The 68th Annual Meeting of Japanese Association for Dental Research

Programme & Abstracts

2020

第68回国際歯科研究学会
日本部会 総会・学術大会

http://jadr68.umin.jp

November 7-8, 2020

Beyond Borders:
From Dental Research to Future Oral Health

PROGRAM AND ABSTRACTS OF PAPERS

JAPANESE ASSOCIATION FOR DENTAL RESEARCH

The 68th ANNUAL MEETING

November 7-8, 2020

Virtual Meeting

CONTENTS

Welcome message

Dear Craniofacial and Dental Scientists:

In behalf of the organizing committee and as the Chairman of the 68th Annual Meeting of Japanese Association of Dental Research (JADR) 2020, I am delighted to welcome every one of you to this meeting. It is our great honor and pleasure to hold such a prestigious meeting. Although conducting this meeting is very difficult because of the COVID-19 pandemic, we express our gratitude to the president of JADR, Professor Satoshi Imazato, and all of our distinguished guests for their attendance.

JADR has contributed to the advancement of basic and clinical research in the dental science field in Japan, in collaboration with the International Association for Dental Research (IADR). We have decided to designate "Beyond Borders: From Dental Research to Future Oral Health" as the main theme of this meeting to expand the basic and clinical research and produce a new translational researc h for spurring evolution in dentistry.

We will invite Dr. Pamela Den Besten (from IADR) and Dr. Joo-Cheol Park (from the Korean Division of the IADR (KADR) as Special Lecturers, who will share with us their future outlook in regard to dental science. As for the Keynote, Professor Richard J. Lamont from the University of Louisville will present an interesting lecture concerning the process of dysbiosis in periodontitis.

We will also organize three symposiums on this particular topic, entitled "At the front-line: Etiology of periodontitis," "Pathophysiological approach from the oral function to systemic diseases" and "Future is now! Stem cell revolution in hard and soft tissue engineering" from which we reach the frontline of periodontitis, oral function from the pathophysiological viewpoint, and stem cell research. We also organized a symposium on neuroscience for young investigators.

Although the meeting will take place online because of the complicated COVID-19 situation, we believe that this meeting will provide an excellent opportunity for all attendees to learn together in the virtual world to evolve dental science through telecommunications beyond the physical distance.

The 68th Annual Meeting of Japanese Association for Dental Research
Congress President: Kazuyuki Ishihara, D.D.S., Ph.D.
Professor Department of Microbiology, Tokyo Dental College

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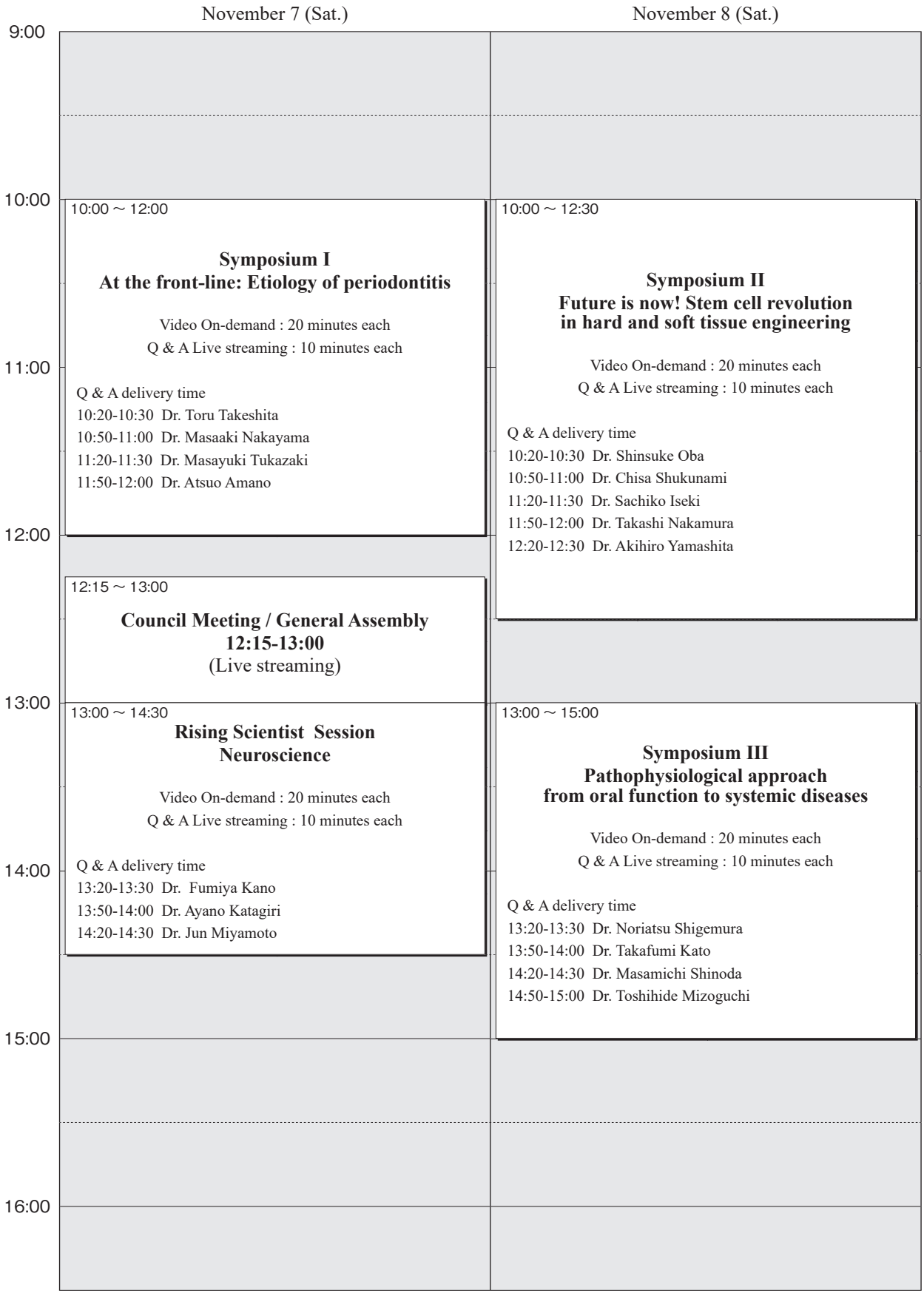
JADR Timetable November 7th (Sat.) November 8th (Sun.)
Video On-demand distribution / Live streaming



JADR Timetable
November 7th(Sat.) - 21st.(Sat.)
Video On-demand and Poster File distribution

Keynote Lecture (Video On-demand distribution)

Prof. Richard J. Lamont
(Department Oral Immunology & Infectious Diseases, University of Louisville, School of Dentistry)
"Porphyromonas gingivalis interactions with epithelial cells in the community context"

Greeting from IADR President (Video On-demand distribution)

Prof. Pamela Den Besten
(President of IADR)

Special Lecture (Video On-demand distribution)

Prof. Joo-Cheol Park
(President of KADR)
"Tubular Dentin Regeneration and Its Clinical Application"

Poster Presentation (On-demand distribution of presentation file (PDF))

November 7th, Saturday, ~ November 21st, Saturday

Keynote Lecture
(Video On-demand distribution)

KL Porphyromonas gingivalis interactions with epithelial cells in the community context

Dr. Richard J Lamont

(Department Oral Immunology & Infectious Diseases, University of Louisville, School of Dentistry)

Greeting from IADR President
(Video On-demand distribution)

G

Dr. Pamela Den Besten

(Department of Orofacial Sciences, School of Dentistry, University of California San Francisco)

Special Lecture
(Video On-demand distribution)

SL Tubular Dentin Regeneration and Its Clinical Application

Dr. Joo-Cheol Park

(Laboratory for the Study of Regenerative Dental Medicine, Department Oral Histology-Developmental Biology, School of Dentistry, Seoul National University)

Symposium I~III, Rising Scientist Session
(Lecture;Video On-demand distribution / Q & A;Live streaming)

November 7th, Saturday

10:00-12:00 Symposium I
"At the front-line: Etiology of periodontitis"

Moderator: Dr. Kazuyuki Ishihara
(Department of Microbiology, Tokyo Dental College, Tokyo, Japan)
Moderator: Dr. Yoshihisa Yamashita
(Section of Preventive and Public Health Dentistry, Division of Oral Health, Growth and Development, Faculty of Dental Science, Kyushu University, Fukuoka, Japan)

SI-1 Bacterial composition of overall oral microbiota associated with periodontal disease and health.

Dr. Toru Takeshita
(Section of Preventive and Public Health Dentistry, Faculty of Dental Science, Kyushu University)

SI-2 The significant role of *Porphyromonas gingivalis* "Gingipains" as a virulence factor on periodontal inflammation

Dr. Masaaki Nakayama[1], Dr. Naoya Ohara[2]
([1]Department of Microbiology, Graduate school of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, [2]Advanced Research Center for Oral and Craniofacial Sciences (ARCOCS), Dental School, Okayama University)

SI-3 Periodontitis as an "osteoimmune" disease

Dr. Masayuki Tsukazaki
(Department of Immunology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo)

SI-4 Epithelial barrier breakdown by *Porphyromonas gingivalis*

Dr. Atsuo Amano, Dr. Hiroki Takeuchi
(Department of Preventive Dentistry, Graduate School of Dentistry, Osaka University)

13:00-14:30 Rising Scientist Session
"Neuroscience"

Moderator: Dr. Tazuko Goto
(Department of Oral and Maxillofacial Radiology, Tokyo Dental College, Tokyo, Japan)

RS-1 Factors secreted from dental pulp stem cells show multifaceted benefits for Synergistically Regenerate Transected Rat Peripheral Nerves by Altering Macrophage Polarity.

Dr. Fumiya Kano
(Department of Tissue regeneration Institute of Biomedical Sciences, Tokushima University Graduate School)

RS-2 Phenotypic and neuroplastic changes in trigeminal nociceptive pathways following trigeminal nerve injury

Dr. Ayano Katagiri
(Department of Oral Physiology, Osaka University Graduate School of Dentistry, Suita, Japan)

RS-3 The human brain and mastication; broad impact on systemic functions

Dr. Jun Miyamoto

(Department of Maxillofacial Orthognathics, Graduate School of Medical and Dental Sciences,
Tokyo Medical and Dental University (TMDU))

November 8th, Sunday

10:00-12:30 SymposiumII
"Future is now! Stem cell revolution in hard and soft tissue engineering"

Moderator: Dr. Toshifumi Azuma
(Department of Biochemistry, Tokyo Dental College, Tokyo, Japan)
Moderator: Dr. Shinsuke Oba
(Department of Cell Biology, Institute of Biomedical Sciences, Nagasaki University)

SII-1 Generation of skeletal cells from pluripotent stem cells

Dr. Shinsuke Oba
(Department of Cell Biology, Institute of Biomedical Sciences, Nagasaki University)

SII-2 Establishment of an *in vitro* culture system for tenogenic/ligamentogenic differentiation using *ScxGFP* iPS cells

Dr. Chisa Shukunami
(Department of Molecular Biology and Biochemistry, Graduate School of Biomedical and Health Sciences, Hiroshima University)

SII-3 Classification and pathological mechanisms of Craniosynostosis based on the differentiation pattern of iPS cells

Dr. Sachiko Iseki
(Section of Molecular Craniofacial Embryology, Tokyo Medical & Dental University Graduate School of Medical and Dental Sciences)

SII-4 Molecular mechanisms of craniosynostosis in Apert syndrome.

Dr. Takashi Nakamura[1], Dr. Hiroyuki Ogura[2]
([1]Department of Biochemistry/Research Branding Project, Tokyo Dental College.,
Department of Orthodontics, Tokyo Dental College.,
[2]Department of Biochemistry/Oral Health Science Center/Research Branding Project, Tokyo Dental College.)

SII-5 Application of iPS cell technologies to treat skeletal disease

Dr. Akihiro Yamashita, Dr. Noriyuki Tsumaki
(Centre for iPS cell Research and Application (CiRA), Kyoto University, Japan)

13:00-15:00 Symposium III
"Pathophysiological approach from oral function to systemic diseases"

Moderator: Dr. Yoshiyuki Shibukawa
(Department of Physiology, Tokyo Dental College, , Tokyo, Japan)
Moderator: Dr. Naofumi Uesaka
(Department of Cognitive Neurobiology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan)

SIII-1 Taste renin-angiotensin system may contribute to the maintenance of sodium homeostasis.

Dr. Noriatsu Shigemura
(Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University,, Research and Development Center for Five-Sense Devices, Kyushu University.)

SIII-2 Pathophysiology of SB: challenges from human and animal studies

Dr. Takafumi Kato
(Osaka University Graduate School of Dentistry, Department of Oral Physiology)

SIII-3 Common pathophysiological features in burning mouth syndrome and irritable bowel syndrome

Dr. Masamichi Shinoda
(Department of Physiology, Nihon University School of Dentistry)

SIII-4 *In vivo* Dynamics of Dental Tissue Regeneration

Dr. Toshihide Mizoguchi
(Oral Health Science Center, Tokyo Dental College, Tokyo, Japan)

Poster Presentation

Dental Materials 1: Ceramic-based Materials

001: Wear Resistance of Novel Machinable Glass Ceramics

S. Kariya, T. Azuma, F. Fusejima
R&D Dept., GC Corporation, Tokyo, JAPAN

Dental Materials 4: Adhesion

002: Evaluation of bonding layer durability on 2-step self-etch adhesive

K. FUJIMORI, K. HIRANO, F. FUSEJIMA
RESEARCH & DEVELOPMENT, GC CORPORATION, TOKYO, Japan

003: Bonding properties of acrylic resin to zirconia and polyaryletherketone (PAEK)

T.-Y. PENG[1], S. SHIMOE[2], D.-J. LIN[1], M. KAKU[2]
[1]School of Dentistry, College of Dentistry, China Medical University, Taiwan R.O.C, [2]Department of Anatomy and Functional Restorations, Hiroshima University Graduate School of Biomedical & Health Sciences, Hiroshima, Japan

004: Bond strength between one-step adhesives and dentin after cyclic loading

T. EGOSHI[1], Y. TAIRA[1], K. SOENO[2], K. KAIDA[1], S. KUBO[1], H. MURATA[3]
[1]Division of Cariology and Restrative Dentistry, Department of Prosthetic Dentistry, Nagasaki University, Nagasaki, Japan, [2]Department of Appried Prosthodontics, Nagasaki University, Nagasaki, Japan, [3]Department of Prosthetic Dentistry, Nagasaki University, Nagasaki, Japan

005: Influence of saliva contamination on adhesion performance of adhesive resin cement

S. MURAKAMI, K. HIRANO, F. FUSEJIMA
RESEARCH & DEVELOPMENT, GC CORPORATION, TOKYO, Japan.

Cariology Research-Demineralization/Remineralization

006: Evaluation of Dentin Anti-Demineralization potential of S-PRG Containing Self-Adhesive Resin Cement

S. ThanNaing[1], N. Hiraishi[1], A. Abdou[1,3], J. Tagami[1]
[1]Cariology and Operative Department, Tokyo Medical and Dental University, Tokyo, JAPAN, [2]Department of Conservative Dentistry, University of Dental Medicine Mandalay, Mandalay, Chanmyathazi, MYANMAR, [3]Biomaterials Department, Faculty of Dentistry, Modern University for Technology and Information, Cairo, EGYPT

007: Preparation of Filler-Dispersed Resin Composite for Additive Manufacturing

P. KARNTIANG[1], H. IKEDA[2], Y. NAGAMATSU[2], H. SHIMIZU[2]
[1]Division of Operative Dentistry, College of Dentistry, Rangsit University, Pathum Thani, Thailand, [2]Division of Biomaterials, Department of Oral Functions, Kyushu Dental University.

008: Enamel Remineralization of TCP with Fluoride in Toothpaste: EPMA Study

H. HAMBA, H. ISHIZUKA, Y. MIYAYOSHI, K. NAKAMURA, T. MURAMATSU
Department of Operative Dentistry, Cariology and Pulp Biology, Tokyo Dental College, Tokyo, Japan

009: EVALUATION OF THE EFFECT OF ZINC-CONTAINING ORAL MATERIALS ON REMINERALIZATION USING IN-AIR MICROBEAM PIXE/PIGE

M. Sakurai[1], Y. Matsuda[2], K. Okuyama[3], H. Yamamoto[2], M. Hayashi[2], T. Saito[1]
[1]Division of Clinical Cariology and Endodontology, Department of Oral Rehabilitation, Health Sciences University of Hokkaido, Tobetsu-cho, Ishikari-gun, Hokkaido, JAPAN, [2]Department of Restorative Dentistry and Endodontology, Osaka University Graduate School of Dentistry, Osaka, JAPAN, [3]Department of Dental Materials Science, Asahi University School of Dentistry, Mizuho, Gifu, JAPAN

Cariology Research-Fluoride & Ca-based Products

010: Dynamics of Ions Artificially Introduced into Caries-affected Dentin

K. NAITO[1], H. YAMAMOTO[1], Y. MATSUDA[2], K. OKUYAMA[3], M. HAYASHI[1]
[1]Department of Restrative Dentistry and Endodontology, Osaka University Graduate School of Dentistry, [2]Division of Clinical Cariology and Endodontology, Department of Oral Rehabilitation, School of Dentistry, Health Sciences University of Hokkaido, [3]Department of Dental Materials Science, Division of Oral Functional Sciences and Rehabilitation, Asahi University School of Dentistry

Dental Materials 2: Polymer-based Materials

011: Physical properties of Resorbable P(LA/CL) bilayer membrane for GBR

Y. SAKAGUCHI, E. ARIMA, K. YAMANAKA, F. FUSEJIMA
GC Corporation, Tokyo, Japan

Prosthodontics Research

012: Influence of Molding Angle on 3D-printed Partial Denture Framework

H. KOBAYASHI[1], A. TASAKA[1], T. SHIMIZU[1], S. HIGUCHI[2], S. YAMASHITA[1]
[1]Department of Removable Partial Prosthodontics, Tokyo Dental College, [2]Wada Precision Dental Laboratories Corporation

Oral Health Research

013: Microbicidal effect and storage stability of neutral electrolyzed water-based gels

Y. NAGAMATSU[1], H. NAGAMATSU[2], H. IKEDA[1], H. SHIMIZU[1]
[1]Division of Biomaterials, Department of Oral Functions, Kyushu Dental University, Fukuoka, Japan, [2]Division of Comprehensive Dentistry, Department of Oral Functions, Kyushu Dental University, Fukuoka, Japan

014: A pilot study of effects on Dentifrice Containing Neem for Oral Malodor, Plaque Adhesion, Gingival Inflammation and Oral Bacteria

M. YASUI, K. SHINADA
Department of Priventive Oral Health Care Sciences, Tokyo Medical and Dental University, Tokyo, Japan

Microbiology/Immunology

015: Antimicrobial effects and mechanical properties of acrylic resin containing aPIZAS

K. INABA[1], H. WATANABE[1], Y. WADA[1], S. WATANABE[1], H. SASAKI[1], H. HIRAMINE[2], M. SASAKI[3], T. NIHEI[1], N. HAMADA[1]
[1]Department of Oral Science, Kanagawa Dental University, Yokosuka, Japan, [2]Department of Highly

Advanced Stomatology, Yokohama Clinical Education Center of Kanagawa Dental University, [3]Nissho Chemistry Corporation

016: Effects of S-PRG eluate on bacterial properties related to oral malodor

S. OMAGARI[1], M. YONEDA[1], N. SUZUKI[2], D. GRENIER[3], S. YAMAMOTO[1], K. YAMADA[1], J. HATAKEYAMA[1], H. MORITA[4], C. KOGA[5], T. HIROFUJI[1]
[1]Section of General Dentistry, Department of General Dentistry, Fukuoka Dental College, Fukuoka, Japan, [2]Section of Oral Public Health, Department of Preventive and Public Health, Fukuoka Dental College Fukuoka, Japan, [3]Groupe de Recherche en Ecologie Buccale, Faculte de medecine dentaire, Universite Laval, Quebec, Canada, [4]The Center for Visiting Dental Service, Department of General Dentistry, Fukuoka Dental College, Fukuoka, Japan, [5]Center for Oral Diseases, Fukuoka Dental College Fukuoka, Japan

017: Evaluation of anti-biofilm effects of bio-active GIC using a bioreactor

T. KOHNO, Y. LIU, F. DENG, R. TSUBOI, H. KITAGAWA, S. IMAZATO
Department of Biomaterials Science, Osaka University Graduate School of Dentistry, Suita, Japan

018: Streptococcus mutans inhibition by toothbrush monofilament with surface pre-reacted glass-ionomer

S. MATAYOSHI, T. KITAMURA, M. OTSUGU, R. NOMURA, K. NAKANO
Department of Pediatric Dentistry, Osaka University Graduate School of Dentistry, Japan

019: Comparison of microbiome organized on denture base materials and hydroxyapatite

Y. NEZU[1], M. RYU[1], K. ISHIHARA[2], T. UEDA[1]
[1]Removable Prosthodontics & Gerodontology, Tokyo Dental College, Tokyo, Japan, [2]Microbiology, Tokyo Dental College

Cariology Research-Microbiological Studies/Biofilm

020: ABC transporter activities in biofilm formation by *Streptococcus mutans*

K. GOTO, M. MATSUMOTO-NAKANO
Department of Pediatric Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Microbiology/Immunology

021: Inhibitory Effect of Cyclodextran on Glucosyltransferase B in *Streptococcus mutans*

H. ASAUMI, K. GOTO, M. MATSUMOTO-NAKANO
Department of Pediatric Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

022: The effects of fatty acid salts against *Streptococcus mutans* biofilm

A. KURAHASHI, K. WATANABE, T. SATO, K. INABA, H. SASAKI, N. HAMADA
Department of Oral Science, Graduate School, Kanagawa Dental University

023: Evaluation of collagen-binding properties of killed Streptococcus mutans strains

Y. SUEHIRO, S. MATAYOSHI, M. OTSUGU, R. NOMURA, K. NAKANO
Department of Pediatric Dentistry, Osaka University Graduate School of Dentistry, Osaka, Japan

024: Screening of S. salivarius isolates with anti-caries activities

H. NAKADE[1], S. ALI[2], D. DUFOUR[2], C. LEVESQUE[2], S. GONG[2]
[1]Faculty of Dentistry, Tokyo Medical and Dental University, [2]Faculty of Dentistry, University of Toronto

025: Characteristics of anti-periodontal bacterial activity in culture supernatant of probiotic

T. KAWAI[1], R. SHIN[2], Y. ITO[2], S. IKAWA[3], T. TANAKA[3], T. OHSHIMA[1]
[1]Department of Oral Microbiology, Tsurumi University School of Dental Medicine, Kanagawa, Japan, [2]ALA Research Institute for Fermentative Microbes, Tokyo, Japan, [3]Technology Research Institute of Osaka Prefecture, Osaka, Japan

Periodontal Research-Pathogenesis

026: Exploring for prevention of periodontal disease using products of the probiotic bacteria, genus Lactobacillus -Targeting the genus Porphyromonas-

Y. SAKAI, T. OHSHIMA, T. KAWAI
Tsurumi University Faculty of Dentistry Department of Dentistry

Microbiology/Immunology

027: *Gemella haemolysans* specifically inhibits the growth of *Porphyromonas gingivalis*

T. MIYOSHI[1], N. YOSHINARI[2], A. YOSHIDA[1]
[1]Department of Oral Microbiology, Matsumoto Dental University, Nagano, Japan, [2]Department of Periodontology, Matsumoto Dental University, Nagano, Japan

028: *Fusobacterium nucleatum* metabolically integrates commensals and pathogens in oral biofilms

A. SAKANAKA[1], M. KUBONIWA[1], S. SHIMMA[2], S. MAYUMI[1], R. LAMONT[3], E. FUKUSAKI[2], A. AMANO[1]
[1]Department of Preventive Dentistry, Osaka University Graduate School of Dentistry, Osaka, Japan, [2]Department of Biotechnology, Osaka University Graduate School of Engineering, Osaka, Japan, [3]Department of Oral Immunology and Infectious Diseases, School of Dentistry, University of Louisville, Louisville, KY, USA

029: Analysis of novel genotypes of Mfa1 fimbriae in *Porphyromonas gingivalis*

K. SAKAE[1], K. NAGANO[2], Y. HASEGAWA[1]
[1]Department of Microbiology, School of Dentistry, Aichi Gakuin University, Aichi, Japan, [2]Department of Microbiology, School of Dentistry, Health Sciences University of Hokkaido, Hokkaido, Japan

030: Role of hyalin-like protein in biofilm formation by *Capnocytophaga ochracea*

T. WARITA[1], K. SHIBAYAMA[1], E. KOKUBU[1], D. KITA[2], Y. KIKUCHI[1], K. ISHIHARA[1]
[1]Department of Microbiology, Tokyo Dental College, Tokyo, Japan, [2]Department of Periodontology, Tokyo Dental College, Tokyo, Japan

031: The periodontopathic bacterium Fusobacterium nucleatum induced proinflammatory cytokine production by human respiratory epithelial cells and in the lower respiratory organs in mice

K. IMAI[1], N. WATANABE[1,2], S. YOKOE[1,2], S. SATO[2]
[1]Department of Microbiology, Nihon University School of Dentistry, [2]Department of Periodontology, Nihon University School of Dentistry

032: IgA Nephropathy-like Lesion Development in Rat Caries Model

S. NAKA[1], K. WATO[2], T. MISAKI[3], Y. NAGASAWA[4], S. ITO[5], R. NOMURA[2], M. MATSUMOTO-NAKANO[1], K. NAKANO[2]
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and Pharmaceutical Sciences, Okayama, Japan, [2]Department of Pediatric Dentistry, Division of Oral Infection and Disease Control, Osaka University Graduate School of Dentistry, Suita, Oska, Japan, [3]Division of Nephrology, Seirei Hamamatsu General Hospital, Hamamatsu, Japan, [4]Division of Kidney and Dialysis, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan, [5]Department of Nephrology anf Endocrinology, National Defense Medical College, Tokorozawa, Japan

033: Effects of *Helicobacter pylori* Infection in Caries-induced Rats

T. KADOTA, Y. OGAYA, R. NOMURA, K. NAKANO
Department of Pediatric Dentistry, Osaka University Graduate School of Dentistry, Osaka, Japan

034: Prevalence of Specific Streptococcus mutans Harbored by Non-alcoholic Steatohepatitis Patients

K. TABATA[1], S. NAKA[1], S. TONOMURA[2,3,4], K. NAKANO[5], M. MATSUMOTO-NAKANO[1]
[1]Department of Pediatric Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan, [2]Department of Neurology, Nara City Hospital, Japan, [3]Department of Neurology, National Cerebral and Cardiovascular Center, Japan, [4]Department of Neurology, Graduate School of Medicine and Faculty of Medicine, Kyoto University, Japan, [5]Department of Pediatric Dentistry, Division of Oral Infection and Disease Control, Osaka University Graduate School of Dentistry, Suita, Osaka, Japan

Pediatric Oral Health Research

035: The Initiation and development of oral microbiome formation in Japanese healthy infants

A. SAKURAI, H. HOMMA, N. NAGAI, T. OTA, S. SHINTANI
Department of Pediatric Dentistry, Tokyo Dental College, Tokyo, Japan

Periodontal Research-Pathogenesis

036: Omics analysis defines differences in microbial community structure between peri-implantitis and periodontitis

K. KOMATSU[1], Y. TAKEUCHI[1], T. SHIBA[1], T. WATANABE[2], M. SHIMOGISHI[3], M. SHIBASAKI[3], T. NEMOTO[1], T. KOYANAGI[1], S. KATAGIRI[1], T. IWATA[1]
[1]Department of Periodontology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan, [2]Department of Chemistry, Nihon University School of Dentistry, Tokyo, Japan, [3]Department of Oral Implantology and Regenerative Dental Medicine, Tokyo Medical and Dental University, Tokyo, Japan

037: Periodontal Bacteria Distribution in Child with Early Primary Tooth Loss

Y. MIYAI, S. NAKA, M. MATSUMOTO-NAKANO
Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

Periodontal Research-Therapy

038: CTLA-4 Reduces Bone Resorption Through the Inhibition of Osteoclast Differentiation

S. NAKANE[1], K. IMAMURA[1], K. ISHIHARA[2,3], A. SAITO[1,2]
[1]Department of Periodontology, Tokyo Dental College, Tokyo, Japan, [2]Oral Health Science Center, Tokyo Dental College, Tokyo, Japan, [3]Department of Microbiology, Tokyo Dental College, Tokyo, Japan

Periodontal Research-Pathogenesis

039: An immunohistochemical study on the expression of bioactive molecules in the mouse kidney in *P. gingivalis* LPS-induced diabetic nephropathy

K. KOICHIRO[1], Y. SAWA[2], T. FUJITA[1], S. TAMAOKI[1]
[1]Section of orthodontics Department of Oral Growth & Development, Division of Clinical Dentistry, Fukuoka Dental College, [2]Deparment of Oral Function & Anatomy, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences

Oral Medicine & Pathology Research

040: Artepillin C regulates extracellular matrix gene expressions in human periodontal fibroblasts by DNA methylation

R. TAKAI[1], O. UEHARA[2], T. MORIKAWA[3], K. YOSHIDA[3], J. SATO[3], Y. ABIKO[3], T. OHTA[1]
[1]Advanced Research Promotion Center, Health Sciences University of Hokkaido, Hokkaido, Japan, [2]Division of Disease Control and Molecular Epidemiology, Health Sciences University of Hokkaido, Hokkaido, Japan, [3]Division of Oral Medicine and Pathology, Health Sciences University of Hokkaido, Hokkaido, Japan

Periodontal Research-Pathogenesis

041: Exosomes from human gingiva-derived MSCs inhibit periodontal bone loss

Y. NAKAO, T. FUKUDA, Y. WATANABE, C. HAYASHI, K. KAWAKAMI, M. TOYODA, H. YAMATO, T. SINJO, U. TANAKA, T. SANUI, F. NISHIMURA
Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

042: PPARγ is required for periodontal ligament cells to retain cemento/osteogeneity

H. YUAN[1], S. SUZUKI[1], A. SATO[1], T. YAMAMOTO[1], E. NEMOTO[1], M. SAITO[2], S. YAMADA[1]
[1]The Department of Periodontology and Endodontology, Tohoku University school of Dentistry, [2]The Department of Operative Dentistry, Tohoku University school of Dentistry

Oral Medicine & Pathology Research

043: Role of IQGAP1- Flightless I interaction in collagen remodeling by fibroblast

K. NAKAJIMA[1], P. ARORA[2], C. MCCULLOCH[2]
[1]Department of Pathology, Tokyo Dental College, Tokyo, Japan, [2]Faculty of Dentistry, University of Toronto, Toronto, ON, Canada

Periodontal Research-Therapy

044: Amelogenin down-regulates MHC class II antigen presentation on macrophages.

K. YOTSUMOTO, T. SANUI, H. YAMATO, U. TANAKA, Y. NAKAO, Y. WATANABE, C. HAYASHI, T. FUKUDA, F. NISHIMURA
Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

Periodontal Research-Pathogenesis

045: Adipose specific C-C motif chemokine ligand (CCL) 19 overexpression drives the mice to both insulin resistance and weight gain.

M. HAYASHI, M. IWASHITA, Y. NISHIMURA, T. SHINJO, T. ZEZE, T. SANO, A. YAMASHITA, F.

NISHIMURA
Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University

Periodontal Research-Therapy

046: Characterization of the *PLAP-1-GFP/CreER* knock-in mouse

T. IWAYAMA[1], K. TOMITA[1], S. MATSUMOTO[1], M. IWASHITA[1], C. FUJIHARA[1], M. TAKEDACHI[1], S. YAMADA[2], S. MURAKAMI[1]
[1]Department of Periodontology, Osaka University Graduate School of Dentistry, Suita, Osaka, Japan, [2]Department of Periodontology and Endodontology, Tohoku University Graduate School of Dentistry, Sendai, Miyagi, Japan

Implantology Research

047: Salivary Alpha-Amylase as a stress marker during dental implant surgeries

A. A. Sabbagh, H. Nakata, A. Abdou, K. Shohei, S. Kuroda
Tokyo medical and Dental University, Tokyo, JAPAN

Oral Medicine & Pathology Research

048: Regional differences in the density of Langerhans cells, CD8-positive T lymphocytes and CD68-positive macrophages

Y. OMINE
Iryouhoujin Syuuyuu-kai, Tsuchiura, Japan

Periodontal Research-Pathogenesis

049: Delayed Wound Healing after Tooth Extraction under Malnutrition

Y. ZHANG[1], H. IDEGUCHI[1], H. AOYAGI[1], K. YAMASHIRO[1], T. YAMAMOTO[1], M. NISHIBORI[2], S. TAKASHIBA[1]
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Oral & Maxillofacial Surgery Research

050: Assessment of Facial Changes after Orthognathic Surgery Using Geometric Morphometrics

S. AL BOUGHA[1], H. NAKANO[2], K. YASUDA[1], T. YAMADA[1], I. TAKAHASHI[3], Y. MORI[1]
[1]Section of Oral and Maxillofacial Surgery-Division of Maxillofacial Diagnostic and Surgical Science - Faculty of Dental Science, Kyushu University, [2]Department of Dentistry and Oral Surgery, Division of Medicine for Function and Morphology of Sensory Organs, Faculty of Medicine, Osaka Medical College, [3]Department of Orthodontics, Faculty of Dental Science, Kyushu University

051: Metformin inhibits oral cancer cell growth by altering glucose metabolism

S. LIU[1], J. WASHIO[1], N. TAKAHASHI[1]
[1]Division of Oral Ecology and Biochemistry, Department of Oral Biology, Tohoku University Graduate School of Dentistry, Sendai, Japan, [2]Department of Head and Neck Oncology, West China Hospital of Stomatology Sichuan University, Chengdu, China

Oral Medicine & Pathology Research

052: *Sirtuin1* DNA hypermethylation may be a biomarker for malignant transformation

S. ISLAM, O. UEHARA, H. MATSUOKA, Y. ABIKO, I. CHIBA
Health Sciences University of Hokkaido

053: Role of EGFR-mediated MOB1 phosphorylation on Hippo pathway regulation

T. ANDO
Center of oral clinical examination, Hiroshima Univeresity Hospital, Hiroshima, Japan

054: Sema3A-AKT Axis In Salivary Gland And Adenoid Cystic Carcinoma Developments

T. FUJIMOTO[1], S. FUJII[2], T. KIYOSHIMA[2]
[1]School of Dentistry, Kyushu University, [2]Laboratory of Oral Pathology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University

Oral & Maxillofacial Surgery Research

055: Effect of Ethinyl Estradiol on Mandibular Condyle of Female Rats

S. YAKLAI, R. PECHAYCO, Y. ABE, A. TAKIZAWA, I. MIZOGUCHI, T. TAKAHASHI, M. CHIBA
Graduate School of Dentistry, Tohoku University

Craniofacial Biology Research

056: Histone methyltransferase SETDB1 negatively regulates PTH/PTHrP receptor in chondrocytes

P. THIHA, N. HIGASHIHORI, S. KANO, K. MORIYAMA
Department of Maxillofacial Orthognathics, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University

Stem Cell Biology Research

057: Generating MSC Spheroids Recovers Stemness Characteristics for Cortical Bone Formation

Y. OHORI, K. NIIBE, H. EGUSA
Division of Molecular and Regenerative Prosthodontics, Tohoku University Graduate School of Dentistry, Sendai, Miyagi, Japan

Craniofacial Biology Research

058: The bHLH transcription factor HAND1 is involved in cortical bone volume through the regulation of collagen expression

N. FUNATO[1], Y. TAGA[3], L. E. LAURIE[1], C. TOMETSUKA[3], M. KUSUBATA[3], K. OGAWA-GOTO[3]
[1]Department of Signal Gene Regulation, Tokyo Medical and Dental University, Tokyo, Japan, [2]Research Core, Tokyo Medical and Dental University, Tokyo, Japan, [3]Nippi Research Institute of Biomatrix, Ibaraki, Japan

Mineralized Tissue

059: Oxygen tension-dependent expression of monocarboxylate transpoter-1 is a prerequisite event for oxidative death of chondrogenic ATDC5 cells induced by interleukin-1β.

M. TANAKA[1], Y. MIYAMOTO[1], K. YOSHIMURA[1], K. SASA[1], K. IKEZAKI[1,2], T. SHIROTA[2], R. KAMIJO[1]
[1]Department of Biochemistry, Showa University School of Dentistry, Tokyo, Japan, [2]Department of Oral and Maxillofacial Surgery, Showa University School of Dentistry, Tokyo, Japan

Pediatric Oral Health Research

060: Effects of Anticancer Drugs on Mouse Tooth Germ Development

Y. TSUNODA[1], S. NAKA[1], R. OKAWA[2], K. NAKANO[2], M. MATSUMOTO-NAKANO[1]
[1]Department of Pediatric Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan, [2]Department of Pediatric Dentistry, Division of Oral Infection and Disease Control, Osaka University Graduate School of Dentistry, Suita, Osaka, Japan

Mineralized Tissue

061: Trehalose, a natural sweetening compound, suppresses osteoclast differentiation.

S. NEGISHI, K. YOSHIMURA, R. KAMIJO
Department of Biochemistry, Showa University School of Dentistry

062: Computational chemistry of phospholipid mineralization

E. S. HARA[1], N. KUNIOSHI[2], M. OKADA[1], T. MATSUMOTO[1]
[1]Department of Biomaterials, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, [2]Department of Materials Science, Graduate School of Fundamental Science and Engineering, Waseda University, Tokyo, Japan

063: Histological study of dentin bridge formed beneath MTA after pulpotomies in rat molars

A. TANAKA, T. TANASE, K. KOJIMA, H. HOMMA, A. SAKURAI, S. SHINTANI
Department of Pediatric Dentistry, Tokyo Dental College, Tokyo, Japan

Implantology Research

064: The effect of micro-sized titanium on osteoblast differentiation

N. OMORI, H. NAKATA, S. KURODA
Department of Oral Implantology and Regenerative Dental Medicine, Division of Oral Health Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University

Dental Materials 5: Biocompatibility, Bioengineering and Biologic Effects of Materials

065: Comparison in physical properties of synthetic carbonate apatite and allogeneic bone graft substitute

N. KIMURA, S. KATO, E. ARIMA, N. KITAMURA, K. YAMANAKA, F. FUSEJIMA
GC Corporation, Tokyo, Japan

066: Novel bio-active adhesive monomer CMET stimulates human dental pulp stem cells differentiation toward odontoblast phenotype: a comparative study

Y.J. QIU, T. SAITO

Division of Clinical Cariology and Endodontology, Department of Oral Rehabilitation, Health Sciences University of Hokkaido, Hokkaido, Japan

067: Hydrogel-based biomechanical environment for understanding Meckel's cartilage fate

M. FARAHAT[1], G. A. S. KAZI[2], E.S. HARA[1], T. MATSUMOTO[1]
[1]Department of Biomaterials, Okayama University, Okayama, Japan, [2]Department of Applied Life Systems Engineering, Graduate School of Science and Engineering, Yamagata University

Diagnostic Sciences

068: Evaluation of Interleukin – 8 In Human Dental Pulp

S. TALWAR[1], R.R. NAWAL[1], A. KAUSHIK[1], R. KONER[2]
[1]CONSERVATIVE DENTISTRY AND ENDODONTICS, MAULANA AZAD INSTITUTE OF DENTAL SCIENCES, New Delhi, DELHI, INDIA, [2]BIOCHEMISTRY, MAULANA AZAD MEDICAL COLLEGE, NEW DELHI, DELHI, INDIA

Pulp Biology & Regeneration Research

069: Interaction analysis of protein S100A7 in dental pulp tissue

M. WATANABE, M. OKAMOTO, H. HAILING, S. MATSUMOTO, K. MORIYAMA, Y. TAKAHASHI, M. HAYASHI
dentistry, Osaka University, Suita, Japan

070: Lyve-1+ Pulpal Macrophages: M2-polarization and Response to Cavity Preparation

K. THOAI, K. TAZAWA, K. NARA, M. FUJII, K. HASHIMOTO, S. NODA, N. KAWASHIMA, T. OKIJI
Department of Pulp Biology and Endodontics, Tokyo Medical and Dental University, Tokyo, Japan

071: DNA methylation of *GJA1*, *BMP2* and *BMP4* in a human cementoblast cell line induced by lipopolysaccharide.

T. MURAMATSU[1], F. KOBAYASHI[2], O. UEHARA[3], C. ITO[4], M. FURUSAWA[2], Y. ABIKO[5]
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072: Effects of Non-thermal atmospheric pressure plasma on human deciduous dental pulp fibroblast-like cells

M. OKUNO[1], K. HARADA[2], S. AOKI[1], S. KAWAI[2], K. ARITA[2]
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Dental Materials 5: Biocompatibility, Bioengineering and Biologic Effects of Materials

073: Effect of Lithium Carbonate on the Healing of Apical Periodontitis

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Pulp Biology & Regeneration Research

074: Large-conductance Ca^{2+}-activated K^+ Channels in Human Cementoblasts

S. KAMATA, M. KIMURA, Y. SHIBUKAWA, S. YAMASHITA
Tokyo Dental College

075: Activation of CGRP receptors increased intracellular cAMP level in odontoblasts

N. SAITO, M. KIMURA, H. MOCHIZUKI, K. KOUNO, M. ANDO, S. OHYAMA, T. ICHINOHE, Y. SHIBUKAWA
Tokyo Dental College, Tokyo, Japan

076: Plasma membrane Ca2+-ATPase regulates dentin formation and mineralization.

H. MOCHIZUKI, M. KIMURA, S. OHYAMA, K. KOUNO, M. ANDO, Y. SHIBUKAWA
Tokyo Dental College

Microbiology/Immunology

077: Functional expression of mechanosensitive ion channel in mouse osteoblasts

S. NAGAI[1], K. KITAMURA[3], M. KIMURA[2], Y. SHIBUKAWA[2], H. YAMAMOTO[3], A. KATAKURA[1]
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Pharmacology/Therapeutics/Toxicology

078: Activation of mechano-sensitive ion channels in cancer cells establishes paracrine network via endothelin signaling

M. ISHIZAKI[1], M. MATSUNAGA[1], T. YAZAKI[1], N. SAITOH[1], S. OHYAMA[2], M. KIMURA[2], Y. SHIBUKAWA[2], T. ICHINOHE[1]
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Neuroscience

079: Mechanical stimulation-induced intercellular communication in trigeminal ganglion neurons

T. YAZAKI[1], M. ISHIZAKI[1], M. MATSUNAGA[1], S. OHYAMA[2], H. KURODA[3], M. KIMURA[2], Y. SHIBUKAWA[2], T. ICHINOHE[1,2]
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080: Piezo1 channel activation evokes mechanosensitive Ca^{2+} signaling in human odontoblast

M. MATSUNAGA[1], M. KIMURA[2], M. ISHIZAKI[1,2], T. YAZAKI[1,2], S. OHYAMA[2], Y. SHIBUKAWA[2], T. ICHINOHE[1]
[1]Dental Anesthesia, Tokyo Dental College, [2]Physiology, Tokyo Dental College

Oral & Maxillofacial Surgery Research

081: Effects of preemptive analgesia with intravenous acetaminophen on postoperat ive pain relief in patients undergoing third molar surgery: a prospective, single-blind, randomized controlled trial

K. KANO[1,2], K. KAWAMURA[3], T. MIYAKE[3]
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Diagnostic Sciences

082: Diagnostic and Prognostic Applications of Artificial Intelligence-based Radiology in Head and Neck Region

M. Poorsattar[1], A. Poursattar Bejehmir[2]
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e-Oral Health Network

083: Revolutionalized Concept of Digitalizing Dental Treatment Contents using Machine Learning

S. OKA[1], K. NOZAKI[2], M. HAYASHI[1]
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Education Research

084: Evaluation of simulation training courses on basic clinical skills for geriatric dentistry for trainee dentists

H. TAKETA[1], T. YOSHIDA[2], A. YABE[1], N. SHIOTSU[1], T. KONO[1], H. SHIRAI[1], Y. TORII[1]
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Behavioral, Epidemiologic and Health Services Research

085: Needs Of Dentists During The Covid-19 Pandemic In Nepal

Y. HARADA[1], D. PRAJAPATI[2], H. IWASHITA[1], T. SUGISHITA[1]
[1]International Affairs and Tropical Medicine, Tokyo Women's Medical University, [2]Community and Public Health Dentistry, Dhulikhel Hospital, Kathmandu University School of Medical Science

086: Oral conditions matter for general health of community-dwelling elderly population

Y. NAKAI[1], Y. NAGATANI[1], M. OZAKI[2], K. OHUCHI[2], H. KINOSHITA[2], A. HIRANO[2], T. YANAGAWA[2]
[1]Department of Dental Hygiene, University of Shizuoka Junior College, Shizuoka, Japan, [2]Shizuoka Dental Association, Shizuoka, Japan

Oral Health Research

087: Comparative study of plaque removal effect on Japanese commercial toothbrushes.

C. HSU, K. SHINADA
Department of Preventive Oral Health Care Sciences, Tokyo Medical and Dental University, Tokyo, Japan

088: Comparing the oral health-related quality of life of people with diabetes and people without diabetes at a Tertiary Hospital in Nigeria

N. A. Ilochonwu
Preventive Dentistry, University of Port Harcourt, Choba, Port Harcourt, Rivers State, NIGERIA

Prosthodontics Research

089: Survey Of Smile Satisfaction And Desired Treatments Among Medical And Dental Students.

T. Khatri[1], S. Butt[2], A. Tufail[1], R. Fatima[1], Z. Irfan[1]
[1]University of Health Sciences, Karachi, Sindh, PAKISTAN, [2]University, Karachi, Sindh, PAKISTAN

KEYNOTE LECTURE

Keynote Lecture
Video On-demand distribution

KL

Porphyromonas gingivalis interactions with epithelial cells in the community context

Richard J. Lamont, Ph. D.
Department Oral Immunology & Infectious Diseases, University of Louisville, School of Dentistry

Oral epithelial cells are central to the maintenance of homeostasis in the face of a diverse and abundant microbial challenge. Bacteria with pathogenic potential, such as *Porphyromonas gingivalis*, induce dysbiotic responses in oral epithelial cells which form the basis for diseases such as periodontitis and, as is becoming increasingly recognized, oral squamous cell carcinoma (OSCC). Virulence of *P. gingivalis*, however, is expressed in the context of heterotypic microbial communities which are comprised predominantly of organisms generally considered commensal, such as the oral streptococci. A major unanswered question therefore, is the extent to which organisms that are commensal in an individual context can potentiate or mitigate pathogenicity when in combination with *P. gingivalis*. We have used RNA-Seq to define gingival epithelial cell pathways regulated in response to *P. gingivalis* alone, but restored toward homeostasis by *S. gordonii.* Such pathways included those involved in proliferation, migration, epithelial mesenchymal transition (EMT), apoptosis, and inflammation; and many of these events revolved around the FOXO1-ZEB2 signaling axis. *P. gingivalis* activated and increased nuclear retention of FOXO1 by dephosphorylation of serine residues, and FOXO1 in turn controlled the production of anti-apoptotic factors such as Bcl-6, and inflammatory mediators including several CXCL family chemokines. FOXO1 also bound to the promoter region of the *ZEB2* gene and increased transcriptional activity. Enhanced production of ZEB2 induced a partial EMT and stimulated the migration of gingival epithelial cells. In the presence of *S. gordonii*, however, there was phosphorylation and activation of the TAK1-NLK pathway. Activated NLK was capable of phosphorylating FOXO1, and thus suppressed activation by *P. gingivalis*. Consistent with this, *S. gordonii* prevented *P. gingivalis* from enhancing epithelial cell migration, proliferation, EMT, resistance to apoptosis and production of inflammatory mediators. The results suggest that on epithelial surfaces a relative decrease in the proportion of *S. gordonii* to *P. gingivalis* would promote phonotypes which are hallmarks of cancer. Conversely, an increase in the relative abundance of *S. gordonii* will inhibit the FOXO1-ZEB2 regulatory axis and disrupt host responses in periodontal tissues.

Brief CV

Professor Richard Lamont graduated with a BSc (Honours) from the University of Edinburgh in 1982 and completed his PhD in Bacteriology from the University of Aberdeen in 1985. He has held academic appointments in various universities in the USA, and currently holds an Endowed Professor and Chair in the Department of Oral Immunology and Infectious diseases at the University of Louisville. He is also a recipient of various awards among which are IADR Distinguished Scientist Awards (1995, 2006), the MERIT award from NIH, and the University of Louisville President's Award for distinguished research (2016). He is a prolific researcher and is currently a principal investigator on 5 grants from NIH, and also an investigator or mentor in 3 other active grants. He has authored over 200 publications in peer-reviewed journals and 5 books. He has been editor in chief of the journal Molecular Oral Microbiology since 2015.

GREETING

Greeting from IADR President
Video On-demand distribution

G

Pamela Den Besten, D.D.S., Ph. D
Professor in the Department of Orofacial Sciences, School of Dentistry,
University of California San Francisco

Pamela Den Besten is Professor in the Department of Orofacial Sciences, School of Dentistry, University of California San Francisco. She directs the Center for Children's Oral Health Research, and co-directs the DDS-PhD and PhD programs in Oral and Craniofacial Sciences within the School of Dentistry and the Graduate Division. Dr. Den Besten is President of the International Association for Dental Research (IADR). She is past Chair of the American Association for the Advancement of Science (AAAS) Section on Dentistry & Oral Health Sciences. In 2009 Dr. Den Besten received the IADR Distinguished Scientist Award in Pulp Biology and Regeneration. She is an AAAS honorary Fellow. Dr. Den Besten has published over 125 scientific manuscripts in peer-reviewed journals, along with 18 book chapters. Her research interests are focused on tooth formation, and in particular enamel and dentin regeneration and biomineralization. She is an international leader in enamel fluorosis research, and studies environmental effects on tooth formation.

SPECIAL LECTURE

Special Lecture
Video On-demand distribution

SL

Tubular Dentin Regeneration and Its Clinical Application

Joo-Cheol Park, D.D.S., Ph. D
Laboratory for the Study of Regenerative Dental Medicine,
Department of Oral Histology-Developmental Biology,
School of Dentistry, Seoul National University, Seoul, Korea

A healthy tooth is composed of nearly 70% dentin enclosing the entire dental pulp, which is a pool of diverse stem cells. Loss of dentin not only generates unpleasant pain but also ultimately leads to the weakening of whole tooth stability due to reduced dentin thickness. Regenerative dental medicine has rapidly progressed since the advancement of stem cell biology and material science. However, more emphasis has been placed on the success of tissue formation than on how well the newly generated tissue retains the original organ structure and function. Based on the knowledge that epithelial-mesenchymal interaction is essential for tooth development, we previously discovered a dental epithelium-derived protein called Copine 7 (CPNE7). CPNE7 is an evolutionarily conserved, calcium-dependent phospholipid-binding protein and consists of two C2 domains in the N terminus and a von Willebrand factor A domain in the C terminus. Secreted from pre-ameloblasts, CPNE7 induces odontoblast differentiation *in vitro* and promotes dentin formation *ex vivo*. As a result, CPNE7 was suggested as a new molecule with the potential to diffuse across the dentin and induce tertiary dentinogenesis. The fact that recombinant CPNE7 is a cell-derived soluble bioactive molecule makes it a promising candidate for use in regenerative dental medicine. Moreover, a synthetic CPNE7-derived oligopeptide, Cpne7-DP, was developed which induces odontoblast differentiation *in vitro* and physiologic dentin formation *in vivo*. Comprehensive evaluation of Cpne7-DP further validated its potential as a bioactive therapeutic agent. Our results suggest that the dual functions of Cpne7-DP in tubular dentin formation and peritubular space occlusion are promising for oral disease-targeted application, especially those involving dentinal loss and sensitivity.

Brief CV

Name:

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Appointment:

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Citizenship: Korean

Education and Training:

1981.03-1987.02	D. D. S. Chosun University, College of Dentistry, Kwang-ju, Korea
1987.09-1989.08	M. S. Chosun University, Graduate School, Kwang-ju, Korea
1987.03-1990.02	Internship and Residency (Oral Pathology) Chosun University, College of Dentistry, Kwang-ju, Korea
1994.03-1996.08	Doctoral Course (equivalent to Ph. D., Oral Anatomy) Seoul National University, Graduate School, Seoul, Korea

Academic Appointments and Career:

1990.02-1993.04	Military service in Korean Army
1997.02-1999.01	Postdoctoral research Fellow (supported by Tokyo Biochemical Research Foundation), Department of Biochemistry & Molecular Biology, Okayama Medical School, Okayama, Japan
1995.05-2007.07	Instructor, Assistant professor, Associate professor and Professor, Department of Oral Histology, College of Dentistry, Chosun University. Gwang-ju, Korea
2003.06-2004.08	Research Associate Professor, Department of Oral Biology, SUNY at Buffalo, NY, USA
2013.01- 2014.12	Associate Dean for Research affairs, School of Dentistry, Seoul National University. Seoul, Korea
2017.02-2019.02	Director, Dental Research Institute, Seoul National University
2007.08-	Associate professor and Professor, Department of Oral Histology-Developmental Biology, School of Dentistry, Seoul National University. Seoul, Korea
2016.07-	Founder & CEO, HysensBio Co., Ltd.
2020.10-	President of KADR

Major Research Interest:

(1) Control of odontoblast differentiation and dentin formation
(2) Nuclear factor I-C and mineralized tissue homeostasis
(3) A periodontitis biomarker, ODAM and junctional epithelium
(4) Dental stem cells and periodontal regeneration

References

Park YH, Lee YS, Seo YM, Seo H, Park JS, Bae HS, Park JC. Midkine promotes odontoblast-like differentiation and tertiary dentin formation. **J Dent Res**. 99(9):1082-1-91, 2020.

Park SH, Lee YS, Lee DS, Park JC, Kim R, Shon WJ. CPNE7 induces biological dentin sealing in a dentin hypersensitivity model. **J Dent Res**. 98(11), 1239-1244, 2019.

Seo YM, Park SJ, Lee HK, Park JC. Copine-7 binds to the cell surface receptor, nucleolin, and regulates ciliogenesis and Dspp expression during odontoblast differentiation. **Sci Rep**. 7(1):11283, 2017.

Choung HW, Lee J-H, Shon WJ, Lee JH, Y. Ku, Park JC. Tertiary dentin formation after indirect pulp capping using protein CPNE7. **J Dent Res**. 95(8):906-912, 2016.

Oh HJ, Choung HW, Lee HK, Park SJ, Lee JH, Lee DS, Seo BM, Park JC. CPNE7, a preameloblast-derived factor, regulates odontoblastic differentiation of mesenchymal stem cells. **Biomaterials** 37:208-217, 2015.

Lee JH, Lee DS, Choung HW, Shon WJ, Seo BM, Lee EH, Cho JY, Park JC. Odontogenic differentiation of human dental pulp stem cells induced by preameloblast-derived factors. **Biomaterials** 32(36):9696-9706, 2011.

SYMPOSIUM

Symposium Ⅰ :
At the front-line: Etiology of periodontitis

Symposium Ⅱ:
Future is now! Stem cell revolution in hard and soft tissue engineering

Symposium Ⅲ:
Pathophysiological approach from oral function to systemic diseases

Rising Scientist Session
Neuroscience

Symposium I
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 7 (Sat.) 10:20-10:30

SI-1

Bacterial composition of overall oral microbiota associated with periodontal disease and health.

Toru Takeshita, D.D.S., Ph. D
Section of Preventive and Public Health Dentistry, Faculty of Dental Science, Kyushu University

Oral cavity is colonized by numerous and diverse commensal microorganisms, which constitute complex microbial communities on various intraoral surfaces. Of them, plaque microbiota that forms on the tooth surfaces and gingival crevices are the cause of one of two major oral diseases, or periodontitis. Currently, little doubt exists that three subgingival plaque bacteria including *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* are prime suspects in periodontitis. Furthermore, recent studies using open-ended molecular approaches and the 16S rRNA gene have identified additional species, including *Filifactor alosis* as the periodontitis-associated taxa. Nevertheless, the bacterial etiology of periodontitis remains unclear, because the onset and progression of periodontitis cannot be simply explained by presence or absence of these taxa.

To obtain deeper insights into oral microbiota composition associated with periodontal disease and health, we conducted a large-scale population-based study of oral microbiota composition using saliva. An important finding was that high bacterial richness in the microbiota constituted by various minority bacteria mainly derived from subgingival sites was significantly associated with gingival inflammation and periodontitis. Another important result was that relative abundances of predominant commensals were significantly associated with the health conditions. Of the two different cohabiting groups of predominant commensals mainly derived from tongue dorsum, a greater relative abundance of group II bacteria such as *Neisseria flavescens* and *Porphyromonas pasteri*, and *Fusobacterium periodonticum* was associated with better health conditions. We introduce our finding on the overall structure of the oral microbiota associated with gingival health obtained by our molecular epidemiological studies.

Brief CV

Toru Takeshita
Section of Preventive and Public Health Dentistry, Faculty of Dental Science, Kyushu University

Education

Undergraduate 1999-2005 Kyushu University, Faculty of Dentistry, D.D.S.
Graduate 2005-2009 Kyushu University Graduate School of Dental Science, Ph.D.

Research Appointment

2007-2009 JSPS Research Fellowship for Young Scientists
2009-2013 Assistant Professor, Kyushu University
2013-current Associate Professor, Kyushu University

Award

2014 Japanese Society of Oral Health “LION AWARD”

Symposium I
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 7 (Sat.)　10:50-11:00

SI-2

The significant role of *Porphyromonas gingivalis* "Gingipains" as a virulence factor on periodontal inflammation

Masaaki Nakayama, Ph. D, Naoya Ohara
1) Department of Microbiology, Graduate school of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, and Advanced Research Center for Oral and Craniofacial Sciences (ARCOCS), Dental School, Okayama Universityl

Periodontal disease is an infectious and inflammatory disease in periodontal tissues, and is also considered to link with systemic diseases such as diabetes, vascular and cardiovascular diseases. *Porphyromonas gingivalis* is well known as one of high-risk pathogens, and its persistent infection may contribute to the destruction of periodontal tissues, eventually also affect the systemic diseases. Periodontal disease has proinflammatory mediators including interleukin-6, TNFα, and prostaglandins (PGs). In our recent research, we have focused on the function of cysteine proteases "gingipains", which are the virulence factors from *P. gingivalis*. and studied the relationship between gingipains and PGE2 production in *P. gingivalis* infection. In this study, human monocytes were challenged with wild-type *P. gingivalis* (wtPg) and gingipains-deficient mutant strain (mtPg) to investigate molecular characterizations of the cellular signaling caused by the host cell-pathogen interactions. We found that gingipains induced COX-2 expression and PGE2 production via activation of MEK/ERK/AP-1 (c-fos/c-Jun) and IKK/NF-κBp65, and their proteolytic activity is crucial to cause these events. Furthermore, we examined the upstream pathways leading to MEK/ERK/AP-1 and IKK/NF-κBp65, and then found the involvement of calcium influx and activation of TLR4 signaling in gingipains-induced signaling pathways for COX-2 expression. It is likely that COX-2 expression/PGE2 production induced by gingipains has several interactions and signaling pathways in the monocytes infected with *P. gingivalis*. Taken together, our study provides the significant role of *P. gingivalis* gingipains in periodontal inflammation.

Brief CV

Masaaki Nakayama
Department of Microbiology, Graduate school of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University

Education:

Undergraduate 1996-2000 Nagasaki University, B.S.
Postgraduate 2000-2002 Nagasaki University, M.S.
Graduate 2002-2006 Nagasaki University, Ph.D.

Research Appointment

2007-2008	Post-doctral fellow, The Institute of Tropical Medicine, Nagasaki University
2008-2010	Post-doctral fellow, National University of Singapore, Cancer Science Institute of Singapore, Singapore
2010-current	Assistant professor, Graduate school of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University

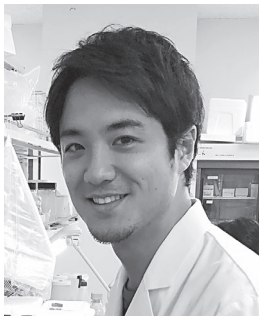
Awards

2015	The 35th Ryobi Teien Memory Fundation Award
2016	The 58th Japanese Association for Oral Biology, Rising Members Award
2016	The 69th Japanese Society for Bacteriology, Chugoku-Shikoku branch, Young Researcher Encouraging Award

Symposium I
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 7 (Sat.) 11:20-11:30

SI-3

Title: Periodontitis as an “osteoimmune” disease



Masayuki Tsukasaki, D.D.S., Ph. D.
Department of Immunology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo

Periodontitis, one of the most common infectious diseases in humans, is a unique “osteoimmune” disease in which antibacterial immune response causes alveolar bone destruction. In 1972, Horton et al. reported that immune cells stimulated by the dental plaque derived from periodontitis patients produce osteoclast-activating factors, providing the first evidence for the osteoimmune dialogue (Horton et al., ***Science*** 1972). However, although the pioneering work in the field of osteoimmunology was conducted in the context of periodontitis, the mechanisms and the role of osteoimmune interaction in periodontitis have been largely unclear until fairly recently.

Using a newly established murine periodontitis model, we showed that the crosstalk between immune and bone cells at the oral barrier plays a critical role in the pathogenesis of periodontitis. Bacterial invasion leads to the generation of specialized immune cells that protect against bacteria by evoking mucosal immune responses as well as inducing bone damage, the latter of which may also contribute to the host defense by removing the infected-tooth. These findings suggest that inflammatory bone destruction, which has been regarded merely as an adverse secondary effect of inflammation, may have evolved as a part of host defense machinery against oral microbiota (Tsukasaki et al., ***Nature Commun*** 2018, ***Nature Rev Immunol*** 2019).

To understand the pathogenesis of and develop future therapeutic strategies for periodontal bone loss, it is vitally important to clarify precise molecular mechanisms underlying osteoclastic bone erosion. Recently, we unveiled the importance of the tight regulation of RANKL activity by the local OPG production in bone and immune systems by generating OPG-floxed mice (Tsukasaki et al., ***Cell Rep*** in press), and deciphered the stepwise cell fate decision pathways during osteoclastogenesis at single-cell resolution (Tsukasaki et al., ***Nature Metabolism*** in revision). In this talk, I will summarize the recent progress of the osteoimmunology field and provide a new perspective on the periodontology from the osteoimmunological standpoint.

Brief CV

Masayuki Tsukasaki
Department of Immunology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo

Education

2013	Graduated from the Showa University School of Dentistry, D.D.S.
2018	Graduated from the Graduate School of Medicine, the University of Tokyo, Ph.D

Research Appointment

2014-2018	Graduate Program for Leaders in Life Innovation (GPLLI), the University of Tokyo
2015-2018	Research Fellow of the Japan Society for the Promotion of Science (DC1)
2018-	Research Fellow of the Japan Society for the Promotion of Science (PD)

Awards

2020	The Japanese Society for Bone and Mineral Research, Rising Stars Grant
2020	The 5th Winter seminar of Japanese Society of Ostoimmunology, Best research award
2019	The Japanese Society for Bone and Mineral Research, 4th Skeletal Science Retreat, Best discussion award
2019	Japanese Association for Oral Biology, Young investigator award
2019	The Oral Medical Science Frontier, Young investigator award
2019	The 47th Annual Meeting of the Japanese Society for Immunology, Best presentation award
2019	The 4th Winter seminar of Japanese Society of Ostoimmunology, Best research award
2018	7th International Conference on Osteoimmunology Travel Award
2018	Japanese Society for Immunology, Tadamitsu Kishimoto International Travel Award
2017	The Advanced Dentistry School, Best research award
2017	The 2nd Winter seminar of Japanese Society of Ostoimmunology, Best research award
2016	The Thailand International Conference on Oral Biology (TICOB) 2016, Best presentation award
2016	6th International Conference on Osteoimmunology, Travel Award
2016	The 1st Winter seminar of Japanese Society of Ostoimmunology, Best research award
2013	Showa University, Kamijo Prize
2012	Japan Student Services Organization (JASSO), Student of the Year, First Prize

Symposium I
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 7 (Sat.) 11:50-12:00

SI-4

Epithelial barrier breakdown by *Porphyromonas gingivalis*

Atsuo Amano, D.D.S., Ph. D. and Hiroki Takeuchi
Department of Preventive Dentistry, Graduate School of Dentistry, Osaka University

Porphyromonas gingivalis is one of keystone pathogens in severe and chronic manifestations of periodontal diseases. A central feature of *P. gingivalis* pathogenicity is dysregulation of innate immunity at the gingival epithelial interface; however, the molecular basis underlying *P. gingivalis*–dependent abrogation of epithelial barrier function remains unknown. Gingival epithelial cells express junctional adhesion molecule (JAM1), a tight junction–associated protein, and JAM1 homodimers regulate epithelial barrier function. We showed that Arg-specific or Lys-specific cysteine proteases (gingipains) secreted by *P. gingivalis* specifically degraded JAM1 at K134 and R234 in gingival epithelial cells, resulting in permeability of the gingival epithelium to 40 kDa dextran, lipopolysaccharide (LPS), and proteoglycan (PGN). A *P. gingivalis* strain lacking gingipains was impaired in degradation of JAM1.

Next, we employed a three-dimensional (3D) multilayered tissue model using epithelial cells coated with fibronectin/gelatin nano-films. Knockdown of JAM1 in monolayer cells and 3D multilayered tissue model also increased permeability to LPS, PGN, and gingipains. Inversely, overexpression of JAM1 in epithelial cells prevented penetration by these agents following *P. gingivalis* infection. Our findings strongly suggest that *P. gingivalis* gingipains disrupt barrier function of stratified squamous epithelium via degradation of JAM1, allowing bacterial virulence factors to penetrate into subepithelial tissues.

If circumstances warrant, we may be able to show the data indicating that *P. gingivalis* breaks through the epithelial barrier using an intercellular, rather than intracellular, pathway to get into the deeper tissues.

Brief CV

Atsuo Amano, D.D.S., Ph.D.
Professor and Chair
Department of Preventive Dentistry, Graduate School of Dentistry, Osaka University

Education and Degree

1984	D.D.S., School of Dentistry, Osaka University
1990	Ph.D., Graduate School of Dentistry, Osaka University

Professional Career

2015 - 2018	Dean, Graduate School of Dentistry and School of Dentistry, Osaka University
2011 - Present	Professor and Chair, Department of Preventive Dentistry, Graduate School of Dentistry, Osaka University
2000 - 2011	Professor and Chair, Department of Oral Frontier Biology, Graduate School of Dentistry, Osaka University
1997 - 2000	Associate Professor, Division of Special Care Dentistry, Osaka University Dental Hospital
1992 - 1994	Postdoctoral fellow, Department of Oral Biology, School of Dental Medicine, State University of New York at Buffalo
1987 - 1997	Assistant Professor, Department of Preventive Dentistry, School of Dentistry, Osaka University

Honors

1998	Yumikura Award in Dental Research of Osaka University
2001	Lion Science Award of Japanese Society of Periodontology
2015	President Award of Osaka University

Symposium II
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 8 (Sun.) 10:20-10:30

SII-1

Generation of skeletal cells from pluripotent stem cells

Shinsuke Ohba, D.D.S., Ph. D.
Department of Cell Biology, Institute of Biomedical Sciences, Nagasaki University

Pluripotent stem cell (PSC)-based differentiation systems are a promising tool for *in vitro* mechanistic studies of developmental processes, disease modeling, drug screening, and stem cell-based therapies. Recent understanding of signaling pathways regulating developmental processes provides us with clues for appropriate inducers for PSC differentiation. We have developed several strategies to generate skeletal cells from mouse and human PSCs under fully defined conditions (*Stem Cell Reports*, 2014; *Sci Adv*, 2017; *Regen Ther*, 2020), based on our previous findings on the Hedgehog signaling-mediated specification of bone-forming cells (*Development*, 2008; *Dev Cell*, 2008; *J Biol Chem*, 2012; *J Biol Chem*, 2013) and canonical Wnt signaling-directed control of pluripotency and differentiation of PSCs (*Stem Cells*, 2013). Our strategies are basically composed of three phases: maintenance of PSCs, induction of mesoderm and skeletal progenitors, and differentiation of the progenitor population into skeletal cells. Induction of mesoderm and skeletal progenitors is achieved *in vitro* under fully defined conditions, where activities of developmentally critical signals are manipulated by small molecules. We have found that the PSC-derived progenitor cells give rise to osteogenic and/or chondrogenic population under appropriate conditions. Thus, the strategies will be a novel platform for biological and pathological studies of skeletal development, screening of therapeutic drugs for the treatment of degenerative skeletal disorders, and regeneration of skeletal tissues.

Brief CV

Shinsuke Ohba
Department of Cell Biology, Institute of Biomedical Sciences, Nagasaki University

Education

2006	Ph.D., Graduate School of Medicine, University of Tokyo
2001	D.D.S., School of Dentistry, Tohoku University

Research and professional experience

2019-	Professor, Department of Cell Biology, Nagasaki University
2017-2019	Associate Professor, Department of Clinical Biotechnology, University of Tokyo
2013-2017	Project Associate Professor, Department of Bioengineering, University of Tokyo
2010-2013	Project Assistant Professor, Department of Clinical Biotechnology, University of Tokyo
2008-2010	Postdoctoral Fellow, Department of Molecular and Cellular Biology, Harvard University
2005-2007	JSPS Research Fellow, Department of Tissue Engineering, University of Tokyo Hospital
2002-2006	Graduate student, Graduate School of Medicine, University of Tokyo
2001-2002	Resident in Oral and Maxillofacial Surgery, University of Tokyo Hospital

Awards

2019	Invited Speaker, 2019 Gordon Research Conference Cartilage Biology and Pathology
2018	JSBMR Research Encouragement Award, Japan Society for Bone and Mineral Research
2017	Paper Award, Japanese Society of Cartilage Metabolism
2016	ASBMR Rising Star Award, American Society for Bone and Mineral Research
2011	Invited Speaker, 2011 Gordon Research Conference Cartilage Biology and Pathology
2005	Outstanding Paper Award, 23th Annual Meeting of Japan Society for Bone and Mineral Research
2004	Outstanding Paper Award, 22th Annual Meeting of Japan Society for Bone and Mineral Research
2004	Award for Excellence, 6th Asian Congress on Oral and Maxillofacial Surgery
2001	DENTSPLY Merit Award, DENTSPLY International Inc.

Symposium II
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 8 (Sun.) 10:50-11:00

SII-2

Establishment of an *in vitro* culture system for tenogenic/ligamentogenic differentiation using *ScxGFP* iPS cells

Chisa Shukunami, D.D.S., Ph. D.
Department of Molecular Biology and Biochemistry, Graduate School of Biomedical and Health Sciences, Hiroshima University

Tendons connect muscles to the skeletal components and function as the mechanical force transmitters, while ligaments bind adjacent bones together to stabilize joints. Cells in tendons and ligaments are specialized fibroblasts known as tenocytes and ligamentocytes. Tenocytes and ligamentocytes are derived from progenitor cells that express the basic helix-loop-helix transcription factor Scleraxis (Scx). A certain population of Scx^+ cells also express *Sry-box 9* (*Sox9*). These $Scx^+/Sox9^+$ cells differentiate into tenocytes and ligamentocytes as well as chondrocytes that contribute to the formation of attachment sites of tendons/ligaments to bone, entheses. Loss of function analysis of *Scx* revealed that *Scx* is required for maturation of tendons/ligaments and the entheses. So far, little is known about the molecular mechanism underlying tenogenic and ligamentogenic lineage commitment and differentiation due to a lack of suitable cell culture system. In this study, we established an *in vitro* culture system that can monitor tenocyte and ligamentocyte differentiation by live imaging using green fluorescence. *ScxGFP* iPS cells were established from fibroblasts isolated from *ScxGFP* transgenic mouse embryos that express *EGFP* in the *Scx*-expressing region during development. We generated chimeric mice to confirm that *ScxGFP* iPS derived cells express green fluorescence upon differentiation into the tenogenic and ligamentogenic cells. We then explored the culture conditions for differentiation of *ScxGFP* iPS cells into tendon/ligament cells monitoring GFP expression. Under optimal conditions, *ScxGFP* iPS cells are differentiated into mesenchymal progenitor cells and then into Scx^+ cells expressing GFP. Scx^+ cells mature to express *tenomodulin* and *Mohawk* at higher levels than tenocytes isolated from limb tendons. Most of Scx^+ cells express Sox9, but *Sox9* expression in $Scx^+/Sox9^+$ cells decreased in association with tenocyte and ligamentocyte differentiation. Thus, taking advantage of *ScxGFP* iPS cells, we have successfully established the culture system that recapitulates the process of tendon and ligament formation *in vivo*.

Brief CV

Chisa Shukunami, DDS, PhD
Department of Molecular Biology and Biochemistry, Graduate School of Biomedical and Health Sciences, Hiroshima University

Education

1985-1991	Undergraduate, Faculty of Dentistry, Hiroshima University
1991-1995	Graduate, Graduate School of Dentistry, Osaka University

Research Appointment

1994-1995	Japan Society for the Promotion of Science (JSPS) fellow (DC2)
1995-1998	JSPS fellow (PD)
1998-2013	Associate Professor, Kyoto University
1999	Human Frontier Science Program short-term fellow, GSF-National Research Center for Environment and Health, Germany
2013-current	Professor, Hiroshima University

Awards

1995	The 3rd Pan Pacific Connective Tissue Societies Symposium Young Investigator Award
2007	Otaka Award, Japanese Society for Matrix Biology and Medicine

Symposium II
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 8 (Sun.) 11:20-11:30

SII-3

Classification and pathological mechanisms of Craniosynostosis based on the differentiation pattern of iPS cells

Sachiko Iseki, D.D.S., Ph. D.
Section of Molecular Craniofacial Embryology, Tokyo Medical & Dental University Graduate School of Medical and Dental Sciences

Craniosynostosis is defined as premature closure of one or more cranial sutures. It is suggested that the condition is induced by accelerated ossification leading to craniofacial skeletal deformities and compromise of cranial space for growing brain. Although it is a multifactorial disease with several risk factors including increased intracranial pressure, the involvement of genetic factors is strongly suggested. The current treatment is mainly surgical resection, and repeated surgeries are often required due to resynostosis. Therefore, prediction of clinical condition is important for treatment planning. However, currently reported gene mutations in craniosynostosis patients explain only 20% of all craniosynostosis cases.

The ability to predict the pathological transition not only facilitates the planning of treatment strategies but also the development of new therapies. Since it is currently impossible to classify patients based on genetic factors alone, we hypothesized that the expression pattern of osteogenic markers and craniosynostosis associated genes in osteobalsts could be used to classify craniosynostosis patients.

Cranial bone-derived osteoblast ossification capacity is known to change with age, therefore It is not easy to compare the pattern of expression of osteogenic markers among patient-derived osteoblasts and control osteoblasts directly.

It has been reported that iPS cells maintain an epigenetic state character from which they are derived, therefore they are prone to easily differentiate into the original cell type.

Using this feature, we will establish a system to establish patient osteoblast-derived iPS cells and redifferentiate them into osteoblasts to compare the expression patterns of osteogenic markers for patient classification.

Brief CV

Sachiko Iseki
Section of Molecular Craniofacial Embryology, Tokyo Medical & Dental University Graduate School of Medical and Dental Sciences

Educations

Undergraduate: 1983-1989 Tokyo Medical and Dental University, Faculty of Dentistry, D.D.S.
Graduate School: 1989-1993 Tokyo Medical and Dental University, Faculty of Dentistry, Ph. D

Research Appointment

Apr 1993	Research assistant, Department of Developmental Biology, Tokyo Medical and Dental University, Japan
May 1993-Mar 1994	Research Associate, Department of Developmental Biology, Tokyo Medical and Dental University, Japan
Apr 1994-Nov 1995	Research Associate, Department of Oral Pathology, Tokushima University, Japan
Dec 1994-Nov 1996	HFSP Long-term Research Fellow, Department of Human Anatomy, University of Oxford, United Kingdom
Dec 1996-Mar 1997	Post-doctoral Research Assistant of Action Research, Department of Developmental Biology, Tokyo Medical and Dental University, Japan
Apr 1997-Mar 1999	Research Associate, Department of Developmental Biology, Tokyo Medical and Dental University, Japan
Dec 1997-Mar 1999	Post-doctoral Research Assistant of Action Research, Department of Human Anatomy, University of Oxford, United Kingdom
Apr 1999-Dec 2007	Research Associate, Department of Molecular Craniofacial Embryology, Graduate School, Tokyo Medical and Dental University, Japan
Jan 2008-	Professor, Department of Molecular Craniofacial Embryology, Graduate School, Tokyo Medical and Dental University, Japan

Symposium II
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 8 (Sun.) 11:50-12:00

SII-4

Molecular mechanisms of craniosynostosis in Apert syndrome.

Takashi Nakamura, Ph. D.
Department of Biochemistry/Research Branding Project, Tokyo Dental College.
Hiroyuki Ogura
Department of Orthodontics, Tokyo Dental College.
Toshifumi Azuma
Department of Biochemistry/Oral Health Science Center/Research Branding Project, Tokyo Dental College.

Apert syndrome(AS) is an inherited disorder caused by *FGFR2* gene mutations and is characterized by craniosynostosis and osseous syndactyly, associated with enhanced osteoblast differentiation. However, no systemic excessive bone formation has been observed in AS patients, and the mechanisms of site-specific osteoblast hyper activation remains unclear. Here, we report that mechanical factors are related to the AS pathogenesis. To elucidate the molecular mechanisms of osteoblast activation in AS, we first established iPS cells from AS patients and analyzed osteoblast differentiation. However, no clear abnormalities were observed in AS-iPS cell derived osteoblasts. Since the bone abnormalities in AS are confined to cranial sutures and fingers, and mechanical stress causes craniofacial deformity in patients, we hypothesized that mechanical stimulation may trigger site-specific hypercalcification. Mechanical stress loading on normal human mesenchymal stem cells (hMSCs) using cell stretching system caused increased *FGF2* gene expression and enhanced proliferation, which was offset by FGF2 neutralizing antibody. Furthermore, FGF2-dependent cell proliferation during the early stages of osteoblast differentiation caused cell condensation, which promoted subsequent osteoblast maturation and calcified nodule formation. This indicates that *FGF2* gene expression is important for the mechanical stress-dependent bone formation. The induction of osteoblast differentiation in AS-iPS cells under mechanical stress showed increased calcification compared to wild-type iPS cells. Namely, mechanical cues increased the local concentration of FGF2, which, in concert with mutant FGFR2, may induce excessive bone formation in AS. These results suggest that the mechanical stress response may be a new therapeutic target for AS.

Brief CV

Takashi Nakamura
Department of Biochemistry/Research Branding Project, Tokyo Dental College.

Education

Undergraduate 1996-2000 Tokyo University of Agriculture, Department of Agricultural Chemistry
Graduate 2000-2005 The University of Tokyo, Graduate School of Agricultural and Life Sciences, Ph.D.

Research Appointment

2005-2006	Postdoctoral fellow, IMCB, The University of Tokyo/ERATO, JST
2006-2007	Research/Teaching assistant, MRI, Tokyo Medical and Dental University
2007-2010	Assistant Professor, MRI, Tokyo Medical and Dental University
2010-2016	Assistant Professor, Keio University School of Medicine/ERATO, JST
2016-2017	Associate Professor, Keio University School of Medicine
2017-current	Associate Professor, Tokyo Dental College

Awards

2004	Young Investigator Award, American Society for Bone and Mineral Research
2005	Encouragement Award, Japanese Society for Bone and Mineral Research
2005	Plenary Poster Award, American Society for Bone and Mineral Research
2014	JSBMR Poster Award, Japanese Society for Bone and Mineral Research
2016	Excellent Presentation Award, Neo Vitamin D Workshop

Symposium II
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 8 (Sun.) 12:20-12:30

SII-5

Application of iPS cell technologies to treat skeletal disease

Akihiro Yamashita, D.D.S., Ph. D., Noriyuki Tsumaki
Centre for iPS cell Research and Application (CiRA), Kyoto University, Japan

The bone has two types of cartilage, articular cartilage and growth plate cartilage. Articular cartilage covers the end of bone and provides lubrication to diarthrodial joints. Trauma or degeneration of articular cartilage causes joint pain during motion, leading to the onset of osteoarthritis. Growth plate cartilage, where the bone grows in children, and its dysfunction due to genetic mutations causes skeletal dysplasia. The conditions that compromise articular cartilage or growth plate cartilage are poorly understood, and curative drugs are not available. iPS cell technologies are being used to study these skeletal diseases. We have been developing a method in which human iPS cells are differentiated into chondrocytes, the cells that constitute cartilage. We are generating effective and safe human iPS cell-derived cartilage as regenerative medicine to treat focal articular cartilage defects. We have confirmed their safeness and efficacy using animal transplantation models. We have also generated iPS cells from patients with FGFR3 chondrodysplasia. We found that chondrocytes derived from patient-iPS cells produce abnormal cartilage formation and thus offer a unique cellular disease model. In this seminar, we will introduce our projects to treat skeletal disease.

Brief CV

Akihiro YAMASHITA, D.D.S., Ph.D.
Department of Clinical Application, Center for iPS cell Research and Application (CiRA), Kyoto University

Education

Undergraduate 1995-2001 Hiroshima University, Faculty of Dentistry, D.D.S.
Graduate 2003-2007 Shiga University of Medical Science, Graduate School of Medical Science, Ph.D.

Research Appointment

2007-2011	Postdoctoral Fellow: University of Calgary, Department of Biochemistry & Molecular Biology, Canada
2012-2015	Postdoctoral Fellow: Kyoto University, Center for iPS Cell Research Application, Japan
2015-current	Assistant Professor: Kyoto University, Center for iPS Cell Research Application, Japan

Awards

2006	AHFMR Postdoc Recruitment Award, Alberta Heritage Foundation for Medical Research, Canada.
2010	Excellent Postdoctoral Fellow Award, SACRI, U of Calgary, Canada
2011	BMB Postdoctoral Fellow Award, Department of Biochemistry and molecular biology, U of Calgary, Canada
2013	Presentation Award, The Japanese Society of Inflammation and Regeneration,
2015	Excellent Poster Award, 18th Takeda Science Foundation Symposium on Bioscience
2015	Incentive Award, Japanese Society for Regenerative Medicine
2016	Japanese Society for cartilage metabolism Award, 21th Japanese Society for cartilage metabolism

Symposium III
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 8 (Sun.) 13:20-13:30

SIII-1

Taste renin-angiotensin system may contribute to the maintenance of sodium homeostasis.

Noriatsu Shigemura[1,2], D.D.S., Ph. D.
1) Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, 2) Research and Development Center for Five-Sense Devices, Kyushu University.

Sodium ion (Na^+) is the principal extracellular cation maintaining body fluid homeostasis and generation of action potentials. Salty taste, one of the five basic tastes (sweet, umami, bitter, sour, and salty), is considered to play essential roles in identifying and ingesting the Na^+ in foods. However, the molecular mechanisms underlying the salt taste reception and its modulation are remained unclear in detail. Regarding the salt taste reception, it has recently been revealed that the epithelial sodium channel (ENaC) α subunit functions as amiloride-sensitive salt taste receptor in taste bud cells. Renin-angiotensin system (RAS) is a major hormone system in the regulation of body fluid and sodium homeostasis. We had reported that a key component of RAS, angiotensin II (AngII) directly acts on taste cells via its receptor AT1, and suppresses amiloride-sensitive salt taste responses. Furthermore, we recently revealed the presence of all RAS components to produce AngII, namely, renin, angiotensinogen, and angiotensin-converting enzymes 1 and 2 (ACE1, ACE2) in mouse taste buds. These results indicate the existence of a local RAS in the taste organ and suggest that taste function may be regulated by both locally-produced and circulating AngII. Such integrated modulation of peripheral salt taste sensitivity by AngII may play an important role in sodium homeostasis.

It is reported that early symptoms of coronavirus disease 2019 (COVID-19) may include a loss of taste or smell, and SARS-CoV-2 uses human ACE2 as the entry receptor on host cells. These results suggest that ACE2 expressed in the taste organs may be involved in the development of the taste loss in COVID-19. In this presentation, we would also like to discuss the possible linkage between the taste RAS and taste loss in COVID-19.

Brief CV

Name: Noriatsu Shigemura, Ph.D
Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, Japan.

Education:

1996: Bachelor of Arts degree in Dentistry (received from Kyushu University) and D.D.S (Doctor of Dental Surgery)
2000: Doctor of Philosophy in Dentistry (received from Kyushu University)

Postgraduate Education:

2000-2001: Postdoctoral associate,
Bio-oriented Technology Research Advancement Institution (NARO),
Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University
(professor: Dr.Yuzo Ninomiya)

Employment History:

2001-2008: Assistant professor
Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University
(professor: Dr.Yuzo Ninomiya)
2008-2015: Associate Professor
Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University
(professor: Dr. Yuzo Ninomiya)
2016-present: Professor
Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University
2018-present: Concurrent post
Research and Development Center for Five-Sense Devices, Kyushu University
(Director of the center: Dr. Toshiro Matsui, Professor, Bioscience & Biotechnology, Faculty of Agriculture, Kyushu University)

Symposium III
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 8 (Sun.) 13:50-14:00

SIII-2

Pathophysiology of SB: challenges from human and animal studies

Takafumi Kato, D.D.S., Ph. D.
Osaka University Graduate School of Dentistry, Department of Oral Physiology

It becomes increasingly recognized that dentists can play roles in diagnosing and managing some sleep disorders, and their orodental consequences. Among them, sleep bruxism (SB) is a common sleep disorder, affecting approximately 20% in children and 10% of adults. However, physiological studies are limited in humans and animals. Polysomnographic studies in humans showed that rhythmic masticatory muscle activity (RMMA) occurred more frequently in patients with SB than in normal subjects. A majority of RMMA occurred during NREM sleep in association with transient arousals and cyclic sleep processes. To further understand the neurophysiological mechanisms of SB, the jaw motor activities were investigated in the naturally sleeping animals. During sleep, animals exhibited a variety of masseter and digastric contractions. Nonetheless, the RMMA episodes were found to occur during NREM sleep: the episodes were associated with cortical and cardiac activations as found in humans. In addition, animals showed phasic bursts of masticatory muscles, mimicking a coordinated pattern of chewing, during REM sleep. To investigate the excitability of masticatory central pattern generator (CPG), cortical descending tract, originating from cortical masticatory area, was electrically stimulated during wakefulness and natural sleep. Stimulations induced rhythmic masseter and digastric contractions during wakefulness and NREM sleep. However, the response threshold was higher and the response latency was longer during NREM sleep than during wakefulness. During REM sleep, stimulation induced rhythmic digastric muscle contractions. The response threshold was higher while response latency was similar in comparison to wakefulness and NREM sleep. These results suggest that distinct modulation of the excitability in masticatory CPG during sleep and wakefulness can underlie the increased occurrence of RMMA during NREM sleep in patients with SB. The above findings suggest that naturally sleeping animals can be used as an experimental model for investigating the pathophysiological mechanism of SB.

Brief CV

Education :

1988-1994 Osaka University Faculty of Dentistry
1994-1998 Osaka University Faculty of Dentistry Graduate School of Dentistry

Employment:

1998-2001: Postdoctoral fellow
2001-2003: Research Assistant
Hôpital du Sacré-Cœur de Montréal, Université de Montréal Faculté de médecine dentaire and Centre recherche en science de neurologique.
2003: Associate Professor, Matsumoto Dental University
2007: Chief, Matsumoto Dental University Hospital Dental Sleep Medicine Clinic
2008: Associate Professor, Osaka University (Department of Oral Anatomy and Neurobiology)
2016-current: Professor, Osaka University (Department of Oral Physiology)

2013-current: Osaka University Hospital Sleep Medicine Center
2016-Osaka University United Graduate School of Child Development

Societies:

IADR Neuroscience group: Secretary/Treasurer (2006-2008), President-elect (2008), President (2009), Immediate past president (2010)

Awards:

1. Prix Jean-Paul Lussier (2001)
2. CADR Postdoctoral Research Award (2001)
3. IADR/Unilever Travel Award (2002)
4. IADR Distinguished Scientist Awards/Young Investigator Award (2005)
5. KAO Health Science Research Award (2011)
6. Yumikura Research Award (2014)

Symposium III
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 8 (Sun.) 14:20-14:30

SIII-3

Common pathophysiological features in burning mouth syndrome and irritable bowel syndrome

Masamichi Shinoda, D.D.S., Ph. D.
Department of Physiology, Nihon University School of Dentistry

Irritable bowel syndrome (IBS), one of the intractable bowel diseases, is known to afflict 10%–15% of the population in developed countries. Intriguingly, burning mouth syndrome (BMS) which is famous as an intractable intra-oral sensory disorder and IBS are characterized by altered sensory qualities, namely pain hypersensitivity in IBS and BMS patients develops without any apparent pathological changes such as nerve injury, inflammation, trauma or malignant tumor in the gastrointestinal tract and oral mucosae. To illuminate these pathogenetic mechanisms, we developed a non-inflammatory model of lower gastrointestinal tract and tongue pain hypersensitivity in the mouse that reproduces these two important features (pain hypersensitivity and non-apparent pathological changes) of IBS and BMS. Firstly, colon pain hypersensitivity in the IBS was virtually absent in $P2X_3$ knockout relative to wildtype mice. Intralumenal release of the endogenous P2X receptor ligand did not differ between wildtype and $P2X_3$ knockout mice or change after the occurrence of IBS. The EC50 of $P2X_3$ ligand for the fast current decreased in dorsal root ganglion (DRG) neurons innervating to gastrointestinal tract mucosa in the IBS. The enhancement of purinergic signaling in DRG neurons may contribute to colon pain hypersensitivity in the IBS. Secondary, we found that the increase of Artemin (Artn) mRNA expression in the tongue mucosa of BMS patients and the significant increase in Artn expression and heat hyperalgesia in the tongue mucosa in BMS model mouse. The transient receptor potential vanilloid 1 (TRPV1) antagonism or Artn neutralization inhibited the heat hyperalgesia. The increase of TRPV1-IR trigeminal ganglion (TG) neuron innervating the tongue was significantly reduced by Artn neutralization in the tongue mucosa. The TG neuronal hyperactivity was also inhibited by Artn neutralization These present findings suggest that the tongue Artn overexpression in the BMS causes heat hyper-responses in TG neurons via the TRPV1 hyperexpression, resulting in tongue heat hyperalgesia.

Brief CV

Masamichi Shinoda
Department of Physiology, Nihon University School of Dentistry

Education

Undergraduate	1992-1998	Tohoku University, School of Dentistry, D.D.S.
Graduate	1999-2003	Nagoya University, Graduate School of Medicine, Ph.D.

Research Appointment

2003-2006	Assistant professor, Nagoya University
2006-2009	Postdoctoral associate, University of Pittsburgh, USA
2009-2011	Assistant professor, Nihon University
2011-2020	Associate professor, Nihon University
2020-current	Professor, Nihon University

Academic activity

Councilor, Japanese Association for the Study of Pain
Councilor, The Physiological Society of Japan
Councilor, Japanese Association for Oral Biology
Board of directors, Japanese Society of Orofacial Pain
Member, The International Association for the Study of Pain
Member, Society for Neuroscience

Awards

2011	The 4th Asian pain symposium outstanding-poster award
2012	Japanese Association for Oral Biology Rising Members Award

Symposium III
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 8 (Sun.) 14:50-15:00

SIII-4

In vivo Dynamics of Dental Tissue Regeneration

Toshihide Mizoguchi, M. Sc., Ph. D.
Oral Health Science Center, Tokyo Dental College, Tokyo, Japan

[Background] Reparative dentin formation is induced in response to severe dental damage. This is a biological defense mechanism to regenerate the damaged hard tissue. In this biological process, dental damage induces odontoblast death, after which dental pulp stem cells differentiate into odontoblast-like cells, contributing to generate reparative dentin. However, how damage induces this regenerative process remains unclear. We hypothesized that odontoblast death induces the regeneration of damaged dental tissue. In this study, we examined the effects of odontoblastic depletion on dentinogenesis activation using a Cre/LoxP-based strategy.
[Methods] (1) To induce cell death specifically in odontoblasts using a Cre/LoxP-based strategy, we confirmed the odontoblast-specific expression of green fluorescent protein (GFP) in type I collagen α *[(Col1 (2.3)]-GFP* mice, in which GFP is expressed under the control of a 2.3-kb fragment of the *Col1* promoter. (2) *Col1 (2.3)-Cre; ROSA26-loxP-stop-loxP-diphteria toxin (DT) receptor (DTR); Col1(2.3)-GFP* mice were generated and administered DT for 1 week to deplete odontoblasts, and the regeneration of odontoblasts and reparative dentin formation were then analyzed.
[Results] (1) The expression of *Col1 (2.3)* promoter-inducible GFP was only detected in odontoblasts in the maxillary first molar, confirming that Col1 (2.3)-Cre was specifically expressed in odontoblasts. (2) Odontoblasts were markedly depleted in maxillary first molars after DT treatment. (3) Depleted odontoblasts significantly recovered in a time-dependent manner. (4) Regenerative odontoblast-like cells generated reparative dentin.
[Conclusion] Dentin formation increased in response to odontoblastic cell death in a genetically modified mouse model. This suggests that there is a dental pulp niche environment regulated by odontoblastic cell death and that this regulatory network is essential for the activation of reparative dentin formation.

Brief CV

Toshihide Mizoguchi
Oral Health Science Center, Tokyo Dental College

Education

1994-1998	Faculty of Life Sciences, Tokyo University of Pharmacy and Life Sciences, B.Sc.
1998-2000	Faculty of Life Sciences, Tokyo University of Pharmacy and Life Sciences, M.Sc.
2002-2005	Faculty of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Ph.D.

Academic Appointments

2000-2006	Research associate, Division of Hard Tissue Research, Institute for Oral Science, Matsumoto Dental University
2006-2018	Senior lecturer, Division of Hard Tissue Research, Institute for Oral Science, Matsumoto Dental University
2011-2014	Postdoctoral fellow, Albert Einstein College of Medicine, Stem Cell Institute
2018-2019	Senior lecturer, Oral Health Science Center, Tokyo Dental College
2019-present	Associate professor, Oral Health Science Center, Tokyo Dental College

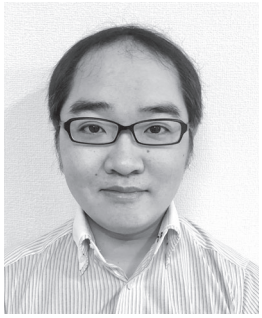
Awards

2014	Travel award, The 11th Bone Biology Forum
2015	Best presentation award, The 1st Japanese Society of Osteoimmunology Meeting
2016	Best presentation award, The 1st Oral Frontier Science Meeting
2016	Research award, The 34th Japan Society for Bone and Mineral Research Meeting

RISING SCIENTIST SESSION

Rising Scientist Session
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 7 (Sat.) 13:20-13:30

RS-1



Factors secreted from dental pulp stem cells show multifaceted benefits for Synergistically Regenerate Transected Rat Peripheral Nerves by Altering Macrophage Polarity.

Fumiya Kano, D.D.S., Ph. D.
Department of Tissue regeneration Institute of Biomedical Sciences, Tokushima University Graduate School

Following peripheral nerve injury (PNI), Schwann cells (SCs) and macrophages cooperatively promote nerve regeneration. The dam- aged axons and myelin sheaths distal to the lesion undergo Wallerian degeneration, in which migrated macrophages remove the myelin and axonal debris from the injury site, while the mature SCs then dedifferentiate and proliferate to form the bands of Bunger, a series of tubular structure that produces trophic factors and extracellular matrix molecules that accelerate axonal regrowth toward their original targets. Recent studies have also shown that fibroblasts and endothelial cells/blood vessels in the PNI promote the sorting and directional migration of SCs, respectively. Thus, the temporal and spatial coordination of multiple cell types promotes peripheral nerve (PN) regeneration. However, while the self-reparative activities of PNs successfully repair crush or cut-induced neuronal injuries, they do not fully repair transection-induced nerve gaps, which are replaced with fibrotic scars.

Stem cell transplantation-based therapy is a promising approach for patients with severe PNI. The transplantation of various types of scaffolds containing bone marrow mesenchymal stem cells, adipose-derived stem cells, umbilical cord-derived mesenchymal stem cells, or dental pulp stem cells into the transected nerves of rodents promotes substantial functional recovery. Notably, in most of these studies, neurological function is recovered primarily through paracrine/trophic mechanisms. Stem cells secrete a broad repertoire of trophic and immunomodulatory factors that can be collected as serum-free conditioned medium (CM). We previously reported that the engrafted stem cells from human exfoliated deciduous teeth (SHEDs) and SHED-CM exert similar therapeutic effects for Central Nervous System (CNS) injuries, acute liver failure, and lung injuries.

However, the therapeutic mechanisms and factors in SHED-CM responsible for PN regeneration are still largely unknown.

In this presentation, we report that SHED-CM regenerates PNs by inducing tissue-repairing M2 macrophages and may provide therapeutic benefits for severe peripheral nerve injuries.

Brief CV

Fumiya Kano
Department of Tissue regeneration Institute of Biomedical Sciences, Tokushima University Graduate School

Education

Undergraduate 2004-2010 Kanagawa Dental College, Yokosuka, Japan, D.D.S.
Graduate 2012-2016 Nagoya University Graduate School of Medicine, Nagoya, Japan, Ph.D.

Research & Professional Experience:

2010-2012	Department of Oral and Maxillofacial Surgery, Nagoya University Hospital
2012-2019	Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine
2019-2020	Specially Appointed Assistant Professor, Tokushima University
2020-	Assistant Professor, Tokushima University

Award:

2015	Excellence Title Award, The 36th Annual Meeting of the Japanese Society of Inflammation and Regeneration.
2015	Travel Award, 12th World Congress on Inflammation
2016	Excellence Title Award, The 37th Annual Meeting of the Japanese Society of Inflammation and Regeneration.
2017	Excellence Title Award, The 63th Annual Meeting of Japanese Society of Oral and Maxillofacial Surgeons

Rising Scientist Session
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 7 (Sat.) 13:50-14:00

RS-2

Phenotypic and neuroplastic changes in trigeminal nociceptive pathways following trigeminal nerve injury

Ayano Katagiri, D.D.S., Ph. D.
Department of Oral Physiology, Osaka University Graduate School of Dentistry, Suita, Japan

Trigeminal nerve injuries are often caused by dental and orofacial surgical procedures. Patients with trigeminal nerve injuries suffer from complex symptoms, such as persistent trigeminal neuropathic pain and affective distress, that interfere with daily activities. The underlying mechanisms linking somatic and affective symptoms remain to be clarified. We investigated the phenotypic and neuroplastic changes in trigeminal ganglion neurons and trigeminal spinal subnucleus caudalis (Vc) neurons in trigeminal neuropathic pain animal models that exhibited mechanical allodynia and heat hyperalgesia.

In intact trigeminal ganglion, Aδ- and C-fiber afferents mainly transmit noxious information, while Aβ-fiber afferents transmit mechanosensitive tactile information. After the trigeminal nerves were injured, Aδ and C afferents became hyper-activated. Moreover, Aβ- afferents have been shown to upregulate calcitonin gene-related peptide (CGRP), a neuropeptide that is generally synthesized and released in nociceptive C afferents. Thus, in addition to Aδ and C afferents, Aβ afferents contribute to abnormal nociceptive signaling in the secondary neurons in the Vc after nerve injury. Vc neurons have projections that ascend to the ventral posteromedial thalamic nucleus (VPM) associated with sensory discrimination and parabrachial nuclei (PBN). After trigeminal nerve injury, C-fiber-mediated nociceptive inputs increased in Vc-VPM and Vc-PBN ascending pathways. However, Aβ-fiber-mediated mechanosensitive nociceptive inputs activated Vc neurons that specifically projected to the PBN. The PBN is a hub of multi-dimensional sensory processing, including visceral malaise, taste, temperature, itch, and affective aspects of pain, to the thalamus, hypothalamus, and extended amygdala. Therefore, our results suggest that phenotypic changes in Aβ afferents in the trigeminal ganglion and neuroplastic changes of Vc projecting to the PBN can underlie the development of pain and affective symptoms after trigeminal nerve injury. The PBN is a crucial structure for future investigations of the interaction between pain and affective problems, such as sleep disturbance and anorexia, in trigeminal nerve injury.

Brief CV

Ayano Katagiri

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1-8 Yamadaoka Suita-shi, Osaka, 565-0871, Japan

EDUCATION and TRAINING

2002 - 2008 Kyushu Dental University, Fukuoka, Japan DDS.
2008 - 2009 Department of Oral and Maxillofacial Surgery,
Nihon University School of Medicine Itabashi Hospital,
Tokyo, Japan Residency
2009 - 2013 Department of Psychosomatic Dentistry,
Graduate School of Medical and Dental Sciences,
Tokyo Medical and Dental University, Tokyo, Japan Ph.D.
2012 - 2014 Department of Diagnostic and Biological Sciences,
University of Minnesota School of Dentistry, Minneapolis, MN
Post-doctoral Fellow

WORK EXPERIENCE

2014 - 2017 Department of Physiology, Nihon University School of Dentistry
Assistant Professor
2017 - present Department of Oral Physiology,
Osaka University Graduate School of Dentistry Assistant Professor

AWARDS

2020 SHISEIKAI Scholarship Fund for basic researcher of medical science, Keiko Watanabe Award

Rising Scientist Session
Lecture: Video On-demand distribution / Q & A: Live streaming
Q & A delivery time: November 7 (Sat.) 14:20-14:30

RS-3

The human brain and mastication; broad impact on systemic functions

Jun Miyamoto, D.D.S., Ph. D.
Department of Maxillofacial Orthognathics, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU)

Mastication is a complex movement coordinated among multiple dentomaxillofacial organs, under control of brain functions based on varieties of inputs arising from mechano- and other sensory receptors richly distributed across dentomaxillofacial structures. Particularly, sensory information from periodontal ligaments is crucial for masticatory motor control, as numbers of animal and human studies demonstrate to the date. Although, among research fields of human cerebral sensori-motor control, "teeth and masticatory movements" has been underemphasized compared to the studies interested in other body parts.

Dentomaxillofacial sensory information, in addition to contribution to masticatory sensori-motor control, also has implications on human cognitive and mental brain functions. That is, tooth loss has been advocated as a risk factor for Alzheimer's disease, or dental sensory information apparently subserves memory functions. As such, tooth-derived sensation appears to have impacts on broad range of systemic functions besides mastication, leaving numbers of subjects for future investigations.

Our department has been studying, using noninvasive brain imaging and eye tracking, the human cerebral organization of information processing for sensation from the dentomaxillofacial region, for masticatory motor control system, and for the effect of mastication on broad systemic functions. Among these, in this presentation, I would like to introduce: 1) reciprocal cortical activation patterns during incisal and molar biting correlated with bite force levels, and 2) the effects of chewing on reducing appetite. These results would not merely elucidate the effects of mastication on brain function, but also pave the way for clarifying the relationship between oral function and general health.

Brief CV

Jun Miyamoto
Department of Maxillofacial Orthognathics, Graduate School of Medical and Dental Sciences,
Tokyo Medical and Dental University (TMDU)

Education

2001	D.D.S,	Tokyo Medical and Dental University
2005	Ph.D.,	Tokyo Medical and Dental University Graduate School

Research and Clinical Appointments

2003-2005	Collaborative Researcher, National Institute for Physiological Sciences, National Institutes of Natural Sciences
2007-2008	Clinical Fellow, Maxillofacial Orthognathics, Tokyo Medical and Dental University Graduate School
2008-2010	Research Fellowship for Young Scientists, Japan Society for the Promotion of Science
2005-current	Visiting Researcher, National Institute of Neuroscience, National Center of Neurology and Psychiatry
2010-current	Assistant Professor, Maxillofacial Orthognathics, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University

Award

IADR Unilever Hatton Divisional Award.
84th General Session & Exhibition of International Association for Dental Research, Brisbane, Australia, 2006.

POSTER PRESENTATION

001: Wear Resistance of Novel Machinable Glass Ceramics

S. Kariya, T. Azuma, F. Fusejima
R&D Dept., GC Corporation, Tokyo, JAPAN

Objectives: In recent years, glass-ceramic blocks for CAD/CAM are clinically well accepted as “Single-Appointment Treatment” material. Conventionally, Feldspar glass ceramics have been frequently selected. Recently, it has been developed a Novel Lithium Disilicate Glass Ceramic which doesn’t require the crystallization process after CAD/CAM fabrication. The purpose of this study was to evaluate the wear resistance of machinable glass ceramic blocks.Methods: The wear resistance properties of a Novel Lithium Disilicate Glass Ceramic Block “Initial LiSi Block” (LS, GC Corp.) and 2 different Feldspar glass ceramic blocks of “GC Initial LRF BLOCK” (LRF, GC Europe N.V.) and “Block V” (BV) were compared by two-body wear test. Polished samples (Φ2.1mm) were prepared with diamond polishing paste “Cera Shine” (GC Corp.). They were set and contacted with hydroxyapatite as antagonist which sintered and polished with #4000 SiC paper in water. The wear test was performed with sliding on antagonist under a load of 0.3kgf with 10,000 cycles. Wear amounts were evaluated by measuring sample height (n=4) before and after wear test. In order to compare the crystal structure and the crystallinity, microscope observation with SEM (SU-70, HITACHI) and Reference Intensity Ratio method (RIR) with XRD (Empyrean, PANalytical) were carried out.Results: The averages of wear amount were LS 1μm, LRF 18μm and BV 39μm respectively and they showed significant difference in each other ($p<0.01$, One-way ANOVA and Turkey test). On the observation of SEM, the crystal type differences were seen between Lithium Disilicate and Feldspar. Moreover, the crystallinity of LS showed apparently higher than both LRF and BV. It was considered that the wear resistance property is influenced by both crystal type and crystallinity.Conclusions: It is considered that LS could be one of the most attractive “Single-Appointment Treatment Glass Ceramic Material”, because of its high wear resistance.

002: Evaluation of bonding layer durability on 2-step self-etch adhesive

K. FUJIMORI, K. HIRANO, F. FUSEJIMA
RESEARCH & DEVELOPMENT, GC CORPORATION, TOKYO, Japan

Objectives: In general, 2-step self-etch adhesives are reported relatively high bond strength. In 2-step self-etch adhesives, they consist with first step PRIMER and second step BOND. The BOND form thicker bonding layer which contributes to make good wettability with hydrophobic composite resin and develop the mechanical strength of entire bonding layer which increase bonding performance. Therefore, durable composition might be suitable for the BOND. However, most of commercial products contain hydrophilic monomers such as HEMA in the BOND which can expect less durability due to its water uptake. We have developed a new 2-step self-etch adhesive (BZF-29) including the BOND with hydrophobic composition and HEMA free. In this study, flexural strength was evaluated on several adhesive to find impact of hydrophobic composition on bonding layer durability.Methods: The BOND of BZF-29 (GC, BZF), Product A (PA) and Product B (PB) were examined. Flexural strength specimens were prepared in accordance with ISO4049:2019. The specimens were immersed in distilled water at 37 degC for 1, 7, 28 and 90 days respectively, and then measured flexural strength by using a universal testing machine (AG-IS, Shimadzu Corporation) (n=5).Results: The flexural strength of PA and PB decreased significantly after 7 days of immersion, whereas that of BZF showed no significant decrease even after 90 days of immersion. PA and PB contain HEMA in the BOND, which can be expected to easily uptake water. Therefore, it is considered that their flexural strength was degraded due to water sorption. On the other hand, BZF has highly hydrophobic composition HEMA free and did not show degradation by immersion.Conclusions: Since the BZF-29 formulate hydrophobic composition and HEMA free on the BOND, its flexural strength did not degrade by immersing in water even for long term. These results indicated BZF-29 can be expected to have excellent long-term durability in clinical practice.

003: Bonding properties of acrylic resin to zirconia and polyaryletherketone (PAEK)

T.-Y. PENG[1], S. SHIMOE[2], D.-J. LIN[1], M. KAKU[2]

[1]School of Dentistry, College of Dentistry, China Medical University, Taiwan R.O.C, [2]Department of Anatomy and Functional Restorations, Hiroshima University Graduate School of Biomedical & Health Sciences, Hiroshima, Japan

Objectives: With the rapid pace of technological development, CAD/CAM technology, low-wax techniques, and digital workflow have become mainstream in laboratory and clinical dentistry. Furthermore, the popularization of aesthetic concepts has raised doubts regarding the use of traditional alloy materials (Co-Cr) in removable partial dentures (RPD). This study aims to discuss the bonding strength of aesthetic dental materials to acrylic resin and to assess the feasibility of using these aesthetic dental materials in RPD applications.

Methods: Five types of testing materials were considered (control: Co-Cr; two zirconia-based materials: yttria-stabilized tetragonal zirconia polycrystal (Y-TZP), and ceria-stabilized tetragonal zirconia/alumina nanocomposite (Ce-TZP/A); two polyaryletherketone (PAEK) materials: polyetheretherketone (PEEK), and polyetherketoneketone (PEKK)). All sample surfaces were prepared via grinding, sandblasting, and priming according to the manufacturer's instructions prior to the acrylic resin polymerization. The surface roughness was measured using atomic force microscopy, and the shear bond strength (SBS) was determined. The obtained data were analyzed using Tukey's HSD (α=0.05). After the SBS test, the interfacial failure modes were observed using a thermal field emission scanning electron microscope.

Results: The PAEK materials showed lower surface roughness than zirconia and Co-Cr alloy ($P<0.05$) after surface pretreatment. Within the material group (zirconia or PAEK), there was no significant difference ($P>0.05$). Zirconia (Y-TZP=12.47±4.8 MPa; Ce-TZP/A=11.80±4.6 MPa) and Co-Cr alloy (11.73±1.82 MPa) had a comparable SBS, while the PAEK materials (PEEK=7.60±2.0 MPa; PEKK=8.38±1.9 MPa) had a relatively lower SBS. Adhesive failure was the dominant failure mode in all the test groups.

Conclusions: The SBS values of the zirconia and PAEK materials with acrylic resins were consistent with the clinical guidelines of ISO 10477 (>5.0 MPa). Within this feasibility study, these aesthetic dental materials were evaluated as potential alternative materials for RPD frameworks.

004: Bond strength between one-step adhesives and dentin after cyclic loading

T. EGOSHI[1], Y. TAIRA[1], K. SOENO[2], K. KAIDA[1], S. KUBO[1], H. MURATA[3]

[1]Division of Cariology and Restrative Dentistry, Department of Prosthetic Dentistry, Nagasaki University, Nagasaki, Japan, [2]Department of Appried Prosthodontics, Nagasaki University, Nagasaki, Japan, [3]Department of Prosthetic Dentistry, Nagasaki University, Nagasaki, Japan

Objectives: The purpose of this study was to investigate the influence of mechanical load cycling on the dentin bond strengths of four different one-step self-etch adhesives.

Methods: Clearfil Tri-S bond (Tri-S, Kuraray Noritake Dental), BeautiBond (B-Bond, Shofu), Bond Force (Bond-F, Tokuyama Dental), and G-Bond Plus (G-Bond, GC) were used as adhesives. Extracted non-carious human molars were sectioned at the cemento-enamel junction to expose the dentin and were ground flat with 600 grit silicon carbide papers. Dentin surfaces were treated according to the manufacturers' instructions for each adhesive and a light-curing resin-composite material was built up on the surface. All specimens were immersed in water at 37 ºC for 24 h. Half of the specimens were tested for micro-tensile bond strength at a crosshead speed of 0.5 mm/min (0-cycle). The remaining half of the specimens were subjected to a vertical load (75.6 N, 1.2 Hz) on the top surface of resin composite with a 15º rotation using an acrylic stylus for 200,000 cycles, after which the micro-tensile bond strengths were determined (200,000-cycle). The data were analyzed by two-way ANOVA and a Tukey-Kramer HSD test at a statistical significance of 0.05.

Results: The mean bond strengths were G-Bond/0-cycle (78.3 MPa), Tri-S/0-cycle (70.9 MPa), B-Bond/0-cycle (67.3 MPa), B-Bond/200,000-cycle (67.2 MPa), Tri-S/200,000-cycle (62.5 MPa), G-Bond/200,000-cycle (54.4 MPa), Bond-F/200,000-cycle (50.0 MPa), and Bond-F/0-cycle (44.7 MPa). Tri-S and B-Bond exhibited relatively high bond strengths before loading that did not significantly change after 200,000 cycles (high). G-Bond had a high bond strength before loading that significantly decreased after 200,000 cycles (slope). Bond-F resulted in a relatively low bond strength before loading that did not significantly decrease after 200,000 cycles (moderate).

Conclusions: The influence of mechanical load cycling on the bond strength between one-step self-etch adhesives and dentin could be divided into three categories: high, slope, and moderate.

005: Influence of saliva contamination on adhesion performance of adhesive resin cement

S. MURAKAMI, K. HIRANO, F. FUSEJIMA
RESEARCH & DEVELOPMENT, GC CORPORATION, TOKYO, Japan.

Objectives:The surface preparation of tooth abutment is important for adhesion performance of adhesive resin cement(ARC), but clinically abutment surface might be accidentally contaminated by saliva(SC), which decrease adhesion performance due to inhibit proper polymerization of ARC at adhesive interface where is difficult to light-cure. We have developed ARC, G-CEM ONE EM, which accelerate polymerization performance even without light-irradiation at adhesive interface in combination with Adhesive Enhancing Primer(AEP, formulated additional polymerization initiator). We evaluated influence of SC on Shear bond strength(SBS) and polymerization rate(PR) of commercially available ARC.Methods:Two commercially available ARC, G-CEM ONE EM with AEP(GO, GC Corporation)and productA were used in this study. Bovine tooth was grinded with 600-grit SiC paper to expose dentin and separated in 2-groups, SC and control. On SC, human-saliva was applied to bonding surface, left to stand for 5seconds and dried with air. Then the bonding area(3.0mm diameter) and cement thickness(0.1mm) were defined. Stainless-steel rod was bonded by ARC. Bonded specimen was stored in 37degC distilled water for 24hours and specimen was subjected to be tested SBS by universal test equipment (SHIMADZU AG-IC, crosshead-speed 1mm/min, N=5). The PR of polymerized ARC specimen was calculated by comparison of C=C bond intensity which in measured by FT-IR(Thermo Fisher Scientific, N=3). Data were statistically analyzed by ONE-way ANOVA(Tukey-Krame).Results:SBS and PR of productA was significantly reduced by SC. On the other hand, SBS and PR of GO was no significant difference between control and SC. This is indicated that SC has impact to decrease SBS and PR of ARC and there was a correlation between PR and SBS.Conclusions:It might suggest polymerization performance at adhesive interface is important to resist against SC and as result, G-CEM ONE EM with AEP which could maintain polymerization performance even with SC had showed no significant decrease of SBS by SC.

006: Evaluation of Dentin Anti-Demineralization potential of S-PRG Containing Self-Adhesive Resin Cement

S. ThanNaing[1], N. Hiraishi[1], A. Abdou[1,3], J. Tagami[1]
[1]Cariology and Operative Department, Tokyo Medical and Dental University, Tokyo, JAPAN, [2]Department of Conservative Dentistry, University of Dental Medicine Mandalay, Mandalay, Chanmyathazi, MYANMAR, [3]Biomaterials Department, Faculty of Dentistry, Modern University for Technology and Information, Cairo, EGYPT

Objectives: to assess the anti-demineralization potential of S-PRG containing self-adhesive resin cement on dentin surfaces. Methods: Fifteen round cavities (3.5 mm diameter and 1.5mm thickness) were prepared on bovine dentin slabs and were filled with three self-adhesive resin cements (n=5); Exprimental silica filler containing cement (Si-based cement, Shofu, Japan), Experimental S-PRG filler containing cement (S-PRG-based cement, Shofu, Japan), and RelyX Unicem2 (F-based cement, 3M ESPE, Germany). After that all specimen surfaces were covered with acid-resistant nail varnish leaving a window area of 4x4 mm2 to expose the dentin surface around the cement. All specimens were subjected to acidic challenge by using remineralizing solution (Simulated Body Fluid-SBF) for 20 hours and demineralization solution (pH 4.5) for 4 hours alternatively (Re-De cycle). Depth of demineralization was assessed using Swept Source Optical Coherence Tomography (SS-OCT) after 7, 14, 21 and 28 days. Data were statistically analyzed using two-way ANOVA followed by multiple comparison with Bonferroni correction.Results: For all groups, Re-De cycles showed significant increase in depth of demineralization after 14 and 28 days. The depth of demineralization for S-PRG-based cement was significantly lower than Si-based cement after 7, 21 and 28 days ($p<0.05$). Conversely, F-Based cement was not significantly different than Si-based cement after 7, 14, and 21 days ($p<0.05$). Demineralization depth minimum limit after 28 days (DML) was around 95 µm. F-based and Si-based cements reached the DML after 16 and 19 days, respectively.Conclusions: S-PRG containing self-adhesive resin cement capable of resisting dentin demineralization after dynamic chemical formation of secondary caries.

007: Preparation of Filler-Dispersed Resin Composite for Additive Manufacturing

P. KARNTIANG[1], H. IKEDA[2], Y. NAGAMATSU[2], H. SHIMIZU[2]

[1]Division of Operative Dentistry, College of Dentistry, Rangsit University, Pathum Thani, Thailand, [2]Division of Biomaterials, Department of Oral Functions, Kyushu Dental University.

Objective: To develop filler-dispersed resin composite for additive manufacturing. Method: STL data for 3D printing of a bar sample was created using ChiTuBox software. A precursor for additive manufacturing was prepared via mixing TEGDMA-UDMA (1:1 ratio), a photo-initiator and silica filler (0.5 micron). There were 5 groups regarding filler load: 0%, 20%, 40%, 60%, 70%. The total number of 36 samples per group were fabricated by a 3D printer and divided into 3 subgroups: (a) immediately received the mechanical properties evaluation (flexural strength, flexural modulus and Vickers hardness); (b) was immersed in water for 2 months before mechanical properties evaluation; (c) received water sorption evaluation. Data were analyzed by 1 or 2way ANOVA followed by Tukey test ($p = 0.05$).Result: Higher filler groups demonstrated higher mechanical properties and lower water sorption values. 70% group had the highest mechanical properties and lowest water sorption values among all groups. Water immersion decreased only flexural strength and Vickers hardness values, but not flexural modulus values.Conclusion: All tested 3D-printed composites have met the requirements of ISO 10477:2018, indicating that the composites could be the potential crown and veneering materials.

008: Enamel Remineralization of TCP with Fluoride in Toothpaste: EPMA Study

H. HAMBA, H. ISHIZUKA, Y. MIYAYOSHI, K. NAKAMURA, T. MURAMATSU

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Objectives: This in vitro study aimed to compare the efficacies of experimental toothpastes containing functionalized tricalcium phosphate (fTCP) with 950 and 1,450 ppm fluoride for enamel remineralization under pH cycling conditions using microcomputed tomography (μCT) and electron probe micro–analyzer (EPMA).

Methods: Bovine enamel specimens were immersed in a demineralizing solution to create enamel subsurface lesions. During pH cycling (12 days), the specimens were assigned into the following three groups based on the type of experimental toothpaste (3M) used: DW (deionized water), TCP + 950F (fTCP with 950 ppm fluoride), and TCP + 1450F (fTCP with 1,450 ppm fluoride). The μCT scans of all specimens were obtained after demineralization and pH cycling. Remineralization percentage (%R) was calculated from the mineral loss (Δ Z) after demineralization and pH cycling. The treated enamel lesions were finally investigated using a scanning electron microscopy (SEM) and EPMA. One-way analysis of variance and post hoc Tukey´s tests were used to analyze %R values.

Results: Mean %R value of DW (10.2%) was significantly lower than those of TCP + 950F (21.8%) and TCP + 1450F (24.7%) groups ($p < 0.05$). However, the TCP + 950F and TCP + 1450F groups did not show significantly different %R values ($p > 0.05$) and showed small crystals on the enamel rods in SEM. Furthermore, subsurface lesions of the TCP + 950F and TCP + 1450F groups showed increased fluoride content in the EPMA line profiles. The TCP + 1450F group tended to have a higher fluoride uptake than the TCP + 950F group according to EPMA mapping.

Conclusions: The experimental toothpastes containing fTCP with 950 and 1,450 ppm fluoride increased the remineralization in the artificial subsurface enamel lesions during pH cycling. Toothpastes containing fTCP with 1,450 ppm fluoride may be more suitable for remineralization in fluoride uptake into the subsurface lesions.

009: EVALUATION OF THE EFFECT OF ZINC-CONTAINING ORAL MATERIALS ON REMINERALIZATION USING IN-AIR MICROBEAM PIXE/PIGE

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Objectives: Zinc, silver, and calcium ions have been explored recently for developing multifunctional bioactive materials. Zinc is reported to have an antibacterial and Matrix Metaroprotase (MMP) inhibitory effect, and a demineralization inhibitory effect. We evaluated the demineralization prevention and zinc (Zn) uptake of an oral material into the dentin using an automatic pH cycling.Methods: Each tooth was divided into four specimens. Their dentin in the buccolingual surfaces was exposed; the other surface was covered with sticky wax. Specimens were demineralized in a buffer solution (0.2 M lactic acid, 3.0 mM CaCl2, 1.8 mM KH2PO4, 2% carboxymethyl cellulose, pH 4.5) at 37°C for 72 h. Moreover, 0.01% zinc chloride from a mouthwash (“Listerine”) and a solution containing 1% zinc chloride and 2% tannic acid were used. Specimens were stored in each material at 37 °C for 24 h. From each tooth, 150 µm sections were prepared. The cut surface and half of the exposed area were covered with sticky wax. The automatic pH-cycling system ((pH 6.8–4.5) simulated daily acid challenges in the oral cavity for two weeks. The zinc and calcium distributions in the carious lesion in each specimen were evaluated using an in-air microbeam PIXE/PIGE system. Multi-elemental sequential analyses were performed by measuring the concentrations of Ca and Zn using the in-air microbeam PIXE/PIGE as per the previous report. For evaluating dentin remineralization, Ca concentrations on the covered and uncovered areas were compared.Results: The Zn concentration in the dentin showed significant differences among the three groups (as per one-way ANOVA and Scheffe multiple comparison tests; $p < 0.05$). The Zn group showed a significant increase in Ca compared with the control group.Conclusions: The data from PIGE and PIXE techniques helped in understanding the efficacy of zinc-containing oral materials for preventing caries. This study was supported by Grants-in-Aid for Scientific Research (17K11712, 17H04382) from the JSPS.

010: Dynamics of Ions Artificially Introduced into Caries-affected Dentin

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Objectives : The aim of this study was to investigate how multi-ions from F-containing materials were incorporated into caries-affected dentin.Methods : Human sound third molars (n=3) were used in this study. The roots were cut perpendicularly to the axis at the cement-enamel junction and exposed the buccolingual dentin surfaces using a low-speed diamond saw. The surface of specimens except the exposed root dentin was covered with sticky-wax. Demineralization was conducted by acetate acid (pH 5.0, 37°C) for 3 days to make the caries-affected dentin. These specimens were covered with following F-containing materials (ZIF-10, Mi varnish® and FRC-02) (provided by GC), then placed in saline water for 3 months 37°C. The materials on specimens were mechanically removed. In-air micro-particle induced X-ray / γ-ray emission (In-air µ-PIXE/PIGE) was performed to measure Ca, F, Zn, Sr concentrations and to record the ion distributions before and after the application of the materials. Then, the specimens were embedded by epoxy resin, polished until being mirror surface. Field Emission Scanning Electron Microscope (FE-SEM) was performed for morphological observation.Results : F uptake was at constant with high concentrations from the surface to approximately 200 µm layer. Zn and Sr were also incorporated into caries-affected dentin, and the high Zn concentration in ZiF-10 was maintained beyond 200 µm. In F concentrations, there was a statistically significant difference between ZiF-10 and the control group. There was no difference in the ion distributions among the materials. Conclusion : The PIXE/PIGE enables us to observe the ionic dynamics of the caries-affected dentin due to high sensitivity to trace elements and measurement in air. The present study suggested that caries-affected dentin differed from the ionic dynamics of sound dentin. This study was supported by Grants-in-Aid for Scientific Research (17H04382, 17K11705) from the JSPS.

011: Physical properties of Resorbable P(LA/CL) bilayer membrane for GBR

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Objectives: Resorbable collagen membrane have been widely used for Guided Bone Regeneration (GBR). However, animal derived collagen may carry a risk of unknown pathogen. In addition, most of the collagen membranes are weak under wet condition and could easily be broken. To overcome these points, we have developed resorbable bilayer membrane using synthetic poly (L-lactide-ε-caprolactone) (PBM) with elastic characteristics. In this study, we aimed to evaluate physical and handing properties of PBM under wet condition.Methods: PLGA membrane (PM) and Collagen membrane (CM) were used as control. Tensile strength was measured by using universal testing machine(CR-500DX, SUN SCIENTIFIC CO., LTD) in dry and wet condition (immersing to saline for 1 minutes). Both end of test specimen (2 mm x 15 mm) was fixed with jig, tensile strength was measured at cross head speed of 20 mm/min until the specimen was broken. Flexibility was evaluated by using jaw model. Test specimens (15 mm x 25 mm) in dry and wet condition was placed on jaw model to confirm whether test specimens could fit the shape of jaw model.Results: [Tensile strength test] PBS showed elastic characteristic and was broken at longer distance and low tensile strength. No difference between dry and wet condition was observed for PBM and PM. Tensile strength of CM decreased under wet condition.[Flexibility] PBM in dry condition didn't fit the shape of jaw model, but PBM in wet condition was able to fit the shape by adhering to the surface. PM didn't fit the shape of jaw model even in wet condition due to the solid characteristic. CM in dry condition didn't fit the shape of jaw model, but CM became softer by immersing to saline and fit the shape of jaw model easily.Conclusions: These results indicate that PBM has good handling in wet condition by maintaining the mechanical strength, which could reduce the technical sensitivity when applying the membrane. Therefore, it is expected that PBM is a clinically useful membrane for GBR.

012: Influence of Molding Angle on 3D-printed Partial Denture Framework

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By the development of the CAD/CAM technology, it became possible to apply selective–laser–sintering <SLS> technique to the framework fabrication of the removable partial denture. However, various factors may affect the accuracy of fabrication because of the complexity of framework design. In this study, it was aimed to clarify the effect of molding angle considered as one of the factors on the shape accuracy of framework fabricated by SLS.
A partial edentulous mandible cast was scanned using dental laboratory scanner. Removable partial denture framework was designed with an Akers clasp on the left first premolar, a ring clasp on the left second molar, a RPI clasp on the right second premolar, and a lingual bar as a major connector <design data>. The framework was fabricated by SLS under two experimental conditions; the molding angle was set to 0 and 45 degrees. Ten frameworks of each conditions were prepared. Heat treatment was performed on the fabricated frameworks at the manufacturer´s recommended temperature. The production data of the framework was captured using a 3D scanner. The design data and the production data of frameworks were then superimposed to compare the shapes of the rest, proximal plate, and the other components of Akers, ring and RPI clasps, center and joining area of the lingual bar by using the 3D data inspection software.
The range of differences for the 0 and 45 degrees conditions were –0.14 to 0.14mm and –0.24 to 0.25mm, respectively. Statistically significant differences were observed at the rest <ring clasp>, the proximal plates <ring clasp and RPI clasp>, connectors, and clasp arms <p<0.05>.
This study showed that the molding angle affects the shape accuracy of the partial denture framework by SLS and suggested that the effect of the setting of support pins depending on the molding angle.

013: Microbicidal effect and storage stability of neutral electrolyzed water-based gels

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Objectives: Electrolyzed waters mainly containing hypochlorous acid have come to be used in dental practice because of their high microbicidal effect. For expansion of their application in dental treatment, we prepared a neutral electrolyzed water-based gel in this study. The prototype gel was examined its microbicidal effect and storage stability to evaluate applicability as an oral care gel. **Methods:** The prototype gel was prepared using with high-concentration neutral electrolyzed water having approximately 1000ppm of available chlorine, distilled water and agar. Seven kinds of test gels were prepared by changing the concentration to 0, 10, 15, 20, 30, 50 and 70ppm. Four microbes, *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans*, were tested. Immediately after preparation, 9.0ml of each test gel was mixed to 1.0ml of bacterial/fungi suspension (2×10^7 cells per ml of phosphate-buffered saline (PBS)), and treated for 3 min. After treatment, 1.0ml of the gel was diluted with PBS, the solution was added to agar culturing media, and incubated. Total number of the surviving bacteria/fungi was calculated from the CFU in the media. As for the gel of 70ppm, the available chlorine concentration was examined during 28-day storage in refrigerator. **Results:** The gel of 15ppm showed significantly higher bactericidal and fungicidal rates (>95% and >80% respectively). As for the gel of 20ppm, the rates were more than 99.999%. Furthermore, no surviving microbes were detected from gels of 30ppm or higher. As for the storage stability, the test gel showed a greater decrease in available chlorine than neutral electrolyzed water during storage. However, it maintained effective available chlorine concentration (>20ppm) having high microbicidal effects during 21-day storages. After 28-day storage, it could maintain more than 15ppm showing enough microbicidal effects. **Conclusions:** It was suggested that the neutral electrolyzed water-based gel has applicability as an oral care gel.

014: A pilot study of effects on Dentifrice Containing Neem for Oral Malodor, Plaque Adhesion, Gingival Inflammation and Oral Bacteria

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Objectives: Azadirachta india (Neem) as an evergreen tree of the genus Azadirachta has been reported about antibacterial and plaque formation inhibitory effects in vitro studies. However, there have been few clinical studies of this dentifrice compared with other dentifrices. The aim of this study was to investigate effects of dentifrice containing neem on oral malodor and antibacterial and plaque inhibitory in vivo as a pilot study.

Methods: Four healthy adults (two males and females) participated in the study. The study was conducted by the crossover method using dentifrices of containing neem, quasi-drug (fluoride and cetylpyridinium chloride containing) and cosmetic were prepared. The subjects were instructed to brush with them at 3 times a day for two weeks. After experimental term, they had a week washout period. At baseline and experimental two weeks after, we evaluated on oral malodor by Breathtron®(YOSHIDA. CO.) and Oral Chroma™(Nissha FIS, Inc.), plaque adhesion (plaque index), gingival inflammation (gingival index, probing pocket depth, and bleeding on probing) and oral bacteria in saliva by Bacterial counter®(PHC Holdings Corp.), Dentocult®-SM and LB(Oral Care. Inc.).

Result: After two experimental weeks, the neem-containing dentifrice showed an improvement tendency in gingival inflammation. On the other hand, the quasi-drug dentifrice showed an improvement tendency in oral malodor. However, there were no significant differences between before and after and 3-experimental groups. This pilot study was carried out by small number of subjects and short test period, so, it should be necessary to increase the number of subjects and the test period in the future.

Conclusion: As a result of using a neem-containing dentifrice for 2 weeks, it may be suggested only an improvement tendency on gingival inflammation, however, there were no significant effects on oral malodor, plaque adhesion, and oral bacteria.

015: Antimicrobial effects and mechanical properties of acrylic resin containing aPIZAS

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Objectives: Denture plaque and poor denture hygiene are associated with stomatitis. They may also serve as a reservoir of potential infectious pathogens, and may contribute to oral malodor. The prevention of microbial adhesion to denture surfaces is essential for maintaining good oral hygiene and preventing plaque accumulation. The purpose of present study was to evaluate the antimicrobial effects and mechanical properties of acrylic resin containing aPIZAS. aPIZAS has been shown to suppress the growth of fungi, bacteria, and algae, and is an antibacterial agent that is used in a wide range of fields. It is also chemically stable and safe.Methods: Acrylic resin disk containing 1.5%, 1.0% and 0.5% aPIZAS were used in this study. Acrylic resin disks containing aPIZAS on mechanical strength was evaluated by Water absorption test and three-point bending test. The antimicrobial effects of aPIZAS and acrylic resin disks containing aPIZAS were performed using *Streptococcus mutans*, *Porphyromonas gingivalis* and *Candida albicans*. The deodorizing activity of acrylic resin disks containing aPIZAS immersed in *P. gingivalis* suspension was also examined.Results: The water absorption, flexural strength and flexural modulus of acrylic resin disks containing aPIZAS were not significantly different from those of acrylic resin disks without aPIZAS. The aPIZAS showed bactericidal activity against *P. gingivalis*, *S. mutans* and *C. albicans*. Bacterial adhesion to the acrylic resin disks containing aPIZAS was significantly lower than those of acrylic resin disks without aPIZAS. Acrylic resin disks containing aPIZAS deodorized methyl mercaptan produced by *P. gingivalis*.Conclusions: Acrylic resin disks containing 1.0% aPIZAS were confirmed to exert antibacterial effects and deodorant effects. Furthermore, the mechanical properties were similar to disks without aPIZAS. These results suggest that acrylic resin disks containing aPIZAS is very useful as a dental material.

016: Effects of S-PRG eluate on bacterial properties related to oral malodor

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Objectives: A multi-ion releasing dental material, surface pre-reacted glass ionomer (S-PRG), is widely used for dental treatments. The S-PRG filler particles are formed by an acid-base reaction between fluoroaluminosilicate glass and polyacrylic acid. The aim of the study was to examine the effects of S-PRG eluate on several bacterial properties, including proteolytic activity, auto-aggregation, interbacterial coaggregation, and volatile sulfur compounds (VSCs) production, which are considered to be related to oral malodor.Materials and methods: The periodontopathic bacteria Porphyromonas gingivalis, Fusobacterium nucleatum and Tannerella forsythia, as well as Streptococcus mutans, S. gordonii, and Escherichia coli were used in this work. In some experiments, protease-deficient mutants of P. gingivalis were also tested. S-PRG eluate was provided by SHOFU INC (Kyoto Japan). The effect of S-PRG eluate on VSCs production by P. gingivalis was assessed using Gas Chromatography. The effects of S-PRG eluate on protease activity was determined by monitoring the degradation of a chromogenic substrate. The effects on auto-aggregation, coaggregation and growth were examined by spectrophotometry.Results: S-PRG eluate inhibited the in vitro production of VSCs. S-PRG eluate inhibited the protease activity of P. gingivalis and boron ion had the strongest suppressive effect. S-PRG eluate induced auto-aggregation of periodontopathic bacteria, while it had few effect on the other bacteria tested. A cell extracts of T. forsythia promoted the growth of P. gingivalis, but the presence of S-PRG eluate suppressed this growth promotion.Conclusions: S-PRG eluate suppressed several properties of P. gingivalis that may interfere with VSCs production. The use of S-PRG may represent a promising strategy to control oral malodor.

017: Evaluation of anti-biofilm effects of bio-active GIC using a bioreactor

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Objectives:
Previously, we reported that the glass-ionomer cement containing BioUnion filler (Caredyne-Restore, GC) demonstrated the effects t o prevent adherence of oral bacteria on its surface due to the release of zi nc ion. Furthermore, we successfully established a new bioreactor system tha t was able to produce biofilm similar to the one formed *in vivo* on re storative materials. The aim of this study was to evaluate inhibitory effect s of Caredyne-Restore against biofilm formation using the new bioreactor.
Methods:
The disc specimens of Caredyne-Restore with a >diameter of 5 mm and a thickness of 1 mm were prepared. A bacterial suspension (approx imately 10^5 CFU/mL) was prepared by diluting bacteria collected f rom human saliva with BHI medium, and dropped at 30 mL/hr onto each specimen fixed in the flow cell of the original bioreactor. The biofilm formed on th e surface was observed and analyzed by using a confocal laser scanning micro scopy with LIVE/DEAD staining. The number of viable bacteria in the biofilm was determined by colony counting. For comparison, a conventional glass-iono mer cement (Fuji VII, GC) and a resin composites (MI Fil, GC) were tested.
Results:
The image analysis revealed that the biofilm formed R>on the Caredyne-Restore was significantly thinner than that of Fuji VII and MI Fil ($p < 0.05$, ANOVA, Tukey´s HSD test). The number of survi ving cells in the biofilm formed on the Caredyne-Restore was significantly l ower than that of Fuji VII and MI Fil ($p < 0.05$). It is considered that the inhibitory effects against bacterial adherence of Caredyne-Restore due to Zn ion-release resulted in less maturation of biofilm on its surface .
Conclusions:
The glass-ionomer cement containing BioUnion fil ler demonstrated the effects to inhibit oral biofilm formation on its surface.

018: Streptococcus mutans inhibition by toothbrush monofilament with surface pre-reacted glass-ionomer

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Objective: Although a toothbrush is the most important self-care dental product, few available have inhibitory effects on oral bacteria. Surface pre-reacted glass-ionomer (S-PRG) filler releases six different ions; fluoride (F-), sodium (Na+), borate (BO33-), aluminium (Al3+), silicate (SiO32-), and strontium (Sr2+), which are known to have antimicrobial activities towards *Streptococcus mutans*, a major pathogen of dental caries. In the present study, monofilaments containing different amounts of S-PRG filler were developed for toothbrushes, with analyses performed to determine their effects on inhibition of *S. mutans*.
Methods: Nylon and polyester monofilaments containing either 20%, 1.4%, or 0% S-PRG filler were prepared. First, each type of monofilament was immersed in sterilized distilled water for 18 hours, then the amounts of the six ions released were measured. Next, each monofilament type was immersed in an *S. mutans* bacterial broth containing 1% sucrose to analyze biofilm formation and bacterial adhesion, after which the monofilaments were removed from the broth and the numbers of *S. mutans* that survived in a dry condition were counted.
Results: As compared to polyester, the nylon monofilaments released larger amounts of ions from the S-PRG filler. Furthermore, bacterial broth with monofilaments containing S-PRG filler showed lower amounts of formed biofilm containing *S. mutans* than broth with monofilaments without the filler. Additionally, *S. mutans* organisms adhered to monofilaments containing S-PRG filler were more easily exfoliated and eliminated in a dry condition as compared to those without the filler. Finally, effects to inhibit *S. mutans* were greater with nylon than polyester monofilaments, whereas no inhibitory effects by either type without S-PRG filler were observed.
Conclusion: Monofilament containing S-PRG filler releases ions that have an inhibitory effect on *S. mutans*. The present findings indicate the effectiveness of this material for use in toothbrushes.

019: Comparison of microbiome organized on denture base materials and hydroxyapatite

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Objectives: Oral microorganisms colonize oral surfaces by forming biofilms. Dentures sometimes act as reservoirs of bacteria in people with insufficient oral hygiene practices. Biofilms on the surface of dentures are thought to increase the risk of aspiration pneumonia in the elderly. However, how oral microbe communities organize into biofilms has yet to be fully clarified. In this study, we investigated the composition of biofilms on denture base materials in vivo.Methods: Thirteen healthy individuals were enrolled in this study. Disk shaped denture material specimens composed of polymethyl methacrylate (PMMA), cobalt-chromium (CoCr) alloy, and hydroxyapatite (HA) were worn by subjects for 48 h attached to an appliance in the oral cavity. The V3-V4 region of 16S rRNA coding sequences from bacteria in the biofilms that formed on the denture materials were sequenced by Mysec. Qiime2 and LEfSe analysis identified the operational taxonomical unit (OTU) in biofilms on each material and characterized the prevenance of the OTUs.Results: The average number of sequence reads per sample was 97,005 ± 91,523. Among the sequences from all samples, 328 OTUs were detected. The α diversity of the sample from CoCr was lower than that of HA; however, the difference was not significant. The composition of the microbiome did not differ at the genus level among the three materials, according to UniFrac analysis. Using LEfSe analysis, 10 genera showed differences between PMMA and HA.Conclusions: This study showed that each denture material supports the formation of a similar microbiome. This result suggests that the choice of material has little effect on the composition of the bacterial flora.

020: ABC transporter activities in biofilm formation by *Streptococcus mutans*

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Objective*Streptococcus mutans*, a biofilm forming bacterium, has been found to possess over 60 ATP-binding cassette (ABC) transporters in gene analysis. ABC transporters have important functions related to gathering nutrients and exporting unnecessary materials, and are considered to be involved in biofilm formation by *S. mutans*. In the present study, we identified ABC transporter-related biofilm formation by the bacterium and analyzed related functions.**Methods**Information regarding the *SMU1520* gene encoding ABC membrane transporters possibly associated with biofilm formation was extracted from a database containing the entire amino acid sequence of the *S. mutans* UA159 strain. To investigate the role of *SMU1520*, we constructed an *SMU1520*-deletion mutant strain (Δ*1520*) of *S. mutans* MT8148. The upstream region of *SMU1520*, an erythromycin cassette gene, and the downstream region were amplified by PCR using specific primers, then those three products were combined and transformed into *S. mutans* MT8148 using a double-crossover recombination method to construct Δ*1520*. To examine bacterial growth, MT8148 and Δ*1520* were grown for 18 hours at 37°C, then inoculated into fresh Todd Hewitt broth at 37°C. Absorbance at 600 nm was measured every 1 hour using a spectrophotometer. Assays of biofilm formation by MT8148 and Δ*1520* were then performed, and biofilm structures were observed using confocal laser scanning microscopy (CLSM).**Results**The growth rate of Δ*1520* was slightly lower than that of MT8148. Furthermore, the quantity of biofilm formed by Δ*1520* was significantly decreased, with a reduction rate of 55%. Additionally, CLSM results demonstrated that biofilm formed by Δ*1520* was more coarse as compared to that formed by MT8148.**Conclusion**The present results suggest that *SMU1520* contributes to bacterial growth and biofilm formation by involving export/import molecules. This study was supported by JSPS KAKENHI grants (20K18757).

021: Inhibitory Effect of Cyclodextran on Glucosyltransferase B in *Streptococcus mutans*

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Objective *Streptococcus mutans*, a primary causative agent of human dental caries, produces three types of glucosyltransferases (GTFs; GTFB, GTFC, GTFD), which synthesize adhesive glucan from sucrose. Among them, GTFB synthesizes the insoluble glucan and has the strongest caries properties. Cyclodextran, a cycloisomaltooligosaccharide (CI) with a structure in which 7 to 12 glucoses are cyclically linked by α-1,6 bonds that has been shown to have inhibitory effects on GTFB, though the mechanism remains to be elucidated. In this study, recombinant GTFB (rGTFB) was used to determine the mode of inhibition of GTFB by CI.Methods Recombinant plasmid pSK6 carrying *gtfB* of *S. mutans* MT8148 was used. *Escherichia coli* XL-2 specimens harboring pSK6 were cultured in Luria-Bertani broth. After harvesting bacterial cells by centrifugation, they were suspended in phosphate buffer, and the suspension was sonicated using an ultra-disrupter. A supernatant of the lysate was obtained by centrifugation, then rGTFB was purified using column chromatography and added to phosphate buffer that included 0-25 mM sucrose and CI at 0% or 0.25%. After 16 hours of incubation, synthesized insoluble glucan was determined based on absorbance at OD550. ResultsThe glucan synthesizing activity of rGTFB reached a plateau when the sucrose concentration was 12.5 mM, both in the presence and absence of CI. In contrast, kinetic analysis showed that the quantity of glucan produced by rGTFB was reduced by addition of CI. ConclusionsKinetic analysis results showed that the glucan-producing activity of rGTFB reached a plateau both with and without CI when the concentration of sucrose was changed. These findings suggest that the inhibitory effect of CI on GTF may be competitive. This study was supported in part by the Fund for Scientific Promotion of Nissin Sugar Co., Ltd., Tokyo, Japan.

022: The effects of fatty acid salts against *Streptococcus mutans* biofilm

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Soap is consisited of several kinds of fatty acid salts. The antimicrobial activity of fattty acid salts against oral bacteria has not been well known. Therefore, we examined the antimicrobial activity of fatty acid salts against oral microorganisms. We used ten species of microorganism (*Streptococus mutans*, *Actinomyces naeslundii*, *Lactobacillus casei*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusbacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*) and nine kinds of fatty acid salts (C4K, C6K, C8K, C10K, C12K, C14K, C18:1K, C18:2K, C18:3K). The antimicrobial activity was examined by minimum bactericidal concentration (MBC) and counting a number of colony forming units (CFU) after treatment with fatty acid salts for 5, 15, 60 minutes. Moreover, cytotoxicity of fatty acid salts on gingival fibroblast was investigated. Additionally, the effect of fatty acid salts against *S. mutans* biofilm was investgated. C12K showed high antimicrobial activity against all oral bacteria and killed at 1.4 mM. Additionally, C18:2K and C18:3K showed high antimicrobial activity against all oral bateria except *A.actinomycetemcomitans*. C8K, C10K, C12K, C14K, C18:1K, C18:2K and C18:3K showed antimicrobial activity against *S.mutans*, *A.actinomycetemcomitans*, *P.gingivalis* after treatment with fatty acid salts for 5 minutes. However, they showed less antimicrobial activity even though they exposed against *C.albicans* for 60 minutes. C4K, C6K and C8K were not observed in the cytotoxic effect on gingival fibroblast at any of concentrations. C10K, C12K, C14K, C18:1K, C18:2K and C18:3K suppressed *S. mutans* biofilm at all concentrations. These results suggest fatty acid salts may be useful for preventing dental caries and periodontal disease and improving oral health.

023: Evaluation of collagen-binding properties of killed Streptococcus mutans strains

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Objective: *Streptococcus mutans*, a major pathogen of dental caries, is regarded as a causative agent of infective endocarditis (IE). Cell surface proteins of *S. mutans*, such as 120 kDa collagen-binding protein (CBP) and 190 kDa protein antigen (PA), have effects on its collagen-binding property and are considered to be involved in IE pathogenicity. In addition, bacterial DNA encoding CBP is frequently detected in heart valve specimens extirpated from IE patients, though live *S. mutans* organisms are rarely isolated. Here, the collagen-binding properties of killed *S. mutans* strains were evaluated, with focus on CBP and PA expression.
Methods: *S. mutans* strains were divided into three groups, CBP+/PA- (n=10), CBP+/PA+ (n=10), and CBP-/PA+ (n=10), then cultured and prepared under 3 different conditions; living, killed by formalin, and killed by autoclave. Next, each strain was added to a 96-well plate coated with type I collagen and cultured at 37 degrees for three hours, then staining with crystal violet was performed. The OD595 value of each *S. mutans* strain was determined, with the value of TW871 (CBP+/PA+) defined as 100%.
Results: As for the living *S. mutans* strains, the average collagen-binding rate in both the CBP+/PA- and CBP+/PA+ groups was approximately 100%, whereas the CBP-/PA+ group showed nearly no collagen-binding ability. When *S. mutans* strains were killed by formalin, the average collagen-binding rate for the CBP+/PA- group was 36.3%, significantly higher than that for the CBP+/PA+ group (12.1%) ($P<0.01$). In contrast, nearly no collagen binding ability was observed in any group after killing by autoclave.
Conclusion: The present results indicate that CBP-positive *S. mutans* strains killed by formalin have a collagen-binding property, with CBP+/PA- strains showing significantly higher collagen-binding than CBP+/PA+ strains. In addition, they suggest that killed CBP-positive *S. mutans* strains without PA expression may have high pathogenicity towards IE.

024: Screening of S. salivarius isolates with anti-caries activities

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Purpose:
To screen for potential probiotic *Streptococcus salivarius* strains with anti-caries activities
Methods:
Tongue and plaque samples were processed for the isolation of *S. salivarius* using culture and the presence of a *S. salivarius* specific gene, gtfk, by PCR. Deferred antagonism assays were next performed to confirm their inhibitory activities against *S. mutans*, *S. pyogenes* and *Micrococcus luteus*. The presence of known bacteriocin genes in these isolates were next studied by PCR using gene-specific primers.
Results:
Two hundred and twenty-nine (229) bacterial isolates were obtained from tongue swabs and plaque of 31 subjects. Among them, 170 (170/229=74%) isolates were determined to be *S. salivarius* based on morphology and the presence of gtfk gene. Seventy-four isolates were found to possess inhibitory activities against the growth of *S. pyogenes* or *Micrococcus luteus*. Only two isolates, 570-P1 and 372-P1, demonstrated inhibitory activities against the growth of *S. mutans* in addition to *S. pyogenes* and *Micrococcus luteus*. Both 570-P1 and 372-P1 contained *salA* but did not carry *salB*, *sal9* and *sldA2* bacteriocins.
Discussion:
570-P1 and 372-P1 are anti-caries *S. salivarius* isolates with potential use as probiotics against dental caries. Although these two isolates contain *salA*, it is known not to have anti-*S. mutans* activities so far. Therefore, it is possible that 570-P1 and 372-P1 produce other bacteriocins that show inhibitory effects on *S. mutans*. Further studies at biochemical and molecular levels, e.g., mass-spectrometric analyses and whole genome sequencing, will be conducted to identify the bacteriocins to understand the molecular regulation of their anti-*S. mutans* activities.

025: Characteristics of anti-periodontal bacterial activity in culture supernatant of probiotic

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Objectives: Recently, the use of probiotics to prevent oral diseases is on focus. However, the probiotic mechanism against periodontal pathogens has not been elucidated. For clarification, we analyzed the characteristics of the active components in the culture supernatant produced by *Lactobacillus fermentum* ALAL020 (Lf020), which has antibacterial activity against *Porphyromonas gingivalis*.Methods: The culture supernatant of Lf020 was heated to 37 °C, 60° C, 100° C and 121° C for 15 minutes to compare the antibacterial temperature sensitivity against the *P. gingivalis* type strain. We also compared the antimicrobial susceptibility to other *P. gingivalis* strains and the inhibitory effect of trypsin-like enzyme (gingipain) activity. Further, we have already reported that the antibacterial components include a cyclic-dipeptide (2016 IADR). Here a synthesized dipeptide was used to test the antibacterial activity against *P. gingivalis* and *Prevotella intermedia* as well as the gingipain inhibitory test.Results: The antibacterial activity of the culture supernatant of Lf020 against *P. gingivalis* was reduced to half by temperature treatment at 37 ° C or higher, but not further even when the temperature was raised. The antibacterial test results with several strains of *P. gingivalis* showed differences in susceptibility. The gingipain inhibitory test suppressed all *P. gingivalis* strains by the Lf020 culture supernatant. The synthetic dipeptide showed antibacterial activity at 5 mg/ml against *P. gingivalis* and *P. intermedia*, but did not have an inhibitory effect on gingipain activity.Conclusions: The culture supernatant of Lf020 was thermostable and effective against all strains of *P. gingivalis*, but there were differences in sensitivity. The synthetic dipeptide showed antibacterial activity at the same concentration as the natural antibacterial dipeptide purified from the culture supernatant of Lf020, but gingipain inhibition was not observed. Therefore, it is likely that other substances suppress *P. ginigvalis* in the culture supernatant of Lf020.

026: Exploring for prevention of periodontal disease using products of the probiotic bacteria, genus Lactobacillus -Targeting the genus Porphyromonas-

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Objectives:There are problems such as allergy and the emergence of drug-resistant bacteria in prevention and treatment with current antibacterial and antiseptic agents against periodontal disease. As one way to overcome them, it was hypothesized that metabolic substances produced by probiotic lactic acid bacteria have the effect of suppressing the growth of periodontal pathogens and suppressing the activity of gingipain (trypsin-like enzyme) and can be used for prevention. However, because lactic acid bacteria produce organic acids, the risk of dental caries was also considered, so we searched for strains which produce effective metabolic products under neutral pH conditions. Methods:The supernatants of 13 test strains of *Lactobacillus* culture in MRS medium (CS) were used in a state of pH7. The minimum inhibitory concentration were determined for three *Porphyromonas* (P. gingivalis, P. salivosa, P. gulae) as a periodontal pathogen. To test the suppression of gingipain (trypsin-like enzyme) activity, each *Porphyromonas* cell extract was mixed with a CS and brought to reaction with BA(P)NA, and measured the absorbance. Results: As a result of the antibacterial activity test, several strains of *Lactobacillus* exhibited growth inhibitory effect on all three *Porphyromonas* genera. When the inhibitory effect on the activity of gingipain, which is one of the main pathogenic factors of *Porphyromonas* was examined, the effect onP. gingivalis was remarkable. Conclusion: For multiple culture supernatants of *Lactobacillus* strains, the usefulness has been confirmed in antibacterial tests. Some of them are different from strains with strong with strong inhibition against gingipain activity, therefore it is considered important to use multiple strains simultaneously in clinical application. Utilizing the supernatant ingredients at a neutral pH as oral probiotics without the risk of caries occurrence could make the suppression of periodontal disease possible.

027: *Gemella haemolysans* specifically inhibits the growth of *Porphyromonas gingivalis*

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A number of bacteria with hemolytic activity to degrade erythrocytes have been reported for serious human infections. However, the characteristics of hemolytic bacteria in saliva have been largely unknown. The purpose of this study is to investigate the characteristics of hemolytic bacteria and clarify the relationship with periodontal disease (PD). Resting saliva was obtained from a total of 15 people, including 10 PD patients and 5 healthy donors. A person with a periodontal pocket with a depth of 4 mm or more was diagnosed as a PD patient. The hemolytic properties of bacteria were investigated through the formation of hemolytic bands by applying saliva on horse blood agar medium and culturing under anaerobic conditions at 37ºC. Identification of hemolytic bacteria was performed based on the 16S rRNA sequence. The proportion of hemolytic bacteria in saliva was carried out by quantitative PCR method. Growth competitive inhibition experiment between bacterial strains was evaluated by culturing under anaerobic conditions at 37ºC. Hemolytic bacteria in saliva were abundant not only in patients with periodontitis but also in healthy subjects. Many of the hemolytic bacteria in saliva are Gemella, and we identified three species of these bacteria, *G. sanguinis, G. haemolysans* and *G. morbillorum*. In addition, *G. haemolysans* was significantly higher in saliva of healthy subjects than in that of periodontitis patients. Furthermore, an experiment of competitive growth inhibition to investigate the relationship between *G. haemolysans* and periodontal-pathogenic bacteria indicated that *G. haemolysans* directly inhibited the growth of *P. gingivalis*. It was revealed that hemolytic *G. haemolysans* is present at a higher ratio in the saliva of healthy subjects compared to PD patients. In addition, the growth of P. gingivalis is directly inhibited by *G. haemolysans*. These results indicate that *G. haemolysans* is an important bacterial species for keeping the oral environment healthy.

028: *Fusobacterium nucleatum* metabolically integrates commensals and pathogens in oral biofilms

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Objectives

Fusobacterium nucleatum, a common oral microbiota constituent in both periodontal health and disease, is well-known for its adhesive properties in oral biofilms; however, metabolic aspects of *F. nucleatum* within polymicrobial communities remain relatively unknown. Previously, we discovered that *Streptococcus gordonii*, an oral commensal, secretes ornithine via an arginine-ornithine antiporter (ArcD), which in turn supports the growth and biofilm development of *F. nucleatum*. Here we introduce another oral commensal, *Veillonella parvula*, further dissect the metabolic interactions mediated by *F. nucleatum* within multi-species consortia and explore their impact on periodontal pathogenesis.

Methods

Intra- and extracellular metabolite changes in *F. nucleatum* when co-cultured with *S. gordonii* (WT or Δ*arcD*) and/or *V. parvula* were examined using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) and ultra-performance liquid chromatography (UPLC). qRT-PCR was used to confirm differential gene expression. Mass spectrometry imaging was performed for polyamine visualization. Biofilm formation was assessed using confocal laser scanning microscopy.

Results

Transcriptional and CE-TOFMS-based metabolomic analyses showed that when co-cultured with *S. gordonii* WT, *F. nucleatum* increased amino acid availability to enhance the production of butyrate and putrescine, a polyamine produced by ornithine decarboxylation. Co-culture with *V. parvula* also increased lysine availability, promoting cadaverine production by *F. nucleatum*. UPLC analyses using an Δ*arcD* mutant confirmed that ArcD-dependent ornithine excretion by *S. gordonii* induces synergistic putrescine production, and mass spectrometry imaging revealed that this metabolic capability creates a putrescine-rich microenvironment inside *F. nucleatum* biofilms. We further demonstrated that polyamines caused significant changes in the biofilm phenotype of a periodontal pathogen, *Porphyromonas gingivalis*, with putrescine being a potent stimulator of biofilm development and dispersal.

Conclusions

Collectively, our results highlight the ability of *F. nucleatum* to induce synergistic polyamine production within multi-species consortia and provide insight into how the trophic web in oral biofilm ecosystems can eventually shape disease-associated communities.

029: Analysis of novel genotypes of Mfa1 fimbriae in *Porphyromonas gingivalis*

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Objectives: *Porphyromonas gingivalis*, a periodontopathogenic gram-negative obligate anaerobic bacterium, generally expresses two types of fimbriae, FimA and Mfa1. Whereas FimA fimbriae are known to have I–V genotypes, the genotypes of Mfa1 fimbriae have not been fully clarified. In recent years, we have analyzed the genotypes of *mfa1* in 84 strains from our laboratory stock and have shown that *mfa1* has at least two different genotypes, namely *mfa70* and *mfa53*. Through a series of analyses, we identified eight strains that could not be classified as either *mfa70* or *mfa53*. In the present study, we performed whole-genome sequencing to classify the genotypes of *mfa1* in these eight strains. Methods: Genomic DNA was extracted from the *P. gingivalis* strains 222, 1436, 1439, B158, EM3, JKG9, JKG10, and Kyudai-3, and sequenced using the Illumina NovaSeq PE150 system. After the raw sequences were trimmed and filtered for quality, the remaining reads were assembled *de novo* using BioBam OmicsBox. Based on the similarity of nucleotide and amino acid sequences, the protein-coding sequences of the strains were compared between the draft genomes using ClustalW. Results: Mfa1 fimbriae were composed of five proteins expressed from the *mfa* cluster (*mfa1–mfa5*) in the type strain of ATCC 33277; however, it was found that the von Willebrand factor-containing protein (VWA) gene corresponding to *mfa5* was present in tandem in the EM3 and JKG10 strains. Notably, VWA was presented alone without *mfa1–mfa4* in the 222 strain. It was also found that only *mfa1* exists independently in the Kyudai-3 strain. Conclusions: We found the diversity of the *mfa* cluster among *P. gingivalis* strains. The genotyping of Mfa1 fimbriae requires sequence analysis to determine the presence or absence of the whole *mfa* cluster, including *mfa1–mfa4* and VWA combination.

030: Role of hyalin-like protein in biofilm formation by *Capnocytophaga ochracea*

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Objectives:*Capnocytophaga ochracea* is a gram-negative rod-shaped bacterium with gliding motility that is a major component of dental plaque biofilms. It has a type IX secretion system that exports proteins, which have gliding motility-associated C-terminal domain. Hyalin-like protein, one of the exported proteins, is a large protein proposed to be involved in quorum sensing according to the Kyoto Encyclopedia of Genes and Genomes, suggesting that this protein may play a role in biofilm formation. In this study, we aimed to investigate the role of *C. ochracea* hyalin-like protein in biofilm formation.Methods: The gene encoding hyalin-like protein and its flanking regions were amplified from *C. ochracea* ATCC 27872 genomic DNA, followed by insertion of the *ermFermAM* cassette into the gene. The resulting fragment was electroporated into *C. ochracea* ATCC 27872 to inactivate the gene by homologous recombination. The hyalin-like protein-deficient mutant was selected and isolated using blood agar plates containing 10 μg/mL erythromycin. The growth rate of the mutant was evaluated by measuring the optical density at 660 nm. The morphology of the biofilm organized by the mutant was evaluated by confocal laser scanning microscopy (CLSM).Results: Insertion of the *ermFermAM* cassette into the target gene was confirmed by PCR using the genomic DNA of the hyalin-like protein deficient mutant, WT-1. The growth rate of WT-1 was almost the same as that of the wild-type strain. The morphology of the WT-1 colony was similar to that of the wild-type strain, but the mutant lost its spreading feature at the edge of the colony. Observations from CLSM indicated that the biofilm organized by the mutant was sparse compared with that organized by the wild-type strain.Conclusions: These data suggest that hyalin-like protein is involved in biofilm formation by *C. ochracea*.

031: The periodontopathic bacterium Fusobacterium nucleatum induced proinflammatory cytokine production by human respiratory epithelial cells and in the lower respiratory organs in mice

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Introduction

Aspiration pneumonia is a major health problem owing to its high mortality rate in elderly people. The secretion of proinflammatory cytokines such as IL-8 and IL-6 by respiratory epithelial cells, which is induced by infection of respiratory bacteria such as Streptococcus pneumoniae (S.p), contributes to the onset of pneumonia. These cytokines thus play a key role in orchestrating inflammatory responses in the lower respiratory tract. Although emerging evidence has revealed an association between aspiration pneumonia and periodontitis, how periodontitis contributes to the onset of aspiration pneumonia remains unclear. Most periodontopathic bacteria are anaerobic and are therefore unlikely to survive in the lower respiratory organs of humans. Hence, we aimed to elucidate whether exposure to heat-inactivated periodontopathic bacteria induces proinflammatory cytokine production by several human respiratory epithelial cells and in the lower respiratory organs and serum in mice.

Materials and Methods

Real-time PCR and ELISA were used to investigate in vitro induction by heat-inactivated periodontopathic bacteria and S.p for mRNA expression and protein production of proinflammatory cytokines by human respiratory epithelial cells. ELISA was also used to determine in vivo induction of cytokine production in the lower respiratory organs and serum of intratracheally heat-inactivated Fusobacterium nucleatum (F.n)-inoculated mice.

Result

Some, but not all, periodontopathic bacteria, especially F.n, strongly induced IL-8 and IL-6 production by BEAS-2B bronchial epithelial cells. In addition, F.n induced IL-8 production by A549 alveolar epithelial cells as well as IL-8 and IL-6 production by Detroit 562 pharyngeal epithelial cells. Furthermore, F.n induced considerably higher cytokine production than S.p. This was also observed in the entire lower respiratory organs and serum in mice.

Conclusion

F.n is a powerful inflammatory stimulant for respiratory epithelial cells and can stimulate cytokine production, thereby potentially contributing to the onset of aspiration pneumonia.

032: IgA Nephropathy-like Lesion Development in Rat Caries Model

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ObjectiveImmunoglobulin A nephropathy (IgAN) is the most frequent type of chronic glomerulonephritis seen worldwide, though its detailed mechanism remains unclear. *Streptococcus mutans* strains with cell surface expression of the collagen-binding protein Cnm are frequently detected in the oral cavity of IgAN patients. In the present study, cnm-positive *S. mutans* was found to be associated with IgAN in a rat caries model.MethodsSpecific pathogen-free Sprague-Dawley rats (males, 15 days old) were inoculated once daily for five consecutive days with 100 µl of a cell suspension containing Cnm-positive *S. mutans* strain SN74R, isolated from the oral cavity of an IgAN patient. Cnm-negative *S. mutans* MT8148R, isolated from the oral cavity of a healthy subject, was administered to control group rats in the same manner. All rats in both groups were provided a powdered caries-inducing diet ad libitum. At 32 weeks after inoculation, the rats were euthanized under ether anesthesia and caries in 12 molar teeth obtained from each were scored. Compositional analysis of collected urine and fluorescent immunostaining of kidney tissues were performed using IgA and C3 antibodies. ResultsThe caries score of the Cnm-negative MT8148R group was significantly higher than that of the Cnm-positive SN74R group (P<0.01). Severe dental caries lesions reaching to the pulp were observed in both groups. The positive rate of hematuria in the Cnm-positive SN74R group was significantly greater than that in the Cnm-negative MT8148R group (P<0.001). Furthermore, immunochemical results demonstrated that the numbers of IgA-positive and/or C3-positive sections were significantly greater in the Cnm-positive SN74R group (P<0.05).ConclusionThe present results suggest that Cnm-positive *S. mutans* strains associated with severe dental caries can trigger an immune response in both kidney and non-kidney tissues, resulting in induction of IgAN-like lesions.This study was supported by JSPS KAKENHI grants (17K11959, 18H03010).

033: Effects of *Helicobacter pylori* Infection in Caries-induced Rats

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Objectives: *Helicobacter pylori* infection, responsible for gastric disease, is considered to occur via the oral cavity and some epidemiological studies have reported that patients with dental caries are more likely to harbor *H. pylori* in the stomach. In the present study, we investigated the effects of *H. pylori* infection on dental caries and stomach condition in caries-induced rats. Methods: Specific-pathogen-free Sprague-Dawley rats (n=28, 18 days old) were divided into 3 groups and fed a caries-inducing diet containing 56% sucrose throughout the experimental period. Group A (n=10) was inoculated with *Streptococcus mutans* strain MT8148R at 1.0×10^8 CFU for 5 consecutive days from 18 days old to induce dental caries, after which 1.5×10^6 CFU of *H. pylori* strain J99 was administered for 5 consecutive days from 75 days old. Group B (n=10) was only administered *S. mutans* at the same timing as Group A, while Group C (n=8) received no bacterial inoculation. All rats were euthanized at 110 days old, and unilateral maxilla and mandibular bones were extirpated. Furthermore, dental caries on occlusal surfaces of molar teeth (6 per rat) were counted by a single dentist. Additionally, stomach tissues were extirpated and stained with hematoxylin and eosin, then deflection of gastric mucosa was scored from 0 to 2 by a single pathologist.Results: The number of dental caries in Group A was greater than that in Group B and significantly greater as compared to Group C ($p<0.01$). Histopathological analysis indicated an average gastric mucosa deflection score in Group A of 1.60, which was significantly higher than that in Group B (0.10) ($p<0.001$) and Group C (0.63) ($p<0.05$).Conclusions: The present findings indicate that *H. pylori* infection in caries-induced rats induces dental caries with greater severity and deflection of gastric mucosa.This study was supported by a JSPS KAKENHI grant (JP 18K17252).

034: Prevalence of Specific Streptococcus mutans Harbored by Non-alcoholic Steatohepatitis Patients

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ObjectiveIn our previous studies, cell surface collagen-binding protein (Cnm)- and 190-kDa cell surface protein antigen (PA)-positive *Streptococcus mutans* strains were shown to induce non-alcoholic steatohepatitis (NASH) aggravation. Here, we assessed saliva samples obtained from nonalcoholic fatty liver disease (NAFLD) patients, including those with NASH and non-alcoholic fatty liver (NAFL), to determine the prevalence of Cnm-and PA-positive *S. mutans* strains, as well as their biological functions. MethodsAfter receiving informed consent for participation, saliva specimens were collected from 40 biopsy-proven NAFLD patients (20 with NASH, 20 with NAFL). Specimens were serially diluted and plated in Mitis-Salivarius agar containing bacitracin, then the number of colonies was determined. Genomic DNA was extracted from *S. mutans* organisms isolated from the plates, then PCR was performed to detect the *cnm*gene encoding Cnm, while PA expression was confirmed by western blotting. Collagen binding ability of the bacterial strains was determined using type IV collagen. ResultsThe number of *S. mutans* in saliva specimens from NASH patients was significantly higher ($P<0.05$), and the percentage of those with NASH who possessed Cnm- and PA-positive strains significantly greater ($P<0.05$), as compared to specimens from NAFL patients. In addition, the type IV binding ability of strains possessed by the NASH patients was significantly greater as compared to the NAFL patients ($P<0.05$). On the other hand, the number of platelets and adiponectin level were significantly lower in NAFLD patients with Cnm- and PA-positive *S. mutans* ($P<0.05$) as compared to NAFLD patients with *S. mutans* negative for both. Similarly, adiponectin level was significantly higher in NASH patients with Cnm- and PA-positive *S. mutans* as compared to NASH patients with *S. mutans* negative for both ($P<0.05$). ConclusionCnm- and PA-positive *S. mutans* strains were frequently detected in NASH patients saliva samples, suggesting that those specific strains are associated with NASH aggravation.

035: The Initiation and development of oral microbiome formation in Japanese healthy infants

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Objectives: With the advent of next-generation gene sequencing, recent studies have advanced current knowledge regarding the microbiota formed in various tissues and organs of the human body. However, few studies have investigated changes in the microbiota of the same infants over a long period of time. The aim of this study is to elucidate how the intraoral microbiota is established in newborns.Methods: The present study was performed following the approval of the Ethics Committee of our college. Saliva and tongue-swab samples of 5 infants had been collected on the day of birth, then at 1 week, 1, 3, 6, and 9 months, and 1 year after birth, and then every 6 months afterwards. Specimens were also collected from their parents when the subjects were 1 year old. Microbiome analysis of the V3-V4 region of bacterial 16S rRNA was performed and sequence reads matching the reference sequence on SILVA database were explored to estimate the lowest possible class. In regard to the richness and diversity of microbiomes, the observed OTUs and Shannon Index were calculated and compared among samples. Results: The relative abundances of Streptococcus were remarkably high in samples collected immediately after birth, but were decreased gradually. On the other hand, the relative abundance of Haemophilus and Rothia was significantly higher at 2 years of age than at birth in lingual swab specimens. It is noteworthy that a PCoA plot and phylogram based on unweighed Unifrac distance showed that the microbiota was evidently diverted in first 2 year of life. No significant difference was observed between the 2-year-olds and adults in regard to the microbiota at genus level.Conclusions: From our findings, lifestyle and dietary habits from birth to 2 years may exert great effects on the risk of dental caries, periodontal disease, and diseases assocated with the oral microbiota in later life.

036: Omics analysis defines differences in microbial community structure between peri-implantitis and periodontitis

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Objectives
Peri-implantitis shows faster progression and less responsiveness to conventional periodontal treatment than periodontitis. This study aimed to reveal that disease-specific species and transcriptional activity lead to disease-specific clinical features using an integrated 16S rDNA sequencing, metagenomic, metatranscriptomic, and network analysis.
Methods
Subgingival plaque samples were collected from 23 subjects with both peri-implantitis and periodontitis sites. Microbial DNA was extracted from each plaque sample and evaluated for microbial species and gene composition by 16S rDNA sequencing and metagenomic analysis. Metatranscriptomic data that we previously reported was also used to evaluate active species and gene activities. The sequences of 16S rRNA region were assigned to the Human Oral Microbiome Database to identify the taxonomic profile. The sequences of mRNA region were assigned to several virulence factor databases, non-redundant sequencing database, and pathway database to identify the microbial function of each disease. The activities of each species and gene were calculated by the comparison of read abundances based on DNA and RNA. The correlations based on the read abundance between each species were evaluated by the sparse correlations for compositional data algorithm program. The microbial co-occurrence networks of peri-implantitis and periodontitis were visualized with Cytoscape.
Results
The highly active species were *Peptostreptococcus stomatis* and *Solobacterium moorei* in peri-implantitis, and *Fusobacterium nucleatum* subsp. *vincentii* and *Peptostreptococcus stomatis* in periodontitis, respectively. The microbial co-occurrence network revealed *Prevotella denticola* and *Solobacterium moorei* had high activity and were significantly correlated in peri-implantitis, whereas none of the species showed significant correlation in periodontitis. Linear discriminant analysis effect size indicated that plr/gapA genes which were microbial virulence factors were significantly enriched in peri-implantitis.
Conclusion
Prevotella denticola and *Solobacterium moorei* were identified as core species specific to peri-implantitis. In addition, plr/gapA genes were putative virulence factors related to the cause of faster disease progression in peri-implantitis than that in periodontitis.

037: Periodontal Bacteria Distribution in Child with Early Primary Tooth Loss

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ObjectiveChildren with early primary tooth loss often have a genetic disease or chromosomal abnormality, which rarely occur in those with no systemic disorder. Periodontal disease with loss of teeth is known to be associated with the presence of subgingival periodontopathic bacteria. In this study, a child with subgingival periodontopathic bacteria but no systemic disorder who showed early primary tooth loss is reported.MethodsA 7-year-old girl was referred to our clinic with severe mobility of multiple teeth. Gingival crevicular fluid (GCF) had been collected from the deepest pockets of both the patient and her mother with paper points. Bacterial DNA was extracted from those specimens and PCR analyses performed for detection of 10 major periodontal pathogens using specific primers. After culturing the samples in blood agar medium, isolated colonies were identified using sequence analysis. ResultsPeriodontal pockets for most mandibular teeth in the patients were 4 mm or greater. Periapical radiography revealed prominent vertical bone resorption in the left first and second primary molar areas. Blood test results supported findings of a systemic inflammatory condition, though no immunological abnormality was seen. PCR analyses of specimens from child and mother detected seven periodontal pathogens in both, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella nigrescens*, *Campylobacter rectus*, *Aggregatibacter actinomycetemcomitans*, *Capnocytophaga ochracea*, and *Capnocytophaga sputigena*, with 2 additional species detected in the mother. Among the isolated bacteria, we observed orange complex (*Prevotella intermedia*, *Campylobacter gracilis*, *Streptococcus constellatus*; 36.6%), green complex (*C. sputigena*; 3.3%), yellow complex (*Streptococcus mitis*, *Streptococcus anginosus*; 36.6%), blue complex (*Actinomyces naeslundii*; 20.0%), and purple complex (*Veillonella parvula*; 3.3%) species.ConclusionThese results suggest that premature tooth loss is related to growth of orange complex periodontopathic bacteria. In addition, mother-to-child transmission of periodontal pathogens is a possible factor for the onset and development of aggressive periodontitis identified in this case.

038: CTLA-4 Reduces Bone Resorption Through the Inhibition of Osteoclast Differentiation

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Objectives: Cytotoxic T lymphocyte associated antigen-4 (CTLA-4) is one of the immune checkpoint receptors expressed on the surface of T cells. It has been reported that CTLA-4 administration suppresses bone resorption as well as inflammation in arthritis model mice. However, information is limited on the effects of CTLA-4 on alveolar bone. The purpose of this study is to investigate the role of CTLA-4 on bone resorption in periodontitis.
Methods: Periodontitis was induced by placing silk ligature on the second molar of C57BL/6 mice. To investigate the effects of CTLA-4, CTLA-4 immunoglobulin fusion protein (CTLA-4-Ig) was administered intraperitoneally. The effect of CTLA-4 on alveolar bone resorption was evaluated by μCT and H-E staining, and the effect on osteoclast differentiation was evaluated by TRAP staining. In vitro, RAW 264.7 cells were treated with RANKL and CTLA-4-Ig and the number of osteoclast-like cells was counted. The expression levels of osteoclast differentiation markers (C-fms, Carbonic anhydrase II, and Cathepsin K) and signaling molecule downstream of CTLA-4 (PP2A) were assessed by qRT-PCR.
Results: CTLA-4 (50 μg/kg) significantly suppressed alveolar bone resorption (65% on the buccal side and 47% on the palate side, $p<0.01$) and decreased the number of TRAP-positive osteoclast-like cells (53%, $p<0.05$) in periodontitis model mice. In vitro, the treatment with CTLA-4 (300 μg/ml) significantly decreased the mRNA expression of C-fms, CaII, and Cat-k (77%, 69% and 41%, respectively, $p<0.001$) and the prevalence of osteoclast-like cells (36%, $p<0.001$), when compared to the RANKL-treated control. The expression level of PP2A was increased (1.4-fold, $p<0.01$) by the treatment with CTLA-4 (300 μg/ml).
Conclusion: It is suggested that CTLA-4 reduces alveolar bone resorption through the inhibition of osteoclast differentiation via activation of PP2A at the site of periodontitis.

039: An immunohistochemical study on the expression of bioactive molecules in the mouse kidney in *P. gingivalis* LPS-induced diabetic nephropathy

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Diabetic nephropathy is a serious complication of diabetes mellitus, which is caused by glomerulosclerosis with renal failure based on the dysfunction of glomerular capillaries. We recently reported that the glomerular endothelium in the glomeruli of diabetic mice expresses toll-like receptor (TLR)2 and TLR4, and that *Porphyromonas (P.) gingivalis* lipopolysaccharide (LPS) induces diabetic nephropathy with urinary protein and the accumulation of type 1 collagen, IL-6, TNF-α, and TGF-β in glomeruli. This study aims to immunohistochemically investigate the expression of leukocyte adhesion molecules, and renal physiologically active molecule FGF23 and SARS-CoV-2 receptor ACE2 in the glomeruli of *P. gingivalis* LPS induced-diabetic nephropathy model mouse. All of the diabetic mice subjected to repeated *P. gingivalis* LPS administrations were euthanized within the survival period of all diabetic mice not administered LPS and within the survival period of all of the LPS-administered non-diabetic mice. The VCAM-1, E-selectin, FGF23, and ACE2 were overexpressed in the glomeruli of *P. gingivalis* LPS induced-diabetic nephropathy in mice although these molecules were not found in LPS-administered normal kidney and non LPS-administered diabetic mice. It is thought that the *P. gingivalis* is one of the critical factors in the diabetic patients.

040: Artepillin C regulates extracellular matrix gene expressions in human periodontal fibroblasts by DNA methylation

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Objectives:
Artepillin C is the major component in Brazilian green propolis. As propolis exhibits antibacterial, antifungal, and antiviral properties, it is often used for the prevention of periodontal diseases. However, the mechanism through which propolis affects the periodontal tissue is still unknown. To elucidate the effect of artepillin C on periodontal tissue, we analyzed the expression level and DNA methylation status in human periodontal ligament fibroblasts (HPDLFs) upon artepillin C treatment.
Methods:
The culture of HPDLFs was repeated alternating 3 days with artepillin C (25 μM/ml, WAKO) and 3 days without artepillin C in DMEM containing 10% FBS for 1 month. Untreated samples were used as controls. We then performed microarray (Agilent technology) and quantitative RT-PCR analyses. The expression profile of the genes analyzed through microarray was classified by functional annotation with keyword analysis in Database for Annotation, Visualization, and Integrated Discovery (DAVID). The selected relevant genes were further analyzed for their DNA methylation status in the promoter regions through methylation-specific PCR.
Results:
Overall, 370 (1.5-fold) upregulated genes and 377 (0.66-fold) downregulated genes were identified upon artepillin C treatment via microarray analysis. Among these, 18 genes belonging to the extracellular matrix were significantly correlated in the keyword analysis in DAVID. Within the extracellular matrix genes, HSPG2 and COL5A3 exhibited significantly altered gene expression patterns and DNA methylation levels.
Conclusions:
These results indicate that artepillin C is responsible for the altered methylation status in the promoter region, resulting in differences in the expression patterns of the extracellular matrix-related genes.

041: Exosomes from human gingiva-derived MSCs inhibit periodontal bone loss

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Objectives: MSCs are known to exhibit anti-inflammatory function besides its multipotency. MSCs from gingiva (GMSCs) are easier to isolate, and appear to secret higher amounts of exosome than other MSCs. Our previous study demonstrated that exosomes from gingiva-derived MSCs (GMSCs) were capable of accelerating wound healing capacity by inducing anti-inflammatory (M2) macrophage polarization, and TNF-α preconditioning further enhanced these effects. In this study, to further gain insight into GMSC-based tissue regeneration, we examined the preventive effect of TNF-α preconditioned-GMSC-derived exosomes against periodontal bone loss and underlying molecular mechanisms.MethodsGMSCs were isolated from human gingival tissues under the approved Institutional Review Board (IRB) protocol at Kyushu University Hospital (2019-374). GMSC-derived exosomes were isolated from the serum-free conditioned media using the MagCapture Exosome Isolation Kit PS. The effect of GMSC-derived exosomes on inflammatory bone loss were examined by ligature-induced periodontitis model in mice under an institutionally approved animal research protocol (Kyushu University, A21-044-1). TNF-α inducible exosomal miRNAs were analyzed by miRNA microarray, and the mechanisms by which inhibitory effect of RANKL expression induced by exosomal miRNA was investigated in LPS-stimulated periodontal ligament cells.ResultsLocal injection of GMSCs-derived exosomes significantly reduced periodontal bone resorption and the number of TRAP-positive osteoclasts in mice, and these effects were further enhanced by preconditioning of GMSCs with TNF-α. GMSCs-derived exosomes reduced expression of RANKL mRNA in mice gingival tissue, while the expression of OPG mRNA was significantly up-regulated. Microarray analysis identified that miR-1260b was one of the most highly up-regulated miRNAs by TNF-α-preconditioning. RANKL expression was regulated by Wnt5a in PDLCs, and exosomal miR-1260b was found to be essential for the inhibition of osteoclastogenesis by targeting the Wnt5a-mediated RANKL pathway.ConclusionsBesides its anti-inflammatory effect, exosomes from TNF-α-treated GMSCs directly prevent periodontal bone loss via miR-1260b-mediated inhibition of osteoclastgenesis.

042: PPARγ is required for periodontal ligament cells to retain cemento/osteogeneity

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Objective: Periodontal ligament tissue acts as a principle cell reservoir when periodontal regeneration therapy has been conducted. Periodontal ligament (PDL) cells actively express osteogenic genes in vitro and in vivo. Thus far, accumulating articles disclose the importance of cell-matrix connection, ligand binding to plasma membrane receptors, and extracellular cellular matrix stiffness, which directly controls the fate and differentiation stage of PDL cells. Recently, intermediate products of biochemical reactions such as TCA cycle, fatty acid β-oxidation, and respiratory chain have been identified as modulator of cellular differentiation. However, it is still unclear whether metabolic pathways involve in the retention of osteogenic capacities in PDL cells. Here, we explore the roles of PPARγ, a nuclear receptor and takes pivotal roles in regulating energy metabolism, for osteogenic capacities of PDL cells.Materials and methods: PPARγ expression was suppressed by 5 independent siRNAs in human PDL (hPDL) cells. hPDL cells were cultured in mineralization induce medium (ascorbic acid and β-glycerophosphate) and cementogenic/osteogenic differentiation was assessed by ALP activity and calcium deposition identified by alizarin red S staining. RNA and anti-PPARγ precipitated chromatin were obtained from siRNA-transfected hPDL cells for RNA-seq and ChIP-seq analyses, respectively.Results: Suppression of PPARγ expression induced decreased ALP activity and suppressed calcium deposition compared with control siRNA-transfected cells. Gene Ontology analyses of RNA-seq revealed that suppressed PPARγ resulted in down-regulation of the genes associated with osteogenic differentiation and metabolic process and up-regulation of the genes associated with inflammatory responses. ChIP-seq disclosed PPARγ binding to known target sites adjacent to metabolic gene loci, however local chromatin area close to osteogenic gene loci was not enriched. Conclusion: PPARγ is indispensable for hPDL cells to possess osteogenic capacities and PPARγ might indirectly regulate osteogenic gene expression possibly through modulating metabolic pathways and their intermediate products.

043: Role of IQGAP1- Flightless I interaction in collagen remodeling by fibroblast

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Objectives: Periodontal connective tissue matrices undergo continuous remodeling to maintain homeostasis. Physiological remodeling is dependent on the degradation of collagen fibers and the phagocytic pathway. A multidomain, small GTPase activating protein IQGAP1 is thought to be involved in the generation of cell extensions through the interaction of cdc42 and the actin binding protein Flightless I (FliI). Here we examined the effect of IQGAP1-Flightless I interaction in collagen remodeling through its impact on the generation of cell extensions and in collagen phagocytosis.Methods: Human gingival fibroblasts (HGFs), IQGAP1+/+ and IQGAP1-/- mouse embryonic fibroblasts were examined by immunoblotting, immunostaining and immunoprecipitation. Collagen-coated beads were used for immunostaining and collagen internalization assay. For the collagen degradation assay, cells cultured in three dimensions on collagen gel were used. Stained cells were observed by confocal microscopy and quantified.Results: IQGAP1 was strongly expressed by HGFs and was localized to vinculin-stained cell adhesions and sites where cell extensions were initiated, which were co-localized with FliI. Immunoprecipitation showed that IQGAP1 associated with FliI. HGFs showed 10-fold increases of collagen binding, 6-fold higher internalization, and 3-fold higher β1 integrin activation between 30 and 180 min after incubation with collagen. Compared with IQGAP1+/+ fibroblasts, deletion of IQGAP1 reduced collagen binding (1.4-fold), collagen internalization (3-fold), β1 integrin activation (2-fold), and collagen degradation (1.8-fold).Conclusions: We conclude that IQGAP1 affects collagen remodeling through its regulation of phagocytic degradation pathways of fibroblast, which may involve the interaction of IQGAP1 with FliI.

044: Amelogenin down-regulates MHC class II antigen presentation on macrophages.

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Enamel matrix derivatives (EMDs)-based regenerative therapy is suggested to promote better healing with minimal inflammatory response after periodontal surgery. We previously found stimulation of monocytes with amelogenin suppresses major histocompatibility complex class II (MHC II) gene expression using microarray analysis. However, the detailed molecular mechanisms for this process remain unclear. Here, we aimed to understand the underlying mechanism and its effects on immune system.THP-1 monocytic cells were stimulated with 50 μM PMA. The cells were pretreated with 10 μg/ml amelogenin for 24 hours, followed by the stimulation with 2.5 μg/ml IFNγ for 24 hours. The cell surface expression of antigens was evaluated by flow cytometry, and signal transduction pathway was analyzed by real-time PCR and western blotting. Cellular uptake of amelogenin was observed by using confocal laser microscopy. Histone modification of class II transactivator (CIITA) promoter region was analyzed by chromatin immunoprecipitation. T cell activation was evaluated by mixed leukocyte reaction (MLR). Amelogenin down-modulated IFNγ-induced cell surface expression of MHC II and this effect was widely conserved across species, as similar effect was confirmed in raw 264.7 murine macrophages. Ameloginin accumulated in the nucleus as early as 15 min following stimulation and inhibited subsequent CIITA expression. Reduced MHC II expression on macrophages pretreated with amelogenin down-regulated the expression of T cell activation markers CD25 and CD69, T cell proliferation, and IL-2 production by allogenic CD4+ T lymphocytes in MLR. H3K27ac and H3K4me3 in CIITA promoter IV region, which are essential for conversion to euchromatin, were markedly suppressed by amelogenin.Amelogenin suppresses MHC II expression by altering chromatin structure and inhibiting CIITA promoter IV transcription activity and attenuates subsequent T cell activation. Therefore, clinically observed better wound healing after the surgery may be, at least in part, explained by the mechanism elucidated in the current study.

045: Adipose specific C-C motif chemokine ligand (CCL) 19 overexpression drives the mice to both insulin resistance and weight gain.

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Objectives: The pathophysiological features of overweight and/or obesity are adipose tissue inflammation and infiltration of activated immune cells, such as macrophages. This results in the increased production of adipokines secreted by adipocytes, as well as additional inflammatory cytokines and chemokines. We previously reported that co-culture of adipocytes and endotoxin-stimulated macrophages significantly increases the expression of C-C motif ligand 19 (CCL19), suggesting that periodontal bacteria-activated circulating monocytic cells may further enhance adipose tissue inflammation upon migration into adipose tissue via its receptor, C-C chemokine receptor type 7 (CCR7). Here, we investigated the effects of CCL19/CCR7 pathway on subsequent metabolic disorders potentially enhanced by periodontal inflammation.MethodsAdipocyte-specific Ccl19 knock-in (KI) mice were generated, and the mice were fed either a normal diet or 40% or 60% fat-diet (FD) to investigate the effects of CCL19 on the induction of inflammation and lipid metabolism.ResultsCcl19 KI mice exhibited increased inflammatory signs in the adipose tissues and enlarged subcutaneous white and brown adipose tissue as compared with wild-type (WT) mice. Ccl19 KI mice were characterized by increased extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation and decreased AMP-activated protein kinase α (AMPKα) phosphorylation in the adipose tissue. Peroxisome proliferators-activated receptor γ coactivator 1α (PGC1α) and uncoupling protein 1 (UCP1) protein expression was significantly reduced in brown adipose tissue of Ccl19 KI mice compared to that in WT mice. These changes were most marked when KI mice were fed a 40% FD, which resembled western style diet. ConclusionsActivation of CCL19/CCR7 pathway in adipose tissue inhibited AMPKα through activating ERK1/2, resulting in impaired lipid metabolism and energy regulation. Thus, subjects preferring western style diet, when accompanied by severe periodontal disease, may suffer from resistance to diet.

046: Characterization of the *PLAP-1-GFP/CreER* knock-in mouse

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Objectives:
Periodontal ligament (PDL) is the fibrous connective tissue between the alveolar bone and cementum. The function of PDL cells was studied extensively in vitro, however, the molecular regulations for homeostasis of PDL in vivo remain elucidative. The inducible Cre-lox system is a valuable tool to study the regulation of the tissue homeostasis in a spatial and time-restricted fashion. We have previously found that PLAP-1/Aspn is highly and specifically expressed in PDL. In this study, we aimed to generate and characterize a mouse line that expresses both green fluorescent protein (GFP) and tamoxifen-inducible Cre recombinase from endogenous PLAP-1 locus.
Methods:
A targeting construct was created that inserts the CreERT2-2A-EGFP-WPRE cassette into the endogenous start codon of the PLAP-1 gene. The construct was electroporated into C57BL/6 embryonic stem (ES) cells, and correctly targeted ES cells were injected into C57BL/6 blastocysts, which were implanted into pseudo-pregnant females. The resulting chimeric mice were bred with C57BL/6, and offspring were analyzed. EGFP expression in periodontal tissue was compared with PLAP-1 endogenous mRNA expression by RNAscope in situ hybridization. Further, the knock-in mice were crossed with Rosa26-tdTomato Cre reporter mice to label PLAP-1 lineage cells. After the development of the periodontium was completed, the mice are treated with Tamoxifen.
Results:
Newly established PLAP-1-GFP/CreER knock-in mouse expressed GFP in most spindle-shaped fibroblastic cells but not in osteoblasts, cementoblasts, and blood vessels in PDL. These signals were matched to in situ expressions of PLAP-1 mRNA expression. Upon crossing with Cre reporter mice and 3-day after tamoxifen treatment, tdTomato expression was matched to GFP expression.
Conclusions:
The fluorescent reporter and lineage tracing analysis showed faithful expression of GFP/CreER under the PLAP-1 promoter in the knock-in mouse. This method can be a valuable tool to i) trace the fate of PDL cells in vivo by crossing with Cre reporter, ii) study gene functions in PDL by crossing with mice with the floxed allele of the targeted gene, and iii) isolate pure PDL cells according to their GFP expression.

047: Salivary Alpha-Amylase as a stress marker during dental implant surgeries

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Objectives: The activity of salivary Alpha-Amylase (sAA) has been thought of as an indicator of mental stress. However, it has not been studied the relationship between sAA levels and mental stress of patients during dental implant treatment. In the present study, we examined the correlation between sAA levels and vital signs changes of the patient during dental implant surgeries.Methods: The total number of patients examined in this study was 44 including 19 for installing dental implants, 22 for second surgery, and 3 for connective tissue graft (17 males and 27 females). Saliva samples were collected before and after surgery to evaluate sAA levels. The heart rate and blood pressure were measured at 3 time points: before, during and after the implant surgery. Data were collected and the correlation between sAA and vital signs were statistically analyzed (α=0.05). Results: Nine patients had high levels in both sAA activity and blood pressure (6 for installing implants and 3 for second surgery) while 19 patients had normal sAA activity but high blood pressure (7 for installing implants and 12 for second surgery). Additionally, 19 patients had high sAA activity but normal blood pressure after surgery. Eight patients had normal BP and sAA. Statistically there is no significant correlation between blood pressure and sAA activity levels before surgery (rs=-0.012, p=0.951) and after surgery (rs=-0.019, p=0.640).Conclusions: sAA activity tend to increase in the after surgery together with the patient’s stress level.

048: Regional differences in the density of Langerhans cells, CD8-positive T lymphocytes and CD68-positive macrophages

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To provide a better understanding of the local immune system in the face and external genitalia, i.e., the oral floor, lower lip, palpebral conjunctiva, anus and penis, we examined the distribution and density of CD1a-positve Langerhans cells, CD8-positive suppressor T lymphocytes and CD68-positive macrophages using specimens from 8 male elderly cadavers.We examined the distribution and density of CD1a-positve Langerhans cells, CD8-positive suppressor T lymphocytes and CD68-positive macrophages using specimens.Most sections were stained with hematoxylin and eosin (H&E), whereas others were used for immunohistochemistry. The primary antibodies used were (1) mouse monoclonal anti-human CD1a (1:100, Dako N1616, Dako, Glostrup, Denmark); (2) mouse monoclonal anti-human CD8 (1:100, Dako N1592); and (3) mouse monoclonal anti-human CD68 KP1 (1:100, Dako M0814). Pretreatment autoclaving was conducted in accordance with the manufacturer's instructions. The secondary antibody (Dako Chem Mate Envison Kit, Dako) was labeled with horseradish peroxidase (HRP), and antigen-antibody reactions were detected via the HRP-catalyzed reaction with diaminobenzidine.Observations and photography were performed using a Nikon Eclipse 80 (Nikon, Tokyo, Japan). The field corresponding to a circle 0.8 mm in diameter contained an almost 1-mm surface length of slightly curved epithelium.The density of suppressor lymphocytes showed a significant correlation between the oral floor and the lip (r=0.78).In addition, the density of either suppressor lymphocytes or macrophages showed a weak correlation between the conjunctiva and the penis (r=0.59). At each of the five sites, there was no evident correlation between the density of Langerhans cells and that of coexisting macrophages or suppressor T lymphocytes.The present observations suggest that the local immune response is highly site-dependent, with a tendency for tolerance rather than rejection.This study was received December 01, 2014; and accepted in August 19, 2015, Anatomy & Cell Biology. In 2018, Japanese article was accepted in the journal of the Tokyo Dental College Society (No.118). The research was also presented in 23rd Japanese Academy of MaxilloFacial Implants, Dec.2019.

049: Delayed Wound Healing after Tooth Extraction under Malnutrition

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Objectives: Malnutrition causes delay in wound healing after tooth extraction. Children in developing country and elderlies even in developed country have risk of this situation. However, it is unclear how inflammation and malnutrition relate each other. High mobility group box-1 protein (HMGB1) is one of the damage-associated molecular patterns, which stimulate inflammasome, then inducing inflammatory response such as secretion of cytokine interleukin-1B (IL-1B). This is an essential response to initiate wound healing. We hypothesized that malnutrition may interfere this cascade, causing abnormal inflammation and ultimately delaying wound healing.
Methods: We employed tooth-extracted mice under malnutrition condition by low-casein diet by comparing to normal diet-fed mice. Malnutrition condition was confirmed by observing weight and triglyceride, albumin, glucose, and total cholesterol in serum after 14-days feeding. In the tooth-extracted socket after 3 and 7 days, the wound tissue was observed by hematoxylin-eosin staining, and analyzed several factors in inflammation-regeneration lineage such as IL-1B and other inflammatory and regenerative related cytokines, mesenchymal stem cells and Th2 cells, myeloperoxidase activity, HMGB1, and ATP, by using RT-qPCR, flow cytometry, *in vivo* molecular imaging analysis system, ELISA, immunohistochemistry, and ATP-dependent glycerol-3-phosphate generation, respectively.
Results & Discussion: Under the malnutrition condition, delayed wound healing was observed with decrease of mRNA accumulation of cytokines for regeneration and stem cells accumulation. Myeloperoxidase and mRNA accumulation of IL-1B were decreased at Day3, but obviously increased at Day7. ATP increased at Day7 than Day3. The accumulation of Th2 cells increased at Day3. HMGB1 increased significantly at Day7. We speculate that in the early stage of inflammation, less ATP and Th2 cells accumulation inhibit the secretion of IL-1B. While in the later stage, the up-regulation of ATP and the significant increase of HMGB1 promote the secretion of IL-1B, which may interfere inflammation resolution and wound healing.
Conclusion: These results suggest that malnutrition alters HMGB1-related inflammation associated with delayed wound healing after tooth extraction.

050: Assessment of Facial Changes after Orthognathic Surgery Using Geometric Morphometrics

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Objectives:Orthognathic surgery results in significant changes in facial profile. Assessment of these changes is difficult due to inter-individual morphological variations. Therefore, Geometric morphometrics is a powerful tool to isolate surgery-induced changes from the normal variation among patients. Our aim in this research is to evaluate changes of the facial soft tissue after orthognathic surgery using geometric morphometrics analysis.Materials & Methods:This study included twenty-seven patients who underwent bilateral sagittal split ramus osteotomy (BSSRO), as a set back surgery. Two CT images were taken from each patient, the first was before surgery (T0), and the second was six months after surgery (T1). Twenty-seven CT images from volunteers with Skeletal Class I were also taken as the control. Three dimensional models were created in 3D modelling software (Mimics; Materialize). These models were imported into 3D surface analyzing software (HBM-Rugle; Medic Engineering) to perform geometric morphometrics analysis and conduct principal component analysis (PCA). PCA measurements were compared using ANOVA test as a statistical analysis.Result:Among fifteen principle components, there were significant differences between T0, T1 and control at the first and second principal components. The first principal component represented a variation in the lower anterior facial height, while the second principal component represented a variation in the anterior-posterior position of chin. The comparison between preoperative group and postoperative group clearly illustrated significant surgical effect of BSSRO among patients with Skeletal Class III. This effect is demonstrated by decrease of lower facial height, and movement of chin and lower lip area toward backward. The surgical effect of BSSRO was suboptimal when postoperative group was compared with control group.Conclusion: Although the patients who underwent BSSRO had significant improvement in the facial profile after surgery, the geometric morphometric analysis detected that additional procedures can be considered to obtain the optimal facial profile.

051: Metformin inhibits oral cancer cell growth by altering glucose metabolism

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Objective
Glucose metabolism is crucial for tumor growth and closely associated with the response to tumor treatment. Metformin, a traditional anti-hyperglycemia drug, has been demonstrated to be beneficial for many malignancies. However, the relationship between its anticancer effect and altered glucose metabolism remains elusive in oral cancer. In the present study, we aim to elucidate the effect of metformin on glucose metabolism of normal cell and oral squamous cell carcinoma cell lines.
Methods
Normal cell (HaCaT) and cancer cells (HSC-2 and HSC-3) were grown with metformin for 4 days and the cell number was determined by cell counting. Cells, treated with or without metformin, were obtained at the logarithmic growth phase and then the real-time monitoring system was applied to detect acid production (lactic acid from the glycolysis and bicarbonic acid from the citric acid cycle) from glucose in a fixed pH environment (pH 7.5).
Results
The cell growth of HSC-2 treated with 1mM metformin was significantly inhibited by 33.1% and 32.2% at 72h and 96h, respectively ($p<0.01$). However, metformin could not inhibit the cell growth of HaCaT and HSC-3. Consistent with the effect of metformin on cell growth, 1mM metformin suppressed the rate of acid production from glucose by 44.6% in HSC-2 ($p<0.05$) but not in HaCaT and HSC-3.
Conclusion
Our results suggest that metformin inhibits the cell growth of HSC-2 by suppressing the acid production from glucose. But metformin shows heterogeneous effect on oral cancer cell lines, which is associated with the altered glucose metabolism. Moreover, monitoring acid production might be used to evaluate the efficacy of metformin. The further study will be required to elucidate the underlying mechanism caused by metformin on glucose metabolism in oral cancer.

052: *Sirtuin1* DNA hypermethylation may be a biomarker for malignant transformation

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SIRT1 has been identified as playing a role in the maintenance of epithelial integrity and its alteration is often related to carcinogenesis. However, the methylation and transcription status of *SIRT1* in oral cancer has not been investigated. We examined the methylation levels of *SIRT1* in oral cancer tissues of betel quid (BQ) chewing and non-BQ chewing patients, and investigated whether the methylation level reflects the transcription level in an *in vitro* model. In addition, we examined *SIRT1* methylation status in smear samples of macroscopically healthy buccal mucosa from individuals with a habit of BQ chewing.The DNA methylation level of *SIRT1* in the tissue samples was analyzed using methylation-specific PCR (qMSP). Gingival epithelial cells were treated with arecoline, DNA methylation, mRNA, and protein expression levels of *SIRT1* were analyzed by using qMSP, quantitative RT-PCR, and western blotting, respectively. DNA methylation levels of *SIRT1* were assessed by using qMSP in the smear samples.DNA methylation of *SIRT1* was significantly higher in tissue samples from BQ chewing patients with oral cancer than in samples from non-chewing oral cancer patients or controls. Our in vitro model showed that hypermethylation is followed by downregulation of the transcriptional level of *SIRT1*. The methylation level of *SIRT1* in macroscopically healthy oral epithelium of BQ chewing subjects is higher than that of non-chewing subjects. The duration of BQ chewing habits was correlated positively to the frequency of *SIRT1* hypermethylation.Our results indicate that DNA hypermethylation of *SIRT1* is involved in the occurrence of oral cancer and that hypermethylation in the oral mucosa of BQ chewers may be a predictive marker for the occurrence of malignant transformation. This is the first report that showed DNA hypermethylation in clinically healthy oral epithelium of BQ chewers. Our study shows evidence that DNA hypermethylation may be an early event of oral carcinogenesis prior to observable clinical changes.

053: Role of EGFR-mediated MOB1 phosphorylation on Hippo pathway regulation

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The Hippo pathway and its downstream targets, the YAP/TAZ coactivators, play a central role in cancer cell growth. The Hippo pathway, composed of MST1/2, LATS1/2 kinases and their adaptor proteins SAV1 and MOB1, is dysregulated and YAP/TAZ are aberrantly activated in oral squamous cell carcinoma (OSCC). However, the genetic alterations triggering YAP/TAZ activation in OSCC remains to be unclear. In this regard, we focused on EGFR, which is amplified and overexpressed in OSCC and regarded as a therapeutic target. But whether EGFR controls YAP activation is still poorly understood. Therefore, the aim is to clarify the role of EGFR on the regulation of Hippo pathway in OSCC. OSCC and lung adenocarcinoma cell lines, and HEK293A cells were used. Western blot, immunoprecipitation, qPCR, immunofluorescence analysis, in vitro kinase assay, proliferation assay were performed. We found that EGFR can directly phosphorylate the core Hippo pathway component MOB1 and inactivates LATS1/2, upstream kinases of YAP/TAZ, thereby promoting the transcription of growth-related genes. Transcriptomic analysis revealed that EGFR inhibitor inactivates YAP/TAZ and suppresses YAP/TAZ-regulated gene expression. Remarkably, loss of LATS1/2, resulting in full activation of YAP/TAZ, was sufficient to confer resistance to erlotinib in OSCC and lung cancer cells with EGFR alterations. Our findings elucidated that EGFR-MOB1-YAP/TAZ plays a pivotal role on cancer cell proliferation in OSCC. Emerging evidence show that YAP/TAZ activation is important for EGFR targeting therapy resistance. Given the evidence and our results, EGFR targeting therapy inactivates YAP/TAZ through MOB1 phosphorylation, then YAP/TAZ are likely to be re-activated by unknown mechanism. Therefore, inhibition of YAP/TAZ in combination with EGFR targeting therapy might be beneficial to prevent treatment resistance, and ultimately to halt cancer progression and recurrence. This project was supervised by J Silvio Gutkind (Principal investigator, University of California, San Diego).

054: Sema3A-AKT Axis In Salivary Gland And Adenoid Cystic Carcinoma Developments

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Recently, we demonstrated that Wnt/β-catenin signaling negatively regulated cellular growth through reduced Semaphorin3A (Sema3A) expression in odontogenic epithelial cells and its involvement in tooth germ development (Sci Rep, 2019). Although the developmental process in salivary gland may share the same mechanisms with tooth germ, the effect of Sema3A signaling on salivary gland development remains unclear. Here, we conducted to investigate the function of Sema3A signaling in salivary gland development and adenoid cystic carcinoma (ACC) cell proliferation.We developed the salivary gland organ culture system with murine submandibular salivary gland (SMG) rudiments at embryonic day 13. In the organ culture system, normal tempo-spatial development of the SMG rudiment or salivary gland organ, which is similar to in vivo situations, was confirmed. The treatment with CHIR99021, a GSK3β inhibitor, activated Wnt/β-catenin signaling but decreased Sema3A expression in the SMG rudiment culture and ACC cell lines. Immunohistochemical analysis demonstrated that Sema3A was highly expressed in the salivary gland epithelial cells. Loss-of-function experiments with a Sema3A inhibitor showed the decrease of total epithelial area, the numbers of buds and proliferating cells, and AKT activation. Consistently, loss-of-function experiment reduced cell proliferation and AKT activation in the ACC cell lines.These results suggest that Wnt/β-catenin signaling reduces Sema3A expression and the Sema3A-AKT axis promotes epithelial cell proliferation to regulate salivary gland development and ACC cell proliferation.

055: Effect of Ethinyl Estradiol on Mandibular Condyle of Female Rats

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Objectives: Idiopathic condylar resorption (ICR) of the mandibular condyle is commonly observed in perimenopausal women. Recent clinical studies have reported a correlation between oral contraceptives (OC) and ICR. This study aimed to establish a young female rat model and examine the effect of ethinyl estradiol (EE), a main component of OC and an active analog of estrogen, on the mandibular condyle.Methods: Seven-week-old female Sprague-Dawley rats with normal estrus cycle were orally treated with peanut oil (PO; vehicle, n = 6) or EE (0.4, 1, 5, 10, and 50 μg/kg/day, n = 6) for 8 weeks. After confirming the estrus stage, blood was collected from the animals for serum 17β-estradiol measurements. The right mandibular condyles and knee joints were subjected to micro-computed tomography, histological staining, and histomorphometric analysis. Total RNA was extracted from the left mandibular condyles and real-time polymerase chain reaction was performed.Results: The bodyweight of the rats gradually increased in all groups. Serum 17β-estradiol levels were significantly decreased in the EE10 and EE50 groups ($p < 0.05$). The EE-treated rats (50 μg/kg/day) demonstrated an increase in bone mineral density (BMD) and a decrease in the hypertrophic layer thickness of the mandibular condylar cartilage (MCC) compared to the PO-treated rats ($p < 0.05$). The BMD, bone volume fraction and trabecular bone number were increased in the EE-treated tibia bone. Moreover, the thickness of the growth plate in the tibia and articular cartilage in the femoral bone decreased significantly ($p < 0.05$).Conclusions: EE administration may suppress the estradiol cycle and inhibit long bone growth, which is similar to the estrogen effect. Moreover, EE may terminate the growth and development of the mandibular condyle prematurely, which may compromise the properties of MCC and lead to ICR.

056: Histone methyltransferase SETDB1 negatively regulates PTH/PTHrP receptor in chondrocytes

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Objectives: SETDB1 is a histone methyltransferase which represses gene expression by catalyzing lysine 9 of histone H3 trimethylation. We previously showed that conditionally inactivation of *Setdb1* only in neural crest-derived cells by crossing *Wnt1-Cre* deleter strain to the floxed allele of *Setdb1* (*Setdb1* CKO) resulted in Meckel´s cartilage enlargement instead of the degeneration that normally occurs in the later stages of mandibular development. Here¸ we show that the proliferation of chondrocytes is regulated by SETDB1 along with the downregulation of PTH/PTHrP receptor.
Methods: *Setdb1* was knocked down or overexpressed in a mouse chondrogenic cell line¸ ATDC5¸ by transfecting the cells with short interfering RNA against *Setdb1* or wild-type *Setdb1* expression vector¸ respectively. The proliferation of cells was detected by BrdU incorporation. RNA was extracted¸ and gene expressions were examined by quantitative RT-PCR. Immunofluorescence staining of paraffin sections of embryonic days 13.5¸ 14.5¸ and 15.5 mice or transfected ATDC5 cells was performed to detect PTH/PTHrP receptor.
Results: *Setdb1* inhibition in ATDC5 cells showed increased proliferation¸ and the expression of *Pthrp* and *Pth1r* was also increased in these cells. Converse results were observed when the wild-type *Setdb1* was overexpressed. Strong staining of PTH/PTHrP receptor was observed in Meckel´s cartilage in *Setdb1* CKO mice on embryonic days 13.5¸ 14.5¸ and 15.5¸ compared to that in their control counterparts in which the staining was barely detected. Similarly¸ *Setdb1* inhibition in ATDC5 cells clearly enhanced the staining of PTH/PTHrP receptor in the cytoplasm and membrane. However¸ PTH/PTHrP receptor was only observed around the nucleus when *Setdb1* was overexpressed.
Conclusions: Increased proliferation of chondrocytes are caused in part by the upregulation of PTH/PTHrP receptor as a result of *Setdb1* inhibition¸ which may lead to abnormal enlargement of Meckel´s cartilage¸ and its prolonged presence in *Setdb1* CKO mice.

057: Generating MSC Spheroids Recovers Stemness Characteristics for Cortical Bone Formation

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Objectives: Alveolar bone resorption limits the effectiveness of dental treatments. Therefore, regenerative approaches have garnered attention. Mesenchymal stem cells (MSCs) represent promising candidates for regenerative therapy. *In vitro* expansion of MSCs is often required prior to *in vivo* transplantation. Gradual loss of stemness via repeated passage may significantly limit the therapeutic potential of MSCs. We previously successfully established a novel three–dimensional (3D) culture method to generate MSC spheroids that maintain multipotency *in vitro*. Objectives of the present study were to optimize 3D culture methods and to investigate regenerative ability of MSC spheroids *in vivo*.

Methods: Human bone marrow–derived MSCs were cultured under adherent conditions for an extended period (16–18 passages) to produce long–term adherent MSCs (lMSC–ad). After expansion, lMSC–ad were harvested and 3D cultured under shaking conditions in neural stem cell medium for 21 days to produce MSC spheroids (lMSC–sph). Cell characteristics were investigated using a differentiation assay, RT–PCR, and histochemistry. For the *in vivo* experiment, cells were implanted into critical sized–femur bone defects of SD–rats. After 3 weeks, femur bones were excised and quantitative assessment of 3D bone morphometric parameters was performed using micro X–ray computed tomography.

Results: Relative to lMSC–ad, lMSC–sph demonstrated marked multipotency recovery, as suggested by high expression of stem cell and anti–inflammatory markers. This phenotypic change may reflect recovery of stem cell functionality. Indeed, a contiguous mineralized structure was observed in cortical regions of lMSC–sph–implanted samples, and average bone mineral density was significantly higher in lMSC–sph–implanted samples (ANOVA; $p < 0.05$). These data suggest that lMSC–sph are superior to lMSC–ad in promoting bone regeneration.

Conclusions: Even after long–term proliferation, MSC spheroids 3D cultured under novel shaking conditions recovered at least some stem cell characteristics and enhanced bone regeneration *in vivo*. This may facilitate alveolar bone regeneration as a form of regenerative dentistry.

058: The bHLH transcription factor HAND1 is involved in cortical bone volume through the regulation of collagen expression

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Objective: Temporally and/or spatially altered expression of the members of the collagen family of genes results in numerous bone defects in humans and mice. However, the mechanisms of collagen expression associated with bone size control remain largely unknown. The basic helix-loop-helix (bHLH) transcription factor, HAND1, is expressed in the developing long bones and is involved in their morphogenesis. To understand the functional role of HAND1 in the postnatal development of long bones, we overexpressed *Hand1* in osteochondroprogenitors and found that the bone volumes of the cortical bones were decreased in *Hand1*-overexpressing mice compared to those in wild-type mice. **Methods:** Using *Hand1*-overexpressing mice conditionally driven by *Twist2-Cre* (*Hand1*$^{Tg/+}$; *Twist2-Cre*), bone staining, histology, and immunohistochemistry, micro-computed tomography, real-time quantitative PCR, and liquid chromatography/mass spectrometry (LC/MS) analysis were performed. **Results:** The continuous expression of *Hand1* downregulated the expression of types I, V, and XI collagen genes in the diaphyses of the long bones, and was associated with decreased expression of *Runx2* and *Sp7/Osterix*, which encode transcription factors for transactivation of fibril-forming collagen genes. Moreover, members of the microRNA-196 family, which specifically target the 3 prime untranslated regions of *Col1a1* and *Col1a2*, were significantly upregulated in *Hand1*-overexpressing mice. Mass spectrometric analysis revealed that the expression ratio of type V and XI collagens increased during postnatal development in the diaphysis of wild-type mice, whereas their increase was delayed in *Hand1*-overexpressing mice. **Conclusion:** Our results demonstrate that HAND1 regulates bone size and morphology through osteochondroprogenitors, at least partially by suppressing the postnatal expression of collagen fibrils in the cortical bones.

059: Oxygen tension-dependent expression of monocarboxylate transpoter-1 is a prerequisite event for oxidative death of chondrogenic ATDC5 cells induced by interleukin-1β.

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Objectives: Articular cartilage is a typical avascular tissue. Its superficial and deeper parts' oxygen tension is estimated to be 6% and 1%. Its destruction begins from the surface in joint disorders including osteoarthritis. We previously reported that monocarboxylate transporter (MCT)-1, a transmembrane transporter for monocarboxylates such as lactate, played an essential role in interleukin-1β(IL-1β)-induced expression of NADPH oxidase (NOX)-2, a reactive oxygen species (ROS)-producing enzyme, and ROS-dependent death of mouse chondrogenic ATDC5 cells cultured in a normal condition (20% oxygen). Here, we investigated the effect of oxygen tension on IL-1β-induced events, including cell death in ATDC5 cells. **Methods:** ATDC5 cells were treated with IL-1β (1 ng/mL) for various periods under 1-20% oxygen. We assessed cell survivability by measuring the optical density after toluidine blue staining of the cells. We also examined the expression of mRNAs quantitatively by real-time RT-PCR. **Results:** IL-1β induced the death of ATDC5 cells under 20% and 6% oxygen but did not under 2% and 1% oxygen. A NOX inhibitor, apocynin, suppressed cell death. IL-1β induced the expression of *Mct1* and *Nox2* mRNAs under 20% oxygen in 6 and 24 hours, respectively, but not under 2% oxygen. On the other hand, a 3-hour incubation with IL-1β upregulated the expression of *Nox2* (inducible nitric oxide synthase) mRNA irrespective of oxygen tension. *Mct1* siRNA suppressed the IL-1β-induced expression of *Nox2*. Inhibition of hypoxia-inducible factor (HIF)-1α did not augment the IL-1β-induced expression of *Mct1* or *Nox2* under 2% oxygen. **Conclusion:** The results indicate that IL-1β induces the expression of MCT-1 in an oxygen tension-dependent manner without regulation by HIF-1α, which precedes NOX-2 expression, ROS production, and cell death in chondrogenic ATDC5 cells. The notions obtained in our study suggest that regulation of MCT-1 in articular cartilage would be a promising approach to treat joint diseases including jaw arthritis.

060: Effects of Anticancer Drugs on Mouse Tooth Germ Development

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Objectives: Chemotherapy at an early age for childhood malignancy causes severe disturbances in odontogenesis such as dental agenesis and microdontia, and also inhibits tooth root development. It has been suggested that these conditions are caused by administration of an alkylating agent for anticancer therapy during the time of formation of permanent teeth. The purpose of this study was to investigate the effects of cyclophosphamide (CPA), an alkylating agent, on development of tooth germs in mice.Methods: Mandibular first molar tooth germs were collected from 16-day-old mouse embryos and cultured using the Trowell method. CPA (0.21 mg/mL) was added on the first day of culture in the experimental group, while nothing was added to the control culture. Histopathological examinations and molecular biological analyses of tooth germs cultured for 7, 14, or 21 days were performed.Results: HE staining revealed a disorganized array of ameloblasts and odontoblasts in the experimental group after 21 days. Also, qRT-PCR results showed increased CK14 expression and decreased vimentin expression at 21 days in the experimental group as compared to the control. In addition, type I collagen expression was decreased after both 14 and 21 days, while fibronectin expression was significantly reduced after 21 days ($P<0.001$) in the experimental group. Furthermore, large numbers of TUNEL-positive cells were found in the epithelial area and tooth papillae after 14 days in the experimental group, with the number increased in the epithelial area after 21 days, whereas those were decreased in dental papillae as compared to 14 days.Conclusions: CPA may inhibit DNA synthesis in mitotic inner enamel epithelial and dental papilla cells, thus causing inhibition of normal growth and differentiation in the tooth germ. In addition, CPA was found to induce histological changes in ameloblasts and odontoblasts, resulting in an abnormal tooth morphology.

061: Trehalose, a natural sweetening compound, suppresses osteoclast differentiation.

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Objectives: Control of osteoclast formation in periodontal disease is an essential issue for patients' QOL. We are interested in the effects of food components on osteoclast differentiation because it would be possible to develop such compounds as agents to control alveolar bone resorption in periodontitis. We reported at JADR 2018 that sucrose inhibited osteoclast differentiation. However, sucrose is one of the causative sugars of dental caries. In the present study, we expanded our research to find dietary sugars that effectively inhibit osteoclast differentiation.Methods: Bone marrow cells obtained from male ddY mice were cultured with M-CSF to propagate macrophages, which were further cultured for 3 days with M-CSF and RANKL to induce their differentiation into osteoclasts in the presence of various sugars. We performed tartrate-resistant acid phosphatase activity staining to detect and count osteoclasts. We assessed the expression of the genes related to osteoclast differentiation by real-time RT-PCR. We also evaluated the effects of sugars on the viability of macrophages by the reduction of MTS reagent. Results: Among the sugars tested, trehalose, a naturally-occurring disaccharide consisting of two α-D-glucose units with α1, α1 glycosidic bond, suppressed osteoclast differentiation induced by RANKL. Trehalose at 5 mmol/L or higher suppressed osteoclast differentiation in a concentration-dependent manner. While 25 mmol/L trehalose completely suppressed osteoclast differentiation, sucrose at the same concentration only partially suppressed it. Mannitol, a sugar alcohol used for osmotic diuretic, at 50 mmol/L did not affect osteoclast differentiation, indicating that change in osmolarity did not explain the effect of trehalose. Trehalose significantly suppressed the expression of Nfatc1, the master gene of osteoclast differentiation. Besides, trehalose did not affect the viability of macrophages.Conclusion: Trehalose inhibited osteoclast differentiation by suppressing the expression of Nfatc1. Its high safety to the human seems preferable for developing an anti-bone resorptive agent applicable to periodontitis patients.

062: Computational chemistry of phospholipid mineralization

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Previously, we have found that cell membrane nanofragments (phospholipids) were the nucleation site for bone formation (Hara ES et al., ACS Biomater Sci Eng, 2018). In addition, we showed that cell membrane nanofragments could promote bone-like mineral (calcium phosphate) formation in only 1 day (Hara ES et al., J Mater Chem B, 2018). However, the mineralization mechanisms of phospholipids are still unclear. This study aimed to evaluate the ability of different phospholipids to mineralize in vitro, and to analyze the energy required for phospholipid cleavage and calcium phosphate formation using in silico quantum chemistry-based tools. Chloroform-dissolved phospholipids were applied onto a glass-bottom dish, and after complete dry, a 2 mM CaCl2 solution was poured into the dish, which was incubated in at 37C, 5% CO2 and 98% humidity for 2 days. The phospholipid/mineral complexes were then collected in small tubes and centrifugally-washed 3 times with ultra-pure water (Milli-Q) and dissolved in 0.1% HCl for quantitative analysis of calcium levels using a simultaneous multi-element analysis atomic absorption spectrophotometer (AAS). A detailed analysis of the cleavage reaction kinetics focusing on the steric factor of each phospholipid molecule was performed to evaluate the affinity of each molecule for binding to calcium ions. The Gaussian16 molecular orbital program was used at the B3LYP/6-31(d, p) level of theory. The results showed that among several phospholipids, phospholipid X showed the highest mineralization ability in vitro, as determined by AAS. Quantum chemical calculations revealed that the mineralization reaction of phospholipid X probably proceeded in the following order: (1) binding of Ca ions (2) removal of the hydrophilic head, (3) removal of the hydrophobic tail, and formation of calcium phosphates. These results reveal important steps of phospholipid mineralization. Future studies are required to clarify the exact mechanisms of phospholipid mineralization.

063: Histological study of dentin bridge formed beneath MTA after pulpotomies in rat molars

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It is known that odontoblasts are differentiated from the undifferentiated mesenchymal cells by stimulation. Recently, a lot of studies on pulpotomy indicate that the clinical results are better using mineral trioxide aggregate (MTA) than CH. Kimura et al. (2016) suggested that transient receptor potential ankyrin (TRPA) channel is involved in dentin formation. However, it is known whether MTA and CH activate TAPA1 channel in vivo. The aim of this study is to clarify the histological differentiation about DB and the association TRPA1 channel using MTA and CH on pulpotomy. Therefore, we made rat models of the pulpotomy at first. The maxilla first molars from postnatal week 7 Wistar rats were performed pulpotomy using MTA with rubber dam isolation and microscope. We fixed the rats after the progress for 4 weeks from surgical operation day. The maxilla including the first molar was embedded in paraffin by a conventional method. The serial sagittal sections of maxilla first molars were made for hematoxyline-eosin staining.The DB was observed in the region facing MTA, and the pulp did not through to outside due to the DB. It was found that the DB included lacuna and dentinal tubules. The dentinal tubules were irregular and abnormal number. Odontoblast-like cells were detected bottom of the DB. There is no inflammation response in the pulp and apical area.Many studies were investigated about MTA in vitro. Although there were studies about direct capping using MTA in vivo, no study about pulpotomy using MTA in vivo. There is one of the causes, it is difficult to perform pulpotomy in the size of rat molar. However, we succeeded in the pulpotomy in this study.We can perform pulpotomy on rat tooth with rubber dam isolation and microscope.

064: The effect of micro-sized titanium on osteoblast differentiation

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Objectives
The purpose of this study is to investigate the effects of micro-sized titanium on osteoblasts and to reveal the mechanism by which the titanium acquires its osseointegration.
Methods
MC3T3-E1 preosteoblastic cell culture was started in 6-well plates with 1×10^5 cells/well and cultured in a growth medium (αMEM, 7 mL), exposed to different amounts of micro-sized titanium particles (0.1 g, 0.5 g and 1.0 g) [Titanium, Powder, 45 μm, 99.9%, FUJIFILM] dialyzed through cellulose tubes [pore size: 5nm, Kenis] in the medium for 24 hours. And then the culture medium was replaced with the same amount of the fresh growth medium as group "GM" or an osteogenic medium as group "OG". Subsequently, ALP staining was performed at 3 and 7 days of culture after changing medium with growth or induction medium. The staining was observed using BZ-X700 [KEYENCE] and the stained area was calculated with Image J software. The post hoc test with one-way ANOVA was statistically processed using the Tukey test with p-value of 0.05.
Results
There was no significant difference in ALP staining by the titanium exposure in all titanium particle amounts in GM whereas the stained area was statistically larger by 0.1 g and 0.5 g of the particles than by 1.0 g and 0 g (negative control) in OG at day 3. Afterward, the stained area was significantly smaller by 1.0 g than by 0.1 g, 0.5 g and 0 g in GM; however, in OG, the stained area enlarged by 0.1g and 0.5 g of the titanium particles than by 1.0 g and 0 g at day 7.
Conclusions
Osteogenic differentiation of MC3T3-E1 cells was promoted by 0.1 g and 0.5 g of micro-sized titanium particle amounts; on the other hand, 1.0 g had no effect on osteoblast differentiation.

065: Comparison in physical properties of synthetic carbonate apatite and allogeneic bone graft substitute

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Objectives: Autogenous bone have been the gold standard for bone augmentation, but invasion to the healthy site remain as a concern. There has been a demand for bone graft substitutes which has potential to be replaced with autogenous bone. We have developed a novel bone graft substitutes "Cytrans Granules (Cyt)" consisting synthetic carbonate apatite, which is the main component of human bone. It is reported that Cyt shows osteoconductivity at similar or higher level of allogeneic bone graft substitutes, but the mechanism behind have not been clarified. In this study, we have compared the composition, crystal structure and resorption property of Cyt to synthetic Hydroxyapatite (HAp) and freeze-dried bone allograft (FDBA) as an allogeneic bone.Methods: Cyt was used as test sample, FDBA and three types of HAp (HAp-1, HAp-2, HAp-3) were used as control. Composition and crystal structure were evaluated by using Fourier transform infrared spectroscopy (FT-IR) and powder X-ray diffraction (XRD). Crushing strength was measured by universal testing machine. Resorption property was measured as amount of calcium ion dissolved from the samples in neutral solution (pH7.3) and weak acidic solution (pH5.5).Results: XRD pattern showed that all samples have apatitic crystal structure. FT-IR spectrum showed that Cyt and FDBA contains carbonate group. Synthetic Hydroxyapatite had higher crushing strength, followed by FDBA and Cyt. Among all samples, dissolution at neutral solution was at low level. In weak acidic solution, Cyt and FDBA showed higher rate of dissolution compared to synthetic Hydroxyapatite.Conclusions: It was confirmed that Cyt and FDBA are Hydroxyapatite containing carbonate group. Cyt had composition, crystal structure and mechanical properties similar to those of FDBA. From the result of dissolution test, Cyt and FDBA were stable in neutral body fluid, and were dissolved in weak acidic solution resembling the pH produced by osteoclast. These results indicate that these two materials could be resorbed by osteoclasts. It is suggested that Cyt has similar characteristics to allogeneic bone, which may be the reason for Cyt to exhibited osteoconductivity at similar or higher level compared to allogeneic bone.

066: Novel bio-active adhesive monomer CMET stimulates human dental pulp stem cells differentiation toward odontoblast phenotype: a comparative study

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Objectives: The present study aims to evaluate the in vitro effect of the novel adhesive monomer CMET, a calcium salt of 4-methacryloxyethyl trimellitate (4-MET), on the proliferation, mineralization and differentiation of human dental pulp stem cells (hDPSCs), comparing with 4-MET, calcium hydroxide (CH), and mineral trioxide aggregate (MTA).**Methods:** hDPSCs were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 50 units/mL penicillin, and 50 μg/mL streptomycin. Mineralization reagents including 10 mM β-glycerophosphate and 50 μg/mL ascorbic acid were incorporated upon confluence. The powder of four tested materials (CMET, 4-MET, CH and MTA) was first dissolved in distilled water (dH_2O) and then was diluted by DMEM to yield final concentrations. Solvent (dH_2O) was used as a control. Cell viability was assessed using CCK-8 assay. Realtime RT-PCR was used to quantify the mRNA expression of odontogenic markers. Mineralization inducing capacity was evaluated by alkaline phosphatase (ALPase) activity and alizarin red S staining. Statistical analyses were performed using one-way ANOVA and *post hoc* Tukey's HSD test, with the significance level at 1%.**Results:** Cell viability was significantly greater in the CMET- and MTA-treated (10%, v/v) groups than that in other groups, higher concentrations of each material exhibited cytotoxicity to different extents ($P < 0.01$). CMET showed the lowest toxic effect on hDPSCs. CMET treatment augmented the mRNA expression levels of odontogenic markers ($P < 0.01$), while no great up-regulation was observed in other three material treated groups. The addition of CMET significantly increased the ALPase activity of hDPSCs on day 14 and 21, then decreased on day 28 ($P < 0.01$). The calcific deposition of hDPSCs was accelerated by the addition of CMET, CH, and MTA on day 30 and 32 ($P < 0.01$), but it was inhibited by 4-MET on both days.**Conclusions:** The present results indicated that the differentiation of hDPSCs was stimulated into odontoblast phenotype by the addition of CMET, while the addition of CH and MTA rather promoted hDPSCs calcific deposition than differentiation into odontoblast. The results provided evidence that CMET is a promising bio-active material in dentin regeneration.

067: Hydrogel-based biomechanical environment for understanding Meckel's cartilage fate

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Objectives:The purpose of this study was to,1.systematically investigate the course of Meckel's cartilage during embryonic development from a biomechanical perspective.2.In vitro mimicking the biomechancial miliu affecting the cartialge development via mechcanically-tuned hydrogel culture systemMethods: 1. Histomophological and biomechanical (stiffness) changes in the Meckel's cartilage were analyzed from embryonic day 12 (E12) to post-natal day 0 (P0).2. E 14 and E15 cartilage rods were isolated and cultured in mechanically-tuned 3D hydrogel culture system (10, 20 and 40 kPa hydrogel). After, 5-7 days in culture, immunofluorescence analysis of metalloproteinase (MMP-1 and MMP-13) was carried out.3. Micro-CT images of the collected samples were obtained to detect the mineralized tissue formation.4. Time-lapse imaging of the development of the cartialge was done to detect chondrocyte cell burstResults: Our results revealed remarkable changes in the morphology and size of chondrocytes, as well as the occurrence of chondrocyte burst, which is an often seen phenomenon preceding endochondral ossification, in the vicinity of the mineralization site. The mechanically-tuned 3D hydrogel culture system results showed that, at the anterior region, a moderately soft environment (10 kPa hydrogel) promoted chondrocyte burst and ossification. On the contrary, at the middle region, a more rigid environment (40 kPa hydrogel) enhanced cartilage degradation by inducing a higher expression of MMP-1 and MMP-13.Conclusions: These results indicate that differences in the biomechanical properties of the surrounding environment are essential factors that distinctly guide the mineralization and degradation of Meckel's cartilage, and would be valuable tools for modulating in vitro cartilage and bone tissue engineering.

068: Evaluation of Interleukin – 8 In Human Dental Pulp

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Objectives: To evaluate and compare the IL – 8 levels in the normal, reversibly inflamed and irreversibly inflamed dental pulpMethods: The study population was composed of 35 patients who underwent dental treatment at the Department of Conservative Dentistry and Endodontics, Maulana Azad Institute of Dental Sciences, New Delhi. Cases subject to testing Group 1 : Intact vital teeth indicated for orthodontic extractions Group 2 : Teeth with reversible pulpitis Group 3 : Teeth with symptomatic irreversible pulpitis Sampling, Transport and Storage of Blood Samples Following access preparation, blood sample was obtained with a micropipette and transferred immediately into numbered reaction tubes (e-cups). Serum was removed, diluted with the appropriate diluents, and assayed immediately. IL - 8 levels were assessed using ELISA according to manufacturer's recommendations.Results: The IL – 8 levels obtained were in picogram/ml and were subdivided as follows: Low level < 100 pg/ml Mid level - 100 – 500 pg/ml High level > 500 pg/ml Out of 35 samples, 04 were normal pulp, 05 were reversible pulpitis and 26 were irreversible pulpitis cases (diagnosed clinically). Results have been provided in the table that is attached with submissionConclusions: Strong correlation was seen between values of IL – 8 and level of pulpal inflammation. In fact, very high value of IL -8 (more than 2000 pg/ml) were seen only in irreversible pulpitis cases and not in reversible pulpitis or normal cases. In few cases of clinically diagnosed irreversible pulpitis, low levels of IL-8 signifies that other inflammatory mediators may also play an important role in pulpal inflammation.

069: Interaction analysis of protein S100A7 in dental pulp tissue

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Objectives: Organic dentin matrix components (DMCs) contain various kinds of bioactive molecules, which can be released by enzymatic digestion of DMCs during caries progression. We previously showed specific molecules from digested DMCs induced by MMP-20 promoted wound healing process of pulp tissue. In the digested DMCs, we found protein S100A7 found was one of the most effective molecules which could induce tertiary dentin formation, but the target molecules in the pulp tissue against this protein was still unclear. In this study, interaction analysis was performed to detect the target molecules of S100A7 using proteomic methods and to investigate the mechanism of the wound healing of dental pulp.Methods: Pull down assay was performed to search for the interacted molecules with S100A7 in dental pulp tissue using rat primary pulp cells from rat incisors and recombinant human S100A7 (PROSPEC, Israel) and, the results were visualized by SDS-PAGE. Then, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed to detect specific molecules comprehensively and quantitatively. To identify the target molecules, proteome analysis software, Scaffold Viewer (Proteome Software, USA), was used to narrow down the candidate target proteins. Then, the focused proteins were analyzed in terms of the function using Gene Ontology categorization.Results: Pull down assay showed several specific proteins were interacted with S100A7. LC-MS/MS revealed 26 molecules might be interacted with S100A7 and showed that the molecules binding to S100A7 could be related to the function of cellular process and biological regulation in Gene Ontology categorization.Conclusions: Some specific candidate molecules were found that might interact with S100A7 in the pulp tissue could facilitate tertiary dentinogenesis.

070: Lyve-1+ Pulpal Macrophages: M2-polarization and Response to Cavity Preparation

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Lymphatic vessel endothelial hyaluronan receptor-1 (Lyve-1) is a CD44 homolog expressed on lymphatic endothelial cells and certain macrophage subpopulations. We have reported that normal rat dental pulp contains Lyve-1+ macrophages co-expressing CD163, a marker of M2 macrophages. The purposes of this study were to examine the phenotype and biological activity of Lyve-1+ macrophages in vitro and responses of Lyve-1+ macrophages to cavity preparation in rat molars.RAW264.7 cells, a murine macrophage cell line, were M2-polarized by MID cocktail (M-CSF, IL-4 and dexamethasone). Lyve-1 was forcibly expressed by transfection of Lyve-1 expression vector into RAW264.7 cells. Lyve-1 mRNA and protein levels were determined by RT-qPCR and western blotting, respectively. The mRNA levels of Arginase1 (Arg1), CD206, Interleukin-1a (IL-1a), IL-1b, IL-10, Metalloproteinase 9 (MMP-9), and vascular endothelial growth factor (VEGF) were measured by RT-qPCR. Lipopolysaccharide (LPS, 100 ng/ml) was used to stimulate RAW264.7 cells. Dentinal cavities were prepared in upper first molars of Sprague Dawley rats and Lyve1, CD68, CD163, and MCH class II was immunolocalized in the pulp with double-immunofluorescence staining. Typical M2 markers (Arg1 and CD206) and Lyve-1 were highly induced in M2-polarized RAW264.7 cells. LPS-stimulation up- and down-regulated pro-inflammatory (IL-1a and IL-1b) and anti-inflammatory (IL-10) cytokines, respectively. Enforced expression of Lyve-1 induced up-regulation of angiogenic factors (MMP-9 and VEGF). Following cavity preparation, Lyve-1+ cells disappeared in the pulp tissue beneath the cavity at 1 day, whereas these cells gradually reappeared and distributed throughout the coronal pulp at 7 days, when odontoblasts showed rearrangement beneath the cavity. Almost all Lyve-1+ cells co-expressed CD68 and CD163, but not MHC class II. Lyve-1 expression on macrophages was associated with M2-plarization and pro-angiogenic factor expression in vitro. The delayed increase of Lyve-1+/CD163+ cells after cavity preparation suggests some association of these cells with reparative responses of the pulp.

071: DNA methylation of *GJA1*, *BMP2* and *BMP4* in a human cementoblast cell line induced by lipopolysaccharide.

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The objective of this study is to examine DNA methylation of *GJA1*, *BMP2* and *BMP4* in human cementoblasts (HCEM) induced by lipopolysaccharide (LPS).HCEM were cultured in osteoinduction medium. After 24 h, *Escherichia coli* LPS (1 μg/mL) was added to the medium, which was changed every 2-3 days. Untreated samples were used as controls. Messenger RNA was extracted after 4 weeks, and quantitative real-time polymerase chain reaction (qRT-PCR) for*GJA1*, *BMP2*, *BMP4* and *DNMT1* was performed. Genomic DNA was extracted after 4 weeks, and quantitative methylation-specific polymerase chain reaction was carried out for *GJA1*, *BMP2* and *BMP4*. To detect mineralization, alizarin red and alkaline phosphatase staining were performed. The cells were also treated with the DNA methyltransferase inhibitor 5-Aza-2'-deoxycytidine (5Aza) and examined. Decreased expression of mRNA was seen in *GJA1*, *BMP2* and *BMP4* after 4 weeks (p<0.05). DNA hypermethylation was detected in *GJA1*, *BMP2* and *BMP4* (p<0.05). Alizarin red staining and alkaline phosphatase staining revealed decreased mineralization levels in HCEM stimulated with LPS. 5Aza abolished the effects of DNA methylation in HCEM stimulated with LPS. These results suggest that long-term LPS stimulation induces DNA methylation of *GJA1*, *BMP2* and *BMP4* in HCEM.

072: Effects of Non-thermal atmospheric pressure plasma on human deciduous dental pulp fibroblast-like cells

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Objective:Stem cells have been a recent focus of regenerative medicine research. They originate from various tissues including human deciduous dental pulp. However, only a very small number of stem cells are present in dental pulp tissue and a large number of cells are needed for clinical application.Non-thermal atmospheric pressure plasma (NTAPP) is a partially ionized gas containing electrically charged particles and radicals at atmospheric pressure. Recent studies reported the beneficial outcomes of NTAPP including wound healing, activation of immune cells, and proliferation of mesoderm-derived adult stem cells in human. Therefore, to evaluate the effects of NTAPP on human deciduous dental pulp fibroblast like cells (hDDPF), we examined the appropriate conditions of NTAPP exposure on hDDPF, and evaluated cell proliferation, and the mRNA expressions that play key functions in maintaining the pluripotency of stem cells.Methods:Cells were obtained from the noncarious deciduous incisor at Osaka Dental University Hospital. All experiments were approved by the Ethical Committee of Osaka Dental University. We used the helium-based NTAPP device and cells were exposed to the indicated dose (2.7 standard liter/min [slm], 20 voltage[V]). The distance between the device and cells was fixed to 20 mm. Cell proliferation was evaluated by a CellTiter96 Aqueous One Solution Cell Proliferation Assay and gene expression analysis was performed using a Step One Plus system.Results:NTAPP for 20 seconds per hour for 3 times efficiently accelerated the proliferation of hDDPF and further exposure reduced the cell proliferative capacity. The relative mRNA expressions of Oct4, Sox2, and Nanog in NTAPP-exposed cells were increased 3 days after exposure, when compared to unexposed control cells.Conclusion:NTAPP can be an efficient tool for expanding the population of hDDPF in vitro for stem cell therapy and regenerative medicine.

073: Effect of Lithium Carbonate on the Healing of Apical Periodontitis

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Objective:We have developed the bioactive root canal medicament which activates immune responses and bone metabolism. *In vivo* experiments using the rat root canal treatment model showed that lithium carbonate (Li_2CO_3) promoted the healing of periapical lesions. The aim of this study is to identify the minimum effective concentration of Li_2CO_3 and verify the safety during application of Li_2CO_3 into root canal.
Methods:The mandibular first molar pulp of 10-week-old Wistar rat was exposed. After 4 weeks from the pulp exposure, four concentrations of Li_2CO_3 (1%, 0.1%, 0.01%, and 0.001%) were applied into the root canals. After 7, 14, 21, and 28 days, the volume of the periapical lesions were measured by micro-CT.
To monitor the blood concentration of Li^+, rats were divided into two groups. One group is the application of Li_2CO_3 into root canals, the other is the systemic administration of Li_2CO_3. Blood samples were collected at 1, 3, 6, 12, 24, 48, and 72 hours, and measured Li^+ concentration.
The study was approved by the research Ethics Committee of the Osaka University, Suita, Japan, and all experiments were performed according to the guidelines related to animal care (AD-26-011-0).
Resuts:There was no significant difference in the volumes of the periapical lesions between four concentrations at 7, 14, and 21 days. At 28 days, the volume of 0.001% Li_2CO_3 was significantly larger than other concentrations.
As a result of the blood concentration monitoring, the systemic administration showed transient increase in the blood concentration of Li^+. On the contrary, in case of the application of Li_2CO_3 into root canal, there was no increase of Li^+.
Conclusion:The experiment indicated that Li_2CO_3 was not sufficiently effective at the concentration of lower than 0.001%. In addition, we showed that the application of Li_2CO_3 into root canal is safe.

074: Large-conductance Ca^{2+}-activated K^+ Channels in Human Cementoblasts

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Objective: Cementum, which is formed by cementoblasts, provides an attachment site for collagen fibers to fix the teeth into the alveolar bone. Ionic signaling associated with cell membrane regulates various physiological and pathological processes in the cell, but their properties in cementum forming cells have yet been remained be clarified. The purpose of this study was to examine the biophysical and pharmacological properties of currents generated by ion channels expressed in human cementoblasts (HCEM).Methods: We measured ionic currents using whole-cell patch-clamp recording. Krebs solution was used as a standard extracellular solution (ECS). Standard intracellular solution (ICS) was composed followings (in mM) ;140 KCl, 10 NaCl and 10 HEPES. We prepared Cs-ICS solution by equimolary replacing K^+ in the ICS with Cs^+. We used non-selective K^+ channel blocker TEA and Ca^{2+} -activated K^+ channel blocker IbTX, apamin and TRAM-34 were added to the ECS.Results: Depolarizing steps from holding potential (Vh) of -70 mV with 10 mV increments evoked outward and inward currents under the ECS/ICS condition. Under the condition of Cs-ICS/ECS, the outward current almost disappeared. Application of TEA and IbTX, reduced the outward current amplitude in the ECS/ICS condition. Apamin and TRAM-34 had no effect on it. The reversal potential was measured by recording the tail current, and the date were fitted to the Goldman-Hodgkin-Katz equation. The results showed that K^+ and Na^+ permeabilities were contributed to the outward current.Conclusion: The results suggested that cementoblasts express large-conductance Ca^{2+}-activated K^+ channels. The currents via the Ca^{2+}-activated K^+ channels were carried by K^+ conductance with background Na^+ conductance.

075: Activation of CGRP receptors increased intracellular cAMP level in odontoblasts

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Objectives : Our previous studies suggested that Ca^{2+} signaling in odontoblasts is involved in dentin formation and generation of dentinal pain. In the studies, we have reported the expression of transient receptor potential vanilloid subfamily member 1 (TRPV1) channels and cannabinoid 1 (CB1) receptors in odontoblasts, and functional crosstalk between them. In odontoblasts, increase in intracellular Ca^{2+} concentration induced by TRPV1 channel activation secretes endocannabinoids. We also demonstrated that cyclic AMP (cAMP) regulates CB1 receptor-TRPV1 channel crosstalk. These results suggest that cAMP may participate in dentin formation and dentinal pain generation. However, the detailed intracellular cAMP signaling pathway in odontoblasts and the role of cAMP in odontoblast function remain unclear. In this study, we examined the cAMP signaling elicited by activation of adenylate cyclase or Gs protein-coupled receptors in odontoblasts.Methods : Odontoblasts were acutely isolated from the incisors of newborn Wistar rats. Odontoblasts were cultured in α-MEM containing FBS for 24-36 hours, and then we added mNeon Green-based cAMP sensor. After culture for 24 hours, intracellular cAMP level was measured from odontoblasts.Results : In the presence of extracellular Ca^{2+}, application of forskolin, an adenylate cyclase activator, dose-dependently increased intracellular cAMP level in odontoblasts. The increases were decreased by repeated application of forskolin. In the presence of extracellular Ca^{2+}, application of CGRP, a CGRP receptor agonist, increased intracellular cAMP level in odontoblasts. The increases were significantly inhibited by application of a selective CGRP receptor antagonist or an adenylate cyclase inhibitor in odontoblasts.Conclusion : These results suggested that activation of adenylate cyclase increased intracellular cAMP level in odontoblasts. We also showed expression of CGRP receptors in odontoblasts. In addition, these results indicate that CGRP receptor activation increased intracellular cAMP level by activation of adenylate cyclase.

076: Plasma membrane Ca2+-ATPase regulates dentin formation and mineralization.

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In odontoblasts, the control of intracellular Ca2+ concentration is important in driving their cellular function, dentinogenesis and sensory transduction generating dentinal sensitivity. The intracellular Ca2+ level is regulated by Ca2+ influx, and mobilization as well as extrusion through plasma membrane Na+-Ca2+ exchanger (NCX) and/or plasma membrane Ca2+-ATPase (PMCA). We previously elucidated functional coupling mechanism between transient receptor potential channels and NCX1 and NCX3 in odontoblasts. On the other hand, Linde et al. (1995) revealed that odontoblasts express PMCA. However, the detailed expression pattern of PMCA, its pharmacological properties and the roles in dentinogenesis remain unclear. Therefore, in this study, we investigated the mRNA expression pattern of PMCAs, and effect of PMCA activity on mineralization mechanism in human dental pulp cells showing odontoblast differentiation (HOB cells). We also examined the pharmacological properties of PMCA. We measured intracellular free Ca2+ concentration ([Ca2+]i) by fura 2 fluorescence. The expression of PMCA1-4 mRNAs in HOB cells was demonstrated by real-time reverse transcription polymerase chain reaction. We also analyzed mineralization efficiencies by alizarin red and von Kossa staining. We observed a little expression of PMCA2 mRNA. In the presence of extracellular Ca2+, the application of hypotonic or alkaline (pH8) solution transiently increased [Ca2+]i in acutely isolated rat odontoblasts. During hypotonic or alkaline solution-induced [Ca2+]i increase, the Ca2+ exclusion efficiency was decreased by PMCA inhibitors. PMCA inhibitors also reduced stainability using alizarin red and von Kossa stains in HOB 28 days culture, indicating that inhibition of PMCA suppressed mineralization levels by HOB cells. These results suggest that PMCA1-4 participate in maintenance of the intracellular Ca2+ concentration in odontoblasts. In addition, they suggest that PMCA in odontoblasts is involved in dentinogenesis under the normal physiological and the pathological condition following mechanical stimulation by hydrodynamic force inside dentinal tubules or alkaline stimulation by application of high pH dental materials to odontoblasts.

077: Functional expression of mechanosensitive ion channel in mouse osteoblasts

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Objectives: Mechanical stress is one of the important regulatory factors in bone homeostasis. Although mechanical stimulation to osteoblasts elicits an increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), their detailed mechanism of the mechanosensitive processes remains unclear. The present study investigated pharmacological properties of direct mechanical stimulation-induced $[Ca^{2+}]_i$ response in osteoblasts. In addition, we examined the immunohistochemical expression of mechanosensitive channels.Methods: Mouse osteoblast-like cells (MC3T3-E1) were cultured in 5% CO_2 at 37 °C, and loaded fura-2/AM for 1 hr. The standard extracellular solution was Krebs solution, and we measured $[Ca^{2+}]_i$ responses during; 1) application of hypotonic Krebs solution or Yoda1 solution, 2) direct mechanical stimulation with a glass micropipette to the single cells with or without extracellular Gd^{3+}, GsMTx4, RN1734, HC030031 or Clemizole. We also studied immunofluorescence of TRPV4, Piezo1 and Piezo2 channel proteins to identify their morphological localization.Results: An application of hypotonic Krebs solution and Yoda1 solution increased $[Ca^{2+}]_i$ in the osteoblasts. Repeated application caused significant desensitizing effects on the $[Ca^{2+}]_i$ increase. When direct mechanical stimulation was applied, $[Ca^{2+}]_i$ was increased, but was not showed significant desensitizing effects. Extracellular Gd^{3+}, GsMTx4 and RN1734 reversibly inhibited mechanical stimulation-induced $[Ca^{2+}]_i$ increases. Immunofluorescent staining showed positive reaction of Piezo1 and TRPV4.Conclusions: These results suggested that Piezo1 and TRPV4 channels were involved in the mechanosensitive machinery of MC3T3-E1.

078: Activation of mechano-sensitive ion channels in cancer cells establishes paracrine network via endothelin signaling

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Intercellular cell-cell communication of cancer cells relates to their metabolism such as, growth, progression, migration and modulation of pain. Both cancer cells and cancer-associated cells have been reported to overexpress endothelin and its receptors within cancer tissue, the autocrine and paracrine effects of endothelin axis involve in proliferation, resistance to apoptosis, migration and invasion. The detailed causes and mechanisms in releasing endothelin still remain unclear, however. We, thus, investigated the mechanical sensitivity of rat squamous cell carcinoma cells and their paracrine communication by diffusible transmitter(s) released from mechanically stimulated cells to examine endothelin system involved in cancer metabolism.Rat squamous cell carcinoma cell lines (SCC-158) were used for this study. We measured intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) by fura-2/AM. $[Ca^{2+}]_i$ changes were recorded during direct mechanical stimulation to single SCC-158 cell. For direct mechanical stimulation, glass micropipette was used to compress the cell membrane to 8 μm for 4 sec. This was done by holding the pipette for 22 sec. During direct mechanical stimulation to SCC-158, we could observe transient $[Ca^{2+}]_i$ increases. Transient $[Ca^{2+}]_i$ increases were also observed in neighboring cells to the stimulated SCC-158. Application of 10 μM Gd^{3+} and 1 μM GsMTX4 inhibited mechanical stimulation-induced $[Ca^{2+}]_i$ increases in stimulated SCC-158. Application of 1 μM BQ-123 (endothelin A receptor inhibitor) did not inhibit $[Ca^{2+}]_i$ increases in stimulated SCC-158 but did those in their neighboring cells.We suggest that SCC-158 express piezo-1 channel. Activation of piezo-1 channels induced endothelin release from the stimulated cells. Released endothelin then activates endothelin A receptors in the neighboring SCC-158. The results suggested that intercellular paracrine communication among cancer cells might play an important role in cancer metabolism by endothelin signaling.

079: Mechanical stimulation-induced intercellular communication in trigeminal ganglion neurons

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Although paracrine communication between neurons and non-neuronal cells has been well described, there has been a little report on the intercellular communication between neurons. We have previously examined intercellular communication between trigeminal ganglion (TG) neurons by patch-clamp recordings. When we applied mechanical stimulation to the single TG neuron, we could record inward current from a stimulated TG neuron, while could not record any responses from neighboring TG neuron. In this study, we thus aimed to clarify whether metabotropic receptor activation was capable to establish inter-neuron communication by diffusible factor(s) released from mechanically stimulated TG neuron or not. For this purpose, we measured intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) from mechanically stimulated TG neurons and its neighboring neurons. TG cells were dissected and isolated from 7-day-old Wistar rats under anesthesia and primary cultured (5% CO_2, 95% Air, 37°C) for 48 hrs. Fura-2, which is a Ca^{2+} fluorescent indicator, was loaded into the cells (1 hr), and $[Ca^{2+}]_i$ was recorded from the fluorescence intensity (at 510 nm) ratio of the excitation wavelengths of 340 nm and 380 nm.When the TG neurons were pushed down by 12 μm using a glass micropipette, we could measure $[Ca^{2+}]_i$ increases simultaneously from stimulated neurons and neighboring neurons. In addition, the increase in $[Ca^{2+}]_i$ did not show desensitization effects even after repeated direct stimulation. In the absence of extracellular Ca^{2+}, $[Ca^{2+}]_i$ increases were not observed. These results suggest that neurons are capable to receive mechanical stimuli and release diffusible intercellular transmitters to establish functional intercellular communication with surrounding neurons.

080: Piezo1 channel activation evokes mechanosensitive Ca^{2+} signaling in human odontoblast

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Odontoblasts play critical roles in dentinogenesis and sensory transduction by stimuli applied to the dentin surface. In a previous study, we reported that direct mechanical stimulation induced Ca^{2+} influx through mechanosensitive transient receptor potential channels in acutely isolated rat odontoblasts. In addition, the Ca^{2+} signaling in odontoblasts participate in reactive dentin formation. On the other hand, we demonstrated, in the trigeminal ganglion neurons-odontoblasts coculture system, suppression of inward current in neighboring trigeminal ganglion neurons following mechanical stimulation to single odontoblasts by mechanosensitive piezo1 channel antagonist. However, in human odontoblasts (HOB) cells, the cellular response elicited by mechanical stimulation, the detailed properties and direct evidence showing expression of piezo channel remain unclear. In the present study, we investigated mechanical stimulation-induced intracellular Ca^{2+} signaling and piezo channel expression in HOB cells. Intracellular free calcium concentration ($[Ca^{2+}]_i$) in HOB cells was measured using fura-2 fluorescence. In the presence of extracellular Ca^{2+}, mechanical stimulation transiently increased $[Ca^{2+}]_i$ in both mechanically stimulated HOB cells and neighboring HOB cells. The increases were not desensitized by repeated stimulation. Membrane deformation using a low-osmotic solution in HOB cells induced transient increases in $[Ca^{2+}]_i$. The increase was inhibited by application of Gd^{3+}, a non-selective piezo channel antagonist. An application of Yoda1, a selective agonist of piezo1 receptors, transiently increased $[Ca^{2+}]_i$ in HOB cells, which was suppressed by extracellular Gd^{3+} and Dooku1, a selective piezo1 channel inhibitor. These results showed that mechanical stimulation evoked intracellular Ca^{2+} signaling in HOB cells, and the Ca^{2+} signaling established intercellular odontoblast-odontoblast communication. The results also suggest that piezo1 channels are expressed in HOB cells.

081: Effects of preemptive analgesia with intravenous acetaminophen on postoperat ive pain relief in patients undergoing third molar surgery: a prospective, single-blind, randomized controlled trial

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Objectives: The efficacy of preemptive analgesia in managing postoperative pain is still controversial. The aim of this study was to compare the efficacy of intravenous (IV) acetaminophen given before or immediately after the surgical extraction of an impacted mandibular third molar. Methods: This prospective, randomized clinical trial included 120 patients. The patients were assigned to receive one of three different protocols: the pre-treatment group (pre-group) received 1000 mg of IV acetaminophen 20 min before the surgery, the post-treatment group (pro-group) received 1000 mg of IV acetaminophen after the surgery, and the no-treatment group (control-group) received nothing. Rescue analgesic medicine (60mg loxoprofen) was issued to each patient with instructions on self-administration if needed. The following data on rescue medication usage was obtained for a 17 hour period following the surgery: (1) the time of the initial loxoprofen treatment and (2) the total amount of loxoprofen consumption. Visual analog scale (VAS) pain scores were obtained from all patients 1, 2, 3, 4, 5 and 15 h after the surgery. Results: There was no significant difference between the three groups in each fixed time interval as measured by the VAS. However, the pre-group demonstrated a significantly lower consumption of rescue analgesic and a later time for the initial administration. Conclusions: Administering IV acetaminophen before third molar surgery provides more effective pain control in contrast to postoperative administrations and no treatments.

082: Diagnostic and Prognostic Applications of Artificial Intelligence-based Radiology in Head and Neck Region

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Objectives: Since the introduction of Artificial intelligence (AI) in early 50' defined as "a field of science and engineering concerned with the computational understanding of what is commonly called intelligent behavior, and with the creation of artifacts that exhibit such behavior", it has witnessed enormous improvement and become more sophisticated and has been used more widely.Methods: This presentation is aimed to elaborate on the most recent dentistry related applications of AI published in literature as a non-systematic narrative review.Results: Machine learning (ML) as a subdivision of AI has been implemented as Genetic Algorithm (GA), the Artificial Neural Network (ANN) to predict the outcome of a genetic disorder or treatment plan aiding scientists and clinicians improve the medical and surgical interventions. A subfield of ML, deep learning (DL) and in particular, convolutional neural networks (CNN) has extended horizons for automatic segmentation of cancerous lesions in head and neck region with promising sensitivity and specificity. CNN is also applied in growth prediction, radiologic anatomic landmark detection, radiologic periodontal bone loss (PBL), caries detection, intraosseous bone lesions, forensic dentistry radiology and metastasis prediction based on CT, CBCT, MRI and histology inputs.Conclusions: According to existing accuracy rate of AI based modeling, an expanding role of such a paraclinical aid for clinical decision is expected in the medical and dental fields at both preclinical and clinical levels.

083: Revolutionalized Concept of Digitalizing Dental Treatment Contents using Machine Learning

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Objectives: Our current study aims at developing a method to encode the treatment and estimate the content of the treatment from the video footage of the tray on which instruments are placed during a dental treatment.Methods: An image recognition algorithm was developed using YOLOv3 to recognize 23 types of dental instruments and hands. Image recognition was performed on all frames of the video of the tray on which instruments were placed during 65 treatments, which consisted of four kinds of treatment contents, and encoded into 24-dimensional vectors with the number of existences of each instrument and hand as elements, and converted them into time series data. One hundred frames were randomly selected from the 65 videos and the average encoding accuracy was determined from the percentage of correct responses of the elements of the 24-dimensional vector representing each frame. The time series data was divided by the presence of a hand, and the average time series data consisting of the average value of the vectors in each section was generated. We trained 1-3 layers of Long Short-Term Memory (LSTM) neural networks with 2 types of time series data as input and 4 types of dental treatment contents as output for 500 epochs, and performed leave-one-out cross-validation every 5 epochs, and obtained the highest accuracy was compared as the accuracy obtained in each neural network.Results: The average coding accuracy was 81.2%.The accuracies of the dental treatment contents classification in the 1-3 layers of LSTM were 66.2%, 70.8% and 67.7% when time series data were used. When average time series data were used, the accuracies were 67.7%, 70.8%, and 69.2%, respectively.Conclusions: We developed a method for encoding dental treatment. From the obtained codes, the contents of the treatment could be classified with about 70% accuracy.

084: Evaluation of simulation training courses on basic clinical skills for geriatric dentistry for trainee dentists

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Objectives: Geriatric dentistry is becoming the most important issue for delivering dental care as Japan transforms into a super-aged society. Although dental care for older adults requires basic dental treatment skills, trainee dentists, who have just acquired a dentist license, are often inexperienced in basic dental care skills. Therefore, we provided a simulation training courses for basic dental clinical skills for geriatric dentistry for trainee dentists. The purpose of this study was to evaluate the simulation training courses using a questionnaire.Methods: We included 43 trainee dentists enrolled in a 1-year postgraduate clinical training course at Okayama University Hospital in 2019. Simulation training courses for basic dental clinical skills such as tooth preparation for various dental restorations, root canal treatment, and oral cleaning for geriatric patients were provided. We administered a questionnaire survey and statistical analysis to investigate the practice environment and effectiveness of simulation training.Results: Almost all trainee dentists thought that the simulation training for preparation and root canal treatment would be useful for clinical practice, although more than half thought that the number of practice sessions for root canal treatment was insufficient. A statistical analysis revealed that the trainee dentists increased their knowledge of dental care for older adults after the simulation training course for geriatric dentistry. In addition, the trainee dentists became interested in the dental treatment of older adults and began to think about contributing to dentistry for older adults in the future.Conclusions: This questionnaire results showed that the trainee dentists felt that the simulation training was useful for clinical practice. It was also suggested that we could perform a higher quality training by improving the training environment.

085: Needs Of Dentists During The Covid-19 Pandemic In Nepal

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Objective: During the Coronavirus disease (Covid-19) pandemic, Nepal decided to continue the dental service with case prioritization and infection control. Because the dental setting is a high risk for cross-infection, the needs of dentists should be identified and addressed. Recent studies illustrated public and private sectors have different practice environments, thus their needs might be different. We aimed to assess the needs of dentists by the practice sectors during the Covid-19 pandemic in Nepal.**Methods:** A cross-sectional online questionnaire survey was conducted from 28th July to 7th August 2020 by distributing the questionnaire through emails and social networking services. It had five sections; basic characteristics, behavior, material availability and affordability, economic and psychological impact, and training and supports. The answers were stratified by the practice sectors (public hospitals and private clinics). Logistic regression was carried out to calculate the odds ratios (OR) of each answer based on the practice sectors, adjusting for sex and age.**Results:** 352 dentists (137 males and 215 females) were included in the analysis. 146 (41.2%) and 200 (56.8%) dentists worked for public hospitals and private clinics, respectively. Fewer dentists in private clinics experienced a lack of personal protective equipment than public hospitals (OR=0.3; p=0.001). Conversely, private clinics had a larger economic negative impact; more dental clinics were permanently closed (OR=2.3; p=0.015) and decreased salary more than 80% (OR=3.5; p<0.001) than public hospitals. Additionally, more dentists in private clinics received Covid-19 training from social media than public hospitals (OR=2.3; p=0.002). Overall, fewer dentists in private hospitals requested material support (OR=0.5; p=0.002) but demanded more for financial (OR=2.3; p=0.010) and technical support (OR=2.2; p=0.012) than dentists in public hospitals.**Conclusion:** Dentists working for public hospitals and private clinics in Nepal had different needs during the Covid-19 pandemic and these needs should be addressed accordingly.

086: Oral conditions matter for general health of community-dwelling elderly population

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Objectives: Deterioration of oral function and general health might be linked in the elderly people, but population-based studies are scarce previously. This study described the oral conditions including its functional aspects, and examined the association between oral conditions and systemic disease in the elderly population. **Methods:** The subjects consist of 20052 75-year-old, and 12410 80-year-old individuals who were dwelling in Shizuoka prefecture, Japan, and participated in the oral health check-ups between 2016-2018, which was provided as a public health service. Number of remaining teeth, carious and periodontal status, masticatory ability, tongue movement, the repetitive saliva swallowing test, mouth dryness, and oral hygiene were examined by the experienced dentists. Information regarding the self-rated oral functions and the presence of systemic disease was collected using the questionnaire which the subjects brought to their dentists when visiting for check-ups. Data on the subjects' body mass index (BMI) and annual medical and dental expenses were obtained from the Shizuoka association of long-term care medical insurance system. Objective oral frailty score was calculated from the results of clinical examination, and Perceived oral frailty score was from the self-rated assessment of the questionnaire. **Results:** 73.0% and 62.1% of the 75- and 80-year-old subjects had 20 or more teeth, respectively. 41.2% and 53.2% showed objective oral frailty (score≥1), and 54% and 56.5% showed perceived oral frailty (score≥1). Objective oral frailty score was positively correlated with perceived oral frailty score, number of present systemic diseases, annual medical and dental expenses. It was negatively correlated with number of caries-free teeth, number of remaining teeth, and BMI.**Conclusions:** The deterioration of oral function was associated with greater risks of having systemic disease and having to pay higher medical and dental expenses in older age. Increasing BMI, retaining more teeth without caries could be important for its prevention. (Support by JSPS KAKENHI 18K02250)

087: Comparative study of plaque removal effect on Japanese commercial toothbrushes.

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Objectives: There are so many shapes, sizes, and styles of toothbrushes on the market. We may know comparative effects about that. This study aims to evaluate the plaque removal effect of commercially various toothbrushes in vitro.

Methods: The toothbrushes were selected Nikkei telecom top-selling products and other different head designs, totally used 11 types. The brands were Clinical Advantage®, System Toothbrush®, Between Lion®, Gum Dental Brush®, Clear Clean Toothbrush®, and Ruscello Toothbrush®. Using 6 (16, 21, 24, 36, 41, and 44) artificial teeth (NISSIN) setting on the dental model, the brushing method was performed for 10 seconds per site with keeping pressure of 150g. The outcome was the removal rate of artificial plaque (NISSIN). Image analysis using software ImageJ was calculated from the remaining area of plaque, by taking photographs before and after brushing. The statistical analysis was used SPSS22.0 (IBM) for comparing.

Results: As for the plaque removal rate in all parts, the mountain-shape cut bristle toothbrush had the highest removal rate (61.2%). On the other hand, the ultrafine bristle toothbrush had the lowest (17.4%) with significant differences between other toothbrushes. These designs, rough surface processing type (59.1%), spherical bristle type (59.1%), and the diamond shape type (59.7%) had better plaque removal effect, although there was no significant difference in this experiment. Nevertheless, almost toothbrushes had more than 50% of plaque remained in the tooth adjacent surface. It seems necessary to use other oral cleaning tools such as dental floss or interdental brush to remove plaque in adjacent surfaces.

Conclusions: The different designs of the commercial toothbrushes like hardness, planting density, tip shape, etc. affected the plaque removal rate. Particularly, the plaque remaining by the ultrafine toothbrushes was large, and there were significant differences statistically between other toothbrushes.

088: Comparing the oral health-related quality of life of people with diabetes and people without diabetes at a Tertiary Hospital in Nigeria

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Objectives: The bidirectional relationship between diabetes mellitus and periodontal disease may negatively influence the oral health quality of life of diabetics. The study aims to compare the oral health quality of life of diabetics and non-diabetics. Methods: A descriptive cross-sectional study involving 115 people with diabetes seen at the Endocrine Clinic and 115 non-diabetics at Dental Centre UPTH. A self-administered questionnaire was employed for this study. The people with diabetes were selected by systematic random sampling method, and the non-diabetics were matched for age and sex from the Oral Diagnosis unit. Data analysis was done with IBM SPSS Statistics version 25. Chi-square and t-tests were done to test for differences and level of significance at 0.05.Results: There were 44 (38.3%) males, 71 (61.7%) females in each group and the mean ages were 56.04 ± 11.67 years for non-diabetics and 55.97 ± 11.48 years for diabetics. Of the 14 subdomains of OHIP-14, only the 'had to interrupt meals' was significantly different between diabetics 17 (14.8%) with impact and non-diabetics 29 (25.2%) without impact (p=0.048). the summated OHIP-14 impact was 27 (23.5%) for diabetics and 32 (27.8%) for non-diabetics (p=0.450). whereas the non-diabetics were found to have slightly higher impacts in nine (64.3%) out of the 14 subdomains of OHIP-14, people with diabetes had 5 (35.7%).Conclusions: The oral health impact of non-diabetics was not significantly different from that of the diabetics. It is imperative to refer people with diabetes for routine oral evaluation and intervention.

089: Survey Of Smile Satisfaction And Desired Treatments Among Medical And Dental Students.

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Objectives: The purpose of this study was to evaluate the factors prompting students' satisfaction with their own smile and their willingness to opt for different treatments to improve their dental appearance.Methods: A cross sectional questionnaire based study was conducted among 400 undergraduate medical and dental students from different universities of Karachi, Pakistan.Only the undergraduate medical and dental students were included while the postgraduate students were excluded. Data was piled up and analyzed by SPSS version 22. A questionnaire was filled by the students responding to dental problems or conditions with which they are not happy and the oral/dental treatments they would opt.Results: The participating candidates were predominantly female(81.5%). The data analysis showed (30.8%) of the students were not happy with their general dental appearance whereas a greater percentage was dissatisfied with their tooth color(37.8%). Tooth whitening was the most desirable treatment (59.8%) followed by orthodontic treatment(32%). Dentures were the least popular option among the young students with only (1.3%).Survey showed that a notable percentage of the population (35.5%) were not willing to bear the expense of the treatments.Conclusions: Majority of the participants were satisfied with their general dental appearance with higher percentage unhappy with their tooth color.Generalized spacing being endemic in Asian population was disregarded by (14.2%) of the students while (19.3%) complained of space among central incisors only. Female students were more agitated concerning their appearance. Out of two student groups, smile assessment was better discerned by dental students.

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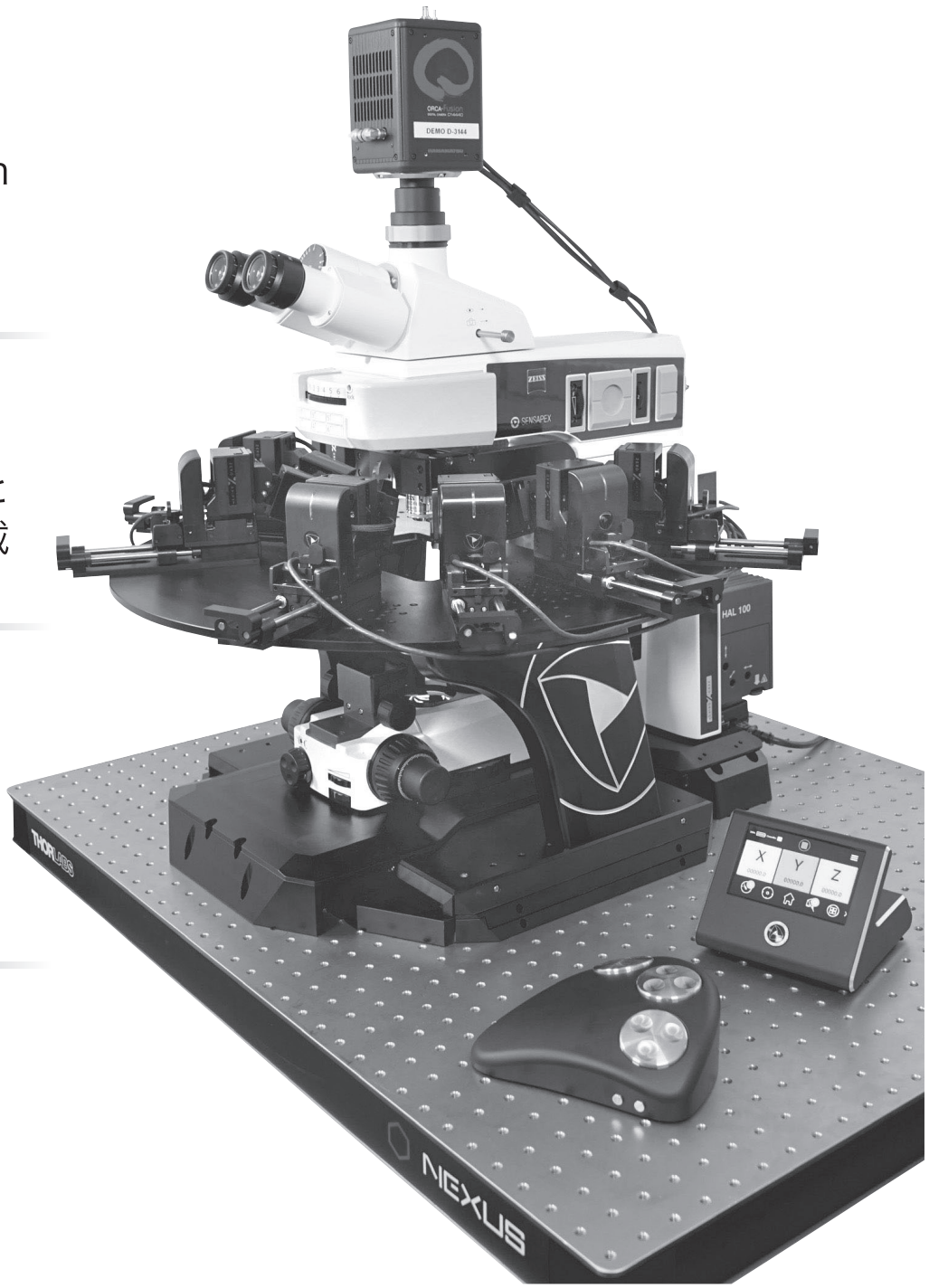

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