell apoptosis. 24 hrs after hypoxia induction, total protein and mRNA samples were collected. Western blot analysis was performed for checking the protein level of hypoxia-inducible factor (HIF) 1 α , a transcription factor that responds to the oxygen level and is involved in cell apoptosis. Real-time PCR was performed for checking the gene expression level of PHD2 (HIF prolyl-hydroxylases 2), the enzyme promoting HIF-1 α degradation. To assess the effects of periostin on the activation of TGF- β 1 which involved in the regulation of HIF-1 α and PHD2 in hypoxic cells, the medium was supplemented latent or active TGF- β , concomitant with the addition of rhPOSTN and further investigated by treating with TGF- β receptor type I blocker. **Results:** The percentage of apoptotic PDL cells under hypoxic condition was significantly decreased in both the rhPOSTN supplemented and the periostin over-expression groups and significantly increased in periostin-silencing group as compared to their control. HIF-1 α protein was altered in association with the percentage of PDL cell apoptosis. Under hypoxic condition, the mRNA level of PHD2 was significantly increased in both the rhPOSTN supplemented and the periostin over-expression groups and significantly decreased in the periostin-silencing group as compared to the control. Treatment with active TGF- β 1 increased the levels of pSMAD2 and HIF-1 α and also the percentage of TUNEL-positive cells. The addition of rhPOSTN inhibited TGF- β 1-induced SMAD2 phosphorylation and also decreased HIF-1 α levels, although these effects of rhPOSTN were seen only under hypoxic conditions. **Conclusion:** Periostin inhibits hypoxia-induced PDL cell apoptosis by decreasing HIF-1 α accumulation in hPDL under hypoxic conditions. Periostin regulates the level of HIF-1 α protein and gene expression of PHD2 by Inhibiting TGF- β /SMAD2 signaling under hypoxia.

Puffy_nanny@hotmail.com **KEYWORDS:** Periostin, Hypoxia, TGF- β

P30) Comparison of Microbial Diversity in Dental Plaque of Severe Childhood Caries Children Pre and Post Treatment by Polymerase Chain Reaction–Denaturing Gradient Gel Electrophoresis Analysis

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Background: To detect whether microbial diversity in dental plaque of severe early childhood caries (SECC) children could change after all the decayed teeth are treated and when recurrent caries emerge, whether associated with the microbial diversity in dental plaque. **Materials and Methods:** 55 SECC children (22 boys, 33 girls) aged 3 to 4 year-old were included in this study from the clinic of the department of pediatric dentistry. The average dt was 8.79 ± 2.86 and ds was 16.14 ± 6.98 . Supragingival plaque was collected from lingual and labial sound surface of all the teeth. All the decayed teeth were treated. 55 children revisited at 3 months post treatment and 51 children revisited at 6 months post treatment. Recurrent caries were recorded and Supragingival plaque was collected. Total DNA was isolated from 161 samples and polymerase chain reaction (PCR)–denaturing gradient gel electrophoresis (DGGE) analysis was conducted using universal primers. **Results:** 28 recurrent caries occurred in 17 of 55 children at 3 months post treatment and 47 recurrent caries occurred in 21 of 51 children. PCR-DGGE profile analysis showed on average, bands number increased significantly, which was 45.11 ± 6.00 pre-treatment and increased to 51.67 ± 5.33 at 3 months and 51.20 ± 6.08 at 6 months post-treatment ($p=0.000$). While occurrence of recurrent caries was not associated with the change of microbial diversity by PCR-DGGE profiling. **Conclusion:** Microbial diversity in dental plaque of SECC children increased at 3 months and 6 months post-treatment compared with pre-treatment, which was not associated with the occurrence of recurrent caries. qinman@gmail.com

Keywords: severe childhood caries (ECC), denaturing gradient gel electrophoresis (DGGE), microbial diversity

P31) Controlled Stemness of Human Periodontal Ligament Stem Cells by c-Kit

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Background: An activation of c-Kit receptor via its ligand, stem cell factor (SCF), regulates functions of cell including proliferation, differentiation, survival, and apoptosis, in various cell types. The roles of c-Kit receptor have been reported in several kinds of human stem cells with strong potential for stem cell regeneration, but not yet determined in human periodontal ligament stem cells (hPDLSCs). The purpose of this study was to investigate the existence and role of c-Kit in hPDLSCs. **Materials and Methods:** c-Kit positive (c-Kit⁺) and main population of hPDLSCs were sorted by fluorescence-activated cell sorting (FACS). Stem cell activities of each population were determined by colony-forming assay (CFA), osteogenic and adipogenic differentiation assays, and alkaline phosphatase (ALP) assay. Unsorted hPDLSCs were transfected with c-kit siRNA and cultured with or without osteoinductive medium (OIM) or adipogenic induction medium (AIM). An ALP activity was measured. The real-time PCR was performed after 72 h-culture to determine mRNA expression of bone-related genes, including osteocalcin (OCN), osteopontin (OPN), and runt-related transcriptional factor-2 (RUNX2), and adipogenic-related genes, including peroxisome proliferator-activated receptor- γ (PPAR γ) and lipoprotein lipase (LPL). **Results:** FACS analysis revealed $0.65 \pm 0.3\%$ c-Kit⁺ population in hPDLSCs (n=7). The highest colony-forming ability was observed in c-Kit⁺ cells, which significantly different from main population. No differences were found between c-Kit⁺ and main population in differentiation assays. c-Kit⁺ cells showed significantly lower ALP activity than that of main population in the absence of OIM. A knockdown of c-Kit mRNA significantly enhanced ALP activity and upregulated gene expression of OCN, OPN, and RUNX2 of hPDLSCs cultured with or without OIM comparing with (siNegative) control. siRNA of c-Kit cultured in AIM also expressed significantly higher level of PPAR γ and LPL gene than that of control group. **Conclusion:** These results suggest the emerging roles of c-Kit/SCF signal in maintaining undifferentiated stage by inhibiting an expression of lineage-specific genes in hPDLSCs.

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Keywords: c-Kit, periodontal ligament, stem cells

P32) Effect of Cobalt Chloride on Stemness in Human Dental Pulp Cells

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Background: hypoxic condition is the one of factors affected stemness of stem cells. Cobalt chloride (CoCl₂), a chemical agent, was used to mimic the hypoxic condition. Therefore, this study aims to investigate the effect of cobalt chloride on the stemness in human dental pulp cells. **Materials and Methods:** human dental pulp cells were cultured with or without cobalt chloride in various concentrations. Cell proliferation was investigated using MTT assay. The expression of stem cell marker genes (Oct-4, Sox-2 and Nanog) was examined using RT-PCR technique. Alkaline phosphatase activity was used to determine osteogenic differentiation. The data were analyzed by one way ANOVA and statistically difference was considered at $p < 0.05$. **Results:** the addition of cobalt chloride (50 μ M) significantly increased the mRNA expression of stem cell markers (Oct-4, Sox-2 and Nanog), while inhibited osteogenic differentiation of human dental pulp cells **Conclusion:** These findings suggested that cobalt chloride could increase stemness of human dental pulp cells. However, further study is needed to investigate the mechanism of cobalt chloride. wantida_s@yahoo.com **Keywords:** Cobalt chloride, Human dental pulp cells, Stemness

P33) Dental pulp dendritic cells migrate to the regional lymph nodes

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Background: The immune system has evolved to recognize microbial attacks and eliminate infections. Dendritic cells (DCs) exist broadly in the skin and mucosa as the front line of defense against microbial pathogens. DC migration to regional lymph nodes (RLNs) is an essential step in adaptive immunity. We previously found two types of DC-like cells in the dental pulp; CD11c⁺F4/80⁻ sentinel DCs and CD11c⁺F4/80⁺ interstitial DCs. Interstitial DCs distributed broadly in the inner pulp and some interstitial DCs moved to the treated side at 2 h expressing CD86. These CD86⁺ cells disappeared at 24 h. These results suggest migration of dental pulp DCs to RLNs. In this study, we identified migratory dental pulp DCs after cusp trimming and acid etching in KikGR mice, in which the photoconvertible fluorescent protein changed from green to red upon violet light exposure.

Materials and Methods: Mesial cusps of lower molars were trimmed and etched by 40% phosphoric acid. At 2 h post-cusp trimming, the trimmed cusp was exposed to violet light for photoconversion. RLN cells at 16 h post-cusp trimming were analyzed by multicolor flow cytometry.

Results: The percentage of photoconverted Kik-red⁺ cells in the RLN at 16 h post-cusp trimming increased tenfold (from 0.002% to 0.02%) after photoconversion. The total number of Kik-red⁺ migrating dental pulp cells was approximately 1000 cells per one side of RLN. Two major cell fractions from the dental pulp had migrated to the RLNs, which showed the following lineage markers in the main and second fractions: CD11c^{high}CD11b⁺⁺Ly6C^{low}Ly6G^{low}F4/80⁺ and CD11c^{med}CD11b⁺⁺⁺Ly6C⁺⁺Ly6G⁺⁺⁺F4/80⁻, respectively. These lineage markers indicate that the former cells were definitively DCs that had migrated through afferent lymphoid vessels, and the latter were granulocytes recruited via blood circulation. Migratory dental pulp DCs expressed the highest levels of CD273 (B7-DC) and CD86 costimulators and MHC class II, suggesting that they are mature DCs.

Conclusion: Using photoconvertible fluoroprotein-knock in KikGR mice, we demonstrated that dental pulp DCs migrate to RLNs at 16 h after cusp trimming and they show a mature functional phenotype. Our results suggest that cariogenic-bacteria-exposed dental pulp DCs migrate to RLNs and trigger adaptive immune responses in RLNs.

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Keywords: dental pulp, dendritic cells, migration

P34) Evaluation and Validation of Vitamin A in Plasma of Children with Non-syndromic Cleft Lip and Palate

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Background: Cleft of the lip and or palate (CLP) is one of the most common congenital craniofacial defects, one sort of which is non-syndromic CLP that belongs to multifactorial disease influenced by interaction of genetic and environmental factors. The basic biologist focused mainly on the gene-based etiologic research, and clinicians focused more on the improvement of surgical or orthodontic treatment. However, there are few study talking about the basic biology of post-born babies. Moreover, some studies showed the nutrition status differ between normal children and those with CLP. Thus we aimed to detect the relationship of nutrition condition with the developmental defect in subjects with CLP, thus to supply symptomatic treatment. **Materials and Methods:** In our study, shotgun proteomics (2D-Nano-LC-ESI-MS/MS) approach was applied to analyze the plasma proteome of all subjects with 13 CLP and 10 controls aged 2-3.5y. Then GO analysis was further conducted. With significant difference in retinol-binding protein 4 (RBP4) between the two groups, affinity-purified mouse monoclonal antibodies were used in western blot analysis to detect the presence of RBP4 in plasma of individuals. Besides, serum vitamin A levels were determined by High-performance Liquid Chromatography (HPLC). **Results:** By shotgun proteomics and GO analysis we successfully detected many significantly different proteins which could be related to CLP, including the proteins involved in lipoprotein metabolic and lipid transport process, retinol transport, insulin-like growth factor-related process and so on. One interesting kind of these was RBP4, the level of which in CLP group was significantly lower than control. A significant difference was also found in vitamin A concentrations which was consistent with the trend of RBP4. **Conclusion:** It indicated that RBP4 and vitamin A were related to the postborn CLP babies and they might present some vitamin A deficiency manifestation. We should pay more attention to the nutrition condition and vitamin A level in the CLP children bodies. Besides, the results might suggest it is necessary to supplement vitamin A at an early stage and remind us to monitor or detect the RBP4 content and other biomarkers.

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Keywords: CLP, Shotgun Proteomics, RBP4

P35) rs929387 of *GLI3* is involved in tooth agenesis in Chinese Han Population

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Background: Tooth agenesis is one of the most common anomalies of human dentition. Recent studies suggest that a number of genes are related to both syndromic and non-syndromic forms of hypodontia. In a previous study, we observed that polymorphism in rs929387 of *GLI3* might be associated with hypodontia in the Chinese Han population based on a limited population. To further confirm this observation, we carried on this study. **Materials and Methods:** we employed 89 individuals diagnosed with sporadic non-syndromic oligodontia (40 males and 49 females) to investigate the relationship between polymorphism in rs929387 of *GLI3* and tooth agenesis. These individuals were analyzed with 273 subjects (125 males and 148 females) diagnosed with non-syndromic hypodontia and 200 healthy control subjects (100 males and 100 females). DNA was obtained from whole blood or saliva samples and genotyping was performed by a Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) method. **Results:** Significant differences were observed in the allele and genotype frequencies of rs929387 of *GLI3*. Distributions of genotypes TT, TC and CC of rs929387 polymorphism were significantly different between the case group and the control group ($p = 0.013$) and C allelic frequency was higher in case group [$p=0.002$, OR = 1.690, 95% CI (1.200-2.379)]. Additionally, our analysis shows that this difference is more pronounced when compared between the male case group and the male control group. The function study suggests that variation in *GLI3* caused by rs929387 leads to a decrease in its transcriptional activity. **Conclusion:** These data demonstrated an association between rs929387 of *GLI3* and non-syndromic tooth agenesis in Chinese Han individuals. This information may provide further understanding of the molecular mechanisms of tooth agenesis. Furthermore, *GLI3* can be regarded as a marker gene for the risk of tooth agenesis.

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