Kyudai Oral Bioscience

&

OBT Research Center

Joint International Symposium 2021

PROGRAM & ABSTRACTS

February 6, 2021
Lecture Room A/B, Faculty of Dental Science,
Kyushu University

KOB 2021

KYUSHU OBT

■ Date:

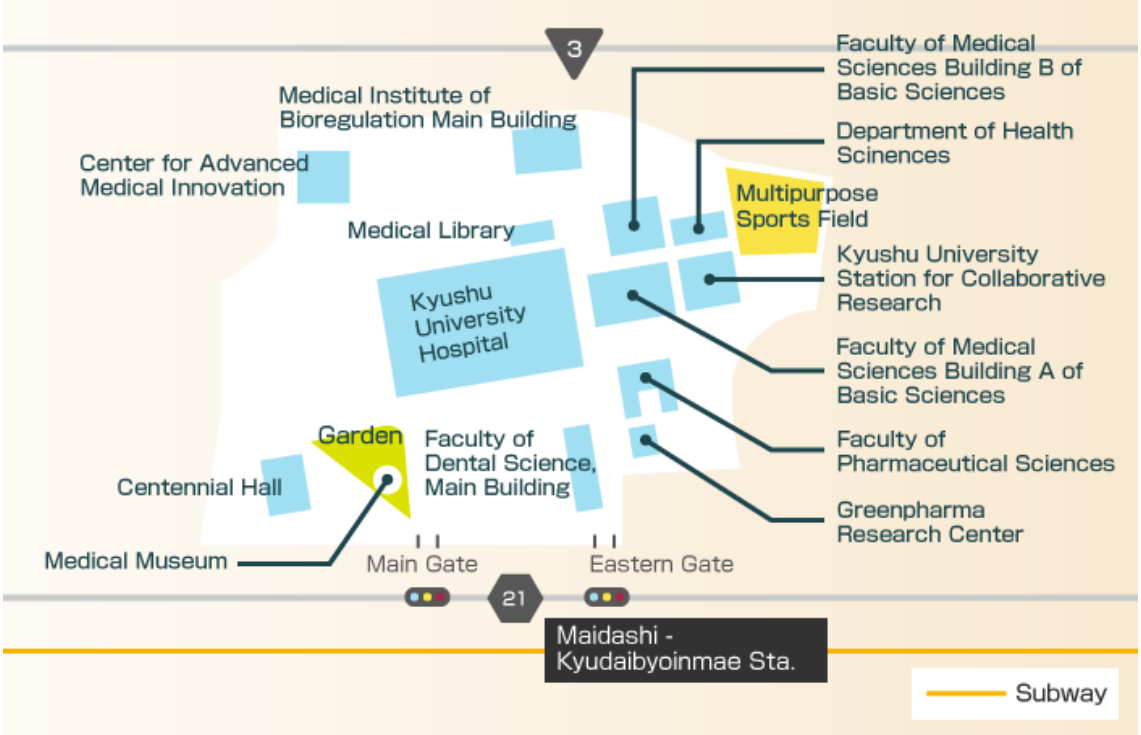
February 6, 2021

■ Zoom Meeting

■ Organization

Kyudai Oral Bioscience

Oral Health・Brain Health・Total Health Research Center



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KOB & OBT Joint International Symposium 2021

PROGRAM

February 6 (Saturday)

Time	Title	Presenter
9:00-9:10	Opening Remark	Prof. Seiji Nakamura
Session 1	**Special Lecture (1)**	**Chair:** Dr. Keisuke Sanematsu
9:10-10:10	An integrative structure of the human sweet taste receptor and protein engineering using deep mutagenesis	Dr. Erik Procko
10:10-10:25	Break	
Session 2	**Graduate Student's Session (1)**	**Chair:** Kozue Yamashita
10:25-10:40	Secreted frizzled-related protein 1 promotes odontoblastic differentiation and reparative dentin formation by regulating Notch signaling in dental pulp cells	Keita Ipposhi
10:40-10:55	Effect of isometric tongue lifting exercise on oral function, physical function, and body composition in the elderly	Asuka Tani
10:55-11:10	Identification of microRNA regulating NF-κB pathway in macrophage	Rongzhi Li
11:10-11:25	The role for non-coding RNAs on pathogenesis of IgG4-related diseases	Soi Kimura
11:25-11:40	Break	
Session 3	**Undergraduate Student's Session**	**Chair:** Dr. Tomoyo Yasukochi
11:40-11:55	Effect of prosthodontic treatment on standing movement function	Yuriko Takamoto
11:55-12:10	Comprehensive analysis of non-essential ribosomal subunits	Ronghao Tang
12:10-12:25	Functional analysis of Semaphorin3A expression, which is an axonal guidance factor, in salivary gland development and adenoid cystic carcinoma cell proliferation	Tatsufumi Fujimoto
12:25-13:15	Lunch Break	
13:15-13:25	**Award Presentation (IF and FWCI Award)**	
Session 4	**Award Lectures (IF Award winners)**	**Chair:** Prof. Hidefumi Maeda
13:25-13:45	Impacts of Pore Architecture on Bone Regeneration in Honeycomb Scaffold	Dr. Koichiro Hayashi
13:45-14:05	Epithelial and Connective Tissue Sealing around Titanium Implants with Various Typical Surface Finishes	Dr. Ikue Narimatsu
14:05-14:25	Disrupted tongue microbiota and detection of nonindigenous bacteria on the day of allogeneic hematopoietic stem cell transplantation	Dr. Saori Oku
14:25-14:45	Activated M2 Macrophages Contribute to the Pathogenesis of IgG4-Related Disease via Toll-like Receptor 7/Interleukin-33 Signaling	Dr. Noriko Ishiguro-Kakizoe
14:45-15:00	Break	
Session 5	**Special Lecture (2)**	**Chair:** Dr. Zhou Wu
15:00-15:40	Glial activation and neurodegeneration in neuroinflammation	Dr. Sadayuki Hashioka
15:40-15:55	Break	
Session 6	**Graduate Student's Session (2)**	**Chair:** Muzhou Jiang
15:55-16:10	Assessment of periodontitis by next-generation sequencing method focusing on subgingival plaque-specific bacteria in saliva	Jiale Ma
16:10-16:25	P. gingivalis LPS Induces Inflammatory Bone destruction and Alzheimer's Disease-Like Brain Pathologies in middle aged mice	Yebo Gu
16:25-16:40	Porphyromonas gingivalis infection induces leptomeningeal cells-mediated synaptic failure in neurons	Wanyi Huang
16:40-16:55	Cathepsin B inhibition blocks neurite outgrowth in cultured neurons by regulating lysosomal trafficking and remodeling	Muzhou Jiang
16:55-17:10	Break	
Session 7	**Special Lecture (3)**	**Chair:** Prof. Fusanori Nishimura
17:10-18:10	FORENSIC ODONTOLOGY IN INDONESIA from A to Z	Prof. Mieke Sylvia
18:10	Closing Remarks	Prof. Eijiro Jimi

ABSTRACTS

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OBT Special Lecture 1

By Dr. Erik Procko

Chaired by Dr. Keisuke Sanematsu

An integrative structure of the human sweet taste receptor and protein engineering using deep mutagenesis

Erik Procko[1]

[1]*Department of Biochemistry, University of Illinois, Urbana, USA.*

In a deep mutational scan, next generation sequencing is used to follow the in vitro selection of a diverse collection of protein variants. By observing which variants increase or decrease in frequency, it becomes possible to determine the relative effects of thousands of protein mutations in a single experiment, provided a suitable selection strategy is available.

In the human heterodimeric sweet taste receptor, surface trafficking of the T1R3 subunit is dependent on co-expression with the T1R2 subunit. A selection and deep mutational scan based on co-trafficking of T1R2-T1R3 to the plasma membrane therefore informs how mutations influence the subunits' physical association. Analysis of nearly 20,000 mutations in T1R2 revealed key contacts to T1R3 within the ligand-binding domain, cysteine-rich domain, and on specific helices of the transmembrane domain. Guided by the mutagenesis data, an integrative structural model of the complete sweet taste receptor in an active conformation was solved. There is a twisted arrangement of domains around a central axis and a continuous folded structure between transmembrane domain loops and extracellular domains, providing an elegant mechanism for how sugar binding to an extracellular site causes conformational changes to propagate to the receptor's cytoplasmic surface for intracellular signaling.

Recently, the same technology developed for deep mutagenesis of the human sweet taste receptor was rapidly repurposed for studying the interaction between the spike of SARS coronavirus 2 and its host entry receptor, ACE2. SARS-CoV-2 is responsible for the complex vasculature disease called COVID-19, which includes unusual neurological symptoms such as loss of taste and smell. A deep mutational scan of ACE2 identified mutations that increase affinity for the viral spike, informing the design of soluble decoy receptors that rival monoclonal antibodies for tight binding and potent neutralization of SARS-CoV-2.

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Graduate Student's Session (1)

Chaired by Kozue Yamashita

Secreted frizzled-related protein 1 promotes odontoblastic differentiation and reparative dentin formation by regulating Notch signaling in dental pulp cells

Keita Ipposhi[1], Atsushi Tomokiyo[2], Taiga Ono[1], Kozue Yamashita[1], M. Anas Alhasan[1], Daigaku Hasegawa[2], Sayuri Hamano[1,3], Shinichiro Yoshida[2], Hideki Sugii[2], Tomohiro Itoyama[1], Marina Ogawa[1], Hidefumi Maeda[1,2]

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[2]Department of Endodontology, Kyushu University Hospital, Fukuoka, Japan;
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Direct pulp capping is an effective treatment for preserving dental pulp against carious or traumatic pulp exposure via the formation of protective reparative dentin by odontoblast-like cells. Reparative dentin formation can be stimulated by several signaling molecules; therefore, we investigated the effects of a secreted frizzled-related protein (sFRP) 1 that was reported to be strongly expressed in odontoblasts of newborn molar tooth germs on odontoblastic differentiation and reparative dentin formation. In developing rat incisors, cells in the dental pulp, cervical loop and inner enamel epithelium, as well as ameloblasts and preodontoblasts weakly expressed sFRP1; however, sFRP1 was strongly expressed in mature odontoblasts. Human dental pulp cells (HDPCs) showed stronger expression of sFRP1 compared with periodontal ligament cells and gingival cells. sFRP1 knockdown in HDPCs abolished calcium chloride-induced mineralized nodule formation and odontoblast-related gene expression and decreased Notch-related gene expression. Conversely, sFRP1 stimulation enhanced nodule formation and expression of Notch-related genes. Direct pulp capping treatment with sFRP1 induced the formation of a considerable amount of reparative dentin in a dose-dependent manner. Our results indicate that sFRP1 is crucial for dentinogenesis and is important in promoting reparative dentin formation in response to injury by regulating Notch signaling in dental pulp cells.

Effect of isometric tongue lifting exercise on oral function, physical function, and body composition in the older people

Asuka Tani[1], Shinsuke Mizutani[1,2], Kiyomi Iyota[1], Harukaze Yatsugi[3], Saori Oku[1], Tianshu Chu[3], Xin Liu[3], Hiro Kishimoto[3,4], Haruhiko Kashiwazaki[1]

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Background: On average, the older people in Japan need long-term care using nursing care insurance for 10 years. Frailty prevention is essential to avoid this substantial need for long-term care. Oral frailty is considered a symptom of pre-frailty; however, a few studies have examined whether an intervention for oral frailty may prevent frailty.
Methods: This interventional study included 49 participants (25 males; aged 65–79 years; mean ± SD, 73.1 ± 3.1 years) from a larger group of participants of the "Itoshima Frail Study," in Itoshima City, Japan. Strength training of the tongue muscle with an isometric tongue lifting tool (Peko-panda®) was conducted for 3 months. Tongue pressure was measured on a monthly basis with a tongue-pressure measuring device. We also measured the number of remaining teeth; tongue and lip motor functions (oral diadochokinesis; ODK); body composition; and physical function, including open-eyed one-leg standing, sit-to-stand motion, and 3m timed up & go (TUG), at baseline and follow up. Data after 3 months of training were compared with data at baseline using the Wilcoxon signed-rank test. The correlation coefficients between the amount of change for each item and change in tongue pressure were assessed using bivariate analysis calculated with Spearman's rank correlation coefficient.
Results: Tongue pressure and ODK significantly improved after the intervention ($p < 0.001$). In terms of physical function, the time for open-eyed one-leg standing, sit-to-stand motion, and 3m TUG significantly improved ($p = 0.004$, $p < 0.001$, $p = 0.019$, respectively). However, the amount of change in tongue pressure was not related to the amount of change in any of the other measured items. In addition, a decrease in subcutaneous fat percentage and an increase in skeletal muscle ratio were observed significantly in the non-frailty group ($p<0.001$, $p=0.036$, respectively), but no significant change was observed in the frailty / pre-frailty group.
Conclusion: Our results showed that isometric tongue lifting exercise is effective in improving oral functions in the older people. This training may also have positive indirect effects on physical function and body composition.

Identification of microRNA regulating NF-κB pathway in macrophage

Rongzhi Li[1], Tomomi Sano[2], Takanori Shinjo[1],
Misaki Iwashita[1], Akiko Yamashita[1], Takao Fukuda[1], Fusanori Nishimura[1]

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We previously detected abnormally dysregulated micro (mi) RNAs in the adipose tissue of mice fed a high-fat-diet (HFD) by microarray analysis. Here, we aimed to elucidate the role of dysregulated miRNAs that were not completely characterized.
First, qPCR was performed to verify the results of the microarray analysis. LPS-stimulated RAW264.7 cells were used to confirm the changes of these miRNAs under inflammatory conditions. Then, miRNA mimic was transfected into RAW264.7 cells to identify the potential targets, and mRNA and protein levels of these target molecules were measured. In addition, expression of inflammatory markers was evaluated in RAW264.7 cells treated with or without miRNA mimic followed by LPS stimulation.
Selected miRNA was significantly down-regulated in adipose tissue of HFD-fed mice compared with normal diet fed mice, as well as in gingival tissue of ligature-induced periodontitis mice compared with their control littermates. Moreover, we noticed a significant reduction of the selected miRNA in RAW264.7 cells stimulated with LPS compared with those without LPS stimulation. mRNA levels of several putative targets were lowered after the transfection of miRNA mimic, but only one putative target molecule exhibited reduction at protein level. Moreover, this selected miRNA appeared to regulate inflammation.
The predicted gene was found to play an important role on NF-κB signaling pathway by subsequent analysis. miRNA mimic transfection into macrophages resulted in reduced expression of target genes as well as altered TNF-α expression. Thus, it is suggested that this miRNA might play a key role in regulating inflammation in adipose and, also, in periodontal tissues.

The role for non-coding RNAs on pathogenesis of IgG4-related diseases

Soi Kimura[1,2], Tomoyo Kawakubo-Yasukochi[1], Masafumi Moriyama[2], Seiji Nakamura[2], Eijiro Jimi[1]

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IgG4-related disease is a disease in which infiltration of IgG4-positive plasma cells into various organs and hyper-IgG4eima are observed. However, its pathogenic mechanism is still unknown. Almost all previous studies on this disease have been performed by immunological approach, and no analysis has yet been performed to the pathogenesis process from normal tissue.

In this study, we focused on microRNA (miRNA) in patient serum and analyzed the pathogenesis mechanism using normal salivary gland cells, in order to identify miRNA(s) involved in the pathogenesis of IgG4-related diseases.

miRNA is a short non-coding RNA (ncRNA) of about 18 to 25 bases, and they are involved in various biological phenomena and pathogenesis by controlling the expression of genes that have a sequence highly complementary to their own base sequence. In particular, circulatory miRNAs exist in body fluids are transported being wrapped in vesicles such as exosomes, and are stable in body fluids such as blood and saliva. Therefore, miRNA is expected as a non-invasive biomarker to grasp the pathogenesis and progression of various diseases.

We perfomed a comprehensive miRNA-mRNA pairing analysis, focusing on patient serum miRNA and intracellular mRNA in rat normal salivary epithelial cells. As a result, the miRNA, highly expressed in patients with IgG4-related diseases, and its target mRNA, localized in salivary gland epithelial cells, were identified. Furthermore, histological analysis revealed that expression pattern of the target factor related with differentiation in pathological region was significantly different from that in human normal salivary gland tissue.

From these results, it was suggested that the ncRNA in serum has an important role in the pathogenesis of IgG4-related diseases.

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Undergraduate Student's Session

Chaired by Dr. Tomoyo Yasukochi

Effect of prosthodontic treatment on standing movement function.

Yuriko Takamoto[1], Kyosuke Oki[2], Mikio Imai[3], Yoko Takemura[3], Yoichiro Ogino[2], Kiyoshi Koyano[2,3]

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Problem/hypothesis; Recently, Japan is experiencing "super-aging" society that is unprecedented in the world. It was reported elderly people with frailty increased risk of falls. However, the appropriate intervention can improve "frail". The objective of this study was to reveal the effect of prosthodontic treatment (restoration of occlusal support) on standing movement. Moreover, it also leads to show the role and the meaning of dentistry in super-aging society.

Methods; The subjects were 48 people and more than 65 years-old who received denture treatment. in University Hospital (Section of Fixed Prosthodontics and Removable Prosthodontics). The subjects were grouped two. One is included 24 subjects who have occlusal support in molar region (Eichner B1～B3), and the other is included 24 subjects who don't have it (Eichner B4～C3). The oral function (the occlusal number of teeth, occlusal area of contact, occlusal force and masticatory performance) and the ability of standing movement(①power, ②speed, ③muscle strength, ④balance) before and after a prosthodontic treatment were evaluated. 1) The occlusal force and the masticatory performance were compared with and without dentures. 2) All subjects were categorized by 2 groups which are with and without occlusal support in molar region and evaluated. Statistical analyses were performed using a Wilcoxon signed rank test.

Results; 1) The occlusal force and the masticatory performance were compared with and without dentures, and the results with dentures were significantly improved compared without dentures ($p<0.05$). Among the evaluation items of standing movement, "balance", "speed" and "muscle strength" were significantly improved with dentures, compared with the condition without dentures ($p<0.05$). 2)The occlusal force and the masticatory performance with dentures were significantly improved compared without dentures in both groups. In the groups of without occlusal support (Eichner B4~C group), a part of masticatory performance was improved with dentures compared without dentures.

Conclusion; It was suggested that the prosthodontic treatments affect not only systemic functions but also patients without occlusal support in the molars.

Comprehensive analysis of non-essential ribosomal subunits

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[2]*School of Dentistry, Kyushu University, Japan.*

The eukaryotic ribosome, which mainly consists of 79 ribosomal proteins (RPs) as well as 4 ribosomal RNAs (rRNAs), is the cellular translational machinery responsible for protein synthesis from messenger RNAs (mRNA). However, emerging evidences reveals that not all ribosomal subunits are essential for ribosome function, and that the ribosome is composed of essential subunits and non-essential subunits that modulate translation efficiency (TE). For instance, the mice lacking RPS19 are embryonic lethal prior to implantation, whereas RPL22-null mice are viable and only show impaired αβ T cell development.

We thus applied the CRISPR-Cas9 knockout screening to comprehensively identify the non-essential subunits based on their viability. As a result, we identified eight non-essential subunits and established their knockout clones, and double-checked their deletion by Western blotting and mass spectrometry. Since the loss of these subunits may affect the TE of specific mRNAs, we are performing ribosome profiling (Ribo-seq) in the knockout cell lines to measure the TE of each mRNAs.

We also focused on tissue-specific non-essential subunits and found that RPL3L, a gene with 74% sequence identity to the RPL3, is specifically expressed in striated muscle tissues such as heart and skeletal muscle. Notably, mutations in RPL3L have been identified in patients with dilated cardiomyopathy (DCM) [Ganapathi M et al, Hum. Genet. (2020)]. Therefore, we generated RPL3L-deficient mice and are currently performing phenotypic analysis by echocardiography and electrocardiography, and analysis of TE changes by Ribo-seq.

In summary, we identified non-essential RPs of constitutive and tissue-specific ribosomes and generated cells and mice lacking these RPs. We are now analyzing these cells and mice to elucidate the relationship of non-essential subunits to biological processes and diseases.

Functional analysis of Semaphorin3A expression, which is an axonal guidance factor, in salivary gland development and adenoid cystic carcinoma cell proliferation.

Tatsufumi Fujimoto[1,2], Shinsuke Fujii[2], Tamotsu Kiyoshima[2]

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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 temporospatial 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.

Mr. Tatsufumi Fujimoto is the best presenter of the Joseph Lister Award 2020 of Japanese Association for Dental Research (JADR).

Award Lectures

Chaired by Prof. Hidefumi Maeda

Impacts of Pore Architecture on Bone Regeneration in Honeycomb Scaffolds

Koichiro Hayashi[1]

[1]*Faculty of Dental Science, Department of Biomaterials, Kyushu University, Japan*

Autografts, allografts, and xenografts are used for treatment for critical-size bone defects. In terms of invasiveness, infection, supply, and cost, however, synthetic materials are preferred as scaffolds for bone regeneration. Synthetic scaffolds should have biocompatibility, high osteoconductivity, bioresorbability at a rate commensurate with bone remodeling, and high mechanical strength. To satisfy these requirements, the design of pore architecture, as well as chemical composition, is critical. Honeycomb construction is considered an ideal pore architecture. In this study, we fabricated honeycomb scaffolds consisting of carbonate apatite, which is analogous to the composition of natural bone, and evaluated the effects of macropores or channels (> 100 μm), micropores (submicron-10 μm), and nanopores (<submicron) on bone regeneration. Carbonate apatite honeycomb scaffolds were fabricated by the following procedures: 1) mixing calcium compounds ($CaSO_4$, $Ca(OH)_2$, or $CaCO_3$) and a wax-based binder; 2) the fabrication of honeycomb green bodies by extruding the mixture; 3) removal of organics from the honeycomb green bodies by heating; 4) the composition conversion of the honeycombs subjected to heat treatment into carbonate apatite via the dissolution-precipitation reactions. For in vivo evaluation, a cylindrical hole (diameter = 6 mm, depth = 5 mm), which represented a critical-sized bone defect, was drilled into the femur condyle of the rabbit. The carbonate apatite honeycomb scaffolds were implanted into the holes. At 4 and/or 12 weeks post-implantation, the condyle of each femur was harvested to perform μ-CT imaging and histological analyses. For evaluating the effects of channel size, honeycomb scaffolds with ~100-, 200-, and 300-μm channels were used. The ~300-μm channels were extensively occupied by new bone, compared to the ~100- and 200-μm channels. The effects of micropores were evaluated using honeycomb scaffolds with ~210-, 200-, and 190-mm^3/g micropore volumes. The replacement of scaffolds by bone was accelerated with increasing micropore volume. Notably, bone marrow-like tissues were formed in the honeycomb scaffolds with ~210-mm^3/g micropore volume. The effects of nanopores were evaluated using honeycomb scaffolds with ~180-, 150-, and 70-mm^3/g nanopore volumes. When the nanopore volume was 150 mm^3/g, osteoclastogenesis and osteogenesis progressed harmonically, resulting in scaffold replacement with new bone.

Epithelial and Connective Tissue Sealing around Titanium Implants with Various Typical Surface Finishes

Ikue Narimatsu[1], Ikiru Atsuta[2], Yasunori Ayukawa[1], Wakana Oshiro[1], Noriyuki Yasunami[1], Akihiro Furuhashi[1], and Kiyoshi Koyano[1]

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[2]*Division of Advanced Dental Devices and Therapeutics, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*

Background: The success of dental implant treatment depends on not only osseointegration around the implant body, but also a soft tissue barrier that protects the underlying hard tissue structures. Numerous surface modification techniques have been introduced to enhance bone contact on the implant surface, but there has been little research on the peri-implant soft tissue seal.

Purpose: This study aimed to investigate the role of epithelial and connective tissues seals around implants with various surface finishes.

Materials and methods: Testing surfaces had been machined (Ms), roughened by sandblasting and acid etching (Rs), treated hydrothermally with $CaCl_2$ (Cs), or anodized (As). (1) *In vitro study:* rat oral epithelial cells (OECs) and fibroblasts were cultured on Ms, Rs, Cs, and As titanium plates. (2) *In vivo study:* implants with Ms, Rs, Cs, and As surfaces were placed in the rat's oral cavity.

Results: (1) *In vitro study:* there was less cell adherence of OECs and more collagen expression when cultured on Rs and As plates than when cultured on Ms and Cs plates. (2) *In vivo study:* Cs group had superior sealing for the implant surface, Ms and Rs implants showed weaker sealing capacity than Cs. After 16 weeks, Rs implants exhibited peri-implant epithelial apical down-growth and had lost bone support, presumably because their resistance to penetration by epithelial attachment was low with this surface type.

Conclusion: Although a smooth surface (Ms, Cs) showed better epithelial attachment, rough surfaces (Rs, As) are more suitable for binding to connective tissue. Strong epithelial attachment to the implant surface seems to be a fundamental first-line of defense against foreign body penetration. Thus selecting suitable surfaces to ensure strong sealing is important for implant success.

Disrupted tongue microbiota and detection of nonindigenous bacteria on the day of allogeneic hematopoietic stem cell transplantation

Saori Oku[1,2,] Toru Takeshita[1,3,] Toshiko Futatsuki[2], Shinya Kageyama[1], Mikari Asakawa[1], Yasuo Mori[4], Toshihiro Miyamoto[4], Jun Hata[5,6], Toshiharu Ninomiya[5,6], Haruhiko Kashiwazaki[2], Yoshihisa Yamashita[1]

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Disruption of the intestinal microbiota caused by intensive chemotherapy, irradiation and antibiotics can result in development of severe gut graft-versus-host disease and infectious complications, leading to poorer outcomes among allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients. Although the oral cavity is also densely colonized by indigenous microorganisms, the bacterial composition in allo-HSCT recipients remains unclear. We determined the tongue microbiota composition of 45 patients with hematological disorders on the day of transplantation and compared them to 164 community-dwelling adults. The V1–V2 regions of the 16S rRNA gene sequences demonstrated that the allo-HSCT recipients had less diverse and distinct microbiota from that of community-dwelling adults. The full-length 16S rRNA gene sequences identified 146 bacterial taxa in the microbiota of allo-HSCT recipients, of which 34 bacterial taxa did not correspond to bacteria primarily inhabiting the oral cavity deposited in the expanded Human Oral Microbiome Database. Notably, the detection of Staphylococcus haemolyticus and/or Ralstonia pickettii was significantly associated with a higher risk of mortality during the follow-up period. These results demonstrate that the oral cavity of allo-HSCT recipients is colonized by a disrupted microbiota on the day of transplantation and suggest that detection of specific nonindigenous taxa could be a predictor of transplant outcome.

Activated M2 Macrophages Contribute to the Pathogenesis of IgG4-Related Disease via Toll-like Receptor 7/Interleukin-33 Signaling

Noriko Ishiguro-Kakizoe[1], Masafumi Moriyama[1,2], Katsuhiro Furusho[1,3], Takuma Shibata[3], Yusuke Murakami[3], Akira Chinju[1], Yuka Gion[4], Miho Ohta[1], Takashi Maehara[1], Akihiko Tanaka[1], Masaki Yamauchi[1], Mizuki Sakamoto[1], Keita Mochizuki[1], Yasuharu Sato[4], Tamotsu Kiyoshima[5], Hidetaka Yamamoto[6], Kensuke Miyake[3], and Seiji Nakamura[1]

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Objectives: IgG4-related disease (IgG4-RD) is a unique inflammatory disorder in which Th2 cytokines promote IgG4 production. In addition, recent studies have implicated the Toll-like receptor (TLR) pathway. This study was undertaken to examine the expression of TLRs in salivary glands (SGs) from patients with IgG4-RD.
Methods: SGs from 15 patients with IgG4-RD, 15 patients with Sjögren's syndrome, 10 patients with chronic sialadenitis, and 10 healthy controls were examined histologically. TLR family gene expression (TLR1 through TLR10) was analyzed by DNA microarray in the submandibular glands (SMGs). Up-regulation of TLRs was confirmed in SGs from patients with IgG4-RD. Finally, the phenotype of human TLR7 (huTLR7)-transgenic C57BL/6 mice was assessed before and after stimulation with TLR agonist.
Results: In patients with IgG4-RD, TLR4, TLR7, TLR8, and TLR9 were overexpressed. Polymerase chain reaction validated the up-regulation of TLR7 in IgG4-RD compared with the other groups. Immunohistochemical analysis confirmed strong infiltration of TLR7-positive cells around ectopic germinal centers (eGCs) and fibrotic areas in the SGs of patients with IgG4-RD. Double immunohistochemical staining showed that TLR7 expression colocalized with $CD163^+$ M2 macrophages. After in vitro stimulation with a TLR7 agonist, $CD163^+$ M2 macrophages produced higher levels of IL-33, which is a Th2-activating cytokine. In huTLR7-transgenic mice, the focus and fibrosis scores in SMGs, pancreas, and lungs were significantly higher than those in wild-type mice ($P < 0.05$). Moreover, the concentration of serum IgG, IgG1, and IL-33 in huTLR7-transgenic mice was distinctly increased upon stimulation with a TLR7 agonist ($P < 0.05$). Immunohistochemical analysis demonstrated focal accumulation of $CD206^+$ cells (marker for M2 macrophage in mice), $IgG1^+$ plasma cells, and $IL\text{-}33^+$ cells around eGCs in the SMGs from huTLR7-transgenic mice.
Conclusion. TLR7-expressing M2 macrophages may promote the activation of Th2 immune responses via IL-33 secretion in IgG4-RD. A more thorough understanding of the role of $TLR7^+CD163^+$ M2 macrophages in IgG4-RD could lead to the establishment of the world's first mouse model of IgG4-RD.

KOB & OBT Joint International Symposium 2021

OBT Special Lecture (2)

Chaired by Dr. Zhou Wu

Glial activation and neurodegeneration in neuroinflammation

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In 1980's, McGeer et al. identified both activated astrocytes and activated microglia in affected regions of a broad spectrum of neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis. They built the neuroinflammation hypothesis on these immunohistochemical findings and the epidemiological finding that the prevalence of AD in rheumatoid arthritis patients, who receive anti-inflammatory therapy, was sixfold spared compared to age-matched general populations. Activated astrocytes could be detrimental to neighboring neurons in neuroinflammatory process. Astrocytes exposed to certain inflammatory stimulants *in vitro* have been shown to release potentially neurotoxic molecules, including inflammatory cytokines, glutamate, nitric oxide and reactive oxygen species. We have previously demonstrated that adult human astrocytes stimulated with the inflammatory cytokine interferon (IFN)-γ, that is predominantly produced by T cells and natural killer cells and is detected only in the pathological brains, exert potent neurotoxicity. This IFN-γ-induced neurotoxicity of human astrocytes was mediated by astrocytic activation of the signal transducer and activator of transcription (STAT) 3 pathway with phosphorylation of STAT3 at tyrosine-705 residue. In addition, the proton pump inhibitors lansoprazole and omeprazole, the histone deacetylase inhibitor suberoylanilide hydroxamic acid, and the L-type calcium channel blockers nimodipine and verapamil significantly reduced the astrocytic neurotoxicity. All the reagents attenuated the IFN-γ-induced phosphorylation of STAT 3 at Tyr705, but not STAT1. Therefore, control of STAT3 activation in human astrocytes may be a promising new therapeutic strategy for various neurodegenerative and neuroinflammatory disorders in which activated astrocytes could contribute to the pathology.

KOB & OBT Joint International Symposium 2021

Graduate Student's Session (2)

Chaired by Muzhou Jiang

Assessment of periodontitis by next-generation sequencing method focusing on subgingival plaque-specific bacteria in saliva

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Saliva contains diverse bacteria shed from various oral sites, including subgingival plaque. Focusing on the total bacterial occupancy in saliva of subgingival plaque-specific bacteria (SUBP bacteria) that live in subgingival environments regardless of virulence is reasonable for detecting periodontitis in salivary testing. This study aimed to validate the clinical utility of the SUBP bacteria in salivary microbiota for assessment of periodontitis by using next-generation sequencing method. We examined stimulated saliva samples collected from 125 subjects who visited three dental clinics. The relative abundances of previously identified 11 SUBP bacteria were determined using 16S ribosomal RNA gene sequencing and the reference-based approach. The prediction performance was evaluated using the receiver operating characteristic (ROC) curve. The SUBP bacteria accounted for a total of 0-15.4% in their salivary microbiota, and the percentage distinguished severe periodontitis patients with ≥15 deep probing sites (≥4mm depth) with 0.90 (95% confidence interval [CI], 0.81-0.98) of sensitivity and 0.70 (95% CI, 0.60-0.80) of specificity (area under the ROC curve [AUC], 0.87). Among 2,047 combinations of 11 SUBP bacteria, in particular, combinations including *Streptococcus constellatus*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* subsp. *vincentii* demonstrated significantly higher AUC values in the detection of severe periodontitis patients. These results suggest that examining the SUBP bacteria in saliva may be useful for screening severe periodontitis patients without periodontal probing.

P. gingivalis LPS Induces Inflammatory Bone destruction and Alzheimer's Disease-Like Brain Pathologies in middle aged mice

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Background: As common issues affecting the elderly, Alzheimer's disease (AD) and bone loss can be clinically exacerbated. However, the mechanism underlying this exacerbation remains poorly understood. In the present study, we tested our hypothesis that periodontitis is involved in the exacerbation, contributing to AD pathologies.
Objective: We tested our hypothesis that periodontitis is involved in the exacerbation, contributing to AD pathologies.
Methods: The bone, memory, and inflammation in bone and brain were examined in 12-month-old mice after systemic exposure to lipopolysaccharide from *Porphyromonas gingivalis* (*Pg*LPS) for 3 consecutive weeks.
Results: Compared with control mice, bone loss in tibia (26% decrease) and memory decline (47% decrease) were induced in mice with a positive correlation after exposure to *Pg*LPS ($r = 0.7378$, $p = 0.0011$). The IL-6 and IL-17 expression in tibia was negatively correlated with the bone volume/total tissue volume ($r = –0.6619$, $p = 0.0052$; $r = –0.7129$, $p = 0.0019$), while that in the cortex was negatively correlated with the memory test latency ($r = –0.7198$, $p = 0.0017$; $p = 0.0351$, $r = –0.5291$). Furthermore, the IL-17 expression in microglia was positively correlated with A42 accumulation in neurons ($r = 0.8635$, $p < 0.0001$). In cultured MG6 microglia, the *Pg*LPS-increased IL-6 expression was inhibited by a PI3K-specific inhibitor (68% decrease), and that of IL-17 was inhibited by IL-6 antibody (41% decrease). In cultured N2a neurons, conditioned medium from *Pg*LPS-stimulated microglia (MCM) but not *Pg*LPS increased the productions of A β PP, CatB, and Aβ42, which were significantly inhibited by pre-treatment with IL-17 antibody (67%, 51%, and 41% decrease).
Conclusion: These findings demonstrated that chronic systemic exposure to *Pg*LPS simultaneously induces inflammation dependent bone loss and AD-like pathologies by elevating IL-6 and IL-17 from middle age, suggesting that periodontal bacteria induce exacerbation of bone loss and memory decline, resulting in AD progression.

Porphyromonas gingivalis infection induces leptomeningeal cells-mediated synaptic failure in neurons

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Background: Synaptic failure is the earliest sign before Alzheimer's disease (AD) onset and closely associated with cognitive decline. Clinical studies have shown that periodontitis is positively correlated with both the onset and pathological progression of AD, and preclinical studies have shown that *Porphyromonas gingivalis* (*P. gingivalis*), the key pathogen in periodontitis, and its virulence factors induced memory decline in mice. However, the mechanisms underlying the involvement of *P. gingivalis* in memory decline remain unclear.

Methods: We used primary leptomeningeal cells and primary cortical neurons to evaluate the effects of leptomeningeal cells on synaptic generation and plasticity after *P. gingivalis* infection in vitro. The expression of the related molecules was examined by mRNA in real time and by protein levels using Western blotting. Pharmacological and genetic approaches were used to explore the mechanism underlying the involvement of leptomeningeal cells in synaptic changes after *P. gingivalis* infection.

Results: NLRP3 inflammasome activation was involved in augmenting the IL-1β secretion by primary leptomeningeal cells after *P. gingivalis* infection, as determined by the knockdown of NLRP3 with siRNA. Cathepsin B (CatB) mediated the activation of both NLRP3 inflammasomes and NF-κB to produce IL-1β by *P. gingivalis*-infected primary leptomeningeal cells, as determined by the pharmacologically specific inhibition of CatB. In contrast, *P. gingivalis*-infected leptomeningeal cells induced an IL-1β-dependent decrease in synaptic molecules in primary cortical neurons, as determined by the pharmacological blockage of the IL-1 receptor. *P. gingivalis*-infected leptomeningeal cells also induced the IL-1β-dependent suppression of BDNF signaling in cultured N2a neurons, a stable mouse neural cell line.

Conclusion: The CatB-mediated IL-1β production was augmented in leptomeningeal cells, resulting in synaptic failure and blockage of BDNF signaling in neurons during *P. gingivalis* infection. These findings highlight a new mechanism underlying the involvement of periodontitis in AD initiation and suggest that CatB may be an early intervention therapeutic target for delaying the onset of AD during *P. gingivalis* infection.

Cathepsin B inhibition blocks neurite outgrowth in cultured neurons by regulating lysosomal trafficking and remodeling

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Background: Lysosomes are known to mediate neurite outgrowth which is essential for neuronal network to enhance brain function. However, the principal lysosomal molecule controlling that outgrowth is unclear.

Methods & Results: We studied primary mouse neurons *in vitro* and found that they naturally develop neurite outgrowths over time and as they did so the lysosomal cysteine protease cathepsin B (CTSB) mRNA levels dramatically increased. Surprisingly, we found that treating those neurons with CA-074Me, which inhibits CTSB, prevented neurites. As that compound also inhibits another protease, we evaluated a N2a neuronal cell line in which the CTSB gene was deleted (CTSB KO) using CRISPR technology and induced their neurite outgrowth by treatment with retinoic acid. We found that CTSB KO N2a cells failed to produce neurite outgrowths but the wild-type (WT) did. CA-074Me is a cell permeable prodrug of CA-074, which is cell impermeable and a specific CTSB inhibitor. Neurite outgrowth was and was not suppressed in WT N2a cells treated with CA-074Me and CA-074, respectively. Lysosome-associated membrane glycoprotein2 (LAMP2)-positive lysosomes traffic to the plasma cell membrane in WT but not in CTSB KO N_2a cells. Interestingly, no obvious differences between WT and CTSB KO N2a cells were found in neurite outgrowth regulatory proteins, PI3K/AKT, ERK/MAPK, cJUN and CREB.

Conclusion: These findings show that intracellular CTSB controls neurite outgrowth and that it does so through regulation of lysosomal trafficking and remodeling in neurons. This adds valuable information regarding the physiological function of CTSB in neural development.

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KOB Special Lecture

By Prof. Mieke Sylvia Margaretha

Chaired by Prof. Fusanori Nishimura

Forensic Odontology in Indonesia from A to Z

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Indonesia is the largest archipelago in the world with over 17,400 numbers of island. The intersection of three major plates, namely the Australian-indian plate, the Eurasia plate, and the pacific plate have caused Indonesia to become one of the countries which prone to natural disasters. It is also supported by the location of Indonesia in the Pacific Circum Series and the smaller Philipine plate.

Indonesia is also a country with the largest Muslim population of all countries in the world. In the Islamic religion, one important funeral rite should be done is the burial of the deceased has to be done immediately or within 24 hours after the time of death. Unfortunately, in the forensic field, the process of identification of the victim needs some extra time and sometimes causes some delayed in the final burial.

There are other challenges for a forensic expert in personal identification in Indonesia due to the high-risk place and lack of ante mortem data. Most of the low society populations in Indonesia do not aware of their oral health and does not know the importance of medical record. Furthermore, dental care is not covered by the insurance company. Because of the several issues, sometimes it is hard to collect the antemortem data in Indonesia. With this in mind, there are several suggestions that we have to solve this problem. First, we will need a specific approach and technique for personal identification which use the photo of the victim when alive and superimposed it with the post mortem photo using smartphone. Second, we should build a new program to educate the society about the importance of medical record used as an ante mortem data in “high-risk area”. With this program, we hope every citizen has a personal medical record so ante mortem data can be obtained easily when needed.

Slowly but sure we also have been doing some excellent researches which can help in personal identification procedure in the future. The development of forensic science studies in Indonesia itself also have been good and have become a government’s concern.

Keywords: Indonesia, Culture, Religion, Specific Approach, Dental Education and Research