Asia-Pacific Conference in Fukuoka 2021

International Symposium on Dental Education during COVID-19

Date: June 25th 2021 (Fri) ~

Held by on demand method
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Srinakharinwirot University
(Bangkok, Thailand)

Royal Thai Consulate-General
(Fukuoka, Japan)

Kyushu Dental University
(Kitakyushu, Japan)

Asia-Pacific Conference in Fukuoka 2021

International Symposium on Dental Education during COVID-19

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June 25th, 2021-

Organizing Committee:

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Organized by Kyushu Dental University

Program

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Dr. Tatsuji Nishihara, Chairman and President, Kyushu Dental University

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2. Mr. Attakarn Wongchanamas, Royal Thai Former Consulate-General in Fukuoka
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Dr. Kenshi Maki, Vice President, Kyushu Dental University

Welcome message

Tatsuji Nishihara, D.D.S., Ph.D.
Chairman and President
Kyushu Dental University

Welcome to Asia-Pacific Conference (APC) 2021. It is our great honor and pleasure to invite you to attend the International Symposium on Oral Education and Research in Kitakyushu, Fukuoka, Japan, 2021. This year we will host an APC online meeting under CIVID-19 crisis. I am inviting you to participate in this exciting project to obtain valuable information on main theme “Dental Education during COVID-19 crisis” as Chairman and President, Kyushu Dental University (KDU).

In this conference, we are delighted to announce the Congratulatory Speeches by Guests of Honor, Dr. Narongsak Laosrisin, Vice President of Administration Affair, Srinakharinwirot University (SWU), Mr. Attakarn Wongchanamas, Royal Thai Former Consulate-General in Fukuoka, and Mr. Kozo Yamamoto, Member of the House of Representatives, ex-Minister of State.

As keynote lectures concerning the dental education under COVID-19 crisis, current status of dental education in Thailand and Japan will be introduced by the speakers, Dr. Nathawut Kaewsutha, Dean, Faculty of Dentistry, SWU and Dr. Shuji Awano, Dean, Faculty of Dentistry, KDU. The other invited speakers, Dr. Wichida Chaweewannakorn, Department of Preventive and Pediatric Dentistry, Faculty of Dentistry, SWU, and Dr. Chuencheewit Thongsiri, Department of Periodontology, Faculty of Dentistry, SWU will introduce the abiding memories during the stay in KDU as graduate students. We are expecting to have active discussion on an education of graduate course in KDU.

We are also planning to have a session for poster presentations and invigorating discussions about the achievement of education and scientific research on oral bioscience. It is our wish to flash an innovative idea into your mind to build true partnership between SWU and KDU.

We thank you in advance for your interest and active participation and look forward to welcoming you to the online Asia-Pacific Conference in Fukuoka 2021.

Congratulatory Speeches by Guests of Honor

Narongsak Laosrisin, D.D.S., Ph.D.
Vice President of Administration Affair
Srinakharinwirot University

Dear Professor Nishihara, Presidents of Kyushu Dental University, Speakers, Distinguished Guests, Colleagues:

First, I would like to thank APC organizing committee for inviting me to give the congratulatory speech in APC2021.

It is my great pleasure and privilege to participate in the APC again. I first joined this conference in 2014. At that time, the MOU between our universities, Srinakharinwirot University and Kyushu Dental University, was just signed in order to create good international relationship and to further strengthen collaborations among our universities.

I would like to extend my thanks and admiration to Professor Nishihara, who has always been a strong driving force behind this international conference which has now expanded to involve many more universities in other Asia-Pacific countries.

Since last year/2020, we have all been facing the same critical world problem, the Corona Virus Pandemic. This crisis brought big changes that affected our ways of living in both good and bad ways. This forces us to rethink how we live and how we can evolve to live in this new-normal society while staying safe from Covid.

As personnel in the academic sector amidst the COVID19 pandemic, we are responsible for implementing new regulations and safety precautions to ensure that our students are able to achieve their learning objectives in dental education in a safe manner. Through sharing of research, knowledge and experiences on how we have all been tackling this issue, I hope that we will be able to develop innovations in dental education during the time of a pandemic. I am most certain that the wisdom and experiences that will be shared by our guest speakers will provide us insights that will help broaden our perspectives in the provision of dental education. I believe that the information discussed in this conference will certainly be meaningful and valuable to all. I would like to express my deep gratitude to distinguished guests and all participants who have contributed and made this meeting successful.

I hope everyone enjoy this virtual conference, and I hope you stay safe in your home countries.

Thank you very much.

Brief CV

Education:

D.D.S. Chulalongkorn University, Thailand		1985
Cert. in Periodontology	Tokyo medical and Dental University, Japan	1990
Ph.D. in Dental Science	Tokyo medical and Dental University, Japan	1990
Thai Board in Periodontics	Thai Dental Council	1997

Position: Associated Professor
Vice President for Administration (2017-2020)
Dean: Faculty of Dentistry (2009-2017)

Other Positions: - President of Thailand Society of Periodontology (2004-2006)
- President elected of Thailand Academy of Periodontology (2008-2010)
- President of Thailand Academy of Periodontology (2010-2012)
- President elected of Thailand Academy of Periodontology (2017-2020)
- President of Thailand Academy of Periodontology (2020- Present)
- Graduate director of Periodontology section, School of Dentistry, Srinakharinwirot University (Present)
- Committee of The Thai Dental council (2009-2017)
- Councilor of the Asian Pacific Society of Periodontology (2003-2019)
- President elected of the Asian Pacific Society of Periodontology (2019-Present)

Congratulatory Speeches by Guests of Honor

Mr. Attakarn Wongchanamas,
Royal Thai Former Consulate-General in Fukuoka

Dr. Tatsuji Nishihara, Chairman and President of Kyushu Dental University, Dr. Narongsak Laosrisin, Vice President of Administration Affairs, Srinakharinwirot University, Mr. Kozo Yamamoto, Member of the House of Representatives, Distinguished Speakers, Ladies and Gentlemen,

It is my great pleasure to be invited as the Guest of Honor to the International Symposium on Dental Education During COVID-19. I would like to appreciate the excellent team of Kyushu Dental University for their strong determination to make this event happen during this difficult time. Amidst the outbreak of Covid-19, digital technology enforces new ways of working and learning. Today event is a good example that represents the “New Normal” pattern in education, in which we are connected by technologies instead of physical interaction. This period of time is also challenging for all of us, to be adaptive to the changing environment. Your presence here is a reflection of your interest in continuing academic partnerships regardless of the obstacles. It is a prove that the ongoing pandemic cannot stop us from learning and cultivating knowledge from each other.

Ladies and Gentlemen,

Thailand and Japan established the diplomatic relations over one hundred and thirty-four years since eighteen eighty-seven. However, the relationship can be traced back nearly 600 years ago since the fifteenth century, through the trading routes between the Ryukyus in Okinawa and the Kingdom of Ayudhaya, the ancient capital of Thailand. Since then, Thailand and Japan have been enjoying the cordial and strong relations through the connection between the Monarchies, the Governments, and people-to-people contacts.

For centuries, the two countries have been cultivating mutual cooperation in all dimensions. In terms of economic, Japan continues to be Thailand’s important strategic partnership in the region. Japan’s investments in Thailand account for thirty-six percent of the total. In term of culture, there have been many activities and cultural exchanges between our two countries. One of the most recent and dynamic events are the Thai Festivals annually organized by the Royal Thai Embassy in Tokyo and the Royal Thai Consulate General in Osaka and Fukuoka. Although the actual festival could not be held this year, due to the spreading of COVID-19, the Thai Embassy in Tokyo and Consulates in Osaka and Fukuoka are joining hands to transform the

typical Thai Festivals into a “New Normal” event, where people can enjoy the glimpse of “Thai-ness” and Thai cultures through a series of online platforms.

Ladies and Gentlemen,

In term of education, the Royal Thai Consulate-General in Fukuoka stands ready to support the partnerships between Thai and Japanese academic institutions in various forms. In this regard, I am pleased to learn that Kyushu Dental University has been actively promoting academic exchanges with several Thai Universities, especially Srinakharinwirot University, one of the most prominent universities in Thailand.

Kyushu Dental University and Srinakharinwirot University have established the MOU to promote joint research and development activities in the fields of Dentistry since the year two thousand-thirteen. Prior to the outbreak of COVID-19, both universities have successfully promoted and organized activities among students exchange programmes and training courses on annual basis.

I truly hope that the global disruption will not last longer, so that more activities will be resumed as normal.

The Symposium today is of great opportunity for excellent experts in dentistry to share knowledges and exchange views on the areas of common interests and could encourage extensive discussion and contribute to the advancement in oral health and dental treatment of our two countries. I also wish the Symposium will be very beneficial for in-depth researches and developments in their respective fields.

Ladies and Gentlemen,

In conclusion, I would like to congratulate once again to Kyushu Dental University and all parties involved in this prestigious International Symposium.

I hope all participants could have fruitful discussion and a great time ahead.

Thank you very much.

Congratulatory Speeches by Guests of Honor

Mr. Kozo Yamamoto
Member of the House of Representatives,
ex-Minister of State

Birth: 8 Aug 1948, Kitakyushu City
Member, House of Representatives
Elected 8 Times / Fukuoka 10th District
Party Affiliation: Liberal Democratic Party (LDP)

Present Posts
Chairperson, Research Commission on the Finance and Banking Systems, LDP
Deputy Chairman, Research Commission on the Tax System, LDP
Chairman of the Subcommittee on Cultural Assets and Shrines & Temples Tourism, LDP

Education
Graduated from University of Tokyo, Faculty of Economics
Graduate School of Management, Cornell University (MBA)
Visiting Scholar, Harvard University's Center for International Affairs (U.S.-Japan Program)

Career
1971 Entered Ministry of Finance
1987 Personal Secretary to Minister of Finance
1991 Lecturer, Kyusyu International University
1993 Elected to the House of Representatives

Past Posts
House
2008 Chairman, Standing Committee on Judicial Affairs
2013 Chairman, Special Committee on Consumer Affairs
2016 Chairman of the Special Committee for Regional Revitalization
2020 Chairman of the Special Committee on Disasters

Administration
2006 Senior Vice-Minister of Economy, Trade and Industry
2016 Minister of State, in charge of Regional Revitalization, Deregulation, etc.

Party
2013 Chairman, Research Commission on Tourism Nation, LDP

Keynote Lectures

Innovation of Dental Education during COVID-19 pandemic in Thailand

Nathawut Kaewsutha, D.D.S., M.P.H., Ph.D.
Dean
Faculty of Dentistry
Srinakharinwirot University

The impact of the COVID-19 pandemic affects dental education in Thailand a lot. The pandemic forced dental schools to suspend their lectures and laboratories to shift to distant learning methods; e-learning has become the only option for continuation of dental education in Thailand.

Almost all lectures (or PBL courses) are switched to online courses to keep social distancing from gathering. Different APPs have been utilized during the pandemic, e.g., ZOOM, Google Meet, Skype, and Microsoft Teams. Fortunately, e-learning has proven to be a successful adjunct and has definitely impacted the environment in which dental students learn. When compared to traditional didactic methods, e-learning can be easily updated and rapidly accessed. Another advantage is that it can easily foster self-learning skills. Indeed, some studies have indicated that self-regulated learning significantly affects academic achievement and learning performance.

However, the sudden shift from traditional teaching methods to more creative distance learning did not allow sufficient time for adaptation of academic staff in Thailand. To learn sufficient knowledge and technology regarding IT will be mandatory in dental education both for educators and students.

The clinical training sessions have been flexible learning methods and that we have ended up finishing the academic year with a substantial degree of cut down on student clinical exposure by approximately 25-30% of their final years of training. The students are divided into different groups to decrease the number and the risk is minimized by standard PPE and good ventilation settings.

However, for future challenges the dental educators should be cautious but not panic, being flexible, and willing to face the changes. Dental education models should be innovated to suit various situations and new technological tools should be applied for dental education.

Brief CV

EDUCATION

2020	Senior Fellow (SFHEA), Advance HE, UK, (PR184125)
2017	Diplomate, Thai Board of Dental Public Health, Thai Dental Council
2015	Doctor of Philosophy (PHD) in Applied Behavioral Science Research, Behavioral Science Research Institute, Srinakharinwirot University
2007	Master of Public Health (MPH), Mahidol University
2005	Doctor of Dental surgery (DDS) 2nd Honors, Srinakharinwirot University

EMPLOYMENT

2017- present	Dean, Faculty of Dentistry, Srinakharinwirot University
2012 - 2017:	Associate Dean for Administrative Affairs, Faculty of Dentistry, Srinakharinwirot University
2009 - 2012:	Head of Dental Public Health Division, Faculty of Dentistry, Srinakharinwirot University
2009 - 2012:	Associate Dean for Students Affairs, Faculty of Dentistry, Srinakharinwirot University
2009	Assistant Dean for Students Affairs, Faculty of Dentistry, Srinakharinwirot University
2016 - 2017:	President for Thai Society for Public Health Dentistry
2015 - 2018:	Deputy Project Manager of Self Care for Kid Project
2015 - Present:	Member for National Alliance for Tobacco Free Thailand
2009 - Present:	Secretary for Srinakharinwirot University Dental Alumni Society
2007 - Present:	Board member for Thai Dentist Against Tobacco Project
2007 - Present:	President for Thai Dentist Student Against Tobacco Project
2007 - 2012:	Board member for Thai Happy Dental Happy Schools Project

Keynote Lectures

Innovation of Dental Education during COVID-19 pandemic in Japan

Shuji Awano, D.D.S., Ph.D.
Dean
Faculty of Dentistry
Kyushu Dental University

Dentistry education has changed according to the transformation of social needs. There are a variety of problems to need the solution in Japanese society, such as a falling birthrate, aging society and shrinking population. Therefore, the social needs to institution of higher education including our university is to foster human resources who can tackle real-world problems with diverse solutions.

Our university has done the review of educational curriculum and environment in recent years. The points of the review are the transformation to outcome-based education and the promotion of active learning in the educational process. We have started the utilization of ICT in lecture for interactive education system and improved the environment for active learning. The active learning is thought to be an important method as the learning strategy for the successful achievement of outcome-based education.

The spread of COVID-19 infection has influenced many activities in the education of university. However, we have an obligation to proceed without stopping the university education while taking measures according to the infection situation even if there is such a situation. The utilization of ICT in education has been essential for the infection control under the infection spread and practically introduced in many situations of the educational process. Consequently, the innovation of dentistry education has led to a result being promoted one step further under the COVID-19 pandemics.

Brief CV

EDUCATION

1986-1992: D.D.S., School of Dentistry, Kyushu Dental College, Japan
1992-1996: Ph.D., Graduate School of Dentistry, Kyushu Dental College, Japan

EMPLOYMENT

1996-1997: Dental Officer, Japan Air Self-Defense Force
1997-2000: Assistant Professor, Kyushu Dental College, Japan
2001-2014: Lecture, Kyushu Dental College, Japan
2015-present: Professor, Kyushu Dental University, Japan
2016-2017, 2019-2020: Vise Director of Kyushu Dental University Hospital, Japan
2020-present: Dean for Faculty of Dentistry, Kyushu Dental University, Japan

Special Lectures

A Life changing experience: the opportunities and challenges of study PhD in Japan

Wichida Chaweewannakorn, D.D.S., Ph.D.
Lecturer
Department of Pedodontics and Preventive Dentistry
Faculty of Dentistry
Srinakharinwirot University

Attending the PhD program at the Department of Developmental Stomatognathic Function Science, Kyushu Dental University is the incredibly valuable and life-changing experiences. While no international students experience in the same ways, education abroad provides me a career opportunity, a global perspective, a long-term partnership and useful clinical and research skills which is extremely rewarding to my entire life.

I was in the program that allowed me to do a joint PhD course between research and pediatric clinical skills. I had a great opportunity to expose to new research environment and methods as well as academics who have different perspective. Moreover, I also had a chance to participate in the international conferences which benefited me greatly including expanding my knowledge and improving my presentation and communication skill. It was not only just do research abroad, but to fulfil the other dimensions including culture, linguistic skill, friendship and personal development.

My research studies focused on the influence of enamel related gene products (ERPs), especially ameloblastin (Ambn) and enamelin (Enam) on osteoclastogenesis. In the past few decades, an enormous body of research on the tooth morphogenesis have provided strong evidence for an association of ERPs with amelogenesis. Indeed, ERPs have been known to contribute not only to enamel biomineralization, but also bone growth and development. In my study, we demonstrated that Ambn and Enam mediated osteoclastogenesis in both direct and indirect pathways. Firstly, Ambn and Enam suppress RANKL expression via down-regulation of ERK1/2 and p38 MAPK signaling pathways, resulting in the reduction in osteoclastogenesis in co-culture system between ST2 cells and bone marrow derived-macrophages. Subsequently, Ambn directly suppresses RANKL-induced osteoclastogenesis by modulating the NFATc1axis.

Brief CV

Education

2015-2018 Doctor of Philosophy in Dental science, Kyushu Dental University Fukuoka, Japan

2006-2012 Doctor of Dental Surgery (Second class Honors), Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand

Employment

2012- Lecturer, Department of Pedodontics and Preventive Dentistry, Faculty of Dentistry, Srinakharinwirot University

Special Lectures

Memoirs of Graduate School of Dentistry at KDU

Chuencheewit Thongsiri, D.D.S., Ph.D.
Lecturer
Periodontal Division
Department of Conservative Dentistry and Prosthodontics
Srinakharinwirot University

The topic of this lecture is “My memories in KDU” which is narrate about my experiences and the activities I have participated during 4 years I studied in Kyushu Dental University.

This lecture including my impressive view points as I am the one of foreigner student.

The 20-minute slides are presented with main content of my stories from the first time I came to KDU until completed Ph.D. program.

Brief CV

Assistant Professor Dr. Chuencheewit Thongsiri (Tip)

Lecturer in the Periodontal Division, Department of Conservative Dentistry and Prosthodontics, Srinakharinwirot University, THAILAND

Completed Graduate school in the Ph.D. program from Kyushu Dental University in March, 2021

Poster Session

※ indicates abstracts applied for the Best Presentation Award.

#1※

Analgesic mechanisms of long residual steroid ointment for oral mucositis

Mako Naniwa[1,2], Chihiro Nakatomi[2], Takuya Tabuchi[3], Sumio Akifusa[1], Kentaro Ono[2]

[1] Division of Oral Health Sciences, Kyushu Dental University

[2] Division of Physiology, Kyushu Dental University

[3] Daiichi Sankyo Healthcare Co. Ltd., Tokyo, Japan,

Oral mucositis is the most common oral disease and leads to pain during meals and speaking and reduce the quality of life of patients. Steroid ointments are commonly prescribed for treatment of oral mucositis. In spite of the long history of the drug usage, analgesic mechanism of steroid ointment in oral mucositis has not been studied in detail. In this study, we examined effects of triamcinolone acetonide with a long residual ointment on oral ulcer-induced pain by our proprietary assay system for conscious rats. From evaluations of physical properties and retention periods in the oral mucosa of human volunteers and rats, we selected the TRAFUL® ointment as a long residual ointment base. In oral mucositis model rats, two-times applications of triamcinolone acetonide with TRAFUL® suppressed inflammatory cell infiltration and prostaglandin E_2 in the ulcer region and inhibited spontaneous nociceptive behavior. The same treatment downregulated the inflammation-inducible genes of COX_2 and TNF-α and upregulated glucocorticoid receptor-inducible anti-inflammatory genes (GILZ, IRAKM and MKP1). Importantly, the coating of triamcinolone acetonide with TRAFUL® also improved oral mucositis-induced mechanical allodynia, which has been reported to be independently on cyclooxygenase. When a shorter residual ointment was used, these effects of triamcinolone acetonide were not elicited or less. In Ca^{2+}-imaging in dissociated trigeminal ganglion neurons, long-term pre-incubation with triamcinolone acetonide inhibited mechanically-activated neuronal response. These results suggests that the representative steroid triamcinolone acetonide suppress oral mucositis-induced spontaneous pain by general genomic actions following glucocorticoid receptor activation, and mechanical allodynia by inhibition of mechanical sensitivity in peripheral nerves. In the point of drug delivery, long residual ointments in the oral mucosa, likely TRAFUL®, is needed to sufficiently elicit the anti-inflammatory and analgesic efficacies. The new experimental condition using TRAFUL® ointment is useful to develop more effective local drug for oral mucositis and periodontal diseases.

#2※

Mechanically-activated PIEZO channels exert extracellular ATP release from human periodontal ligament fibroblasts

Seiwa Horie[1,2], Chihiro Nakatomi[1], Chia-Chien Hsu[1], Mako Naniwa[3], Aoi Morii[1,2], Tatsuo Kawamoto[2], and Kentaro Ono[1]

[1] Division of Physiology, Kyusyu Dental University

[2] Division of Orofacial Functions and Orthodontics, Kyusyu Dental University

[3]School of Oral Health Sciences, Kyusyu Dental University

In the periodontal ligament, extracellular ATP releases by mechanical force contributes initial orthodontic pain and persistent tooth movement via bone remodeling. However, mechano-receptor in periodontal ligament fibroblasts has not been revealed to date. PIEZO channels are recently-identified mechano-sensitive channels. In this study, we examined functional expression of PIEZO channels in human periodontal ligament fibroblasts (HPdLF).

In quantitative mRNA expression assay, PIEZO1 and PIEZO2 mRNAs were relatively higher and lower in HPdLF than human dorsal root ganglia and oral keratinocytes. Immunofluorescent study using PIEZO1 and PIEZO2 antibodies demonstrated protein expressions of the both channels in HPdLF. The PIEZO1 agonist Yoda-1 induced Ca^{2+} response in HPdLF and ATP releases, dose-dependently. The Yoda-1-induced ATP release was suppressed by the vesicular nucleotide transporter inhibitor clodronic acid, the pannexin 1 inhibitor probenecid and the connexin 43 inhibitor meclofenamic acid. In our developed cell pressure system using balance weights of 2 g, pressure stimulation induced ATP release from HPdLF. The pressure-induced ATP release was suppressed by clodronic acid, probenecid, meclofenamic acid, likely to Yoda-1, and also inhibited by ruthenium red and GsMTx-4, blockers for mechano-sensitive channels, involving PIEZO channels.

These results suggest that mechanically-activated PIEZO channels exert extracellular ATP release from HPdLF via vesicular storages and through pannexin and connexon routes, probably resulting in pain induction and bone remodeling.

#3※

Bacteria removal effect of cleaning fixed orthodontic appliances with neutral electrolyzed water

Yasuhiko Akama[1,2], Yuki Nagamatsu[2], Hiroshi Ikeda[2], Kayoko Kuroishi[1], Kaori Gunjikake[1],

Hiroshi Shimizu[2], Tatsuo Kawamoto[1]

[1] Division of Orofacial Functions and Orthodontics, Department of Health Promotion, Kyushu Dental University

[2] Division of Biomaterials, Department of Oral Functions, Kyushu Dental University

During orthodontic treatment with fixed appliances, it is not easy to clean narrow areas between bracket and wire and tooth surface around bracket for patients, especially pediatric patients. In order to construct a new simple and safe cleaning method, we focus on neutral electrolyzed water (NW) due to its wide antimicrobial spectrum and no negative effect on human enamel. In this study, the bacteria removal effect of cleaning treatment with NW to a metal bracket ligated to wire was examined to evaluate applicability in dental practice.

A Co-Cr alloy wire cut into 2 cm length was ligated to a metal bracket to prepare a specimen. The specimen was contaminated by 15-h immersion in a bacteria suspension (*Streptococcus mutans* in Brain Heart Infusion). After contamination, it was cleaned by brushing with an interdental brush, water flow washing or water jet washing with an oral irrigator using NW (NW_{30} or NW_{100}: 30ppm or 100ppm in available chlorine concentration). The treated specimen was ultrasonically cleaned in sterile phosphate-buffered saline for 5 min to collect bacteria, and each extract solution was added to an agar medium, and incubated for 48 h at 37°C. After incubation, the total number of surviving bacteria on each specimen was calculated from the colony forming unit on the agar plate (n=5). As comparisons, the specimens treated with tap water or commercial mouthwash, Listerine or Concool were also tested.

The number of surviving bacteria after water jet washing was significantly less than those after other treatments ($p<0.05$). The combination of NW (NW_{30} or NW_{100}) and water jet washing had a significantly higher bacteria removal effect than other combinations ($p<0.05$).

The water jet washing with NW showed an excellent ability to remove *Streptococcus mutans*. These findings suggest that NW might be applicable for cleaning fixed orthodontic appliances contaminated with oral bacteria.

#4※

Development of a novel training model tooth with grindability equivalent to human enamel

Jumpei Tokunaga[1,2], Hiroshi Ikeda[2], Yuki Nagamatsu[2], Shuji Awano[1], Hiroshi Shimizu[2]

[1]Division of Clinical Education Development and Research, Department of Oral Functions, Kyushu Dental University.

[2]Division of Biomaterials, Department of Oral Functions, Kyushu Dental University.

Clinical training of tooth preparation for dental students have been carried out using phantom system. In this system, training model teeth made from resin-based material have been widely used for past decades. However, grinding sensation of a commercial training teeth differs from that of natural human teeth. This difference makes dental students find it difficult to simulate real grinding sensation of tooth preparation. The purpose of this study is to develop novel training model tooth with grindability equivalent to human enamel.

The model teeth material was prepared in our laboratory by using the modified sol-gel process via sintering and polymer infiltration processes [1]. Enamel of human extracted wisdom teeth and commercial model teeth（A5AN-500-#36, Nissin Dental Products INC） were used as comparison samples. Vickers hardness of each sample was examined using a hardness tester. Grindability of each sample was evaluated by using the self-made grinding device equipped with a diamond point. Herein we define that the grindability is amount of volume loss by the grinding test. A questionnaire for grinding sensation of each sample was conducted on ten dentists who has been licensed for more than three years.

Vickers hardness of the prepared material (312±27) was closer to that of enamel (348±26) than that of commercial one (42±4). The grindability of the prepared material ($1.27\pm0.8 mm^3$) was close to that of enamel ($1.41\pm0.1 mm^3$) with comparison of that of commercial one ($11.11\pm3.5 mm^3$). The machinability of the prepared material and enamel did not change by increasing numbers of the test, while that of commercial one gradually decreased. The questionnaire showed that the grinding sensation of the prepared material was similar to that of enamel. In summary, the prepared material was equivalent to human enamel in terms of hardness and grindability.

Reference

[1] Kawajiri Y et al., Materials 14 (2021) 1182

#5※

The analysis of Mash1-expressing cell lineage in taste bud organoids

Kae Matsuyama, Shinji Kataoka, Takashi Toyono, Yuji Seta

Division of Anatomy, Department of Health Promotion, Kyushu Dental University

Taste buds, which are taste sensory end organs, are composed of several distinct type cells. Mash1 is a transcription factor and known efficient for differentiating stem cells into neurons. We demonstrated that Mash1 is expressed in subsets of mature taste cells and basal cells in adult taste buds. However, it remains unclear whether Mash1 regulates the differentiation of both type II and III or only type III taste cells. In this study, we explored the cell lineage of Mash1-expressing cells utilizing taste bud organoid culture.

To investigate from developing taste buds, we used Mash1-Cre^{ERT2} / CAG-floxed neo-tdTomato mouse line. In this mouse line, Mash1-expressing cells are labeled by tdTomato expression after administration of tamoxifen. We gave tamoxifen orally to pregnancy mice and collected newborn mice on postnatal day7. Immunostaining of circumvallate papillae showed that many of tdTomato+ cells coexpressed Car4 (type III taste cell marker) and a few of them coexpressed gustducin (type II taste cell marker) in initially developed taste buds of newborn mice. To trace Mash1-expressing cell lineage *ex vivo*, taste bud organoids were cultured from the transgenic mice. For Cre activation, we added hydroxy tamoxifen to culture medium including fresh cells at the first culture day. Immunostaining of tamoxifen-treated organoids showed that tdTomato+ cells coexpressed Car4 and a subset of them coexpressed gustducin. Furthermore, we made taste bud organoids from Mash1-Cre^{ERT2} / CAG-floxed neo-diphtheria toxin A (DTA) mice. After tamoxifen treatment, DTA leaded Mash1-expressing cells to cell death. And we found out that the generation of Car4+ cells were significantly suppressed in organoids lacking Mash1-expressing cells.

These results suggest that Mash1 may play a role in the differentiation of gustducin-expressing type II taste cells in addition to type III taste cells.

#6※

A novel three-dimensionally printable polymethyl methacrylate-based resin

Kentaro Hata [1, 2], Hiroshi Ikeda[2], Yuki Nagamatsu[2], Chihiro Masaki[1], Ryuji Hosokawa[1], Hiroshi Shimizu[2]

[1]Division of Oral Reconstruction and Rehabilitation, Department of oral functions, Kyushu Dental University

[2]Division of Biomaterials, Department of oral functions, Kyusyu Dental University

Polymethyl methacrylate (PMMA)-based resins have been used as a material of prosthesis for past many decades. Along with recent advance of CAD/CAM system, considerable effort has been paid to the research on specific materials for an additive manufacturing (3D-printing) process. However, a 3D-printable PMMA-based resin has not been developed yet. The purpose of this study is to develop a novel PMMA-based resin with printable and usable mechanical properties.

A liquid-PMMA precursor was prepared by mixing appropriate amount of PMMA powder, MMA, EGDMA and photo-initiator. Bar-shape specimens were printed by using a stereolithography 3D-printer with the prepared liquid-PMMA precursor. After the printing, the printed object was subjected to post-cleaning with MMA and post-curing using a light irradiator. The printed samples were characterized by three-point bending test, Vickers hardness test and shear bond strength test to a commercial auto-polymerizing PMMA-based resin.

Flexural strength of the printed sample containing 64 % EGDMA showed the highest in flexural strength (96.2 ± 12.9 MPa) among printed samples. Hardness of the 3D-printed PMMA increased with increasing of EGDMA content. As the result, the printed sample with 72% EGDMA showed the highest in Vickers hardness value (26.2 ± 4.3 HV). It was significantly higher than that of the commercial auto-polymerizing PMMA-based resin (12.5 ± 1.8 HV). Shear bond strength of the printed sample after the 10,000-times-thermocycling test gave 9–15 MPa, which was comparable to that of auto-polymerizing PMMA-based resin (15.6 ± 5.1 MPa). Failure mode of almost samples showed cohesive failure.

The novel PMMA-based resin was compatible to the commercial auto-polymerizing PMMA in flexural strength and shear bond strength, and was higher in hardness. The present PMMA-based resin has a potential to be applied to dental prostheses for 3D printing.

#7※

PLECTIN, a SRC oncogene-binding protein, promotes the growth and adhesion of malignant melanoma via regulation of SRC activity.

Kana Mizuta[1,2], Takuma Matsubara[1], William N. Addison[1], Tatsuki Yaginuma[3], Izumi Yoshioka[2], Shoichiro Kokabu[1]

[1] Division of Molecular Signaling and Biochemistry, Kyushu Dental University

[2] Division of Oral Medicine, Kyushu Dental University

[3] Division of Maxillofacial Surgery, Kyushu Dental University

【Objectives】

Malignant melanoma (MM), occurring in the oral mucosa and skin, metastasizes at an early stage leading to poor prognosis. The oncogene *Src* promotes proliferation, adhesion and invasion of MM. Drugs targeting SRC have side effects since SRC also plays essential roles in healthy tissue. Therefore, a better understanding of novel SRC targets is necessary. Recently, we identified plectin as a SRC-binding and SRC-activating protein. However, the role of plectin in MM is still unclear. In this study, we investigated the function of plectin in MM cells.

【Methods & Results】

NCBI GEO database showed that plectin expression levels were higher in MM than in normal skin. Plectin-deficient MM B16 cells (PKOs) were generated by CRISPR gene editing. PKOs displayed an elongated spindle shape with long actin fibers whereas control cells were more rounded with short actin fibers. Cell proliferation, measured by WST-8 assay, was lower in PKOs than in control cells. Expression level of *CyclinD1* was also elevated in PKOs compared to control cells. In an *in vivo* tumor formation assay, there was no difference between tumor volume of PKO cells or control cells. However, tumor density was reduced in PKO cells and H&E-stained sections of the tumors revealed that PKO cells had larger spacing between adjacent cells. Furthermore, in a spheroid formation assay, PKOs formed larger spheroids with a similar sparse cell-spacing phenotype. PKO cells also exhibited reduced cell adhesion in a fibronectin cell adhesion assay. By western blotting we observed that PKO cells have lower SRC activity than control cells. Finally, overexpression of SRC in PKOs restored the abnormal cytoskeleton morphology and reduced cell attachment.

【Conclusion】

These results suggest that plectin promotes the proliferation and adhesion of MM via regulation of SRC activity.

#8※

Nobiletin promotes osteoblast differentiation via suppressing NFkB signaling.

Thira Rojasawasthien[1,2], William Addison[2], Takuma Matsubara[2], Keisuke Nakashima[1], Shoichiro Kokabu[2]

[1] Department of Periodontology, Kyushu Dental University

[2] Division of Molecular Signaling and Biochemistry, Kyushu Dental University

Background

Inflammation can affect bone homeostasis by stimulating osteoclasts and inhibiting the function of osteoblasts. Periodontitis is one of the inflammatory bone diseases. NF-kB signaling induced by inflammatory cytokine such as tumor necrosis factor alpha (TNF-α) play important role in the development of periodontitis. Nobiletin (5,6,7,8,3,4 -hexamethoxy flavone), a citrus polymethoxy flavonoid, extracted from *Citrus depressa.* Previous studies have shown that Nobiletin exhibits anti-proliferative and apoptotic effects on various cancer cells and inhibits inflammation pathways. However, the effects of Nobelitin on osteoblastic differentiation and on bone formation have not been studied fully.

Methods

MC3T3-E1 cells at $5x10^4$ cells/cm^2 were cultured in osteogenic differentiation medium in various concentrations (0, 1, 5, 10 μM) of Nobiletin with TNFα (5ng/ml). The proliferation of MC3T3-E1 cells was assessed using Cell Counting Kit-8. Osteoblast differentiation was induced by treating cells with ß-glycerophosphate (5 mM) and L-ascorbic acid (50 μg/ml). Osteoblast differentiation was assessed by measurement of alkaline phosphatase (Alp) activity, alkaline phosphatase staining, Alizarin Red staining was used to detect the mineralized nodules. Also, quantification of the mRNA levels of osteoblast marker genes such as runt-related transcription factor 2 (*Runx2*), osterix (*Osx*), alkaline phosphatase (*Alpl*), osteocalcin (*Ocn*), collagen type I alpha(*col1a*) and Interleukin 6 (*IL-6*).

Result

ALP activity detection, Nobiletin had different reversal effects on reducing damage of MC3T3-E1 induced by TNF-α and 10 μM of Nobiletin had most improvement effect. Also, in ALP and Alizarin red showed staining deepened after adding Nobelitin. The expressions of the osteogenesis-related gene *ALP, OCN, Col1a* were increased by Nobiletin and decrease IL-6 at same concentration. TNF-α at high concentration decreased the expression of osteogenesis related gene, but we found that osteogenesis-related genes of MC3T3-E1 may be upregulated when exposed to Nobiletin.

Conclusion

Nobiletin could cancel the suppressive effect of TNF-α on osteoblast differentiation.

#9※

KIF22, a kinesin-like protein, is essential for cell proliferation in ATDC5 chondrocyte-like cells.

Hiroka Kawaue[1,2], Takuma Matsubara[1], William N Addison[1], Katsura Saeki[2], Koji Watanabe[2], Kenshi Maki[2], Shoichiro Kokabu[1]

[1] Division of Molecular Signaling and Biochemistry, Department of Health Promotion, Kyushu Dental University

[2] Division of Developmental Stomatognathic Function Science, Department of Health Promotion, Kyushu Dental University

Spondyloepimetaphyseal dysplasia with joint laxity type 2 (SEMDJL2[MIM 603546]) is characterized by a flat face, generalized joint laxity and a short stature with shortening of both trunk and limbs. SEMDJL2 is caused by point mutations in *KIF22*, a gene encoding a kinesin motor protein. The missense mutations occur in the motor domain of KIF22 and are considered to attenuate KIF22 function. Although the SEMDJL2 phenotype is evident in cartilage and bone, the physiological and pathological role of KIF22 is not well understood. Here in this study, we investigated the function of KIF22.

Quantitative PCR analysis of *Kif22* tissue distribution showed that *Kif22* was highly expressed in cartilage-rich femoral head and growth plate tissue. Moreover, KIF22 immunostaining detected high levels of KIF22 in proliferation-zone chondrocytes of mouse tibia. This data, taken together with the SEMDJL2 phenotype, suggests that KIF22 has a function in chondrocytes of the proliferating zone. Thus, we investigated the role of KIF22 in chondrocytes using ATDC5 cells, a pre-chondrocyte-like cell line. First, *Kif22* was knocked down in ATDC5 cells by shRNA and cell proliferation examined by WST-8 assay. The number of living cells was decreased in *Kif22* knock-down cells compared to control cells. We next performed LDH assay, TUNEL assay and immunostaining of cleaved caspase 3 to examine apoptosis. There was no significant difference in LDH levels nor the numbers of apoptotic cells between control and *Kif22* knock-down cells. Consistent with this, KIF22 overexpression increased the number of living cells compared to control cells.

These results suggest that KIF22 promotes chondrocyte cell proliferation. Therefore, the clinical abnormalities in SEMDJL2 relating to cartilage and limb hypoplasia may be due to defective chondrocyte proliferation resulting from KIF22 loss of function.

#10※

Short-time sterilization of the dental instruments by radical vapor reactor

Kaede Okita[1,2], Yohei Nakamura[2] , Ryota Yamasaki[2], Tatsuya Sakakura[3], Aki Kawano[4], Yoshiyuki Takatsuji[3], Tetsuya Haruyama[3], Yoshie Yoshioka[2], Wataru Ariyoshi[2]

[1]Department of Oral Health, Kyushu Dental University

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

[3]Division od Functional Interface Engineering, Department Biological Systems and Engineering, Kyushu Institute of Technology

[4]Department of Oral Health Sciences, Kobe Tokiwa University

Dental instruments such as dental bur, endodontic files, scalers , and dental vacuum suction are required complete sterilization because of protecting infection to the other patient. Generally, these instruments are sterilized by autoclave or ethylene oxide gas. However, these sterilization method takes long time, high energy, or producing hazardous waste. Therefore, short time, low energy, no hazardous waste, and perfect sterilization method are required at dental fields.

In this experiment, the caries-related bacterium *Streptococcus mutans* was used. A radical vapor reactor (RVR) can sterilization the bacteria by producing high concentration of reactive oxygen species. In this paper, we identified that RVR can sterilize effectively (i. e less than 10 min and green technology) the dental instruments. This system is used under room temperature and atmospheric pressure. Therefore, it is available to use at low cost, low energy, and low environmental load. Furthermore, we have demonstrated that even packed dental instruments can be completely sterilized in 10 minutes of RVR treatment.

RVR require only O_2 gas and, water, therefore it is low cost, low energy, and low environmental load. Hence, the sterilization technique that using RVR is effectiveness for dental field since the dental instruments that need sterilization are produced in large amount every day. This RVR sterilization technique can be applied in various, e. g, medical instruments such as a catheter and pacemaker, baby bottle and pacifier, and shoes sterilize and deodorize, so we propose a technique that can be executed in a short time with less impact on the environment and the usefulness of RVR sterilization.

#11※

A phosphatase regulatory protein PPP1r18 inhibits NFATc1 activation and osteoclast differentiation via c-fos dephosphorylation

Kazuma Yasuda[1,2], Takuma Matsubara[1], William Addison[1], Tatsuo Kawamoto[2], Shoichiro Kokabu[1]

[1] Division of Molecular Signaling and Biochemistry, Kyushu Dental University

[2] Division of Orofacial and Functional Orthodontics, Kyushu Dental University

Orthodontic tooth movement is induced by osteoclastic bone resorption on the compression side. Control of osteoclasts in orthodontic treatment is important in treatment outcome because too much activated osteoclasts resorb tooth root and alveolar bone and induce associated gingival recession. NFATc1, the essential transcription factor in osteoclastogenesis, is expressed and localized in nuclear by RANKL. After that, NFATc1 makes complex with the transcription factor c-fos and transcribe the target genes such as Acp5, Ctsk and also auto-regulate NFATc1 expression as well. We previously reported PPP1r18 inhibited attachment to bone matrix and subsequent bone resorption by mature osteoclasts. We also got the data PPP1r18 has potential to regulate NFATc1. To explore this, we studied the effect of PPP1r18 in NFATc1 expression and activation.

We stimulated the osteoclast precursor cell line, RAW264.7 cells by RANKL after PPP1r18 introduction and found NFATc1 expression level and number of osteoclasts were decreased by PPP1r18. We next examined PPP1r18 effect on NFATc1 transcription activity by determined NFATc1 target genes' mRNA expression by qPCR and luciferase activity responsible for NFAT response elements. PPP1r18 suppressed both mRNA expression including NFATc1 and luciferase activity upregulated by NFATc1 overexpression. On the other hand, PPP1r18 mutant that did not have phosphatase activity did not suppress NFATc1 activity and osteoclast differentiation. These results indicate phosphatase activity of PPP1r18 suppress NFATc1 activity. We next examined how PPP1r18 regulates NFATc1 transcription activity. PPP1r18 suppressed constitutively activated of NFATc1 that is always localize in nuclear. This suggests NFATc1 is not direct target of PPP1r18. Thus, we focused the coactivator of NFATc1, c-fos. PPP1r18 wild type suppressed c-fos phosphorylation and nuclear localization. Interestingly, overexpression of c-fos canceled the suppression of NFATc1 activity and osteoclast differentiation by PPP1r18.

Altogether, PPP1r18 suppresses osteoclast differentiation by disturbing NFATc1 and c-fos complex through dephosphorylation of c-fos.

#12※

Mandibular deformation in edentulous patients treated with implant supported fixed prosthesis.

Sirapat Thongpoung[1], Chihiro Masaki[1], Tomotaka Nodai[1], Takashi Munemasa[1], Taro Mukaibo[1], Yusuke Kondo[1], Ryuji Hosokawa[1]

[1] Department of Oral Reconstruction and Rehabilitation, Kyushu Dental University Kitakyushu City, Fukuoka, Japan

Abstract

Purpose: Mandibular deformation during mouth opening has been reported in dentate patients; however, the incidence in patients with edentulous mandible requiring implants has not been clarified. This study examined mandibular deformation during mouth opening in edentulous patients treated with implant-supported fixed prosthesis using strain gauges to identify factors that affect deformation.

Methods: A total of 20 patients with fully edentulous mandible who required either 4 or over 6 implants were included. In patients with 4 implants (n = 13), the most distal implants were placed between mental foramen (premolar area). In patients with 6 or more implants (n = 7), the most distal implants were placed distal to mental foramen (Molar area). Mandibular deformation during mouth opening was measured in 2 directions with strain gauges; specifically, it was measured in the anteroposterior direction as well as lateral direction between the most distal implants in the left and right sides (arch width). Mandibular anatomy was also measured using computed tomography data.

Results: Arch width reduction during mouth opening ranged from 47.38 - 512.80 μm. The mean values were 100.07 μm in premolar area and 286.05 μm in molar area. Deformation range was 0.12 - 15.14 μm in the anteroposterior measurement direction. Furthermore, the degree of deformation was significantly greater in the molar region than in the premolar region in both directions ($p < 0.01$). Furthermore, a significant positive correlation was noted between arch width reduction in the premolar area and the ratio between symphyseal bone height and width ($p = 0.0003$, $r = 0.72$).

Conclusions: We demonstrated that reduction of arch width was greater in the molar area than in the premolar area in patients with edentulous mandible. Our findings indicated that mandibular arch width reduction during mouth opening may be greater in the mandibular symphyseal bone shape as it is longer and thinner.

#13※

Molecular mechanism of -glucan-induced suppression of NFATc1 expression in osteoclasts

Ayaka Koga[1, 2], Yoshie Nagai-Yoshioka[2], Ryota Yamasaki[2], Wataru Fujii[3], Wataru Ariyoshi[2]

[1] School of Oral Health Sciences, Kyushu Dental University

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

[3] Unit of Interdisciplinary Education School of Oral Health Sciences Faculty of Dentistry Kyushu Dental University

Immunoreceptors expressed on osteoclast precursor cells modulate osteoclast differentiation and bone resorption activity. Dectin-1 is a lectin receptor for β-glucan and is specifically expressed on osteoclast precursor cells. Our previous studies demonstrated that curdlan inhibits nuclear factor of activated T cells (NFATc1) expression in a dectin-1-dependent manner and inhibits osteoclast differentiation induced by receptor activator of nuclear factor-kappa B (NF-kB) ligand (RANKL). In this study, we investigated the molecular mechanism by which curdlan suppresses NFATc1 expression suppression.

First, we focused on the transcription factor B lymphocyte induced maturation protein 1 (Blimp-1), which represses the negative regulators of NFATc1 transcription such as B cell lymphoma 6 (Bcl6), interferon-regulatory factor 8 (IRF8) and V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MafB). Real-time PCR analysis revealed that Blimp-1 expression in osteoclast progenitor lineage RAW264.7 is overexpressing the dectin-1 receptor (d-RAW cells) was not repressed by administration of curdlan suggesting that down-regulation of NFATc1 by curdlan was not dependent on Blimp-1 expression.

Next, we examined the effect of curdlan on the activation of NF-kB signaling induced by RANKL. NF-kB is important for the initial induction of NFATc1 induced by RANKL. Westen blotting analysis revealed that RANKL-induced NF-kB p65 translocation into the nucleous in d-RAW cells was suppressed by curdlan. To evaluate whether this inhibitory effect depends on dectin-1 expression, we examined vector control cells (c-RAW cells) in comparison with d-RAW cells. Surprisingly the inhibitory effect of curdlan on NF-kB activation in c-RAW cells was comparable to d-RAW cells, suggesting that inhibitory effect of curdlan on NF-kB activation is not dependent on the interaction with dectin-1.

From these results, we plan to investigate which receptors are responsible for the inhibitory effect of curdlan on osteoclastgenesis.

#14※

The relationship between the dynamics of TNF-α and its soluble receptors in saliva and periodontal health state

Kibune R.[1], Muraoka K.[1], Tokunaga J.[1], Morishita M[1], Awano S.[1]

[1]Division of Clinical Education Development and Research, Kyushu Dental University

[Introduction] Soluble tumor necrosis factor receptor type 1 and 2 (sTNFR-1 and -2) are reported to protect during clinical inflammation against excessive TNF-α, a primary mediator of systemic responses to infection. In this study, sTNFR-1 and -2 in saliva aimed to verify whether to be related to the regulation of TNF-α during inflammatory periodontal diseases.

[Materials & Methods] The study population consisted of 28 adult patients (11males and 17females), who visited the Kyushu Dental University Hospital. Their average age was 64.7 ± 15.8 years (mean ± SD). Periodontal examination, such as probing pocket depth (PPD), clinical attachment level (CAL), and bleeding on probing (BOP), were performed. Periodontal inflamed surface area (PISA) of each subject was calculated using CAL, gingival recessions and BOP. Before the periodontal examination, samples of stimulated saliva were collected by chewing sugar free and odorless gum. Levels of TNF-α, sTNF-R1 and sTNF-R2 in saliva samples were determined by ELISA kits. Additionally, levels of total protein (TP) in each saliva samples was determined by using protein assay kit.

[Results] The proportion of TNF-α, sTNF-R1, and sTNF-R2 against TP (/TP) in saliva had a significant correlation with each other (the Spearman correlation analysis). Additionally, there was a significant positive correlation between PISA and sTNF-R1/TP, and sTNF-R2/TP in saliva. The stepwise multiple regression analysis revealed that PISA was significantly associated with sTNF-R2/TP in saliva. Furthermore, TNF-α/TP was not significantly associated with PISA.

[Conclusion] This study suggested that the expansion of periodontal inflammation may induce the increase of sTNF-R1 and R2 in saliva, and that induced sTNF-R may be associated with regulation of TNF-α.

#15※

Specific role of *Msx1* during mouse mandibular development

Saori Shibuya[1,2], Mitsushiro Nakatomi[2,3], Kae Matsuyama[2], Shinji Kataoka[2], Takashi Toyono[2], Yuji Seta[2], Tatsuo Kawamoto[1]

[1] Division of Orofacial Functions and Orthodontics, Kyushu Dental University

[2] Division of Anatomy, Kyushu Dental University

[3] Department of Human, Information and Life Sciences, University of Occupational and Environmental Health

In the process of maxillofacial development, the right and left mandibular processes grow forward and fuse at the midline of the tip. Failure of this fusion process can result in a midline cleft of the lower lip and/or the mandible in a severe case or so-called split jaw in a mild case. However, its molecular mechanisms have been largely unknown. The *Msx1* gene, which encodes a homeobox-type transcription factor, plays an important role in craniofacial development, including the lip, jaw, palate, and teeth. *MSX1* mutations in humans are known to be responsible for cleft lip, palate, and congenital defects of teeth. However, the function of *Msx1* in the formation of the median mandible has not been fully examined in experimental models. Therefore we aimed to elucidate the role of *Msx1* in the fusion of the mandible in this study.

Msx1$^{+/-}$ male and female mice were mated and fetuses were sampled from embryonic day (E) 11 to newborn. The normal expression patterns of *Msx1* during mandibular development were analyzed by whole-mount *in situ* hybridization and X-gal staining. Morphological phenotypes of *Msx1*$^{-/-}$ mice were compared with wild-type littermates by external observation using stereo-optical microscopy and scanning electron microscopy as well as histological observation using frontal sections.

Msx1 was strongly expressed in the tip of the developing mandible. In the control group (wild-type and *Msx1*$^{+/-}$), the median tip of the mandible was fused and pyramidal, whereas the same region was not fused and remained bifurcated in *Msx1*$^{-/-}$ fetuses. Histological analysis revealed that Meckel's cartilage was also unfused and bifurcated in *Msx1*$^{-/-}$.

These results suggest that the function of *Msx1* is critical for the normal fusion process of the right and left mandibular primordia. In humans, mutations of *MSX1* may affect the proper formation of the median mandible.

#16

Differences in the factors associated with tongue pressure between Japanese children with Class I and Class II malocclusions

Yuko Fujita[1], Kenshi Maki[1]

1 Division of Developmental Stomatognathic Function Science, Department of Health Promotion, Kyushu Dental University

The relationship between tongue pressure and masticatory performance in cases of Class II malocclusion during the mixed dentition period has not been clarified. The aim of this study was to determine differences in tongue pressure-related factors, including maxillofacial morphology and masticatory performance, between Class I and Class II malocclusions during the mixed dentition period.

A total of 56 children with Class I malocclusion (12 boys, 16 girls) or Class II malocclusion (16 boys, 12 girls) with mixed dentition were included in the present study. Height, body weight, hand grip strength, maximum tongue pressure, masticatory performance, and the number of decayed, missing, and filled teeth were measured in all participants. Their lateral cephalograms were also evaluated. The means of all measurements were compared between Class I and Class II malocclusions. Pearson's correlation coefficients were used to determine associations between maximum tongue pressure and other variables for each type of malocclusion. The maximum tongue pressure and hand grip strength in the Class II malocclusion group were significantly lower than those in the Class I malocclusion group (both, $p < 0.05$). The maximum tongue pressure was significantly positively correlated with hand grip strength, masticatory performance, and SNB (sella, nasion, B point) angle in the Class I group (all, $p < 0.05$), and with height, body weight, and labial inclination of the central incisors in the Class II group (all, $p < 0.05$).

The maxillofacial morphometric factors associated with tongue pressure were clearly different between cases of Class I and Class II malocclusion with mixed dentition. Masticatory performance and tongue pressure were significantly positively correlated in cases of Class I malocclusion, but not in cases of Class II malocclusion.

#17

Disinfection effects of oral care gel easily prepared by high-concentration neutral electrolyzed water

Yuki Nagamatsu[1], Hiroshi Nagamatsu[2], Hiroshi Ikeda[1], Hiroshi Shimizu[1]

[1]Division of Biomaterials, Department of Oral Functions, Kyushu Dental University

[2]Division of Comprehensive Dentistry, Department of Oral Functions, Kyushu Dental University

Several types of hypochlorous acid water prepared by electrolysis (electrolyzed waters for disinfection) have been used in dental practice due to their high microbicidal effect and biological safety. We especially focus on neutral type, namely neutral electrolyzed water (NW), because of no affection to human enamel and less metal corrosion compared with acidic types. For utilizing its high antimicrobial effect more widely in oral care treatment, we prepared three NW-based gels (NWGs) with different concentrations using a high-concentration NW (approximately 1000ppm) and agar powder which was chosen for its availability as well as easy handling, and evaluated their bactericidal activity and stability of disinfection components.

NWGs (NWG_{10}, NWG_{30} and NWG_{70}) with varying available chlorine concentration (10, 30 and 70ppm) and distilled water gel (DWG) were prepared with NW (150ppm) and/or distilled water (DW) by cooling after mixing with agar sol. Each gel (9.0 mL) was contacted with 1.0 mL of bacterial suspension (*Staphylococcus aureus* or *Streptococcus mutans*, 2×10^7/ml of PBS) by mixing them, after 3-min contact, total number of the surviving bacteria in each gel was examined with the agar plate method.

NWGs having 30ppm or more of available chlorine showed significantly higher bactericidal activities than NWG_{10} and DWG to both bacteria strains ($p<0.05$, *Tukey* test *after one way ANOVA*) at the preparation day without decrease of available chlorine concentration. After 4-week storage, NWG_{30} could not maintain its bactericidal activity due to decrease or almost loss of all available chlorine concentration; however, NWG_{70} maintained high bactericidal activities even if its concentration decreased to 15ppm by storage.

It is suggested that a gel easily prepared utilizing solation of agar with a neutral hypochlorous acid water might be applicable for a dental gel due to maintaining its bactericidal activity for a certain period by controlling its available chlorine concentration.

#18

Promoter analysis for human *TAS1R1* umami receptor gene in the human fungiform taste cells

Takashi Toyono[1], Yui Obikane[2], Kae Matsuyama[1], Shinji Kataoka[1], Ryuji Hosokawa[2], Yuji Seta[1]

[1] Division of Anatomy, Department of Health Promotion, Kyushu Dental University

[2] Division of Oral Reconstruction and Rehabilitation, Department of Oral Functions, Kyushu Dental University

The TAS1R family (TAS1R1, TAS1R2 and TAS1R3 receptors) has a role in the detection of sweet and umami tastes in taste buds. The heterodimer of TAS1R1 and TAS1R3 plays a role as an umami receptor. The functions of these receptors as the taste receptors have been revealed. However, the mechanisms of transcriptional regulations of TAS1R family have not been elucidated. In this study, we examined the function of *TAS1R1* promoter in the human primary fungiform taste cells.

The 5'-rapid amplification of cDNA ends (RACE) analysis was performed to determine the transcription start sites (TSSs) of the *TAS1R1* gene. The amplified fragments acquired by 5'-RACE were subcloned and sequenced. Sequencing of 18 clones identified six distinct sites for the initiation of *TAS1R1* transcripts, starting at positions 35, 37, 57, 61, 65, and 67 bp upstream of ATG in the first exon. These sites were designated Site 1–6, respectively. Site 5 is the major TSSs, represented in the 6 clones. Among these sites, Site 5 was indicated as +1. The initiator lies around the TSS. The downstream promoter element (DPE) lies 30 bp downstream of the TSS. For Site 5, the initiator and DPE were found between −2 to +33. Luciferase reporter assays showed that a 201-bp region upstream of the ATG start codon of *TAS1R1* had a promoter activity. GT box is the recurring motif of SP/KLF family members in promoters. Site-directed mutagenesis of GT box in *TAS1R1* promoter significantly reduced promoter transactivation. The GT box in the *TAS1R1* promoter was conserved in the many mammalian species. These results show that the GT box in the *TAS1R1* promoter plays a role in the transcriptional activation of *TAS1R1* promoter.

#19

The gubernaculum tracts in maxillary anterior teeth and specialties in mesiodens on CT

Masafumi Oda, Tatsurou Tanaka, Shinobu Matsumoto-Takeda, Nao Wakasugi-Sato, Yutaro Nagasaki, Yasuhiro Morimoto

Division of Oral and Maxillofacial Radiology, Kyushu Dental University

Objectives: To evaluate the characteristics of the gubernaculum tracts in anterior maxillary teeth on the images of multidetector computed tomography and cone beam computed tomography. And the secondary objective was to elucidate the distinctive characteristics of the gubernaculum tracts in mesiodens.

Study Design: The characteristics of gubernaculum tracts in anterior maxillary teeth were retrospectively analyzed by using multidetector computed tomography and cone beam computed tomography. The gubernaculum tracts of the anterior teeth and the gubernaculum tracts of mesiodens were examined. The parameters studied were detection ratio, long axis, short axis, angle against the tooth axis and connecting area of the gubernaculum tracts.

Results: The detection ratio of gubernaculum tracts in impacted mesiodens was significantly lower than in regular teeth. The average sizes of the gubernaculum tracts were elucidated. Most of the detectable gubernaculum tracts in the inverted mesiodens were derived from the incisive canal. In contrast, all of the regular teeth and mesiodens except for inverted were from the alveolar crest. Most of the connecting area of major gubernaculum tracts to tooth was crown area, but those of inverted mesiodens was the cervical or root area.

Conclusions: We exhibited the standard sizes of the gubernaculum tracts of the anterior maxillary teeth and the specialties of the gubernaculum tracts of mesiodens.

#20

Development of a Novel Filler-Dispersed Resin Composite for 3D Printing

Pirat Karntiang[1,2], Hiroshi Ikeda[1], Yuki Nagamatsu[1], Hiroshi Shimizu[1]

[1] Division of Biomaterials, Department of Oral Functions, Kyushu Dental University

[2] College of Dental Medicine, Rangsit University

Objective: This study aimed to develop a novel filler-dispersed resin composite for 3D printing. Mechanical properties (MP) and physicochemical properties (PP) were evaluated.

Materials and Methods: Precursors for additive manufacturing were prepared via mixing TEGDMA-UDMA (1:1 ratio), photo-initiator (BAPO) and silica filler according to the experimental group. There were 2 types of filler and 4 percentages for each filler, (A) microfiller: 20%; 40%; 60%; and 70%, (B) nanofiller: 10%; 20%; 30%; and 40%. Unfilled resin was used as a control. Samples were fabricated by 3D printer (n=36) and divided into 3 subgroups: (a) immediately received MP (flexural strength (FS), flexural modulus (FM) and Vickers hardness (VH)) evaluation; (b) immersed in water for 2 months before MP evaluation; and (c) received PP (two-month water sorption (WS), water solubility (WL)) evaluation. Data were analyzed by one- or two-way ANOVA followed by Tukey's test ($p = 0.05$).

Results: For both filler types, higher filler-content groups demonstrated better MP and PP. Conversely, high filler precursors also had high viscosity, making the precursors difficult to manipulate and creating voids in the printed specimens. For microfiller, 60% and 70% groups demonstrated best MP and PP. For nanofiller, 30% and 40% groups demonstrated best MP and PP. Water-immersion decreased FS and VH, but not FM for both filler types. Comparing between same filler-content groups, microfiller groups exhibited higher VH, lower FM and WS than nanofiller groups, but FS was not significantly different. Overall, 70%-microfiller group had the best MP (FS = 178.59±14.9MPa, FM = 7.83±1.34GPa and VH = 82.11±1.94) and PP (WS = 19.5±0.81μgmm^{-3}, WL was not detected) among all groups.

Conclusion: Microfiller groups showed comparable or better properties than nanofiller groups. For both types of filler, higher filler content tends to demonstrate better mechanical and physicochemical properties. This material seems promising and is worth further research and development.

#21

Magnesium hydroxide nanoparticles kill *Escherichia coli* exponential or persister cells physically

Ryota Yamasaki [1], Yohei Nakamura [1], Kaede Okita [1], Daisuke Kudo [2], Dao Nguyen Duy Phuon [2], Yoshihito Iwamoto [2], Yoshie Yoshioka [1], Wataru Ariyoshi [1]

[1] Division of Infections and Molecular Biology, Department of Health Promotion, Kyushu Dental Uni-versity, Kitakyushu, Fukuoka 803-8580 Japan

[2] Kyowa Chemical Industry Co., Ltd., Hayashida-cho, Sakaide, Kagawa, 762-0012 Japan

Abstract (12pt, 300 words or less)

Magnesium hydroxide nanoparticles are widely used in medicinal and hygiene products because of their low toxicity, environmental friendliness, and low cost. Here, we studied the effect of the size of magnesium hydroxide nanoparticles on their antibacterial activity using nanoparticles of three different sizes NM80, NM300, and NM700. NM80 (D50 = 75.2 nm) showed higher bactericidal effect against Escherichia coli than larger nanoparticles (D50 = 328 nm (NM300) or 726 nm (NM700)). Moreover, NM80 showed a high bactericidal effect against not only exponential cells but also persister cells, which are difficult to eliminate due to their high tolerance to antibiotics. NM80 killed strains in which the magnesium transport genes were knocked out, and its bactericidal effect was similar to that on the wild type. This ruled out the involvement of chemical bactericidal action. The physical bactericidal action was confirmed using scanning electron microscopy, which showed that *E. coli* cells treated with NM80 were directly injured.