PROGRAM AND ABSTRACTS OF PAPERS

JAPANESE ASSOCIATION FOR DENTAL RESEARCH

56th ANNUAL MEETING
November 29-30, 2008
School of Dentistry, Aichi-Gakuin University
Nagoya, Japan
PROGRAM AND ABSTRACTS OF PAPERS

JAPANESE ASSOCIATION FOR DENTAL RESEARCH

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November 29-30, 2008
School of Dentistry, Aichi-Gakuin University
Nagoya, Japan
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List of Sponsors 131
**MESSAGE**

Welcome to the 56th Annual Meeting of Japanese Association for Dental Research (JADR) in Nagoya, Japan

Haruo Nakagaki,  
President of the 56th Annual Meeting of Japanese Association for Dental Research, Nagoya, 2008

I am very honoured to organize the 56th Annual Meeting of the Japanese Association for Dental Research (JADR) which will be held on the 29th (Sat) and 30th (Sun) November 2008 here in Nagoya, Japan.

JADR started in 1954 as the Japanese division of the International Association for Dental Research (IADR) which started in 1919 in U.S.A. JADR publishes the Journal of Dental Research. We recently celebrated the 50th Anniversary of the JADR in 2006.

In 2007, the 55th Annual Meeting of JADR was successfully held in Tsurumi University, School of Dentistry, organized by Professor Nobuko Maeda. This time would like to have a “Zen-like”, simple meeting, because our University belongs to a Buddhist sect called “Sotoshu Zen” funded by Dogen Zenji.

We have 3 special lectures; Professor Jacob ten Cate, President of IADR; Professor Yoshiaki Kawashima, Aichi-Gakuin University and Professor Byung-Moo Min, President of KADR.

We have 2 symposia, the first is “Oral Biofilm Today” with 5 speakers: Professor Emeritus C. Robinson, UK; Professor L. Samaranayake, Hong Kong; Professor L. Stösser, Germany; Professor N. Takahashi, Tohoku University Graduation School, Japan, and Professor N. Hanada, Tsurumi University, Japan. The other symposium is “Neural Regulation of Bone Metabolism” with 4 Japanese speakers; Professor A. Togari, Aichi-Gakuin University; Professor S. Tanaka, Tokyo Medical and Dental University, Graduate School; Professor N. Udagawa, Matsumoto Dental University and Associate Professor T. Goto, Kyushu Dental College. There will be 101 presentations in total.

I do hope that this meeting results in fruitful discussion and stimulates all participants, for further research activities. I am sure that you will find it to be peaceful here at the close of the beautiful autumn in Japan.

Aichi-Gakuin University and all the members of the School of Dentistry welcome you and look forward to seeing you all here in Nagoya.

Finally, I would like to express my sincere gratitude for all of your good-will and support in organizing the meeting in Nagoya.
BOARD MEMBERS
OF
JAPANESE ASSOCIATION
FOR DENTAL RESEARCH

President · · · · · · · · · · · · · · · · · · · · · · · · · · Y. ODA
President-elect and Secretary · · · · · · · · · · · · · · · · · · · · Y. TAKANO
Immediate Past President · · · · · · · · · · · · · · · · · · · · K. OHYA
Treasurer · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · S. MURAKAMI
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Director · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · Y. NAKAJIMA
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Director · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · T. YAMAMOTO
Auditor · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · Y. ABIKO
Auditor · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · · S. SHIZUKUISHI

Chairperson, Organizing Committee · · · · · · · · · · · · · · · · · · H. NAKAGAKI
Secretary-general · · · · · · · · · · · · · · · · · · · · · · · · · K. KATO
General Information

56 Annual Meeting of Japanese Association for Dental Research
November 29 Sat.– 30 Sun., 2008
Aichi-Gakuin University Kusutomo Campus
1–100 Kusumotori-cho, Chikusa-ku, Nagoya 464–8650
TEL: 052–751–2561(Extension1352) Fax: 052–752–2566
http://wwwsoc.nii.ac.jp/jadr/jadr56/index.html

Dear Participants
1. On–site registration will be handled at the reception desk on the lobby of the Main Hall (the entrance to Room A) from 8:30 both on November 29th and 30th.
2. You are requested to report to the reception desk to make sure that your registration has been correctly made. All student members are requested to present their ID. You are requested to wear your name plate inside the venue and at the friendship party.
3. You are all welcome to the JADR friendship party to be held at Hotel Rubura Ohzan from 18:30 on November 29th. On–site registration for the party is also possible.
4. All announcements to the participants will be made on the bulletin board only not through the microphone.
5. Smoking is prohibited in kusumoto campus. Eating and drinking are also prohibited.

Instructions for Poster Presenters
1. All presentations will be in poster form and will be arranged in three parallel sessions. The posters are to be placed in Room B (2nd floor, Lecture room 323), C (2nd floor, Lecture room 325) and D (1st floor, Student common room) of the 3rd Building in Kusumoto Campus, Aichi–Gakuin Univ.
2. The space available for each poster is 900 mm wide×1800 mm high. A0 size posters (841 mm×1189 mm in portrait format) are thus convenient.
3. All posters will be mounted on the numbered poster boards using drawing pins, which will be provided by the congress staff at the venue. Posters should be made from paper or thin cardboard. Heavy board materials may be difficult to keep in position on the poster panel.
4. Next to the title, place a small photograph of the presenter, so that he/she can be identified by delegates.
5. Indicate the source of any funding for the study on the poster.
6. Studies on human subjects: Add a declaration that permission was obtained from an institutional ethical committee and that subjects (or their guardians) gave written, informed consent.
**Poster viewing**

1. Posters should be put up as early as possible, preferably between 8:00 and 11:00 on the day of presentation, to make it possible that posters will be available for viewing through the ‘poster viewing’ periods.

2. Presenters should stand next to their poster, to be available for discussion with delegates, during the ‘designated session’ periods which are indicated in the program booklet. For details when the designated session period is scheduled for your poster refer to the programme which will be listed on the web site and in the JADR Programme Book provided at registration.

**Poster discussions**

1. The discussions of the posters will take place in the rooms where your poster has been displayed.

2. The total time allotted to discussion of each poster is 8 minutes. Please pay careful attention to the following notes, to maximise discussion time:

   1) Each presenter should verbally summarise their poster in no more than 3 minutes. This presentation can be assisted by a maximum of 3 pictures made using MS power point 2003 for Windows. This power point file should be prepared in advance and send it as an attachment to the secretariat (kazkato@dpc.aichi-gakuin.ac.jp.) before 21 November, 2008. On the pictures, the font size should be 28 point or larger and any animation effects should be avoided.

   2) The aim of the short presentation is to remind the audience of the aims and main conclusions of the study.

   3) Do not attempt a verbal version of the poster.

   4) Assume that your audience has already viewed the poster and simply aim to refresh their memories of the important points.

   5) Make the message on the pictures as simple as possible: use short statements or simple, clear figures to make your points. Do not present lots of numbers.

   6) Following the verbal presentation, 5 minutes will be available for discussion with the audience.

3. In the case of Hatton Award poster presentation, the total time for discussion is 15 minutes, including the verbal presentation for no more than 8 minutes using a maximum of 6 pictures and discussion with the audience for 7 minutes.

4. The posters have to be removed after the sessions of poster discussions.

**Dear Chairpersons**

There will be no timekeeper.

The responsibility of proceeding with the program as scheduled will be all left in your hands.
2nd Meeting Room

- JADR Young Investigator Award Committee (Nov. 29th)
- JADR Board Meeting (Nov. 29th)

Headquater

Room A
Main Hall

Cloak Room

Exhibition
Cafeteria

Room B
Poster Session

Room C
Poster Session

Room D
Poster Session

JADR2008 Floor Map
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<th>Pharmaceutical build. cafeteria</th>
<th>N0.2 build. Room 221</th>
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November 29th, Saturday, Room A

● 9:00–10:00 Special Lecture 1

Moderator: Professor Yutaka Oda (Tokyo Dental College, Japan)

L1 New developments in dentistry and IADR
Professor JM (Bob) ten Cate (Academic Center for Dentistry Amsterdam)

● 10:00–12:00 Symposium1 “Oral Biofilm Today”

Moderator: JM (Bob) ten Cate (President of IADR) Haruo Nakagaki (Aichi-Gakuin University, Japan)

S1–1 Mass transfer within natural plaque biofilms: the role of plaque architecture
Professor Colin Robinson (Emeritus Professor of Leeds Dental Institute, UK)

S1–2 Can biofilms kill you?
Professor Lakshman Samaranayake (Faculty of Dentistry, University of Hong Kong, Hong Kong)

S1–3 Fluoride content of dental plaque
Professor Lutz Stösser (Jena University, Germany)

S1–4 Ecological dynamics of caries–associated oral biofilm: involvement of mutans streptococci and non–mutans bacteria
Professor Nobuhiro Takahashi (Division of Oral Ecology and Biochemistry, Dept. of Oral Biology, Tohoku University Graduate School of Dentistry, Japan)

S1–5 Oral biofilm formation, what goes on afterwards.
Professor Nobuhiro Hanada (Dept. of Translational Research, Tsurumi University, Japan)

● 13:30–14:30 Special Lecture 2

Moderator: Professor Haruo Nakagaki (Aichi-Gakuin University, Japan)

L2 Laminin–derived peptides: their biomedical applications and signaling pathways
Professor Byung-Moo Min (Department of Oral Biochemistry and Program in Craniomaxillofacial Reconstruction Science, Seoul National University School of Dentistry, Seoul, Korea)
November 30th, Sunday, Room A

**9:00–10:00 Special Lecture 3**
Moderator: Professor Yoshiro Takano
(Tokyo Medical and Dental University, Japan)
L3 Nanomedical system developed with PLGA nanosphere platform
Professor Yoshiaki Kawashima
(Dept. of Pharmaceutical Technology, School of Pharmaceutical Science, Aichi–Gakuin University, Japan)

**10:00–12:00 Symposium 2 “Neural Regulation of Bone Metabolism”**
Moderator: Professor Keiichi Ohya (Tokyo Medical and Dental University, Japan)
Professor Akifumi Togari (Aichi–Gakuin University, Japan)

S2–1 Direct implication of peripheral sympathetic nerve system in bone metabolism
Professor Akifumi Togari
(Aichi–Gakuin University, Japan)

S2–2 Central control of bone remodelling
Associate Professor Shu Takeda
(Tokyo Medical and Dental University, Japan)

S2–3 Possible role of L-glutamate signaling in bone remodeling
Professor Nobuyuki Udagawa
(Dept. of Biochemistry, Matsumoto Dental University, Japan)

S2–4 Sensory neuropeptides modulate bone remodeling
Associate Professor Tetsuya Goto
(Division of Anatomy, Kyushu Dental College, Japan)
Poster Presentation 1 November 29th, Saturday, Room B, C, D

14:30–15:58 (Room B)  The Young Investigator Award

Chairperson: Dr. N. Maeda

001 Three-dimensional & layered culture method for tooth induction and development
T. NOTANI, M.J. TABATA, O. BABA and Y. TAKANO
Sec. of Biostructural Science, Tokyo Medical and Dental University Graduate School, Japan

002 Treponema denticola dentilisin involvement in periodontopathic bacterial coaggregation
Y. SANO, M. MIYAMOTO, R. ITO, N. MATSUMOTO, M. YAKUSHIJI, S. SHINTANI and K. ISHIHARA
Tokyo Dental College, Japan

003 Preferred-chewing-side-dependent two-point discrimination and cortical activation of tactile tongue sensation
A. MINATO1, T. ONO1, J. MIYAMOTO1, E. HONDA2, T. KURABAYASHI1 and K. MORIYAMA1
1Tokyo Medical & Dental University, Japan 2Tokushima University, Japan

004 Sodium fluoride induces apoptosis in human gingival epithelial cell line
M. HERAI, T. MURATA and K. YAEGAKI
Nippon Dental University, Japan

005 Impaired tooth development in reduced scale medaka mutant (rs-3)
A.D.S. ATUKORALA1, K. HIGUCHI1, M.J. TABATA1, O. BABA1, H. MITANI2 and Y. TAKANO1
1Section of Bio Structural Science, Tokyo Medical & Dental University Graduate School, Tokyo, Japan 2Department of Biological Sciences, Graduate School of Science, University of Tokyo, Japan

Chairperson: Dr. Y. Takano

006 Initial response of osteoblast-like cells to zirconia and titanium
Kagoshima University, Japan

007 Nociceptive stimulation induces satellite glial cell activation
K. GUNJIGAKE, T. GOTO, K. NAKAO, T. ISHIBE, S. KOBAYASHI and K. YAMAGUCHI
Kyushu Dental College, Japan

008 Simvastatin enhances differentiation of human dental pulp stem cells
Y. OKAMOTO1, W. SONOYAMA1, M. ONO1, K. AKIYAMA2, T. FUJISAWA1, M. OSHIMA1,
Y. TSUCHIMOTO¹, Y. MATSUKA¹, T. YASUDA¹, S. SHI² and T. KUBOKI ¹
¹Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan ²University of Southern California School of Dentistry, U.S.A.

Electrolyzed water with functional–chlorine effectively penetrates deep into cariogenic biofilms
A. OKADA¹, K. MATIN², Q.H.M.S. ZAMAN³, N. HANADA³ and J. TAGAMI³
¹Tokyo Medical and Dental University and National Institute of Public Health, Japan ²Cariology and Operative Dentistry, Tokyo Medical and Dental University, Japan ³Department of Translational Research, Tsurumi University, Japan

Human gingival fibroblasts release HMGB1 through active and passive pathways
K. FEGHALI, K. IWASAKI, K. TANAKA, M. KOMAKI and Y. IZUMI
Tokyo Medical & Dental University, Japan

Nicotine induction of CCN2: from smoking to periodontal fibrosis
H. TAKEUCHI¹, S. KUBOTA², E. MURAKASHI¹, Y. ZHOU², M. TAKIGAWA² and Y. NUMABE¹
¹Dept. Periodontology, School of Life Dentistry The Nippon Dental University, Japan ²Dept.Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan

● 14:30–15:42 (Room C) Pharmacodynamics / Microbiology & Oral Microbiology / Neurology
Chairperson: Dr. N. Ogi

012 Antifungal susceptibility of C. albicans and its hyphal mutants
T. WATAMOTO¹, C.J. SENEVIRANTNE¹, J.A.M.S. JAYATILAKE¹, H. EGUSA², H. YATANI² and L.P. SAMARANAYAKE¹
¹University of Hong Kong, Hong Kong ²Osaka University, Japan

013 Antitumor effects of bleomycin by electrochemotherapy using local electric pulses
Y. SUGITA, M. JINNO, M. TAKAYAMA, Y. HONDA, W. YOSHIDA, K. KUBO, E. SATO and H. MAEDA
Aichi–Gakuin University, Japan

014 Use of fentanyl with a pharmacokinetic during oral surgery
N. KATO, J. HARADA, Y. WATANABE, T. TACHI and M. MATSUURA
Aichi–Gakuin University, Japan
Chairperson: Dr. Y. Yoshida

015 Development and verification of a rapid oral bacteria detection system
T. KIKUTANI¹, F. TAMURA¹, S. SEKINO², A. HISANO¹, K. KONISHI¹, M. YOSHIDA¹, R. HAMADA³, A. TAKAGI³, T. INAGUCHI³, H. KAYANAKA³ and K. NISHIWAKI²
¹Nippon Dental University Hospital, Japan ²Nippon Dental University, Japan ³Nippon
Cleaning efficacy of an experimental rinsing/disinfecting solution
S. UNO, J. SUGIZAKI, M. MORIGAMI and T. YAMADA
Toranomon Hospital, Japan

The capacity to produce hydrogen sulfide in *Streptococcus intermedius*
S. ITO, Y. YOSHIDA, T. Sasaki, K. KUNIMATU and H. KATO
Iwate Medical University, Japan

IL-6 Signaling in gingival epithelial cells by *Candida albicans* Infection
L. ZHANG, I. LI, Y. TANAKA, N. KUBOYAMA, Y. ABIKO
Nihon University, Japan

Chairperson: Dr. K. Hiraba

An optimal umami taste stimulating system for brain mapping
Y. NAKAMURA\(^1\), T. GOTO\(^1\), K. TOKUMORI\(^1\), T. YOSHIURA\(^2\), K. KOBAYASHI\(^3\), Y. NAKAMURA\(^4\), H. HONDA\(^5\), Y. NINOMIYA\(^6\) and K. YOSHIURA\(^1\)
\(^1\)Kyushu University, Japan \(^2\)Department of Clinical Radiology, Graduate School of Medical Sciences, Kyushu University, Japan \(^3\)Department of Medical Technology, Kyushu University, Japan \(^4\)Department of Medical Technology, Kyushu University Hospital, Japan \(^5\)Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, Japan

Hypoglossal motoneuronal activities induced by NMDA in brainstem slice preparation from newborn rats.
K. ARAKI, N. KATAKURA, K. SHIMOZATO and K. HIRABA
Aichi-Gakuin University, Japan

**14:30–15:50 (Room D) Prosthodontics Research / Mastication & Occlusion**
Chairperson: Dr. T. Kawai

Functional aspects of treatment with single implant–supported crowns
K. GOSHIMA\(^1\), M. BAKKE\(^2\), M. LEXNER\(^2\), C. THOMSEN\(^3\), H. MIURA\(^4\) and K. GOTFREDSEN\(^5\)
\(^1\)Tokyo Medical & Dental University, Japan \(^2\)University of Copenhagen, Faculty of Health Sciences, Denmark \(^3\)University of Copenhagen, Denmark \(^4\)Copenhagen Dental School, Denmark

Predicting the retentive force of FRC clasps by nonlinear FEA
H. MARUYAMA, Y. NISHI, C. KISHITA, K. TSURU and E. NAGAOKA
Kagoshima University Graduate School of Medical and Dental Sciences, Japan

Structurally compromised roots restored with four post and core systems
Y. FUKUI, W. KOMADA, K. YOSHIDA, S. OTAKE and H. MIURA
Tokyo Medical and Dental University, Japan
Chairperson: Dr. T. Kuboki

024 Effect of conscious clenching on simple calculation task
T. MIZUMORI, Y. KOBAYASHI, S. INANO, M. SUMIYA, F. MURASHIMA and H. YATANI
Osaka University, Japan

025 Relation between upper and lower molars during functional mandibular movement
H. OKAYASU, D. OKADA, C. SHIN, Y. KIZUKI, K. KAWASHIMA and H. MIURA
Tokyo Medical and Dental University, Japan

026 Influence of asymmetric mandible on masticatory performance
T. HASHIMOTO1, S. KURODA2, T. KATAOKA3, H. OIKAWA1, S. MIYAZAKI4,
T. YAMASHIRO5 and T. YAMAMOTO1
1Tohoku University, Japan, 2Tokushima University, Japan, 3Okayama University,
Japan, 4Kagoshima University, Japan

027 Disk movements during fictive mastication under bite-raised condition in rabbits
T. MORITA, H. MARUO, T. FUJIWARA, T. NEGORO, K. KURITA, S. GOTO and K. HIRABA
Aichi-Gakuin University, Japan

028 Effects of using teething food for infants during weaning
Y. MIWA1, K. KITADA2, Y. UCHIKAWA3 M. YAMAZAKI4 T. FUJITA1 T. OHO2 and I. SATO1
1Nippon Dental University, Japan 2Kagoshima University Graduate School of Medical
and Dental Sciences, Japan 3Nippon Dental University Hospital, Japan

029 Finite element analysis of ceramic inlays restored posterior teeth
L. SANDU, F. TOPALA, S. POROJAN and C. BORTUN
Victor Babes University of Medicine and Pharmacy Timisoara, University School of
Dentistry, Romania

030 3D reconstructions for numerical simulations of prosthetic restorations
S. POROJAN1, L. SANDU1, F. TOPALA1 and N. FAUR2
1Victor Babes University of Medicine and Pharmacy Timisoara, University School of
Dentistry, Romania 2Politehnica University Timisoara, Romania

16:45–17:33 (Room B)  JADR Travel Award / Dental Education

031 Site–specific colonization and genotypic diversity of S. mutans in different individuals.
(TW1) Q.Z. JIANG and Q. ZHANG
School of Stomatology, Wuhan University, Wuhan, China

032 Implementation of the KTSND questionnaire on Australian dental undergraduates
(TW2) Huang B1, K Inagaki2, C Yoshii3, M. Kano2, H Nakagaki2, T, Noguchi2
1School of Dentistry, The University of Western Australia, Australia 2Aichi–Gakuin
University, Japan 3University of Occupational and Environmental Health, Japan

033 Mutans streptococci and caries experience in Mongolian children
(TW3) Soyolmaa MASHBALJIR4,*, Hulan ULAMNEMEKH1, Mungunsetseg LUVSAN1,
Altansukh TSEND-AYUSH and Sarantuya JAV
1School of Dentistry, Health Sciences University of Mongolia, Mongolia 2Dept. of Genetics and Molecular Biology, Health Sciences University of Mongolia, Mongolia

Chairperson: Dr. H. Ito

034 “Social Nicotine Dependence” of dental undergraduates in Japan
K. INAGAKI1, B. HUANG2, T. NOGUCHI1, F. YOSHIMURA1, I. MORITA1, H. NAKAGAKI1, T. KOIDE1, T. HANIOKA3, C. YSHII4 and M. KANO4
1Aichi-Gakuin University, Japan 2University of Western Australia, Australia 3Fukuoka Dental College, Japan 4Division of Respiratory Disease, University of Occupational and Environmental Health Japan, Japan 5Department of Internal Medicine, Shinnakagawa Hospital, Japan

035 The change of emotionally intelligence quotient through the PBL
H. KATSURAGI1, M. IGARASHI1, I. KAGEYAMA1, T. SEKIMOTO1, Y. MIYAGAWA2, F. WATANABE1, K. FUJI1, M. MIZUTANI, D.D.2 and S. NAKAHARA1
1Nippon Dental University, Japan 2The Nippon Dental University School of Dentistry at Niigata, Japan

036 A system for supporting independent learning in dental education
W. YOSHIDA, K. KUBO, Y. SUGITA, E. SATO, T. NOGUCHI, T. KAWAI and H. MAEDA
Aichi-Gakuin University, Japan

●16:45–17:41 (Room C) Mineralized Tissue 1 / Periodontal Research 1
Chairperson: Dr. J Tabata

037 Micro–CT analysis of the mandibles and femurs in warfarin–administered Rats
M. ONOHARA1, S. KAWAMOTO2 and E. NAGAOKA2
1Kagoshima University Graduate School of Medical and Dental Sciences, Japan 2Kagoshima University, Japan

038 Remineralization of primary tooth dental enamel of Down syndrome
T. OKAMOTO, S. TSUBOI, J. INUKAI, Y. KUSABE, O. FUKUTA, H. NAKAGAKI and T. TSUCHIYA
Aichi–Gakuin University, Japan

039 TMR and micro–Raman studies on enamel bleaching with remineralization treatment
I. IWAYA, Y. MUKAI and T. TERANAKA
Kanagawa Dental College, Japan

040 Provisional mineralization layer in the predentin of murine teeth
M. AHMAD, H. ISEKI, O. BABA, M.J. TABATA and Y. TAKANO
Tokyo Medical & Dental University, Japan

Chairperson: Dr. K. Yamazaki

041 EF–TEM cytochemical observation of elongated rete ridges in gingival hyperplasia
K. MORIGUCHI, Y. MITAMURA, M. FUKUDA, H. MAEDA, T. NOGUCHI and N. OHNO
Aichi–Gakuin University, Japan
042 Adrenomedullin inhibits CXCL10 production by human gingival fibroblasts
I. HOSOKAWA, Y. HOSOKAWA, K. OZAKI, H. NAKAE and T. MATSUO
The University of Tokushima, Japan

043 Role of Thy-1 in collagen phagocytosis by human gingival fibroblasts
K. TAKANO, M. KOBAYASHI, M. MIYATA, R. MURAYAMA and M. YAMAMOTO
Showa University, Japan

16:45–17:41 (Room D) Oral surgery / Cariology, Biofilm
Chairperson: Dr. T. Goto

044 A case of ameloblastic carcinoma in the mandible
K. KUBO¹, W. YOSHIDA¹, Y. SUGITA¹, E. SATO¹, Y. KAZAOKA², S. YAMADA²
and H. MAEDA¹
¹Aichikaguin University, Japan ²Aichi Medical University Hospital, Japan

045 Primary treatment for TMJ pain and trismus
Y. HATTORI, K. KURITA, A. ATSUSHI, Y. ITOH, N. KONDO, M. SHIMIZU,
T. YAJIMA, H. NABESHIMA, N. KUROYANAGI, N. OGI and M. IZUMI
Aichi-Gakuin University, Japan

046 Coronectomy for the management of mandibular third molars
Y. HATANO¹, Y. KUROIWA¹, K. KURITA¹, E. ARIJI¹ and H. YUASA²
¹Aichikaguin University, Japan ²Branch Hospital of Tokai Municipal Hospital, Japan

Chairperson: Dr. M. Fujitani

047 Advantages of using two-photon laser scanning microscopy for biofilm imaging
S. TAKENAKA¹, B. PITTS², R. WAKAMATSU¹ and T. OKIJI¹
¹Niigata University, Japan ²Center for Biofilm Engineering, Montana State
University, U.S.A.

048 Influence of alkali-ion water on removing cariogenic biofilms by jet-washer
K. MATIN¹, M. GYO¹, A. OKADA¹, K. SHIDA¹, M. NAGAYAMA³, Y. SAIHARA³ and J. TAGAMI¹
¹Tokyo Medical and Dental University, Japan ²Tokyo Medical and Dental University and National
Institute of Public Health, Japan ³Panasonic Electric Works Co., Ltd., Japan ⁴Tokyo Medical &
Dental University; G-COE Program at TMDU, Tokyo Medical & Dental University, Japan

049 Impact of mineral supplementation to acidic solutions on enamel erosion
N. ISHIZUKI, D. INABA and M. YONEMITSU
Iwate Medical University, Japan

050 Streptococcal distribution within plaque formed on enamel with glass–ionomere cement
T.T. TRAN¹, K. KATO¹, H. NAKAGAKI¹, Y. KAWAMURA² and T. SATO³
¹Dept. of Preventive Dentistry and Dental Public Health, Aichi-Gakuin University,
School of Dentistry, Japan ²Dept. of Microbiology, Aichi-Gakuin University, Japan
³Dept. of Dentistry Tohoku University Graduate School of Dentistry, Japan
**Poster Presentation 2 November 30th, Sunday, Room B, C, D**

● 14:00–15:15 (Room B)  
**Presentation by Candidate for Hatton Award 2009**

Chairperson: Dr. S. Imazato, Dr. N. Takahashi

051 Phylogenetic analysis of the gbpC and dbi genes among mutans streptococci.  
(H1) Y.KOJIMA and Y.SATO.  
Tokyo Dental College, Japan

052 Antibacterial effects of MDPB against anaerobes associated with endodontic infections.  
(H2) N. IZUTANI¹, S. IMAZATO¹, Y. TAKAHASHI¹, S. EBISU¹ and R.R.B. RUSSELL²  
¹University Graduate School of Dentistry, Japan, ²Newcastle University, UK

053 Transcriptional regulation of BRAK/CXCL14, a tumor-suppressing chemokine.  
(H3) R. Komori*, S. Ozawa, Y. Kato, H. Shinji, S. Kimoto, and R. Hata  
Kanagawa Dental College, Yokosuka, Japan

054 Invasion of host cells by membrane vesicles of Porphyromonas gingivalis.  
(H4) N. Furuta* and A. Amano  
Dept Oral Frontier Biology, Osaka Univ Grad School Dent, Japan

055 Amelogenin is a potent inhibitor of odontoclastic root resorption.  
(H5) Y. YAGI¹, N. SUDA¹, Y. YAMAKOSHI², O. BABA¹, and K.MORIYAMA¹  
¹Tokyo Medical and Dental University, Tokyo, Japan ²University of Michigan, Ann Arbor, MI

● 14:00–15:04 (Room C)  
**Cell Biology & Pulp Biology / Geriatric Dentistry & Oral Rehabilitation**

Chairperson: Dr. H. Maeda

056 Direct neurite–osteoclast cell communication in co-culture system  
K. OBATA, S. SUGA, S. GOTO and A. TOGARI  
Aichi-Gakuin University, Japan

057 Effects of HClO incorporated electrolyzed water on proliferation of KB–cells  
Q.H.M.S. ZAMAN¹, K. MATIN², A. OKADA¹, N. HANADA³ and J. TAGAMI¹  
¹Tokyo Medical and Dental University & National Institute of Public Health, Japan  
²Tokyo Medical and Dental University, Japan ³Tsurumi University, ⁴Tokyo Medical and Dental University & G-COE Program at TMDU, Japan

058 A histological study of human dental pulp–derived odontogenetic cells  
S. KUM Abe, M. NAKATSUKA, K. TAKAMA, T. MIKAMI and Y. IWAI  
Osaka Dental University, Japan

059 Enhanced wound healing by matrix metalloproteinase–3 after dental pulp amputation  
K. AMANO¹, M. NAKASHIMA², L. ZHENG², K. IOHARA², H. MATSUI¹, M. YAMASAKI¹, K. MATSUSHITA² and H. NAKAMURA¹
TNF-α enhances MMP-2 production in deciduous dental pulp fibroblasts

N. WATANABE, K. WATABNABE, S. SHIRASU, K. DAITO and M. DAITO
Osaka Dental University, Japan

Chairperson: Dr. N. Hanada

An oral rehabilitation robot for muscle massage

Y. ARII1, A. KATSUMATA2, N. OGI1, M. IZUMI1, S. SAKUMA1, Y. IIDA2, K. KURITA1, H. ISHIH2, A. TAKANASHI2 and E. ARII1
1Aichi-Gakuin University, Japan 2National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, Japan

Oral health care using a rapid oral bacteria detection system

F. TAMURA1, T. KIKUTANI1, H. KATAGIRI1, T. HANAGATA2, M. YOSHIDA3, R. HAMADA4, T. INAGUCHI1, A. TAKAGI1, T. YONEYAMA3, M. KODAMA4 and M. SUDA2
1Nippon Dental University Hospital, Japan 2Yamanashi Dental Association, Japan 3Hirosima University, Japan 4Panasonic Shikoku Co, Japan 5Yoneyama Dental Clinic, Japan 6Nippon Dental University, Japan 7The Nippon Dental University School of Dentistry at Tokyo, Dental Hospital, Japan

Oral health and ADL in 90-years old people in Japan

F. AIZAWA1, M. KISHII1, M. YONEMITSU1, D. INABA1, M. TAZAWA1, K. SAIGO1, T. SATO1, M. HAKOZAKI2
1Iwate Medical University, Japan 2Iwate Dental Association, Japan

14:00–15:12 (Room D) Operative Dentistry / Dental Material 1

Chairperson: Dr. M. Fujitani

FEL showed dental tissue specificity of laser ablation

T. SAKAE1, Y. NUMATA1, Y. SATO1, H. OKADA2, H. YAMAMOTO4, T. KUWADA5, T. SAKAF6, K. NOGAMI7, Y. HAYAKAWA2, T. TANAKA1, K. HAYAKAWA2 and I. SATO2
1Nihon University School of Dentistry at Matsudo, Japan 2Nihon University, Japan

Comparison of fatigue and tensile strength of radicular dentin

T. INOUE, M. SAITO, F. NISHIMURA and T. MIYAZAKI
Showa University, Japan

Bleaching efficiency employing various light units and irradiation time

M. NAYIF1, M. OTSU1, A. KISHI1 and J. TAGAMI2
1Tokyo Medical & Dental University, Japan 2Tokyo Medical and Dental University & G-COE Program at TMDU, Japan

The newly developed root canal filling materials

H. WANIBE1, M. YAMAMOTO1, N. KITAMURA1, K. NAKATA1, T. KAWA1 and H. NAKAMURA1
1Aichi-Gakuin University, Japan 2Aichi-Gakuin University School of Dentistry, Japan
Microleakage of gutta percha and resilon at different canal length
S. WIMONCHIT and R. CHALERMNONTAGARN
Srinakarinwirot University, Thailand

Shaping ability of two rotary Ni–Ti instruments in curved canals
C. PIYACHON and P. WANGWATTANAPISARN
Srinakarinwirot University, Thailand

Chairperson: Dr. S. Ban

Bond strengths of resin composite to caries-affected root canal dentin
S. OTAKE, K. YOSHIDA, Y. FUKUI, W. KOMADA and H. MIURA
Tokyo Medical and Dental University, Japan

Effect of microperoxidase primer on bonding three dentin adhesive systems
Y. TAIRA and K. SOENO
Nagasaki University, Japan

Resin bonding to dentine after casein phosphopeptide–amorphous calcium phosphate treatments
V. SATTABANASUK¹, M. BURROW², Y. SHIMADA³ and J. TAGAMI³
¹Srinakarinwirot University, Thailand ²University of Melbourne, Australia ³Tokyo Medical & Dental University, Japan

16:15–17:28 (Room B) Periodontal Research 2 / Mineralized Tissue 2
Chairperson: Dr. H. Nakamura

Effects of obesity on oxidative stress in rat gingiva
T. TOMOFUJI¹, T. SANBE¹, N. TAMAKI¹, D. EKUNI¹, K. KASUYAMA², M. UMAKOSHI², K. IRIE¹, T. AZUMA¹, J. MURAKAMI², S. KOKEGUCHI¹, T. YAMAMOTO¹, and M. MORITA²
¹Okayama University Graduate School of Medicine, Japan Dentistry and Pharmaceutical Sciences, Japan ²Okayama University, Japan

Oral infection of Porphyromonas gingivalis induces pro–atherogenic change in mice
T. MAEKAWA, N. TAKAHASHI, Y. AOKI, H. MIYASHITA, K. TABETA and K. YAMAZAKI
Niigata University Center for Transdisciplinary Research, Japan

Effect of non–surgical periodontal therapy on plasma reactive oxygen metabolites
N. TAMAKI, R. YAMANAKA, D. EKUNI, T. TOMOFUJI, T. YAMAMOTO and M. MORITA
Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan

Expression of collagenases in development of rat periradicular lesion
H. MATSUI, K. AMANO, M. YAMASAKI, K. NAKATA and H. NAKAMURA
Aichi-Gakuin University, Japan

Salivary cortisol and Th1/Th2 cytokines of patients with complaint halitosis
M. FUKUI, T. BAATARJAV, K. KATAOKA, D. HINODE and H. ITO
Tokushima University, Japan
078 Facilitated anion delivery through human enamel and dentin with AC–iontophoresis
  H. IKEDA and H. SUDA
  Tokyo Medical & Dental University, Japan

079 TNF–α–induced bone resorption model using CHP nanogel in mice
  K. NAGANO, K. AOKI, A.H. MIAN, N. ALLES, A. SHIMODA, N. MORIMOTO,
  K. AKIYOSHI and K. OHYA
  Tokyo Medical & Dental University, Japan

080 Osteoclast differentiation during tooth eruption in microphthalmic mice
  T. TAKAHASHI, N. USHIJIMA, Y. NODASAKA and T. IIZUKA
  Hokkaido University, Japan

081 Down-regulation of RANKL/NF–κ B by β–TCP implant in dog jaw
  J. ZHAO¹, T. WATANABE² and Y.ABIKO³
  ¹Nihon University, Japan ²Kanagawa Dental College, Japan

●16:15–17:35(Room C)  Mineralized Tissue 3 / Dental Material 2
  Chairperson: Dr. K. Yaegaki

082 Stem cell–like characteristics of dental pulp and bone marrow cells
  N. ISHKITIEV⁴, V. MITEV², B. CALENIC¹, T. NAKAHARA¹ and K. YAEGAKI¹
  ¹Nippon Dental University, Japan ²Medical University - Sofia, Bulgaria

083 α 3 β 1 integrin–mediated MC3T3–E1 osteoblasts adherence induced by interleukin–1 α
  T. TOMYAMA¹, K. NARUSHI⁴, K. OMORI² and H. MAEDA¹ and S. TAKASHIBA¹
  ¹Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical
  Sciences, Japan ²Okayama University Hospital of Medicine and Dentistry, Japan

084 Oral malodorous compound suppresses the proliferation of osteoblasts
  H. II, T. IMAI, T. KAMODA, T. MURATA, T. TANAKA, T. SATO and K. YAEGAKI
  Nippon Dental University, Japan

085 Roles of AMPK in osteogenic differentiation
  T. KASAI, K. BANDOW, S. KAWAMOTO, E. NAGAOKA and T. MATSUGUCHI
  Kagoshima University, Japan

086 Effect of daidzein together with raloxifene in osteoblast–like cells
  M. NOMURA, A. KAWAMOTO and Y. KOMASA
  Osaka Dental University, Japan

087 Establishment of human osteoblasts culture system obtained from aged donors.
  M. AINO¹, M. SAITO², S. MURAKAMI², T. NOGUCHI¹ and T. YONEDA²
  ¹Aichi–Gakuin University, Japan ²Osaka University, Japan

  Chairperson: Dr. M. Hattori

088 Long term clinical evaluation of glass–fiber–reinforced restorations
  T. ABE, T. SUMI, T. INUKAI and Y. ITO
Aichi-Gakuin University, Japan

089 Influence of light–polymerization systems on surface properties of indirect composite
M. MURAKAMI¹, H. KOIZUMI¹, M. NAKAZAWA¹, M. KOCHI¹, N. TANOUE², M. FURUCHI¹ and H. MATSUMURA¹
¹Nihon University, Japan ²Nagasaki University, Japan

090 Properties of dental zirconia changed with sintering temperature
S. BAN, N. TAKEUCHI, Y. OKUDA, M. NODA, H. KONO and H. SATO
Kagoshima University, Japan

091 Breaking strength of an experimental mobile mandibular advancement splint
N. TANOUE, K. NAGANO, S. YANAMOTO and A. MIZUNO
Nagasaki University, Japan

●16:15–17:35(Room D)  Gene Expression / Diagnosis
Chairperson: Dr. F. Yoshimura

092 Corticosteroid affects upregulated expression of beta–defensins in keratinocytes
M. HORIUCHI, M. SAITOH, S. NAKAMURA, M. NISHIMURA, M. YAMAZAKI, Y. KURASHIGE, T. KAKU, S. IGARASHI and Y. ABIKO
Health Sciences University of Hokkaido, Japan

093 Gene expression profiling of LPS responsive genes in human trophoblast
Y. LI, R. CHO, Y. SATO, N. KUBOYAMA and Y. ABIKO
Nihon University Nihon University School of Dentistry at Matsudo, Japan

094 DNA sequence of γ–glutamyl transpeptidase from Actinobacillus actinomycetemcomitans Y4
R. MINEYAMA¹, A. TAKAO² and N. MAEDA²
¹Nippon Dental University, Japan ²Tsurumi University, Japan

095 Proteomic analyses of a two–component regulator mutant of tannerella forsythensis
D. NIWA¹, K. NISHIKAWA², Y. MURAKAMI¹, H. NAKAMURA³ and F. YOSHIMURA³
¹Department of Endodontics and microbiology Aichi–Gakuin University, Japan ²Aichi–Gakuin University School of Dentistry, Japan ³Aichi–Gakuin University, Japan

096 DNA microarray analysis of dental pulp exfoliated from deciduous teeth
R. HARADA, K. WATANABE, S. SHIRASU, M. KATO and M. DAITO
Osaka Dental University, Japan

097 Expression of toll–like receptors during development of sialoadenitis in mice
S. SHIMIZU, M. SAITOH, Y. KURASHIGE, M. NISHIMURA, M. HORIUCHI, T. KAKU and Y. ABIKO
Health Sciences University of Hokkaido, Japan

Chairperson: Dr. D. Inaba

098 Inflammatory markers and stress index in sleep apnea syndrome
K. TOYOSHIMA, M. KIMURA, A. YAMAGUCHI and K. SHIBASAKI
Niipon Dental University, Japan
099 Analysis of the transmitted–light through the human teeth in vivo
   M. IKAWA
   Tohoku University, Japan

100 Measurement and clinical significance of uric acid in saliva
   M. KIMURA, R. IKARASHI, K. TOYOSHIMA, A. YAMAGUCHI, T. WATANABE,
   K. HASEGAWA and K. SHIBASAKI
   Nippon Dental University, Japan

101 Study of mandibular dental arch form by Fourier analysis
   M. NAKATSUKA, S. KUMABE, K. TAKAMA, H. MIKAMI and Y. IWAI
   Osaka Dental University, Japan
SPECIAL LECTURES
New developments in dentistry and IADR

Professor JM (Bob) ten Cate

President International Association for Dental Research (IADR)
Academic Center for Dentistry Amsterdam
Royal Netherlands Academy of Arts and Sciences

Dental caries has long been one of the most prevalent diseases of mankind. In spite of the successes in caries prevention, the disease is still far from eradicated. The detrimental effects are many: Apart from the suffering of individual patients, the costs of treatment and retreatment are substantial and to this indirect costs should still be added. These include loss of working hours and school days and ineffectiveness due to pain suffered. Globally there is a clear difference in caries prevalence and incidence between various countries, typically characterized by their respective economic conditions. This all implies that there is a continued need to study dental caries and evaluate new methods of caries prevention.

Research in recent years has brought important paradigm shifts in caries etiology, prevention and management. New insights have been gained in the etiology of dental caries with considerable advancements being made in bacterial genomics, ecology and the study of bacterial susceptibility to antimicrobials. Another paradigm shift is that restorative treatments are no longer seen as the generally preferred treatment mode and that an invasive treatment should be chosen only after thorough preventive interventions. While fluoride is still the most effective agent used in caries prevention, new avenues including calcium containing applications and antimicrobials are studied. Bio- and nanotechnology have also entered in the dental field with fascinating new technologies being considered. In this arena stem cell- and bioengineering are considered as ultimate treatment option for patients.

The International Association for Dental Research (IADR) has continued to be the preferred platform for dental scientists to share their new findings and increased attention is given to the international character of the organization. This implies more emphasis on the regional promotion of dental science and support for individual members. Global IADR meetings are planned on all continents in the next decade.
Brief CV

Dr. J.M. (Bob) ten Cate is Professor of Preventive Dentistry at the Academic Center for Dentistry Amsterdam (ACTA), and a former Dean of ACTA and Director of the Netherlands Institute for Dental Sciences.

His multidisciplinary international research group focuses on caries prevention and oral infectious diseases, with special emphasis to modes of action of fluorides and antimicrobials. He has published over 200 full length papers in refereed international journals and numerous publications aimed at general practitioners and chapters in textbooks. He has lectured in many countries around the world.

For his research he received the ORCA-Rolex Prize (1986), the Yngve Ericsson Prize for Prevention (2000) and the IADR Distinguished Scientist Award in Caries Research (2003). In 2007 he was awarded a Professorship with the Royal Netherlands Academy of Arts and Sciences. After serving in various capacities in the organization, he is currently the World President of the International Association for Dental Research (IADR).
Laminin-derived Peptides: their biomedical applications and signaling pathways

Professor Byung-Moo Min
President of KADR
Department of Oral Biochemistry and
Program in Craniomaxillofacial Reconstruction Science,
Seoul National University School of Dentistry, Seoul Korea

Laminin is a heterotrimeric glycoprotein specific to the basement membrane and has many biological functions, including cell adhesion, migration, cell proliferation, differentiation, neurite outgrowth, angiogenesis and tumor invasion. Laminin-5 (an isoform consisting of the laminin α3, β3, and γ2 chains), the major ligand for keratinocyte adhesion in the epidermis, promotes keratinocyte survival \textit{in vivo} and \textit{in vitro}. Laminin-5 and α3β1 integrin promote keratinocyte survival; however, the downstream signaling pathways for laminin-5/α3β1 integrin-mediated cell survival has not been fully established.

Here, we expressed the five human laminin-5 α3 chain globular (LG) domains as monomeric, soluble fusion proteins, and examined their biological functions and signaling. Recombinant LG3 (rLG3) protein, unlike rLG1, rLG2, rLG4, and rLG5, played roles in cell adhesion, spreading, and integrin α3β1 binding. More significantly, we identified a novel motif (PPFLMLKSTR, aa 1312-1323; Ln5-P4 peptide) in the LG3 domain that is crucial for these responses. Studies with the synthetic peptides delineated the Ln5-P4 peptide within LG3 domain as a major site for both integrin α3β1 binding and cell adhesion. Substitution mutation experiments suggested that the Arg residue is important for these activities. Since the final goal of peptide development is biomedical application, it is important to understand the effects of a peptide on cellular processes when the peptide is used in tissue engineering scaffolds. We reported that laminin-5, rLG3, and Ln5-P4 coated onto chitin microfibrous matrices, which are used as a wound dressing material, promoted cell growth and inhibited apoptosis through the integrin and the focal adhesion kinase (FAK)/PI3K/Akt signaling pathways in human epidermal keratinocytes. The downstream signaling pathways mediated by Akt survival signaling are diverse. Therefore, to identify targets modulated by Ln5-P4, we compared the protein profiles of vehicle- and Ln5-P4-treated cells using 2D-PAGE and mass spectrometric analysis. Our results indicated that Ln5-P4 stimulation induced several 14-3-3 isoforms, including ζ and . Because more than 50 signaling proteins have been reported as 14-3-3 ligands, multiple interactions between 14-3-3 proteins and Akt-phosphorylated proteins are expected in survival signaling pathways; however, the nature of these multiple interactions within survival signaling pathways remain unknown. Further, downstream signaling pathways for α3β1 integrin-mediated cell survival have not been fully elucidated. We revealed the unexpected finding of multiple interactions between 14-3-3 isoforms and proapoptotic proteins in the survival signaling pathway. Ln5-P4 peptide within human laminin-5 α3 chain promoted cell survival and anti-apoptosis by inactivating the functions of Bad and YAP. This effect was achieved through the formation of 14-3-3ζ/p-Bad and 14-3-3ζ/p-YAP complexes, which was initiated by α3β1 integrin and focal adhesion kinase.
(FAK)/phosphatidylinositol 3-kinase (PI3K)/Akt signaling. These complexes resulted in cytoplasmic sequestration of Bad and YAP and their subsequent inactivation. An increase in Akt1 activity in cells induced 14-3-3ζ and , p-Bad and p-YAP, leading to promotion of cell survival, whereas decreasing Akt activity suppressed the same proteins and inhibited cell survival. In an *in vivo* experiment, we covered full-thickness skin wounds of rabbits with Ln5-P4-coated matrices and found that Ln5-P4 markedly promoted wound healing and accelerated re-epithelialization in comparison to a vehicle-treated control.

To our knowledge, this is the first report demonstrating that the Ln5-P4 peptide within the LG3 domain is a novel motif that is capable of supporting integrin α3β1-dependent cell adhesion and spreading. These results also reveal a new mechanism of cell survival whereby the formation of 14-3-3ζ/p-Bad and 14-3-3ζ/p-YAP complexes is initiated by laminin-5 stimulation via the α3β1 integrin and FAK/PI3K/Akt signaling pathways, thereby resulting in cell survival and anti-apoptosis.

**Brief CV**

1980   D.D.S.Seoul National University School of Dentistry, Seoul, Korea  
1985   M.S.Graduate School, Seoul National University School of Medicine, (Biochemistry)  
1989   Ph.D.Graduate School, Seoul National University School of Medicine, (Biochemistry)  
1981-1983   Teaching Assistant, Department of Oral Biochemistry, Seoul National University, Korea  
1986-1989   Instructor, Department of Oral Biochemistry, Seoul National University School of Dentistry, Korea  
1990-1993   Visiting Assistant Professor, Section of Oral Biology, UCLA School of Dentistry, U.S.A.  
1989-1994   Assistant Professor, Department of Oral Biochemistry, Seoul National University, Korea  
1994-1998   Visiting Associate Professor, Section of Oral Biology, UCLA School of Dentistry, U.S.A.  
1996-1998   Visiting Associate Professor, Department of Oral Biochemistry, Seoul National University, Korea  
2000-2000   Visiting Professor, Dental Research Institute, UCLA School of Dentistry, U.S.A.  
1999-present   Professor and Chair, Department of Oral Biochemistry and Program in Craniomaxillofacial Reconstruction Science, Seoul National University School of Dentistry, Seoul, Korea

**Field of research interest**

1. Oral Oncology  
2. Molecular Mechanisms for Differentiation and Senescence of Normal Human Keratinocytes  
3. Skin and Nerve Regenerations
Nanomedical system developed with PLGA nanosphere platform

Professor Yoshiaki Kawashima
Department of Pharmaceutical Technology, School of Pharmaceutical Science,
Aichi-Gakuin University, Nagoya, Japan

Introduction

In this lecture, a new nanomedical system in 21st century developed by using a biodegradable polymer nanosphere platform will be discussed mainly on drug delivery system and further applications to such as gene delivery, cosmetics, medical device and dental system for celebrating this international symposium held at Aichi Gakuin University in Nagoya, Japan.

In our project, PLGA, poly(lactide-co-glycolide) was chosen as a polymeric material, because of its superior biodegradability and biocompatibility recognized widely and approved by FDA. We have established the preparation method of PLGA nanosphere platform as a preferable carrier for peptide, oligonucleotide, pDNA, siRNA and others. At beginning the unique behavior of nanoparticulate system appeared in vivo are discussed to understand the mechanism of PLGA nanosphere interacting with biological tissue. By reducing the size of PLGA nanosphere to the level of nm in dimension < 100-500 nm, unexpected new function for delivery, such as adhesion to mucous layer, penetration into biological membrane, enhancing endocytosis and etc. can be provided resulting in improved absorption or uptake into the target tissue and the cell respectively. An alternative administration way to injection of such carrier has been explored to improve patient compliance and benefit. Non invasive delivery way such as dermal, oral, nasal and pulmonary delivery systems should be desired form to meet the requirements from patients.(1)

Established PLGA nanosphere platform by polymeric spherical crystallization process

PLGA nanospheres (ca. 250nm in diameter) with drug were prepared by the polymeric spherical crystallization process, i.e. emulsion solvent diffusion (ESD) method in water. (2) When the surface modification of nanospheres was required to facilitate bioadhesive properties, chitosan was coformulated in the dispersing aqueous medium to coat the surface of nanosphere produced simultaneously with the adsorption of chitosan. The resultant PLGA nanospheres were powdered by freeze drying. Gene-drug carriers were prepared also by using ESD method. NFkB decoy oligodeoxynucleotide (NDON) was encapsulated in the PLGA nanosphere. pDNA (pCMV-luciferase) was encapsulated in the PLGA nanosphere by the modified emulsion solvent diffusion method in water with cationic complexing agent for pDNA such as DOTAP;N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethyl ammonium and chitosan dissolved in the pDNA solution. The rest of procedure was followed to that of PLGA nanosphere with insulin.

Non invasive delivery of PLGA nanosphere loaded with peptide

The chitosan-coated PLGA nanospheres containing 500 IU/kg calcitonin, administered intragastrically to fasted male Wistar rats, prolonged dramatically the reduction in blood Ca level over 36 hr compared with 12 hr for
the uncoated nanospheres. The increased residence time of nanospheres at the small intestinal wall as a result of their strong mucoadhesion and deeper penetration into the mucus layer should significantly improve the extent of drug absorption12 hr.(3)

Distribution of the PLGA nanospheres deposited in the lung was determined after intratracheal administration of nanosphere composite into a Wistar male rat by using a syringe method. Deposited percentage of nanospheres on trachea and alveoli of soft nanocomposite granule and soft ordered mixture of nanocomposite were more than 80% and 65%, respectively. It was found that the surface modified nanosphere with chitosan prolonged the residence time at the deposited site compared with unmodified one. Pharmacological effects with the nanosphere composite of insulin loaded PLGA nanospheres were investigated with the same manner as employed in vivo deposition test. The nanosphere composite showed prolonged pharmacological effect compared with the insulin solution following i.v. or i.t. administration.

**Cellular uptake and transfection efficiency of pDNA loaded PLGA nanosphere in vivo**

The cellular uptake of PLGA nanosphere into A549 cell increased with decreasing the particle size of the PLGA nanosphere. The surface modification of PLGA nanosphere with chitosan enhanced the cellular uptake in A549 cell, irrespective of the particle size. The transfection efficiency of pDNA loaded PLGA nanosphere could be increased by increasing the amount of pDNA introduced into the cell, which could be determined by the pDNA loading efficiency. Whereas the chitosan coated DOTAP-pDNA complex loaded PLGA nanosphere increased the luciferase activity with incubation time. Therefore the highest transfection efficiency was obtained by introducing the chitosan modified PLGA nanosphere with the DOTAP-pDNA complex. The transfection efficiency of PLGA nanosphere in the A549 cell was found as a function of incubation time. After attaining a maximum luciferase activity a prolonged slightly decreasing activity was appeared. This finding suggested that the chitosan coated PLGA nanosphere uptaken in the cell released the DOTAP-pDNA complex, resulting a prolonged luciferase activity. In vivo transfection efficiency of PLGA nanosphere loaded with DOTAP-pDNA complex was investigated at 24hr after administrating intratracheally it in ICR female mice. It was found that the luciferase activity of pDNA was increased significantly by encapsulating the complex of DOTAP-pDNA in PLGA nanosphere.

**New nanomedical system (NMS) developed with PLGA nanosphere platform**

The present PLGA nanosphere platform was found to be widely applicable to open new medical principle and system to create multifunctional delivery system.

PLGA nanospheres have been used as useful drug carriers such as to protect against enzymatic degradation, to prolong the residence time by adhesion at absorption site and the releasing drug, leading to improvement in the absorption of drug. It was recently found that PLGA nanospheres were capable of penetrating the subcutaneous membrane. We have developed a new functional skin care cosmetics, “NanoCrysphere®” with pro-vitamin C loaded PLGA nanospheres, which was launched on the market in 2005. In this lecture, how new functions of PLGA nanospheres were provided and applied to create new function. Crude drug extracts were encapsulated in the nanospheres for developing hair-growing cosmetics, which will be launched on the market. New nano-medical device is going to be developed with PLGA nanosphere coated inner surface of stent for next generation Drug Eluting Stent, establishing a new cardiovascular therapeutic system.

Such nanoparticulate system should be paid more attention to develop new drug delivery devices for dental application, as found suitable for incorporation into a hydrogel matrix. Novel nanocomposite materials for dental use are designed by nanotechnology in future as discussed in the lecture.
### Brief CV

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<td>B. Pharm.</td>
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### Fields and Areas of Specialization

Particulate design engineering for drug delivery systems including spherical crystallization, agglomeration, microencapsulation, nanosphere

### Award

1. The Best Paper Award, 2003 from the Academy of Pharmaceutical Science Technology, Japan
2. PSWC Research Achievement Award, 2004 at the Pharmaceutical Science World Congress, FIP

### Publication

- Original Research Papers, 274
- Review Papers, 72
- Editor and Coauthors of book, 51
- Patent, 35
SYMPOSIUM
Mass transfer within natural plaque biofilms: the role of plaque architecture

Professor Emeritus Colin Robinson
Division of Oral Biology, University of Leeds, UK

Transfer of materials into and out of dental plaque is a pivotal determinant of plaque behaviour since it involves the entry of bacterial cell nutrients and therapeutics including fluoride and the elimination of waste products. Dietary components, including acid producing carbohydrates and acid itself, are of particular importance. Using the plaque device described by Robinson et al 1997 [1], it has been possible not only to produce plaque on natural enamel surfaces in vivo, but also to recover this plaque intact, to study its architecture [2,3], bacterial species distribution [4] as well as the uptake and distribution of materials within it [1,5,6]. During short times of exposure similar to those associated with tooth brushing, penetration of fluoride, sucrose, phosphate and triclosan was limited to the outer one third or half of the plaque biofilm [1, 5]. Comparison of penetration profiles with plaque architecture strongly suggested that the penetration was related to the surface area/mass ratio of the biomass [1, 2, 6]. This suggests that penetration into and uptake by biomass is a rate limiting step. More detailed analysis revealed that while showing similar penetration profiles, distribution within the plaque varied. Amine fluoride seemed to be limited to the edges of the biomass at the borders of channels within the plaque i.e. these molecules did not seem readily to enter the plaque biomass [6]. Triclosan on the other hand seemed to penetrate biomass quite well [6]. This implied that nonpolar molecules penetrate biomass better than molecules with higher polarity. Efforts to improve fluoride penetration using low pH to generate less polar HF, however, reduced uptake even further (Tokura T., Robinson C. et al unpublished). Since low pH does not seem to dramatically affect plaque biofilm architecture [7], this suggests that chemical interactions between penetrant and biomass may be also important. Changes to plaque architecture were, however, achieved using detergent suggesting this may be a way forward to changing penetration patterns [7].

Brief CV

Colin Robinson holds a BSc and PhD (1968) in biochemistry from the University of Leeds UK. After a period as lecturer in biochemistry and oral biology, he was awarded a readership (1985) and a personal chair (1990) in Oral Biology at the Leeds Dental Institute.

He became Head of the department of Oral Biology in 1985 and Director of Research in 1992. During this period he also served as Pre-Clinical Dean. He retired formally in April 2007 and remains active as Professor Emeritus.

Professor Robinson has served as board member, secretary general and President (1999) of ORCA (European Organisation for Caries Research), President of the British Society for Dental Research (British Division IADR). He was Chairman of the Association for Basic Science Teachers in Dentistry, a group now with European status and remains an honorary member. He was Chairman of the Mineralised Tissue Groups of both BSDR and IADR and retains honorary membership. He has served on a number of national and international committees including the National Research Assessment Exercise, Dental Panel and is currently panel member for Grant awards for the MRC, UK, MRC Canada and NICDR. He is a member of the MRC UK College of Experts. The ORCA Rolex prize for outstanding caries research was received in 1982 and the IADR distinguished scientist award for biomineralisation in 1993. For services to medical science he was elected a founder fellow of The Academy of Medical Sciences UK in 1997.

Professor Robinson’s main interests lie in biomineralisation processes particularly dental tissues, dental caries and fluoride. More recently an interest in oral biofilms has produced novel data on human plaque architecture and the dynamics of mass transfer through it.

He has produced over 300 peer reviewed publications in these fields of research.
Can biofilms kill you?

Professor Lakshman Samaranayake
Dean and Chair of Oral Microbiology
Faculty of Dentistry, The University of Hong Kong, Hong Kong

Biofilms are ubiquitous in nature. It is now known that bacteria and fungi mostly exist attached to surfaces, in the biofilm phase in contrast to their suspended or planktonic phase existence. This is true for most infections of humans, including oral diseases. Thus, the two commonest human afflictions and the primary diseases of the oral cavity, caries and periodontal disease, are caused by oral biofilms—which were traditionally called dental plaque. There is a growing body of data that periodontal diseases, can have profound effects on total health including cardiovascular disease, adverse pregnancy outcomes, diabetes, pulmonary disease and stroke.

Apart from these systemic effects, oral biofilms per se are now known to possess intriguing properties that have clinical implications. For instance, biofilm phase bacteria and fungi (particularly Candida species) compared with planktonic phase organisms are recalcitrant to antibacterials, and antifungals, respectively. Hence, the possible reason why it is not easy to eradicate dental plaque or chronic candidal infections (especially in compromised individuals) purely by chemical or antibiotic treatment. Moreover, emerging data indicate other intriguing dimensions of the behavior of biofilm organisms such as their ability to ‘cross-talk’, through chemical messengers between the biofilm community dwellers. Indeed, more recent evidence points to the possibility of chemical messengers between the biofilm residents and even host cells.

Yet, the association between biofilms and either local or systemic diseases they cause is not straightforward. This presentation will provide an overview of the role of oral biofilms and how they affect human morbidity and mortality outcomes.
Brief CV

Professor Samaranayake is the Dean and, Chair of Professor of Oral Microbiology at the Faculty of Dentistry, University of Hong Kong and, the Director of the Prince Philip Dental Hospital. He has held teaching/honorary consultant positions at the University of Glasgow, UK, University of Alberta, Canada and the University of Peradeniya, Sri Lanka.

He has also served as a Director of the FDI World Dental Federation and the Chairman of its Science Commission. Currently he is an Honorary Professor at the Eastman Dental Institute, London. His research is mainly focused on infectious diseases and he has been the recipient of both the ‘Outstanding Researcher Award’ and the ‘Outstanding Research Student Supervisor Award’ of the HKU.

The author or co-author of over 400 research/review articles, Professor Samaranayake has also written 28 book chapters and eight books/monographs some translated into five languages. He has lectured in all five continents, serves on the editorial boards of more than 14 journals and, is a World Bank Consultant on problem based learning.
The major reason for the decline in dental caries in the industrialised world during the last decades is believed to be the widespread use of fluoride dentifrices. Throughout the world fluoride toothpaste is by far the most widely used method of applying fluoride. There is reported the mean reduction in caries increment amounts to 25 % and is saving at the most approximately 0.7 carious surfaces per year in children or young adolescents. F-toothpaste is the almost perfect delivery system to the specific site of action, to the biofilm of dental plaque at the tooth surface. Fluoride - present in the aqueous phase at the crystallite surface - plays a determining role in the inhibition of enamel demineralization and in the stimulation of remineralisation.

One of the primary determinants of the preventive efficacy of toothpastes is its fluoride concentration because there is a clear dose response relationship between the concentration of fluoride contained in toothpastes and anticaries efficacy. Today it is estimated, that over the range of 1000 – 2500 ppm F there is a 6 % improvement in efficacy for every 500 ppm increase in fluoride concentration of preventive preparation.

The paper to be presented summarizes the results acquired with different NaF-concentrations (250-5000 ppm F) and with different F-compounds (NaF, NaMFP, Amine Fluoride [AmF] all with 1.250 ppm F) on the F retention in dental plaque. A double-blind cross-over protocol with subjects aged 26 – 58 years was used. Manual toothbrushing was performed twice a day for three minutes; the protocol started for each dentifrice with a fluoride free 7 day wash out phase. No approximal cleaning was carried out during the test period. Interdental plaque was sampled using F-free dental floss 30 minutes or 12 h after brushing at day 4 or 7 respectively. The amount of plaque was weighed and then F was analysed using an ion selective electrode (Orion 9609). The amount of plaque collected ranged from 3.5 to 6.5 mg. After fluoride free wash-out interdental plaque contained 3.35±1.79 ppm F and showed 8.3 ppm F (NaMFP), 14.5 ppm F (NaF) or 16.6 ppm F (AmF) 30 minutes after brushing. The last two means differ significantly from baseline (Tukey test: p>0.05). 12 h after brushing F concentrations returned to a level noticed as average for self-care with fluoride toothpaste and amounted to 8.35 ppm F (NaF) or 7.64 ppm F (AmF). NaMFP or F-free toothpastes caused lower values of 3.87 or 3.35 ppm F respectively. NaF-toothpaste with 250 ppm did not increased the fluoride concentration above baseline values significantly (4.41ppm). 3000 and 5000 ppm considerably forced up the fluoride concentration of dental plaque to 20.5 or 27.6 ppm respectively.

In summary the fluoride content of dental plaque could be estimated with high accuracy if plaque is sampled carefully without contamination by saliva. The ionized fluoride content of dental plaque is much higher than in saliva and directly depends on the supply by selfapplication with toothpaste. The fluoride content in dental plaque after toothbrushing is elevated at least for 2 – 4 hours. Na-MFP toothpaste caused lower ionized fluoride levels in dental plaque. NaF and AmF caused similar fluoride increase in dental plaque. 250 ppm F in toothpaste for children do not
raise the F content in dental plaque but 500 ppm F exhibits a significant effect on plaque and enamel. The content of fluoride of dental plaque could be an objective reflection of the individual oral hygiene regimen and probably a useful tool in caries risk prediction.

**Brief CV**

1962-67 Study of Dentistry (Volgograd/Russia)

1967-68 One year postgradual biochemical course in Volgograd (Russia);

1969 Academic degree “Candidate of Biol. Science” (in Russia) - (German equivalent - Dr.rer.nat);

1969-75 Assistant at the Institute of Medical Biochemistry, University of Halle

1973-75 (parallel) Study of Medicine at the University of Halle (Germany);

1976-85 Medical Univ. of Erfurt, School of Dentistry, Dept Preventive Dentistry, Research subunit

1985 Dr.med.habil.: “Cariogenic significance of acid production and acid tolerance of S. mutans”.

1993 Professor of Experimental and Preventive Dentistry; School of Dentistry, Univ. of Erfurt

1995 Director of the Dept Preventive Dentistry & Pedodontics, Dental School, Univ. of Jena.

Research interests:  
- Acid production and acid tolerance of oral streptococci,
- Caries model on conventional and germ-free rats,
- Intra-oral pH measurement for the estimation of cariogenicity of food-stuffs and their components,
- Experimental and clinical evaluation of caries protective properties of preventive measures and substances,
- Caries risk assessment studies.
Ecological dynamics of caries-associated oral biofilm: involvement of mutans streptococci and non-mutans bacteria

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The oral biofilm supports a “micro-ecosystem” of bacteria that exhibit a variety of physiological characteristics. Particularly, microbial acid production from carbohydrates in the oral biofilm and the resultant decrease in pH are responsible for demineralization of the tooth surface and the subsequent formation of dental caries.

Among the bacteria in the oral biofilm, mutans streptococci have received the most attention as the pathogen responsible for dental caries. This is because, (1) mutans streptococci are frequently isolated from caries lesions; (2) mutans streptococci are highly acid-productive (acidogenic) and acid-tolerant (aciduric); and (3) mutans streptococci produce water-insoluble glucan, which promotes bacterial adhesion. However, recent studies have revealed that the level of mutans streptococci is not high in caries-associated biofilms, especially the biofilms covering non-cavitated stages of caries lesion, white spot lesions. Instead non-mutans bacteria including non-mutans streptococci and actinomycyes comprise the predominant part of the microflora. Mutans streptococci as well as lactobacilli increase in number as the lesion matures, particularly in cavitat ed lesions. These findings suggest that mutans streptococci are not initiators of caries formation but promoters of caries progression. In addition, more recent studies have shown that not only mutans streptococci and lactobacilli but also bifidobacteria, other aciduric bacteria, are frequently isolated from cavitated lesions.

On the basis of “ecological plaque hypothesis”, it is possible to explain the ecological dynamics of caries-associated oral biofilm as follows: non-mutans streptococci and actinomycyes are predominant in the oral biofilm associated with healthy tooth surface, probably because these bacteria are more versatile in metabolic characteristics and adhesive to saliva-coated tooth surface than other oral bacteria. Non-mutans streptococci and actinomycyes are able to produce acids from carbohydrates and lower the environmental pH enough to demineralize tooth surfaces. In addition, the environmental acidification by microbial acid production increases the acid-productivity (acidogenicity) and acid-tolerance (acidurance) of these bacteria through a series of acid-adaptive responses including the induction of H⁺-ATPase, alkaline-producing enzymes and stress-proteins. These phenomena may result in the initiation of dental caries. The bacterial acid-adaptation does not only enhance the cariogenic potential of oral biofilm but also triggers the acid-selection of bacteria. The acid-selection may result in the emergence of more aciduric bacteria such as mutans streptococci, lactobacilli and bifidobacteria in the oral biofilm.

In this scenario, the acid-adaptation of non-mutans streptococci and actinomycyes play a critical role for the
initiation of dental caries. Once the acidic environment has been established, the acid-selection occurs: mutans streptococci and other aciduric bacteria such as lactobacilli and bifidobacteria may increase and act as promoters of lesion progress. It is known that the pH levels of active and cavitated caries lesions are maintained around 4, irrespective of sugar intake. Under such highly acidic conditions, only very aciduric bacteria such as mutans streptococci, lactobacilli and bifidobacteria can survive and cause progression of caries lesions. This may be the reason why these bacteria comprise high proportions in cavitated lesions, and thus, high proportions of mutans streptococci and/or other aciduric bacteria can be considered markers of dental caries.

In this symposium, I will discuss a viewpoint of the caries process from ecological dynamics of oral biofilm. In the future, it would be getting less meaningful to discuss which bacterium is more associated with caries, but getting more important to know how microbial members of oral biofilm are involved in the caries process.

References:

Brief CV
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1998.04 - 2001.02 Associate Professor, Tohoku University School of Dentistry
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1998.10 to date Councilor, Japanese Association for Oral Biology
1998.12 to date Director, Cariology Today in Japan
2001. 4 to date Councilor, Tohoku Dental Society
2001. 4 - 2003. 4 Director, Tohoku Dental Society
2001. 4 to date Councilor, Tohoku Division of Japanese Biochemical Society
2002. 4 to date Director, Japanese Association for Oral Biology
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Oral microbiologists made huge effort to solve the question where did biofilm come from? We already have precise knowledge that the $gtf$ genes, Quorum-sensing genes and various important phenomenon for example Mfa1-SspB binding are related to oral biofilm formation. However, it seems to fail to solve what goes on afterwards? Oral biofilm; a living slime layer made of billions of bacterial cells, salivary proteins, and bacterial wastes and polysaccharides can easily reach a thickness on the teeth surfaces, and, over time, biofilm can become mineralized, eventually turning to dental calculus.

Mechanism of bioformation of dental calculus should be complicated, because there are several hundred of bacterial species.

Corynebacterium matruchotii, known as calcified bacteria, is a microbial inhabitant of the oral cavity probably associated with dental calculus formation. It produces membrane-associated proteolipid capable of inducing hydroxyapatite formation in vitro. This proteolipid was already purified from chloroform:methanol extracts by chromatography on Sephadex LH-20 and migrated on SDS-polyacrylamide gel electrophoresis at 6-9 kDa. The purified proteolipid induced calcium precipitation in vitro.

Furthermore, nanobacteria is a new additional candidate of promoter of dental calculus. The presence of nanobacteria discovered during the last decade in various pathogenic calcification such as kidney stones, gallstones and atherosclerosis. In 1998, Kajander and Çiftçioglu published a paper of "nanobacteria." Nanobacteria appear as self-propagating calcifying macro-molecular complexes found in human blood and saliva. It may be responsible for the formation of dental calculus which has a similar stone formation process of other ectopic calcifications. In 2000, Cisar et al. found that mineralization previously attributed to nanobacteria may be initiated by nonliving macromolecules derived from human saliva and dental plaque biofilm. Our lab group is now going on the isolation and purification of the substance of calcification of nanobacteria. Infection of nanobacteria may be considered to be a risk factor for the periodontal diseases.

Infection of nanobacteria may promote the biofilm calcification.
Brief CV

Professor Nobuhiro Hanada (Department of Translational Research, School of Dental Medicine, Tsurumi University, Yokohama, Japan) is a former Director of National Institute of Public Health.

His research focuses on the elimination of oral pathogenic microorganisms, especially the mutans streptococci and *Porphyromonas gingivalis*. He has published over 100 full length papers in refereed international journals. He also published over 100 Japanese journals and textbooks. He has lectured around in various Japanese Dental Universities and Japanese and Korean Dental Associations.

In 1999, he was appointed a member of governmental committee of "People's Health Promotion Campaign for the 21st Century (Health Japan 21)" and the Eminent Persons' Council on the New Health Frontier Strategy (2007).

For his research he received a FDI/Unilever Poster Award in Sweden (2008). Currently, he is a committee member of the International Association for Dental Research (IADR) and a trustee of Japanese Association for Dental Research (JADR).
Direct implication of peripheral sympathetic nerve system in bone metabolism

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Bone remodelling is the physiological process consisting of two balanced opposing activities: the formation of new bone by osteoblasts and the resorption of old bone by osteoclasts. Osteoblastic and osteoclastic activities are regulated by several systemic hormones, including parathyroid hormone, 1,25-dihydroxyvitamin D₃, calcitonin, glucocorticoids, sex steroids, and thyroid hormones, and pathological disturbances in the production or activities of these hormones lead to pathological conditions in the skeleton. Moreover, various factors are produced by bone cells themselves and others are released by non-bone cells in the vicinity. Such autocrine and paracrine factors include cytokines, growth factors, and prostaglandins. To some extent, the release of these local factors is controlled by the systemic hormones. In addition to endocrine and paracrine/autocrine mechanisms, bone remodelling, like most other homeostatic functions, is revealed to be also under sympathetic control.

The vertebrate skeleton is richly innervated with adrenergic and peptidergic nerve terminals, and these play important roles in bone remodelling. In recent studies, it has been evident that bone cells are equipped with functional receptors for several neuro-osteogenic factors; and, therefore, it has been proposed that signalling molecules in the nervous system may participate in the control of bone metabolism and that consequently a neuro-osteogenic network may exist, similar to the previously proposed neuro-immune and neuro-immune-endocrine interactions. Recent studies have generally shown that increased sympathetic nervous activity causes bone loss via an increase in bone resorption and a decrease in bone formation. Increased bone resorption is based on the stimulation of both osteoclast formation and osteoclast activity. These effects are associated with β2-adrenergic activity toward both osteoblastic and osteoclastic cells. Such findings indicate that β-blockers may be effective against osteoporosis, in which case there is increased sympathetic activity.

In recent years, it has been demonstrated that human osteoblastic as well as osteoclastic cells are equipped with adrenergic receptors and neuropeptide receptors and that they constitutively express diffusible axon guidance molecules that are known to function as a chemoattractant and/or chemorepellent for growing nerve fibers. These findings suggest that the extension of axons of sympathetic and peripheral sensory neurons to osteoblastic and osteoclastic cells is required for the dynamic neural regulation of local bone metabolism. However, while several studies have shown a functional nerve-bone cell interplay, whether both osteoblastic and osteoclastic cells activation occurs as a direct response to neuronal activation or requires an intermediary cell is unclear. Therefore, we examined direct nerve-osteoblastic cell communication using an in vitro co-culture model comprising mouse osteoblastic cells, MC3T3-E1, and neurite-spouting mouse superior cervical ganglia. Our findings demonstrate...
that osteoblastic activation, as judged by intracellular Ca\(^{2+}\) mobilization, can be a direct consequence of contact with a specific activated nerve fiber. Moreover, we provide evidence that this osteoblastic activation was mediated, at least in part, by the noradrenaline acting through \(\alpha_1\)-adrenergic receptors.

Here I will describe our current understanding of the adrenergic effect on bone resorption based on a variety of *in vitro* and *in vivo* studies. In addition, I will introduce the direct implication of peripheral sympathetic nerve system in bone metabolism.

**Brief CV**

1974 -1978 Ph.D. Degree, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan
1978-1985 Research Associate at Laboratory of Cell Physiology, Department of Life Chemistry, Graduate School at Nagatsuta, Tokyo Institute of Technology, Yokohama, Japan
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1984-1989 Lecturer at Department of Pharmacology, School of Dentistry, Aichi-Gakuin University, Nagoya, Japan
1989-1996 Associate Professor at Department of Pharmacology, School of Dentistry, Aichi-Gakuin University, Nagoya, Japan
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1996-present Professor at Department of Pharmacology, School of Dentistry, Aichi-Gakuin University, Nagoya, Japan
Bone mass is maintained by the balance between osteoblastic bone formation and osteoclastic bone resorption. It has been generally assumed that bone remodeling is mainly controlled by the local environment, i.e., autocrine or paracrine mechanisms. Increasing evidences that neurons and neurotransmitters are intimately involved in bone remodeling shed light on a novel regulatory mechanism for bone homeostasis. We have uncovered that leptin, an adipocyte-derived anorexigenic hormone, regulates bone mass through its receptor located in the central nervous system. Leptin is a 16kDa peptide hormone synthesized by adipocytes and it affects appetite and energy metabolism through its binding to the leptin receptor located in the hypothalamus. ob/ob mice that lack functional leptin are obese and sterile. In spite of hypogonadism, the most common cause of osteoporosis, ob/ob mice and db/db mice that lack a functional leptin receptor display high bone mass. Further analysis reveals that, while leptin receptor-deficient osteoblasts proliferate and differentiate normally, an intracerebroventricular (ICV) infusion of leptin to ob/ob mice or wild-type mice, even with a minimal dose that does not affect body weight, decreases bone mass.

Along with its anorexigenic effect, leptin exerts various physiological roles including sympathetic nervous system (SNS) regulation. Moreover, many osteoblasts reside next to sympathetic neurons in bone marrow and also express the beta2-adrenergic receptors (adrb2) specifically, indicating the interaction of SNS and bone remodeling. Indeed, mice treated with isoproterenol, a beta agonist, display a massive decrease in bone mass and mice that are blocked SNS signalling either genetically (adrb2-deficient mice or dopamine beta hydroxylase-deficient mice) or pharmacologically (wild type mice treated with beta blocker) all exhibit a high bone mass phenotype due to an increase in bone formation. These mice are also protected from the inhibition of bone formation by leptin. Thus, SNS is a major, if not the only, pathway that is responsible for the inhibitory role on bone formation by leptin.

Leptin and SNS also regulate osteoclastic resorption. In addition to increased bone formation, adrb2-deficient mice also display decreased bone resorption and possess fewer numbers of osteoclasts. Indeed, SNS and b2AR activation activates protein kinase A, and this in turn phosphorylates ATF4, an essential transcription factor for osteoblastic differentiation and function that induces Rankl (receptor activator of NFkappaB ligand) expression. Thus, leptin regulates bone resorption as well, through its action on osteoblasts.

Recently, we demonstrated that another neuropeptide regulate bone mass centrally: NeuromedinU is a neuropeptide which inhibits food intake by a leptin-independent mechanism. Accordingly, Nmu-deficient mice are hyperphagic and obese and, interestingly, present a high bone mass phenotype with an isolated increase in bone formation, similar to ob/ob mice. This phenotype is not cell-autonomous, since Nmu-deficient osteoblasts are...
indistinguishable from wild-type osteoblasts in vitro. Treatment of wild-type osteoblasts with NMU does not affect proliferation or differentiation, suggesting the central nature of NMU to regulate bone formation. In line with that and as in the case with leptin, NMU icv to Nmu-deficient mice and wild-type mice decreases their bone formation and bone mass. Importantly, leptin ICV infusion or b2AR agonist isoproterenol treatment does not decrease bone mass in Nmu-deficient mice, demonstrating that NMU mediates the action of leptin and SNS in the regulation of bone formation. Further analysis reveals that NMU in the hypothalamus affects only the negative regulator of osteoblast proliferation, namely the molecular clock.

Thus, like all other homeostatic functions, bone remodelling is under the control of hypothalamus. A growing amount of reports describing “unexpected” bone phenotypes in mutant mice deficient for neuropeptides or neurotransmitters has now established a new research area linking skeletal and neuronal biology.

Brief CV
1992 M.D. University of Tokyo, School of Medicine
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2004 Associate Professor (Junior), Department of Orthopaedic Surgery, 21 Center of Excellence Program, Graduate School, Tokyo Medical and Dental University
2005-2008 Associate Professor (Senior), Department of Orthopaedic Surgery, 21 Center of Excellence Program, Graduate School, Tokyo Medical and Dental University
2008-present Associate Professor, Department of Orthopaedic Surgery, 21 Center of Excellence Program, Graduate School, Tokyo Medical and Dental University

Awards:
President Book Award of American Society for Bone and Mineral Research (1997)
Young Investigator Award of Japanese Society for Bone and Mineral Metabolism (1997)
Young Investigator Award of American Society for Bone and Mineral Research (2000)
Corresponding author for the presentation: Most Outstanding Abstract Award for the 29th American Society for Bone and Mineral Research annual meeting (2007)
Possible role of L-glutamate signaling in bone remodeling

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The vesicular glutamate transporter (VGLUT) is responsible for vesicular storage of L-glutamate, and plays an essential role in L-glutamate-mediated chemical transduction in the central nervous system and some peripheral tissues. Recently evidence has accumulated to suggest the occurrence of L-glutamate-mediated signaling in bone cells, because osteocytes, osteoblasts and osteoclasts express plasma membrane type glutamate transporter(s) and receptor(s). However, it is unknown which type of cells in bone secretes L-glutamate and how the secretion is regulated. We investigated L-glutamate signaling in murine bone using VGLUTs as a marker for glutamate-secreting cell, and obtained the following results: (1) Among three isoforms of VGLUT, VGLUT1, 2 and 3, VGLUT1 is specifically expressed in mature osteoclasts as revealed with RT-PCR, Northern blot, Western blot and immunohistochemical analyses. (2) VGLUT1 and FITC-labeled degradation products are co-localized in the same vesicles, indicating the association of VGLUT1 with transcytotic vesicles in bone resorbing osteoclasts. (3) The VGLUT1 is active and actively transports L-glutamate upon the addition of ATP. (4) L-Glutamate and the bone degradation products are co-secreted through exocytosis upon depolarization with either KCl or ATP. (5) The metabotropic glutamate receptor 8 (mGluR8) was expressed in mature osteoclasts. Upon stimulation of the receptor with ACPT-I, a specific agonist, inhibits secretion of both L-glutamate and bone degradation products through an inhibitory cAMP cascade. (6) Trabecular bone volume of femora from VGLUT1 knockout mice was lower than wild-type mice. These results strongly support the idea that L-glutamate is stored in transcytotic vesicles and co-secreted with bone degradation products through transcytosis from osteoclasts. The L-glutamate signaling may be involved in autoregulation of bone resorption followed by regulation of transcytosis.

Furthermore, we investigated the mechanism of L-glutamate secretion from osteoclasts and the cause of osteopenia induced by VGLUT1 deficiency in mice in more detail. The results obtained from this work are as follows: (1) we first examined whether the signaling pathway of cAMP-dependent protein kinase (PKA) is involved in the secretion of L-glutamate from osteoclasts. Osteoclasts obtained from the co-culture of mouse osteoblasts and bone marrow cells were treated with KT-5720, a PKA inhibitor, and stimulated with KCl or ATP to induce L-glutamate secretion. KT-5720 scarcely inhibited the KCl and ATP-induced L-glutamate secretion from osteoclasts. (2) To determine the cause of bone loss in VGLUT1 deficiency in mice, we measured serum levels of TRACP5b (a marker of bone resorption) and alkaline phosphatase (a marker of bone formation) in 8-week-old VGLUT1 KO and wild-type mice. Serum levels of TRACP5b in VGLUT1 KO mice were significantly higher than those in wild-type mice. Serum alkaline phosphatase activities were also increased in VGLUT1 KO mice.
These results suggest that osteoclasts secrete L-glutamate through PKA independent pathway, and that bone loss in VGLUT1 KO mice is caused by an increase in bone resorption. The results also suggest that the lack of L-glutamate-mediated signals in bone induces a high turnover bone state.

Brief CV

1992  Ph.D. Graduate School of Showa University, Tokyo, Japan
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1987  D.D.Sc. Matsumoto Dental University, Nagano, Japan
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1992-1994  Research Associate, Department of Biochemistry, School of Dentistry,
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Awards:
2005  Lion Award (The Japanese Association for Oral Biology)
2002  Fuller Albright Award (The American Society of Bone and Mineral Research)
1995  Young Investigator Award (The American Society of Bone and Mineral Research)
1993  Society Prize (The Japanese Association for Oral Biology)
1990  Young Investigator Award (The Japanese Society of Bone and Mineral Research)
Sensory neuropeptides modulate bone remodeling

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Sensory neuropeptides, especially tachykinins and calcitonin gene-related peptides (CGRP), are widely distributed in the body and act as neurotransmitters and neuromodulators. The tachykinin family consists of at least five peptides, including substance P (SP), neurokinin A (NKA), and neurokinin B (NKB). The biological effects of SP, NKA, and NKB are mediated through neurokinin (NK)-1, NK-2, and NK-3 receptors, respectively. Tachykinins are associated with various biological processes, including pain transmission, neurogenic inflammation, smooth muscle contraction, and vasodilation. SP, a well-characterized tachykinin found mainly in unmyelinated c-fibers, is synthesized in the dorsal root ganglion and transported primarily to the periphery. Though bone displays abundant sensory neuronal innervation, particularly in the bone marrow of the patella and epiphyses, whether osteoblasts possess sensory neuropeptide receptors was unclear. In 1998, we demonstrated NK-1 receptor expression in rat osteoblasts by immunohistochemistry; however, the expression of NK-1 receptor in osteoblastic cells was refuted based on PCR data from other groups. Recently, we detected NK-1 receptor expression in mature primary osteoblasts, but not in immature primary osteoblasts or an osteoblastic cell line. Interestingly, hematopoietic growth factor inducible neurokin-1 type (HGFIN), which shares sequence similarity with NK-1 receptor, is expressed in benign cells but not in malignant tumor cells. The association of NK-1 receptor expression with osteoblastic cell malignancy or differentiation requires further study. Among the tachykinins, SP and NKB stimulate bone formation by primary osteoblastic cells. Semi-quantitative RT-PCR indicated that SP stimulated the mRNA expression of osteocalcin in osteoblasts; however, it did not stimulate the mRNA expression of runX2 or type I collagen. Given that NKB is believed to act on the central nervous system, whether NKB modulates peripheral bone metabolism is unclear. These findings suggest that sensory neuropeptides, or at least SP, directly modulate osteoblastic bone formation. Regarding the effects of tachykinins on osteoclastic bone resorption, SP was found to stimulate osteoclastic bone resorption via NK-1 receptor in vitro. SP stimulates both osteoclast formation and the bone resorptive activity of osteoclasts. Though in vitro studies have clearly shown the stimulation of osteoclastic bone resorption by SP, evidence of the involvement of tachykinins in osteoclastic bone resorption in vivo is lacking. The indirect effects of SP on bone metabolism have been examined. Synovial fibroblastic cells derived from rat knee joint expressed NK-1 receptor; moreover, the proliferation and expression of RANKL in those cells was stimulated by SP, which suggests that the release of SP from peripheral nerve endings during chronic arthritis is a risk factor for the development of arthritis. Sensory neuropeptides, such as SP and CGRP, are also associated with bone remodeling in response to mechanical stress during orthodontic tooth movement. In the presence of neuropeptides, the level of OPG decreased synergistically with compression. Neuropeptides stimulated RANKL expression without compression, whereas they decreased the
mRNA expression of RANKL with compression. These results indicate that periodontal ligament cell compression induces the up-regulation of RANKL and down-regulation of OPG, whereas neuropeptides suppress the compression-induced expression of RANKL. These findings suggest that the dull pain induced during orthodontic treatment may be involved in the stimulation of bone remodeling by mechanical forces. Since SP and NK-1 receptor knockout mice exhibited no morphological variation, SP or NK-1 receptor may not be essential for development. Neuropeptides from sensory neurons probably modulate or activate bone metabolism in response to nociceptive stimuli. Sensory neuropeptides are crucial for biological defense as well as the maintenance of local homeostasis.

**Brief CV**

1982-1988, Faculty of Dentistry, Kyushu University, DDS.
1988-1992, Graduate School of Dentistry, Kyushu University, Ph.D.
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1994-1996, University of Toronto, Canada, Post Doctoral Fellowship
1992 Kyushu University Dental Hospital, Pediatric Dentistry, Clinical Fellow
1992 Kyushu University, Faculty of Dentistry, Department of Oral Anatomy, Assistant Professor
2001 to date, Kyushu Dental College, Division of Anatomy, Associate Professor
POSTER PRESENTATION
001 Three-dimensional & layered culture method for tooth induction and development

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Objectives: For in vitro resustrction of tooth development, which undergoes a series of epithelial-mesenchymal interactions, a novel three-dimensional & layered culture method (TDL culture) was introduced and tested on odontogenic cells derived from rat incisor teeth.

Methods: Epithelial cells and mesenchymal cells were prepared from the mandibular incisors of 10-day-old rats and the former cells were inoculated to the collagen solution within a φ 4.5mm plastic cylinder. After the collagen gel had been polymerized, dental epithelial cells were layered on the gel containing dispersed mesenchymal cells, thus combining the three-dimensional culture of dental mesenchymal cells with layered culture of dental epithelial cells, which we designated the 3D & layered culture (TDL culture). One day after inoculation, the TDL gel was removed from the cylinder and floated in the medium (TDL floating culture). The TDL gels were then fixed, embedded, and processed for routine histological examinations. The cells inoculated to collagen-coated 96well-plate for the mono-layered cultures were used as control.

Results: During TDL culture, the gel shrank to 1/3-1/5 in size depending on the cell density. Undistorted shrinkage of the gel and cell layers was attained when the proportion of epithelial/mesenchymal cell density was 1:2. Proliferation, differentiation, and morphology of both types of cells differed depending on the culture medium used, but cells in TDL culture generally showed better morphology compared to that in mono-layered culture. Further, matrix-like structure was deposited at the interface between the layers of epithelial and mesenchymal cells. Processes of mesenchymal cells extended vertically to the epithelial cell layer in the upper marginal region of the gel but much less in the deeper layers away from the interface.

Conclusion: Our TDL culture is suitable for both epithelial and mesenchymal cells prepared from rat incisors, and useful for in vitro analyses of epithelial-mesenchymal interactions.

002 Treponema denticola dentilisin involvement in periodontopathic bacterial coaggregation

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Objectives: Treponema denticola, Tannerella forsythia and Porphyromonas gingivalis are frequently coisolated from periodontal lesions, and this combination of microorganisms has become known as the ‘red complex’. Coaggregation plays a key role in the organization of biofilm by these microorganisms. Although several adhesion factors have been identified for T. denticola, the ligand by which it achieves coaggregation remains to be determined. The purpose of this study was to clarify the component involved in coaggregation between T. denticola and T. forsythia.

Methods T. forsythia ATCC43037 and T.denticola ATCC35405, ATCC33520, K1, KpSano and DMSP3 were used in this study. T.denticola K1 and KpSano are dentilisin-deficient mutants constructed by homologous recombination of wild-type strains T.denticola ATCC35405 and ATCC33520, respectively. T.denticola DMSP3 is a major outer sheath protein-deficient mutant constructed from T.denticola ATCC35405. Coaggregation between T. denticola and T. forsythia was evaluated by measuring OD660 using a spectrophotometer. The molecular mass of the ligand involved in coaggregation between T.denticola and T. forsythia was determined by overlay assay.

Results Wild-type strains T.denticola ATCC35405 and ATCC33520 coaggregated with T. forsythia ATCC43037, whereas T.denticola K1 and KpSano decreased coaggregation reaction compared with wild-type strain about 55% and 45%, respectively. On the other hand, coaggregation was observed between T.denticola DMSP3 and T. forsythia ATCC43037.

Conclusion The results suggest that dentilisin is involved in coaggregation between T.denticola and T. forsythia.
Preferred chewing-side dependent two-point discrimination and cortical activation of tactile tongue sensation

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Objectives: The purpose of this study was to investigate 1) whether the sensory threshold of the tongue differed according to the side, and 2) whether the pattern of hemispheric cortical activation by tactile tongue stimulation differed, with special attention to the preferred chewing side (PCS).

Methods: Ten healthy adults participated in the study. The PCS was determined with the use of a mandibular kinesiograph. Experiment 1 (behavioral study): The mean thresholds of the two-point discrimination (TPD) of the anterior, canine and posterior regions on both sides of the tongue, and those between PCS and non-PCS in each region were statistically compared. Experiment 2 [functional magnetic resonance imaging (fMRI study)]: Tactile stimulation was delivered to the either side of the tongue with acrylic balls via a mandibular splint. The runs were measured with the use of a T₂*-weighted gradient echo-type echo planar imaging sequence in a 1.5T scanner. Activated voxel numbers in the bilateral S₁ were statistically compared.

Results: Experiment 1: The threshold of TPD increased in order of the anterior, canine and posterior regions. Moreover, it was significantly smaller on the PCS than the non-PCS in both the canine and posterior regions. Experiment 2: There was a marked difference in the activated pattern localized in S₁ contralateral to the stimulated side: a broader activation area with greater fMRI signal intensity in S₁ contralateral to the PCS was found. The activated voxel numbers in S₁ contralateral to the PCS was significantly greater than that in S₁ contralateral to the non-PCS.

Conclusion: The present study shows that the PCS is associated with the asymmetric tactile sensation and cortical activation of the tongue. The sensory acuity of the tongue on the PCS may play an important role in a functional coupling between the jaw and tongue to maximize the efficiency of chewing.

Sodium fluoride induces apoptosis in human gingival epithelial cell line

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Objectives: Sodium Fluoride (NaF) is one of the most popular compounds in preventive dentistry, however, high concentration and/or chronic intake of fluoride may be toxic to human tissues. On the other hand, apoptosis of gingival crevicular epithelial cells plays an important role in the process of gingival conditions. In this study, we determine if fluoride induces apoptosis in human gingival epithelial cells (HGEC), and elucidate its mechanisms.

Methods: HGEC (Ca9-22, HSRRB, Osaka) were incubated in 100, 150, 200 or 250ppm of NaF medium at 37oC. Concentrations of NaF were much lower than concentrations in clinical dose. After 24h incubation, HGEC were double stained with Annexin V and 7-AAD to detect apoptosis, and analyzed using a flow cytometer. Key apoptotic enzymes, caspase-8 and 9, and reactive oxygen species (ROS) also assessed by a flow cytometer using fluorescent markers. Fragmentation of DNA, Caspase-3, and cytotoxicity (LDH activity) were determined using ELISA.

Results: The levels of LDH and ROS increased with concentration dependently. Early apoptosis and caspase-3 activity were significantly increased in 150 and 200ppm NaF that in comparison with 100, 250ppm NaF and control. DNA fragmentation were also showed 5-7 times higher than the control. At 150 and 200ppm NaF, caspase-3, 8 and 9 activities were significantly increased by 40-50%.

Conclusion: The present results demonstrated that the range of NaF concentration causing apoptosis in HGEC is 150-200ppm. Results of caspase activities and ROS assay indicated that one of the apoptotic pathway caused by fluoride is mitochondrial-mediated.
Impaired tooth development in reduced scale medaka mutant (rs-3)

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Background Both scales and teeth are thought to derive from exoskeleton of primitive vertebrates. However, no concrete evidence for the hypothesis is currently available. Medaka (Oryzias latipes) has oral and pharyngeal dentitions and is recently recognized as fine animal model to examine ontogeny and evolution of tooth. A mutation of medaka reduced scale-3 (rs-3) is characterized by lack of scales leaving few large-sized irregular-shaped scales around the dorsal fin and along the lateral lines. The causative gene for this defect encodes ectodysplasin A receptor (Edar). Ectodysplasin A (Eda) pathway is known to play an important role in ectodermal organogenesis including that of teeth in humans.

Objective To examine the influence of Edar deficiency in oral and pharyngeal teeth in rs-3 medaka, and gain insights into putative evolutional pathways from scales to teeth.

Methods Adult rs-3 and normal medaka of both sexes were used for the analysis. After counting scale numbers, the entire numbers of oral and pharyngeal teeth were counted in each medaka. Scales, jaws and tooth-bearing pharyngeal bones were embedded in plastic and processed for histological and histochemical analyses.

Results The number of functional teeth was much smaller in rs-3 medaka compared with that of normal medaka in both oral and pharyngeal regions. Magnitude of tooth loss, structural changes and erratic arrangement of teeth were more prominent in the pharyngeal regions relative to those in the jaws. The number of remaining scales varied considerably among the individual rs-3 medaka but no apparent correlation was noted among the scale number, oral tooth number and pharyngeal tooth number.

Conclusion These data indicate that lack of Eda receptor impairs tooth formation in medaka, most prominently in the pharyngeal regions where teeth are thought to develop through endodermal epithelial-mesenchymal interactions.

Initial response of osteoblast-like cells to zirconia and titanium

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Objectives: From more than 20 years ago, biocompatibility of zirconia has been investigated as a dental implant material in vitro and in vivo. Recently, Ceria stabilized zirconia/alumina nanocomposite (NANOZR) was introduced as dental ceramics. We have been mainly studied its attractive mechanical properties. The aim of this study was to investigate the biocompatibility of osteoblast-like cells attachment with the surface of NANOZR and yttria stabilized zirconia (3Y-TZP) in comparison to a commercially pure titanium (Ti).

Materials and Methods: Disks of NANOZR, 3Y-TZP, and Ti were used in this study. Osteoblast-like cells (MC3T3-E1) were placed on NANOZR, 3Y-TZP and Ti in 24-well tissue culture plates. Ti was employed as a control specimen. Cell attachment in each well was measured using a kit (Cell-counting kit-8). Cell morphology was observed by scanning electron microscopy (SEM). Integrin α5 and α1 expression were evaluated by flow cytometric analysis after 3-hour incubation. For actin staining, cells were incubated with Alexa 465-phalloidin for 1, 3 and 6h.

Results Time-dependent attachment of MC3T3-E1 was observed in all three materials. SEM photographs demonstrated that cells on all the plates attached and spread well. MC3T3-E1 expressed integrin α5 and α1, and showed typical long and straight stress fibers running across the cell body after 6-hour incubation on all the plates. There were no significant differences in the attachment among the three materials.

Conclusions: MC3T3-E1 attached on zirconia plates as well as titanium. There were no significant differences in the cell attachment among NANOZR, 3Y-TZP and Ti, and indicating that NANOZR and 3Y-TZP were as biocompatible as Ti.
Nociceptive stimulation induces satellite glial cell activation

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Objectives: In response to oral nociceptive stimulation, trigeminal ganglion (TG) neurons produce various neuropeptides that are secreted retrogradely and modulate inflammation. Neurons in the TG are surrounded by satellite glial cells (SGCs), which passively support the function of the neurons; however, little is known about the interactions between SGCs and TG neurons after peripheral nerve injury. To examine the effect of nerve injury on SGCs, we investigated the relationship between cell injury and satellite cell activation using rat TG neurons and SGCs following upper molar extraction.

Methods: Rats were anesthetized and their upper molars were extracted. Three, seven, or ten days after extraction, the animals were perfused transcardially with 4% paraformaldehyde under deep anesthesia with diethyl ether and the TG was removed. Cryosections of the ganglia were immunostained with antibodies against glutamine synthetase (GS), a glial cell marker, glial fibrillary acidic protein (GFAP), a marker of activated SGCs, protein-gene product 9.5 (PGP-9.5), a neuronal cell marker, and ATF3, a marker of damaged neurons.

Results: After tooth extraction, the number of neurons enclosed by GFAP-immunoreactive (IR) SGCs increased in a time-dependent manner in the maxillary nerve region of the TG. Three days after extraction, ATF3-IR neurons appeared in the maxillary nerve region that persisted until day 10. The ATF3-IR neurons were surrounded by GFAP-IR SGCs. After extraction, ATF3-IR neurons were not detected in the mandibular nerve regions; however, the number of GFAP-IR SGCs was increased in both maxillary and mandibular nerve regions.

Conclusions: Our results suggest that peripheral nerve injury affects the activation of TG neurons and the SGCs around the injured neurons: moreover, our data suggest the existence of a neuronal interaction between maxillary and mandibular neurons via SGC activation.

Simvastatin enhances differentiation of human dental pulp stem cells

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Objectives: Statin, HMG-CoA reductase inhibitor, is known to promote osteogenesis. However, it is not clear whether statin promotes dentinogenesis, as well. The purpose of this study was to investigate the effects of statin on the behavior of human dental pulp stem cells (DPSCs). Methods: DPSCs were isolated from human third molars under approved guidelines and informed consent. DPSCs at 3 - 6 passages were used in this study and cultured with 1 μM simvastatin (statin), one of the commonly used statins. Cell proliferation was evaluated with MTS assay, and cell cycle was analyzed with FACS. Differentiation of DPSCs was confirmed with quantitative RT-PCR through the expression levels of osteocalcin (OCN) and dentin sialophosphoprotein (DSPP). Furthermore, DPSCs treated by statin was transplanted into immunocompromised mice to evaluate their hard tissue formation in vivo. BMP-2 (100 ng/ml) was used as a positive control. Statistical differences were analyzed by one-way ANOVA, followed by post-hoc test. Results: Statin suppressed cell proliferation significantly (p <0.0001, at day-5 culture against PBS), but BMP-2 did not. It is confirmed with FACS that this suppression was the result from accumulation in the sub-G1 phase of cell cycle. Gene expression analysis revealed that both OCN and DSPP were upregulated significantly when DPSCs were cultured for 14 days with statin (p <0.0001, against PBS). The upregulation caused by statin was much effective compared to that by BMP-2. From the results of in vivo transplantation, it is revealed that statin accelerated hard tissue formation. Conclusion: These results indicate that statin is a candidate for enhancer of DPSCs differentiation, possibly lead to acceleration of dentinogenesis. Supported by JSPS grant (17209062) and MEXT grant (19689038).
009  **Electrolyzed water with functional-chlorine effectively penetrates deep into cariogenic biofilms**

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**Objective** This study was designed to evaluate the efficacy of electrolyzed waters containing different functional-chlorine concentration levels on cariogenic bacteria and biofilm. **Methods:** Electrolyzed hypochlorite water (PerfectPerioTM water: PPW, Noguchi Dental Medical Research Institute: Noguchi-DMRI, Japan). PPW was diluted: x2 (PPW-2), x4 (PPW-4), x6 (PPW-6) and x10 (PPW-10). Milli-Q water and 0.5% NaOCl were included as controls. Viability of the bacterial cells was assessed by staining with BacLightTM Bacterial Viability Kit followed by fluorescence microscopy and counting colony forming units (CFU/ml) after 10sec PPW treatment. Proteins of PPW treated Streptococcus mutans MT8148 (S. mutans) were inspected by using SDS-PAGE and two-dimensional gel electrophoresis (2DE). Also, biofilms were grown on equal-shaped bovine enamel coupons using four species of oral streptococci (S. mutans, Streptococcus sobrinus, Streptococcus gordonii and Streptococcus mitis) at 37°C for 12 hrs in an oral biofilm reactor. After treatment with PPW the bacteria were separated from three different levels (upper, middle and bottom) of the biofilms by shaking and CFUs/ml counted in each level. Further, to neutralize the functional-chlorine, PPW was diluted with solution of sodium ascorbate (pH7.5). **Results:** Viability test and CFU data showed that almost all bacteria were killed by the diluted PPW down to the concentration of 100ppm. Protein analysis indicated that S. mutans proteins were damaged on PPW treatment. Interestingly, no colony formation could be detected even in case of samples were plated from the most bottom part of biofilms. Further, it was confirmed that the functional-chlorine concentration in PPW was reduced to 2ppm from 600ppm by sodium ascorbate, but maintained low alkali pH level. **Conclusion** PPW maintained as potential bactericide even after penetrating deep into the bottom of the cariogenic biofilms. Also, it was found that sodium ascorbate solution effectively neutralizes PPW. This study was supported by G-COE Program, IRCMSTBD at TMDU and Noguchi-DMRI.

010  **Human gingival fibroblasts release HMGB1 through active and passive pathways**

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**Objectives:** High mobility group box 1 (HMGB1), known widely as a nuclear protein, acts as a proinflammatory cytokine extracellularly. HMGB1 can be actively secreted by monocytes stimulated with lipopolysaccharides (LPS), TNF-α or IL-1. It can also diffuse passively from necrotic cells. In periodontitis, HMGB1 was strongly detected in the gingival crevicular fluid and gingival epithelial cells were able to secrete it after being stimulated with TNF-α, suggesting a possible contribution of HMGB1 in the progression of periodontitis. In this study, we investigated whether human gingival fibroblasts (HGF) can produce HMGB1 after cell stress such as stimulation with LPS from periodontopathogens and necrotic and apoptotic cell death. **Methods:** HGF from healthy periodontal tissue were cultured and stimulated with LPS from Aggregatibacter actinomycetemcomitans (A.a), Porphyromonas gingivalis (P.g), and Escherichia coli (E.coli). We also initiated apoptotic and necrotic cell death in HGF. The amounts of HMGB1 released from stimulated or dying cells were measured by ELISA. **Results:** A significantly higher amount of HMGB1 diffused from necrotic and apoptotic fibroblasts compared to control. LPS from A.a, P.g and E.coli significantly induced the production of HMGB1 in a dose and time dependent manner. After 6 hours of stimulation with A.a LPS, HMGB1 was visible in the cytoplasm of cells. However, its location was mainly nuclear after 24 hours. The cytotoxicity assays showed that the amounts of LPS used were not cytotoxic indicating that HMGB1 can be actively secreted by stimulated cells. **Conclusions:** LPS from two major periodontopathogens A.a and P.g induced HMGB1 secretion from HGF. Apoptotic and necrotic cell deaths resulted as well in the enhancement of HMGB1. Our results suggest that HMGB1 may be an important factor in the aggravation of periodontal disease.
Nicotine induction of CCN2: from smoking to periodontal fibrosis

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Objectives: In clinical cases, gingival hyperplasia is often observed among smokers. However, there has been no report that indicates distinct relation between the thickening/fibrosis of periodontal tissue and habitual smoking. The aim of this study was to investigate the effects of nicotine on the production of a profibrotic molecule, connective tissue growth factor (CCN2/CTGF) in human gingival fibroblasts (HGFs) and periodontal ligament (PDL) cells. Methods: HGFs and PDL cells were isolated from normal periodontal tissues. Subconfluent cells were stimulated with 0 (control), 0.1, 1 or 10 μg/ml nicotine for 12-48 h. Effect of nicotine on CCN2/CTGF and type I collagen mRNA expression was evaluated by RT-PCR. Production/distribution of CCN2/CTGF protein in the cells was observed using immunofluorescence microscopy. Effect of nicotine on CCN2/CTGF, type I collagen, MMP-1 and TGF-ß1 production was quantitatively analyzed by ELISA. Cell growth assay and morphological analysis were also performed. Results: Proliferation of both cells and expression of Ccn2/Ctgf mRNA in HGFs were slightly increased at 0.1 μg/ml of nicotine (p<0.05). Interestingly, 1 μg/ml nicotine increased the production of CCN2/CTGF protein in both cells without increasing the mRNA expression (p<0.05). Immunofluorescence analysis revealed higher protein levels at 1 μg/ml in both cells compared to control. At 1 μg/ml, type I collagen mRNA (p<0.01), protein (p<0.05) and TGF-ß1 levels were increased, whereas MMP-1 was not significantly induced. Vacuolization and attenuated proliferation were observed at the same dose. Conclusion: This is the first study to show a relationship between CCN2/CTGF and nicotine in periodontium. Our previous study indicated that CCN2/CTGF is induced by TGF-ß1 (Takeuchi et al. 2008, in press). Since CCN2/CTGF is a profibrotic molecule, our results suggest that periodontal fibrosis is promoted by long-term synergistic induction of CCN2/CTGF by 2 distinct molecules in smokers: one is exogenous nicotine, and the other is endogenous TGF-ß1 from inflammatory tissues.

Antifungal susceptibility of C. albicans and its hyphal mutants

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Objectives: Biofilm formation is a major virulence attribute of Candida species which confer higher antifungal resistance to this mode of growth. We investigated the role of transcriptional regulators Efg1 and Cph1 in relation to the antifungal susceptibility of Candida in the planktonic, adhesion and biofilm modes. Methods: A wild type strain of C. albicans and its hyphal mutants were used to evaluate the kinetics and antifungal susceptibility of caspofungin (CAS), amphotericin B (AMB), nystatin (NYT), ketoconazole (KTC) and flucytosine (5FC). Standard CLSI method was used for determination of planktonic MIC. Then, Candida biofilms were developed on polystyrene wells and MIC determined using a standard XTT assay. Subsequently, antifungal susceptibility testing was performed for higher inoculum size (1×10⁷ cells/ml) of planktonic Candida. Furthermore, Candida adhesion phase was also subjected to antifungal susceptibility testing. Results: Compared to planktonic mode, adhesion and biofilm mode of growth were resistant to all the antifungals tested. Fungistatic drugs KTC and 5FC were not effective against high density planktonic cultures, adhesion or biofilm modes of Candida. Although Δ efg/efg1 and ΔΔ cph/cph1 efg/efg1 mutants formed less biofilm than wild type C. albicans SC5314, they were similarly resistant to CAS. However, latter mutants were more sensitive to AMB and NYT. Conclusions: These data indicate that adhesion per se confer fungi a somewhat higher resistance to antifungals which further argument in the biofilm mode. However, the present study also shows that filamentation is not an absolute prerequisite for antifungal resistance in the biofilm mode of Candida.
Antitumor effects of bleomycin by electrochemotherapy using local electric pulses

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Objectives: In this study, the effects of electrochemotherapy with bleomycin on human uterine squamous cell carcinoma was investigated metrically and histologically.

Methods: Uterine squamous cell carcinoma was transplanted into the dorsal subcutaneous tissue of BALB/c nude mice. After transplantation, the animals were randomly divided into four equal groups. These groups received either no treatment (D-E-); 30mg/kg body weight bleomycin treatment without electroporation (D+E-); electroporation without bleomycin treatment (D-E+); or 30mg/kg body weight bleomycin treatment followed by electroporation (D+E+).

Results: These treatment performed four times of 0, 1, 7, 8 days, and the tumor volume were measured every other day for 14 days, and were investigated histologically. Electrical pulse treatment together with bleomycin injection markedly reduced the size of the tumor, whereas bleomycin injection or electrical pulse treatment alone did not.

Conclusions: These results clearly indicate that the antitumor effect of bleomycin on human uterine squamous cell carcinoma was considerably potentiated or enhanced by the administration of local electrical pulses at low voltages.

Use of fentanyl with a pharmacokinetic during oral surgery

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Objectives: The use of opioids in general anesthesia is a key component in the current notion of balanced anesthesia. The aims of this study was to investigate the efficacy of using a pharmacokinetic simulation model for the administration of fentanyl in the oral surgery.

Methods: Sixty two patients (ASA1~2) undergoing oral surgery (extraction for impacted teeth, cystectomy, mandibular osteotomy for prognathism, neck dissection and the others) were enrolled in the study. Anesthesia was induced with propofol (target concentration at 3~5ug/ml), fentanyl 1.5~3.0ug/kg, and with vecuronium bromide 0.1mg/kg in order to facilitate endotracheal intubation. After the patients' tracheas were intubated anesthesia was maintained with 0.6~1.5% sevoflurane and 50% oxygen. Fentanyl was administered intermittently to maintain an effect site concentration at 1~2.5ng/ml using a pharmacokinetic simulation software (Palmakokinetics).

Results: The time required for emergence (from the end of surgery to extubation) was 12.6±7.3 minutes. The respiratory rates after five minutes from extubation were 12.5±2.9 breaths/min. The effect site concentration were 1.01±0.25ng/ml at this point. No adverse reactions such as respiratory depression were observed during the study. A few patients needed analgesic in the ward.

Conclusions: We suggest that the simulation model of fentanyl can accurately predict its effect site concentration, which enables to use the opioids without the risks of its adverse reactions such as respiratory depression, thereby paving the way of opioid-based anesthesia to the area of oral surgery.
Development and verification of a rapid oral bacteria detection system


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Objectives: A rapid oral bacteria detection system was newly developed by Panasonic Shikoku Electronics Co., Ltd. for evaluation of oral hygiene by measuring the amount of oral bacteria. This device employed a dielectrophoretic impedance measurement (DEPIM) method that enabled measurement of the amount of oral bacteria easily and quickly (approximately within several tens of seconds), without using any reagents. The aim of this study was to examine the efficacy of this device for clinical application.

Methods: As an exploratory experiment, various concentrations of Escherichia coli K-12 strain as a standard specimen was measured using this device to confirm the measurement performance. After confirmation, we detected bacteria obtained from the dorsum of the tongue in 98 healthy adults and 251 dependent elderly persons, and evaluated the correlation between the measurements obtained by the DEPIM method and the existing culture method. In addition, we examined the effect of the pressure strength during sampling specimens on the number of bacteria obtained.

Results: The correlation coefficient between the bacterial concentrations calculated by the existing culture method and the DEPIM method in healthy adults and dependent elderly persons was R = 0.86 and R = 0.85, respectively, showing a high correlation. The number of oral bacteria in dependent elderly persons showed stronger correlation than that in healthy adults. In addition, there was a relationship between the pressure strength during sampling specimens and the number of oral bacteria. The number of oral bacteria increased with an increase in pressure strength.

Conclusions: It was suggested that the rapid oral bacteria detection system is effective for monitoring oral hygiene for clinical application.

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Cleaning efficacy of an experimental rinsing/disinfecting solution

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Objectives: The aim of the study was to evaluate the cleaning efficacy of an experimental rinsing/disinfecting solution (RDS) for general dental use on proteins and lipids. Methods: Dye solution containing a commercial mayonnaise and blue dye was painted onto the surface of alginate impression, resinous denture, self-cured resin and Au-Ag-Pd alloy as cast. RDS was applied onto surfaces for 30 sec and rinsed with water for 30 sec. The remained dye was removed with acetone and determined by means of photospectrometer. Clearance rate was calculated by the following formula; (w-x)/w×100(%), where x=y/2.3716, w: painted dye solution (g), y: absorbance at 630nm, x: remained dye solution (g). For control, the surface was water-rinsed for 60 sec.

Results: Clearance rates (RDS/control (%)) for alginate impression, resinous denture, self-cured resin and alloy were 98.5/6.2, 99.3/51.0, 99.3/53.3 and 99.9/63.9, respectively. Significant difference was found between RDS and control (p<0.05). It has been reported RDS has no influence on the accuracy of impression materials and disinfected effect against S. aureus, C. albicans, S. mutans, A. viscosus, P. gingivalis and A. actinomycetemcomitans. The cleaning effect for proteins and lipids may facilitate the disinfecting effect of RDS. Conclusion: RDS has a promising cleaning and disinfecting efficacy for dental materials.
017  The capacity to produce hydrogen sulfide in *Streptococcus intermedius*

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**Objectives:** Hydrogen sulfide (H2S) is a toxic gas that induces the modification and release of hemoglobin in erythrocytes. The protein encoded by *lcd* catalyzes β-elimination of L-cysteine to produce H2S. *Streptococcus intermedius* belongs to the anginosus group of streptococci together with *S. anginosus* and *S. constellatus*, both of which were shown to have a high capacity to produce H2S. In contrast, the capacity in *S. intermedius* remains to be elucidated. In this study, we evaluated the capacity of H2S production in *S. intermedius* and determined its molecular basis. **Methods:** The capacity of crude extract to produce H2S was compared among 29 strains of *S. intermedius*, 6 strains of *S. gordonii*, 6 strains of *S. anginosus* and 6 strains of *S. constellatus*. The *lcd* gene was sequenced and cloned, and the encoded protein was purified for enzymatic characterization. The quantification of *lcd* were performed by real-time PCR. **Results:** The crude extracts from *S. intermedius* strains mostly have a high capacity to produce H2S, whereas 3 of 29 crude extracts from *S. intermedius* strains had no significant capacity. Expectedly, *lcd* from the three strains, which were *S. intermedius* strains ATCC 27335, IMU151 and IMU202, contained mutations or small deletions. The capacity in strain ATCC 27335 was restored by repairing *lcd* in its genomic DNA. The kinetic properties of the purified protein encoded by the repaired *lcd* gene were comparable to those of native proteins from H2S-producing strains, while the truncated protein from strain ATCC 27335 had no enzymatic activity. However, real-time PCR analysis indicated that *lcd* in strains ATCC 27335, IMU151 and IMU202 is transcribed and regulated in a manner similar to that in the H2S-producing strains. **Conclusion:** Molecular basis of H2S production in *S. intermedius* was determined.

018  IL-6 Signaling in gingival epithelial cells by *Candida albicans* infection

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**Objectives:** The presence of yeasts in periodontal pockets is well known, and *Candida albicans* is the most commonly isolated species from the oral cavity. The immune response and antifungal activity of oral keratinocytes play a key role in host defense against *C. albicans* infection. It has been reported that *C. albicans* challenged IL-8 production in human gingival epithelial cells (HuGE), however, the molecular mechanism of *C. albicans* infection in periodontal diseases is unknown. To clarify the role of *C. albicans* in periodontitis, gene expression profiles in *C. albicans*-infected HuGE were monitored. **Methods:** Primary human gingival epithelial cells were cultured from healthy human gingival tissues, and stimulated with *C. albicans* ATCC90029 for 8 hrs. Total RNA was extracted and monitored mRNA levels using Affymetrix GeneChip (Human Genome U133 plus 2.0 array, ca. 47,000 genes). GeneChip data was analyzed by GeneSpring software and Ingenuity Pathway Analysis (IPA) system. Altered mRNA levels in GeneChip results were confirmed by RT-PCR and real-time PCR. Rat gingival tissues were challenged with *C. albicans* live cells, and gingival tissues were collected, and then immunohistochemical examination was carried out. **Results:** GeneChip results demonstrated that many gene expressions were altered by *C. albicans*, including up-regulated IL-6. IPA analysis reveals that IL-6 signal pathways is involved in induction of IL-8 production through NF-IL6. Strong immunoreactivity against IL-6 was observed in the rat gingival epithelium by *C. albicans* infection. **Conclusion:** *C. albicans* infection may contribute to the modulation of periodontitis through activation of the IL-6 signal pathway in gingival epithelium.
An optimal umami taste stimulating system for brain mapping

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Objectives: fMRI studies of taste tend to decrease specificity of the results by head movements associated with swallowing. The purpose of this study was to develop a new system that spreads taste stimuli on the dorsal surface of the tongue as widely as possible and also prevents swallowing to perform functional brain mapping of the umami taste sense.

Methods: The delivery system of taste solution consisted of an intra-oral and an extra-oral device. The intra-oral device was assembled with four solution-inlet tubes and an outlet tube that were attached to an individual mouthpiece. The outlet tube was connected to a continuous suction apparatus. Healthy volunteers (5 male and 3 female, age range 19-28 years) participated in this study. The Human Experimentation Committee of Kyushu University approved all experimental procedures. The taste stimulus was umami (0.1M monosodium L-glutamate and 0.005M inosine monophosphate), salty (0.1M sodium chloride), and a tasteless control solution containing the main ionic components of saliva (25 mM KCl plus 2.5 mM NaHCO₃). All images were acquired with a whole-body 3.0-T MRI scanner. Image processing and data analysis were performed using the Statistical Parametric Mapping 5 software package (Wellcome Department of Cognitive Neurology, London, UK).

Results: This device delivered the taste solution on most of the dorsum part of the tongue and prevented swallowing. The head motions of all subjects were less than 1.5 mm in translation and less than 3.0 degrees in rotation in all sessions. The area activated by umami was located on the putative human primary taste cortex (uncorrected P < 0.001).

Conclusion: This intra-oral device stimulated the subject’s tongue as much as possible under constant conditions, and suppressed each subject’s head movements sufficiently. This system will make it possible to perform precise functional brain mapping of the taste cortex induced from the umami substance.

Hypoglossal motoneuronal activities induced by NMDA in brainstem slice preparation from newborn rats.

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OBJECTIVE: Tongue muscles innervated by the hypoglossal nerve play an important role to ensure airway patency and nutrition intake. Although both suckling in neonates and mastication in adults involve rhythmic tongue movements, early development of the brain includes dramatic changes in morphology and function. Since these oscillations may differ in location where they are organized and mechanisms which they are generated. Several lines of evidences indicate that masticatory rhythm is generated in the pons and medulla, mean while NMDA-induced sucking like activities are induced in the medulla itself. We investigated the NMDA-induced XII m activities using an in vitro preparation. METHOD: NMDA-induced activities in hypoglossal motoneurons (XII m) were examined using whole cell patch-clamp recording in coronal brainstem slices obtained from neonatal rats (P0-P7). We observed XII m activities induced by bath-application of NMDA with or without TTX. RESULTS: In current-clamp mode, NMDA application produced membrane depolarization followed by continuous firing. In a few XII m (3/33), rhythmic membrane potential changes superimposed of burst activity were observed after continuous firings. TTX application did not block membrane depolarization but firing. In voltage-clamp mode, NMDA application induced rhythmic membrane current changes at -65 mV of holding potential. The rhythmic current changes were enhanced by additional application of bicuculline (Bic) and strychnine (Stry). Negative slope conductance of N-shaped I-V relation obtained under NMDA application enhanced in simultaneous application of Bic and Stry. CONCLUSION: These data suggest that XII m per se enables to evoke rhythmic activities during NMDA receptor activation, and that activated NMDA receptors exist in more distal dendrites than inhibitory amino acid receptors reside. During early development, motoneurons would have supplementary roles of the rhythm formation using respiration, suckling or swallowing, which roles may escape from the front stage after the maturation.
Functional aspects of treatment with single implant-supported crowns

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Background: No comprehensive patient-centered and clinical evaluations of the functional effect of treatment with implant-supported single crowns (ISSC) have been reported previously.

Objective: To investigate whether treatment with ISSC affect patient-centered outcome and masticatory function.

Material and methods: In 9 females and 9 males (32±10 yr) all with agenesis of permanent teeth and treated with 1-4 metal-ceramic crown (68\% in the premolar region), the treatment effect and masticatory function were assessed. The evaluation was done twice, once after implant placement shortly before crown cementation and the second time one month after cementation. The examination included questionnaires (including Oral Health Impact Profile (OHIP-49)), and functional examination with 0.05 mm plastic strips, Dental Prescale Film and Occluser system, Xylitol color-changeable gum, and slices of Granny Smith apple.

Results: The patients' satisfaction with treatment was high and they experienced a significant overall improvement of their oral health-related quality of life (on average 13\% reduction in total OHIP scores). The cementation of the crowns was associated with significant increase in: the number of near occlusal tooth contacts, the contact area, the bite force, and the chewing ability and performance. Furthermore, the load on the ISSC was about half the load of corresponding natural teeth and the occlusal load center moved significantly posterior. There was a significant, positive correlation between number of tooth contacts and (1) occlusal contact area, (2) bite force and (3) chewing performance.

Conclusion: Both on subjective and clinical levels treatment with ISSCs improved oral function and masticatory parameters significantly.

Predicting the retentive force of FRC clasps by nonlinear FEA

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Objectives: The aim of this study was to predict the retentive force of clasps made of a glass fiber-reinforced composite (FRC) material by nonlinear finite element analysis (FEA). Methods: The geometry of an abutment tooth, a lower second premolar surrounded by a circumferential clasp arm, was set as a barrel-shape. The basic clasp arm shape was without taper, and was 2.60 mm wide and 1.30 mm thick (Bb). These geometries were consistent with our earlier study on the retentive force of FRC clasps. FRC was treated as an elastic orthotropic material. All nodes at the 1/3 lower apical side of the abutment tooth were restrained, and forced displacements of 5 mm were applied to the nodes at the base of the clasp arm in the removal direction. The friction coefficient was set to 0.31. The relationship between the displacement of the tip of the clasp arm in the removal direction and the retentive force calculated as the sum of the nodal reaction forces at the base of the clasp arm was plotted. Similarly, retentive forces of modified clasp arms were also calculated. Widths and thicknesses of the modified clasp arms were as follows: 2.60 mm and 0.65 mm (a), 2.60 mm and 1.95 mm (c), 1.30 mm and 1.30 mm (A) and 3.90 mm and 1.30 mm (C). Results: The retentive force of the basic clasp arm (Bb) was 6.36 N, in good agreement with the value obtained from our experimental measurements. The retentive forces of modified clasp arms were 1.00 N (a), 16.30 N (c), 3.36 N (A) and 8.90 N (C). Conclusions: The retentive force of glass fiber-reinforced composite clasps could be quantitatively predicted by nonlinear FEA. This study was supported by a Grant-in-Aid for Scientific Research (No.19791452) from the Japan Society for the Promotion of Science.
023  Structurally compromised roots restored with four post and core systems

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Objective  This study was aimed to evaluate the fracture resistance of structurally compromised restored teeth with flared root canals using four different post and core build-up methods.

Methods  After root canal treatment, 32 bovine mandibular incisor roots were shaped into uniform configuration simulating human mandibular premolar roots on a lathe. The root canals were enlarged to leave approximately 0.8mm thick of dentin walls.

The roots were divided into four types of restorations: cemented cast post and cores (Castwell M.C. 12% Gold, GC Corp., Tokyo, Japan [M]), built up with dual-cured resin composite (Clearfil DC Core Automix, Kuraray Medical Inc., Tokyo, Japan [CR]), built up with resin composite in combination with prefabricated glass fiber posts (FibreKor Post, Pentron Co, Wallingford, USA [FRC]), and reinforced with a thick layer of dual-cured composite before fabrication of small-diameter tapered cast post and cores [CRM].

All the specimens were embedded in acrylic resin up to 2mm below the cervical line and subjected to a static loading test with a crosshead speed of 10 mm/min at a 45 degrees angle to the long axis of the root until failure occurred. The failure modes were observed after the testing. The data were analyzed using one-way ANOVA and Dunnett’s T3 test (a=0.05).

Results  CRM group (719.38±196.73 N) showed significantly high fracture resistance compared to the other groups (M: 429.56±82.43 N, CR: 349.56±66.21 N, FRC: 398.94±112.71 N) (P<0.05). There were no significant differences among M, CR, and FRC groups. In all specimens, fracture lines extending over 2mm below the cervical line were observed.

Conclusion  The CRM restoration showed the better structural properties for structurally compromised roots with no ferrules, though all methods in this study showed non-restorable fracture modes.

024  Effect of conscious clenching on simple calculation task

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Objectives  To investigate the benefit of conscious clenching during a simple calculation task.

Methods  Twenty normal subjects (mean age 26.8 years, sd 2.0 years; 9 females and 11 males) were recruited from our department. Exclusion criteria were: 1) engaging occupational mental arithmetic duties; 2) missing posterior teeth (excluding third molars); and 3) having other dental or medical problems including orofacial pain. They were told to refrain from smoking and consuming caffeine or alcohol for three hours before the test. Surface EMG was recorded from the left masseter muscle by a portable EMG system (Bagnoli-2 Handheld, Delays, USA). EMG data were sampled at 512 Hz by a digital data acquisition system (UAS-108S, Unique Medical, Japan). Sampled data were rectified and integrated. 100-square calculation of addition of two-digit numbers in three minutes was adopted for the mental arithmetic task. Four sets of calculation were performed. The first set was conducted for familiarization. The second was conducted in usual jaw condition. The third and fourth sets were randomly conducted with no contact of teeth or rhythmical clenching under a conscious effort during calculation. Intermission of one minute was taken between each set of calculation. The number of right answers was counted as the measure of accuracy and quickness of mental arithmetic.

Results  EMG activities were almost doubled during conscious clenching. No significant change of calculation outcome was observed between no tooth contact condition and clenching condition (no contact: 68.8, sd 12.2; clench: 66.5, sd 12.7).

Conclusions  In normal subjects, tooth clenching showed no beneficial effect such as improved calculation outcome. Although this study measured only short term effects of clenching on a simple calculation task, the results show that clinicians can encourage their patients to keep free from clenching of teeth.
025  Relation between upper and lower molars during functional mandibular movement

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Objectives: Most researches about the mastication were investigated in the later stage prior to deglutition, for the mandibular movements of this stage were stable compared with those of early stage. However, in the early stage of the mastication, much masticatory force to comminute foods will be required and this stage plays a very important role in the mastication process. This study investigated the relation between upper and lower molars in the main occluding area during the first stroke of mastication. Methods: The subjects were five adults with the normal dentition. Study models were fabricated and occlusal surface of them were measured with the three-dimensional shape measurement system in each subject prior to the experiment. Five trial of detecting main occluding area were performed with 4.0mm length temporary filling materials in each subject. During these trials, six-degree-of-freedom mandibular movements were measured simultaneously. In the molar region, the contact area at the intercuspal position within main occluding area and corresponding "A" and "B" contact area were selected. The reference points UA and UB were selected for the surface of upper casts, and LA and LB belong to the surface of lower casts. The occlusal surface of the cast model were manipulated on the personal computer according to the data of mandibular movement measurement and the distance between reference point UA and LA, UB and LB were calculated (|A|, |B|). In every 0.5 mm, from the intercuspal position to the 3.0 mm away from this position (at the edge of lower left incisor) during biting, the ratio of |A|/|B| were calculated. Results: The mean values of each subject showed 1.003 ±0.007. Conclusions: These results reflected that the occlusal contacts of upper and lower molars come into contact approximately vertically each other during comminuting foods in the first stroke of mastication.

026  Influence of asymmetric mandible on masticatory performance

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Objective: In patients with asymmetric mandible, the peripheral sensor information is different between deviated and non-deviated side, therefore accommodative mechanism should occur for peripheral asymmetries in central generator. If masticatory performance is adapted to asymmetrical peripheral structure, its cycle shape and chewing velocities should do complementary work to maintain a chewing rhythm. The purpose of this study was to determine the validity of this hypothesis. Methods: The subjects consisted of 3 groups: symmetric skeletal Class I group, asymmetric skeletal Class III group with asymmetric mandible and unilateral crossbite, symmetric skeletal Class III group, respectively consisted of 13 males and females. An optoelectric jaw-tracking system was used to record jaw movements. Each subject performed unilateral chewing of 5g of gummy jelly. The following parameters were calculated: maximum cycle excursive ranges, duration of opening, closing, and intercuspal phases and one chewing cycle; maximum opening and closing velocities; gapes at the maximum velocities; and maximum gapes at each measuring point. In addition, we divided mechanically cycle pattern into normal pattern and reverse pattern. Results: The maximum cycle excursive range in the anteroposterior direction at the lower incisor point during unilateral chewing was larger in both the symmetric and asymmetric Class III groups than that in the normal group. Moreover, in asymmetric Class III group, it was longer in vertical direction during on the non-deviated side chewing. Additionally, maximum opening and closing velocities, and maximum gapes on the non-deviated side showed larger values during unilateral chewing. The ratio of the reverse-sequence cycle shape was highest when chewing on the deviated side in the asymmetric Class III group. Conclusion: Consequently, the subjects with asymmetric mandible showed asymmetrical cycle excursion diverse from those in the normal group, however, cycle duration maintained a certain chewing rhythm by changing chewing velocities.
Disk movements during fictive mastication under bite-raised condition in rabbits

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Objective In mastication of solid food, occlusion is effectively increased vertically by the food itself interposed between the opposing molars. The aim was to examine the bite-raising effects of solid food on movements of the condyle and articular disk of the temporomandibular joint (TMJ). To simulate the bite-raising effect, a removable splint was interposed between the opposing molars during fictive mastication induced by electrical stimulation of the cortical masticatory area of anaesthetized rabbits.

Methods EMG activity of the masseter (MS) and lateral pterygoid (LP) muscles was simultaneously recorded along with movement of the condyle and the disk during fictive mastication. Movement of the condyle and the disk in the sagittal plane was directly video-recorded at a time resolution of 8 ms with a high speed CCD camera. For the direct video imaging of the disk, a lateral one thirds of articular eminence of the temporal bone which makes upper and lateral roof of the upper articular cavity of the TMJ was partly removed and lateral portion of the disk was exposed.

Results The bite-raising splint on the working side induced an unusual movement of the working-side condyle, which was transient and consisted of postero-inferior movement appearing in the second half of the occlusal phase. The disk showed also transient deviation approximately 0.35 mm postero-inferior to the trajectory typically observed under pre bite-raising condition. The unusual movement of the condyle and disk was also found on the balancing side, but quite different in nature and degree from those on the working side. Conclusion: The transient unusual postero-inferior movement of the condyle and disk in the occlusal phase should be noteworthy in terms of the TMJ disorder because of generation of wide space between the condyle and articular eminence, into which the posterior part of the disk could possibly slip off anteriorly.

Effects of using teething food for infants during weaning

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Objectives: It has been reported that certain percentage of pre-school children have immature behavior at chewing and swallowing. In Japan, teething food or teething ring are known as ‘HAGATAME’ and they are believed to strengthen the teeth and chewing function of the children. The purpose of this study was to examine the relationship between the use of this teething food and the development of chewing and swallowing ability of infants.

Methods A survey using questionnaire was performed for three hundred and forty healthy mothers and children (4-12 years of age) in Tokyo, Kagoshima and Akita (Japan) and complete answers were obtained from 70 subjects (20.5%).

Results Sixty-three percent of the subjects answered that they used teething food or teething ring during weaning. There was a tendency that children who used teething food or teething ring started weaning earlier (P <0.1, Student’s t test).

Conclusion The results suggest that the use of the ‘HAGATAME’ food during the food transition period affect the development of chewing and swallowing ability. This study was supported by the grant in aid for food and health research by YAZUYA CO., LTD.
Finite element analysis of ceramic inlays restored posterior teeth

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Objectives: The aim of the study was to investigate the effect of preparation design on stress distribution in posterior teeth with different class II cavity preparations restored with ceramic inlays, in order to evaluate and compare stress distributions under occlusal loads.

Methods: 3-D models of posterior teeth were created: intact teeth; unrestored teeth with class II cavity preparations with different tapers (between 0 and 10 degree); the same teeth restored with ceramic inlays. The geometries of the teeth were constructed by 3D scanning using a manufactured device. Files were imported in LeiosMesh (Enhanced Geometry Solutions Corporations, Italy), where the point clouds from the teeth surfaces were cleaned and assembled, and then merged to create a complete model. NURBS surfaces were built and imported in Rhinoceros (McNeel North America) modeling program. The 3D teeth models were used as a support for inlay modeling. These were exported in ANSYS finite element analysis software (Ansys Inc., Philadelphia, USA), to be used for structural simulations. Each model was subjected to a force of 200 N directed to the occlusal surface. Stresses were calculated in the tested inlays, and tooth tissues.

Results: In teeth restored with ceramic inlays, the von Mises equivalent stress values were higher than in intact teeth. High stresses were located at the junction of the butt joint margin inlay and enamel. The values depend on the preparation shape and decrease with the increase of the taper.

Conclusions: The finite element study provides a biomechanical explanation for inlays restored teeth. Within the limitations of this method, it resulted that ceramic inlays do not restore the original strength of the teeth, and the preparation shape is decisive for the stress values and distribution. (ID_1264)

3D reconstructions for numerical simulations of prosthetic restorations

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Objectives: The purpose of the study was to reconstruct 3D teeth models and prosthetic restorations in order to develop numerical simulations.

Methods: Plaster teeth were scanned rotary and from a single plane using LPX-1200 Picza Laser Scanner (Roland DG Corporation, Japan). The point clouds from the teeth surfaces were used to extrapolate the shape of the object, in order to create continuous surfaces by reconstruction. The collected data were used to construct three dimensional models using Rhinoceros (McNeel North America) NURBS (Nonuniform Rational B-Splines) modeling program. These solids were used as a support for further modeling of prosthetic restorations (inlays, onlays, dental clasps). Resulted objects were exported in ANSYS finite element analysis software (Ansys Inc., Philadelphia, USA), to be used for structural simulations.

Results: Generated stresses were calculated numerically and plotted graphically. Results were displayed as colored stress contour plots to identify regions of different stress concentrations. The finite element method allowed the calculation of the stresses, through equivalent stress and of the flexibility through calculation of the displacements.

Conclusions: This in vitro study demonstrated that structural analysis of dental restorations may offer a powerful tool in selection of an adequate design according to each clinical case. (ID_1264).
Site-specific colonization and genotypic diversity of *S. mutans* in different individuals.

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The aim of this study was to clarify the distribution and genotypic diversity of mutans streptococci on different tooth sites in caries-free and caries-affected individuals. Fourteen subjects, aged 22-24 years, were examined. Salivary levels of mutans streptococci, caries prevalence, oral hygiene habits and status of tooth surfaces sampled were recorded. Plaque samples were obtained from three sites, the buccal smooth surface of the right upper teeth, the fissures (B) of the non-caries occlusal surfaces (O) and carious lesions (C). Up to 10 colonies/site were isolated when present and genotyped by arbitrarily primed PCR (AP-PCR) analysis. All 49 samples obtained were culture-positive. Mutans streptococci in 47 samples originate from all 14 subjects. Mutans streptococci were not detected in 2 samples from caries-free sites. Four samples, obtained from one individual, showed both *S. mutans* and *S. sobrinus* isolates. Among the 410 isolates were 40 AP-PCR profiles representing. Caries-free subjects have a larger number of genotypes of *S. mutans*. In caries-affected subjects major frequency of genotypes found in the other sites was also observed in caries. *S. mutans* genotypes were more prevalent in caries-free individuals than in caries-affected individuals. The frequency of genotypes which found in saliva between caries-free and caries-affected individuals was significantly different. This study was supported by the scientific and technological project of Hubei province, Grant 2005AA304B13.

Implementation of the KTSND questionnaire on Australian dental undergraduates

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Objectives: To assess the reliability of the English version of the Kano Test for Social Nicotine Dependence (KTSND) questionnaire in order to establish the association between age, gender, smoking status, relationship with smokers and KTSND scores, in a sample of Australian dental undergraduates.

Methods: A sample of 255 dental undergraduates at the University of Western Australia was used. Each was examined with an English version of the KTSND questionnaire twice in an interval of a month.

Results: The prevalence of smoking among Australian dental undergraduates was 4.7% (95% CI=2.6%, 8.3%). Seven out of the ten questions in the English version of the KTSND questionnaire (Q1, Q3, Q4, Q5, Q7, Q8, Q10) showed an adequate test-retest reliability (Cronbach’s alpha≥0.64). The internal consistency of the 10 questions was 0.69 and it reached to a maximum at 0.77 when only six questions (Q3-Q8) were included. Current smokers showed stronger belief in Q3-“cigarettes bring enjoyment of flavour and stimulation” (OR=2.42, 95% CI=1.05, 5.56, p=0.037), Q5-“cigarette smoking enriches some smokers’ life” (OR=3.15, 95% CI=1.25, 7.89, p=0.015), Q7-“cigarettes can relieve stress” (OR=6.30, 95% CI=1.67, 23.75, p=0.007), and Q8-“cigarettes help smokers’ brain work better” (OR=3.73, 95% CI=1.46, 9.57, p=0.006). All other questions failed to differentiate between smokers and non-smokers (p≥0.202). Age, gender, relationship with smokers, and years of dental study were not associated with smoking status (p≥0.445).

Conclusions: The prevalence of smoking was lower among Australian dental undergraduates than general population. Smoking status was not associated with age, gender, relationship with smokers and years of dental study in this sample. The reliability of the English version of the KTSND questionnaire is adequate. Future investigation in its validity is indicated.

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033 Mutans streptococci and caries experience in Mongolian children.
(TW3)

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Objective Streptococcus mutans and Streptococcus sobrinus have been implicated as the principal etiological agents of dental caries in humans and experimental animals. The objective of this study was to detect S. mutans and S. sobrinus by PCR, and to compare their presence with the incidence and risk of dental caries in Mongolian children.

Materials and methods: Dental examination and caries risk assessment using the Cariostat test were carried out on 421 Mongolian children. The subjects comprised 3 groups: children aged 6-30 months, preschool children aged 3-5 years and school children aged 12-15 years. Oligonucleotide primers designed on the basis of nucleotide sequences of dextranase genes were used for amplification of species-specific amplicons of S. mutans and S. sobrinus. The presence of bacteria was checked by PCR.

Results and conclusion: In children aged 6-30 months, 45% were positive for S. mutans alone, 9% were positive for S. sobrinus alone, 18% were positive for both S. mutans and S. sobrinus, and 28% were negative for both strains. However in preschool children, 63.4% were positive for S. mutans alone, and 36.6% were positive for both S. mutans and S. sobrinus. In school children, 75% harboured S. mutans only, and 25% both S. mutans and S. sobrinus. The dmft/DMFT scores of children positive for both S. mutans and S. sobrinus were significantly higher than those positive for S. mutans alone in all groups of children. The detection of S. sobrinus increased statistically significantly with increasing caries risk levels in all children (p<0.01). The results indicate that children harbouring both S. mutans and S. sobrinus have a significantly higher incidence of caries than those with S. mutans alone, and that mutans streptococci colonization increased with increasing age.

034 “Social Nicotine Dependence” of dental undergraduates in Japan

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Objectives: Smoking behavior persisted due to psychological and physical dependence. A new questionnaire, “the Kano Test for Social Nicotine Dependence (KTSND)”, has been developed. Psychological nicotine dependence can be assessed with the KTSND questionnaire which is composed of ten questions with a total score of 30. This study aimed to establish the association between age, gender, smoking status, relationship with smokers and KTSND scores, in a sample of Japanese dental undergraduates.

Methods: A sample of 798 dental undergraduates at the Aichi Gakuin University, aged 18 to 46 years (21.4 ± 2.8 years), was used. Each was assessed with a KTSND questionnaire. Results: The sample included 97 smokers (12.3%), 39 ex-smokers (4.9%), and 654 non-smokers (82.8%). Two hundred and twenty-four students (32.7%) inhaled second-hand smoke at home. The total KTSND score was 11.8 ± 6.3 in this sample. According to smoking status, the KTSND scores were 15.9 ± 6.2 in smokers, 14.9 ± 6.3 in ex-smokers, and 11.1 ± 6.0 in non-smokers. Smokers’ and ex-smokers’ KTSND scores were significantly higher than those in non-smokers (p < 0.01). Those who received second-hand smoke at home showed higher KTSND scores than their counterparts (12.9 ± 6.3; 11.5 ± 6.1, p < 0.01). Male students demonstrated higher KTSND scores than female students (12.4 ± 6.6; 10.6 ± 5.3, p < 0.01). Conclusion The prevalence of smoking in Japan was lower among dental undergraduates than general population. The total KTSND score was associated with smoking status, relationship with smokers and gender. This study confirmed the differentiability of the KTSND questionnaire. Acknowledgement: This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Health, Labour and Welfare (H18-Cancer-youth-004) and a 2008 Japan Society for Tobacco Control Grant.
The change of emotionally intelligence quotient through the PBL

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Objectives: PBL-tutorial (PBL) was based on adult learning theory and it was helpful for the student by student centered and long-life learning. But it seemed to be difficult and take much time to evaluate how growing of students through the PBL. The purpose of this study was to analyze the influence of PBL on the emotional intelligence quotient of students.

Methods In Nippon Dental University at Niigata, PBL was started at 2004 for 3rd year students. Approximately 95 students were random separated by drawing to 12 small groups with a tutor. Our PBL-tutorial program was composed of group session (80 min) with a tutor, self-assessment of group session, self-learning (120 min) and individual self-assessment with a tutor. Estimation of our PBL was based on the observation record of group session, portfolio of self-learning and modified essay question. At 2007, we had 101 3rd year students and conducted a simplified emotional intelligence quotient (EQ) assessment before and after the PBL program. Each assessment was tabulated and analysis of Wilcoxon test by Stat view software.

Results: There was no significant difference between before and after the PBL as a whole in the EQ ability-based model such as perceiving emotions, using emotions, understanding emotions and managing emotions. However the students were separated into four groups (analyzer, controller, promoter, and supporter) by communication style, and the promoter type students (n=24) showed significant difference in perceiving emotions between before (21.3±2.9) and after (22.5±3.2) the PBL. Other three group's students showed no significant difference in the EQ between before and after the PBL.

Conclusion: These result suggested that only 6 months experience of PBL have a possibility to influence the students EQ ability and students will grow up by themselves through the PBL.

A system for supporting independent learning in dental education

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Objectives: Our educational program “A supporting system with archive of dentistry for independent learning from student education to lifelong education” was adopted as one of supporting program for contemporary education needs in 2007, by the Ministry of Education, Culture, Sports, Science and Technology in Japan. Using Information and Communication Technology (ICT) our program was developed for students and medical worker and teachers in dentistry. Students can use it for their independent learning, and others can get academic information from it as their lifelong education. This program gives knowledge and information, not only it but also this program brings up ability for practical use and guidance ability of the ICT. We report about summary of this program and our use.

Methods Our archive is based on many questions and answers about dentistry and adjacent medical field need for dentist. When want to learn, the user can access anywhere anytime. Students can use this for school days, and dentists can use this through a life. Therefore, we propose this to all people of this field as a new method of the independent learning.

Results: From last year, we are using this system for the lecture and practice of oral pathology for the third grade dental students. A result of assessment by them were nice. We could feel that they were able to deepen the understanding of the lecture and practice. Furthermore, it seems that the standard level of the dental education improves if these archives are opened to the public more widely in the future.

Conclusion This program provides the opportunity to learn it to all people who are trying to study by this system, including students and graduates of our university. As a result, our program can contribute to bring up excellent dentists and medical staffs.
037 Micro-CT analysis of the mandibles and femurs in warfarin-administered Rats

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Objective This study was designed to investigate whether an administration of warfarin causes morphological changes of the mandibles and femurs in rats. Methods Sixteen male Sprague-Dawly rats, at 12-week-old were divided into 2 groups, and received daily injection of either sodium warfarin (0.18mg/Kg, WF group) or physiologic saline (PS group) for 4 weeks. The other 8 rats were sacrificed at day 0 as a baseline control. Mandibles and bilateral femurs of all rats were removed and subjected to morphological analysis. Micro-CT (Scan Xmate-E80, Comscantechno) scanning was accomplished to investigate micro architecture of right halves of femurs and mandibles using a 3D structural analysis software (TRI/3D-BON, RATOC). Trabecular bone volume (TBV) and cortical bone volume (CBV) were measured separately in the epiphysis of femurs. For measurement of TBV of mandibles, the region of interesting (ROI) was determined in the frontal section at the second molar. Results In femurs, TBV of the femoral metaphysis were obviously smaller in WF group than that in PS group (p=0.03). In mandibles, a tendency of TBV to decrease was seen in WF group, but significant difference was not found in comparison with PS groups. In WF group, a correlation of TBV of femurs with TBV of mandibles was found (Pearson's correlation coefficient: 0.758, p=0.029). Conclusion These results suggest that TBV of femurs and mandibles of rats are reduced in 4 weeks by daily administration of warfarin.

038 Remineralization of primary tooth dental enamel of Down syndrome

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Objectives The purpose of this study was to investigate the difference of remineralization on primary tooth enamel between Down syndrome and control. Methods The tooth samples were 9 extracted Down syndrome's primary molars (Down group) and 11 control primary molars (Control group). They are healthy teeth without dental caries. Firstly, the specimen was demineralized and remineralized. Then they were processed for analysis. Secondly, Transverse Microradiography was used for image analysis. After that, we measured the lesion depth(ld), mineral loss value(ΔZ), maximum mineral value(Vmax), minimum mineral value(Vmin), Depth of maximum mineral value (Vmax(ld)) and Depth of minimum mineral value(Vmin(ld)). Then remineralization rates were calculated. Finally, the data was analyzed using Mann-Whitney U test. Results The data of the demineralization test of ld and ΔZ were compared and the results showed that the calcification degree of the Down group was lower than the Control group. While the Vmax value of the dental enamel surface was lower than the Control group, the other values did not show any significant differences. The data of the remineralization test of ld and ΔZ were compared and the results showed that the calcification degree of the Down group was lower than the Control group. While the Vmin and Vmin (ld) value was inferior to the Control group. Furthermore, comparison of the remineralization rates of ld and ΔZ both groups showed no differences. However, the Vmax value was larger in the Down group, while the Vmin (ld) value was smaller than the Control group. Conclusions This study concluded a low calcification degree of enamel of Down syndrome in both remineralization and demineralization test. However, the mechanism of remineralization of Down syndrome might be different from the Control.
TMR and micro-Raman studies on enamel bleaching with remineralization treatment
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Objectives: The aim of this study was to examine influences of remineralizing solution on the enamel surface properties and expect the effects of saliva against long-period bleaching. Methods: Bovine enamel specimens with 2 x 3mm windows were divided into following groups. As a control group, enamel specimens were immersed into a remineralizing solution simulating saliva (1.5mM CaCl2, 0.9mM KH2PO4, 130mM KCl, 20mM Hepes, pH 6.5) for 12 weeks. As experimental group (I), HiLiteTM was applied on the window once a week according to the manufacturer's instructions and was repeated for 12 weeks. During this period, the specimens were immersed in deionized water. As experimental group (II), HiLite™ was applied in the same way as the control group, however the remineralizing solution was used for immersing. After the experimental period, mineral profiles and integrated mineral loss (IML) were measured by transversal microradiography (TMR). Simultaneously, nano-indentation testing was performed across the lesion into the underlying sound enamel. Furthermore, to determine modifications of the molecules in the enamel structure, Raman microspectrometry was performed. The IML was analyzed statistically using ANOVA and Games-Howell test at p<0.05 (n=6). Results: The distinct sub-surface lesion was observed in the experimental group (I), however any lesion was not observed in the experimental group (II) and the control group. The IML indicated similar values during these groups (p>0.05). Also, the cross-sectional nano-hardness profiles of the control and the experimental group (II) were resembles. However, the Raman spectrum of the group (II) indicated the phosphate peak of surface of the lesions more intensively compared with the control. Conclusions: These results indicate that remineralization solution simulating saliva contributes not only recovering the mineral loss from enamel during the long-period bleaching with 35% H2O2 but also stabilizing of enamel crystals nearby the surface by exchanging the constituent elements.

Provisional mineralization layer in the predentin of murine teeth
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Background: Mineralization of circumpulpal dentin has been shown to progress at the mineralization front, where predentin matrix is abruptly converted to almost fully mineralized dentin. Goldberg et al. (1996) claimed existence of lightly mineralized layer in predentin immediately adjacent to the mineralization front, and suggested that dentin mineralization is a rather transient process. These authors described the lightly mineralized layer as “metadentin” and that metadentin is recognizable in anhydrously processed specimens. Objectives: The purpose of the study is to clarify the rather illusive, transient predentin layer along the dentin mineralization front by light and electron microscopy, in an aim to gain more insights in the mechanism of dentin mineralization in rodent teeth, based on fine structural analyses.

Methods: Young and adult Wistar rats were fixed by conventional vascular perfusion with 4% paraformaldehyde (PFA) or a mixture of 2% glutaraldehyde and 3% PFA in 0.1M cacodylate buffer (pH 7.4). Lower incisors were dissected, immersed in the same fixative overnight and embedded in EPON 812 with or without OsO4 post-fixation. Semi-thin sections were stained with toluidine blue and some were processed for von Kossa staining to test mineral deposition in predentin layers. Ultrathin sections were cut with ethylene-glycol in trough to prevent mineral loss from the specimen.

Results: Light microscopy confirmed lightly metachromatic and weakly von Kossa-positive layer in the predentin of crown-analogue portion of incisors, and its absence in the root-analogue. Thickness of the transient layer varied from none to 3 micrometer in the crown-analogue of the individual teeth. Electronmicroscopy confirmed mineral deposition in von Kossa positive portions. Specialized predentin layer was not confirmed in the rat molars.

Conclusions: These results indicate region-specific as well as tooth-type-specific diversity in the process of appositional mineralization of circumpulpal dentin in rat teeth.
041  **EF-TEM cytochemical observation of elongated rete ridges in gingival hyperplasia**

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Objectives: Gingival overgrowth is a side-effect of calcium channel blocker medications, characterized by an accumulation of collagenous components within the gingival connective tissue and epithelial hyperplasia with elongated rete ridges and various degrees of chronic inflammatory infiltration. Human polymorphonuclear leukocytes (PMN) stimulated to phagocytosis or other chemical stimuli were reported to exhibit extensive deposits indicative of H2O2 production. These deposits were localized on the plasma membranes adjacent to neighbouring cells and on the phagosomal membranes of PMN means of a cerium method. We report the use of energy-filtering transmission electron microscopy (EF-TEM) to visualize the invisible enzyme reaction products. **Methods:** For the histochemical localization of cerium perhydroxide, gingival tissue sections of patients were treated with 1.0 mM CeCl3, and 10 mM NaN3. Following the cytochemical reaction procedures mentioned above, tissue pieces were fixed in 2% GA-2% pFA at 4°C for 60 min, and embedded in Quetol 653. We used an EF-TEM (LEO LIBRA 120) operated at 100 kv. To analyze ESI, energy-filtered images of each element were recorded by slow scan CCD camera linked to a computer. The ESI of the Ce element were obtained from the DEmax at 907 eV of M4 edge. To quantitative identify the weak edges, the background was stripped using a two-window method. ESI analysis of phosphorus (P) element (DEmax 153.5 eV of L2,3 edge) was also performed. **Results:** No reaction product was found in PMN and intact epithelium. Ce precipitations could be visualized on the free surface of the plasma membrane in epithelium of elongated rete ridges. Precipitation reaction was not scavenged by the addition of catalase (an inhibitor of H2O2). An ESI analysis shows the distribution of Ce elements clearly correspond with that of P elements. **Conclusion:** Such findings support the concept that Ce deposits react with P residues of atrophic plasma membranes.

042  **Adrenomedullin inhibits CXCL10 production by human gingival fibroblasts**

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Objectives: Adrenomedullin (AM) is a peptide that has multiple regulatory functions. Several studies indicate that AM could act as an endogenous immunomodulatory factor. Our aim was to elucidate the effects and mechanism of AM on the CXC chemokine ligand (CXCL) 10 production, which are involved in Th1 response by human gingival fibroblasts (HGFs). **Methods:** The HGFs that grew from three clinically healthy gingival tissues were cultured primarily on plastic dishes in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and antibiotics at 37°C in humidified air with 5% CO2. Confluent cells were transferred and cultured for use in the present study. We examined the expressions of AM, AM receptors, calcitonin receptor-like receptor (CRLR), receptor activity-modifying protein (RAMP)2 and 3 in HGFs culture by RT-PCR. HGFs were stimulated with tumor necrosis factor (TNF)-α, AM, folskolin (adenylate cyclase activator) and cyclic AMP (cAMP), and the CXCL10 production by HGFs was evaluated by RT-PCR and/or ELISA. In some experiments, we pre-incubated HGFs with H89 (protein kinase A inhibitor) prior to the stimulation. **Results:** HGFs expressed AM and AM receptors, CRLR and RAMP2, mRNA constitutively. Meanwhile, RAMP3 mRNA was not expressed in HGFs. AM treatment inhibited CXCL10 production by TNF-α-stimulated HGFs. H89 treatment cancelled the inhibitory effects of AM on CXCL10 production from TNF-α-stimulated HGFs. Moreover, folskolin and cAMP suppressed CXCL10 production by TNF-α-stimulated HGFs. **Conclusions:** From these results, AM inhibited CXCL10 production by TNF-α-stimulated HGFs via cAMP/PKA pathway activation. These findings indicate that the effects of AM on CXCL10 production by HGFs are related to lymphocyte infiltration, particularly Th1-type T cells, into the gingival tissue with periodontal disease. Recently, it has been reported that Th1-type T cells were involved in bone resorption in periodontal diseased tissue. Therefore, AM could play a role as therapeutic approach to treatment of periodontitis.
Role of Thy-1 in collagen phagocytosis by human gingival fibroblasts

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Introduction: Thy-1, a 25-37 kDa glycosylphosphatidylinositol (GPI)-anchored glycoprotein, is expressed on many cell types. Since Thy-1 affects widespread nonimmunologic functions such as cellular adhesion, migration and proliferation, this molecule may be an important regulator of cell-cell and cell-matrix interactions. We investigated here a role of Thy-1 in collagen phagocytosis by human gingival fibroblasts (HGF) and its mechanisms.

Methods: HGF were separated into Thy-1High and Thy-1Low subpopulations using a magnetic bead selection. Following incubation of each subset with collagen-coated fluorescent beads, number of beads bound to cell layer was counted, and internalization of beads was analyzed using a flow cytometer. After HGF were pretreated with anti-Thy-1 antibody (AS02) in the presence or absence of PI3K inhibitors or Cytochalasin D, actin reorganization and phosphorylation of Akt were assessed by rhodamine-phalloidin staining and Western blotting, respectively, and further the binding and internalization of bead were determined.

Results: Thy-1High HGF were >90% positive for Thy-1, and Thy-1Low HGF were <10% positive. Thy-1High HGF showed higher levels of bead binding and its internalization than Thy-1Low HGF. AS02 treatment stimulates actin reorganization, Akt phosphorylation and subsequent bead phagocytic route. Pretreatment with PI3K inhibitors or Cytochalasin D inhibited the AS02 actions.

Conclusion: Thy-1 positively regulates collagen phagocytosis by HGF. Further, the PI3K/Akt signaling pathway is thought to partially mediate the Thy-1-activated collagen phagocytic pathway, which may be dependent on actin cytoskeletal rearrangement.

A case of ameloblastic carcinoma in the mandible

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Objectives: We report a case of ameloblastic carcinoma in the mandible.

Methods: The patient was a 66-year-old male, who had been aware of the tumor in the right side of the mandible for about six months and underwent partial resection of the mandibular bone. X-ray examination revealed a radiolucent lesion and marked expansion of cortical bone.

Results: The resected tumor was solid mass with cystic lesion. Histologically, the tumor was composed of large polygonal cells that exhibited solid, and palisading patterns were easily found. Nests and islands of epithelium within collagenous stroma are composed a peripheral layer of polarized cells enclosing stellate to basaloid cells. And nests had a marked keratinization. Individual cellular features include pleomorphism, frequent mitotic figures, indistinct cell membranes, focal necrosis and loss of cellular cohesion. Immunohistochemically, tumor cells were positive for cytokeratin 13, cytokeratin 14, cytokeratin 19 and AE1/AE3. MIB-1 labelling index was 9.3%.

Conclusion: The postoperative histopathological diagnosis was ameloblastic carcinoma in the mandible.
Primary treatment for TMD with TMJ pain and trismus

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Objectives: The aim of this study was to evaluate the first and second treatments in the patients with TMJ pain and trismus.

Methods: TMJ pain and trismus levels were classified into none, slight, moderate, and severe using visual analog scales. Ninety-eight patients with moderate or severe TMJ pain and trismus were classified into disk displacement with reduction (DDwR), disk displacement without reduction (DDw/oR), and osteoarthritis (OA) using MRI. As the first treatment, non-steroidal anti-inflammatory drug (NSAIDs) were given with mouth opening exercise for two weeks. Arthrocentesis was performed as the second treatment in the patients who had no effect from the first treatment.

Results:
1. The improvement rates of the first treatment: DDwR: 89%, DDw/oR: 51%, and OA: 61%.
2. The improvement rates of the second treatment: DDwR: 50%, DDw/oR: 71%, and OA: 56%.
3. The total improvement rates: DDwR: 94.5%, DDw/oR: 86%, and OA: 83%.

Conclusions: The results suggested that the first and second treatments are effective for TMD with TMJ pain and trismus.

Coronectomy for the management of mandibular third molars

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OBJECTIVES: The close anatomical relationship between the roots and the inferior alveolar canal (IAC) often causes nerve injury following the extraction of lower third molars. Coronectomy or intentional partial odontectomy is a technique that may avoid such nerve injury.

METHODS: We used dental computed tomography (dental CT) to examine all patients who attended our department for treatment to mandibular third molars from January 2006 to March 2008 and who had radiological findings of proximity to the IAC. 274 mandibular third molars had a close relationship with the IAC on dental CT. We performed coronectomy for those patients (n=126) giving informed consent for the procedure. The other cases (n=148) were treated traditionally by removal. We evaluated cases that had over 3 months of follow up.

RESULTS: In the extraction group, 7 patient showed signs of nerve injury. 1 patient showed neurapraxia in the coronectomy group and became asymptomatic within a month. 11 patients from the coronectomy group which indicated infectious signs were treated by removing the remaining roots. No nerve injuries were found in these 11 patients.

CONCLUSION: Coronectomy appears to be a useful alternative for mandibular third molars that have a close anatomical relationship to the IAC. Because of the postoperative root migration from the IAC, it also has the advantage of facilitating the safe secondary removal of any remaining roots without nerve injuries.
047 Advantages of using two-photon laser scanning microscopy for biofilm imaging

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Objectives: To compare single-photon and two-photon laser scanning microscopy with regard to the quality of fluorescent images of model oral biofilms.

Methods: Biofilms were grown in glass capillary biofilm reactors. Images were collected using a Leica TCS-SP2 AOBS confocal microscope with or without the use of a two-photon excitation option (Leica/Spectra Physics MaiTai 2-photon system: Ti/Sapphire 780- to 920-nm infrared laser). To examine photobleaching and photodamaging, three-species (Streptococcus oralis, Streptococcus gordonii and Actinomyces naeslundii) biofilms were stained with Calcein-AM (CAM), and average fluorescence intensity of the same field irradiated every 30 s was calculated. Three-dimensional reconstruction was carried out using MetaMorph software for biofilms of a gfp-expressing strain of Pseudomonas aeruginosa on which fluorescent beads (15 μm) were immobilized, and Staphylococcus epidermidis biofilms stained with BacLight LiveDead.

Results: After 20 min irradiation, average fluorescence intensity showed a 20.4% decrease with the single-photon system, whereas only an 8% decrease was recorded with the two-photon system. Thus, the intensity was significantly high when the two-photon system was used (p<0.05, Greenhouse-Geisser test). Three-dimensional reconstruction demonstrated that deformation artifact of the beads on the P. aeruginosa biofilms was minimal for the two-photon system. Moreover, some parts of S. epidermidis biofilms that appeared hollow using the single-photon system were proved to be solid when observed with the two-photon system. Thus, the overall resolution appeared better for the two-photon system.

Conclusion: The two-photon laser scanning microscopy provided better three-dimensional resolution with deeper light penetration and reduced photobleaching and photodamaging, and thus may open new possibilities for biofilm imaging.

048 Influence of alkali-ion water on removing cariogenic biofilms by jet-washer

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Objective: Jet-washers are being used to remove dental plaque from the tooth surfaces utilizing force of water jet at home. In a previous study we reported that alkali-ion water (AW) can disintegrate glucan from cariogenic biofilms with or without a driving force (Gyo M et al. IADR, Brisbane, 2006). Therefore, it was hypothesized that AW would influence the removal of cariogenic biofilms by a Jet-washer and thus investigated. Methods: Sucrose dependent biofilms were grown on equal-sized bovine enamel coupons (surface ground with #1200 silicon carbide paper) using four species of oral streptococci (Streptococcus mutans, Streptococcus sobrinus, Streptococcus gordoni and Streptococcus mitis) at 37°C for 20 hrs in an oral biofilm reactor. AW (TK7705, Panasonic, Japan) with different pH levels was used to remove biofilms at two different force levels of a Jet-washer (Doltz, EW1250, Panasonic, Japan) for 10sec and tap water (TW) was used as control. That was followed by quantitative analysis of retained biofilms. SEM observation and Real-time PCR were also performed. Results: In almost all cases biofilms were removed from the contact point of the water jet. On Two-way ANOVA analysis it was found that smaller amounts of glucan and bacterial cells were retained when AW was used compared to TW. SEM photomicrographs also revealed that comparatively more clean enamel surface could be visible in case of AW compared to TW. However, in some cases a large amount of biofilms were removed by TW with the application of Jet-washer. Conclusion: Depending on the application Jet-washer found effective in removing cariogenic biofilms from slightly rougher enamel surfaces. However, in comparison AW appeared to be more influential than TW and that may be effective in terms of caries prevention in the long run. This study was supported by G-COE Program, IRCMSTBD at TMDU and Panasonic Electric Works Co., Ltd. Japan.
049  Impact of mineral supplementation to acidic solutions on enamel erosion

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Objectives: The aim of this study was to examine the impact of mineral/fluoride supplementation to acidic solutions on inhibition of enamel erosion in vitro as measured by quantitative light-induced fluorescence (QLF, Inspektor Dental Care BV, The Netherlands).

Methods: Bovine enamel blocks (n=6/group) were used and 15-min treatment with 0.1% citric acid (pH 2.74) at 37°C was employed as erosion model (control). Experiment 1: Enamel samples were exposed to 0.1% citric acid or beverage with or without various Ca sources as CaCl2, fluoride as NaF and buffering agent as NaHCO3. Experiment 2: The specimens were incubated in human saliva samples at 37°C for 2 h to form pellicle and then exposed to 0.1% citric acid with or without Ca (Ca/P = 0.3). Experiment 3: The specimens were treated with 0.1% citric acid and were immersed in saliva samples at 37°C for 24 h. Erosion was assessed by relative fluorescence reduction from sound level (ΔF, %) using QLF.

Results: In the Experiment 1, all the samples except for the beverage without Ca source (ΔF = -8.2 ± 0.7%) showed significantly lesser ΔF values compared with the control (ΔF = -11.2 ± 4.5%; p<0.05; Tukey-Kramer test) indicating inhibition of erosion. In the Experiments 2 and 3, there was no significant difference in ΔF values among the groups tested.

Conclusions: In conclusion, it was suggested that Ca and fluoride can protect enamel from erosion when these components react with acidic solutions or beverages.

050  Streptococcal distribution within plaque formed on enamel with glass-ionomer cement

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Objectives: The aim of this study was to investigate the distribution pattern of streptococci within dental plaque formed in vivo on enamel filled with glass-ionomer cement (Fuji VII, GC) and compared to that on enamel without any fillings.

Methods: Biomass was collected by plaque generation devices which were set on upper molars of twenty consenting volunteers. Plaque biofilms were allowed to develop on enamel surfaces with and without Fuji VII restoration (GIC and Enamel groups respectively). After seven days, the devices were taken out, frozen dry and embedded in resin. Embedded plaque samples were then sectioned using an ultra-microtome. Sections stained with neutral toluidine blue were used the evaluation of biomass volume using an image analyzer. The level of streptococci was measured by TaqMan real-time PCR.

Results: There were no significant differences between the two groups in both biomass density and number of bacteria within dental plaque. The level of bacteria varied widely among subjects. The distribution of streptococci in layers within 400 μm biomass depth from enamel interface showed that the level of streptococci were significant lower in the first layer closed to enamel surface compared with the subsequent layers only in the GIC group (Wilcoxon test, p<0.01) and not in Enamel group.

Conclusion: It can be suggested from this study that the biofilms closed to GIC restorations are not habitats favorable for the growth of oral streptococci.
Phylogenetci analysis of the gbpC and dbl genes among mutans streptococci

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Objectives: The glucan-binding protein C gene, gbpC, solely involved in dextran-dependent aggregation (ddag) of Streptococcus mutans was previously identified. Streptococcus sobrinus, which exhibits more active ddag than S. mutans, has recently been found to harbor 4 gbpC gene homologues (gbpC1, gbpC2, dblA, dblB) in contrast to S. mutans. We could not discuss whether these genes are paralogous or orthologous, unless sequence analyses of these genes in a species (e.g. Streptococcus criceti) relative to S. sobrinus were performed. Therefore, we needed to identify these genes in S. criceti. Methods: Primers to amplify internal fragments of the S. criceti gbpC homologues were the same as those used for S. sobrinus. Based on nucleotide sequence information obtained from the fragments, a PCR-based genome walking strategy was employed to amplify the entire regions of the gene homologues. The amplified fragments were purified and sequenced. Results: The tandem dblA and dblB genes were located in S. criceti in the same arrangement as those in S. sobrinus. The S. criceti dblA gene encoded a 1093 aa protein and similarity between two DblA proteins amounted to 71.4% identity. The S. criceti dblB gene encoded a 1717 aa protein and similarity between two DblB proteins involved 80.2% identity. In contrast to tandemly located S. sobrinus gbpC1 and gbpC2 genes, the S. criceti gbpC1 gene was absent upstream from the gbpC2 gene. The S. criceti GbpC2 protein contains 637 aa and similarity between these two GbpC2 proteins amounted to 61.4% identity. Phylogenetic analysis using the ClustalW program (DDBJ) revealed that genetic distances of the dblA or dblB genes between these two species are closer than those between the dblA and dblB genes in each species. Conclusions: These results suggest that the dblA and dblB genes from S. sobrinus are likely orthologous to those of S. criceti.

Antibacterial effects of MDPB against anaerobes associated with endodontic infections.

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Objective: Dentin primer incorporating an antibacterial monomer MDPB has been demonstrated to show cavity disinfecting effects. MDPB is expected to be useful for achieving a resin-based root canal filling system with antibacterial activity, which contributes to better prognosis of endodontic treatment. In this study, antibacterial effects of unpolymerized MDPB against anaerobes associated with endodontic infections were investigated.

Methods: The sensitivity of three anaerobes (Enterococcus faecalis SS497, Fusobacterium nucleatum 1436, and Prevotella nigrescens ATCC33563) to MDPB was examined by agar-disc diffusion tests. The MIC and MBC values of MDPB were determined and compared with those of cetylpyridinium chloride (CPC), chlorhexidine diacetate (CHX), and metronidazole (MTZ). Rapid killing effects of MDPB against planktonic cells were examined by a cell number counting method, and bactericidal effects against biofilm cells were assessed by a viability staining method using the biofilm of each bacterium prepared on a collagen disc.

Results: MDPB produced inhibition zones against all of the bacteria by agar-disc diffusion tests. The MIC/MBC values of MDPB for the three species were much smaller than MTZ, although slightly greater than CPC or CHX. For all planktonic bacteria, significant reduction in viable cell numbers was obtained by contact with 250 μg/mL of MDPB for 20 sec (p<0.05, Fisher's PLSD tests). Forty sec contact with 500 μg/mL or 20 sec contact with 1000 μg/mL of MDPB resulted in more than 90% killing. Biofilm cells of all species were completely killed by application of 1000 μg/mL of MDPB for 60 sec.

Conclusion: MDPB was found to have strong antibacterial effects against three anaerobes associated with endodontic infections, and such effects are rapidly exhibited even against biofilm cells, suggesting the usefulness of application of MDPB to resin-based materials for endodontic filling.

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**053** Transcriptional regulation of BRAK/CXCL14, a tumor-suppressing chemokine.

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**Objectives:** In the process of searching for in vivo suppressors of oral tumor progression, we found that activation of epidermal growth factor receptor signaling significantly down-regulated the expression of the chemokine BRAK/CXCL14, and that the forced expression of BRAK in head and neck squamous cell carcinoma (HNSCC) cells decreased the rate of tumor formation and size of tumor xenografts (Ozawa, Kato, Komori et al., Biochem. Biophys. Res. Commun. 2006). In addition, in the following study, we found that an increase in the expression of the BRAK/CXCL14 gene was essential for suppression of tumors by gefitinib, an inhibitor of the epidermal growth factor receptor (EGFR) (Ozawa, Kato, Komori et al., 85th IADR/UNILEVER HATTON AWARDS COMPETITION, 1st Place, 2007). Therefore understanding the mechanism of BRAK gene expression is very important.

**Methods** In order to clarify the mechanism of gene expression of BRAK, we searched for the transcriptional start site of the gene by 5' Rapid Amplification of cDNA Ends (5'-RACE method) and examined promoter/enhancer sequences by using various luciferase reporter gene constructs.

**Results:** The transcriptional start site was found to be in the previously reported exon 1 region (+284) of the gene. Determination of luciferase activities by use of deletion and/or mutation constructs clarified that a TATA-like sequence, TATTAA, was essential for the transcription of the gene. The AP-1 binding sequence was necessary for stimulating the expression of the gene. The tandem GC boxes 3 and 4 were essential for transcriptional stimulation of the gene by gefitinib.

**Conclusions:** Our data indicate that the TATA-like TATTAA sequence forms an essential part of the promoter of the BRAK/CXCL14 gene and that the tandem GC boxes located down stream of it represent a cis-element that regulates gene expression by gefitinib. This is the first report describing a cis-element of the gefitinib-regulated gene.

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**054** Invasion of host cells by membrane vesicles of Porphyromonas gingivalis

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Most Gram-negative bacteria including Porphyromonas gingivalis (Pg) produce outer membrane vesicles (MVs) which are natural vehicles, or bacterial “bombs,” for directed intercellular transport of bacterial virulence factors into host cells and tissues. Pg-MVs retain a full complement of outer membrane constituents including LPS, gingipain (Rgp and Kgp) and fimbriae. Although Pg-MVs are suspected to invade various host cells, it is absolutely unknown how Pg-MVs invade the cells. **Objectives:** We here analyzed the invasion of host cells and cellular impairment by Pg-MVs. **Methods:** HeLa cells were incubated with Pg-MVs prepared from strains ATCC33277 and OMZ314, and gingipain mutants. The intracellular localization of Pg-MVs was analyzed with a confocal laser microscopy. **Results:** The experiments, using dominant negative cell lines, showed that Pg-MVs were endocytosed dependently on specific cellular machineries such as cholesterol-rich lipid rafts, Rac1, actin, cholesterol and PI3P. Pg-MVs were also found to be transported to early endosomes, and eventually sequestered by lysosomes for 90 minutes of incubation. However, the MVs resisted lysosomal degradation for over 24 hours. Loss of Rgp activity apparently diminished the MVs invasion, whereas that of Kgp did not. Invading MVs degraded cellular transferrin receptor, a carrier protein for transferring, whereas Drgp-MVs did not. Focal adhesion complex proteins (FACP; paxillin and focal adhesion kinase) were also degraded by MVs, resulting in impairment of cellular migration and proliferation. Interestingly, Kgp deficiency inhibited the FACP degradation by intracellular MVs. **Conclusions:** These results show that Pg-MVs invade host cells via endocytosis pathway and Pg-MVs likely serve as specifically targeted transport vehicles that mediate entry of bacterial virulence factors such as gingipains. The present findings demonstrate a novel role of Pg-MVs in etiology of periodontitis.
**Amelogenin is a potent inhibitor of odontoclastic root resorption**

(H5)

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Objective: Remarkable root resorption was reported in amelogenin-null mutant mice, suggesting this enamel protein as a possible negative regulator of root resorption. It is quite interesting to ask whether amelogenin has an inhibitory action on root resorption. The aim of this study is to clarify the effect of amelogenin on odontoclastic root resorption, in vivo, and on the odontoclast differentiation, in vitro.

Methods: For in vivo analysis, an experimental root resorption model, in which maxillary first molars (M1) of rats were extracted and replanted after air drying, was developed. The root surfaces of M1s were treated with or without amelogenin before the replantation. Rats were sacrificed 7 days after the replantation. The mesial roots area in M1s were used for the histomorphometric analysis (n=7). To examine the odontoclastic differentiation in culture, spleen cells from 5-week-old male mice were co-cultured with human periodontal ligament cells for 7 days. TRAP-positive cells which were larger than 10μm were identified as the odontoclastic cells. Human odontoclasts were isolated from shedding deciduous teeth and cultured for 24 hrs. In both systems, the porcine amelogenin lacking exon 4, P173, or its recombinant form, P172, was added.

Results: In vivo, the application of amelogenin dramatically suppressed the root resorption. Amelogenin significantly decreased the values of the odontoclast surface, odontoclast number, and resorbed dentin (p<0.01). In the co-culture system, the addition of P173 or P172 did not show significant suppression on the odontoclastic cell formation. In the human isolated odontoclast culture, most of TRAP-positive cells formed clusters and many cells were immunopositive to CD51/CD61 antigen. Interestingly, both P172 and P173 significantly decreased the number of TRAP-positive isolated odontoclasts (p<0.01).

Conclusion: These findings strongly suggest that amelogenin inhibit odontoclastic root resorption via suppressing the number of odontoclasts in the late phase of the differentiation.

**Direct neurite-osteoclastic cell communication in co-culture system**

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Objective: It has been well known that sympathetic nerve system implicate in bone metabolism. Recently, we have demonstrated that nerve-osteoblastic cells communication can directly occur using our in vitro co-culture models comprising mouse nerve and osteoblastic cells. Our previous studies also showed that osteoclastic cells equipped with adrenergic and peptidergic receptors and that β-adrenergic agonists directly stimulate bone-resorbing activity in matured osteoclasts. In the present study, we examined the functional interaction between nerve and osteoclastic cells. Methods: Primary cultures of superior cervical ganglia neurons were dissected from newborn mice. Osteoblastic cells were used RANKL-induced TRAP-positive multinucleated cells in murine macrophage-like cell line RAW264.7. For co-culture experiments, nerve cells were added to 72-h-old cultures of RAW264.7 with soluble RANKL. After co-culture for 3days, cells were treated with culture medium containing the calcium fluorophore Fluo-3, nerve-osteoclastic cell units were monitored intracellular Ca2+ mobilization by confocal laser scanning microscopy. Results: We found that the addition of scorpion venom (SV) elicited nerve cells activation via intracellular Ca2+ mobilization and, after a lag period, osteoclastic Ca2+ mobilization. SV did not have any direct effect on osteoclastic cells in absence of nerve cells. These results suggested that osteoclastic cells activation occurred as a direct response to neuronal activation. We also observed that treatment with an α1-adrenergic agonist, phenylephrine evoked a transient Ca2+ mobilization in Fluo-3-loaded osteoclastic cells. Conclusion: Using our in vitro co-culture models, we demonstrated that nerve-osteoclastic cell communication can occur without intervening cells and that this osteoclastic activation might be mediated by α1-adrenergic receptor. These results implicate the direct action of the peripheral sympathetic nerve system in bone metabolism.
057 Effects of HCIO incorporated electrolyzed water on proliferation of KB-cells

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Objectives: Recently, a new electrolyzed water; PerfectPerio\textsuperscript{TM} water (PPW; Noguchi Dental Medical Research Institute, Tochigi, Japan,) was introduced containing high concentrations of free chlorine (Okada et al.,\textsuperscript{86th IADR}) to control oral infections. However, the effects of PPW on the oral tissue have not been evaluated yet. Thus, the aim of this study is to investigate cytotoxic effects of PPW on human epithelial cell line (HeLa-KB).

Methods: An MTT assay was employed to evaluate the effects of the PPW on KB cell proliferation and a permeability test was performed using fluorescence microscopy after staining with a LIVE/DEAD BacLight\textsuperscript{TM} Bacterial Viability Kit. The KB cells were grown (1x10\textsuperscript{4} cells/well) and incubated for 1-9 days. On the 3rd day of culture, 10sec treatment was performed with different functional chlorine concentrations of PPW; PPW1 (600ppm), PPW2 (300ppm), PPW4 (150ppm) and PPW6 (100ppm). Mineral water (MW), Listerine and 0.12\%NaOCl were used as controls. MTT data were taken on day 1, 3, 5, 7 and 9 after treatment using 20\textmu l of MTT solution in each well, followed by further incubation for 4 hours. Results: Statistically significant increase in Optical Density (OD) was observed in KB cells treated with PPW4 and PPW6. However, OD remained unchanged in PPW1 and PPW2 groups. Permeability tests displayed absolute co-relationship with MTT data as most of the cells fluoresced green indicating integrity in the nuclear membrane in case of PPW4 and PPW6 which was almost same as untreated and MW treated control groups on the 5th day after treatment. Conclusion The results indicate that the 4 or 6 times dilution of original PPW concentrations can be considered for prophylactic treatment to control cariogenic and periodontal infections and would serve as very useful baseline information for further clinical studies. This study was supported by G-COE Program, IRCMSTBD at TMDU and Noguchi-DMRI.

058 A histological study of human dental pulp-derived odontogenetic cells

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Objective: We reviewed the histology of the same HDPD cells in a collagen type I gel scaffold cultured in conditioned Dulbecco’s Modified Eagle Medium (DMEM).

Methods: The collagen gel was paved on collagen type I floor and cultured with \beta-GP(-) DMEM and was designated to be the control group.

Results: Immunohistochemical studies of HDPD cells-and-collagen mixture cultured in \beta-GP(+) DMEM (experimental group) demonstrated differentiation of odontoblast-like cells, which secreted DSP, DSPP and collagen types I and III in the ECM. Fine structure TEM study observed they were flat and polygonal cells with many elongated cellular processes. In accordance with the increase of ALP activity and expression of DSPP mRNA of the HDPD cells, vital staining demonstrated hard tissue formation in ECM of the \beta-GP(+) experimental group. The TEM study also revealed initial matrix vesicle-related and subsequent collagen-related mineralization in the experimental group. Results of this in vitro study elucidate that odontogenic HDPD cells cultured in the \beta-GP(+) DMEM have same properties as the odontoblast-like cells observed in our previous in vivo transplantation study.

Conclusion: The HDPD cells differentiate and initiate dentin formation in the either collagen type I or gelatine scaffolds, but they showed different morphology in either in vivo or in vitro environments.
Enhanced wound healing by matrix metalloproteinase-3 after dental pulp amputation
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Objectives: Matrix metalloproteinases (MMPs) play critical roles in morphogenesis, angiogenesis, wound healing and tissue remodeling in the physiology and pathology of inflammation and cancer. When the pulp is damaged or exposed, pulp cells have the potential to differentiate into dentin-forming odontoblasts. However, the mechanism of angiogenesis during pulp wound healing still remains unclear. We hypothesized that a member of the matrix metalloproteinases, MMP-3, may play a role in pulp wound healing. Methods: Dental pulp tissues were isolated immediately and 12, 24, 48 and 72 hours after injury and real-time RT-PCR of MMP-3 was performed. Immunohistochemical analysis of MMP-3 was performed 24 and 72 hours after injury. The proliferative, migrative and anti-apoptotic effects of MMP-3 were examined in vitro. MMP-3 was applied to the amputated pulp, and newly formed blood vessels at 24 hours were quantitatively analyzed by staining with BS-1 lectin. Reparative dentin formation was compared with a PBS control at 72 hours after treatment. Results: MMP-3 mRNA was upregulated at 24 hours after pulp injury. MMP-3 protein was localized in endothelial and/or endothelial progenitor cells in injured pulp in vivo. MMP-3 showed mitogenic, chemotactic and anti-apoptotic effects on human umbilical vein endothelial cells (HUVECs) in vitro. Furthermore, the application of MMP-3 protein to the amputated pulp enhanced angiogenesis and reparative dentin formation in vivo. Conclusion: These results suggest that MMP-3 released from endothelial and/or endothelial progenitor cells in injured pulp might play critical roles in angiogenesis and pulp wound healing.

TNF-α enhances MMP-2 production in deciduous dental pulp fibroblasts
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Objectives: Inflammation in deciduous dental pulp tissue is associated with tissue degradation. The matrix metalloproteinases (MMPs) are believed to participate in its destruction. The elevated levels of some MMPs have been reported in inflamed pulp and periapical lesions. In inflamed pulp, many kinds of inflammatory cytokines such as TNF-α are released from inflammatory cells. In this study, we examined whether TNF-α affected the production of MMP-2 in deciduous dental pulp fibroblasts and its signaling pathway. Methods: Dental pulp fibroblast cultures were established from cells growing out of the dental pulp tissue of deciduous teeth. Cell proliferation was evaluated using the CellTiter 96® AQueous. The production of MMP-2 in deciduous dental pulp fibroblasts and its signaling pathways were evaluated utilizing gelatin zymography and western blotting analysis. Results: TNF-α increased the production of MMP-2 in a dose-dependent manner in deciduous dental pulp fibroblasts. LY294002 and Wortmannin, which are PI3-K inhibitors, inhibited MMP-2 production induced by TNF-α in deciduous dental pulp fibroblasts. Moreover, in deciduous dental pulp fibroblasts cultured with TNF-α, AKT was phosphorylated in a time dependent manner with the maximum phosphorylation at 30min, and LY294002 and Wortmannin abolished phosphorylation of AKT in TNF-α-stimulated deciduous dental pulp fibroblast. Conclusion: These results suggest that during pulp inflammation, TNF-α may enhance pulp tissue destruction in part by regulating MMP-2, and AKT pathway is involved in the MMP-2 production in deciduous dental pulp fibroblast.
An oral rehabilitation robot for muscle massage

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Objectives: This study was aimed to determine the suitable condition for masseter and temporal muscles massage using a specially fabricated robot named the "Waseda-Asahi Oral-Rehabilitation Robot No. 1 (WAO-1)" and to evaluate its effects on patients with TMJ dysfunction associated with myofascial pain.

Methods: WAO-1 was composed by two 6-degree of freedom (DOF) arms with plungers attached at the end-effector. Massage was applied to the patient by controlling the force and position of the plunger (virtual compliance). Three massage pressures (1, 6-8, and 10 N) were tested in twelve healthy volunteers. All volunteers were examined on the mouth opening, and asked to record subjective evaluations regarding comfortableness, warmth and ease of mouth opening using the visual analogue scale (VAS). The thickness and intramuscular appearances were evaluated using ultrasonography.

After the determination of massage pressure, the robot was applied to some patients. The massage pressure applied started at 6 N and was increased gradually. One-minute massage was performed alternately for the masseter and temporal muscles and a treatment session consisted of seven repeated massages. This treatment was performed three times every two weeks. The maximum mouth opening was measured before and after massage together with subjective evaluations including pain.

Results: On subjective evaluation, most volunteers showed the highest VAS score for comfortableness at 6-8 N, while the warmth and ease of mouth opening scores were the highest at 10 N. The VAS after massage was slightly related to muscle thickness.

The maximum mouth opening increased and the pain decreased in all patients after treatment.

Conclusion: This robot appeared to be a potential tool for the treatment of TMJ dysfunction associated with myofascial pain.

Oral health care using a rapid oral bacteria detection system

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Objectives: Management of oral health care requires a high quality team-care system consisting of multidisciplinary medical professionals and facility staff, with a PDCA cycle care plan based on the proper assessment of oral hygiene and function and risk management. In this study, we verified the efficacy of the management of oral health care by dental hygienists using a rapid oral bacteria detection system at an institution for dependent elderly persons.

Methods: A total of 60 subjects, living at an institution for dependent elderly persons in Yamanashi Prefecture, participated in this study (mean age: 85.6 years, 12 men and 48 women). The study was performed three times in series: in July, 2007 (1st intervention), in November, 2007 (2nd intervention: monitoring), and in March, 2008 (last intervention). Dental hygienists prepared an interventional oral care plan for the subjects, based on the risk assessment of each subject's oral region, and conducted oral care intervention in cooperation with the facility staff. We used a rapid oral bacteria detection system, which was newly developed, for evaluation of oral hygiene.

Results: In 48 subjects in whom all three assessments could be made, the number of oral bacteria at the final intervention had significantly decreased (p<0.05) compared to that at the first intervention. For 13 of 34 subjects who were at high risk at the first intervention, the care plan was reconsidered at the second intervention for monitoring. As a consequence, it was observed that the number of oral bacteria had decreased at the last intervention.

Conclusion: This study suggested that the rapid oral bacteria detection system is effective for management of oral health care, providing a clear indication of improvement of oral hygiene.

This study was partly supported by a Grant-in-Aid from the Japanese Ministry of Health, Labour and Welfare (H19-choju-011) in 2007.
063  Oral health and ADL in 90-years old people in Japan

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Objectives: The purpose of this research is to analyze the relations between the ability of daily living activities (ADL) in 90-years old people and the change of the present number of teeth from 85 to 90 years old.

Methods: Questionnaire survey and dental check-up were executed for the 90 years old people who lived in Iwate Prefecture. The questionnaire consists of 57 items concerning lifestyle, oral hygiene, etc. The questions about lifestyle based on the Tokyo Metropolitan Institute of Gerontology (TMIG) index of competence were used.

Results: The average number of present teeth among 102 subjects was 3.36±5.91. The subjects were 41 dentulous (male: 25, female: 16) and 61 edentulous (male: 13, female: 48), and the number of dentulous subjects in male were more than in female (p<0.01). We classified the subjects into two groups (dentulous and edentulous), and analyzed the difference of lifestyle. As a result, a high level of the intellectual activity was shown in dentulous subjects was admitted (p<0.01). We examined relations between the change of the number of present teeth and ADL of 89 subjects who took the dental check-up at 85 years old. They were classified into three groups (edentulous at 85 years old: 52, edentulous before 90 years old: 4, dentulous at 90 years old: 33). As a result of MANOVA and the multiple comparison test, it was shown that the total score of TMIG index of competence of the dentulous subjects was intentionally higher compared with the subjects who were edentulous at 85 years old (p<0.01).

Conclusions: It was shown that ADL of dentulous subjects was high. ADL of senior people may be able to be maintained if enough oral function will be provided. The result of this cohort survey from 80 to 90 years old will be analyzed in the future.

064  FEL showed dental tissue specificity of laser ablation

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Objectives: Laser ablation is widely applied to dental clinics. Mechanism of laser ablation, however, has not been clarified, and lined up many candidates. Free electron laser (FEL) is featured by variable wavelength. The aim of this study was clarify the optimum wavelength for dental hard tissue ablation.

Methods: FEL was generated at LEBRA, Nihon University, at the wavelength range from 2.0 micron to 6.0 micron. Polished tooth sections were irradiated by LEBRA-FEL at the varied conditions. Pits formed on the surface of teeth were analyzed by a laser displacement meter (LT-8010, KEYENCE, Osaka).

Results: Optimum wavelength for the dental hard tissues ablation was at about 3.0 micron, but there was slight but significant difference in the optimum ablation wavelength for dental enamel and dentin, and also for enamel-dentin junction region. The formed pit shape varied not only in depth but also in width. Thermal damage was not observed around and in the pits.

Conclusion: Varied wavelength using FEL revealed that the variation in optimum wavelength for dental hard tissues. Mechanism of pit formation on the dental hard tissue surface may be due to plasma ablation rather than mechanical or heat ablation. Micro-water combustion theory should be re-discussed because of the variation in optimum wavelength. This study was supported in part by a Grant-in-Aids for Scientific Research, MEXT, Japan (17591927).
065  **Comparison of fatigue and tensile strength of radicular dentin**

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**Objectives:** This study carried out a comparison of the fatigue and tensile strengths of bovine radicular dentin.

**Methods:** Forty bovine lower central incisors were used, twenty teeth for the fatigue test and twenty for the tensile test. Dentin slabs 1mm in thickness were cut from the teeth using a low-speed cutting machine. The slabs were sectioned from the radicular dentin of the tooth. A dentin slab was harvested from each tooth. After preparation, a rectangular block (3.0 x 3.0 mm) was cut from each dentin slab, and dumbbell-shaped specimens with a narrow central portion (1.0 x 1.0 x 1.5 mm) were obtained using an air turbine hand piece attached to a profiling machine. For the fatigue test, specimens were mounted on a servo-hydraulic test machine in 37°C Hank’s balanced saline solution with a haversines cyclic load of 1 Hz for frequency, maximum load from 35 to 50 MPa and minimum load 5 MPa. The staircase method was employed to determine the fatigue strength and the standard deviation. A texture analyzer was used for the tensile test. Mean fatigue and tensile strengths were compared statistically by one-way ANOVA and Fisher's PLSD test (p<0.05). Fracture surfaces were observed by scanning electron microscopy.

**Results:** The mean fatigue strength and tensile strength were 44.3 and 87.4 MPa, respectively. There was a difference of fracture surfaces between the fatigue and tensile test groups; the fracture surfaces of the fatigue test were smooth while those of the tensile test were rough.

**Conclusion:** The fatigue strength of radicular dentin is significantly lower than the tensile strength. In this study the ratio of fatigue strength to tensile strength was 0.5.

066  **Bleaching efficiency employing various light units and irradiation time**

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**Objectives:** To evaluate the bleaching efficiency of various light units utilizing bleaching agent containing titanium dioxide photocatalyst (TiO2).

**Methods:** Labial surfaces of forty bovine incisors were ground flat, polished and stained with black tea for one week. A bleaching agent (Pyrenees, Mitsubishi Gas Chemical, Japan) contains low concentration H₂O₂ and TiO₂ was applied on the stained labial surface followed by light irradiation for either one or five min with different light sources and intensities: (Optilux 501, Kerr, Danbury, USA; Hyper Lightel, Ushio, Gonma, Japan; G-light, GC, Tokyo, Japan; and LEDemetron 1, Kerr, Danbury, USA). In one min group the treatment was repeated fifteen times while for five min ten times. CIE L*a*b* were measured with dental chromameter (ShadeEye NCC, Shofu Inc., Japan). Data were analyzed with ANOVA followed by Tukey’s HSD test and T-test (α = .05).

**Results:** All lights induced significant change in color lightness at both times of irradiation (p<0.05), except for Hyper Lightel at 5 min because continuous activation was not applicable. Hyper Lightel showed significantly highest bleaching effect followed by LEDemetron 1 and Optilux that not different from each other in one minute activation group, however, no difference was observed in 5 min. At each application, a higher lightness was recorded in specimens irradiated for five minute than one min (p<0.05) irrespective of light source.

**Conclusion:** The bleaching efficiency using Pyrenees was light source, intensities and irradiation time dependent.
The newly developed root canal filling materials

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Objectives: Resin-based sealer do not completely adhere to each other or to the root canal walls, thus resulting in insufficient sealing and microbial leakage. The purpose of this study was to develop a new adhesive root canal filling material containing EMA resin and to assess its physical properties.

Methods: (1) Preparation of specimens: EMA resin was prepared by mixing the following components: (monomer) 60% methacryloxyethyl methyl succinate, 40% 1,6-hexanediol dimethacrylate, 0.6% DL-comphorquinone and 3.0% dibutyltinlaurylate and (polymer) polyethylmethacrylate. And ethanol was added at various concentrations into the EMA resin. (2) Elastic modulus: These specimens were measured with INSTRON 4481 and the elasticity modulus was calculated. (3) Longitudinal weight changes: Each specimen was kept into the bottle in the temperature room without the lid. The change in weigh was measured t every 24 hours. (4) Adhesive test: A gap (0.5mm) was made between the 2 specimens. Resin-based sealer was placed between 2 specimens. And so, those were measured with INSTRON 4481, and the tensile strength of specimens was calculated. Then, the surface texture of the specimen were observed. (4) FT-IR: The molecular bonding characterization of the specimen including ethanol was analyzed and compared with the non-ethanol specimen.

Results: The EMA resin-based material showed an ethanol dose-dependent decrease in the elastic modulus value. The weight of specimen decreased as the time erupted, and the phenomenon was remarkable as the concentration of ethanol getting higher. The adhesive strength of the EMA resin was also weakened dose-dependently by the ethanol, and was significantly higher than that of the GP. EMA resin was not reacted chemically with Ethanol according to the results from FT-IR.

Conclusions: The newly developed EMA resin-based material is suitable for clinical use as a root canal filling material.

Microleakage of gutta percha and resilon at different canal length

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The sealing of root canal filling is proportional to the length of the remaining filling. Reduction of gutta percha filling to 5 mm is considered as a safe margin. Advances in dentin bonding technology have led to the development of new root canal filling, Resilon. Objective To assess the efficacy of two root canal filling, gutta percha and Resilon, to preserve apical seal after post space preparation. Methods: Twenty four single rooted teeth with 14 mm long were prepared with step back technique using K-flex file and gate glidden drill. Apical enlargement was performed to #60 file while the coronal orifice was controlled to diameter of 2 mm. All roots was assigned randomly to two experimental groups of 12 each. Group I was obturated with Resilon/RealSeal, while the other was obturated with gutta percha/ZOE sealer. After that, the root canal filling in both groups was removed 10 mm and 12 mm. All teeth at each length of root canal filling were subjected to fluid filtration analysis to measure volume of fluid flow rate (nl/sec). Data was analyzed statistically using repeated analysis of variance. Results: After removal of root canal filling, the fluid flow rate increased statistically significant (p<0.05). However, the groups obturated with Resilon/RealSeal showed significantly lower fluid flow rate than the others, irrespective of post space preparation length. Conclusion: Reduction of root canal filling, both Resilon and gutta percha, affect the apical microleakage; however, Resilon seemed to perform better apical seal than gutta percha.
069  **Shaping ability of two rotary Ni-Ti instruments in curved canals**

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**Objectives:** To compare the shaping ability of K3 and Easy RaCe rotary Ni-Ti instruments in curved canals with the crown down preparation technique. **Methods:** Forty mesiobuccal and mesiolingual root canals (average degree of curvature 22.5°, average radius of curvature 7.45 mm) of extracted mandibular first molars were used. Twenty root canals were instrumented using the K3 and the others were instrumented using the Easy RaCe. The cross-sectional shapes of each canal at three different levels were evaluated before and after instrumentation by using Endo block system. At each level two parameters were evaluated: the centering ability of instruments and the roundness of canals. In addition, the time required to prepare the canals was also evaluated. **Results:** There was no significant difference between the mean centering ratio of K3 and Easy RaCe groups in every level of root canal at p>0.05. There was no significant difference in the roundness in the coronal and middle sections but the canals instrumented with the Easy RaCe were significantly rounder than those of the K3 in the apical sections (p<0.05). For the preparation time, the Easy RaCe was significantly faster than the K3 (p<0.05). **Conclusion:** When compared to the K3, the Easy RaCe created better root canal preparation at the apical third level with less time.

070  **Bond strengths of resin composite to caries-affected root canal dentin**

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**Objectives:** The purpose of this study was to evaluate the bond strengths of dual-cure resin composite to caries-affected root canal dentin with different caries removal techniques. **Methods:** Fifteen freshly extracted single root teeth with carious root canal dentin and five sound teeth were used in this experiment. Each root was longitudinally hemisected and excavated according to the following criteria.  
(1) Dye-stain 1 (excavated until stain free)  
(2) Dye-stain 2 (excavated until pale-pink-stained)  
(3) Probing (excavated until hard to a sharp probe)  
(4) Control (pulpal dentin walls were polished flat)  

On dye-stained groups, infected dentin stained with Caries Detector solution (Kuraray Medical, Japan) was removed with a water-cooled low-speed steel round bur. On probing group, infected dentin was removed by bur excavation until the dentin surface became hard to a sharp probe. Prepared dentin was bonded with Clearfil Mega Bond (Kuraray Medical, Japan). Composite build-up was performed with Cleafil DC Core automix (Kuraray Medical, Japan). All specimens were stored in water at 37 °C for 24 hours in darkness and specimen was shaped hourglass configuration with a cross-sectional area of approximately 1mm² for microtensile testing. The specimens were loaded in a microtensile testing device (AUTOGRAPH AGS-H, Shimadzu, Japan) at a crosshead speed of 1.0 mm/min. Data were analyzed by one-way ANOVA and Tukey's HSD test (p<0.05). **Results:** Microtensile bond strength was following (MPa):

<table>
<thead>
<tr>
<th>Group</th>
<th>Bond Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye-stain 1</td>
<td>47.7±6.4 a</td>
</tr>
<tr>
<td>Dye-stain 2</td>
<td>52.3±5.9 a</td>
</tr>
<tr>
<td>Probing</td>
<td>71.1±9.8 b</td>
</tr>
<tr>
<td>Control</td>
<td>71.9±8.2 b</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant difference. **Conclusion:** The caries removal technique affected the microtensile bond strength. Probing group showed higher tensile strength than used the solution group.
071 Effect of microperoxidase primer on bonding three dentin adhesive systems
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Objectives: The purpose of this study was to evaluate the efficacy of an experimental primer for increasing the bond strength between dentin and three different types of adhesive systems. Methods: The primer consisted of a microperoxidase (MP-11), 2-hydroxyethyl methacrylate, and water. The adhesive systems prepared were an etch-and-rinse system using a dentin conditioner containing 10wt% citric acid and 3wt% ferric chloride and 4-META/MMA-TBB resin (10-3/Super-Bond, Sun Medical), a self-etching system composed of a self-etching primer and a resin-composite luting agent (Panavia F2.0, Kuraray Medical), and a self-adhesive system composed of a resin-composite luting agent (SA Luting, Kuraray Medical). These products were used in conjunction with the MP-11 primer, and were designated as 10-3/MP-11/Super-Bond, MP-11/Panavia F2.0, and MP-11/SA Luting, respectively. The dentin surfaces of human premolar teeth were ground with #600 wet silicon carbide paper, modified with the dentin conditioners alone and also with the primer added, and then an acrylic rod was bonded to a defined bonding area (2 mm in diameter) of each specimen. Shear bond strengths were determined after 24 hours of storage in water. Data were analyzed using ANOVA and a multiple comparison test (n=9, p<0.05). Results: The highest mean bond strengths were obtained with 10-3/MP-11/Super-Bond, followed by 10-3/Super-Bond, MP-11/Panavia F2.0, MP-11/SA Luting, Panavia F2.0, and SA Luting (Table). It is suggested that the additional use of the MP-11 primer improved the bond strength of the etch-and-rinse, self-etching, and self-adhesive systems to dentin. Table. Means and standard deviations of shear bond strengths (MPa).

072 Resin bonding to dentine after casein phosphopeptide-amorphous calcium phosphate treatments
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Objective Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has an ability to enhance enamel and dentine remineralisation and thus has been incorporated in new medicaments for such clinical problems as tooth hypersensitivity and early carious lesions. The current study investigated whether the application of CPP-ACP in the proprietary paste, Tooth MousseTM, would influence the subsequent resin adhesion to dentine. Methods Bonding effectiveness was assessed by microtensile bond strength test and scanning electron microscopy (SEM). A three-step etch-and-rinse adhesive (OptiBond FL: Kerr, USA) or a two-step self-etch adhesive (Clearfil SE Bond: Kuraray Medical, Japan) was bonded to three groups of dentine surfaces: no treatment; 5 minutes or 5 days CPP-ACP application. The microtensile test was performed and bond strength data were analysed using ANOVA and Tukey-Kramer post hoc test. Etching characteristics on respective dentine surfaces and resin-dentine interfaces were observed under SEM. Results Bond strengths of Clearfil SE Bond to dentine were similar for all tested groups. OptiBond FL; however, showed lower bond strengths following the application of CPP-ACP, Tooth MousseTM, for both time periods. Under SEM observations, the CPP-ACP treated dentine displayed a layer of residue or precipitate attached to the surfaces after the phosphoric acid treatment. The sectioned specimens also showed up the resin-dentine interface with less intertubular linking of resin tags, which could mean some mineralisation of these channels, hence preventing resin infiltration. Conclusion The presence of CPP-ACP on the dentine surface may compromise bonding effectiveness of etch-and-rinse adhesive system. However, CPP-ACP application may be beneficial to the dentine bonding of self-etch system, as the chemical interactions between calcium and functional monomers of the adhesives might be enhanced to some degree.
**Effects of obesity on oxidative stress in rat gingiva**

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Objectives: Studies have suggested a correlation between obesity and periodontal disease. It has been shown that oxidative stress is involved in progression of periodontitis. The purpose of this study was to investigate the effects of obesity on gingival oxidative stress in rat periodontitis model.

Methods: The obese Zucker rats (n= 14) and their lean littermates (n= 14) were divided into two groups of 7 rats, respectively. In one of each group, periodontitis was ligature-induced for 4 wks, while the other group was left unligated. In order to examine gingival oxidative stress, the level of 8-hydroxydeoxyguanosine (8-OHdG) and the ratio of reduced/oxidized glutathione were determined. Serum level of hydroperoxides was measured by a free radical electric evaluator. In addition, the gingival gene expression pattern related to oxidative/metabolic stress, inflammation and cell behavior was evaluated using real-time polymerase chain-reaction array technology.

Results: In comparison with the lean rats without periodontitis, the obese rats without periodontitis showed a 57% increase in 8-OHdG level (p< 0.05) and an 18% decrease in reduced/oxidized glutathione ratio (p< 0.05) in the gingival tissue, with increasing serum level of hydroperoxides (p< 0.05). The obese rats with periodontitis also increased 8-OHdG level by 61% (p< 0.05) and decreased reduced/oxidized glutathione ratio by 37% (p< 0.05) compared to the lean rats with periodontitis. Furthermore, in the periodontal lesions, the expression of multiple cytochrome P450 genes were more than 2-fold down-regulated by obesity.

Conclusions: Obesity induces gingival oxidative stress by increasing serum hydroperoxides. In addition, a reduced capacity for xenobiotic detoxification in the gingival tissue may be involved in augmentation of periodontitis-induced oxidative stress in the obese model.

**Oral infection of Porphyromonas gingivalis induces pro-atherogenic change in mice**

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Objectives: Results of epidemiological studies suggest that periodontal infection may play a role in systemic conditions such as cardiovascular diseases. However, there is no evidence for a causal effect. The aim of the present study was to determine whether oral infection of Porphyromonas gingivalis (P. gingivalis) could induce atherogenic changes in mice. Methods: After pretreatment with antibiotics-containing drinking water for 10 days and subsequent 3-days antibiotic-free period, C57BL/6J mice were infected with live P. gingivalis W83 (10⁹ CFU in 100 μl of PBS with 2% carboxymethylcellulose) orally using feeding needle. Infection was repeated ten times at 3-days intervals. Control mice received the antibiotic pretreatment and then sham infected. After 2days of last infection, the mice were euthanized and tissues and sera were obtained. Alveolar bone resorption was assessed by X-ray micro-CT. Serum hs-CRP and IL-6 were measured by ELISA. Gene expression profiles of aorta and liver were analyzed by DNA micro array and quantitative real-time PCR.

Results: The Experimental group demonstrated significantly higher serum hs-CRP and IL-6 compared with the control group in addition to an elevated alveolar bone resorption. Gene expression analysis revealed that periodontal infection up-regulated the expression of several genes involved in the atherogenesis such as CCL2 and CRP in the aorta and the liver, respectively. Furthermore, several genes associated with regulation of blood pressure and insulin resistance were down-regulated. Conclusion: Periodontal infection does affect pro-atherogenic change in various tissues through modulating the gene expression profiles suggesting causal association between periodontitis and systemic diseases.
**075 Effect of non-surgical periodontal therapy on plasma reactive oxygen metabolites**

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Objectives: Periodontitis induce excessive production of reactive oxygen species in periodontal lesions, which might lead to systemic oxidative stress. However, little is known about the relationship between periodontitis and systemic oxidative stress. Recently, the method of measuring reactive oxygen metabolites (ROM) in blood samples has been introduced as a useful method for evaluating the oxidative stress in the body. The main components of ROM are hydroperoxides, which are intermediate oxidative products of lipids, peptides, amino acids and DNA. The aim of this longitudinal study was to investigate the effect of non-surgical therapy on plasma ROM level in chronic periodontitis patients.

Methods: Fifteen chronic periodontitis subjects (mean age: 39.3 years) were monitored at baseline prior to non-surgical therapy (full-mouth scaling and root planing) and 1 and 2 months after the therapy. Plasma was obtained at the baseline examination and at re-assessments in addition to periodontal parameters. Plasma was also obtained from 15 control subjects without periodontitis (mean age: 39.5 years). Plasma level of ROM was determined with a free radical electric evaluator, according to the analysis procedures.

Results: At baseline, chronic periodontitis patients had higher plasma ROM level (417.5±56.2 CARR U) than control subjects (325.9±33.7 CARR U; P<0.001). Probing pocket depth, clinical attachment level, bleeding on probing and plaque control record in chronic periodontitis patients were significantly improved after 1 and 2 months treatment, which were accompanied by a significant reduction of plasma ROM level (317.4±47.9, 288.3±58.6 CARR U; P<0.001).

Conclusions: In chronic periodontitis patients, non-surgical periodontal therapy was effective in improving clinical parameters and in reducing plasma ROM. The improvement of chronic periodontitis could offer the clinical benefits on decreasing systemic oxidative stress.

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**076 Expression of collagenases in development of rat periradicular lesion**

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Objective: Matrix metalloproteinases (MMPs) degrade the extracellular matrix (ECM) in chronic inflammation and bone-destructive lesions. The objective was to elucidate the expression of collagenases (MMP-8 and MMP-13) in experimentally induced rat periapical lesions.

Methods: Rat periapical lesions were induced in male Wistar rats (n=30, 8 weeks of age) by unsealed pulp exposure of the lower first molars. The animals were humanely sacrificed at 0 (nonexposure control), 1, 2, 3, 4, and 6 weeks after exposure and the lower jaws were extracted. The left molars were used for immunohistochemical staining and the right molars were employed for reverse transcription-polymerase chain reaction (RT-PCR) analyses. In the histological sections, the sizes of periapical lesions were measured. Then, the periapical tissue of the mesial root was histopathologically observed and the numbers of cells immunopositive for MMP-8 and MMP-13 that appeared in the lesions were counted.

Results: Gene expression of MMP-8 was gradually increased compared to the normal state from 1 to 4 weeks, but slightly decreased at 6 weeks. Gene expression of MMP-13 was largely increased compared to the normal state from 1 to 2 weeks. At 3, 4, and 6 weeks, the expression was stronger. The size of periapical lesions gradually increased from 1 to 4 weeks, but decreased at 6 weeks. There were significant differences between normal and pulp exposure groups. Regarding immunohistochemistry, the number of MMP-13 cells expressing was significantly higher than that of MMP-8 cells expressing at 1 and 2 weeks, but not at 3, 4, and 6 weeks.

Conclusion: Expressions of MMP-8 and MMP-13 were increased as rat periapical lesions extended. It was shown that MMP-13 was expressed earlier in comparison with MMP-8. These results suggest that MMP-8 and MMP-13 might be involved in the development of rat periapical lesions.
Salivary cortisol and Th1/Th2 cytokines of patients with complaint halitosis

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Objective: The aim of this study was to determine the levels of cortisol and Th1/Th2 cytokines in saliva samples from patients with various forms of halitosis and then to analyze their possible relationship to mental stress against halitosis.

Methods: Forty patients examined at the Breath Odor Clinic of our university hospital, were classified into the two categories, genuine halitosis (GH) and psychosomatic halitosis (PH), on the basis of the results of organoleptic measurement and gas chromatography. The PH group consisted of pseudo-halitosis and halitophobia patients. Twenty-one healthy volunteers without complaint of halitosis were involved as the control group. Resting saliva was collected and concentration of cortisol was measured by ELISA. Levels of Th1/Th2 cytokines (IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-13, IFN-γ, GM-CSF, TNF-α) in saliva were simultaneously determined using Bio-plex® suspension array system.

Results: Significantly higher levels of cortisol were observed in PH group in compared to the GH group and control group (p<0.05). When comparison was made between patients with high salivary cortisol levels and those with low levels, a higher prevalence of subjects positive for a Th2 cytokine IL-5 was shown in the former group.

Conclusion: The results suggest that the level of salivary cortisol is elevated in PH patients in comparison with those in GH patients and control subjects. The anxiety for oral malodor would stimulate the cortisol production and this may, in turn, alter the balance between Th1/Th2 cytokines.

Facilitated anion delivery through human enamel and dentin with AC-iontophoresis

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Objectives: Enamel is a defensive barrier to external irritation although it is permeable to monomeric substances. The aims of the present study were to analyze the relation between the concentration and electrical conductance of lidocaine hydrochloride and to quantitatively evaluate enamel/dentin permeability using AC iontophoresis. Methods: Electrical impedance of different concentrations of lidocaine hydrochloride was measured at a fixed temperature using a bipolar platinum electrode. The relation between concentration and conductance of the solution was examined. Two chambers were used; one was filled with extrapure water and the other 2 or 50% of lidocaine hydrochloride. Six premolars were extracted for the orthodontic treatment. The fresh tooth crowns were transversely cut at enamel/cementum junction and fixed between the two chambers with two O-rings. Lidocaine hydrochloride was put in the enamel side chamber and extrapure water was in the dentin side. Simulated hydrostatic pulp tissue pressure was applied to extrapure water. Change in the concentrations of lidocaine hydrochloride was measured every 2 minutes with a platinum recording electrode positioned in the center of the extrapure water. Two platinum plates parallel to each other were set at both ends of the chambers for stimulation using amplified AC sine. Passive diffusion without iontophoresis was used as control. After measuring electrical impedance, we examined the enamel surface of sample tooth crowns using scanning electron microscopy. Results: One tooth crown that had enamel cracks and showed prominently higher conductance was excluded from the experimental samples. Electrical conductance (G, mho) correlated closely to the concentration (x, mmol/L) of lidocaine hydrochloride (G=2.16x2+0.0289x+0.000376, r²=0.999). Lidocaine hydrochloride passed through enamel and dentin increased with time against the dentinal fluid flow caused by simulated pulpal tissue pressure. Conclusions: Diffusion of lidocaine hydrochloride through enamel and dentin increased in quantity using AC iontophoresis.
079 TNF-α-induced bone resorption model using CHP nanogel in mice

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Objectives: TNF-α is a pivotal cytokine in lipopolysaccharide (LPS)-induced bone resorption. We have previously found that LPS-induced bone resorption is significantly increase in TNF receptor type 2 (TNFR2)-deficient mice compared to the wild type mice. It is, however, difficult to clarify the mechanism of bone resorption induced by LPS since many cytokines are secreted after LPS injection. Therefore we tried to establish a simple bone resorption model using TNF-α. Nanogel of cholesterol bearing pullulan (CHP) have developed as nanocarrier which can form complex with hydrophobic drugs or protein for the control release. In this study, we tested whether the CHP-TNF-α complex could induce bone resorption or not and tried to establish the TNF-α-induced bone resorption model. Methods: Five-week-old male TNFR2-deficient mice were used. Either 2 μg of TNF-α in 50 μl PBS or TNF-α/CHP solution was injected once a day for 4 days subcutaneously onto the calvaria of each mice and sacrificed 24h after the last injection. Calvariae were dissected and fixed for 3 days. Then they were subjected to μCT analysis and to the bone mineral density (BMD) measurement using dual x-ray absorptiometry. Results: 3D-μCT reconstruction images showed many resorption pits on calvariae in the TNF-α/CHP injected group, but not in the TNF-α injected group. The calvarial BMD in the TNF-α/CHP injected group was significantly decreased compared to the vehicle/CHP injected group (12.2 ± 0.1 vs 11.27 ± 0.55 (mg/cm², p<0.05), but not in the TNF-α injected group vs vehicle injected group. (11.9 ± 0.4 vs 12.4 ± 0.5 (mg/cm², p=0.23)) Conclusion: The bone resorption model by using CHP nanogel might be suitable for evaluating the TNF-α-induced bone resorption.

080 Osteoclast differentiation during tooth eruption in microphthalmic mice

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Objectives: Microphthalmic (mi) mouse is an osteopetrotic mutation characterized by reduced osteoclastic bone resorption. This mutation has been shown to result from abnormalities in the microphthalmia transcription factor (MITF). The MITF is thought to be essential for osteoclast differentiation, but the site-specific effects on osteoclasts are not fully elucidated in this mutant. The objective of this study was to investigate disorders of osteoclast differentiation in mi mice. Methods: Mice from normal and mutant group were sacrificed 7, 10, 14 days after birth, then tibia and maxilla including the first molar were excised. Samples were examined histologically and ultrastructurally. Results: In both tibia and maxilla, the number of osteoclast in mi mice decreased and size of cell was smaller than those of normal mice. Although mutant osteoclast showed generally mononuclear and less activity for TRAP staining, some osteoclasts at the alveolar bone were multinuclear and heavy intensity for TRAP activity. Ultrastructurally, osteoclasts at the alveolar bone in mi mice were multinuclear, including cytoplasmic vacuolization, but these osteoclasts attached tightly at the bone surfaces and had poorly developed ruffled borders and clear zone compared to those of normal mice. Conclusion: These data demonstrate that the initial osteoclast differentiation including cell-fusion and TRAP activity is induced at alveolar bone surface during tooth eruption in mi mice, but these osteoclasts show impaired bone resorption due to poorly developed ruffled borders. It suggests that MITF is essential for full development of osteoclast.
081  Down-regulation of RANKL/NF-κB by β-TCP implant in dog jaw

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Objectives: Successful remodeling in the bone surrounding dental implants requires the coordinated activities of both osteoblasts and osteoclasts. Bone induction by different calcium phosphate biomaterials has been previously reported, and β-tricalcium phosphate ceramic (β-TCP) has shown osteoinductive activity in implant therapy. However, little is known about the molecular basis for mechanisms responsible for β-TCP in bone formation. To study the effects of β-TCP on mineralization, the gene expression profiles of dog jawbone tissue implanted with β-TCP were examined. Methods: The premolars of beagle dogs were extracted. After 3 months, β-TCP was implanted into cylindrical artificial bone defects (4.5 x 8 mm). After 7 and 14 days, all specimens were taken out, and total RNA was isolated from bone tissues using Multi-Beads Shocker. After amplification of mRNA, gene expression profiles were examined using Affymetrix GeneChip (Canine Genome 2.0 Array, ca. 38,000 genes) system. GeneChip data was analyzed using GeneSpring software and imported into Ingenuity Pathways Analysis (IPA). Results: At 7 days, 2,224 up-regulated genes and 2,503 down-regulated genes, at 14 days, 2,108 up-regulated genes and 5,304 down-regulated genes were observed (p<0.05). β-TCP altered many gene expressions, and decreased RANKL, IL-1, MYD88, TRAF2, RIP, and NF-κB1 gene expressions, which were involved in NF-κB signaling pathway. IL-1 is proinflammatory cytokine that is a potent stimulator of bone resorption and an inhibitor of bone formation. RANKL is a key mediator of osteoclast formation, function, and survival. Our findings suggest that β-TCP may inhibit the differentiation of osteoblast precursors to osteoclasts through reduction of RANKL and NF-κB expressions and promote matrix bone mineralization and calcification.

082  Stem cell-like characteristics of dental pulp and bone marrow cells

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Objectives: The stromal compartment of mesenchymal tissues is considered to harbor stem cells that display extensive proliferative capacity and multilineage potential. Stromal stem cells offer a potentially large therapeutic potential in the field of regenerative medicine. The present study is a comparison of stem cell like properties of CD 117 positive Mesenchymal cell cultures from Dental Pulp and Bone Marrow. Methods: Cell cultures were isolated from Milk Tooth Pulp. Bone Marrow cells were kindly provided by Dr. H. Ishikawa, Nippon Dental University. Cells in the cultures were separated by Magnetic Activated Cell Separation (MACS, Miltenyi Biotec). Further cell cultures, enriched in CD 117 positive cells, were obtained. Expression of CD117, CD44H, Oct ¾, alpha Feto-Protein and Serum Albumin were characterized in both cell lines by immunofluorescency and flow-cytometry and they were compared. Then the stem-cell-like characteristics of the two cell cultures were assessed. Results: Both Mesenchymal cell lines were proven to be positive for Pluripotent cell markers CD117, CD44H, Oct ¾. Enriching the cell cultures with CD 117 positive cells lead to increase of the number of cells in the cultures, expressing the other two stem cell related markers. The cells did not express alpha Feto-Protein and Serum Albumin. Conclusions: The present results demonstrate the increase of the stem cell markers in both Bone Marrow Mesenchymal Cell and Milk Tooth Pulp Mesenchymal Cell cultures after Magnetic Activated Cell Separation of CD 117 positive cells. It shows that the levels of stem cell markers of Milk Tooth Pulp Mesenchymal Cell are higher than Bone Marrow Mesenchymal Cells and is a basis for further investigations pre-requisite to establishing differentiation protocols and assessing the two CD 117 positive cell lines differentiation abilities.
α3β1 integrin-mediated MC3T3-E1 osteoblasts adherence induced by interleukin-1α

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Objective Osteoblasts play a central role in bone formation during healing phase of periapical periodontitis. Adhesion of osteoblasts to extracellular matrix proteins initiates bone formation at periapical lesion. Integrins compose a superfamily of cell surface receptors involved in cell-cell and cell-matrix adhesion. We have previously reported that interleukin (IL)-1α increased during periapical healing phase in a rat experimental periapical lesion (Arias et al, J Endod, 2007). However, biological roles of IL-1α on periapical healing are still unknown. In the present study, we investigated the IL-1α-induced integrin expression and adherence in MC3T3-E1 osteoblasts to clarify the possible mechanisms of osteoblasts adhesion-mediated periapical healing.

Methods Mouse osteoblastic cell line, MC3T3-E1 (Riken), was treated with IL-1α (0-10 ng/ml; R&D) for 24 hours, and the cells (1 × 10⁶ cells) were seeded in a 6-well tissue culture plate. Cell morphology was observed by phase-contrast microscopy. Non-adherent cells were removed by washing 3 times with phosphate-buffered saline solution, and adherent cells were counted using hemocytometer. Next, cells were treated with IL-1α for 24 hours, and the total cell lysates were collected for detecting integrin subunits α3 and β1 using Western blotting. In addition, to investigate the effects of p38 MAPK pathway on integrin expression, the specific chemical inhibitor SB203580 (10 μM; Calbiochem) was used.

The attachment of MC3T3-E1 osteoblasts to the culture plates after stimulation with IL-1α increased significantly compared to that of non-stimulated cells (Student’s t-test, p<0.05). Furthermore, IL-1α enhanced the expression of integrin subunit α3, but not β1, in MC3T3-E1 osteoblasts, and SB203580 dramatically suppressed IL-1α-induced expression of integrin subunit α3.

Our findings suggest that the enhancement of α3 integrin expression via p38 MAPK pathway by IL-1α may represent an important mechanism for adherence of MC3T3-E1 osteoblasts in periapical healing.

084 Oral malodorous compound suppresses the proliferation of osteoblasts

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Objectives Oral malodorous compounds including hydrogen sulfide (H2S) are periodontal pathogenic. The aim of the study was to determine the effect of H2S on cell proliferation of human osteoblasts and on the mitogen-activated protein kinase in a signaling transduction pathway.

Methods The human osteoblasts (NHOst)(Cambrex) and murine osteoblasts (MC3T3-E1) were cultured in the osteoblast growth medium supplemented with 10% fetal bovine serum in 5% CO2 at 37 °C. The growth factors were removed from the medium. During experiment, then the cells were cultured in the presence of H2S gas(0-100ng/ml). Cell proliferation was measured by [3H]thymidine incorporation in the 5% TCA insoluble fraction. The phosphorylation of Erk1/2 and p38 were determined with Western blot analysis.

Results After treatment of the NHOst cells with H2S for 24 hours, [3H]thymidine incorporation into the DNA was decreased significantly and H2S dose-dependently. At a concentration of 100 ng/ml H2S, [3H] thymidine incorporation decreased by 79% compared to the controls. Similar reduction was observed in MC3T3-E1 cells. The effects of H2S on signal transduction pathways for controlling cell proliferation were also determined in NHOst cells. Phosphorylation of p38 was induced by H2S. The phosphorylation of Erk1/2 was dose-dependently increased by H2S. The phosphorylation was started within 10 min after starting H2S incubation. Then, the phosphorylation slightly decreased. Activation of ERK1/2 induced by H2S was inhibited with the ERK1/2 specific inhibitor U0126.

Conclusion These results demonstrated that H2S inhibits cell proliferation of human osteoblasts through the MAPK cascade.
085 Roles of AMPK in osteogenic differentiation

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Objectives: AMP-activated protein kinase (AMPK), an α/β/γ-heterotrimeric kinase, is an energy sensor maintaining the energy homeostasis within cells. However, its role in bone remodeling is not known. In this study, we explored the roles of AMPK in osteoblasts by analyzing its effects on osteogenic differentiation in vitro. Materials and methods: MC3T3-E1 cells and primary osteoblasts derived from calvaria of C57BL/6 mice were induced to differentiate by a combination of 50 μg/ml VitC and 5 mM β-glycerophosphate in the presence or absence of metformin, a specific stimulator of AMPK. AMPK expression and phosphorylation levels during differentiation were analyzed by Western blotting. Osteogenic differentiation was evaluated by Alizarin red staining. The mRNA expression of AMPK subunits was analyzed by RT-PCR. Gene expression of osteogenic differentiation markers including transcription factors was analyzed by Northern blotting. Results: The protein and mRNA expression levels of AMPK subunits were not significantly altered during osteogenic differentiation. In contrast, phosphorylation of AMPKα subunit, which represents the activated form of kinase, was progressively downregulated in the course of differentiation. Consistently, stimulation of MC3T3-E1 cells with metformin during osteogenic differentiation significantly inhibited matrix mineralization and mRNA expression of osteogenic differentiation markers including RUNX2, osteopontin, osteocalcin, and bone sialoprotein. Conclusions: Our present data have suggested an inhibitory role of AMPK on osteogenic differentiation.

086 Effect of daidzein together with raloxifene in osteoblast-like cells

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Objectives: Osteoporosis is caused by decrease in bone mass resulting from estrogen deficiency. Hormone replacement therapy has been widely used for preventing and treating osteoporosis. However, the side effect including breast cancer is known to be accompanied. Daidzein (Dz) is a phytoestrogen derived from soy isoflavone, and raloxifene (Ral), which is a selective estrogen receptor modulator (SERM), is now accepted as anti-resorptive agents. We hypothesized that taking SERM with dietary intake of isoflavones could have a mutually potentiating effect in postmenopausal women with osteoporosis. The purpose of this study is to investigate the effect of Dz together with Ral, which act as a different anti-resorptive agents, in osteoblasts. Methods: Osteoblastic KUSA-1 cells were grown in culture medium with Dz and/or Ral. The differentiation and mineralization of osteoblasts were determined using alkaline phosphatase (ALP) activity and von kossa staining. Osteocalcin production in culture supernatant was evaluated by ELISA. Results: The ALP activity was expressed significantly in the presence of Dz (10-6M) and Ral (10-7M) at 14 d. Osteocalcin production was slightly increased with both agents at 14d and 21d. At 21 days von kossa staining was increased in the presence of Dz with or without Ral. Conclusion: These results suggested that the effect of Dz was stable in osteoblasts in the presence of Ral.
Establishment of human osteoblasts culture system obtained from aged donors.

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Establishment of human osteoblasts that retain bone-forming capacity is one of the prerequisites for successful bone regeneration therapy. Since the demand for bone regeneration considerably increases in elderly, use of osteoblasts derived from these aged individuals should significantly facilitate bone regeneration therapy. However, isolation of osteoblasts from elderly is difficult. Here we show a novel culture technique of human osteoblasts isolated from alveolar bones of elderly donor. We attempted to isolate osteoblasts using alveolar bones of 52-, 53- and 66-year-old donors. Those bones sequentially digested and primary human alveolar bone osteoblasts (HAOBs) were isolated. HAOBs from all of the individuals were successfully expanded and grow more than 60 population doublings (PDs). HAOBs exhibited high alkaline phosphatase activity and mineralized nodule formation and expressed osteoblast marker genes such as runx2, osterix, osteocalcin and bone sialoprotein upon treatment with rhBMP-2. To examine bone forming capacity of HAOBs in vivo, HAOBs at 6 PDs were subcutaneously transplanted into the dorsal skin in severe combined immunodeficiency (SCID) mice. Histological examination revealed that the transplanted HAOBs formed bone tissues 4 weeks after the transplantation. In conclusion, our results show that the culture technique used here allows us to successfully grow human osteoblasts of aged donors. They also suggest that HAOBs are a useful cell source for future application to cell based bone regeneration therapy for local bone diseases such as periodontal diseases.

Long term clinical evaluation of glass-fiber-reinforced restorations

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Objectives: The advantages of aesthetic restoration materials are good biocompatibility and lack of toxicity compared to metal restorations. Glass-fiber-reinforced composite material Targis/Vectris (Ivoclar-Vivadent, FL) were observed using a microscope and the composition was analyzed. As a result, it was judged that the material was good for clinical use. This material was used in dental treatments in 1997. However, the Glass-fiber-reinforced restorations often fractured. The purpose of this study was to evaluate the clinical strength of the Glass-fiber-reinforced restorations by Cohort analysis. Methods: The Glass-fiber-reinforced restorations were made according to the manual of the manufacturer. 29 patients who had 73 the Glass-fiber-reinforced restorations were investigated on by questionnaire survey, from a period of June 25th, 1999 up to September 7th, 2000 at Aichigakuin University dental hospital. Results: These restorations were investigated after a maximum of 8 years. 35 out of 73 (47.9%) of the restorations were fractured, including cracks and chipping. 6 out of 14 inlays were fractured (42.9%). 14 out of 43 crowns were fractured (32.5%). 15 out of 16 bridges were fractured (93.8%). Survival rate was calculated according to the Kaplan-Meier Product-Limit method. 94% of crowns, 82% of inlays and 56% of bridges will still have broken in 3 years time, and 71% of crowns, 57% of inlays and 12% of bridges will still have broken in 6 years time. According to log-rank test when the risk of hazard is 3%, there is no significant difference between crowns and inlays, but, there is a significant difference between crowns and bridges, and between inlays and bridges. Conclusion Clinicians should not use materials developed by new concept or techniques too quickly. Instead we think that long-term clinical evaluation is needed for safety and durability.
089 Influence of Light-polymerization systems on surface properties of indirect composite

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Objectives: The aim of the current study was to evaluate the influence of laboratory polymerization systems on Knoop hardness, toothbrush abrasion resistance, and gloss change of an indirect composite. Methods: An indirect composite (Sinfony) was polymerized with nine polymerization modes using the five polymerization systems (Visio system, Hyper LII, Pearlcure Light, Twinkle MIII, and UniXS II). After light exposure, Knoop hardness number, wear depth, and change of gloss were determined (n=6). The results were analyzed by one-way ANOVA and Tukey HSD test (P=0.05 level). Worn surfaces were also observed by scanning electron microscope. Results: Specimens polymerized with the Hyper LII (120 s) and Pearlcure Light (120 s) units demonstrated greatest hardness number, whereas specimens polymerized with Visio system and UniXS II (60 s) units demonstrated the smallest value. The highest wear resistance to toothbrush abrasion was recorded with six groups (Hyper LII 60 and 120 s, UniXS II 120 s, Pearlcure Light 60 and 120 s, Twinkle MIII 120 s) and the lowest wear resistance was obtained with two groups (Visio system and UniXS II 60 s). Gloss of the composite did not depend on the polymerization modes before wear testing. The surface gloss was significantly reduced for all polymerization modes by toothbrush dentifrice abrasion, and gloss values of worn surface were significantly different by polymerization modes. Conclusion It can be concluded that the Sinfony composite is polymerized properly by means of high-intensity light polymerization units.

This work was supported in part by Special Research Grant for the Development of Distinctive Education from the Promotion and Mutual Aid Corporation for Private School of Japan (MM, 2008).

090 Properties of dental zirconia changed with sintering temperature

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Objectives: The aim of this study is to investigate the effect of sintering temperature on properties of dental zirconia.

Materials and Methods: Y-TZP block (ZENO Zr disc, Wieland) was sliced to plates and bars. The plates and bars were fired at 1000, 1100, 1200, 1350, 1450, and 1500°C for 2 h. After the sintering, the dimensions of plates were determined with a digital caliper and calculated the sintering shrinkage. Vickers hardness was determined under 1-kgf load and 3-point bending strengths were measured with 10-mm span. The apparent grain size (D) was determined by the lineal intercept method from SEM photographs of thermally etched surfaces of each zirconia. To evaluate low temperature degradation, the plates were stored in an autoclave maintained at 134°C for 5 h. The contents of monoclinic phase were determined using Toraya’s equation through X-ray diffraction patterns.

Results: Sintering shrinkage and Vickers hardness increased with the sintering temperature up to 1350°C and did not changed above it. The apparent grain size and 3-point bending strength continuously increased with the sintering temperature up to 1500°C. Monoclinic phase contents of zirconia fired at 1450 and 1500°C increased after the autoclaving, whereas no change in the X-ray diffraction patterns of zirconia fired below 1350°C were observed. It suggests that the durability against low temperature degradation of zirconia decreased with the sintering temperature.

Conclusions: It is concluded that the sintering of dental zirconia block finishes at 1350°C for 2 h and the grain growth rate increases above it. Although the bending strength increased with the sintering temperature, the durability against the low temperature degradation decreased with it.

Acknowledgment: This study was partially supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (No.18390521).
091  Breaking strength of an experimental mobile mandibular advancement splint

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Objectives: The mandibular advancement splint (MAS) usually serves patients with sleep-disordered breathing in advancing and fixing the mandible forward. The conventional rigid MAS restricts the movement of the mandible, and this immobility sometimes produces discomfort, including temporomandibular disorder. With the intention of making the MAS more comfortable, a simple method for fabricating a mobile MAS was devised by use of a connector made from a polyethylene toothed belt.

Methods: The experimental connector was contrived to be easily constructed, inexpensive, and small for use as an intraoral MAS. To evaluate the durability of the trial MAS system, the axial and diagonal tensile breaking strengths for the MAS using high-density (HDPE) or low-density polyethylene (LDPE) lateral toothed belts were compared to those for a conventional mobile MAS (Silensor).

Results: Compared with Silensor system, the experimental MAS system exhibited sufficient breaking strength, especially when a diagonal tensile load was applied to mimic mandibular lateral translation (HDPE-polycarbonate, 81.0 N; LDPE-polycarbonate, 55.8 N; Silensor, 35.9 N).

Conclusions: The experimental connecting system is thought to have the possibility of the clinical application. To make the connector stronger for clinical use, HDPE should be selected.

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092  Corticosteroid affects upregulated expression of beta-defensins in keratinocytes

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Objectives: Human beta-defensins (hBDs) belong to a group of antimicrobial peptides expressed mainly in epithelial cells, which contribute to both innate and adaptive immunity. Stresses affect multiple components of the immune systems. Human stress response is orchestrated by the hypothalamic-pituitary-adrenal axis acting via corticosteroids. Human keratinocytes recognize microbes via toll-like receptors (TLR) through which hBD expressions are upregulated. The present study examined whether corticosteroid affected the upregulated expression of hBDs by the stimulation with TLR agonists. Methods: Human normal keratinocytes (NHEK) were grown in DMEM supplemented with 10% FBS or in hydrocortisone-free keratinocyte growth medium containing 1.8mM Ca2+ (KBM, Switzerland) supplemented with Utrroser G (Life sciences NY). The cells were treated in the presence or absence of 10, 1 and 0.1μM Dexamethasone (Dex: corticosteroid, Sigma-Aldrich) for 24hr, followed by 24 hr with or without TLR agonists. TLR2 agonist (108 cells/ml HKLM, InvivoGen CA) and TLR4 agonists (100ng/ml LPS, InvivoGen) are used. Total RNAs were extracted from the cultured cells. Expression of hBD-1, -2 and -3, TLR2 and 4 in NHEK cells were observed by RT-PCR and quantitative RT-PCR using TaqMan probes (Applied Biosystem, CA). Several experiments were performed, all in triplicate. The relative expression of each mRNA was calculated as the ΔΔCt using the formula described by Livak et al. (Methods 2001). The data was analyzed using one-way ANOVA. Differences between experimental groups were considered statistically significant at the p<0.05 levels.

Results: TLR2 agonists induced upregulation of hBD-1, -2 and TLR4 agonist induced upregulation of hBD-2 and -3 in NHEK. The expression levels of hBDs were significantly higher in the groups with Dex and agonists than those in the groups with Dex or agonist alone (p<0.05). Conclusion: The results indicate that corticosteroid may affect upregulated expression of hBDs induced by TLRs.
093 Gene expression profiling of LPS responsive genes in human trophoblast

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Objectives: There is growing evidence that a number of complex human systemic diseases are caused by periodontal diseases. It has been suggested that maternal periodontal disease and incident progression are significant contributors to obstetric risk of preterm low birth weight. To elucidate the molecular basis of the mechanism, human trophoblast cell (BeWo) were treated with LPS isolated from Actinobacillus actinomycetemcomitans, and gene expression profiles were monitored using DNA microarray technology. Effect of A. actinomycetemcomitans LPS administration to pregnant rats on low birth weight was also examined. Methods: BeWo were treated with A. actinomycetemcomitans LPS, and mRNA isolated, then mRNA levels were monitored using Affymetrix GeneChip (Human Genome U133 plus 2.0 array, ca. 47,000 genes). GeneChip data was analyzed using GeneSpring software and imported into Ingenuity Pathway Analysis (IPA) database to find the signal pathways involved. Altered mRNA levels in GeneChip results were confirmed by RT-PCR and real time PCR. Pregnant rats were intravenously injected with LPS and the body weight of newborn rats was measured. Then TUNEL method was used for detection of the apoptotic cells in placenta tissue of pregnant rats treated with A. actinomycetemcomitans LPS. Results: Intravenous administration of LPS resulted in newborn rats with a low birth weight and higher occurrence of apoptotic cells. GeneChip analysis showed that LPS treatment altered many gene expressions. IPA analysis for the function of reproductive system development revealed decreased mRNA levels of MSH5, FGFR1 and SMAD4. RT-PCR and Real-time PCR analysis successfully confirmed these mRNA level changes from GeneChip data. Conclusion: Since MSH5 play important roles in germ cells differentiation, further FGFR1 and SMAD4 are involved in cell proliferation, A. actinomycetemcomitans infection may induce apoptosis and inhibition of the proliferation of placenta cells through altering of these reduced gene expressions and contribute to low birth weight.

094 DNA sequence of γ-glutamyl transpeptidase from Actinobacillus actinomycetemcomitans Y4

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A γ-glutamyl peptide-hydrolysing enzyme obtained from A. actinomycetemcomitans Y4 hydrolyses a substrate for γ-glutamyl transpeptidase (GGT) and the enzyme activity is specifically inactivated by γ-glutamyl affinity reagents. But some properties of the bacterial enzyme are different from those of other GGTs. Objectives In order to compare the predicted amino acid sequence and DNA sequence of the bacterial enzyme with those of other GGTs, we undertook the subcloning and sequencing of the corresponding gene. Methods Bacterial cells were cultured at 37 °C for 20 h in an atmosphere of 10 % CO2 and 90% in BHI broth. Bacterial cells were harvested, washed with buffered saline and then treated with lysozyme and RNase. The cells were lysed with SDS and digested with proteinase K. Total DNA was extracted with phenol and chloroform. PCR primers were designed on the basis of DNA sequence of A. actinomycetemcomitans HK1651 GGT gene. Amplification products were electrophoresised in a 2% agarose gel. The PCR product was inserted pETBlue-1 Vector with Perfectly Blunt Cloning Kits. DNA sequencing was analyzed by using ABI PRISM 310NT genetic analyzer. Results The amino acid sequences of the bacterial enzyme and E. coli K12 GGT were 58.6 % similar. Also higher similarity (64.7 %) was observed between the small subunits which γ-glutamyl-binding subsites localize. Alignment of the DNA sequences of the bacterial enzyme and E. coli K12 GGT revealed identity of 71.8 %. Evolutionary tree constructed by UPGMA method indicated that the bacterial enzyme is more closely related to E. coli K12 GGT than to F. nucleatum GGT and S.aureus. Conclusions It was suggested that γ-glutamyl peptide-hydrolysing enzyme which we reported, might be GGT-like enzyme. However further investigations, such as expression of the GGT gene, are necessary.
Proteomic analyses of a two-component regulator mutant of Tannerella forsythensis

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Tannerella forsythensis is a Gram-negative anaerobe closely associated with adult periodontitis, and is also detected with high frequency from the root canals with periradicular lesions. The virulence of a pathogenic bacterium like this could be modulated with two-component regulatory systems. The type strain ATCC43037 has at least 15 open reading frames encoding two-component regulators (RR) in the genome. Of these, TF0022 has a unique domain organization with a fusion of a sensor kinase and a RR. The purpose of this study was to identify the genes regulated by TF0022.

Cells were grown in trypticase soy broth supplemented with 2.5% Fildes extract (Oxoid) and 10 μg/ml N-acetyl muramic acid. TF0022 mutants with an insertion of the ermF-ermAM cassette were generated using an allelic exchange procedure. Utilization of carbon sources was analyzed with a BIOLOG AN MicroPlate. Total protein samples from 5-day cultures of each strain were separated in a rehydrated Immobiline DryStrip (GE), followed by SDS-PAGE. The CBB-stained gels were scanned and analyzed with ImageMaster 2D Platinum software (GE). The protein spots were cut out from the gels and digested with trypsin, then analyzed with a 4800 MALDI TOF/TOF Analyzer (Applied Biosystems). RT-PCR analyses were conducted with SuperScript III (Invitrogen), random primers, and the primer sets designed for the genes encoding differentially produced proteins.

Disruption of the TF0022 locus caused a decreased growth rate and a change in utilization of carbon sources. Comparative proteomic analyses revealed that the production levels of at least four proteins were increased, whereas one protein was decreased in the TF0022 mutant. RT-PCR analyses showed that some of them were regulated at the transcriptional level.

TF0022 could act as both a positive and a negative transcriptional regulator under the standard culture conditions.

DNA microarray analysis of dental pulp exfoliated from deciduous teeth

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Objectives: Dental pulp plays an important role in tooth vitality. Previous studies have indicated that stem cells can be isolated from dental pulp, and dental pulp exfoliated from deciduous teeth has become a useful alternative for dental tissue engineering because of its higher proliferation rate. In the present study, we analyzed the differences in gene expressions between human dental pulps exfoliated from deciduous and permanent teeth by DNA microarray assays.

Methods: Dental pulp fibroblasts were established from cells growing out of the dental pulp tissue of deciduous and permanent non-carious teeth. For microarray assays, total RNA was isolated from each sample using the Trizol reagent. To confirm the microarray results, insulin-like growth factor-binding protein 5 (IGFBP5) and vascular endothelial growth factor A (VEGFA) showing higher mRNA expression levels were selected and analyzed for their mRNA levels by RT-PCR.

Results: A scatter plot of mRNA levels based on fluorescent signals in human dental pulps from deciduous and permanent teeth indicated a dispersed distribution pattern. In a scatter plot of the genes, 2573 genes were expressed at 2-fold higher levels in dental pulp from deciduous teeth compared with permanent teeth. The RT-PCR results indicated that both the IGFBP5 and VEGFA mRNA levels were upregulated by about 3-fold in dental pulp from deciduous teeth compared with permanent teeth. Thus, the RT-PCR results for these genes were consistent with the microarray data.

Conclusion: The dental pulps in deciduous and permanent teeth differ significantly with regard to their developmental processes, tissue structures and functions. Thus, the present findings using DNA microarray analyses to detect differences in the gene expressions of deciduous and permanent teeth may be useful for dental pulp tissue engineering.
097  **Expression of toll-like receptors during development of sialoadenitis in mice**

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**Objectives:** Toll-like receptors (TLR) play a fundamental role in the activation of the innate immune system during infection. Although expression of TLRs has been detected in the salivary gland, there is little information on the expression profiles in the salivoadenitis. Non-obese diabetic (NOD) mice spontaneously develop sialoadenitis, producing a condition that resembles Sjogren's syndrome. The present study analyzed the expression patterns of TLR-2, -3, -4, -7, -8 and -9 during the development of sialoadenitis in NOD mice.

**Method:** Female NOD mice (CleaJapan) were used in the present study. Submandibular glands were dissected from the mice at 4, 8, 10, 12, 14 and 16 weeks of age (n=5 in each group). Expression of TLR-2, -3, -4, -7, -8 and -9, and myeloid differentiation factor 88 (MyD88) was observed by RT-PCR and quantitative RT-PCR using TaqMan probes (Applied Biosystem, CA). Several experiments were performed, each in triplicate. The relative expression of each mRNA was calculated as the \(\Delta\Delta C_t\) using the formula described by Saitoh et al. (Med Mol Morphol 2007). The data was analyzed using one-way ANOVA. Differences between experimental groups were considered statistically significant at the p<0.05 levels. Immunohistochemical staining for TLR-7 and -9 was carried out using anti-TLR-7 and -TLR-9 antibodies (Hycult Biotech.)

**Results:** The submandibular glands show no indication of inflammatory reactions in 4- and 8-week-old mice. Ten-week-old mice generally had one focus of lymphatic infiltration per lobule. Expression levels of TLR-7 and -9 at 14 and 16 weeks were significantly higher than at 4 or 8 weeks (p<0.01). Immunohistochemical positives for TLR-7 and -9 were observed in the foci of lymphatic infiltration.

**Conclusion:** The results indicate that TLR-7 and -9 may be upregulated during the development of autoimmune sialoadenitis.

098  **Inflammatory markers and stress index in sleep apnea syndrome**

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**Objectives:** We have reported that the disease-severity of sleep apnea syndrome (SAS) is closely associated with platelet function and various cytokines. Polysomnography (PSG) is essential for diagnosis and prediction of disease-severity of SAS. However, the monitoring by PSG is obliged to the prolonged restraint with indispensable hospitalization. In this study, we investigated whether platelet activation (PA), interleukin-18 (IL-18), high sensitive C-reactive protein (h-CRP) and stress index correlate with diagnosis and prediction of disease-severity of SAS.

**Methods:** Ninety-nine patients were divided into four groups (non-SAS, SAS-mild, SAS-moderate and SAS-severe) by apnea-hypopnea index (AHI). PA was analyzed by flow cytometry. IL-18 and h-CRP were measured by ELISA and Behring Nepherometer II, respectively, and stress index was quantified using Diacron Reactive Oxygen Metalites test (d-ROMs).

**Results:** (1) Positive correlation was recognized between AHI, and IL-18 and PA (p<0.05). (2) Positive correlation was recognized between h-CRP, and PA and d-ROMs (p<0.05). (3) Significant difference was recognized between non-SAS and SAS-severe in IL-18 (152.2 pg/ml vs. 192.8 pg/ml, p<0.05). (4) Significant difference was recognized between non-SAS and SAS-total in h-CRP (0.06627 mg/dl vs. 0.11720 mg/dl, p<0.05). (5) Significant difference was recognized between non-SAS, and SAS-mild and SAS-sever in h-CRP (0.06627 mg/dl vs. 0.09996 mg/dl and 0.15653 mg/dl, p<0.05). (6) Significant difference was recognized between SAS-mild and SAS-sever in PA (2.73% vs. 5.27%, p<0.05). (7) There was no significant sexual difference in all data.

**Conclusion:** h-CRP and IL-18 were useful marker for diagnosis and prediction of disease-severity of SAS. On the contrary, stress index was not useful at this point. However, stress index was reflecting the inflammatory status of SAS in relation to h-CRP.
Analysis of the transmitted-light through the human teeth \textit{in vivo}

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Objectives: The author reported the colorimetric analysis of the transmitted-light through human extracted tooth crown to the contents of the root canal. The purpose of the present study was to examine the applicability of colorimetric analysis to the human teeth \textit{in vivo}.

Methods: This study was approved by the Tohoku University Graduate School of Dentistry Research Ethical Committee. The purpose and the method of the present study were explained to the subjects (n = 11) and written informed consent was obtained from all of them. For this purpose human upper central incisors (19 vital teeth and 3 non-vital teeth) were examined. Infrared and green laser lights simultaneously illuminated the labial surface of the tooth crown via two optical fibers (outer diameter: 0.5 mm). The transmitted-light through the tooth crown was collected from the palatal surface of the examined tooth and led to the light detector via another optical fibers. The intensities of transmitted infrared light (TIL) and the transmitted green light (TGL) were simultaneously measured.

Results: The intensities of TIL and TGL were smaller when the lights were illuminated and collected from the cervical area than the incisal area. The intensities of TIL and TGL were significantly smaller with the non-vital teeth than the vital teeth (p<0.05, Mann-Whitney U-test).

Conclusion: The results indicated that the analysis of TLI through tooth crown was applicable to pulp vitality diagnosis.

Measurement and clinical significance of uric acid in saliva

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Objectives: Hyperuricemia has been increasing among middle-aged people with change of eating habits and lifestyle. If hyperuricemic state has been continued for a long time, uric acid will deposit to the joint or the kidney, and the symptoms of gout will be developed. In this study, we examined the clinical significance of the measurement of uric acid in saliva, after investigating correlation of the uric acid concentration in serum and saliva.

Methods: One hundred ninety-seven healthy volunteers and outpatients were enrolled in this study (male: 158, 39.8 +/- 15.4 years, female: 39, 40.9 +/- 16.7 years). For treatment of hyperuricemia, 28 and 12 patients had taken benzbromarone and allopurinol, respectively. Daily changes of the uric acid concentration in serum and saliva were examined in six healthy volunteers. Two milliliters of blood and saliva were collected in the morning and quantitative analysis of uric acid was carried out by enzyme method.

Results: (1) The uric acid concentration in serum and saliva showed a significant correlation in 197 subjects (r= 0.307, p<0.01).
(2) Higher significant correlation was recognized in the uric acid concentration between serum and saliva in 164 who excluded the subjects whose uric acid value in saliva was higher than serum (r=0.47, p<0.001).
(3) In 26 (92.8%) of 28 treated with benzbromarone, the salivary uric acid concentration was higher than serum.
(4) In 12 treated with allopurinol, the serum uric acid concentration was higher than saliva.
(5) The uric acid concentrations in serum and saliva were high at early morning and fell in the evening.

Conclusion: The measurement of salivary uric acid was useful for diagnosis of hyperuricemia, and saliva had played an important role in uric acid excretion. In addition, benzbromarone may greatly be participating in the mechanism of uric acid excretion in salivary glands.
Study of mandibular dental arch form by fourier analysis

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Objective  We studied morphological characteristics of 53 dental arches by Fourier series of measured values. Vectors \( r \theta n \) (\( n = 1, \cdots, 7 \)) were represented by lines connecting \( O \) and the reference points. Fourier analysis was conducted on a polar coordinate system developed from the vectors. Fourier coefficient and amplitudes were analyzed for evaluation of mandibular dental arch forms.

Results  1) Each mandibular dental arch form was reproduced by 1st to 4th of Fourier harmonics. 2) Significant differences of the constant value and the amplitude of the 1st Fourier harmonic (representing the arch size and form) were not observed in the analysis. In contrast, the amplitudes of the 2nd, 3rd and 4th Fourier harmonics concerning the arch forms, showed significant differences between the mandibular dentitions. 3) The amplitudes of the 2nd, 3rd, and 4th Fourier harmonics were identified closely correlating with length/width ratio, the curvature of anterior teeth and the curvilinear contour of the dental arch, respectively.

Conclusions  1) Establishment of Fourier series is a significant method to reproduce mandibular dental arch forms. 2) Characteristics of different dental arch forms are distinctively found in each Fourier harmonic. The present analysis obtains results similar to our previous studies on characteristics of the mandibular dentitions by correlation and principal component analyses.
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第56回JADR会長：中垣晴男
第56回JADR準備委員長：加藤一夫
楠元キャンパス
薬学部／歯学部／短期大学部／歯学研究科／歯科技工専門学校／法人本部

【主なアクセス】
地下鉄東山線「本山駅」1番出口から徒步約5分

【所在地】
〒464-8650 名古屋市千種区楠元町1－100 TEL：052-751-2561

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第56回国際歯科研究学会日本部会（JADR）総会・学術大会

会期 2008年11月29日（土）、11月30日（日）
会場 愛知学院大学歯学部（楠元キャンパス）
〒464-8650 名古屋市千種区楠元町1-100
愛知学院大学歯学部口腔衛生学講座内
TEL:052-751-2561（内線1352）、FAX:052-751-2566
http://wwwsoc.nii.ac.jp/jadr/jadr56/index.html

ご参加の皆様へ
1. 当日登録は、11月29日、30日ともに8時30分より、記念講堂（A会場）内にあるロビーにて行います。
2. 受付では必ず登録手続き、あるいはその確認をしてください。また学生会員の方は全員、学生証の提示をお願いいたします。なお、会場内および懇親会場では必ずネームプレートをご用意ください。
3. 11月29日18時30分から、ルプラ王山にて懇親会を開催いたします。当日申し込みも受け付けます。どうぞ多数ご参加ください。
4. 参加者への連絡は掲示で行いますので、受付前の掲示板をご覧ください。（館内放送での呼び出しは行えませんのでご了承ください。）
5. 本総会・学術大会の学会場となっている楠元キャンパスは敷地内全面禁煙ですので、ご協力のほどお願いします。また、会場内での飲食は禁止しておりますのでご協力ください。

シンポジストの皆様へ
1. 発表は原則として英語でお願いいたします。
2. 発表はすべてPCプレゼンテーションとし、専用のPCとプロジェクターを1台ご用意いたします。（原則として持ち込みPCでのご出力は受け付けません。）通常のスライドは用意しません。専用のPCはWindows XPで、パワーポイント2003を使用します。ご発表の30分前までにA会場においてスライド受付にて試写をお願いします。

ポスター発表の方法について
1. すべての発表はポスター形式とし、愛知学院大学楠元学舎3号館にあるB会場（2階323教室）、C会場（2階325教室）、D会場（1階学生談話室）の3会場で同時に行います。
2. ポスターのサイズは幅90cm、高さ180cm以内です。A0サイズ（841×1189mm）が便利です。
3. ポスターは画鋲でとめて下さい。画鋲は会場にて用意をいたします。ポスターは紙または薄手のポール紙で作成して下さい。重量のある物のポスターボードへの掲示はご遠慮下さい。
4. 発表者がわかるようにタイトルの横に発表者の写真をお貼りください。
5. ポスターには、研究の基本的な情報をすべて入れて下さい。
6. ヒトを使った研究では、倫理委員会の承認とインフォームドコンセントについて明記して下さい。
7. 学会での公式言語は日本語と英語です。ただしポスターとスライドの言語は全て英語です。
8. 発表者は当日11：00〜13：00の間に各会場にて登録およびパワーポイントの試写を行い、リボンを受け取ってください。
ポスタービューアイング
1. ポスターの掲示は、発表当日の 8:00 から 11:00 の間に、ポスタービューアイングの時間にポスターガ供覧できるようにして下さい。
2. 発表者は参加者と討論できるように、抄録集に示した“指定時間”の間はポスターの脇に待機して下さい。ポスターに予定されている“指定時間”の詳細については、ホームページや抄録集に掲載されたプログラムから確認して下さい。

ポスターディスカッション
1. ポスターディスカッションは、ポスター会場で行います。
2. 1 項当たりの発表と討論は合わせて 8 分間（発表 3 分＋討論 5 分）です。討論の時間が十分確保できるように、次の点に注意して下さい。
   1) 各発表者は 3 分以内でポスターの概要を口頭発表して下さい。発表の際には、最大 3 コマまでのパワーポイントスライドが使用可能です。事前にパワーポイントファイル（PowerPoint 2003 for Windows を使用のこと）を作成の上、11 月 21 日までに添付ファイルとして事務局（kazkato@dpc.aichi-gakuin.ac.jp）へ送付して下さい。スライドでは 28 ポイント以上のフォントを使用し、アニメーション効果は使わないで下さい。
   2) この短い発表で、参加者が研究の目的と主な結論を思い出させるようにして下さい。
   3) 単なるポスターの口頭発表にしないで下さい。
   4) 参加者は既にポスターを見ているものと考え、研究の重要なポイントを想起させるようにして下さい。
   5) スライド上の内容はなるべく簡単にして下さい。文章を短くしたり、分かりやすい図を用いて下さい。多くの数値を加えないで下さい。
   6) 口頭発表の後、5 分間の質疑応答を行います。
3. Hatton Award のポスター発表に限り、パワーポイントスライドは使用せず発表と討論は 15 分間です。8 分以内で口頭発表した後、7 分間で質疑応答を行って下さい。
4. ポスターディスカッションの終了後、ポスターを撤去して下さい。

特別講演、シンポジウムの座長の先生方へお願い
1. 演題の進行および討論の司会はすべて座長にお願いいたします。
2. 進行係は設けませんので、時間を厳守していただきますよう、お願いいたします。

ポスター発表の座長の方へお願い
1. 座長の方は、会場の受付（発表者・座長受付）で、事前（15 分前まで）に座長の確認をお願いします。その際、配付資料がある場合や、発表の変更がある場合は、係からお伝えします。
2. 発表会場では、次座長席を用意しておりますので、時間になりましたら、移動をお願いします。
3. 進行係を設けますが、時間内に終了するようご協力をお願いいたします。

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4号館3,4F理工科専門学校

A会場
110周年記念講堂

B会場

企業展示

展示
休憩・食事

クロック

3号館

2号館

クロック

1号館
法人本部

守衛室

体育館

グランド

歯学・薬学
図書館
情報センター

A会場
講演会場

D会場

Poster Session

C会場

Poster Session

B会場

Poster Session

— 113 —
第56回JADR総会・学術大会  2008年11月28日（金）

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<th>法人本部講堂</th>
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<th>3号館1階学生談話室</th>
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<th>ルブラ王山</th>
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| 10:00         |        |        |                |                |        |        |                |                |        |
| 11:00         |        |        |                |                |        |        |                |                |        |
| 12:00         |        |        |                |                |        |        |                |                |        |
| 13:00         |        |        |                |                |        |        |                |                |        |
| 14:00         |        |        |                |                |        |        |                |                |        |
| 15:00         |        |        |                |                |        |        |                |                | 理事会 |
| 15:00〜17:00  |        |        |                |                |        |        |                |                |        |
| 16:00         |        |        |                |                |        |        |                |                |        |
| 17:00         |        |        |                |                |        |        |                |                | 会場準備・設営 |
| 18:00         |        |        |                |                |        |        |                |                |        |
| 19:00         |        |        |                |                |        |        |                |                | 理事懇親会 |
| 18:00〜20:00  |        |        |                |                |        |        |                |                |        |
| 20:00         |        |        |                |                |        |        |                |                |        |
# 第56回JADR総会・学術大会 2008年11月29日（土）

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<th>歯学部第3会議室</th>
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<td>9:00</td>
<td>特別講演1 (President of IADR)</td>
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<td>10:00</td>
<td>シンポジウム1 Oral Biofilm Today</td>
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<td>9:00</td>
<td>特別講演3 (川島 直男教授)</td>
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<td>10:00</td>
<td>シンポジウム2 (神経活動による骨代謝抑制)</td>
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11月29日（土） 第1日 A会場

●9:00-10:00 特別講演1 (IADR 会長特別講演)

座長：小田 洋(会長)

L1 New developments in dentistry and IADR
Professor JM (Bob) ten Cate (Academic Center for Dentistry Amsterdam)

●10:00-12:00 シンポジウム1

Oral Biofilm Today 座長：Professor JM (Bob) ten Cate (President of IADR)
中垣 晴男(愛知学院大学)

S1-1 Mass transfer within natural plaque biofilms: the role of plaque architecture
Professor Colin Robinson (Leeds大学)

S1-2 Can biofilms kill you?
Professor Lakshman Samaranayake (Hong Kong大学)

S1-3 Fluoride content of dental plaque
Professor Lutz Stösser (Jena大学)

S1-4 Ecological dynamics of caries-associated oral biofilm: involvement of mutans streptococci and non-mutans bacteria
高橋信博(東北大学大学院)

S1-5 Oral biofilm fromation, what goes on afterwards.
花田信弘(鶴見大学)

●13:30-14:30 特別講演2 (KADR 招待講演)

座長：中垣 晴男

L2 Laminin-derived peptides: their biomedical applications and signaling pathways
Professor Byung-Moo Min (Department of Oral Biochemistry and Program in Craniomaxillofacial Reconstruction Science, Seoul National University School of Dentistry, Seoul Korea)
11月30日（日） 第2日 A会場

●9:00–10:00 特別講演③

L3 Nanomedical system developed with PLGA nanosphere platform

川島 嘉明（愛知学院大学）

●10:00–12:00 シンポジウム2

Neural Regulation of Bone Metabolism（神経活動による骨代謝制御）

座長：大谷啓一（東京医科歯科大学）
戸塚彰史（愛知学院大学）

S2-1 末梢交感神経系の骨代謝への直接的関わり
戸塚彰史（愛知学院大学）

S2-2 中枢神経系による骨代謝調節
竹田 秀（東京医科歯科大学）

S2-3 骨リモデリングにおけるグルタミン酸の役割
宇田川信之（松本歯科大学）

S2-4 感覚神経系と骨代謝
後藤哲哉（九州歯科大学）
ポスター発表 11月29日（土） 第1日 ポスター会場

ポスター発表
The Young Investigator Award
●14:30~15:58（B会場）

座長：前田 伸子

001 歯の形成誘導を目的とするラット歯胚由来上皮と間業細胞の三次元・積層培養法の開発
野谷 拓也、田畑 純、馬場 麻人、高野 吉郎
東京医科歯科大学大学院歯学総合研究科硬組織構造生物学分野

002 Treponema denticola の dentilisin は歯周病原菌の共凝集に関与する
佐野 由美子、宮本 亜一、伊藤 理恵子、松本 直、薬師寺 仁、新谷 誠康、石原 和幸
東京歯科大学

003 習慣性咀嚼側に依存した舌の2点弁別能および舌触覚による大脳皮質賦活
渋 亜紀子 1、小野 卓史 1、宮本 亜一 1、戸田 栄一 2、倉林 亨 3、森山 啓司 1
東京医科歯科大学大学院歯学総合研究科顎顔面歯学分野、2 徳島大学大学院ヘルスバイオサイエンス研究部口腔顎顔面放射線医学分野、3 東京医科歯科大学大学院歯学総合研究科口腔放射線医学分野

004 フッ化ナトリウムによるヒト歯肉上皮由来細胞株のアポトーシス誘導
戸来 真由美、村田 貴俊、八重垣 健
日本歯科大学生命歯学部衛生学講座

005 鰓欠損メタ変異体(rs~3)に見られる歯の変異
アトゥカララ A. デヴィ セウワンディニ 1、樫口 和代 1、田畑 純 1、馬場 麻人 1、
三谷 啓志 2、高野 吉郎 1
東京医科歯科大学大学院歯学総合研究科硬組織構造生物学分野、
東京大学大学院新領域創成科学研究科先端生命科学専攻動物生殖システム分野

座長：高野 吉郎

006 ジルコニア及びチタン上における骨芽細胞様細胞の初期反応
山下 大輔 1、町倉 三保 1、宮本 元治 1、武内 博信 1、竹内 尚士 1、河野 博史 2、
野口 和行 1、伴 清治 3
鹿児島大学大学院歯学総合研究科歯周病学分野、2 鹿児島大学病院歯科総合診療部、3 鹿児島大学 大学院医歯学総合研究科歯科生体材料学分野

007 侵害刺激によって三叉神経節衛星細胞は活性化する
郡司掛 香織 1、後藤 哲哉 2、中尾 加代子 3、石部 徹 4、小林 繁 2、山口 和憲 1
九州歯科大学顎口腔機能矯正学、2 九州歯科大学顎頭部構造解析学、3 九州歯科大学分子情報生化学、4 九州歯科大学形態機能再建学

008 シンパスタチンによるヒト歯髄幹細胞の分化制御に与える影響
岡本 洋介 1、園山 岳 1、大野 充昭 1、秋山 謙太郎 2、藤澤 拓生 1、大島 正充 1、
次亜塩素酸電解水の効果的調査バイオフィルムへの浸透作用
岡田 彩子 1, マティン カイルー 2, ザマン ショウカット 1, 花田 信弘 3, 田上 順次 4
1 東京医科歯科大学院生体機能学研究科歯周病学分野/国立保健医療科学院口腔保健部、2 東京医科歯科大学院生体機能学研究科歯周病学分野、3 鶴見大学歯科医学部歯周病学講座、4 東京医科歯科大学歯科歯周病学分野

ヒト歯肉線維芽細胞はHMGB1を能動的、受動的に放出する
フェガリ カリン 1, 岩崎 剣吾 1, 田中 敬子 1, 小牧 基浩 2, 和泉 雄一 1
1 東京医科歯科大学歯科医学部歯周病学講座、2 東京医科歯科大学

歯周組織においてニコチン誘導性CCN2/CTGFが与える影響：喫煙と線維化との関連について
武内 寛子 1, 久保田 聡 2, 村松 悦子 1, Zhou Yi 2, 渋川 正春 2, 沼部 幸博 1
1 日本歯歯大学生命歯学部歯周病学講座、2 岡山大学大学院歯歯学総合研究科

Pharmacodynamics / Microbiology & Oral Microbiology / Neurology
※14:30~15:42（C 会場）
座長：小木 信美

C. albicans とその変異体における抗真菌薬感受性
綿本 隆生 1, Seneviratne C. Jayampath 1, Jayatilake J. A. M. Sumedha 1, 江草 宏 2, 矢谷 博文 2, Samaranayake Lakshman P. 1
1 Oral BioSciences, Faculty of Dentistry, University of Hong Kong, 2 大阪大学大学院歯歯学研究科歯歯科補綴学第一教室

低電圧電気穿孔法によるプレオマインの抗腫瘍効果
杉田 好彦、神野 正人、高山 光平、本田 元匠、吉田 和加、久保 勝俊、佐藤 恵美子、前田 初彦
愛知学院大学歯科医学部口腔病理学講座

口腔外科手術におけるフェンタニル投与時の薬物動態シミュレーションの有用性
加藤 尚子、原田 純、渡辺 賀子、城 毅、松浦 雅代
愛知学院大学歯科医学部附属病院麻醉学講座

口腔内細菌数測定器の開発と検証
菊谷 武 1, 田村 文彦 1, 畑野 健 2, 久野 彰子 2, 古西 清司 4, 吉田 光由 5,
濱田 了 6, 高木 愛理 6, 稲口 智也 6, 萩中 寿恵 1, 西脇 恵子 1
1 日本歯科大学附属病院口腔介護・リハビリテーションセンター、2 日本歯科大学生命歯学部歯周病学講座、3 日本歯科大学附属病院総合診療部、4 日本歯科大学生命歯学部歯歯生物医学講座、5 広島市総合リハビリテーションセンター、6 バナソニック四国エレクトロニクス（株）ヘルスケア開発センター
016 試作洗浄・除菌液の洗浄効果
宇野 滋、杉崎 順平、森上 誠、山田 敏元
虎の門病院歯科

017 Streptococcus intermedius の硫化水素産生能の解析
伊東 俊太郎 1、吉田 康夫 2、佐々木 隆子 2、國松 和司 1、加藤 裕久 2
1 岩手医科大学歯学部歯科保存学第二講座、2 岩手医科大学歯学部歯科薬理学講座

018 歯肉上皮細胞への Candida albicans 感染が IL-6 シグナルパスウェイに与える影響
張 林 1、李 鶯 1、田中 陽子 2、久保山 昇 3、安孫子 宜光 1
1 日本大学栃戸歯学部生化学・分子生物学、2 日本大学栃戸歯学部障害者歯科、3 日本大学栃戸歯学部分子薬理学

座長：平場 勝成

019 ヒト脳機能マッピングのためのうまみ刺激提示システム
中村 優子、後藤 多津子、德森 謙二、吉浦 敬、小林 幸次、中村 泰彦、本田 浩、二ノ宮 裕三、吉浦 一紀
九州大学

020 ラット新生仔脳幹スライス標本における NMDA 誘発舌下神経運動ニューロン活動
荒木 一将 1、片倉 伸郎 2、下郷 和雄 1、平場 勝成 2
1 愛知学院大学歯学部顔面外科学講座、2 愛知学院大学歯学部生理学講座

Prothodontics Research / Mastication & Occlusion
●14:30-15:50(D 会場)

座長：河合 達志

021 インプラントクラウンの機能評価
五島 健一 1、Bakke Merete 2、Lexner Michala 2、Thomsen Carsten 2、三浦 宏之 1、Gotfredsen Klaus 2
1 東京医科歯科大学歯学口腔機能研究科歯機能保存学分野、2 コペンハーゲン大学

022 非線形有限要素解析による FRC クラスプの維持力の予測
丸山 浩美、西 慎宏、木下 智恵、水流 和徳、長岡 英一
鹿児島大学大学院医歯学総合研究科

023 漏斗状根管における支台築造法の検討
福井 雄二、駒田 豊、吉田 恵一、大竹 志保、三浦 宏之
東京医科歯科大学歯学部歯学総合研究科歯機能保存学分野

座長：窪木 拓男

024 意識的なかみしめの単純計算作業への効果
瑞森 崇弘、小林 美保、稲野 眞次、角谷 誠和、村嶋 史子、矢谷 博文
大阪大学大学院歯学研究科顎口腔機能再建学講座

025 機能運動時の上下顎間咬合関係
岡安 晴生、岡田 大嶌、進 千春、木津喜 裕子、川島 久美子、三浦 宏之
東京医科歯科大学大学院医歯学総合研究科摂食機能保存学分野

026 下顎骨の非対称が咀嚼運動に及ぼす影響
橋本 隆志¹, 黒田 晋吾², 片岡 伴記³, 及川 拓¹, 宮脇 正一⁴, 山城 隆³, 山本 照子¹
¹東北大学大学院歯学研究科顎口腔歯正学分野, ²徳島大学大学院ヘルスバイオサイエンス研究科顎口腔再建医学講座口腔顎顔面歯正学分野, ³岡山大学大学院医歯薬総合研究科顎顔面口腔歯正学分野, ⁴鹿児島大学大学院医歯学総合研究科顎顔面育成学分野

027 ウサギ咀嚼様運動における片側咬合挙上時の関節円板の運動
森田 匠¹, 丸尾 尚伸³, 藤原 琢也¹, 根来 武史³, 栗田 賢一², 後藤 滋巳³, 平場 勝成¹
¹愛知学院大学歯学部生理学講座, ²愛知学院大学歯学部顎口腔外科学講座, ³愛知学院大学歯学部歯科歯正学講座

028 離乳期の乳幼児における歯固め食品使用の影響
三輪 容子¹, 北田 勝浩³, 内川 喜盛¹, 山崎 昌彦³, 藤田 俊哉¹, 斎保 孝彦³, 佐藤 巖¹
¹日本歯科大学生命歯学部解剖学第一講座, ²日本歯科大学附属病院小児歯科歯科, ³鹿児島大学大学院医歯学総合研究科予防歯科学分野

029 Finite element analysis of ceramic inlays restored posterior teeth
L. SANDU, F. TOPALA, S. POROJAN, C. BORTUN
Victor Babes University of Medicine and Pharmacy Timisoara, University School of Dentistry

030 3D Reconstructions for numerical simulations of prosthetic restorations
S. POROJAN¹, L. SANDU¹, F. TOPALA¹, N. FAUR²
¹Victor Babes University of Medicine and Pharmacy Timisoara, University School of Dentistry, ²Politehnica University Timisoara

JADR Travel Award / Dental Education
●16:45-17:33(B 会場)
座長: 稲垣 幸司

031 Site–specific colonization and genotypic diversity of S. mutans in different individuals.
Q.Z. JIANG, Q. ZHANG
School of Stomatology, Wuhan University, Wuhan, China

032 Implementation of the KTSND questionnaire on Australian dental undergraduates
Huang B¹, K Inagaki², C Yoshii³, M.Kano², H. Nakagaki², T. Noguchi²
¹School of Dentistry, The University of Western Australia, ²Aichi–Gakuin University, ³University of Occupational and Environmental Health

033 Mutans streptococci and caries experience in Mongolian children
Soyolmaa MASHBALJIR¹*, Hulan ULAMNEMEKH¹, Munguntsseg LUVSAN¹, Altansukh TSEND–AYUSH², Sarantuya JAV²
¹School of Dentistry, Health Sciences University of Mongolia, ²Dept. of Genetics and Molecular Biology, Health Sciences University of Mongolia
日本の歯学部学生の社会的ニコチン依存度

座長: 伊藤 博夫

034
日本の歯学部学生の社会的ニコチン依存度
稲垣 幸司 1、野口 俊英 2、吉村 文信 3、森田 一三 4、中垣 晴男 4、小出 龍郎 5、
塚岡 隆 6、Huang Boyen 7、吉井 千春 8、加濃 正人 9
1 愛知学院大学短期大学部歯科衛生学科、2 愛知学院大学歯学部歯周病学講座、
愛知学院大学歯学部微生物学講座、4 愛知学院大学歯学部口腔衛生学講座、
愛知学院大学保健センター、7 福岡歯科大学口腔衛生学講座、8 西オーストラリア大学歯
学部小児歯科学講座、産業医科大学呼吸器内科、新中川病院内科

PBL チュートリアルによる学生の EQ 能力の変化について

座長: 伊藤 博夫

035
PBL チュートリアルによる学生の EQ 能力の変化について
葛城 啓彰 1、五十嵐 勝 1、影山 隆男 3、関本 恒夫 1、宮川 行夫 1、渡邊 文彦 1、
藤井 一紀 1、水谷 太尊 1、中原 泉 2
1 日本歯科大学新潟生命歯学部 PBL 教育委員会、2 日本歯科大学

歯科教育における自立学習支援システム

座長: 田畑 純

036
歯科教育における自立学習支援システム
吉田 和加 1、久保 勝俊 1、杉田 好彦 1、佐藤 恵美子 1、野口 俊英 2、河合 達志 3、前田 初彦 1
1 愛知学院大学歯学部歯周病学講座、2 愛知学院大学歯学部歯周病学講座、3 愛
知学院大学歯学部歯科理学講座

Mineralized Tissue 1 / Periodontal Research 1

座長: 田畑 純

037
ワーサリリン投与ラットにおける下顎骨と大腿骨のマイクロCT解析

座長: 伊藤 博夫

038
ワーサリリン投与ラットにおける下顎骨と大腿骨のマイクロCT解析

座長: 田畑 純

039
再石灰化処理を併用した漂白歯冠質の TMR およびラマン分析

座長: 田畑 純

040
再石灰化処理を併用した漂白歯冠質の TMR およびラマン分析

座長: 山崎 和久

041
EF–TEM cytochemical observation of elongated rete ridges in gingival hyperplasia

座長: 山崎 和久

042
adrenomedullin はヒト歯肉線維芽細胞の CXCL10 産生を抑制する

座長: 山崎 和久
下顎骨に発生した ameloblastic carcinoma の1例
久保 慎俊 1, 吉田 和加 1, 杉田 好彦 1, 佐藤 恵美子 1, 風岡 宣恵 2, 山田 史郎 2, 前田 初彦 1
1 愛知学院大学歯学部口腔病理学講座, 2 愛知医科大学病院歯科口腔外科

頸関節痛と関口障害を有する頸関節症患者に対する初期治療の効果
服部 雄紀 1, 栗田 賢一 1, 田島 毅士 1, 伊東 優 1, 近藤 倫弘 1, 清水 幹雄 1, 矢島 慎弥 1, 鍋島 弘充 1, 黒柳 章雄 2, 小木 信美 2, 泉 雅浩 3
1 愛知学院大学歯学部顔口歯外科学講座, 2 愛知学院大学歯学部顔面外科学講座, 3 愛知学院大学歯学部歯科放射線学講座

歯冠部切除術 Coronectomy の下顎第三大臼歯への臨床応用
波多野 裕子 1, 黒岩 裕一郎 1, 栗田 賢一 1, 有地 榮一郎 2, 湯浅 秀道 3
1 愛知学院大学歯学部顔口歯外科学, 2 愛知学院大学歯学部歯科放射線科, 3 東海産業医療団中央病院

バイオフィルムの観察・解析における2光子レーザー顕微鏡の有用性
竹中 彰治 1, Pitts Betsey 2, 若松 奈佳 1, 興地 隆史 1
1 新潟大学大学院医学総合研究科口腔学分野, 2 モンタナ州立大学バイオフィルムセンター

アルカリ電解水供給ジェットウォッシャーのう蝕バイオフィルム剝離に及ぼす影響
マティン カイルール 1, 早川 万里子 1, 豊田 彩子 2, 志田 嘉奈子 1, 永山 正仁 4, 小原 康弘 4, 田上 順次 3
1 東京医科歯科大学大学院口腔学分野, 2 東京医科歯科大学大学院口腔学分野/国立保健医療科学院口腔保健部, 3 東京医科歯科大学大学院口腔学分野/東京医科歯科大学 G-COE プログラム, 4 バナソニック電工株式会社電器 R&D センター美容科学研究所

エナメル質酸化症における酸性溶液へのミネラル添加の効果
石附 法子, 稲葉 大輔, 米満 正美, 岩手医科大学歯学部予防歯科学講座

Streptococcal distribution within plaque formed on enamel with glass–ionomere cement
T.T. TRAN 1, 加藤 一夫 1, 中垣 晴男 1, 河村 好章 2, 佐藤 拓一 3
1 愛知学院大学歯学部口腔衛生学講座, 2 愛知学院大学薬学部微生物学講座, 3 東北大学大学院歯学研究科歯科学
ポスター発表 11月30日（日） 第2日 ポスター会場

2009 Hatton Award 国内候補者発表
●14:00~15:15（B会場）

051 Phylogenetic analysis of the gbpC and dbl genes among mutans streptococci.
(H1) 小島 佑貴、佐藤 裕
東京歯科大学

052 Antibacterial effects of MDPB against anaerobes associated with endodontic infections.
(H2) 泉谷 尚美 1、今里 聡 1、高橋 祥根 2、恵比須英之 1、R.R.B. RUSSELL 2
1・大阪大学大学院歯学研究科口腔分子感染制御学講座、2Newcastle University, UK

Transcriptional Regulation of BRAK/CXCL14, a Tumor-Suppressing Chemokine.
(H3) 小森 令旨、小澤 重幸、加藤 靖正、進士 久明、木本 茂宏、畑 隆一郎
神奈川歯科大学小児歯科学講座

054 Invasion of host cells by membrane vesicles of Porphyromonas gingivalis.
(H4) 古田 信道、天野 敦雄
大阪大学大学院歯学研究科先端機器情報学教室

055 Amelogenin is a potent inhibitor of odontoclastic root resorption.
(H5) 八木 優子 1、須田 直人 1、山越 康雄 2、馬場 麻人 3、森山 啓司 1
1・東京医科歯科大学大学院医歯学総合研究科顎面矯正学分野、2University of Michigan, Ann Arbor, MI、3東京医科歯科大学大学院医歯学総合研究科硬組織構造生物学分野

Cell Biology & Pulp Biology / Geriatric Dentistry & Oral Rehabilitation
●14:00~15:04（C会場）

056 共培養実験系を用いた神経細胞と破骨細胞の細胞間相互作用
小畑 孝二 1、須賀 智子 2、後藤 滋巳 2、戸井 彰史 1
1・愛知学院大学歯学部薬理学講座、2愛知学院大学歯学部歯科矯正学

057 電解水に含まれる有効塩素の上皮細胞（KB-cells）増殖に及ぼす影響
ザマン ショウカット 1、マティン カイルール 2、岡田 彩子 1、花田 信弘 3、田上 順次 4
1・東京医科歯科大学大学院歯科矯正学分野/国立保健発達科学研究機構口腔保健部、2
東京医科歯科大学大学院歯科矯正学分野/鶴見大学歯学部探索歯学講座、4東京医科歯科大学大学院歯科矯正学分野/東京医科歯科大学 G-COE プログラム

058 タイプ1コラーゲン包埋培養したヒト歯髄由来細胞の組織学的研究
隅部 俊二、中塚 美智子、高間 敬子、三上 淑子、岩井 康智
大阪歯科大学歯学部歯科醫

059 ラット歯髄創傷治癒過程における MMP-3 の機能解析
天野 一晴 1、中島 美砂子 2、鄭 力 2、庵原 耕一郎 2、松井 寛敬 1、山崎 雅弘 1、
松下 健二1, 中村 洋2
1 愛知学院大学歯学部歯内治療学講座, 2 国立長寿医療センター研究所口腔疾患研究部

060 TNF-α: 刺激乳歯歯髄来源線維芽細胞における MMP-2 産生
渡邉 記代, 渡邉 京子, 白数 慎也, 大東 晃好, 大東 道治
大阪歯科大学小児歯科

座長: 花田 信弘

061 オーラル・リハビリテーションリボットによる筋マッサージ
有地 淑子1, 資又 明敏2, 小木 信美3, 泉 雅浩1, 佐久間 重光4, 飯田 幸弘2, 栗田 賢一5, 石井 美之6, 高西 淳夫5, 有地 榮一郎7
1 愛知学院大学歯学部歯科放射線学講座, 2 朝日大学歯学部口腔病態医療学講座, 3 愛知学院大学歯学部頸顔面外科学講座, 4 愛知学院大学歯学部冠・義歯学講座, 5 愛知学院大学歯学部頸口腔外科学講座, 6 早稲田大学理工学術院

062 口腔内細菌数測定装置を利用した口腔ケア・マネジメント
田村 文彦1, 菊谷 武1, 片桐 陽香1, 花形 哲夫2, 吉田 光吉3, 濱田 浩4, 高木 愛理4, 米田 哲也4, 水山 武雄5, 隼子 実穂1, 須田 牧夫1
1 日本歯科大学附属病院口腔介護・リハビリテーションセンター, 2 山梨県歯科医師会, 3 広島大学大学院, 4 バナソニック四国エレクトロニクス(株)ヘルスケア開発センター, 5 米山歯科クリニック

063 90 歳高齢者の口腔内状況と日常生活活動能力との関連性
相澤 文恵1, 岸 光男1, 稲葉 大輔1, 米満 正美1, 田沼 光正1, 西郷 慶悦2, 佐藤 保2, 箱崎 守男2
1 岩手医科大学歯学部予防歯科学講座, 2 岩手県歯科医師会

Operative Dentistry / Dental Material 1
● 14:00-15:12 (D 会場)

座長: 富士谷 盛興

064 レーザー・ブレーザーの歯の組織特異性の自由電子レーザーによる解明
寒河江 登志朗1, 沼田 靖子2, 佐藤 由紀江3, 岡田 沢之4, 山本 浩嗣3, 桑田 隆生1, 境 武志4, 野上 杏子5, 早川 恭史5, 田中 俊成5, 早川 建5, 佐藤 勇4
1 日本大学松戸歯学部組織・発生・解剖学, 2 日本大学松戸歯学部顔面・口腔・義歯・リハビリテーション学, 3 日本大学松戸歯学部口腔病理学, 4 日本大学総合研究大学院, 5 日本大学理工学部

065 歯根象牙質の疲労および引張特性
井上 利志子, 齊藤 誠, 西村 文夫, 宮崎 隆
昭和大学歯学部歯科理工学教室

066 照射器の種類と照射時間が漂白効果に及ぼす影響
Nayif Ma’an1, 大槻 昌幸1, 岸 綾香1, 田上 順次2
1 東京医科歯科大学大学院医歯学分野, 2 東京医科歯科大学グローバル COE プログラム

— 126 —
067 試作レジン系根管充填材に関する基礎的研究
　鶴部 春昌 1、山本 光徳 1、北村 成孝 1、中田 和彦 1、河合 達志 2、中村 洋 1
1 愛知学院大学歯学部歯内治療学講座、2 愛知学院大学歯学部歯科理工学講座

068 Microleakage of gutta percha and resilon at different canal length
S. WIMONCHIT, R. CHALERMMONTAGARN
Srinakarinwirot University

069 Shaping ability of two rotary Ni-Ti instruments in curved canals
C. PIYACHON, P. WANGWATTANAPISARN
Srinakarinwirot University

座長：伴 清治

070 龋蝕罹患根管壁象牙質に対するコンポジットレジンの接着強さ
　大竹 志保、吉田 恵一、福井 雄二、駒田 亘、三浦 宏之
東京医科歯科大学大学院歯学総合研究科歯科機能保存学分野

071 ３種類の象牙質接着システムの接着に対するマイクロレベルオキシダーゼプライマーの効果
　平 曜輔、添野 光洋
長崎大学大学院歯学薬学総合研究科歯薬化学分野

072 カゼインホスホペプチド-アモルファスカルシウムホスファートを塗布した象牙質へのコンポジットレジンの接着について
　V. SATTABANASUK 1, BURROW MICHAEL 2, 島田 康史 3, 田上 順次 3
1 Faculty of Dentistry, Srinakarinwirot University, 2 School of Dental Science, The University of Melbourne, 3 東京医科歯科大学歯学部歯科制御学分野

座長：神保 徹之

Periodontal Research 2 / Mineralized Tissue 2
●16:15-17:28（B 会場）

073 ラット歯肉の酸化ストレスに対する肥満の影響
　友藤 孝明 1、三部 俊博 1、玉木 直文 1、江國 大輔 1、粕山 健太 1、馬越 通弘 1、入江 浩一郎 1、東 哲司 1、村上 純 2、苔口 進 3、山本 龍生 1、森田 学 1
1 岡山大学大学院歯学薬学総合研究科防歯科学分野、2 岡山大学大学院歯学薬学総合研究科歯科機能学分野、3 岡山大学大学院歯学薬学総合研究科歯科微生物学分野

074 Porphyromonas gingivalis 感染が冠動脈疾患リスクに及ぼす影響
　前川 知樹、高橋 直紀、青木 由香莉、宮下 博考、多部田 康一、山崎 和久
新潟大学超研研究機構

075 血漿中の活性酸素種濃度に対する非外科的歯周治療の効果
　玉木 直文、山中 玲子、江國 大輔、友藤 孝明、山本 龍生、森田 学
岡山大学大学院歯学薬学総合研究科歯科防歯科学分野

076 ラット根尖病変成立過程におけるコラーゲナーゼ発現
　松井 要敬、天野 一晴、山崎 雅弘、中田 和彦、中村 洋
愛知学院大学歯学部歯内治療科
口臭有訴者の唾液中コルチゾールおよびTh1/Th2 サイトカインの分析
福井 誠 1, Baatarjav Tselmej 1, 片岡 宏介 1, 日野出 大輔 2, 伊藤 博夫 1
1 徳島大学大学院 HBS 研究部予防歯学分野、2 徳島大学歯学部口腔保健学科口腔保健基礎学講座

座長：山本 照子

ヒトエナメル質と象牙質を通過する陰イオン量は交流イオン導入法により増加する
池田 英治、須田 英明
東京医科歯科大学大学院歯学総合研究科歯科生物学会分野

CHP nanogel を用いたマウス TNF-α 誘導骨吸収モデルの検討
永野 健一 1, 青木 和広 1, ミアン フセイン 1, アレキ ニール 1, 下田 麻子 2、
森本 萌行 2, 土谷 一成 2, 大谷 啓一 1
1 東京医科歯科大学大学院歯学総合研究科歯科生物学会分野、2 東京医科歯科大学
大学生体材料工学研究所有機材料分野

mi マウスの歯芽萌出過程における破骨細胞性骨吸収について
高橋 智美、牛島 夏未、野田 新悦、飯塚 正
北海道大学大学院歯学研究科学術支援部

犬頸骨へのβ-TCP インプラントによる RANKL/NF-κB 発現の陰害
趙 剣 1, 渡辺 孝夫 2, 安孫子 宜光 1
1 日本大学松戸歯学部、生化学・分子生物学講座、2 神奈川歯科大学解剖学講座

# Mineralized Tissue 3 / Dental Material 2

●16:15~17:35（C 会場）

歯髄細胞および骨髄細胞の幹細胞様性質について
ISHKITIEV NIKOLAY 1, MITEV VANYO 2, CALENIC BOGDAN 1, 中原 貴 3, 八重垣 健 1
1 日本歯科大学生命歯学部衛生学講座、2 Medical University - Sofia、3 日本歯科大学
生命歯学部発生・再生医科学講座

インターロイキン1αは、骨芽細胞様細胞 MC3T3-E1 におけるα3β1 インテグリンの発現を増
強し、細胞接着を促進する
富山 高史 1, 成石 浩司 2, 大森 一弘 2, 前田 博史 1, 高柴 正悟 1
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附属病院歯周科

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伊井 久貴、今井 敏夫、鴨田 剛司、村田 貴俊、田中 とも子、佐藤 勉、八重垣 健
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Gene Expression / Diagnosis
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1 愛知学院大学歯学部歯科治療学講座、2 愛知学院大学歯学部微生物学講座
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大阪歯科大学歯学部歯学科
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<thead>
<tr>
<th>年齢</th>
<th>〜3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>成人</th>
<th>高齢者</th>
</tr>
</thead>
<tbody>
<tr>
<td>ミラノールの種類</td>
<td>1g分包（フッ素 250ppm）</td>
<td>1.8g分包（フッ素 450ppm）</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1包の使用日数例</td>
<td>1回5mLの使用 → 40日分</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1包の使用日数例</td>
<td>1回7mLの使用 → 28日分</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1回10mLの使用で20日分</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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<th>承認・調剤・届出番号</th>
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<td>歯科用アクリル系レジン</td>
<td>医療機器製造業者番号 218AZX0088400</td>
</tr>
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